Effect of *Choerospondias axillaris* (Roxb.) B. L. Burtt & A. W. Hill (Lapsi fruit) on growth, biochemical and immuno-haematological performance in fishes

A THESIS SUBMITTED TO THE
CENTRAL DEPARTMENT OF ZOOLOGY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY
NEPAL

FOR THE AWARD OF
DOCTOR OF PHILOSOPHY
IN ZOOLOGY

BY

SHUBHA RATNA SHAKYA

APRIL-2019

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DECLARATION

Thesis entitled "**Effect of *Choerospondias axillaris* (Roxb.) B. L. Burtt & A. W. Hill (Lapsi fruit) on growth, biochemical and immuno-haematological performance in fishes**" which is being submitted to the Central Department of Zoology, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Prof. Dr. Shyam Narayan Labh, Head (RMC), Department of Zoology, Amrit campus, Tribhuvan University.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

……...……

Shubha Ratna Shakya

RECOMMENDATION

This is to recommend that **Shubha Ratna Shakya** has successfully carried out his final research work entitled "**Effect of *Choerospondias axillaris* (Roxb.) B. L. Burtt & A. W. Hill (Lapsi fruit) on growth, biochemical and immuno-haematological performance in fishes**" for the award of Doctor of Philosophy (Ph.D.) in **Zoology** under my supervision. To my knowledge, this work has not been submitted for any other degree.

He has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph. D. degree.

…….....

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April 2019

LETTER OF APPROVAL

Date: 10 April, 2019

On the recommendation of Prof. Dr. Shyam Narayan Labh, this Ph. D. thesis submitted by Shubha Ratna Shakya, entitled "**Effect of *Choerospondias axillaris* (Roxb.) B. L. Burtt & A. W. Hill (Lapsi fruit) on growth, biochemical and immuno-haematological performance in fishes**" is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, Tribhuvan University.

.....

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Date: 10 April, 2019

ABSTRACT

Aquaculture is probably the fastest growing food-producing sector that accounts for 50% of the world's fishes that are used for food. Fish contributes over 20% of the animal protein intake for more than 2.6 billion people around the world. Hence, fish and fisheries make a major contribution to nutritional security and the fight against hunger and poverty in Asia. Immunostimulants are attractive substances that activate the immune system to prevent diseases and improve the body's natural resistance to various viral and bacterial infections. These biologically active substances are products derived from natural sources or are synthetically made with different chemical properties and mechanisms of action. Lapsi, *Choerospondias axillaris*, is an indigenous fruit tree of Nepal found growing within 900-2000 m above sea level in many parts of the country. Nepal is unique for processing and use of Lapsi fruits, which are rich in vitamin C content. Lapsi fruits are consumed fresh, pickled and processed for preparing a variety of sweet and sour, tasty food products locally called Mada and candy. It is grown in 301 Village Development Committees of 29 hill districts of Nepal for socio-economic purposes. Like other medicinal fruits, lapsi also acts as immunostimulants and enhances the immunity of fish in aquaculture.

Considering Lapsi's potential to boost immune response in fishes, three experiments were designed to investigate the effect of *Choerospondias axillaris* (Roxb.) B. L. Burtt & A. W. Hill (Lapsi fruit) on growth, biochemical and immuno-haematological performance in three fish species: indigenous major carp rohu *Labeo rohita,* common carp *Cyprinus carpio,* and rainbow trout *Oncorhynchus mykiss* under three climatic conditions, namely Gunjnagar, Chitwan for Tarai climate, Kathmandu Valley in the Aquaculture Research Laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur for hilly climate and Sosod Trout Farm at Ranipauwa, Nuwakot, Trishuli, Nepal, for Himalayan climate, respectively. In each experiment six different doses of lapsi-incorporated diets were prepared as T1, T2, T3, T4, T5 and T6 containing 0, 100, 200, 400, 800 and 1600 mg kg^{-1} ethanol extract of lapsi fruits along with other usual ingredients (i.e., fish meal, wheat flour and cod liver oil). Fish were fed at 3% of their body weight daily at 9 a.m. and 4 p.m. for 90 days and at the end survival rate, growth performances (i.e., weight gain, SGR and FCR) were measured. Total protein, albumin, globulin and A/G ratio in blood serum, brain and muscles were quantified. To understand the haemato-immunological parameters, SGOT, SGPT and ALP in the liver and gills and complete blood profile were monitored. The weight gain and SGR increased as the doses of lapsi fruit extract increased while the reverse was found for FCR. As the doses of

lapsi fruit extract increased in the diets, SGOT, SGPT and ALP levels decreased in all three experiments. Blood profile was always in the normal range. In conclusion, the 400 mg kg^{-1} ethanol extract of lapsi fruits-incorporated diet was found to be beneficial for fish growth, enhanced immunity and low mortality. Lapsi's vitamin C content might be the main contributor in enhancing the quality of fish feed, thereby resulting in favorable fish performance. Thus, farmers can use lapsi-supplemented fish feed, particularly during climate change, to maximize production of local fish species. In Nepal, aquaculture is still in its infancy, but has huge potential for development. However, information regarding the effect of lapsi on various species of fish in Nepal and other countries is limited.

LIST OF ACRONYMS AND ABBREVIATIONS

AA	Ascorbic acid
A/G	Albumin globulin ratio
AGDP	Agriculture Gross Domestic Product
ALP	Alkaline phosphatase
AST	Asperate amino transferase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
BHT	Butylatedhydrotoluene
CMC	CarboxymethlylCellulose
DM	Dry Matter
DO	Dissolved Oxygen
DMRT	Duncan's multiple range test
DNPH	2, 4-dinitrophenylhydrazine
DPPH	2, 2, diphenyl-1 picryl-hydrazyl
EDTA	Ethylene diethyl tetra acetic acid
FCR	Feed Conversion Ratio
SGR	Specific Growth Rate
GLO	Gulonolactone oxidase
HMG/N	His Majesty Government of Nepal
LDH	Lactate dehydrogenase
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
pH	Pouvoir hydrogen (Power of hydrogen)
NFE	Nitrogen-free extract
NRC	National Research Council
PBS	Phosphate Buffer Saline
PCV	Packed cell volume
PER	Protein efficiency ratio
PNPP	p-Nitrophenyl Phosphate
Pg	Picogram

LIST OF SYMBOLS

@	at the rate of
µ	micro
×	multiplication
<	less than
º	degree
%	percent or percentage
&	and
=	equal to
/	per
±	plus/minus
♂	male
♀	female

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CHAPTER 1

1. INTRODUCTION

1.1 Background

Aquaculture is the farming of all forms of aquatic animals and plants in fresh, brackish and marine environments under controlled conditions. It is the fastest-growing food-producing sector of agriculture worldwide that provides half of all fish food for human consumption (Allison, 2011). Fisheries remain important sources of food, nutrition, income and livelihoods for millions of people around the world (FAO, 2014). However, world per capita fish supply has outpaced population growth and reached a record high of 20 kg in 2014 (double the level of the 1960s), due to vigorous growth in aquaculture (Troell *et al.,* 2013). In the last two decades, dramatic growth in aquaculture production has boosted average consumption of fish and fishery products worldwide (FAO, 2016).

1.2 Aquaculture in Nepal

Aquaculture in Nepal started in the mid 1940s on a small scale with pond culture of indigenous major carp seed from India (FAO, 2016). Since then, significant progress has been made in the field of aquaculture with every passing decade. Briefly, successful farming of Common carp *Cyprinus carpio* took place in the 1950s and 1960s, of three exotic Chinese carp species: silver carp *(Hypophthalmichthys molitrix*), bighead carp *(Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idellus*) in the 1970s, of three indigenous major carps: rohu *(Labeo rohita),* mrigal (*Cirrhinus mriga*la) and catla (*Catla catla*) in the 1980s, and of Silver barb in the 1990s. Rainbow trout (*Oncorhynchus mykiss*), a cold-water fish, was introduced in the 1960s, and reintroduced in 1970s, and 1980s.

At present, approximately 5% of Nepal is occupied by different freshwater aquatic habitats where 239 fish species are reported to thrive (FishBase, 2018). Among the 239 fish species, 224 are indigenous and 15 are exotic. Freshwater fisheries are mostly dominated by capture fisheries of indigenous fish species from rivers, lakes and paddy fields. Majority of pond fish production takes place in the Terai plain where 94% of the fish ponds are located.Tribal groups such as Tharu, Majhi, Malaha, Dunuwar, Kewat, Bote, Musahar, Mukhiya, Darai, Kumal, Dangar, Jalari Rai and other poverty-laden tribes are traditionally

involved in capture fishery for their livelihood and food sources (Gurung *et al.,* 2005; Dahal *et al.,* 2013).

Fish farming has traditionally been practiced by a few tribal groups in Nepal. Aquaculture in Nepal started in the mid 1940s on a small scale with pond culture of indigenous major carp seed from India (FAO, 2016). Common carp *Cyprinus carpio* was first introduced in the 1950s and its farming became very popular in the private sector after its successful breeding in the 1960s. Major progress in aquaculture was made in the 1970s with the introduction and farming of three exotic Chinese carp species: silver carp *(Hypophthalmichthys molitrix*), bighead carp *(Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idellus*). These carps were successfully breeded in captivity. Similarly, the successful induced breeding of three indigenous major carps: rohu *(Labeo rohita)*, mrigal (*Cirrhinus mriga*la) and catla (*Catla catla*) were done in the 1980s under Aquaculture Development Project supported by the Asian Development Bank (ADB) and the United Nations Development Programme (UNDP).

1.3 Aquaculture Production Statistics in Nepal

Aquaculture contributes 2.68% to Nepal's Agricultural Gross Domestic Production (AGDP). Rivers and streams are major sources of capture fishery covering 395,000 ha of natural water resources in the country (DoFD, 2003-2004). Around 5, 83, 467 people are directly engaged in aquaculture and capture fishery with net fish production of 83,897 MT (aquaculture practices 62,897 MT and capture fisheries 21,000 MT) in the year 2016. Cold water fish production from race way ponds stands about 300 MT (DoFD, 2017). The Government of Nepal identified fisheries/aquaculture as one of the prominent sub-sector for poverty reduction in Nepal and aims to make Nepal a self-sufficient country in fish production. To achieve this ambitious target, the current production level needs to increase by many folds.

1.4 Trade and Economy

Most fish produced are sold as fresh fish in market while remaining is preserved by smoking and sun drying. Fish consumption trend is increasing in Nepal day by day. The present consumption of fish is 2.96 kg per capita in Nepal (DoFD, 2015-2016). Price of exotic rainbow trout is comparatively higher than local species (Rai *et al*., 2008). There is

high demand of fish in the market but the country's internal production is not sufficient to meet the demand. Thus, most of the fishes are imported from India and China (Dahal, 1998). Fish market infrastructure is available in few cities including Kathmandu. Certification procedure for fresh fishes and labeling systems are not developed but monitoring is done by Municipality consumer's forum and the Department of Food Technology and Quality control on a random basis.

1.5 Management Issues and Policies

Fisheries and aquaculture have high growth potential but there is low organizational stature in Nepal. The modern aquaculture and capture fishery practices contribute nearly 1.00 % of the Gross Domestic Production (GDP) and 2.68 % of the Agriculture GDP (Gurung *et al*., 2005). This positive achievement of the sub-sector suggests its popularity among farmers. Similarly, Government of Nepal has recognized the importance of fisheries and aquaculture for nutritional supply and poverty reduction. The primary objective of the national fisheries and aquaculture policy is to contribute economic growth and poverty reduction through inclusive, equity-based and Ecosystem Approach of Aquaculture (EAA). Specific laws and legislation on aquaculture development have to be formulated or enforced to build capacity and facilitate entrepreneurship, especially in context of the World Trade Organization (WTO). Besides, Best Management Practices also need to be identified and adopted to achieve sustainable growth (Gurung *et al*., 2012). Development, production strategies and policies for aquaculture are strongly affected by the country's budgetary plan, which targets production and gross domestic production (GDP) (FAO, 2006).

1.6 Challenges in Fish Production

Aquaculture is the main source of livelihood for many low-income families in Nepal (Shakya and Labh, 2014). Aqua farmers are facing challenges of disease outbreaks such as bacterial, fungal, viral and protozoans (Labh *et al*., 2014). Thus, farmers are in great loss due to exposure of fish in culture to pathogens and ineffective prophylactic treatment with antibiotics (Wattes *et al*., 2001). Thus, expected net production is low and is unable to fulfill the market demand. Lack of skilled and experienced manpower, poor management and unhygienic practice of aquaculture are major causes of disease outbreak in fishes (Gurung and Basnet, 2003). Low monetary investment, lack of good quality cage and net materials (Gurung *et al*., 2005) are other challenges of aquaculture in Nepal. Disease like fin rot,

hepatoma, fungal problems in fertilized eggs, presence of watery fluid in stomach and physical disorders like blunt snout, twisted alevins, abnormal gills, degenerated operculum and blindness are responsible for high mortality in rainbow trout culture (Gurung and Basnet, 2003).

1.7 Use of Medicinal Herbs in Aquaculture

Herbs and herbal products are used to overcome stress and as growth promoter, appetite stimulator, immunostimulators in aquaculture. The herbs also have aphrodisiac and antimicrobial properties in fisheries due to the presence of various active components such as alkaloids, terpenoids, pigments, phenolics, tannins, steroids flavonoids and essential oils (Citarasu, 2010). Herbal extracts are used as immunostimulants to culture fish because they are easily obtained and act against a broad spectrum of pathogens. Most herbs and herbal extracts are administered orally. Immunostimulants are a safer alternative to antibiotic in curing diseases. Immunostimulating components such as poly-saccharides, proteins, alkaloids, flavonoids, vitamin E, minerals and fatty acids are used for a long time without resistance or accumulation of residues and are both beneficial and harmless to humans and other animals (Ardo *et al*., 2008; Divyagnaneswari *et al*., 2007; Khanna *et al*, 2007).

Many plant-derived compounds have non-specific immunostimulating effects in animals with more than a dozen evaluated in fish and shrimp (Citarasu *et al*., 2006; Sakai, 1999). Immunostimulant incorporated diet fed to fish and shrimps led to better performance of haematological, biochemical and immunological parameters (Citarasu *et al.,* 2006). Immunostimulants enhance non-specific defense mechanism, their capacity for disease resistance and provide complete protection against certain pathogens (Citarasu *et al.*, 2002, 2006). In fish, immunostimulants enhance the phagocytic capacity of neutrophils and lymphocytes, stimulate secretion of cytokines from lymphocytes, coordinate cellular and humoral immunity and evoke antibody and complement responses (Qin 2000; Sahoo and Mukherjee, 2001; Wang *et al*., 2001). Growth rates, survival rates and disease resistance have significantly improved by supplementation of immunostimulants in fish diet.

1.8 Status of Lapsi (*Choerospondias axillaris*) fruits in Nepal

Lapsi (*Choerospondias axillaris*) is a large, deciduous fruit-bearing tree of the family Anacardiaceae. A native of the hilly regions of Nepal (850–1900 m above sealevel), but is

also found in India, China, Thailand, Japan and Vietnam. Lapsi wood is used as light construction timber and fuelwood; seed stones are used as fuel in brick kilns and the bark has medicinal value (Nguyen *et al*., 1996). Lapsi is rich in vitamin C content (Shah, 1978). It is generally consumed fresh and processed to prepare a variety of sweet and sour, tasty food products called Mada and Achar locally (Labh *et al.,* 2015). Nowadays, other products such as candy, jam, squash and powder are also prepared from Lapsi and its fruits are used in rituals as an offering to the Gods & Goddesses.

Lapsi is cultivated in 301 Village Development Committees (VDCs) in 29 hill districts of Nepal (Paudel, 2003). Lapsi trees are found in small patches in forests in mid hills of central Nepal and scattered in private farmlands and at different religious sites. It is a potential agro-forestry tree species to generate income and to provide nutrients in the mid hills of Nepal. With increase in demand for lapsi fruit, popularity of lapsi tree has also increased. Lapsi has become a commercially important tree mainly in districts surrounding Kathmandu valley. The increased demand for lapsi as a fruit in Kathmandu led the forestry program to expand the tree plantation. Since then, lapsi farming has become attractive for cultivation. The annual transaction of lapsi fruit in Kathmandu alone is estimated to be worth over 50 million Nepalese Rupees (approximately US$0.65 million (BM, 1999). Lapsi has great potential as a cash-generating tree for hill farming communities in Nepal (Gautam 1997; Paudel and Parajuli, 1999), thus reducing farmers' reliance on subsistence food production and improving their welfare (Tomich *et al.,* 1994).

1.9 Lapsi as an Immunostimulant and Antioxidant

In Mongolia, fruits of *Choerospondias axillaris* are used to treat myocardial ischemia, to calm nerves, to ameliorate blood circulation and to improve microcirculation (Dai *et al*., 1992; Shi *et al*., 1985). Fruits of *C. axillaris* contain phenolic and flavonoid compounds (Lian *et al.,* 2003) that possess antioxidants properties (Wang *et al.*, 2008). Researches show that flavonoids content of *C. axillaris* could inhibit dexamethasone-induced thymocyte apoptosis (Li *et al.,* 1998). Ao *et al*. (2007) have reported that flavonoids content of *C. axillaris* could attenuate the serum levels of creatine kinase (CK), CK-MB and lactate dehydrogenate (LDH) in isoproterenol-induced myocardial infarction (MI) injury in rats. Quantification of lapsi fruit's antioxidant properties showed that lapsi fruits possess strong antioxidant power with higher inhibition of DPPH radical as recorded in ethanolic extracts (98%), followed by

ascorbic acid (95%) and aqueous (91%) extract (Labh *et al.*, 2015). Lapsi fruits contain exploitable and potent antioxidant molecules and could be promoted as a prospective dietary supplements and a Nutraceutical for both human and animal use.

1.10 Statement of the Problem

Fishes in culture that are stressed and increasingly exposed to pathogens are given either antibiotics or vaccines. But prophylactic treatment with antibiotics is no longer acceptable and ineffective for many pathogens (Wattes *et al*., 2001). Alternative strategies are essential to prevent diseases and sustain production. Immunostimulants enhance immunocompetence and disease resistance (Ganguly *et al*., 2010). When used as dietary supplements, some immunostimulants increase disease resistance in fish by improving the immune defense system (Ye *et al.,* 2011). Several immunostimulants stimulate phagocytes, natural killer cells, complement, lysozyme, and antibody responses. Many reports on immunostimulants originated from plant, animal and bacterial sources are available. These stimulants are safer than antibiotics and live vaccines on both the consumers and the environment (Anderson, 1992). Since Lapsi *Choerospondias axillaris* (Roxb.) fruits are rich in vitamin C content (Shah, 1978; Paudel *et. al.,* 2002a; Labh *et al.,* 2015), its inclusion in the diet allows it to prove beneficial to the health sector (Paudel and Parajuli, 1999).

1.11 Rationale of the Study

Fishes cultured under high density conditions have impaired immune responses as a result, pathogens flourish and chances of disease outbreak increase (Van Muiswinkel *et al*., 1985). Control of infectious diseases in fish and maintenance of their health are most essential to the sustainable development of aquaculture. Diseases in fishes are controlled and treated by chemotherapy or vaccination or by a combination of both. Using chemical drugs to treat and control diseases in fish culture contaminate and damage the ecosystem. Chemical drugs are expensive and have negative impacts such as accumulation in tissue residues, development of drug resistant strains, immunosuppressant and reduced consumer preference. Vaccination of all fishes in ponds or race ways is a very tedious job. Moreover, commercial vaccines are expensive and not available against all the emerging diseases (Raa *et al*, 1992).

To promote sustainable aquaculture, there is a need to find a disease preventive measure that is eco-friendly, cheaper and practically feasible. Using medicinal fruits as

immunostimulants to prevent fish from infectious diseases and herbal compounds to activate fish immune functions (Sahoo and Mukherjee, 2001) are the only options at present. Feeding lapsi fruit incorporated diet may protect fishes from diseases during culture. Lapsi fruits are cheaper and can be easily adopted by aqua farmers. Hence, an attempt has been made to study the effect of Lapsi, *Choerospondias axillaris* (Roxburgh, 1832), on growth, biochemical and immuno-haematological performance in some fishes.

1.12 Objectives of Study

The main objective of this research is to study the effect of lapsi *Choerospondias axillaris* (Roxburgh, 1832) fruits on growth, biochemical and immuno-haematological performance in common carp, rohu and rainbow trout while the specific objectives are as follows:

1. To analyze the percent survival, percent weight gain (WG%), specific growth rate (SGR) and feed conversion ratio (FCR) of common carp, rohu and rainbow trout fed varied doses of lapsi fruits along with other ingredients.

2. To quantify the concentrations of vitamin C (L-ascorbic acid) in brain, liver and blood serum of common carp, rohu and rainbow trout fed varied doses of lapsi fruits along with other ingredients.

3. To estimate the concentrations of total protein, albumin and globulin in brain, body muscles and blood serum of common carp, rohu and rainbow trout fed varied doses of lapsi fruits along with other ingredients.

4. To access the serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) in liver, gills and blood serum of common carp, rohu and rainbow trout fed varied doses of lapsi fruits along with other ingredients.

5. To understand the effects of lapsi on some hematological parameters in common carp, rohu and rainbow trout.

CHAPTER 2

2. LITERATURE REVIEW

Aquaculture is the farming of aquatic animals and plants in fresh, brackish and marine environments for economic profit. The main goals of aquaculture industry are to increase growth rate of fish and to protect their health (Yilmaz *et al.*, 2015). However, diseases outbreak among fishes is a major challenge. The high susceptibility of fish to stress and rapid spread of diseases in water have forced aqua-culturists and aqua-scientists to concentrate their efforts on maintaining fish in good health to achieve sustainable economic growth performance. Growing healthy fish requires developing strong defense mechanisms against pathogen invasion (FAO, 2014). Fish cannot synthesize vitamin C due to lack of L-Gulonolactone oxidase (GLO). Hence, there is a need to supplement fish feed with vitamin C. Literature survey explains that fruits of lapsi contains high amount of vitamin C.

2.1 Use of Medicinal Plants in Fish Immune System

Fish is a heterogeneous group of agnathans (lampreys and myxines), condryctians (sharks and rays) and teleosteans (bony fish). The immune system present in multi-cellular organisms can differentiate between "self" and "non-self" agents. The system protects the host from infectious diseases and develops neoplastic cells (Roitt *et al*., 1998). As in all vertebrates, fishes also have specific and non-specific immune mechanisms for defense that enable them to survive in hostile environments. When pathogens enter the body, non-specific immune mechanisms may be sufficient to stop infection. If not, disease will develop and the specific immune mechanisms will get involved. If the fish survives, it will be protected against re-infection of the same pathogen, owing to development of a specific immunological memory.

Immune systems have been investigated in a small number of approximately 20,000 fish species (Zelikoff, 1994). Since the 1970s, most immunological studies have been conducted in fishes such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), common carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*) and bluefin tuna (*Thunnus maccoyii*). The most advanced teleost species do not possess bone marrow or lymph nodes, but have functionally equivalent haematopoetic tissue primarily in areas of the

kidney, spleen, and thymus. In addition, fishes also have white blood cells that are morphologically and functionally similar to mammalian lymphocytes, granulocytes, and monocytes (Zelikoff *et al.,* 1991; Enane *et al.*,1993).

Non-specific immune reactions in fishes are general responses to injury and/or invasion by organisms from outside. Phagocytosis and inflammation are two non-specific responses that appear to be universal in fishes (Corbel, 1975). Macrophages, along with neutrophils and non-specific cytotoxic cells, are the principal cell types that carry out non-specific immune reactions in fishes.

2.1.1 Non-specific immune mechanism

Non-specific immune mechanism, also called natural or innate immunity is the host defense mechanism that responds to pathogens in a generic way (Alberts *et al*., 2002). The mechanism recognizes specific antigen and is the dominant system of the host defense in most organisms (Litman *et al*., 2005). This system does not confer long lasting immunity against pathogens. Non-specific immune mechanism is the first and foremost important response in fishes, which includes physical barriers such as skin, scales and mucus (Fletcher, 1982). In contrast to higher vertebrates, fishes are free-living organisms depending on their non-specific immune system for survival (Rombout *et al.,* 2005).

Non-specific immunity is a fundamental defense mechanism in fishes. It also plays a key role in the acquired immune response and homeostasis through a system of receptor proteins. These receptor proteins identify molecular patterns of pathogenic micro-organisms, including polysaccharides, lipopolysaccharide, peptidoglycan bacterial DNA, viral RNA and other molecules that are not present normally on the surface of multi-cellular organisms.

Non-specific response is divided into physical barriers and cellular and humoral immune responses. These non-specific immune response parameters include growth inhibitors, lytic enzymes, the classic complement pathways, the alternative and lectin pathway, agglutinins and precipitins (opsonins and primary lectins), antibodies, cytokines, chemokines and antibacterial peptides. External factors such as temperature changes, stress

management and density can influence non- specific immune response (Magnadottir, 2006, 2010).

2.1.2 Specific immune mechanism

Specific immune mechanism, also called adaptive immunity requires a specific recognition of antigen to defend. It has two basic parts: cellular and humoral responses that show specificity and memory (Van-Muiswinkel, 1992). The responses are temperature dependent (Bly and Clem, 1992). Pathogens contain antigens such as viral particles, bacteria and fungi. These antigens bind with lymphocytes in blood and other body secretions that produce a large amount of antibody or immunoglobulin against the invaded antigens. Unlike other vertebrates, fishes produce only one immunoglobin (Jurd, 1985). These antibodies circulate and attack the antigen molecules, binding directly with them to produce neutralized antigen-antibody complex.

Fishes possess two types of lymphocytes that result in two types of immune responses (Jurd, 1985). Humoral immunity is mediated by B-lymphocytes that are transformed into plasmocytes in response to an antigen. T-lymphocytes are responsible for cell-mediated immunity. T-lymphocytes produce soluble lymphocytes that activate macrophages to enhance their ability to kill intracellular pathogens. The immune system requires the host to produce a wide array of proteins to counter challenges. Thus, any nutritional imbalance that influences protein synthesis may lead to functional impairment of certain defense mechanisms (Lall and Oliver, 1993).

2.1.3 Medicinal plants as immunostimulants

Medicinal plants have been used in human medicine as immune boosters for millennia. Furthermore, they are alternatives to antibiotics in aquaculture (Van Hai, 2015). A large number of medicinal plants with antiviral, antibacterial, and antiparasitic properties are used in the treatment of and control of many diseases in animals and fishes (Duke, 1987). Herbal products are cheaper, safer and have greater accuracy than chemotherapeutics. These products used as anti-stress agent, growth promoter, appetite stimulator, tonic, antimicrobial and immunostimulator have significantly influenced cultured fishes. Herbs contain active chemical components such as alkaloids, flavonoids, phenolics, terpenoids, steroids, pigments,

and essential oils (Citarasu *et al.,* 1998, 1999, 2001, 2002; Sivaram *et al*., 2004) that can increase the body's natural resistance to infection and prevent and treat various diseases (Devasagayam and Sainis, 2002). These components have the ability to scavenge free radicals.

Use of immunostimulants also as an alternative to chemotherapeutics and antibiotics, is attracting the attention of many researchers. In this context, many have focused on the use of medicinal plants and animal-based products as potential therapeutic measures for modulating the immune response to prevent and control fish diseases. Many researchers have discussed the possible uses of naturally available herbal plants such as *Ocimum sanctum* (Tulsi), *Phyllanthus emblica* (Amla), *Azadirachta indica* (Neem), *Solanum trilobatum* (Purple Fruited Pea Eggplant), *Eclipta alba* (Bhringraj), *Zingiber officinale* (Ginger), *Echinacea purpurea (*Purple coneflowers), *Allium sativum* (Garlic), *Camellia sinensis* (Green tea), *Aloe vera* (Ghyukumari), *Cynodon dactylon* (Bermuda Grass), and *Choerospondias axillaris* (Nepalese Hog Plum) and animal-based products like chitin, chitosan and fermented products of chicken egg etc. (Bairwa *et al.,* 2012; Shakya and Labh, 2016).

2.2 Use of Medicinal Plants In Fish Diseases

A large portion of the world population, especially in developing countries depends on the traditional system of medicine to treat diseases. Several hundred plant genera are used medicinally and are a vital source for potent and powerful drugs. A wide variety of secondary metabolites present in plants contain chemicals such as tannins, alkaloids and flavonoids, which act against different diseases (Pandey and Madhuri, 2010; Ravikumar *et al.,* 2010). Unfortunately, the parasitic outbreaks act as an important limiting factor for aquaculture businesses. Pinkate *et al.* (2003) reported that every tilapia fish (*O. niloticus*) raised by farmers in Chiang Mai, Thailand has a *Trichodina* parasite infection. Indian almond (*Terminalia catappa*) and garlic (*A. sativum*) are used to treat trichodiniasis caused by *Trichodina* sp. in tilapia (*O. niloticus*) fingerlings. Both Indian almond and garlic have low acute toxicity to tilapia fingerlings.

Immunostimulatory effects of dietary intake of *Viscum album*, *Urtica dioica* and *Z. officinale* extracts on rainbow trout (*O. mykiss*) have also been studied (Süheyla, *et al.*, 2003). Christybapita *et al.* (2007) observed the immunostimulatory effect of aqueous extracted *E.*

alba leaf in tilapia (*O. mossambicus*) and found enhancement of non-specific immune responses against *A. hydrophila* infection. According to Winkaler *et al.* (2007), *A. indica* extract can be successfully used in aquaculture to control fish predators. Sharma *et al.* (2010) observed stimulatory effect of *Withania somnifera* (Ashwagandha) root on immunity against *A. hydrophila* infection in *L. rohita* fingerlings. Abdul Kader Mydeen and Haniffa (2011) reported that *A. indica* leaf's aqueous extract could effectively control *A. hydrophila* infection in *C. carpio*. Furthermore, *Enterobacter* sp. and *Escherichia coli* bacteria, isolated from *Amphiprion sebae* showed 15 mm zone of inhibition against neem extract. The antimicrobial activity of aqueous extract of *A. indica* (leaf), *Solanum torvum* (fruit coat) and *C. longa* (rhizome) against in vitro growth of *A. hydrophila*, isolated from infected fresh-water fish, *C. striatus* was also noticed by Abdul Kader Mydeen and Haniffa (2011). Kolkovski and Kolkovski (2011) reported that some herbal extracts are very effective against gills and skin flukes like *Benedenia seriolae*. Nargis *et al.* (2011) studied the immunostimulant effects of the dietary intake of *A. sativum* and *Vitex negundo* extracts on fingerlings of *L. rohita*. Ravikumar *et al*. (2011) reported medicinal plants such as *A. indica, Cinnamomum verum* and *Eupatorium odoratum* exhibited excellent antibacterial activity against 10 bacterial pathogens from diseased ornamental fishes.

2.2.1 Plants as antioxidant agents

Antioxidants are compounds that inhibit the initiation or propagation of oxidative chain reactions and delay or inhibit the oxidation of lipids or other molecules (Velioglu *et al.*, 1998). Antioxidants counter harmful reactive oxygen species (ROS) or free radicals that are constantly generated in living organisms during regular metabolism, especially under the stressful conditions thereby causing extensive damage to tissues and biomolecules resulting in various disease conditions. Medicinal plants are employed as an alternative source of medicine to mitigate diseases associated with oxidative stress (Roja and Rao, 2000).

Fish from the intensive culture systems are continuously exposed to several forms of stressors including chemical, biological and physical disturbances, which lead to significant physiological and biochemical changes. Changes in biochemical condition that challenges homeostasis can be considered as an overall effect of stress and consequently a threat to the fish health.

Many herbs contain high amount of antioxidant compounds that help organisms deal with oxidative stress caused by free radical damage and help improve the general physiological condition of fishes (Ali *et al*., 2008; Chakraborty and Hancz, 2011). Metwally (2009) found that fishes fed with *A. sativum* supplemented diets had significantly reduced glucose concentration in blood serum. It also increased the activity of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase (SOD) and catalase (CAT) in *O. niloticus.* The result was in agreement with Li *et al*. (2008) findings where the activities of CAT and SOD increased significantly and activities of malonaldehyde diethyl acetal decreased in the allicin supplemented group (Li *et al*., 2008). Shahsavani *et al*. (2010) showed that dietary supplementation with organosulfide allicin at 10 mg/kg in common carp was effective in reducing lead accumulation in the liver, kidney, brain, bone and blood. The mechanism behind this effect might be due to metal-chelating ability of allicin which in turn leads to reduced lead burden in the tissues (Chakraborty and Hancz, 2011).

Wu *et al*. (2007) observed the effects of *Astragalus membranaceus, Portulaca oleracea, Flavescent sophora* and *A. paniculata* on stress resistance and immunological parameters of *C. carpio*. Result showed that herbal extracts acted as an antistresser and inducer to serum lysozyme activity, SOD, NOS, total serum protein levels, globulin and albumin of the fishes. *C. carpio* fed with supplemented diets of 1.0–2.0% anthraquinone extract from rhubarb (*Rheum officinale*) for 10 weeks were able to mitigate the negative effects of crowding stress. The results showed lower blood cortisol, glucose and hepatic malondialdehyde levels but higher hepatic CAT and SOD activities compared to the control group after exposure to crowding stress for 1 and 7 days (Xie *et al*., 2008).

Herbs-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavonols, catechins, anthocyanins and proanthocyanins possess redox properties and could delay or prevent the onset of degenerative diseases. Such properties allow them to act as hydrogen donors, reducing agents, hydroxyl radicals or superoxide radical scavengers (Marwah *et al*., 2007) and may increase immune factors, thus indirectly raising resistance in fishes to various stresses (Chakraborty and Hancz, 2011). Phenolic components such as flavonoids (Pietta, 1998), phenolic acids and phenolic diterpenes (Shahidi *et al.,* 1992) present in medicinal plants are antioxidant compounds that may help protect cells against oxidative damage caused by free-radicals (Kahkonen *et. al.,*1999). Many herbs and medicinal plants such as oregano, rosemary, sage, *Ocimum* sp

(Samson *et al*., 2007), *Alpina calcarata*, *Jatropa multifida*, *Hyptis suaveolens*, *Solanum indicum*, *Clitorria ternate* and thyme have antioxidant properties. *Ocimum* sp contains a wide range of essential oils rich in phenolic compounds and other natural products including flavonoids and anthocycnins (Joshi *et al.*, 2011). The high phenolic and flavonoid content of *Osmium canum* explain its high free radical scavenging activity.

Phytochemicals such as vitamin E, vitamin C, beta carotene, flavonoids, and phenolic acid present in fruits and herbs are effective and potential source of natural antioxidant (Wang, 2003). Ascorbic acid or Vitamin C is considered as the most important water soluble antioxidant that is capable of neutralizing reactive oxygen species (ROS) before lipid peroxidation is initiated (Afolabi, 2009). Lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body including collagen, bone matrix and tooth dentine (Hunt *et al.*, 1980). The presence of adequate amount of vitamin C in the leaves of *Ocimum* spp is an indication of the ability to prevent the formation of carcinogens and scavenge free radicals that are formed during metabolic processes in fishes.

The antioxidant properties from the methanol extracted of 180 selected oriental herbs have been determined by Kim *et al.* (1994). Among the herbs extracted, 44 species had antioxidant activities. *Psoralea corylifolia* L. (Malaytea Scurfpea), *Epimedium koreanum* Nakai, *Syringa dilate* Nakai (Clove), *Prunus mume* (Mumeplant), and *Aconitum rocyanum* Raymund (Arbor Monkshood) showed particularly high antioxidant activity (Wang, 2003). Likely, Zheng and Wang (2001) studied the antioxidant capacity of 39 culinary and medicinal herbs and found that *Catharanthus roseus*, *Thymus vulgaris*, *Hypericum perforatum*, and *Mentha piperita* had highest antioxidant values. Rosmarinic acid and luteolin are the major constituents in *Salvia officinalis* which has medicinal properties for the treatment of various diseases (Areias *et al.*, 2000).

2.2.2 Medicinal Plants as antimicrobial agents

Numerous investigations have shown antimicrobial potential of herbs as an alternative biomedicine in aquaculture (Zheng *et al*., 2009). Either extracted to essential oil or aqueous or solvent extracts, herbs have excellent antimicrobial properties since they can inhibit various microorganisms. The antibacterial active principles of herbals may break the cell wall, block protein synthesis and DNA synthesis, inhibit enzyme secretions and interfere with

the signaling mechanism of quorum sensing pathway (Chitrasu, 2010). Syahidah *et al*. (2012, 2013) found antibacterial potential of aqueous and methanolic extracts of *Cosmos caudatus, Piper betle, Justicia gendarussa, Curcuma mangga*and *Zingiber zerumbet* against *A. hydrophilla, Pseudomonas* sp and *Streptococcus agalactiae.* Methanol extract of *Piper betle* demonstrated the highest level of antibacterial activity. Zilberg *et al*. (2010) showed positive results when dried leaf and leaf extract of *Rosmarinus officinalis* inhibited *Streptococcus iniae* in *Oreochromis* sp. The essential oil extracted from this herb also showed a wide spectrum of antibacterial properties (Mangena and Muyima, 1999; Viuda-Martos *et al*., 2008). Rosmarinic acid, the major polyphenol of *R. officinalis* (Del Bano *et al.,* 2003), is an antibacterial agent (Petersen and Simmonds, 2003) that accounts for the antibacterial activity of *R. officinalis* against *S. iniae.*

Similarly, chamomile extract was used successfully as an antibacterial agent against *Streptococcus agalactiae,* where the minimal inhibitory concentration was 6.25mg/ml (Abdelhadi *et al*., 2012). Moreover, Alsaid *et al*. (2010) reported that pathogens such as *Mycobacterium* sp, *Staphylococcus* sp, *Enterococcus* sp, *Pseudomonas* sp. and *Micrococcus* sp could be effectively inhibited by (*Cinnamomum* sp.) cinnamon's extract. The cinnamon's extract contain eugenoll, cinnamic acid and cinnamaldehyde. Its potent antimicrobial activity could be attributed to phenolic compounds, and also eugenoll that inhibits production of certain enzymes needed for growth of microorganisms (Parasa, 2012).

Some herbs and plant extracts are known to prevent and control infectious microbes in culture systems. Abdel-Tawwab *et al*. (2010) investigated the survival of Nile tilapia (*O. niloticus*) challenged by pathogenic *A. hydrophila* after 12 weeks of feeding diet supplemented with green tea (*C. sinensis*). Results showed that green tea supplemented diet could prevent tilapia from Aeromoniosis. This result was in agreement with a study where administration of herbal supplemented diets showed a mortality reduction and resistance against *A. hydrophila*in tilapia fed with ethanolic extract of guava, *Psidium guajava* (Pachanawan *et al*., 2008). Another research proved that *P. guajava* was also able to eliminate *Vibrio* infection in Black tiger shrimp (*P. monodon)* more effectively than with antibiotic oxytetracycline (Direkbusarakom, 2004).

Ethanol, methanol and hexane extracts from *Ocimum basilicum* were investigated for their in vitro antimicrobial properties against 146 microbial organisms. The hexane extract

showed a stronger and broader spectrum of antibacterial activity (Adiguze *et al*., 2005). Indian almond, *Terminalia catappa*, extract is used against *A. hydrophila* in tilapia. The growth of two strains of *A. hydrophila* was inhibited at a concentration of 0.5 mg ml^{-1} (Chitmanat *et al.*, 2005).

2.2.3 *Medicinal plants as antifungal agents*

Herbs are also used in managing fungal infections in fishes. Gormez and Diler (2012) successfully controlled the fungal pathogen, *Saprolegnia parasitica* by using essential oils of black tyme (*Thymbra spicata* L.), oregano (*Origanu monites* L.) and savory (*Saturejat ymbra* L.). Ilondu *et al.* (2009) demonstrated that the potency of inhibition of *Saproleginia parasitica* growth increased with increasing concentration of *Vernonia amygdalina* extract in fishes. The fishes fed with control diet had fluffy tufts of fungus growing on their bodies after 28 days. It was also elucidated that *V. amygdalina* has potential in suppressing fungal growth in *C. gariepinus*. Sharif Rohani *et al*. (2013) found that essential oil extracted from *Z. multiflora* has a significant antifungal effect and eliminated *Candida albicans* and *Fusarium solani* in cultured shrimp. Herbal extracts involve fungal cell wall breakage, altering permeability, affecting metabolism, RNA and protein synthesis which leads to death.

2.2.4 . Medicinal Plants as antiviral agents

Herbal plants and their extracts are used in controlling viruses. Olive tree leaf (*Olea europaea*) extracts successfully controlled *Salmonid rhabdovirus*, and Viral Haemorrhagic Septicaemia virus (Harikrishnan *et al*., 2010 a & b; Micol *et al*., 2005). *Punica granatum* solvent extracts showed antiviral effectiveness against Lymphocystis Disease virus (LDV) in *Paralichthys olivaceus* (Harikrishnan *et al.*, 2010a). Direkbusarakom (2004) found that in shrimp supplementing ethanol extracted *Clinacanthus nutans* with polyvinylpyrolidone increased resistance to the Yellow Head virus (YHV). Balasubramanian *et al*. (2007) reported that the use of petroleum ether, benzene, diethyl ether, chloroform, ethyl acetate, methanol and ethanol extracts of 20 species of Indian traditional medicinal plants such as *Aegle marmelos, C. dactylon, Lantana camara, Momordica charantia* and *Phyllanthus amarus* have antiviral activity against White Spot Syndrome Virus (WSSV). This finding was similar to the recent report by Yogeeswaran *et al.* (2012) where methanolic extracts of herbal immunostimulants such as *Acalypha indica, C. dactylon, Picrorrhiza kurrooa, W. somnifera* and *Z. officinalis* when incorporated in shrimp diets and fed for 60 days after vaccination,

successfully protected them from WSSV. Generally, herbal active compounds inhibit or block the transcription of the virus to reduce its replication in host cells, thereby enhancing host's non-specific immunity (Citarasu, 2010).

2.2.5 Medicinal plants as anti-parasitic agents

In farm fishes, herbs and their products are used in the treatment of parasitic diseases such as myxobolasis, trichodiniasis, gyrodactylosis, argulosis, and scuticociliates (Micol *et al.,* 2005; Harikrishnan *et al.,* 2010 a, b). Garlic extract has been reportedly effective against intestinal protozoan parasites such as *Opalina ranarum, O. dimidicita, Balantidium entozoon, Trypanosoma, Leishmania, Leptomonas, Crithidia* (Reuter *et al.,* 1996), *Entaemoeba histolytica* and *Giardia lamblia* (Ankri and Mirelman, 1999).

Madsen *et al.* (2000) demonstrated that raw and squeezed garlic at 200 ppm had potential to treat Trichodiniasis in eel fish. According to Chitmanat *et al.* (2005) crude extracts of either Indian almond (*Terminalia catappa*) or garlic at 800 ppm was able to eliminate all *Trichodina* sp from tilapia after two days of treatment. This is similar to Pandey's (2013) findings. Bartolome *et al.* (2010) also reported that garlic extract at 10–100% could effectively control or delay *Ichthyopthirius multifiliis* infection, the most pathogenic parasites affecting freshwater fishes. Yao *et al*. (2010) recorded decreased in number of *Ichthyopthirius multifiliis* in gills of infected grass carp, *Ctenopharyngodon idella* treated with leaves of *Macleaya cordata.*

Additionally, crude extracts of green tea were reported to be useful in controlling the flagellate fish parasites, *Ichthyobodon ecator* in Chum salmon, *Oncorhynchus keta* and Masu salmon, *O. masou* (Suzuki *et al.,* 2006). Abdel-Hadi (2007) has successfully used purified *Commiphora myrrha* extract in the feed to treat and control the monogenetic trematodes (gill flukes) infesting the gills of common carp fingerlings. However, he reported that the *Commiphora myrrha* extract had no effect when added to water and impaired survival of the fishes because the extract caused white clouds that precipitated on the gills of carp fingerlings thereby blocking the process of oxygen exchange.

2.2.6 *Medicinal plants as appetite stimulators*

Many reports have documented the effect of herbs and their extracts as appetite stimulators in fishes and in aquaculture. The flavor of herbs influence eating patterns, total feed intake, stimulates the secretion of saliva, digestive enzymes, bile and mucus (Lee and Gao, 2012). Olfactory feed ingredients enhance fish to consume more feed than normal (Adams, 2005). Various extracts from herbs and spices are reported to stimulate action on gut secretions or have a direct bactericidal effect on gut microflora. Use of hot spices from peppers (e.g., capsaicin and piperine) and other essential oils (e.g., cinnamon) containing cinnamaldehyde have been demonstrated to stimulate salivation (amylase production). The increases in enzyme production improved digestibility and availability of nutrients from feedstuffs (Chesson, 1987).

Livol-incorporated diet stimulated digestive enzyme activity and led to increased consumption in *L. rohita* (Maheshappa, 1993). Papain present in papaya leaf meal increased protein digestion, food conversion ratio, specific growth rate and weight gain in the 16% unsoaked papaya meal diet fed to *Penaeus monodon* postlarvae (Penaflorida, 1995). Venketramalingam *et al.* (2007) reported a significant improvement in digestive enzyme activities in post-larvae of *P. monodon* when fed with herbal appetizer, *Zingiber officinalis* enriched Artemia. Garlic contains an active allicin which can induce fishes to ingest and increase feed intake. Harada (1990) found that garlic had a strong food calling effect on Oriental weather loach (*Misgurnus anguillicaudatus*) and Japanese amberjack (*Seriolaquin queradiata*). This is similar with what was reported by Lee and Gao (2012) on most aquatic animals including *Pelodiscus sinensis*, *C. idellus*, *C. carpio*, *Carassius auratus* and *O. niloticus.*

2.3 Use of Medicinal Plants as Fish Growth Promoter

Fishes of commercial importance are farmed in captivity under controlled conditions to fulfill the demand of white meat for human consumption. Fish production is maximized by increasing weight of individual fish (Schuchardt *et al.,* 2008). Artificial feed used in aquaculture improves fish growth and maximizes weight in a short period of time (Bhosale *et al.,* 2010). New substances are added in fish feed to improve feed conversion efficiency that result in fish growth (Fernández-Navarro *et al.,* 2006). Many studies have shown that inclusion of herbs in fish diet has positive effects on fish growth and health.

Excess use of antibiotics, hormones and other synthetic drugs to control diseases and improve fish growth in aquaculture has led to emergence of drug resistant bacteria, production of toxic substances harmful to environment and human health (Esiobu *et al.,* 2002) and suppression of host immunity (Panigrahi and Azad, 2007). Thus, their use has been criticized all over the world (Baruah, 2008). Since herbs are cheaper, eco-friendly and have with minimum side effects, herbs are used as alternative to antibiotics in fish health management. The World Health Organization (WHO) encourages supplemental diets incorporated with medicinal herbs or plants and minimum use of chemicals in fish diet (Dada, 2015). In this context, herbs and herbal products can be used in fish diet to increase feed consumption in cultured fish (Levic *et al.,* 2008).

Various herbs such as *Hygrophila spinosa, Withania somnifera, Zingiber officinalis, Solanum trilobatum, Andrographis paniculata, Psoralea corylifolia, Eclipta erecta, Ocimum sacnctum, Picrorhiza kurooa, Phyllanthus niruri* and *Tinospora cordifolia* are used to reduce stress, increase immunity and control bacterial activities. *Penaeus monodon* that were in culture fed with diet containing these herbs improved growth under culture condition (Citarasu *et al.,* 2002). Similarly, garlic, onion, marjoram, caraway, basil, anise, fennel, licorice, black seed and fenugreek are known to promote growth (Sivaram *et al.,* 2004), feed conversion (Shalaby, 2004), improve protein digestibility and retain energy (El-Dakar *et al.,* 2004a; 2004b) in aquatic animals. Promising results were achieved when Harada (1990) used garlic as stimulatory effect on olfaction instead of chemotherapeutics on Oriental weather loach (*Misgurnus anguillicaudatus*) and Japanese amberjack (*Seriolaquin queradiata*) (Harada, 1990). This is similar to findings of Lee and Gao (2012) on *Pelodiscus sinensis, C. idellus, C. carpio, Carassius auratus and O. niloticus*. Allicin is an active compound of garlic which induces increase in feed intake. Zeng *et al*. (1996) also reported that adding 50 mg $kg^{-1}$of synthesized allicin to tilapia diet increased its weight gain by 2-3% after 45 days of culture. Uses of other culinary herbs such as red clover (*Trifolium pratense*), caraway (*Carum carvi*) and basil (*Ocimum basilium*) have shown positive results as growth promoting agents in *O. niloticus* (Ahmad and Abdel-Tawwab 2011). Methanol extract of green tea (*Camellia sinensis*) enhanced growth, survival rate, feed utilization and protein content in black rockfish (*Sebastess chlegeli*) (Hwang *et al.,* 2013). Garlic supplemented diet improved weight gain (WG) and specific growth rate (SGR) in *O. niloticus* (Soltan and El-Laithy, 2008). Feed containing 3% garlic powder improved WG, feed efficiency (FE), and SGR in *O. niloticus* (Shalaby *et al.,* 2006). In the same species high growth rate was observed with feeding diet

that contained 2.5% garlic (Diab *et al.*, 2002). Garlic supplemented diet increased weight and SGR in tilapia (Abou-Zeid, 2002). Diet containing 3.2% garlic powder showed best growth in *O. niloticus* (Metwally, 2009). Similarly, *O. niloticus* that were fed with garlic supplemented diets showed significant improvement in weight gain, feed conversion and protein efficiency (Abdel-Hakim *et al.*, 2010). Rainbow trout fed with 1.0% garlic showed increased growth and improved feed utilization (Nya and Austin, 2009). *L. rohita* that was given herbal supplemented diet improved feed consumption which resulted in better growth due to high protein synthesis (Johnson and Banerji, 2007).

Leaves of *Sesbania grandiflora, Moringa oleifera, Coleus aromaticus, Ocimum basilium* and *Solanum verbascifolium* have been found to promote growth in *Oreochromis mossambicus* (Karpagam and Krishnaveni, 2014). *O. mossambicus* that were fed with diet containing *Moringa oliefera* showed maximum increased weight and SGR. Maximum increase in length was observed in fishes that were fed with *Ocimum basilicum* supplemented diet. Thus, plant ingredients are included in fish diet to promote better growth. Red clover (*Trifolium pretense*) in fish diet enhanced growth in *Oreochromis aureus* (Turan, 2006). Juvenile pike perch (*Sander lucioperca*) that were fed on diets supplemented with medicinal plants grew faster than those fed with the control diet (Zakes *et al*., 2008). In common carp *C. carpio*, guppy *Poecilia reticulata*, cichlid *Cryptoheros nigrofasciatus*, and red seabream *Pagrus major* diet supplemented with medicinal plants improved growth. The use of Ginseng herb (Ginsana G115) in diet also enhanced the growth in *Oreochromis niloticus* fingerlings (Yilmaz *et al.*, 2006; Cek*et al*., 2007a, 2007b; Ashraf and Goda, 2008). The use of antibiotics can be replaced by optimized dose of garlic to enhance growth performance and meat quality (Shakya and Labh, 2014). Metwally (2009) recommended garlic supplementation in fish feed to promote growth and increase survival rate. Inclusion of *Phyllantus emblica* in fish feed at any doses results in maximum growth (Sivagurunathan *et al*., 2012). John *et al.*, (2007) used four different plants such as *Eichinacea purpurea, Allium sativum, Nigella sativa,* and *Origanum marjoranaas* as feed additives, which enhanced growth and improved survival of *O. niloticus*.

In *Catla catla*, 5% inclusion of *Cynodon dactylon* in the diet improved growth, feed efficiency, body composition, digestive enzyme and anti-protease activity (Kaleeswaran *et al.*, 2011). Significant increase in SGR, FCR and 100% survival rate in the experimental groups were due to the presence of essential amino acids in *C. dactylon* (Stewart, 2011).

Catla catla fed with *C. dactylon* incorporated diet showed the increased activity of digestive enzymes such as amylase and protease which enhanced digestion and absorption of nutrients essential for fish growth (Kaleeswaran *et al.*, 2011). *O. niloticus* fingerlings fed with dietary herbal powder (Superliv®) improved weight gain, FCR, PER, and SGR (Adekunle, 2012). Superliv® powder contains bioflavonoids with estrogenic activity stimulated growth in common carp (Kocour *et al*., 2005).

Sambhu and Jayaprakash (2000) recommended using 1% of Livol (IHF-1000) to enhance maximum growth and improve nutrient digestibility in prawn. Livol (IHF-1000) is a purely herbal product containing different plant ingredients that improve digestion thereby leading to better growth in cultivable fishes (Unnikrishnan, 1995; Jayaprakash and Euphrassia, 1997). Maheshappa (1993) studied the effect of Livol (IHF-1000) on *L. rohita*. The Livol incorporated diets stimulated digestive enzyme activity and led to increased consumption.

Fishes fed with different doses of ImmuPlus improved growth and inflammatory responses (Priyadarshini *et al*., 2012). Higher growth in fish fed with ImmuPlus is a result of better utilization of feed due to improved secretion of digestive enzymes and higher deposition of fats and protein in carcass (Priyadarshini *et al*., 2012).

Herbal products, stressol-I- and stressol-II-enriched *Artemia nauplii* fed with *Penaeus indicus* postarvae significantly increased growth, efficiencies and reduced osmotic stress (Chitra, 1995). Tefroli contains ingredients such as *Tephrosia purpurea*, *Eclipta alba, Phyllanthus niruri*, *Andrographis paniculata*, *Ocimum sanctum* and *Terminalia chebula* enriched with Artemia. *Penaeus monodon* postlarvae fed with Tefroli improved survival, growth and moulting efficiencies. Also, Trasina, a commercial herbal product, enriched with Artemia when consumed by *P. monodon* postlarvae improved the growth and stress efficiencies significantly (Rani, 1999). Various herbal products such as *Hygrophila spinosa, Withania somnifera, Zingiber officinalis, Solanum trilobatum, A. paniculata, Psoralea corylifolia, Eclipta erecta, Ocimum sacnctum, Picrorhiza kurooa, P. niruri, Tinospora cordifolia*, purified Silajit and cod-liver oil have the characteristics of growth promotion, anti-stress, immunostimulation, and anti-bacterial. These preparations had a good influence in the *Penaeus larviculture* (Citarasu *et al.*, 1998, 2002). Papaya leaf meal contains papain, an enzyme which increases protein digestion, FCR, SGR and weight gain in 16% unsoaked

papaya meal diet which was fed to *P. monodon* postlarvae (Penaflorida, 1995). The dietary ginseng herb (Ginsana G115) greatly enhanced the growth performance, diet utilization efficiency and haematological indices in Nile tilapia fingerlings (Ashraf and Goda, 2008). *Quillaja saponins* have the potential to increase growth in culture fish species and reduce their metabolic rate in tilapia (Francis *et al.,* 2005). These herbal growth promoters help to induce transcription rate, which leads to increased RNA, total amino acids and finally, increased proteins production in the cells.

2.4 Use of Medicinal Plants in Fish Haematological and Biochemical Parameters

Hematological and biochemical parameters are influenced by environmental and physiological factors and are important indicator of fish health. These parameters are used assessing health, nutritional status, physiological disturbances and pathological changes in fishery management and diseases investigation in intensive aquaculture (Gabriel *et al.,* 2004; Satheesh Kumar *et al.,* 2011). Many factors such as stress due to capturing, transportation, sampling, fish species, age, sex, the cycle of sexual maturity, nutritional status, population density, and disease changes fish hematology (Arnold, 2005; Fazio *et al.* 2016). Changes in haematological parameters, such as haemoglobin content, hematocrit (Hct) and the number of erythrocytes, can be used to monitor stress caused by pollutants such as heavy metals (Romani *et al.,* 2003; Barcellos *et al.,* 2004). The most common parameters consistently influenced by diet are Hct and Hb levels (Ologhobo, 1992). Ferguson *et al.* (2010) reported some variation (but not significant) in Hb and Hct content among the control and groups fed diet enriched with probiotics in *O. niloticus*. Moreover, blood parameters of fish are highly sensitive to environmental changes. Quality of water, oxygen, temperature and salinity are directly reflected in blood parameters (Luskovav 1997; Sheikh and Ahmed 2016), as well as basic ecological factors such as feeding regime and stocking density (Což-Rakovac *et al*. 2005; Ferri *et al.* 2011).

Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Svoboda, 2001; Witeska, 2003). It has also been linked as one major factor of disease outbreak, low productivity and mortality in aquaculture. The most common hematological variables measured during stress included red and white blood cells count, hemoglobin content, hematocrit value and red blood cells indices.

Plasma proteins also termed as serum proteins or blood proteins, transport of lipids, hormones, vitamins and metals in the circulatory system and regulate the functioning of a cellular activity and the immune system. Serum albumins account for 55% of blood proteins transports lipids and steroid hormones. Globulins make up 38% of blood proteins transport ions, hormones and lipids to assist in immune function. Fibrinogens are 7% of the blood proteins and convert fibrinogen to insoluble fibrin in blood clotting. The remaining of plasma proteins (2%) is made up of regulatory proteins such as enzymes, pro-enzymes and hormones. All blood proteins except for the gamma globulins are synthesized in liver. Immanuel *et al.* (2009) observed increased in protein content in *O. mossambicus* after feeding diet supplemented with acetone extracts of four medicinal plants (*C. dactylon, A. marmelos, W. somnifera* and *Z. officinales*). Globulins such as gamma globulin are essential for maintaining a healthy immune system. Albumin–globulin ratio is a measurable humoral component of the non-specific defenses. Serum albumin and globulin values were always higher in fish treated with different immunostimulants than those in the control (Choudhury *et al.,* 2005). Kaleeswaran *et al.* (2010) observed significantly higher serum protein, albumin and globulin in *C. catla* that were fed with *C. dactylon* ethanol extract supplemented diet. Increases in the serum protein, albumin and globulin levels are associated with a stronger innate response in fishes (Wiegertjes *et al.,* 1996). Manju and Nair (2004) have reported an increase in serum protein and albumin content in *A. testudineus fed* with aqueous leaf, stem and root extracts of *A. marmelos*. Many medicinal plants are basically used as feed supplements for fish growth or for medicinal purposes thereby becoming involved in a cascade of physiological reactions, that in turn lead to the alteration of haematological and serum biochemical parameters (Ewuola and Egbunike, 2008).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Experimental Design and Set Up

3.1.1 Selection of sites and study area

Three experiments were conducted at the following locations: First experiment in the Wet Research Laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur; Second experiment in Corona of Aquaculture, Gunjanagar, Chitwan and the third experiment in Sosoda Trout farm situated in Ranipouwa, Nuwakot.

3.1.1. a Site for Experiment 1: Central Department of Zoology in T. U., Kirtipur

Tribhuvan University, CDZ is located in Kirtipur Municipality of the Kathmandu District and lies in the hilly region. Kirtipur lies between longitude 27˚ 38’ 30” and 27˚ 41’ 30” E and latitude 85˚ 13’ and 85˚ 19’ N, at altitudes ranging from 1284 m to 1524 m above mean sea level. The Kathmandu Metropolitan City lies in the northern side, while the Bagmati River separates the Lalitpur district on the eastern side.

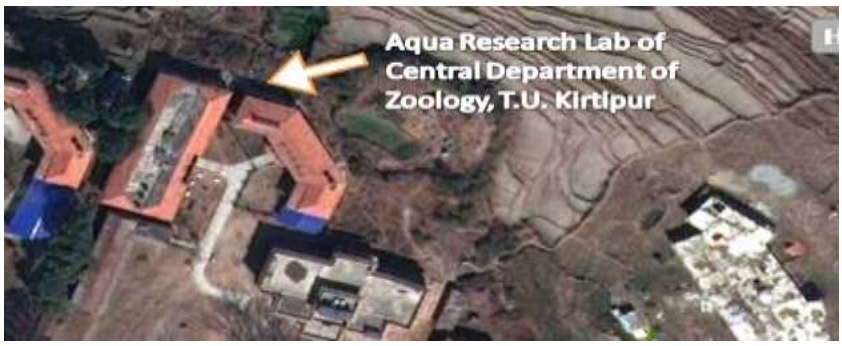

Figure 3.1 Satellite view of Aqua Research lab, Central Department of Zoology, Tribhuvan University, Kirtipur

3.1.1.b Site for Experiment 2: Corona of Aquaculture at Gunjnagar, Chitwan, Nepal

Corona of Agriculture Fish Farm lies in Gunjanagar VCD in Chitwan Municipality of Chitwan district of southern Nepal. The farm spreads over an area of 4 ha has a total of 12 ponds of different sizes for various purposes. It lies within 84°25’ and 27° 42’’ E and N longitude and latitude respectively.

Figure 3.2 Satellite view of Corona of Aquaculture at Gunjanagar, Chitwan, Nepal

3.1.1. c Site for Experiment 3: Sosoda Trout Farm, Ranipauwa, Nuwakot, Nepal

Figure 3.3 Satellite view of Sosoda Trout Farm, Ranipauwa, Nuwakot, Nepal

Nuwakot district is located in Bagmati zone in Mid Development Region of Nepal. It lies within latitude 27°48’ N to 28°06’ N and longitude 84°58’ E to 85°30’ E with an altitude from 518 m to 4876 m. Sindhupalchowk and Kathmandu districts are to the East; Dhading to the West; Rasuwa to the North; and Dhading and Kathmandu to the South. Besides the agriculture farming, small scale livestock is the main source of occupation and livelihood of the majority of population. Ranipauwa is existing key culture center for trout fish at elevation of 1800 m.

3.1.2 Fish species: sources and maintenance

Common carp *Cyprinus carpio*, major carp rohu *Labeo rohita*, and rainbow trout *Oncorhynchus mykiss* were used as test fish species in experiments 1, 2 and 3, respectively. Common carp fingerlings were procured from central Fisheries laboratory, Balaju, Kathmandu, while rohu from Corona of Agriculture farm, Gunja Nagar, Chitwan, and rainbow trout from Sosoda Trout farm, Ranipouwa, Nuwakot, Nepal.

3.1.3 General classification of the fishes used

General classification of common carp, rohu and rainbow trout are given below:

Cyprinus carpio (Linnaeus,1758***)***

Kingdom : Animalia

Phylum : Chordata

Sub Phylum : Vertebrata

Series : Pisces

Class : Teleostomi

Order : Cypriniformes

Family: Cyprinidae

Genus: ***Cyprinus***

Species: ***carpio***

Labeo rohita (Hamilton,1822)

Kingdom : Animalia

Phylum : Chordata

Sub Phylum : Vertebrata

Series : Pisces

Class : Teleostomi

Order : Cypriniformes

Family : Cyprinidae

Genus: ***Labeo***

Species: ***rohita***

Oncorhynchus mykiss (Walbaum,1792)

Kingdom : Animalia

Phylum : Chordata

Sub Phylum : Vertebrata

Series : Pisces

Class : Teleostomi

Order : Salmoniformes

Family : Salmonidae

Genus: ***Oncorhynchus***

Species: ***mykiss***

3.1.4 Experiment design and set-up

In all three experiments there were six treatments in triplicate forms; hence fishes were divided into eighteen groups. In experiment 1 eighteen rectangular glass aquaria (100 L capacity) were kept in indoor culture conditions for the application of six different diets each with replicate forms. Glass aquaria were filled with 80 l of dechlorinated water and covered with nylon net to prevent the escape of fish. Adequate aeration facility was provided in each aquarium. About 400 healthy farm-raised fingerlings of *Cyprinus carpio* were procured from the nursery ponds of Central Fisheries Laboratory at Balaju, Kathmandu, Nepal. Fingerlings were packed in 10 l polyethylene bags filled with oxygenated water and were transported to Central Department of Zoology at Kirtipur, Kathmandu. After that 270 fingerlings (1.7±0.37 g) were selected and distributed randomly in 18 glass aquaria at the rate of 15 fingerlings in each aquarium.

In experiment 2 eighteen rectangular nylon happas (1m×1.5m×1m) were suspended in the experimental pond using ropes and bamboos for fixing them properly. 270 fingerlings of rohu (3.43±0.13 g) were selected and distributed randomly into 18 happas at the rate of 15 fingerlings in each happa.

In experiment 3 the main source of water was the stream which was perennial with clean and cold water suitable for trout culture. Altogether eighteen square nylon cages (1m × 1m × 1m capacity) were placed in the cemented race way pond with an inlet and an outlet for flow of water. The cages were placed in three parallel rows with six in each row. About 270 trouts (37.24±0.39 g) were selected and distributed randomly into 18 cages at the rate of 15 trout in each cage. T represents treatment and R represents replicate.

Replicates	**Treatments**					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
R1	T_1R_1	T_2R_1	T_3R_1	T_4R_1	T_5R_1	T_6R_1
R2	T_1R_2	T_2R_2	T_3R_2	T_4R_2	T_5R_2	T_6R_2
R3	T_1R_3	T_2R_3	T_3R_3	T_4R_3	T_5R_3	T_6R_3

3.1.5 Collection and identification of lapsi fruits

Lapsi *Choerospondias axillaris* (Roxb.) fruits used in the experiment were obtained from local market, and confirmed by the National Herbarium and Plant laboratories, Department of Plant Resources, Ministry of Forests and Soil Conservation, Government of Nepal located at Godavari, Nepal. (Appendices – Annexure – II)

3.1.6 Preparation of lapsi pulp

Lapsi fruits were taken to laboratory soon after their collection. Crude extract of lapsi pulp was prepared using ethanol (70%) as described by Arabshahi-Delouee and Urooj (2007). A known quantity (10 g) of lapsi powder was transferred to 500 ml conical flask (500 ml) and 500 ml of 70 % ethanol was added. The flask with its content was sealed using a cotton plug and aluminum foil and kept in an orbital shaker for 48 hrs. The mixture was then filtered using Whatman filter paper No.1 and the filtrate was centrifuged at 10,000 rpm for 5 minutes to collect the supernatant. The supernatant was concentrated at 70° C using a water bath. Finally, a greasy substance (crude extract) of the lapsi pulp was obtained and transferred to a screw-cap bottle and stored at 4° C until use.

3.1.7 Preparation of lapsi supplemented artificial diets

Dry fishes from the local market were grounded in a grinder and sieved (mesh size: 500μ). The obtained fish powder was then dried in sun and stored. Ingredients were procured from the local market and grounded in a grinder and then sieved (mesh size: 500μ). The fish powder was mixed thoroughly with wheat flour, other ingredients and lukewarm water to make dough which was steamed for 30 minutes to make gelatinous. The dough was left to cool after which cod liver oil, sunflower oil, premix of vitamins and minerals, Betaine Hydrochloride, Butylatedhydroxytoluene (BHT) and Carboxymethylcellulose (CMC) were added and mixed. For the control diet, no lapsi extract was added, but for treatment diets 0.1, 0.2, 0.4, 0.8 and 1.6 % of lapsi pulp were added to the final dough separately. The final gelatinous dough was again mixed and passed through a feed maker using a 1 mm die. The obtained threads were spread on a white cardboard paper and kept in an oven at 30 °C for drying. Finally, the dried threads were chopped into small pieces of pellets and passed through a sieve to obtain equal sized particles.

Table 1 Ingredients and proximate composition of experimental diets (%)

Ingredients	Experimental diets (% inclusion)					
	T1	T2	T3	T4	T5	T6
Fish Meal†	29.31	29.31	29.31	29.31	29.31	29.31
Soya meal‡	14.52	14.52	14.52	14.52	14.52	14.52
Groundnut oil cake†	9.17	9.17	9.17	9.17	9.17	9.17
Rice Powder†	14.16	14.16	14.16	14.16	14.16	14.16
Wheat Flour†	14.43	14.43	14.43	14.43	14.43	14.43
Corn flour†	11.37	11.37	11.37	11.37	11.37	11.37
Sunflower oil†	3	3	3	3	3	3
Cod liver oil†	2	2	2	2	2	2
Vitamin & Mineral Premix§	1	1	1	1	1	1
C. axillaris extract†	0	0.01	0.02	0.04	0.08	0.16
Betain Hydrochloride††	0.02	0.02	0.02	0.02	0.02	0.02
BHT(Butylatedhydroxytoluene)††	0.02	0.02	0.02	0.02	0.02	0.02
CMC (Carboxymethylcellulose) ††	1	0.99	0.98	0.96	0.92	0.84
Total	100	100	100	100	100	100

Using standard calculation, altogether six practical diets (40% protein) were prepared for each experiment. Diet 1 was control diet (0.0 g kg^{-1}) without any lapsi pulp where as in the remaining five diets (0.1 g kg^{-1}, 0.2 g kg^{-1}, 0.4 g kg^{-1} , 0.8g kg^{-1} and 1.6 g kg^{-1}) lapsi pulps were supplemented against NFE along with other important ingredients. All six diets were prepared and called T1, T2, T3, T4, T5 and T6, respectively (Table 1). All six types of test diets were stored at normal temperature until used.

3.1.8 Culture period and conditions

Fishes were acclimatized for one week and were fed the control diet. For indoor culture lab dissolved oxygen was maintained above 5 mg l^{-1} with help of aerators. Faecal matter was siphoned every evening at 6 p.m. Two third of water was replenished at weekly intervals. Dechlorinated tap water was used in the first experiment. For cage culture and raceway cage culture the entire system was natural. Temperature, pH and dissolved oxygen were measured on weekly intervals.

3.1.9 Feeding and duration of experiments

The total time period for each experiment was 90 days. Fishes were fed with either control diet at the rate of 3% of their body weight. Their daily feed was divided into two equal parts and was fed twice daily, once in the morning (9 a.m.) and next in the afternoon (4 p.m.). Five fishes were randomly weighed every fifteen days to adjust feeding rate.

3.1.10 Sample collection and examination

After the experiment was complete, individual fish length (cm) and weight (g) increment were recorded using a measuring scale and digital balance. Fish mortality was monitored to determine the survival rate during the experimental periods.

3.2 Growth Performance

Sampling for growth was done at every 15 days to assess body weight of the fishes. Fishes were starved overnight to measure weight in a digital balance. Growth performance was assessed by using the following parameters:

3.2.1 Percentage weight gain

The percentage weight gain was calculated using the following formula:

$$\text{Weight Gain (\%)} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$

3.2.2 Specific growth rate (SGR)

The Specific Growth Rate was calculated using the following formula:

$$\text{SGR} = \frac{\text{Loge Final weight} - \text{Loge Initial weight}}{\text{Number of Days}} \times 100$$

3.2.3 Feed conversion ratio (FCR)

The Feed Conversion Ratio was calculated using the following formula:

$$FCR = \frac{\text{Feed given (Dry weight)}}{\text{Body weight gain (Wet weight)}}$$

3.2.4 Fish survival (%)

Difference in number of fishes between stocking and at harvest was determined for the estimation of survival. This was expressed in percent of the initial number of fishes.

3.3 Biochemical Parameters

3.3.1 Proximate analysis of experimental diets

Proximate analysis of the 6 experimental diets was determined following the methods described in AOAC (1995) at Fish Nutrition Laboratory, ICAR-CIFE, Versova, Mumbai, India.

3.3.1.1 Moisture

Moisture content of experimental diets was determined by taking a known weight of powdered sample in tarred aluminium dishes and heated in a hot air oven at $105^{o} \pm 2$ ºC C till a constant weight was obtained. The moisture contents were expressed in percentage and calculated from loss in weight of the sample using the formula below:

$$\text{Moisture (\%)} = \frac{W2 - W1}{W2 - W} \times 100$$

Where W2 and W1 are weights of aluminium dishes with the samples before and after heating respectively. W is the weight of empty aluminum dish.

3.3.1.2 Crude Protein (CP)

Crude protein of experimental diets was determined by Kjeldahl method using Tecator kjeltec autoanalyser (Foss, Warrington, UK) using titration. The crude protein percentage was obtained by multiplying the nitrogen percentage with a factor of 6.25.

$$\text{Crude Protein (\%)} = N_2\,(\%) \times 6.25$$

3.3.1.3 Ether Extract (EE)

Ether extract of dried experimental diets was estimated using Soxhlet apparatus with petroleum ether (boiling point 40-60 ^{0}C) as the solvent. The empty thimble was weighed (W1) and known sample (10 g) was added and weighed (W2) again. Weight of the empty flask was noted (W) and the thimbles were placed in Soxhlet apparatus and extracted with petroleum ether for 14-16 hours. The excess solvent in the flask was evaporated on a steam bath and the residual fat was dried at 80 ^{0}C in hot air oven for 1 hour, cooled in a desiccators, weight of the flask (W3) was noted. The EE was calculated using the formula below:

$$\text{Ether Extract (\%)} = \frac{W3 - W}{W2 - W1} \times 100$$

3.3.1.4 Crude Fibre (CF)

Crude fibre was estimated using fibra plus instrument. Sample (1 g) was weighed into a glass crucible, weight noted (W) and the crucible fixed into the digestion tubes. Thereafter, 1.25% sulphuric acid (200 ml) was added and heated at 400 ^{o}C for 30 min. The crucibles were cooled and acid solution was filtered off. The residue was washed thrice with hot distilled water. The same procedure was repeated with 200 ml of 1.25 % of sodium hydroxide. The crucibles were removed and kept for drying in air oven at 100 ºC for 1 h. Thereafter, the crucibles were cooled in a desiccator. The cooled crucibles were weighed (W1) and kept for ashing in muffle furnace at 550 $\pm$ 5 ºC for 4 h. After ashing the crucibles were cooled in a desiccator and weighed (W2). The results were expressed in percentage crude fibre as follows:

$$\text{Crude Fibre (\%)} = \frac{W1 - W2\ (g)}{W\ (g)} \times 100$$

3.3.1.5 Total Ash (TA)

Total ash content of the experimental diets was estimated by AOAC (1984) procedure. The clean silica crucible was kept in a muffle furnace at 400 ^{0}C for half an hour, followed by cooling in a desiccator. Weight of the empty crucible was noted (W1).

Thereafter, known weight of dried sample (5 g) was taken in a crucible and weight was noted (W2). The crucible with the sample was heated over bunsen burner till the sample got charred and later transferred to muffle furnace maintained at 650 ± 10^0 C incerated for 5 hours, cooled and weighted (W3). The ash contents in diets were expressed in percentage total ash as follows:

$$\text{Total Ash (\%)} = \frac{\text{W3} - \text{W1 (g)}}{\text{W2} - \text{W1 (g)}} \times 100$$

3.3.1.6 Organic Matter

Organic matter of experimental diets was calculated by subtracting the ash (%) from 100.

3.3.1.7 Total Carbohydrate (TC)

The total carbohydrate (TC) of the experimental diets was calculated by subtracting the percentage of other nutrients from 100 (Hasting, 1967).

Total Carbohydrate (TC) % = 100 – (Crude Protein % + Ether Extract % + Total Ash %)

3.3.2 Antioxidant Activities of Lapsi Fruit

Antioxidant activities of lapsi fruits were done using standard protocols at Division of Fish Nutrition, Biochemistry and Physiology, ICAR-CIFE, Versova, Mumbai, India.

3.3.2.1 Estimation of Total Phenols

Total phenols in the lapsi pulp were estimated using the method proposed by Mallick and Singh (1980) as described below. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent to produce a blue-coloured complex in an alkaline medium, which can be measured in a spectrophotomet at 650 nm.

(a) Reagents

1. Ethanol (80%)
2. Folin-Ciocalteau reagent (1N)
3. Sodium carbonate (20%)
4. Standard catechol solution (100µg/ml in water)

(b) Procedure

Sample of 0.5 g was homogenized in 10 × volume of 80% ethanol, and the homogenate was centrifuged at 10,000 rpm for 20 minutes. The extraction was repeated with 80% ethanol. Supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipetted out and the volume in each tube was made up to 3.0 ml with distilled water. Folin-Ciocalteau reagent (0.5ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650 nm in a spectrophotometer (Genesys10-S, USA) against a blank reagent. Standard catechol solutions (0.2-1ml) corresponding to 2.0-10µg concentrations were also treated as above. Concentration of phenols is expressed as mg/g lapsi pulp.

3.3.2.2 Estimation of Flavonoids

Flavonoids present in the lapsi fruit was extracted and estimated using a method proposed by Cameron *et al*. (1943). Flavonoids react with vanillin to produce a coloured product that can be measured in a spectrophotometer.

a) Reagents

1. Vanillin reagent (1% in 70% sulphuric acid)
2. Catechin standard (110µg/ml)

b) Extraction of flavonoids

Samples of lapsi fruit pulp (0.5g) were first extracted with ethanol: water mixture first in a 2:1 and second in 1:1. Extracts were shaken well and allowed to stand overnight. Supernatants were separated and the volume was measured. The supernatant was concentrated and then used for the assay.

c) Procedure

A known volume of the extract was pipetted and evaporated to dryness. Vanillin reagent (4.0 ml) was added and the tubes were heated in a boiling water bath for15 minutes. Varying concentrations of the standard were also treated in the same manner. Optical density was read in a spectrophotometer (Genesys 10-S, USA) at 340nm. A standard curve was constructed and the concentration of flavonoids in each sample was calculated. Values of flavonoids were expressed as mg g^{-1} sample.

3.3.2.3 DPPH Radical Scavenging Activity

Radical scavenging activity of the lapsi fruit extract was tested against 2,2-diphenyl-1- picryl-hydrazyl (Sigma-Aldrich) radical (DPPH) as described by Brand-Williams *et al.* (1995) with slight modification. Each lapsi fruit extract sample's stock solution (0.5g/ml) was diluted to final concentrations of 0.005, 0.01, 0.015, 0.02 and 0.025 g/ml in ethanol and water. Ten microlitre (10µl) of crude lapsi fruit extract was placed in test tubes and 2ml of 0.06M DPPH solution in methanol was added. Test tubes were incubated for 30 minutes in the dark at room temperature and the UV absorbance was read at 517 nm. A blank solution containing the same amount of methanol and DPPH was prepared and measured. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Measurements of samples were taken in triplicate and the mean values were calculated. The % radical scavenging activity was calculated using the following formula:

$$\text{DPPH Radical Scavenging Activity (\%)} = \left[\frac{Ao - As}{Ao}\right] \times 100$$

Where Ao is the absorbance of positive blank (without sample) and As is the absorbance of the lapsi fruit pulp extract solution (tested sample).

3.3.2.4 Estimation of ascorbic acid (vitamin C) from lapsi fruits extract

For proximate analysis ascorbic acid was analyzed using a spectrophotometer as described by Roe and Keuther (1943). On treatment with activated charcoal, ascorbate is converted into dehydroascorbate that reacts with 2, 4-dinitrophenyl hydrazine to form

osazones. These osazones when dissolved in sulphuric acid produce an orange coloured solution that can be measured in a spectrophotometer at 540 nm.

a) Reagents

1. TCA (4%)
2. 2, 4-dinitrophenyl hydrazine reagent (2%) in 9N H_2SO_4
3. Thiourea (10%)
4. Sulphuric acid (85%)
5. Standard ascorbic acid solution: 100µg / ml in 4% TCA

b) Extraction of ascorbic acid

Ascorbate was extracted from 1g of the lapsi fruit pulp extract using 4% TCA and the volume was made up to 10ml with the same. The supernatant obtained after centrifugation at 2000 rpm for 10 minutes was treated with a pinch of activated charcoal and shaken vigorously using a cyclomixer and kept for 5 minutes. Charcoal particles were removed by centrifugation and aliquots were used to estimate ascorbic acid.

c) Procedure

Standard ascorbate ranging between 0.2-1.0ml and 0.5ml and 1.0ml of the supernatant were taken and the volume was made up to 2.0 ml with 4% TCA. DNPH reagent (0.5ml) was added to all tubes followed by 2 drops of 10% thiourea solution. Contents were mixed and incubated at 37°C for 3 hours resulting in the formation of osazone crystals. The crystals were dissolved in 2.5ml of 85% sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after a addition of sulphuric acid. The tubes were cooled on ice and absorbance was read at 540 nm in a spectrophotometer (Genesys 10-S, USA).A standard graph was constructed using an electronic calculator set at linear regression mode. Concentration of ascorbate in samples were calculated and expressed in terms of mg g^{-1}.

3.3.3 Collection of Tissues and Estimation of Vitamin C

At the end of the experiment randomly selected fishes from all the treatments (T1 to T6)) were anaesthetized using MS 222 (0.2% solution) for 2-3 minutes. Fishes were then dissected and tissues such as brain and liver were immediately removed aseptically. Vitamin

C was estimated from brain and liver only. A 5% tissue homogenate was prepared in chilled sucrose buffer (0.25M sucrose (MW=342), 3 mM $CaCl_2$, 1 mM Tris pH 8.0, 0.5% NP-40). The whole procedure was done under ice cold condition. Homogenized samples were centrifuged at 10,000 rpm for 10 minutes at 4°C and supernatants were collected in 10 ml vials and stored in deep freezer (-20°C) for further assay.

Assay of vitamin C was based on a method described by Dabrowski and Hinterleitner (1989). Pre-weighed tissues were homogenized in ice-cold 250 mM HClO4 containing 5% trichloro acetic acid (TCA) and 0.08% ethylenediaminetetra acetic acid (EDTA). Homogenates were centrifuged at 27000 rpm for 30 min at 4ºC. 25 µl of 0.2% dichlorophenolindophenol (DCIP) was added to the 250 µl of deproteinised sample and to a blank. The mixture was then incubated at 37°C for 1 h after which 25 µl of 1% potassium bromate ($KBrO_3$) was added and the mixture was incubated at 37°C for a another 1 h. The blank was prepared in the same manner as described above but using only deproteinising buffer. After incubating for 1 h at room temperature, 250 µl of 2% thiourea in 5% meta-phosphoric acid was added followed by an equal volume of 2% of 2, 4-dinitrophenylhydrazine (DNPH) in 12M H_2SO_4. All samples were incubated for 3 h at 60°C after which 0.5 ml of ice-cold 18M H_2SO_4 was added. Samples were transferred into Eppendorf tubes and centrifuged at 11300 rpm for 3 min. Absorbance was recorded at 524 nm with a spectrophotometer. Standard (20-200 µg/ml) was prepared with vitamin C (l-ascorbic acid from Hi-media).

3.4 Immuno-Haematological Parameters

3.4.1 Collection of Blood Sample from Fish

After the feeding trial, analysis of biochemical and immuno-haematological parameters were performed. Fish from each replicate of a group were netted and anaesthetized with 50 mg/l of tricaine methanesulfonate (MS 222, Sigma Chemical Co. St. Louis, MO, USA). 1-2 ml of blood was drawn from the vena caudalis with disposable hypodermic needle (26 gauge). Half of the blood samples were then transferred immediately to sterile penicillin vial containing a pinch of lithium heparin powder and shaken gently and kept at 4°C for haematological profiles. For serum separation, the remaining blood samples were transferred to sterile Eppendorf tubes without anticoagulant and then stored at -20°C for

immunological assay. The stored blood serum and blood plasma were used for further analysis.

3.4.2 Estimation of Total Protein from Fish

Concentrations of total protein levels in tissues such as brain, body muscles and blood serum were determined using a spectrophotometer by the biuret method (Henry *et al.*, 1957). 1000 µl Biuret reagent (Aspen laboratories Pvt. Ltd., India) was added to 20 µl of sample and incubated for 5 min at room temperature. Absorbance was recorded at 546 nm against a blank reagent. The concentration of sample was expressed as g/dl for serum.

Concentration of total protein in test sample (g/dl) =

$$= \frac{\text{Aborbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.4.3 Estimation of Albumin from Fish

Concentrations of albumin levels in fish tissues like brain, body muscles and blood serum were determined in a spectrophotometer using BCG method (Webster, 1977). 1000 µl Biuret reagent (Aspen Laboratories Pvt. Ltd., India) was added to 20 µl of sample and incubated for 5 min at room temperature. Absorbance was recorded at 630 nm against a blank reagent.

Concentration of albumin in test sample (g/dl)

$$= \frac{\text{Aborbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.4.4 Estimation of Globulin

Serum globulin was calculated as shown below.

Globulin (g/100ml) = Total protein – Albumin

3.4.5 Serum glutamic oxaloacetic transaminase (SGOT)

SGOT was determined using Reitman and Frankel's (1957) method. 500 µl reagent from a kit (Transasia Biomedical Limited, India) was added to 50 µl of serum and was mixed well. After 60 minutes of incubation at 37ºC absorbance of sample was recorded at 340 nm against a blank reagent.

$$\text{SGOT (IU/L)} = \frac{(\Delta \text{ Activity/min.}) / \text{T.V. X } 10^3}{\text{S.V.} \times \text{Absorptivity x P}}$$

Where,

T.V.	=	Total reaction volume (µl)
S.V.	=	Sample volume (µl)
Absorptivity	=	millimolar absorptivity of NADH at 340 nm = 6.22
P	=	Cuvette light path (cm) = 1cm
Activity of SGOT	=	Δ Abs/min x 1768

3.4.6 Serum glutamate pyruvate transaminase (SGPT)

SGPT was also determined using Reitman and Frankel's (1957) method and calculated the same way as SGOT.

3.4.7 Alkaline phosphatase (ALP)

Alkaline phosphatase (ALP) was determined by the p-Nitrophenyl Phosphate (PNPP) method (Reitman and Frankel, 1957) using kit from Bayer Diagnostics India Ltd., India. 1 ml reagent was added to 30 µl of serum and incubated for 10 min. at 37ºC. The Absorbance was recorded at 405 nm against a blank reagent.

$$\text{ALP (IU/l)} = \frac{\text{Aborbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.4.8 Haematological Parameters

For hematological determinations, total 1 ml blood was collected from each sample and a drop of blood was smeared on a grease free clean glass slide. Haemoglobin, differential cell counts (erythrocytes, leucocytes, lymphocytes, neutrophils and monocytes) and the hematocrit (Hct) or packed cell volume (PCV) were assayed on the same day of sampling. The rest of the samples were stored overnight at 4°C for further study. On the next day blood serum was obtained by centrifugation at 3000 rmp for 15 min and was used for further assay.

Leishman's stain is generally used to stain blood smears in order to differentiate and identify leucocytes. It has excellent staining quality as it provides a better stain for cytoplasmic details and granules. Leishman's stain is prepared using 1.5 gm Leishman powder in 1 litre of methyl alcohol. Hence, in order to determine differential leucocytes count, standard laboratory procedure was followed. A drop of blood was placed on a clean glass slide and the blood smear was made with the help of a spreader slide placed at an angle of 45° and then stained by Leishman's stain for 5-7 minutes. The excess stain was drained, washed with tap water, air dried before observing under microscope. By using 40 × objective lens, 100 leucocytes were counted in the blood smear of each sample. The percentage of each of the leucocyte (lymphocytes and monocytes etc.) was determined.

3.4.8.1 Haemoglobin (Hb)

Hemoglobin was determined using the Cyanmet-haemoglobin method (Larsen and Snieszko, 1964). 0.02 cm^3 of blood was mixed with 4 cm^3 Drabkin's reagent. The solution was gently mixed by inversion and was allowed to stand for 10 minutes for full conversion of haemoglobin to Cyanmet-haemoglobin. Transmittance was read on a spectrophotometer at 540 nm and the reading was converted to haemoglobin concentration in mg/dl with help of commercially available Cyanmet-haemoglobin standards.

3.4.8.2 Erythrocyte and leukocyte counts

For erythrocyte count, blood samples were diluted in Hendricks' fluid (1: 200) and cells counted on a Neubauer-haemocytometer (Dacie and Lewis, 1966). For leukocyte count, blood samples were diluted in Shaw's solution (1:100).and counted using similar procedure.

3.4.8.3 Hematocrit or Packed cell volume

Blood was drawn into a microhaematocrit tubes and centrifuged for 5 minutes at 10500 rpm. Readings were recorded with the help of a microhaematocrit reader and expressed as the volume of the erythrocytes per 100 cm^3. Snieszko (1960) reported the use of this procedure, mainly used in human hematology, in fishery research and management was reported by. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by using Dacie and Lewis (1984) formulae.

3.4.8.4 Mean corpuscular volume (MCV)

MCV is the average volume of a single red blood cell (RBC) in cubic microns (μm^3). It is expressed in fentoliter (fL) and computed as follows:

$$\text{MCV} = \frac{\text{PCV (\%)}}{\text{RBC (millions x cu mm)}} \times 100$$

3.4.8.5 Mean corpuscular haemoglobin (MCH)

MCH is the average amount of haemoglobin in a single RBC expressed in picogram (10^{-12} gm) and computed as:

$$\text{MCH} = = \frac{\text{Hb } \left(\frac{\text{g}}{\text{dl}}\right)}{\text{RBC (millions x cu mm x } 10^6)} \times 100$$

3.4.8.6 Mean corpuscular haemoglobin concentration (MCHC)

MCHC is the average concentration of haemoglobin in a single RBC. It is expressed as g/dL and computed as:

$$\text{MCHC (g/dL)} = \frac{\text{(Hb g/dL)}}{\text{PCV (\%)}} \times 100$$

3.5 STATISTICAL ANALYSIS

All data were presented as means ± standard error (n = 3). Statistical analyses for all experiments were performed using SPSS 20. Duncan's multiple range test (DMRT) was used for post hoc comparison of mean (P<0.05) between different variables.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Antioxidant Properties of Lapsi Fruits

Significant ($P< 0.05$) results for antioxidant properties of lapsi were observed and as the concentration of extracts were increased the antioxidant compounds such as phenolic, flavonoids, 2, 2, diphenyl-1 picryl-hydrazyl (DPPH) and Ascorbic acid (AA) also increased. It clearly shows that lapsi fruits are rich with antioxidant compounds and useful for fish growth.

Table 2 Antioxidant properties of ethanol extracts of lapsi fruits (*C. axillaris*) collected from the local market of Kathmandu

ANTIOXIDANTS				
µg of Lapsi fruit in ml extract	**Phenolic**	**Flavonoids**	**2, 2, diphenyl-1 picryl-hydrazyl**	**Ascorbic acid**
10	$0.58\pm0.10_a$	$18.26\pm0.56_a$	$6.17\pm0.38_a$	$6.41\pm0.60_a$
20	$1.30\pm0.13a_b$	$24.37\pm0.71_b$	$10.43\pm0.60_b$	$11.00\pm0.45_b$
40	$2.16\pm0.18_c$	$39.11\pm1.40_c$	$30.75\pm0.64_c$	$26.29\pm0.33_c$
80	$6.02\pm0.83_d$	$47.17\pm1.56_d$	$52.04\pm0.68_d$	$48.17\pm0.16_d$
160	$8.92\pm0.66_e$	$58.91\pm3.66_e$	$67.47\pm0.58_e$	$62.38\pm0.53_e$
320	$13.26\pm0.62_f$	$71.23\pm0.98_f$	$79.37\pm0.25_f$	$78.60\pm0.24_f$
640	$23.98\pm0.90_g$	$88.74\pm0.48_f$	$92.98\pm0.68_g$	$95.77\pm0.20_g$

4.2 Proximate Analysis of Fish Feed

Proximate analysis of fish feed was carried out through proper biochemical analysis before the feeding trial. Dry matter, moisture, crude protein (CP), ether extract (EE), crude fibre (CF), total ash (TA), Nitrogen-free extract (NFE) and gross energy were studied through standard protocols during the feed preparation and found that all the nutrients were at optimum level considered for a normal growth of the fish (Table 3).

Table 3 Proximate analysis of fish feed

Ingredients (g/kg)	T1 (0.0% LE)	T2 (0.1% LE)	T3 (0.2% LE)	T4 (0.4% LE)	T5 (0.8% LE)	T6 (1.6% LE)
Dry Matter	91.63 $\pm 0.09_{a}$	91.71 $\pm 0.10_{ab}$	91.72 $\pm 0.01_{ab}$	91.76 $\pm 0.01_{abc}$	91.79 $\pm 0.01_{bc}$	91.83 $\pm 0.01_{bc}$
Moisture	8.37 $\pm 0.09_{c}$	8.29 $\pm 0.10_{bc}$	8.28 $\pm 0.01_{bc}$	8.24 $\pm 0.01_{abc}$	8.21 $\pm 0.01_{ab}$	8.17 $\pm 0.01_{ab}$
Crude protein	34.94 $\pm 0.05_{c}$	35.22 $\pm 0.04_{c}$	36.48 $\pm 0.05_{e}$	37.74 $\pm 0.05_{f}$	35.92 $\pm 0.14_{d}$	36.27 $\pm 0.05_{e}$
Ether Extract	12.35 $\pm 0.07_{c}$	12.71 $\pm 0.05_{d}$	13.05 $\pm 0.07_{de}$	13.40 $\pm 0.07_{ef}$	13.74 $\pm 0.07_{f}$	13.42 $\pm 0.27_{ef}$
Crude Fibre	7.51 ±0.23	7.62 ±0.14	7.63 ±0.20	7.74 ±0.06	7.50 ±0.26	7.43 ±0.21
Total Ash	13.54 $\pm 0.14_{d}$	13.47 $\pm 0.08_{d}$	12.54 $\pm 0.12_{c}$	12.24 $\pm 0.05_{bc}$	12.13 $\pm 0.51_{bc}$	11.72 $\pm 0.31_{b}$
NFE	31.66 $\pm 0.22_{d}$	30.97 $\pm 0.03b_{cd}$	30.31 $\pm 0.30_{b}$	28.89 $\pm 0.16_{a}$	30.72 $\pm 0.33_{bc}$	31.17 $\pm 0.17_{cd}$
Gross energy	332.29 $\pm 0.62_{a}$	335.27 $\pm 0.50_{b}$	338.08 $\pm 0.55_{c}$	340.94 $\pm 0.55_{d}$	343.81 $\pm 0.55_{e}$	346.67 $\pm 0.55_{f}$

Note: Different letters in the same column is subscript (a, b, c, d, e & f) indicate the significant statistical difference (p<0.05, Tukey's test) during One Way ANOVA.

4.3 Performance of common carp *Cyprinus carpio* (Linnaeus, 1758) (Cyprinidae) fed varied doses of ethanol extract of Lapsi *Choerospondias axillaris* fruit' pulp (LFP)

4.3 (A) Growth Performances

4.3.1 Survival rate

Cent per cent survival rate was observed in T3 and T4 diet fed common carp and 97.78±2.22, 97.17±2.22 and 95.56±2.22, 91.11±2.22 in T5, T6, T2 and T1 diet fed fish respectively (Fig. 4.3.1)

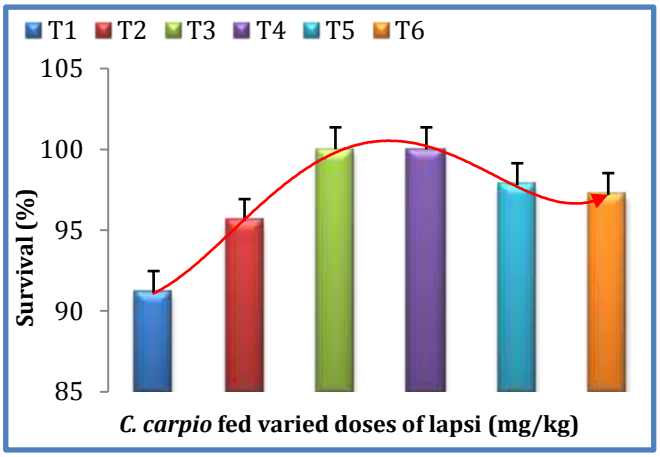

Figure 4.3.1 Survival rate of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.2 Final average weight gain and weight gain (%)

The average weight of common carp in the beginning of the experiment was 1.7±0.37 g. After 90 days of culture, a direct relationship was found between final average body weight of common carp and diets containing different doses of ethanol extracts of lapsi fruit's pulp (LFP). The final average weight gain of common carp fed with diet T4 was found significantly (P<0.05) high (7.14±0.016c g) followed by diets T3 (6.75±0.011b g), T5 (6.71±0.013b g), T2 (5.89±0.041a g) and control diet T1 (5.56±0.031a g). Final average weight gain of common carp fed with T4 diet was 28.34 % higher to that of control diet fed fish while it was 11.29 times higher as compared to initial average body weight (Fig. 4.3.2). The weight gain % was significantly high (P<0.05) in T4 (420.21±0.23c) diet fed common carp followed by 397.32±0.44b, 394.38±0.16b, 349.02±0.53a, 346.66±0.41a and 327.25±0.87a in T3, T5, T6, T2 and T1 diet fed fish respectively (Fig. 4.3.2a).

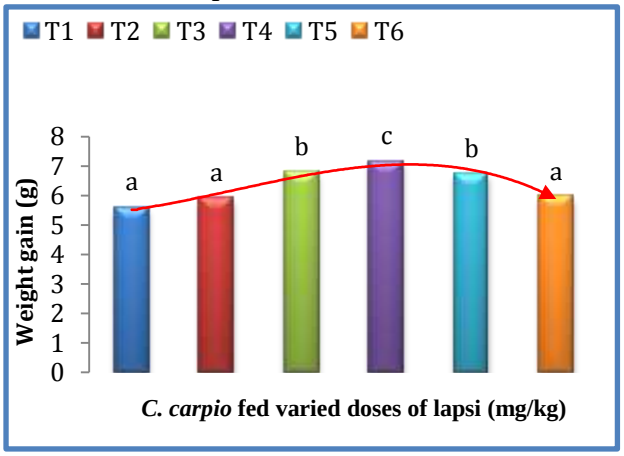

Figure 4.3.2 Final average weight gain of *C. carpio* fed with diet containing six different doses of lapsi fruits

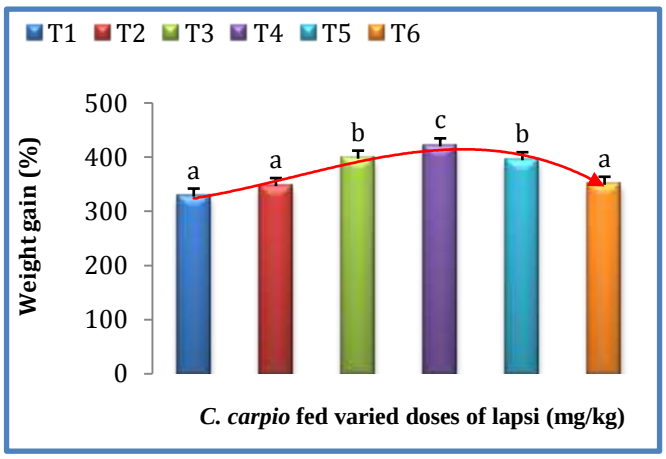

Figure 4.3.2a Percentage of final average weight gain of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.3 Specific growth rate (SGR)

A direct relationship was found between SGR and LFP doses. However, SGR was significantly (P<0.05) high in common carp fed with diet T4 (1.83±0.67d) followed by T5 (1.77±0.17c), T6 (1.67±0.38b), T3 (1.78±0.13c), T2 (1.66±0.35ab). The lowest SGR was found in control diet fed fish T1 (1.61±0.24a). The SGR was 13.43 % higher in common carp fed with T4 diets compared to that of control T1 diet fed fish (Fig. 4.3.3).

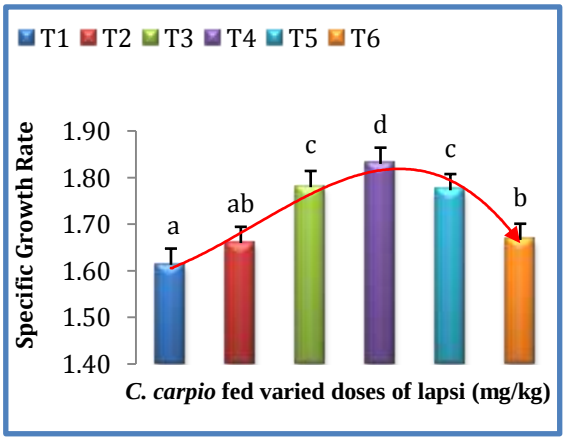

Figure 4.3.3 Specific growth rate of *C. carpio* fed with diet containing six different doses of lapsi fruits

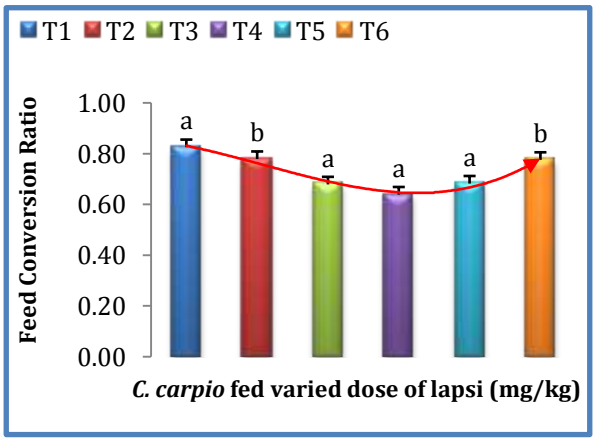

Figure 4.3.4 Feed conversion ratio of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.4 Feed conversion ratio (FCR)

An inverse relationship was found between FCR and LFP doses. FCR level was found high in common carp fed with T1 (0.82±0.033a), followed by fish fed with T2 (0.78±0.057b),

T6 (0.77±0.011b), T3 (0.68±0.071a), T5 (0.68±0.031a). The lowest FCR was recorded in T4 (0.64±0.012a) diet fed fish (Fig. 4.3.4).

4.3 (B) Biochemical Parameters

The amount of vitamin C in blood serum, brain and liver of common carp fed with six different diets were recorded.

4.3.5 Vitamin C in Blood Serum

A direct relationship was found between the doses of LFP in diets and concentration of vitamin C in blood serum of common carp. The concentration of vitamin C in blood serum was significantly (P<0.05) higher (88.90±1.28f µg/dl) in fish fed with T4 diet followed by fish fed with diets T5 (82.24±1.28de µg/dl), T6 (75.59±1.28cd µg/dl), T3 (67.49±1.63bc µg/dl) and T2 (65.17±1.64b µg/dl). It was minimum in control diet (T1) fed fish (54.16±1.29a µg/dl). 64.15% higher vitamin C level was recorded in fish fed with T4 (800 mg vitamin C kg^{-1}) diet compared to that of control fish (Fig.4.3.5).

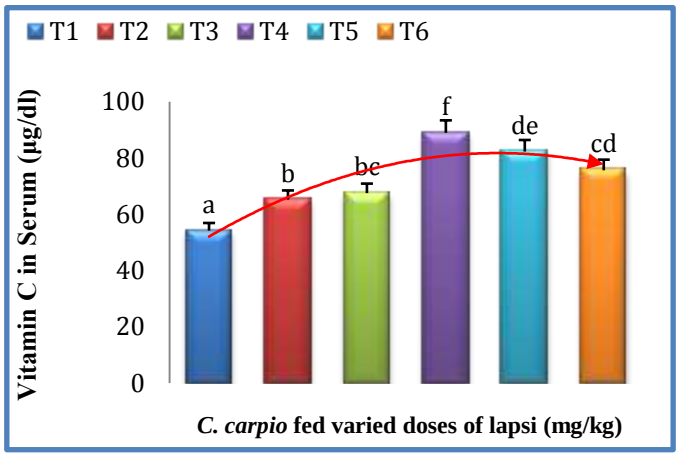

Figure 4.3.5 Vitamin C in blood serum of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.6 Vitamin C in Brain

The Concentration of vitamin C in brain was significantly (P<0.05) higher in common carp fed with diet T4 (74.50 ±1.33e µg/mg) followed by T6 (64.62±1.69d µg/mg), T5 (64.10±1.68d µg/mg), T3 (53.06 ±1.65c µg/mg) and T2 (45.76±1.45b µg/mg) diets fed fish. Minimum vitamin C concentration was

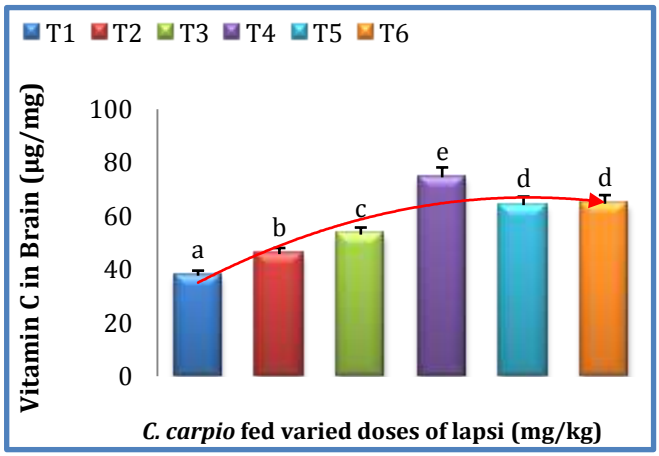

Figure 4.3.6 Vitamin C in brain of *C. carpio* fed with diet containing six different doses of lapsi fruits

found in control diet (T1) fed fish (37.68±1.73a µg/mg). Vitamin C concentration was 97.71% higher in the fish fed with T4 diet compared to that of control fish (Fig.4.3.6).

4.3.7 Vitamin C in Liver

Significantly (P<0.05) higher concentration of vitamin C in liver was recorded in common carp fed with diet T4 (82.77±1.28e µg/mg) compared to the others. The concentration of vitamin C in carp fed with diets T5, T6, T3 and T2 were 76.11±1.28de µg/mg, 69.46±1.28cd µg/mg, 61.36±1.63bc µg/mg and 59.04±1.64b µg/mg, respectively. The lowest concentration of vitamin C (48.03±1.29a µg/mg) was recorded in carp fed with diet T1. Vitamin C concentration was 72.34% higher in carp fed with T4 diet compared to that of control carp (Fig. 4.3.7).

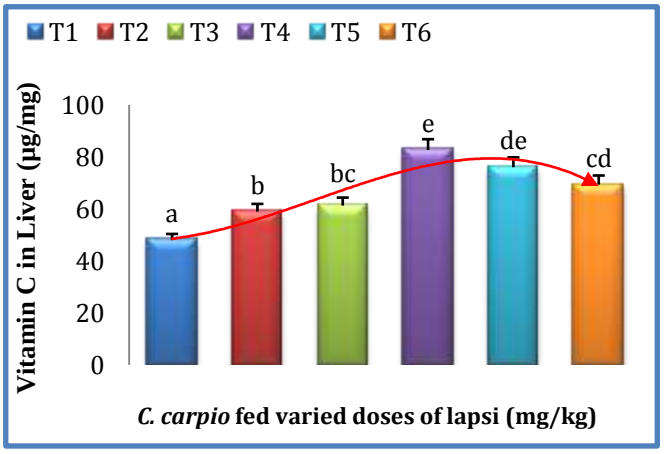

Figure 4.3.7 Concentration of vitamin C in liver of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3(C) Immuno-Haematological Parameters

4.3. C-1 PROTEIN PROFILE

4.3.8 Total Serum Protein

A direct relationship was found between doses of LFP and concentration of total serum protein (TSP) in blood of common carp. The concentration of TSP was significantly (P<0.05) higher in the blood sample of carp fed with diet T4 (32.38±1.48e µg/dl) followed by carp fed with diets T3 (28.42±1.22d µg/dl), T5 (23.87±0.69c µg/dl), T2 (18.93±1.98c µg/dl), T6 (15.57±1.204b µg/dl) and

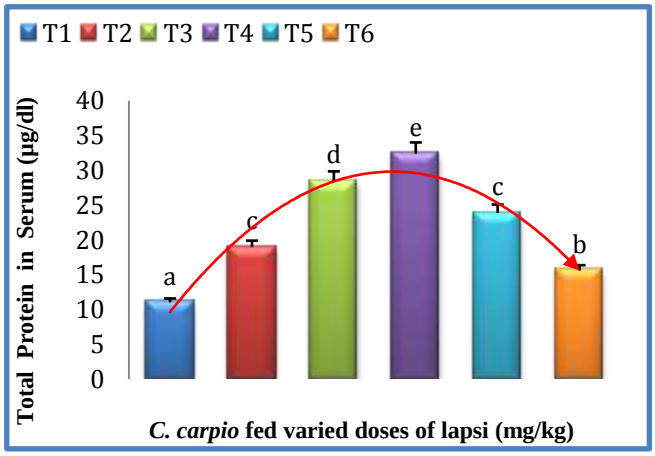

Figure 4.3.8 Concentration of Total serum protein in blood of *C. carpio* fed with diet containing six different doses of lapsi fruits

minimum was in control diet fed carp T1 (11.01±1.27a µg/dl). Serum protein level was 2.7 times higher in carp fed with T4 diet compared to that of control carp (Fig.4.3.8).

4.3.9 Total Protein in Brain

The concentration of total protein in brain increased as the doses of LFP in diets increased. The concentration of total protein in brain was significantly (P<0.05) high in common carp fed with diet T4 (15.66±0.48d µg/mg) followed by T5 (11.23±0.37ab µg/mg), T3 (10.71 ±0.83c µg/mg), T6 (7.31±0.74b µg/mg), T2 (7.21±0.81b µg/mg). It was minimum in control diet T1 (4.52±0.48a µg/mg) fed carp. Total protein concentration in brain was 4.14 times higher in the carp fed with T4 diet compared to that of control (Fig.4.3.9).

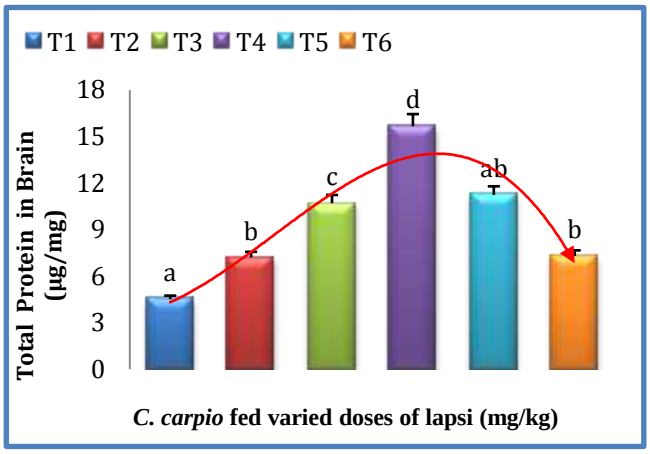

Figure 4.3.9 Concentration of Total protein in brain of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.10 Total Protein in Liver

A direct relationship was found between different doses of LFP in the diet and concentration of total protein in liver of common carp (Fig.4.3.10). The concentration of total protein in liver was significantly (P<0.05) higher (31.66±0.11d µg/mg) in carp fed with diet T4 followed by carp fed with diets T3 (29.76±0.44c µg/mg), T2 (27.17±0.32bc µg/mg), T5 (26.3±0.18b µg/mg) and T6 (25.23±0.43b µg/mg). It was minimum in control diet (T1) fed carp (19.32±0.14a µg/mg). 40.6 to 63.9 % higher total protein level was recorded in carp fed with T4 diet compared to that of control.

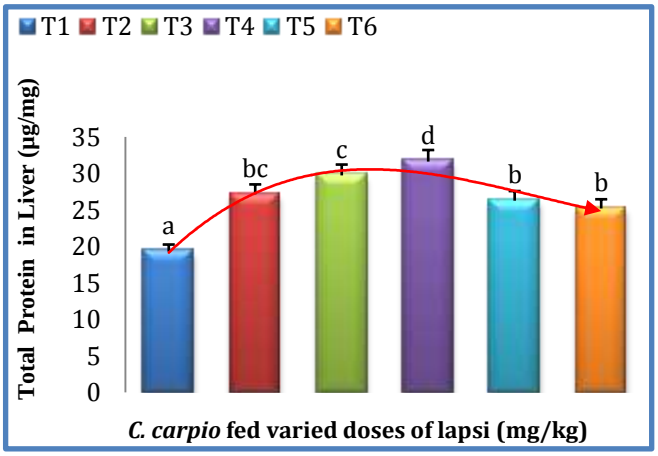

Figure 4.3.10 Concentration of Total protein in liver of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.11 Total Protein in Muscles

Like in liver and brain, concentration of total protein in muscles was found increased with increase in doses of LFP extracts in diet (Fig. 4.3.11). The concentration of total protein in muscles was significantly (P<0.05) high in carp fed with diet T4 (18.41 ±0.762e µg/mg) followed by carp fed with diet T3 (14.74±0.602d µg/mg), T5 (13.95 ±0.376a µg/mg), T2 (10.49 ±0.515c µg/mg) and T6 (6.54±0.493b µg/mg). The minimum was in carp fed with control diet T1 (6.32±0.098b µg/mg). 2.9 times higher LFP level was recorded in carp fed with T4 diet compared to that of control.

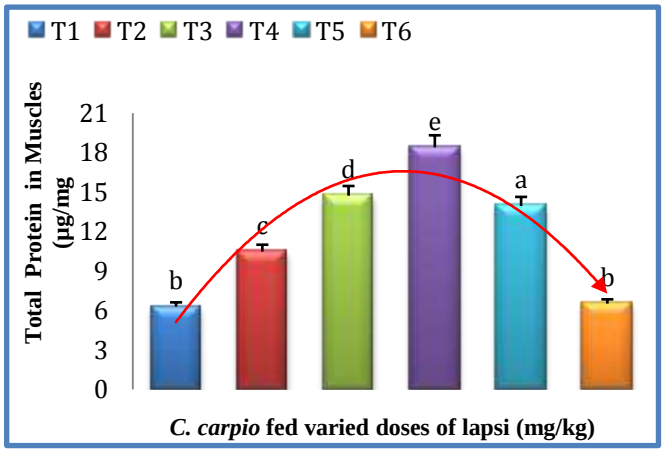

Figure 4.3.11 Concentration of Total protein in muscles of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.12 Albumin in Blood Serum

The concentration of albumin in blood serum was significantly (P<0.05) high in common carp fed with diet T4 (12.51±1.410e µg/dl) followed by T3 (11.04±2.092d µg/dl), T2 (7.72±1.264c µg/dl), T5 (6.37±0.842c µg/dl) and T6 (4.88±1.671b µg/dl). It was minimum in control diet T1 (2.55±0.526 a µg/dl) fed carp. The albumin concentration in blood serum 1.92 times higher in T4 as compared to that of control carp (Fig. 4.3.12).

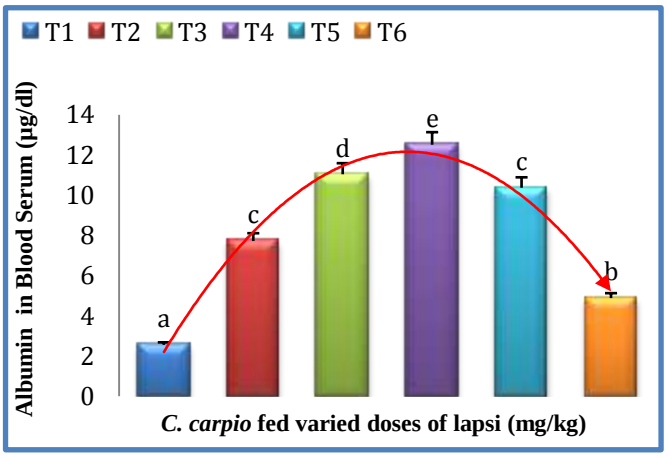

Figure 4.3.12 Concentration of Albumin in blood serum of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.13 Albumin in Brain

A direct relationship was found between different doses of LFP in diets and concentration of albumin in the brain of common carp. The concentration of albumin in brain was significantly (P<0.05) higher (5.39±0.015d µg/mg) in carp fed with diet T4 followed by carp fed with diets T3 (3.65±0.347c µg/mg), T6 (2.70±0.105b µg/mg), T2 (2.23±0.015b µg/mg) and T5 (1.50±0.036a µg/mg). It was minimum in control diet (T1) fed carp (1.73±0.133a µg/mg). 7.32 times higher albumin level was recorded in carp fed with T4 diet compared to that of control carp (Fig.4.3.13).

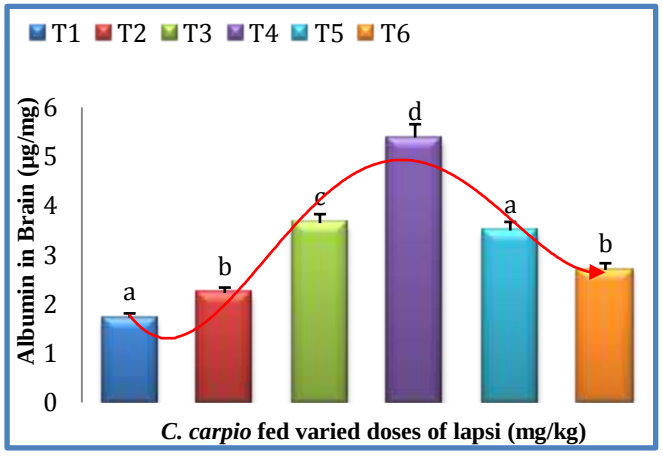

Figure 4.3.13 Concentration of albumin in brain of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.14 Albumin in Liver

Similar results were recorded in albumin concentration in liver of common carp fed with different doses of LFP. The albumin concentration was found high in liver of carp fed with diet T2 (7.93±0.47e µg/mg) followed by carp fed with diets T3 (7.43±0.29d µg/mg), T5 (6.98±0.63bc µg/mg), T4 (6.14±0.26b µg/mg), T6 (5.94±0.22b µg/mg) and control diet T1 (4.15±0.51a µg/mg) (Fig.4.3.14).

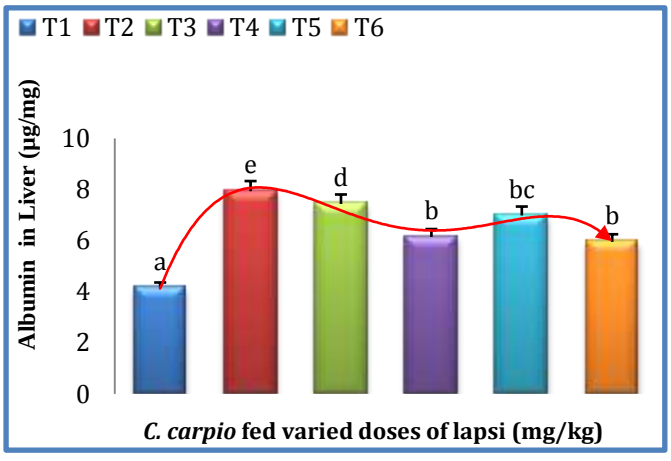

Figure 4.3.14 Concentration of albumin in liver of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.15 Albumin in Muscles

Similar results were recorded in albumin concentration in muscles of common carp fed with different doses of LFP. The albumin concentration was found high in muscles of carp fed with diet T4 (7.27±0.56d µg/mg) followed by carp fed with diets T5 (6.58±0.12a µg/mg), diet T3 (5.64±0.35c µg/mg), T2 (5.13±0.35c µg/mg), T6 (4.13±0.38b µg/mg) and control diet T1 (2.37±0.23ab µg/mg). It was 91% higher in liver of T2 diet fed carp as compared to control (Fig.4.3.15).

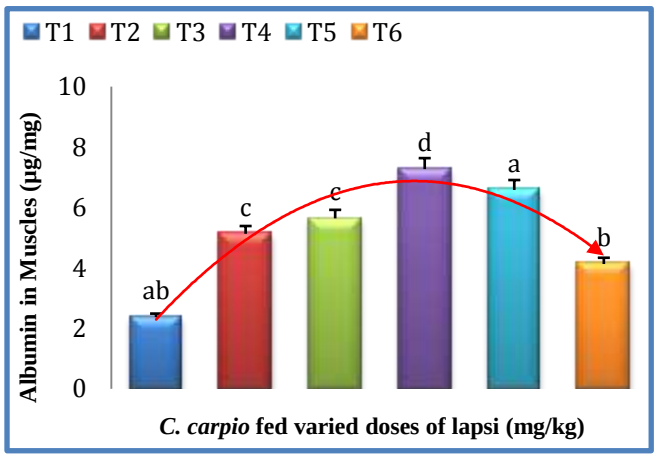

Figure 4.3.15 Concentration of albumin in muscles of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.16 Globulin in Blood Serum

A direct relationship was found between doses of LFP in diets and concentration of globulin in blood serum of common carp. The concentration of globulin in blood serum was significantly (P<0.05) high (19.87±0.476d µg/dl) in carp fed with diet T4 (Fig. 4.3.16) followed by T3 (17.39±2.181c µg/dl), T5 (11.50±0.468b µg/dl), T2 (11.21±3.24b µg/dl) and T6 (10.68±0.46b µg/dl). It was minimum in carp fed with control diet T1 (4.46±1.73a µg/dl). The concentration of albumin level was 2.47 times higher in T4 diet fed carp as compared to that of control carp.

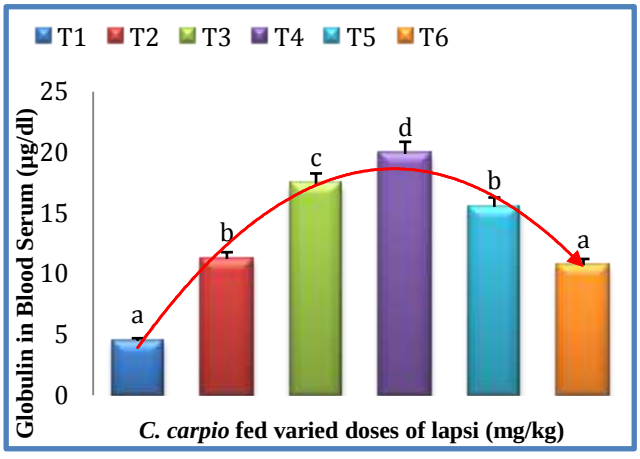

Figure 4.3.16 Concentration of globulin in blood serum of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.17 Globulin in Brain

A direct relationship was found between different doses of LFP in diets and concentration of globulin in brain of common carp. The concentration of globulin in brain was significantly (P<0.05) higher (10.27±0.47d µg/mg) in carp fed with diet T4 followed by carp fed with diets T3 (7.05±0.51c µg/mg), T2 (4.97±0.81b µg/mg), T6 (4.60±0.63b µg/mg) and T5 (3.73±0.34ab µg/mg). It was minimum in control diet (T1) fed common carp (2.79±0.35a µg/mg). 3.41 times higher globulin level was recorded in common carp fed with T4 diet as compared to T1 diet fed common control carp (Fig.4.3.17).

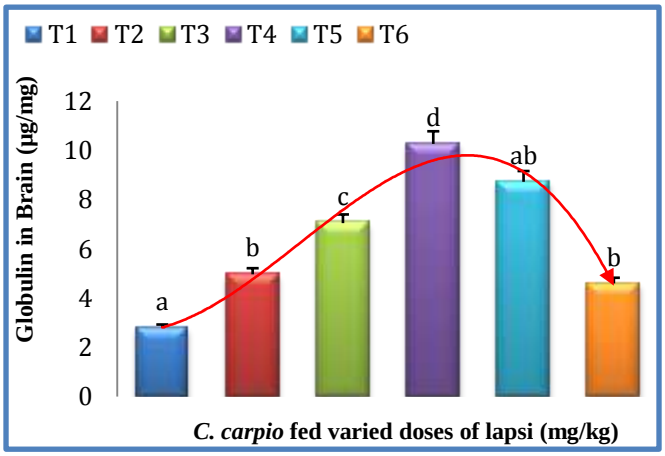

Figure 4.3.17 Concentration of globulin in brain of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.18 Globulin in Liver

Similar results were recorded in globulin concentration in liver of common carp fed with different doses of LFP. The globulin concentration was found high in liver of carp fed with diet T4 (15.52±0.63d µg/mg) followed by carps fed with diets T3 (12.33±0.26c µg/mg), diet T5 (9.32±0.22b µg/mg), T6 (9.28±0.51b µg/mg) and T2 (9.28±0.51b µg/mg). It was minimum in control diet T1 (5.17±0.47a µg/mg) fed carp. 2.54 times higher globulin level in liver was recorded in T4 diet fed carp as compared to control carp (Fig.4.3.18).

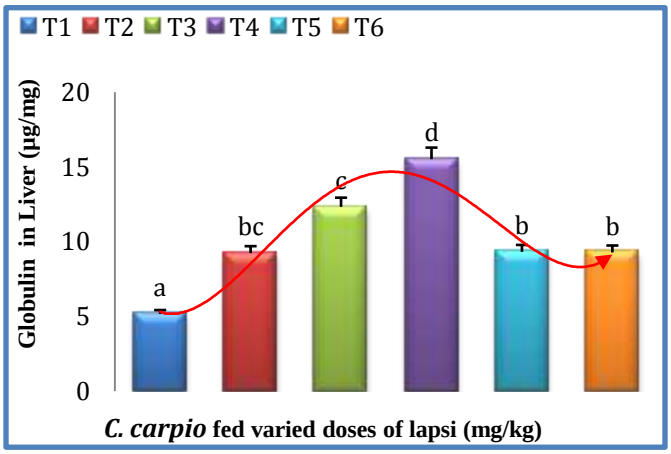

Figure 4.3.18 Concentration of globulin in liver of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.19 Globulin in Muscles

Globulin concentration in muscles was found significantly ($P< 0.05$) high in common carp fed with diet T4 (11.14±0.89e µg/mg) followed by carps fed with diets T3 (9.10±0.26d µg/mg), T5 (7.37±0.33b µg/mg) and T2 (5.49±0.38c µg/mg), T6 (3.41±0.21ab µg/mg). The minimum globulin concentration was in control diet T1 (3.95±0.26b µg/mg) fed carp. 4.67 times higher globulin level was in muscles of carp fed with T4 diet as compared to control carp (Fig.4.3.19).

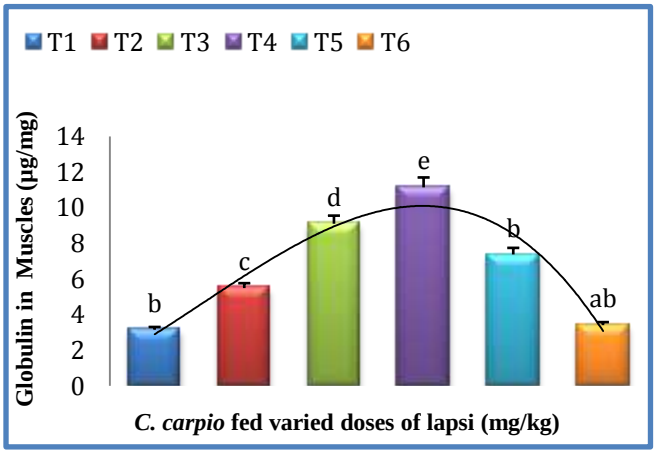

Figure 4.3.19 Concentration of globulin in muscles of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.20 Ratio of Albumin and Globulin (A/G) in Blood Serum

The A/G ratio in blood serum was higher in common carp fed with diet T2 (0.71±0.356). The ratio A/G was 0.64±0.18 in T3, 0.63±0.75 in T4, 0.61±0.338 in T1, 0.56±0.089 in T5 and 0.46±0.173 in T6 diets fed carps. The lowest A/G ratio was found in T6 diet fed carp. The A/G ratio was 37.6% higher in T2 diet fed carp as compared to control carp (Fig. 4.3.20).

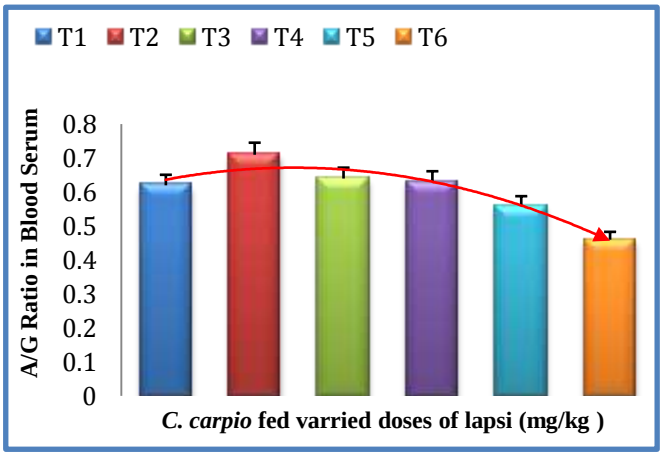

Figure 4.3.20 Ratio of albumin and globulin in blood serum of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.21 Ratio of Albumin and Globulin (A/G) in Brain

The A/G ratio in brain was higher in common carp fed with diet T1 (0.63±0.034c). The ratios were 0.60±0.058bc in T6, 0.53±0.023abc in T4, 0.52±0.023abc in T3, 0.47±0.072ab in T2 and 0.41±0.035a in T5 diets fed carp (Fig. 4.3.21). The lowest A/G ratio

in brain was found in T5 diet fed carp. The ratio was 53.7% higher in T1 diet fed group as compared to T5 fed carp.

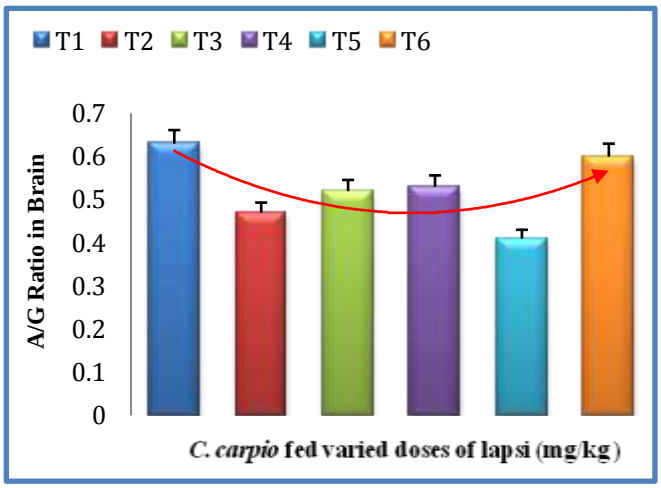

Figure 4.3.21 Ratio of albumin and globulin in brain of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.22 Ratio of Albumin and Globulin (A/G) in Liver

The A/G ratio in liver was higher in common carp fed with diet T2 (0.85±0.22d). The ratios were 0.82±0.63c in T1, 0.74±0.29bc in T5, (0.62±0.51b) in T3 and (0.39±0.47a) in T4 diets fed common carp (Fig. 4.3.22). A/G ratio was 1.5 fold higher in T2 diet fed carp as compared to T4 diet fed carps.

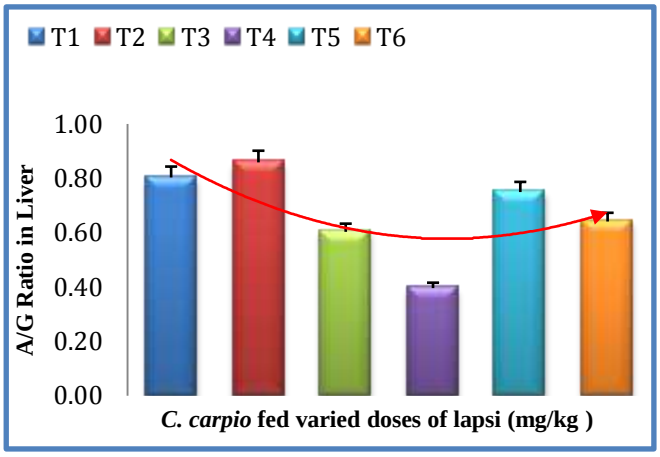

Figure 4.3.22 Ratio of albumin and globulin in liver of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.23 Ratio of Albumin and Globulin(A/G) in Muscles

The A/G ratio in muscles was higher in common carp fed with diet T6 (0.92±0.113). The ratio was 0.92±0.091 in T2, 0.69±0.119 in T6 followed by 0.67±0.095 in T4, 0.62±0.027 in T5 and 0.61±0.104 in T1 control diet fed common carp. The ratio was 50.8% higher in T6 diet fed common carp compared to T1 diet fed common carp (Fig. 4.3.23).

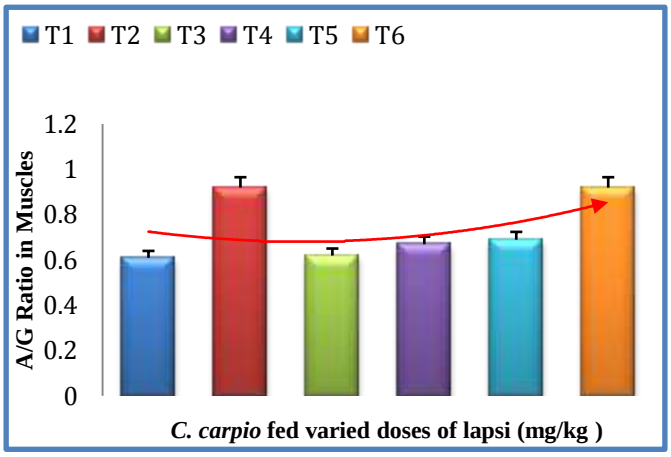

Figure 4.3.23 Ratio of albumin and globulin in muscles of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3. C-II. ENZYME PROFILE

4.3.24 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Blood Serum

An inverse relationship was observed between doses of LFP and SGOT level in blood serum of common carp. The SGOT level was found significantly (P<0.05) high in blood serum of carp fed with control diet T1 (94.10±3.07d IU/L) followed by T2 (80.52±3.16c IU/L), T3 (65.50±2. 88b IU/L), T4 (38.62±0.90a IU/L), T5 (35.64±1.21a IU/L) and the lowest was in carp fed diet T6 (31.23±2.78a IU/L). SGOT level was three fold higher in control diet fed carp as compared to T6 (Fig.4.3.24).

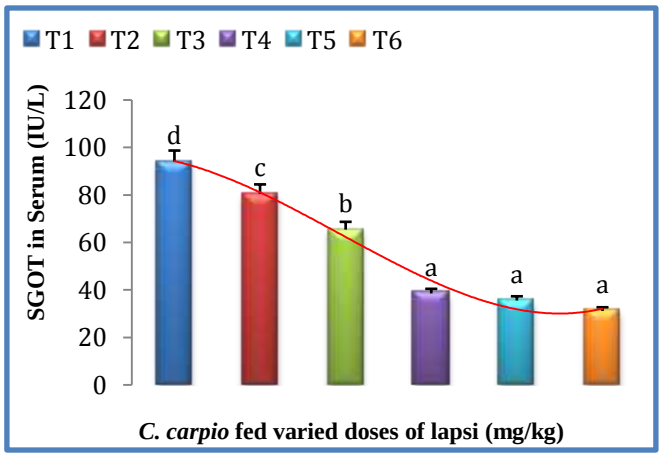

Figure 4.3.24 Serum glutamic oxaloacetic transaminase (SGOT) level in blood serum of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.25 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Liver

SGOT level was found significantly (P<0.05) high in liver of common carp fed with control diet T1 (51.19±1.276d IU/L) followed by T2 (44.55±1.276c IU/L), T6 (42.81±1.49d IU/L), T5 (37.73±1.441e IU/L), T3 (31.66±1.214b IU/L) and T4 (16.12±0.837a IU/L). SGOT level was more than threefold higher in control diet (T1) diet fed common carp as compared to that of T4 carp(Fig.4.3.25).

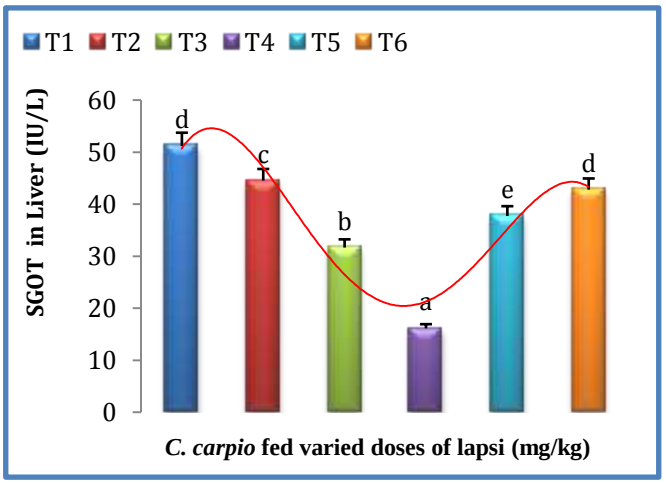

Figure 4.3.25 Serum glutamic oxaloacetic transaminase (SGOT) level in liver of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.26 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Gills

An inverse relationship was observed between doses of LFP in diets and SGOT level in the gills of common carp (Fig.4.26). SGOT level was found significantly (P<0.05) high in gills of carp fed with control diet T1 (76.55±0.925c IU/L) followed by T5 (72.76±1.88c IU/L), T6 (63.18 ±0.693b IU/L), T2 (59.57±0.696b IU/L), T3 (44.06 ±6.503a IU/L) and the lowest was in carp fed diet T4 (35.95±0.696a IU/L). The SGOT level was more than threefold higher in control (T1) diet fed carp as compared to T4 carp (Fig.4.3.26).

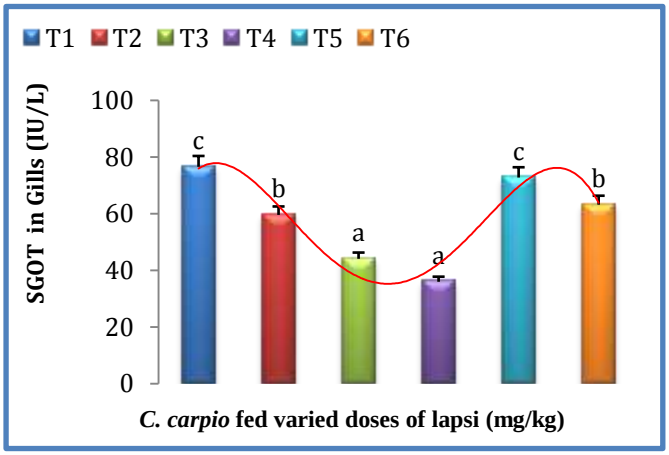

Figure 4.3.26 Serum glutamic oxaloacetic transaminase (SGOT) level in gills of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.27 Serum Glutamic Pyruvate Transaminase (SGPT) in Blood Serum

An inverse relationship was observed between doses of LFP and SGPT level in blood serum of common carp (Fig.4.27). SGPT level was found significantly (P<0.05) high in blood serum of carp fed with control diet T1 (47.76±2.90d IU/L) followed by T2 (45.99±3.34d IU/L), T4 (34.27±0.27c IU/L), T3 (31.17±0.88c IU/L), T5 (19.96±1.28b IU/L) and T6 (13.34 ±0.21a IU/L) fed carp. SGPT level was five times higher in control diet fed carp as compared to T6 carp (Fig.4.3.27).

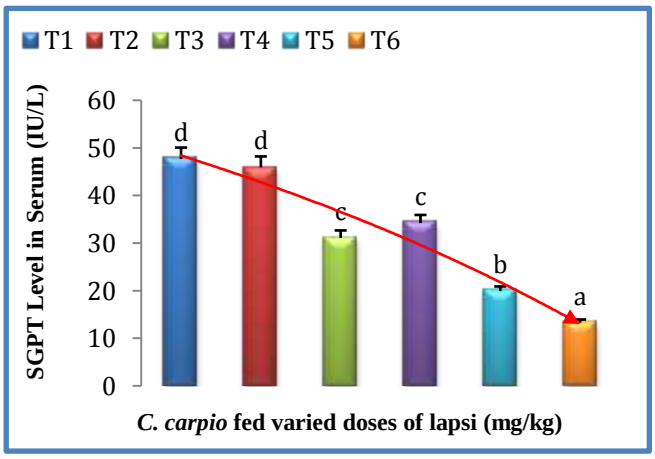

Figure 4.3.27 Serum glutamic pyruvate transaminase (SGPT) level in blood serum of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.28 Serum Glutamic Pyruvate Transaminase (SGPT) in Liver

SGPT level was found significantly (P<0.05) high (71.3%) in liver of common carp fed with control diet T1 (105.32±2.32e IU/L) followed by T2 (95.24±1.61d IU/L), T3 (86.83±1.69c IU/L), T6 (81.94 ±1.72c IU/L), T5 (75.61 ±1.37b IU/L) and the lowest was in carp fed diet T4 (61.58±1.11a IU/L). The SGPT level was 1.3 times higher in control diet fed common carp compared to T4 (Fig.4.3.28).

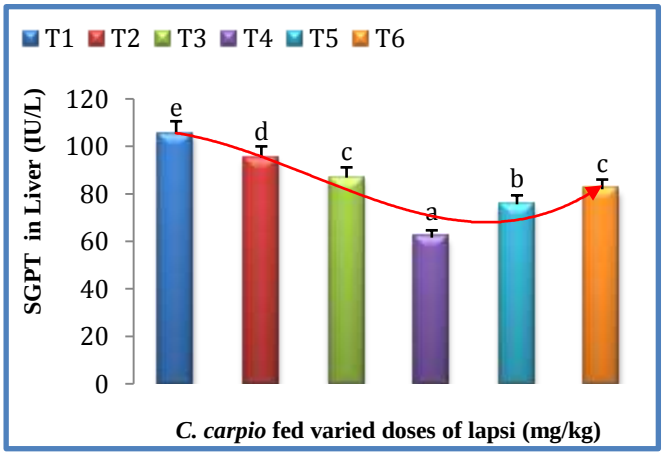

Figure 4.3.28 Serum glutamic pyruvate transaminase (SGPT) level in liver of *C. carpio* fed with diet containing six different doses of lapsi fruits.

4.3.29 Serum Glutamic Pyruvate Transaminase (SGPT) in Gills

SGPT level was found significantly (P<0.05) high (71.3%) in gills of common carp fed with control diet T1 (76.15±1.35e IU/L) followed by T6 (69.16±1.34d IU/L), T2 (62.17 ±1.34c IU/L), T5 (55.55±3.32b IU/L), T5 (75.61±1.37b IU/L) and the lowest was in carp fed diet T4 (37.34±0.81a IU/L). The SGPT level was 1.56 times higher in control diet fed common carp as compared to T4 carp (Fig.4.3.29).

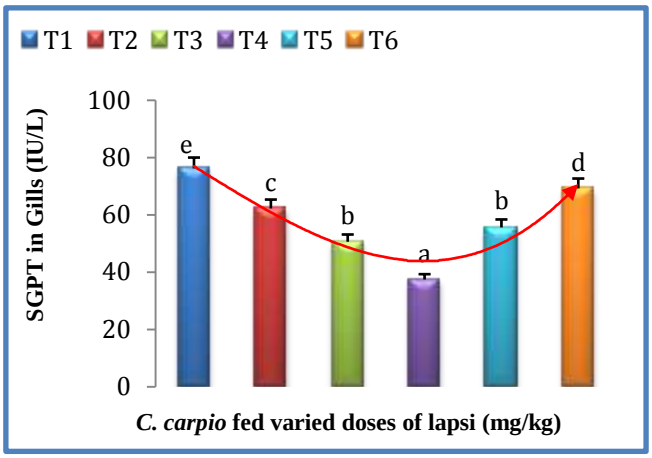

Figure 4.3.29 Serum glutamic pyruvate transaminase (SGPT) level in gills of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.30 Alkaline Phosphatase (ALP) in Blood Serum

ALP level was significantly (P<0.05) high (134.46±6.27a IU/L) in blood serum of common carp fed with diet T1 followed by T6 (123.07±5.42cd IU/L), T3 (119.48 ±2.71bc IU/L), T5 (113.26 ±0.55bc IU/L), T2 (109.60±1.181b IU/L) and T4 (93.89 ±3.51d IU/L) diets fed common carp. ALP level in the blood serum was 1.43 times higher in control diet fed carp as compared to that of T4 carp (Fig.4.3.30).

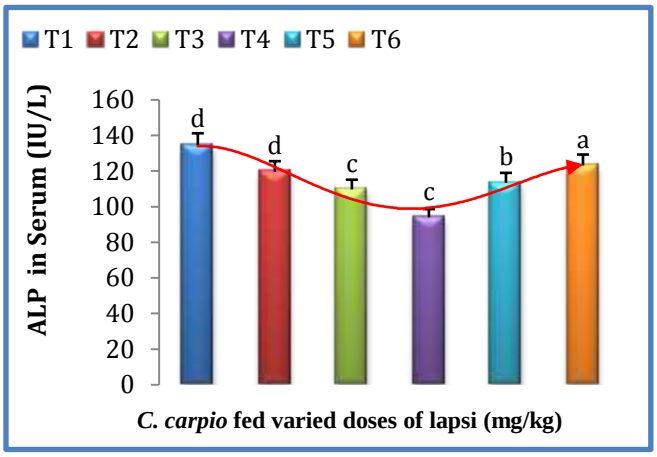

Figure 4.3.30 Alkaline phosphatase (ALP) level in blood serum of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.31 Alkaline Phosphatase (ALP) in Liver

ALP levels was found high (1.16 times) in the liver of common carp fed with diet T1 (383.13±1.51f IU/L) followed by diet T2 (348.09±15.374e IU/L), diet T3 (256.89±1.516d IU/L), diet T6 (158.32±8.184c IU/L), diet T5 (149.01 ±1.516b IU/L) and T4 (125.49 ±1.445a

IU/L) diets fed rohu. ALP level in liver was significantly higher (P<0.05) in control diet (T1) fed carp as compared to T4 diet fed common carp (Fig.4.3.31).

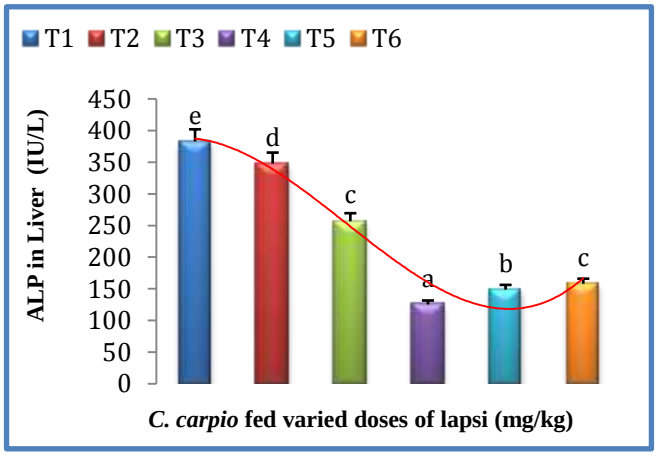

Figure 4.3.31 Alkaline phosphatase (ALP) level in liver of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.32 Alkaline Phosphatase (ALP) in Gills

The concentration of ALP level in gills was significantly (P<0.05) higher (275.25±1.51f IU/L) in the common carp fed with diet T1 followed by carps fed with diets T2 (213.66±1.69e IU/L), T3 (190.36±5.53d IU/L), T6 (160.45±2.17c IU/L) and T5 (147.09 ±2.43b IU/L). It was minimum in T4 diet fed carp (133.72±2.57a IU/L). Two times higher ALP level was recorded in gills of carp fed with T1 diet as compared to others. The ALP level in gills was (1.77) significantly higher (P<0.05) in control diet (T1) fed carp as compared to T4 carp (Fig.4.3.32).

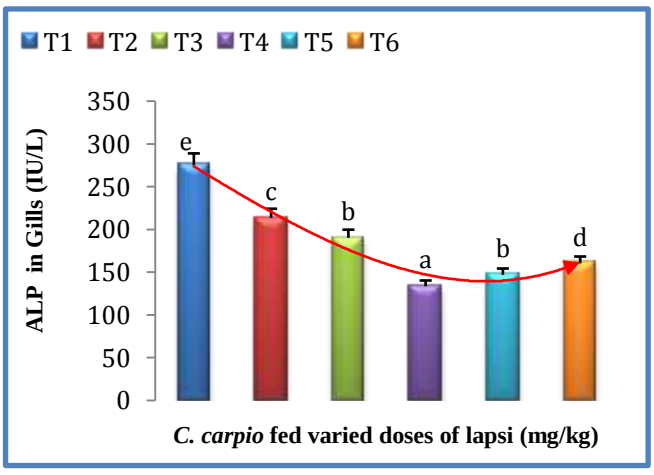

Figure 4.3.32 Alkaline phosphatase (ALP) level in gills of *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3. C-III BLOOD PROFILE

a) Complete Blood Counts

4.3.33 Haemoglobin

Haemoglobin concentration in blood (Fig.4.3.33) was significantly (P<0.05) higher in common carp fed with diet T4 (18.68±0.69 mg/dl) as compared to other treated and control diets fed carps. The concentration of haemoglobin in T5, T6, T3, T2 and T1 diets fed carps were 15.21±0.69, 11.55±0.78, 7.43±0.87, 5.19±0.60 and 3.38±0.83 mg/dl respectively. The concentration of haemoglobin in T4 diet fed common carp was 8.45 times higher than T1 diet fed common carp.

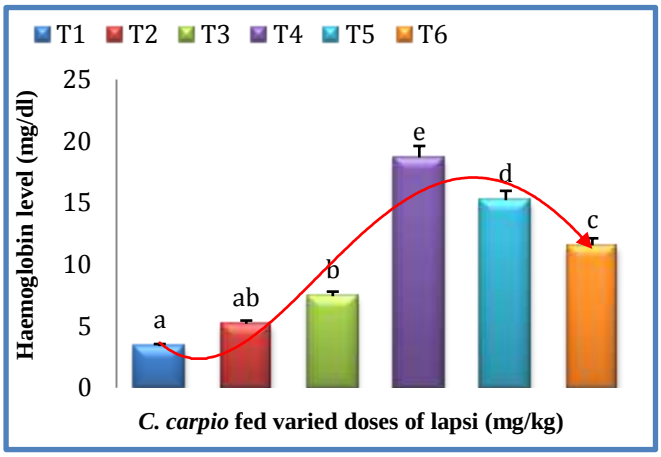

Figure 4.3.33 Haemoglobin level in C. carpio fed with diet containing six different doses of lapsi fruits

4.3.34 Erythrocytes (RBCs)

The number of RBCs in blood was significantly (P<0.05) higher in common carp fed with diet T2 (2.92±0.01 million/mm^3) as compared to other treated and control carps. The number of erythrocytes in T4, T6, T5, T3 and T1 diets fed carps were 2.90±0.04, 2.74±0.05, 2.73±0.06, 2.73±0.02 and 1.80±0.12 million/mm^3, respectively. The number of RBCs in T2 diet fed fish was 1.2 times higher as compared to T1 carps (Fig.4.3.34).

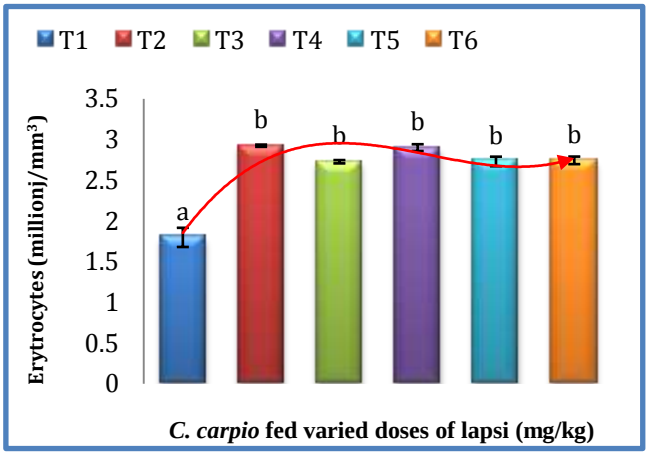

Figure 4.3.34 Erythrocytes in *C. carpio* fed with diet containing six different doses of lapsi fruits.

4.3.35 Leucocytes (WBCs)

The number of WBCs in blood (Fig.4.3.35) was significantly (P<0.05) higher (11040±77.38 10^3 /m^3) in common carp fed with diet T2 as compared to other treated and control groups. The numbers of leucocytes in T5, T1, T6, T3 and T4 diets fed carps were 9700±68.59, 9700±68.59, 8568±67.60, 8568±67.29, and 7840±77.38 10^3/mm^3 respectively. The number of WBCs in blood was 1.2 times higher in carp fed with T2 diet compared to T4 diet fed carps.

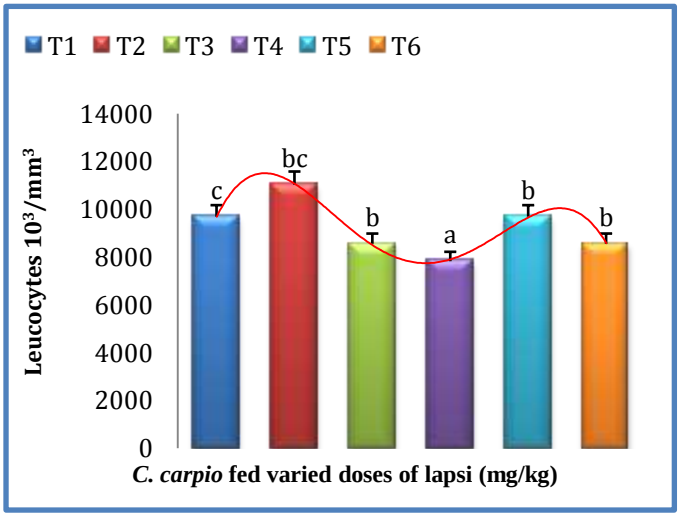

Figure 4.3.35 Leucocytes in *C. carpio* fed with diet containing six different doses of lapsi fruits

b) Absolute Values

4.3.36 Haematocrit or Packed Cell Volume (PCV)

The PCV level in blood (Fig.4.3.36) was significantly (P<0.05) higher in common carp fed with diet T4 (29.27±1.10 %) as compared to other treated and control groups. The PCV level in T5, T3, T6, T2 and T1 diets fed carp were 27.43±0.87, 27.60±0.38, 26.59±0.38, 26.29±0.62 and 21.43±2.24 % respectively.

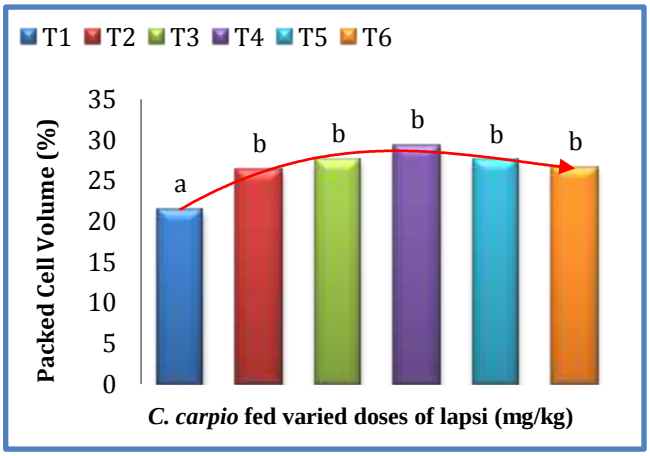

Figure 4.3.36 Packed Cell Volume level in *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.37 Mean Corpuscular Volume (MCV)

Significantly (P<0.05) higher MCV level in blood (Fig.4.3.37) was recorded in common carp fed with diet T4 (149.33±4.67µm³). The MCV levels were 135.33±2.33, 118.67±3.28, 116.00±4.51, 114.67±1.20 and 112.00±0.58 µm³ in T3, T6, T5, T2 and T1 diets fed carps, respectively. The MCV level in blood of T4 diet fed carp was 1.3 times higher as compared to carp fed with T5 diet fed common carp.

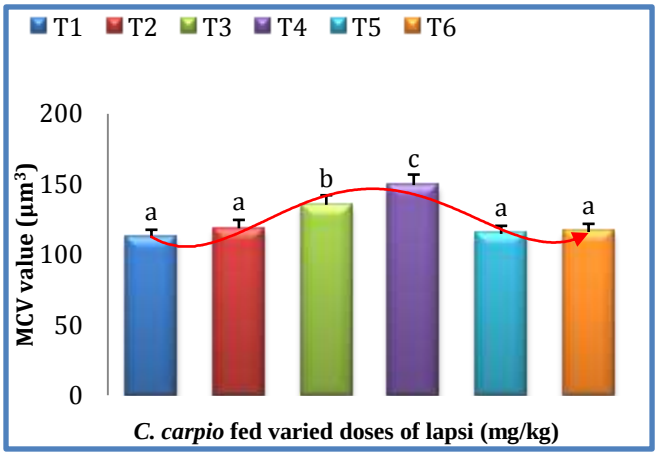

Figure 4.3.37 MCV level in *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.38 Mean Corpuscular Haemoglobin (MCH)

Significantly (P<0.05) higher level of MCH (49.35±1.66 pg) was recorded in common carp fed with diet (T4) compared to others (Fig.4.3.38). The MCH levels were 47.34±1.26, 47.31±0.44, 46.57±1.95, 42.63±0.16 and 36.25±0.77pg in T2, T5, T3, T6 and T1 diets fed common carp, respectively. MCH level was one time higher in control diet fed common carp compared to the treated groups.

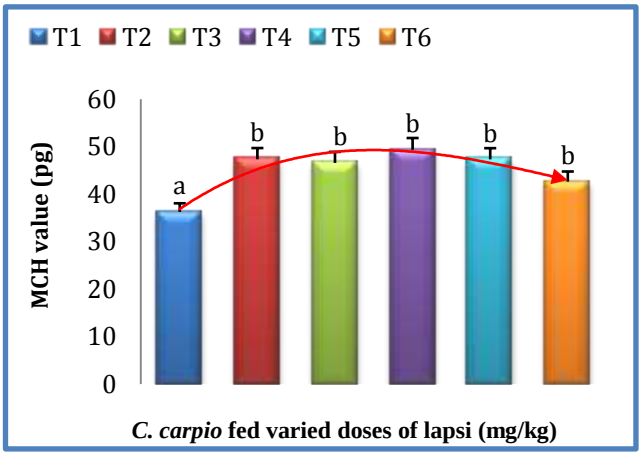

Figure 4.3.38 MCH level in *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.39 Mean Corpuscular Haemoglobin Concentration (MCHC)

Significantly (P<0.05) higher percentage of MCHC in blood was recorded (Fig.4.3.39) in common carp fed with diet T4 (36.30±0.63%) compared to the control (T1) carp. The percentage of MCHC in carp fed with diets T3, T5, T6 and T2 were 35.33±0.35, 34.77±0.24, 34.46±0.40 and 34.47±0.39 %, respectively. The lowest MCHC level (32.77±0.23 %) was recorded in carp fed with diet T1.

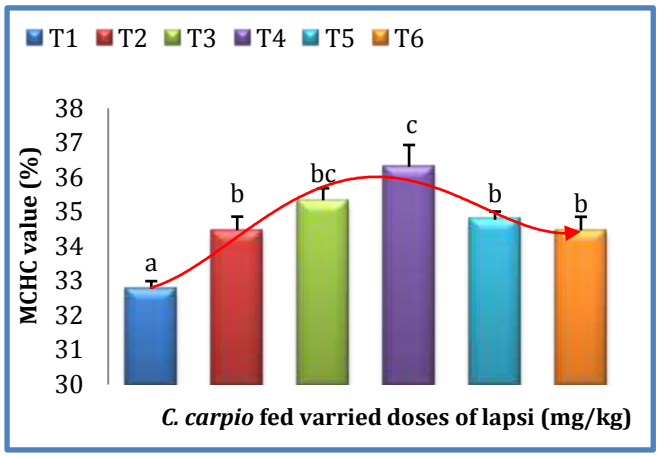

Figure 4.3.39 MCHC level in *C. carpio* fed with diet containing six different doses of lapsi fruits

c) Differential counts

4.3.40 Neutrophils (%)

Neutrophils % in blood (Fig.4.3.40) was significantly (P<0.05) higher (65.67±6.36 %) in the common carp fed with diet T5. Neutrophils % in T2, T6, T3, T4 and T1 diets fed common carp were 63.67±6.36, 63.67±4.31, 62.67±4.98, 53.00±6.35 and 43.00±3.35%, respectively.

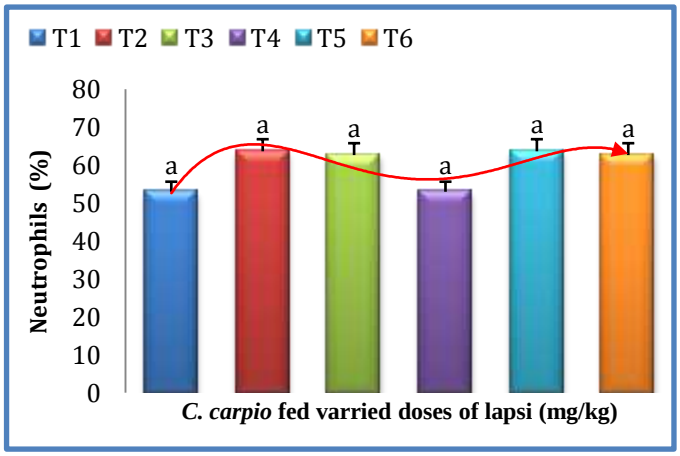

Figure 4.3.40 Neutrophils (%) in *C. carpio* fed with diet containing six different doses of lapsi **fruits**

4.3.41 Lymphocytes (%)

Lymphocytes % was significantly (P<0.05) higher (30.33±5.78%) in carp fed with diet T4 (Fig.4.3.41). Lymphocytes % in T3, T6, T5, T2, and T1 diets fed carp were 26.33±3.93, 26.33±1.93, 25.67±7.69, 24.67±7.69 and 23.33±5.78 %, respectively.

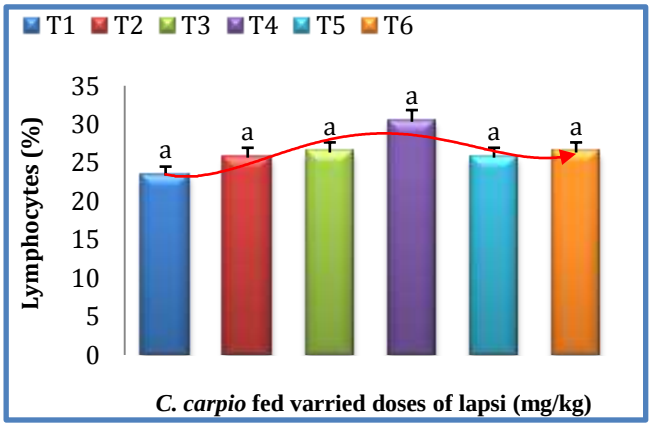

Figure 4.3.41 Lymphocytes (%) in *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.42 Monocytes (%)

Monocytes % was significantly (P<0.05) higher (3.43±1.31%) in common carp fed with diet T5 (Fig.4.3.42). The percentage of monocytes in T4, T6, T3, T2, and T1 diets fed common carp were 3.43±0.94, 3.13±0.48, 2.97±0.48, 2.87±1.31 and 2.13±0.94 %, respectively.

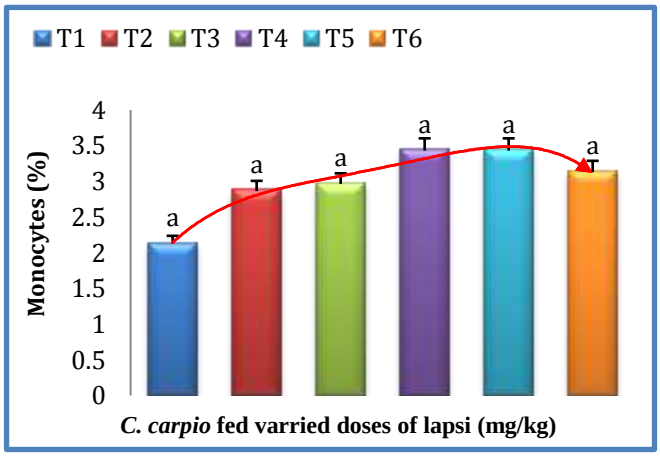

Figure 4.3.42 Monocytes (%) in *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.43 Eosinophils (%)

Significantly (P<0.05) higher eosinophil level was recorded (Fig. 4.3.43) in fish common carp fed with T6 diet (1.00±0.26) compared to the treated and control groups. Among the treatments, the value of eosinophils % in common carp fed with diets T4, T3, T5 T2 and T1 were 0.97±0.01, 0.90±0.20, 0.83±0.24, 0.83±0.24 and 0.73±0.22 respectively.

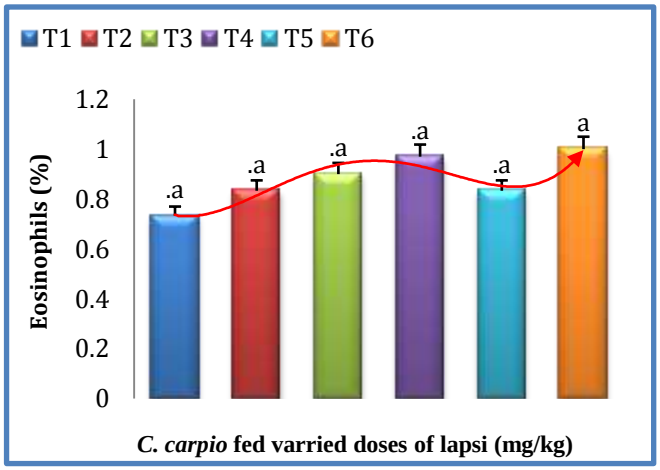

Figure 4.3.43 Eosinophils (%) in *C. carpio* fed with diet containing six different doses of lapsi fruits

4.3.44 Basophils (%)

Significantly (P<0.05) higher basophils % (0.07±0.09) was recorded in common carp fed with diet T4. In T5 diet fed carp, basophils level was 0.06±0.18 followed by 0.06±0.03 (T2), 0.06±0.18 (T3) and 0.05±0.22 (T6) diets fed common carp. The lowest basophils level was found in carp fed with diet T1 (0.03±0.03) (Fig. 4.3.44).

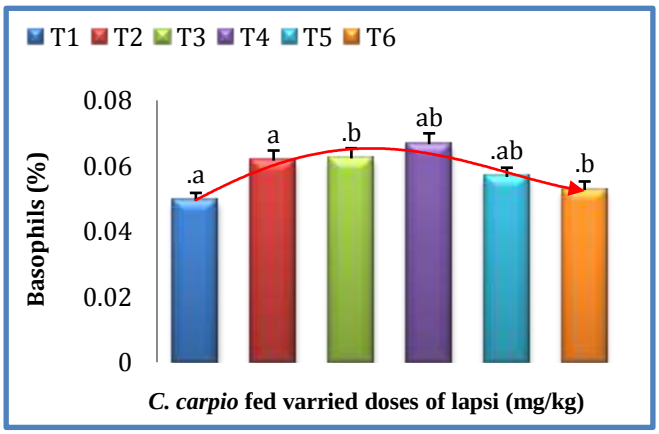

Figure 4.3.44 Basophils (%) in *C. carpio* fed with diet containing six different doses of lapsi fruits

4.4 Performance of Indigenous major rohu *Labeo rohita* Hamilton, 1822 (Cyprinidae) fed varied doses of ethanol extract of Lapsi *Choerospondias axillaris* fruit' pulp (LFP)

4.4 (A) Growth Performances

4.4.1 Survival Rate

Cent per cent survival rate was observed in T4 and T5 diet fed groups. For T2, T3 and T6, the survival rate was 97.78±6.65 % compared to 93.33±11.54 % in T1 group (Fig. 4.4.1).

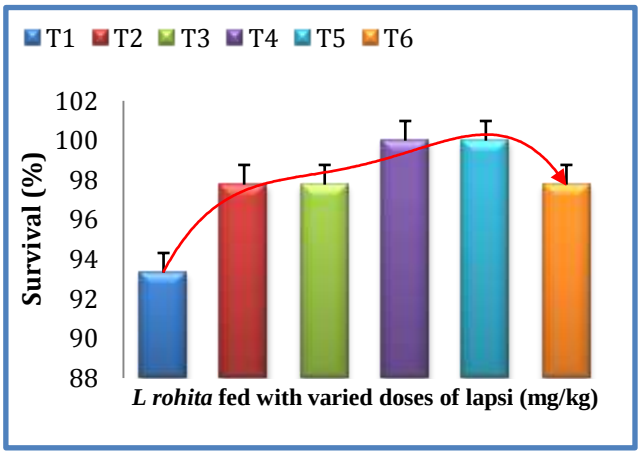

Figure 4.4.1 Survival rate of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.2 Final Average Weight Gain and Weight Gain (%)

The average weight of rohu in the beginning of experiment was 3.43 ± 0.13 g. After 90 days of culture, a direct relationship was found between final average weight gain of rohu and diet containing different doses of LFP.

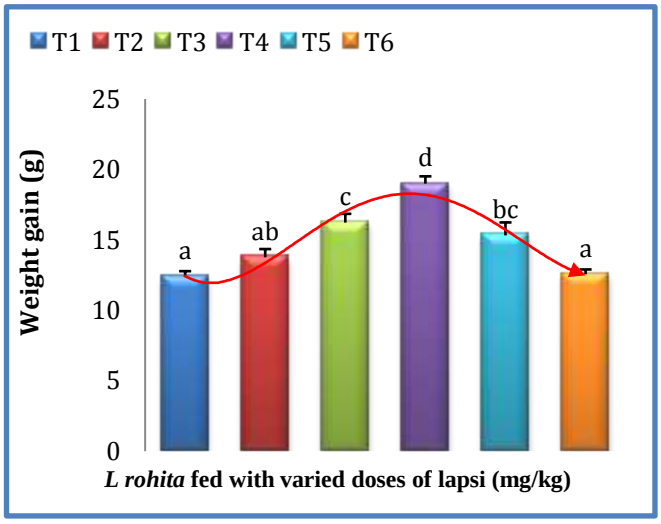

Figure 4.4.2 Final average weight gain of *L. rohita* fed with diet containing six different doses of lapsi fruits

The final average weight gain of rohu fed with diet T4 was found significantly (P<0.05) high (19.06±0.51d g) followed by groups fed with diets T3 (16.23±0.62c g), T5 (15.32±0.92bc g), T2

(13.76±0.58ab g) and T6 (12.55±0.34a g) and T1 (12.35±0.12a g). Final average weight of fish fed with T4 diet was 53.78 % higher compared to control group (Fig. 4.4.2).

The weight gain % was found high in T4 (554.44±16.13d) diet fed rohu followed by fish fed with diets T3 (472.62±18.11c), T5 (446.46 ±29.25bc), T2 (397.95 ±18.46ab), T6 (366.31±9.28a) and control diet T1 (359.73±11.098a). Similarly, 54.13 % increased in weight gain % was observed in fish fed with T4 diet as compared to control group (Fig. 4.4.2a).

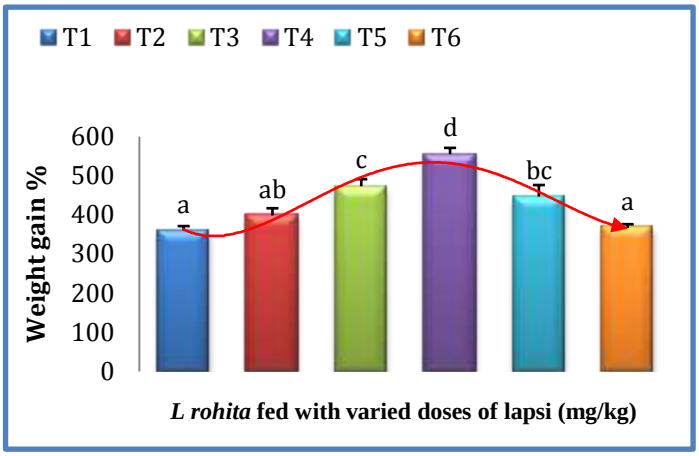

Figure 4.4.2a Weight gain percentages of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.3 Specific Growth Rate (SGR)

A direct relationship was found between SGR and LFP doses (Fig. 4.47). SGR was significantly (P<0.05) high in rohu fed with diet T4 (2.08±0.027d) followed by rohu fed with diets T3 (1.94±0.037c), T5 (1.88±0.068bc), T2 (1.78±0.041ab), and T6 (1.71±0.021d). Lowest SGR was found in rohu of control group T1 (1.69±0.026a). SGR was 23.03 % higher T4 diet fed rohu compared to control group (Fig. 4.4.3).

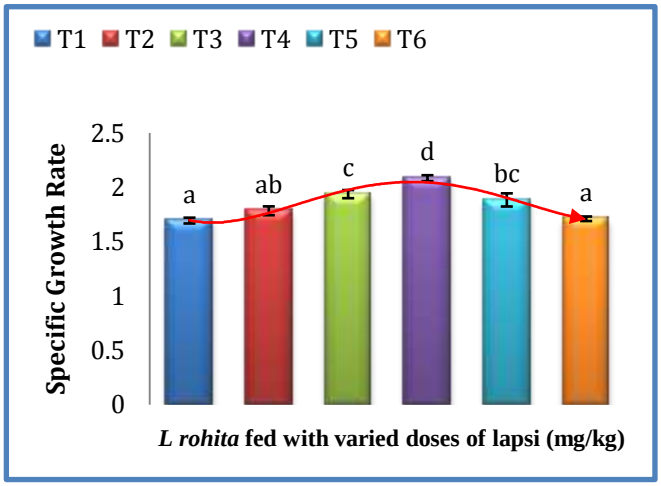

Figure 4.4.3 Specific growth rate of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.4 Feed Conversion Ratio (FCR)

An inverse relationship was found between FCR and LFP doses in diets. FCR level was found high in rohu fed with diet T1 (0.75±0.023a) followed by rohu fed with diets T6 (0.74±0.018d), T2 (0.68±0.032cd), T5 (0.61±0.048bc), and T3 (0.57±0.021b). Lowest FCR was

recorded in T4 (0.49±0.015a) diet fed rohu. FCR was 53.74 % lower in rohu fed with T4 diet compared to control diet fed rohu (Fig. 4.4.4).

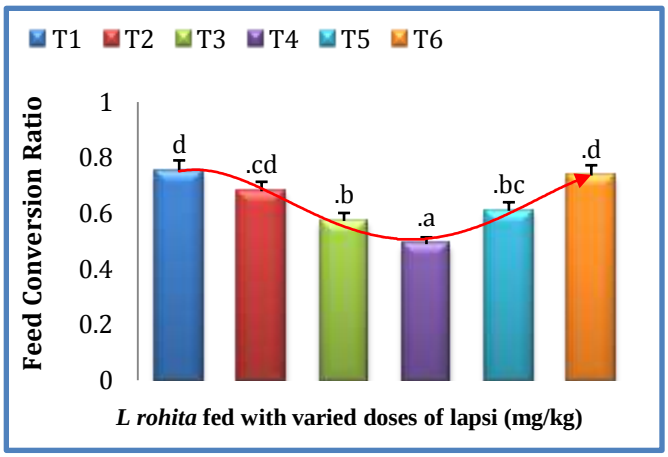

Figure 4.4.4 Feed conversion ratio of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4 (B) Biochemical Parameters

Vitamin C in blood serum, brain and liver of rohu in treatment and control groups were recorded.

4.4.5 Vitamin C in Blood Serum

A direct relationship was found between LFP doses in diets and concentration of vitamin C in blood serum (Fig. 4.4.5). Concentration of vitamin C in blood serum was significantly (P<0.05) higher (15.38±0.19e µg/dl) in rohu fed with T4 diet followed by rohu fed with diets T5 (14.46±0.16d µg/dl), T6 (13.34±0.16c µg/dl), T3 (12.52±0.25b µg/dl) and T2 (12.16±0.39b µg/dl).

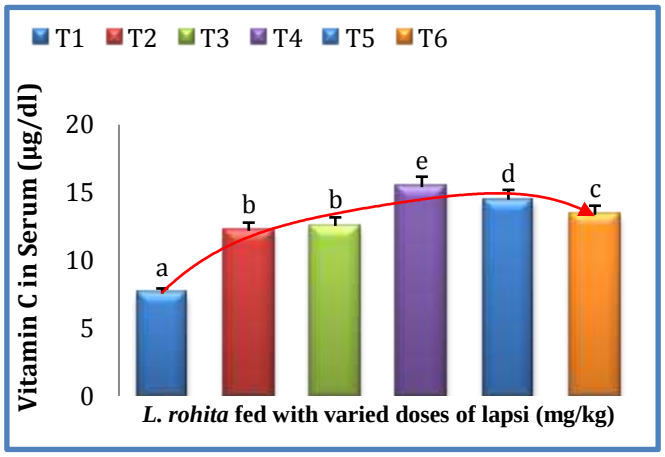

Figure 4.4.5 Vitamin C in blood serum of *L .rohita* fed with diet containing six different doses of lapsi fruits

The control diet fed rohu had the least amount of vitamin C in blood serum (9.54±0.22a µg/dl). 61.27 % higher vitamin C level was recorded in fish fed with T4 diet as compared to rohu fed with T1 diet.

4.4.6 Vitamin C in Brain

Concentration of vitamin C in brain was significantly (P<0.05) higher in rohu fed with diet T4 (91.20±1.21d µg/mg) followed by rohu fed with diets T3 (81.87 ±1.81c µg/mg), T2 (78.93±0.65c µg/mg), T5 (65.87±3.41b µg/mg) and T6 (59.02±1.35a µg/mg). The control diet (T1) fed group had the lowest vitamin C concentration in the brain (57.37±1.17a µg/mg). Vitamin C concentration was 58.95 % higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.6)

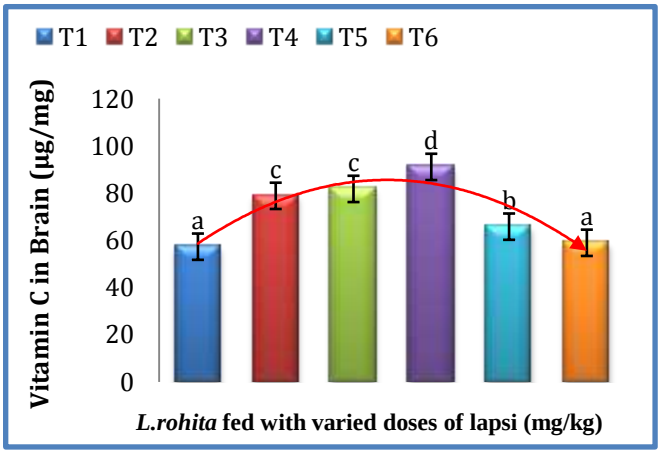

Figure 4.4.6 Vitamin C in brain of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.7 Vitamin C in Liver

Significantly (P<0.05) higher concentration of vitamin C in liver was recorded in rohu fed with diet T4 (191.83±1.97c µg/mg) compared to rohu fed with treated and control diets. Concentration of vitamin C in rohu fed with diets T3, T6, T2 and T5 were 148.51±3.25b µg/mg, 142.63 ±7.72b µg/mg, 136.72 ±0.36ab µg/mg and 135.49±4.94ab µg/mg respectively. The lowest concentration of vitamin C (127.52±1.93a µg/mg) in liverwas recorded in rohu fed with T1 diet. Vitamin C concentration was 50.43% higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.7).

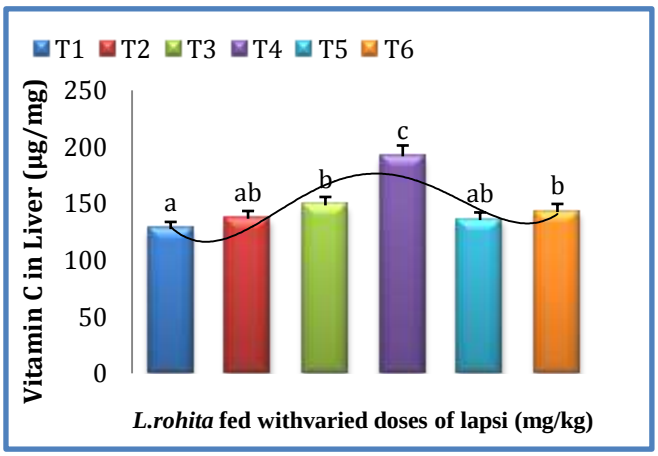

Figure 4.4.7 Concentration of vitamin C in liver of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4 (C) Immuno-Haematological Parameters

4.4. C-I PROTEIN PROFILE

4.4.8 Total Serum Protein

A direct relationship was found between LFP doses and concentration of total serum protein (TSP) in the blood of rohu. Concentration of serum protein was significantly (P<0.05) higher in blood sample of rohu fed with diet T4 (17.13±0.45c µg/dl) followed by rohu fed with diets T3 (14.95 ±0.95bc µg/dl), T6 (14.41 ±0.38bc µg/dl), T5 (13.74±0.22bc µg/dl) and T2 (13.09±0.31b µg/dl). Lower serum protein concentration was in rohu fed with control diet T1 (10.21±0.61a µg/dl). Serum protein level was 67.69 % higher in rohu fed with T4 diet compared to rohu fed with control diet T1 (Fig. 4.4.8).

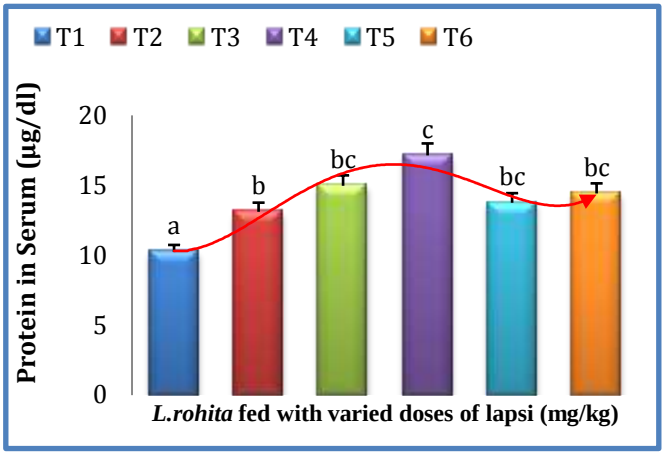

Figure 4.4.8 Concentration of Total serum protein in blood of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.9 Total Protein in Brain

The concentration of total protein was significantly (P<0.05) high in brain of rohu fed with diet T4 (47.96±0.27c µg/mg) followed by rohu fed with diets T6 (35.66±1.92c µg/mg), T5 (33.87±1.24b µg/mg), T3 (32.73±2.22b µg/mg), and T2 (30.27±4.02a µg/mg). Rohu fed with control diet T1 had the lowest protein concentration (21.76±5.21a µg/mg). Total protein concentration in brain was 3.07 times higher in rohu fed with T4 diet compared to rohu fed with control diet (Fig. 4.4.9).

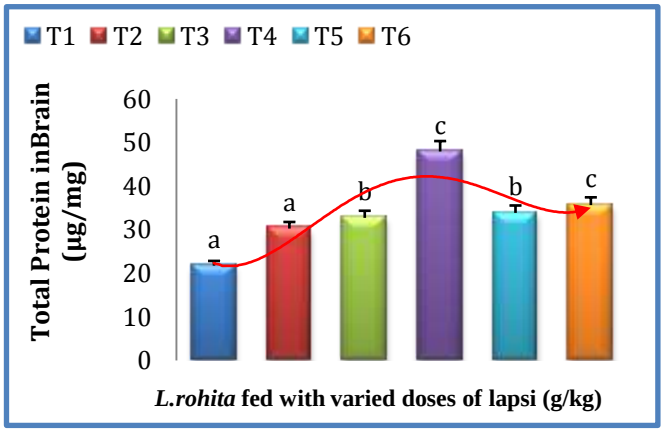

Fig.ure 4.4.9 Concentration of Total protein in brain of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.10 Total Protein in Liver

A direct relationship was found between LFP doses and concentration of total protein in liver of rohu. Concentration of total protein in liver was significantly (P<0.05) higher (22.80±0.35d µg/mg) in rohu fed with diet T4 followed by rohu fed with diets T2 (20.19±0.29c µg/mg), T5 (17.26±0.44bc µg/mg), T3 (17.14±0.43b µg/mg) and T6 (14.75±0.34b µg/mg). Lowest liver protein concentration was measured in control diet (T1) fed rohu (14.74±0.53a µg/mg). Total protein level was 54.65 % higher in rohu fed with T4 diet compared to rohu fed with control T1 diet (Fig. 4.4.10).

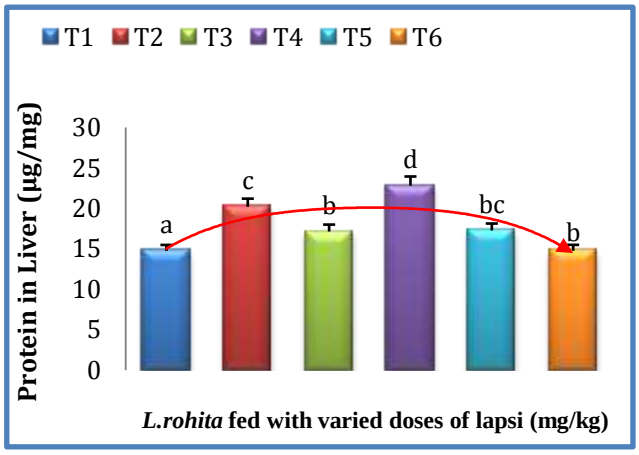

Figure 4.4.10 Concentration of Total protein in liver of *L.rohita* fed with diet containing six different doses of lapsi fruits

4.4.11 Total Protein in Muscles

Similar to brain and liver, concentration of total protein in muscles of rohu was found to increase with increasing LFP doses in the diets. Concentration of total protein in muscles was significantly (P<0.05) high in rohu fed with diet T5 (15.37±0.79c µg/mg) followed by rohu fed with diets T4 (15.08 ±0.29c µg/mg), T6 (14.14±0.12c µg/mg), T3 (10.16±0.16b µg/mg) and T2 (8.16±0.24b µg/mg) and control diet T1 (4.77±0.27a µg/mg). Total protein concentration in muscles was 3.13 times higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.11).

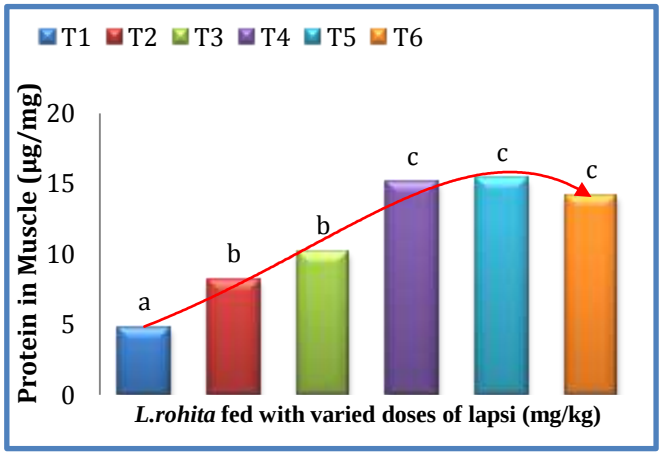

Fig.ure 4.4.11 Concentration of Total protein in muscles of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.12 Albumin in Blood Serum

Concentration of albumin in blood serum was significantly (P<0.05) high in rohu fed with diet T4 (6.64±0.59b µg/dl) followed by rohu fed with diets T5 (6.05±0.23b µg/dl), T3 (5.98±0.33b µg/dl), T6 (5.71±0.67a µg/dl) and T2 (5.70±0.54ab µg/dl).). Lowest albumin concentration was recorded in rohu fed with control diet T1 (4.20±0.11a µg/dl). Albumin concentration in blood serum was 1.62 times higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.12).

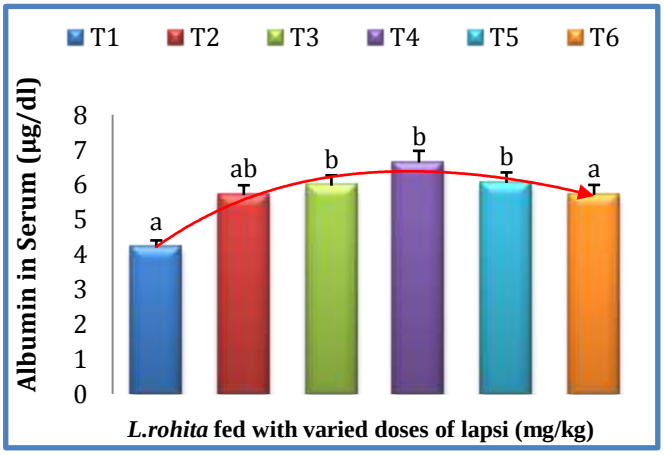

Figure 4.4.12 Concentration of Albumin in blood serum of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.13 Albumin in Brain

A direct relationship was found between LFP doses in diets and concentration of albumin in the brain of rohu. Concentration of albumin in the brain was significantly (P<0.05) higher (19.18±0.11c µg/mg) in rohu fed with diet T4 followed by rohu fed with diets T6 (14.25±0.74b µg/mg), T5 (13.55±0.49b µg/mg), T3 (13.09±0.047b µg/mg) and T2 (12.11±0.28ab µg/mg). Lowest albumin concentration was measured in the control group T1 (8.71±0.67a µg/mg). Albumin concentration in brain was 3.08 times higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.13).

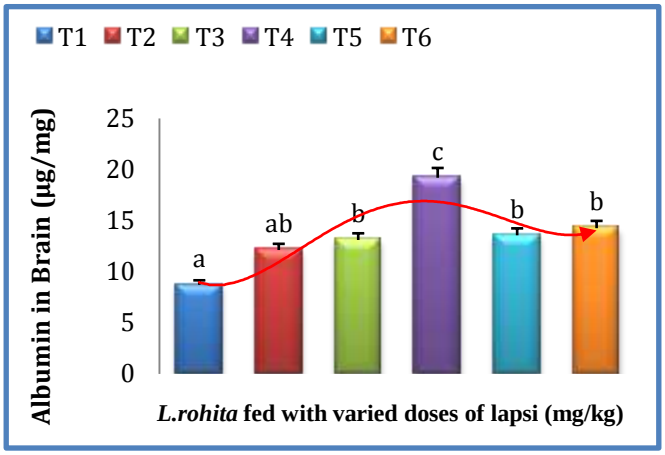

Figure 4. 4.13 Concentration of albumin in brain of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.14 Albumin in Liver

Similarly, albumin concentration was high in liver of rohu fed with diet T4 (9.12±0.17b µg/mg) followed by rohu fed with diets T2 (8.08±0.12b µg/mg), T5 (6.90±0.55aµg/mg), T3 (6.86±0.57a µg/mg), T6 (5.94±0.22b µg/mg). Liver albumin concentration was lowest in the control diet fed rohu (5.90±0.21a µg/mg). Liver albumin concentration was 54.58 % higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.14).

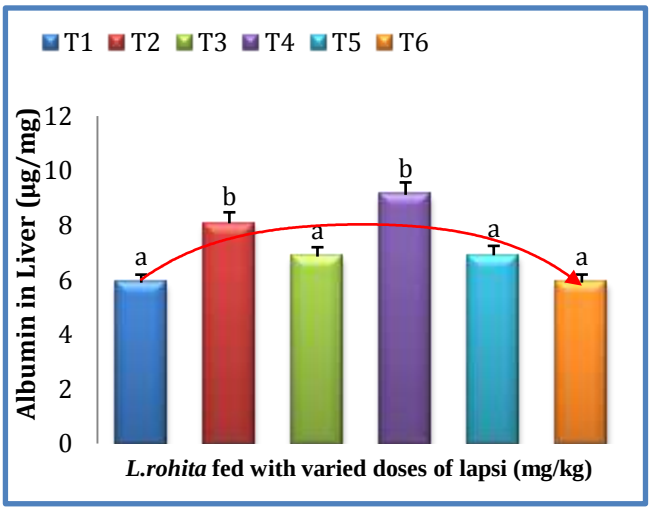

Figure 4.4.14 Concentration of albumin in liver of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.15 Albumin in Muscles

Albumin concentration was high in muscles of rohu fed with diet T4 (6.27±0.25c µg/mg) followed by rohu fed with diets T5 (5.95 ±0.24c µg/mg), diet T6 (5.62 ±0.41c µg/mg), T3 (4.06±0.46b µg/mg), T2 (3.26±0.49b µg/mg) and T1 (1.94±0.13a µg/mg). Albumin concentration was 3.2 times higher in rohu fed with T4 diet as compared to rohu fed with control (T1) diet (Fig. 4.4.15).

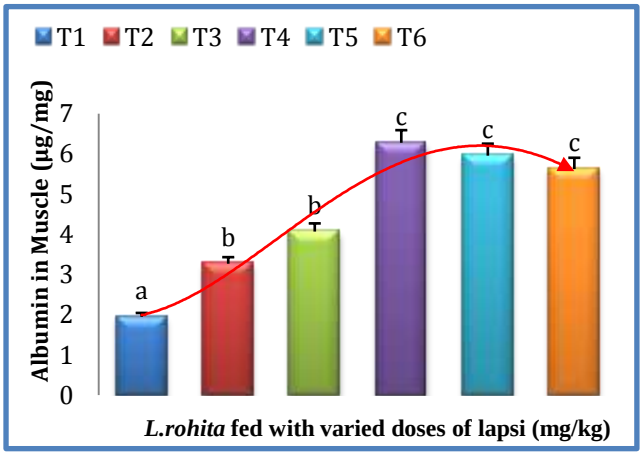

Figure 4.4.15 Concentration of albumin in muscles of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.16 Globulin in Blood Serum

A direct relationship was found between LFP doses and concentration of globulin in blood serum of rohu. Concentration of globulin in blood serum was significantly (P<0.05) high (10.48±0.89c µg/dl) in rohu fed with diet T4 followed by rohu fed with diets T3 (8.98±0.96bc µg/dl), T6 (8.69±0.29bc µg/dl), T5 (7.68±0.46ab µg/dl) and T2 (7.39±0.40ab µg/dl). Blood globulin concentration was measured lowest in control diet (T1) fed rohu

(6.02±0.68a µg/dl). Concentration of albumin in blood serum was 3.25 times higher in T4 diet fed rohu compared to rohu fed with control (T1) diet (Fig.4.4.16) .

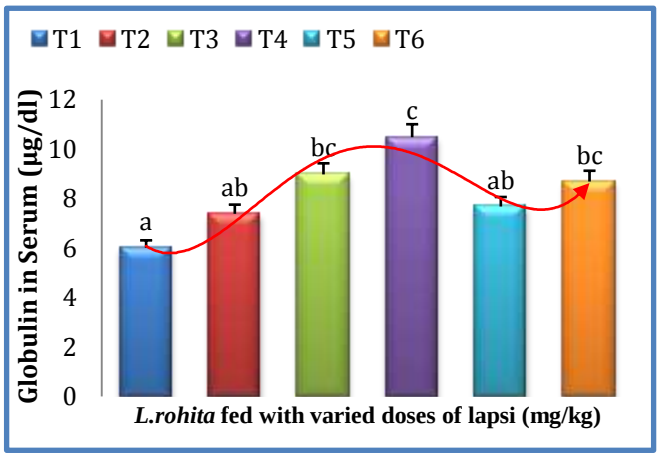

Figure 4.4.16 Concentration of globulin in blood serum of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.17 Globulin in Brain

A direct relationship was found between LFP doses and concentration of globulin in brain of rohu. Concentration of globulin in brain was significantly (P<0.05) higher (28.77±0.16b µg/mg) in rohu fed with diet T4 followed by rohu fed with diets T6 (21.41±0.15c µg/mg), T5 (20.32±0.74b µg/mg), T3 (19.64±0.33b µg/mg) and T2 (18.16±0.41ab µg/mg). Lowest brain globulin concentration was found in the control diet (T1) fed rohu (13.05±0.12a µg/mg). Globulin level in brain was 3.11 times higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.17).

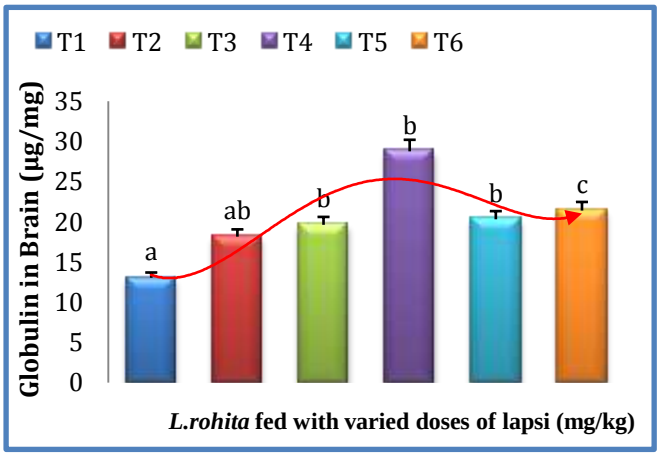

Figure 4.4.17 Concentration of globulin in brain of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.18 Globulin in Liver

Similar results were recorded globulin concentration in liver of rohu fed with LFP doses. The globulin concentration was found high in liver of rohu fed with diet T4 (13.68 ±0.18b µg/mg) followed by rohu fed with diets T2 (12.12 ±0.17b µg/mg), T5 (10.36±0.86a µg/mg), T3 (10.28 ±0.86a µg/mg) and T6 (8.85±0.25b µg/mg). Control diet T1 fed rohu had the lowest liver globulin (8.84±0.32a µg/mg). Globulin concentration in liver was 1.4 times higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.18).

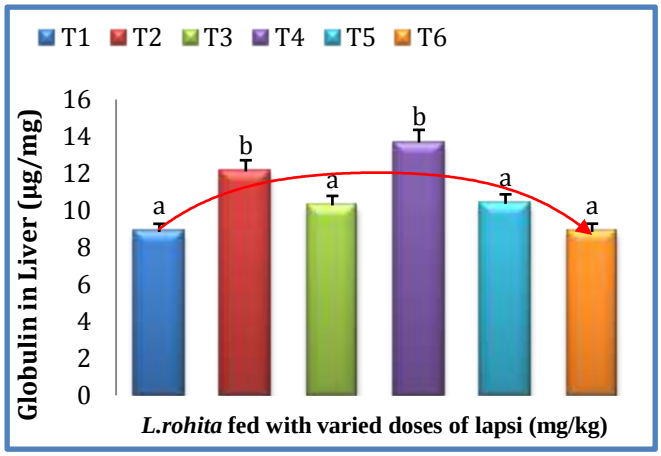

Figure 4.4.18 Concentration of globulin in liver of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.19 Globulin in Muscles

Globulin concentration in muscles was significantly (P<0.05) high in rohu fed with diet T5 (9.42± 0.67c µg/mg) followed by rohu fed with diets T4 (8.81±0.36c µg/mg), T6 (8.53±0.71c µg/mg), T3 (6.09± 0.69b µg/mg) and T2 (4.89±0.74b µg/mg). Lowest globulin concentration was in the control diet T1 (2.83±0.14a µg/mg) fed rohu. Globulin concentration was 2.83 times higher in muscles of rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.19).

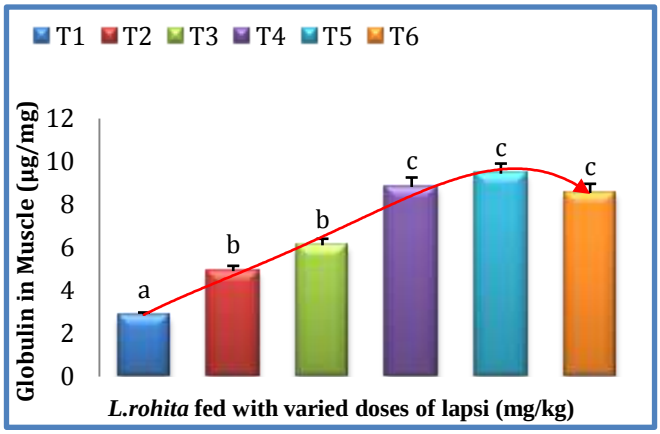

Figure 4.4.19 Concentration of globulin in muscles of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.20 Ratio of Albumin and Globulin (A/G) in Blood Serum

The ratio A/G in blood serum was higher in rohu fed with diet T5 (0.79±0.075). The A/G ratio was 0.77±0.037 in T2, 0.69±0.086 in T1, 0.67±0.055 in T3, 0.66±0.054 in T6 and 0.63±0.017 in T4 diets fed rohu (Fig. 4.62). Lowest A/G ratio was found in T4 diet fed rohu. A/G ratio was 21% lower in T4 diet fed rohu compared to rohu fed with control (T1) diet (Fig. 4.4.20).

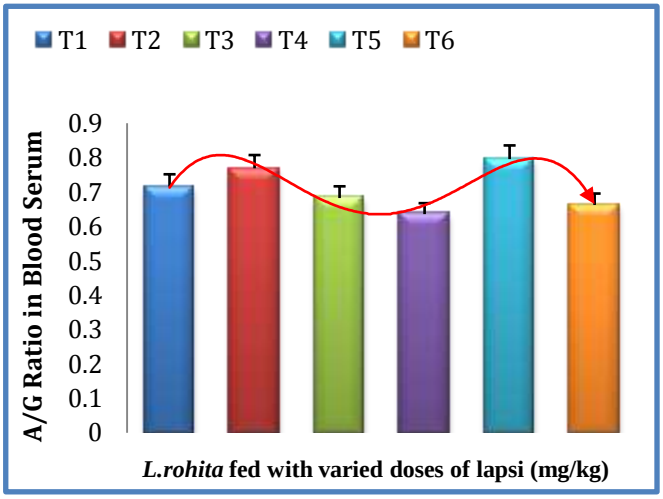

Figure 4.4.20 Ratio of albumin and globulin in blood serum of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.21 Ratio of Albumin and Globulin (A/G) in Brain

The A/G ratio in brain was higher in rohu fed with diet T5 (0.62±0.033). The ratios were 0.61±0.032 in T1, 0.57±0.084 in T3, (0.53±0.048) in T2, (0.47±0.072ab) in T6 and (0.51±0.012 in T4 diets fed rohu. Lowest A/G ratio in brain was found in T4 diet fed rohu. A/G ratio was 29.1% higher in T5 diet fed rohu compared to rohu fed with T4 diet (Fig. 4.4.21).

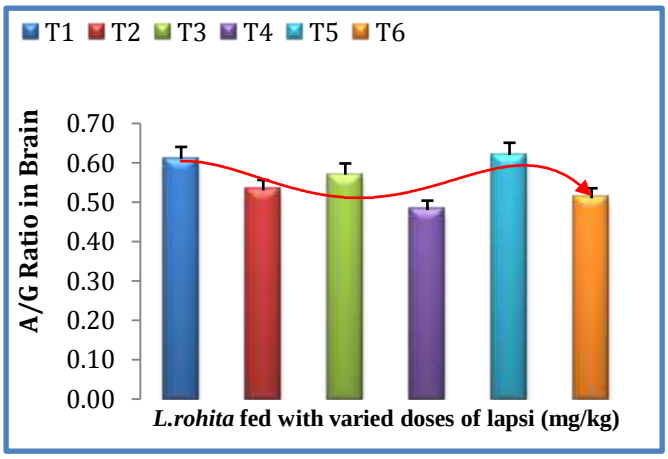

Figure 4.4.21 Ratio of albumin and globulin in brain of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.22 Ratio of Albumin and Globulin (A/G) *in Liver*

The A/G ratio in liver was highest in rohu fed with diet T3 (0.69±0.026), The ratios were 0.67±0.012 in T4, 0.65±0.018 in T5 and 0.62±0.024 in T1, (0.62±0.014) in T6 and (0.61±0.024) in T2 diets fed rohu. A/G ratio was 0.5 fold higher in T3 diet fed rohu compared to rohu fed with control diet T1 (Fig. 4.4.22).

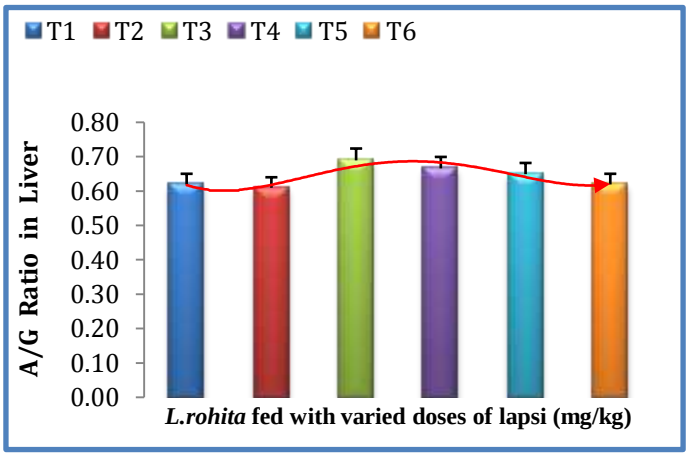

Figure 4.4.22 Ratio of albumin and globulin in liver of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.23 Ratio of Albumin and Globulin (A/G) *in Muscles*

The A/G ratio in muscles was highest in rohu fed with diet T4 (0.78±0.072). The ratio was 0.71±0.016 in T3, 0.69±0.011 in T1, 0.64±0.048 in T5, 0.63±0.024 in T6 and 0.62±0.012 in T2 diets fed rohu. A/G ratio was 25.8% higher in T4 diet fed rohu compared to control T1 rohu (Fig. 4.4.23).

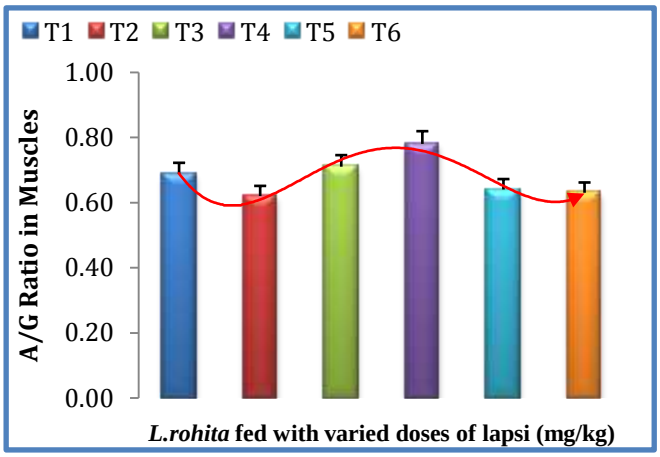

Figure 4.4.23 *Ratio* of albumin and globulin in muscles of rohu fed with diet containing six different doses of lapsi fruits

4.4. C-II ENZYME PROFILE

4.4.24 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Blood Serum

SGOT level was found significantly ($P<0.05$) high in blood serum of rohu fed with control (T1) diet (63.08±0.98d IU/L) followed by T2 (62.28 ±0.28d IU/L), T4 (62.28 ±0.24c IU/L), T3 (53.35±0.96d IU/L), T5 (44.79±0.74b) and T6 (24.05±0.41a IU/L) diets fed rohu.

SGOT level was 2.6 times higher in control (T1) diet fed rohu compared to rohu fed with T6 diet (Fig.4.4.24).

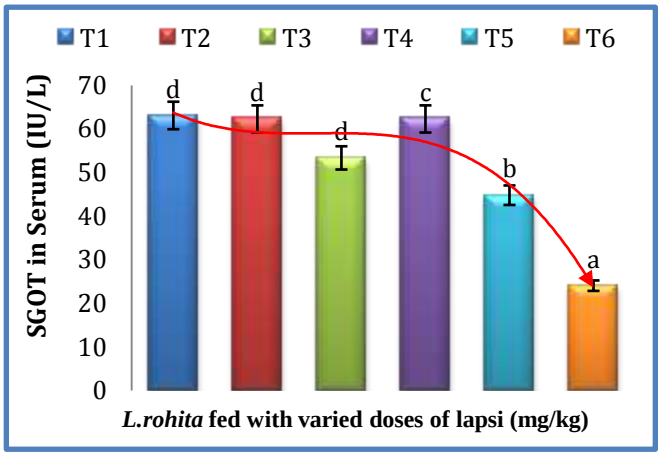

Figure 4.4.24 Serum glutamic oxaloacetic transaminase (SGOT) level in blood serum of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.25 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Liver

SGOT level was found significantly (P<0.05) high in liver of rohu fed with control diet T1 (123.57±0.38f IU/L) followed by T2 (118.05±0.74e IU/L), T3 m(112.44±0.63d IU/L), T5 (107.03±0.38c IU/L), T6 (101.83±0.32b) and T4 (95.98±0.18a IU/L) diets fed rohu. SGOT level in liver was 1.21 times higher in control diet (T1) fed rohu compared to rohu fed with T4 diet (Fig.4.4.25).

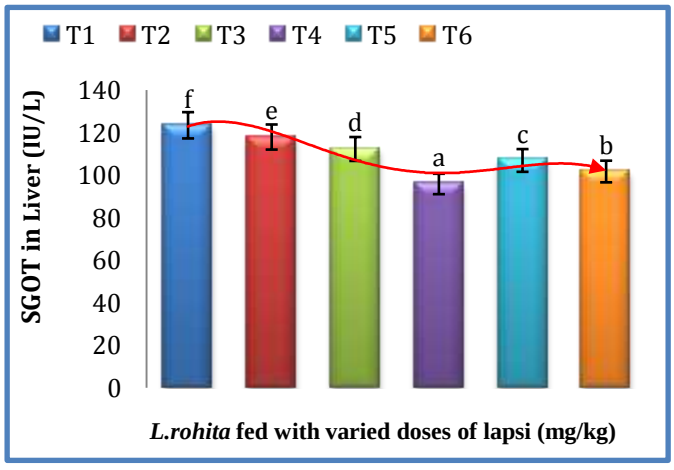

Figure 4.4.25 Serum glutamic oxaloacetic transaminase (SGOT) level in liver of *L.rohita* fed with diet containing six different doses of lapsi fruits

4.4.26 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Gills

SGOT level was found significantly (P<0.05) high in gills of rohu fed with control diet T1 (76.59±0.84f IU/L) followed by T2 (67.72±0.85e IU/L), T4 (64.67±0.62d IU/L), T5 (62.80±0.65c IU/L) and T6 (55.42±0.25b) diets fed rohu. The lowest SGOT in gills was found in rohu fed with diet T3 (54.92±0.14a IU/L). SGOT level in gills was 1.38 times higher in control diet (T1) fed rohu compared to rohu fed with T3 diet (Fig.4.4.26).

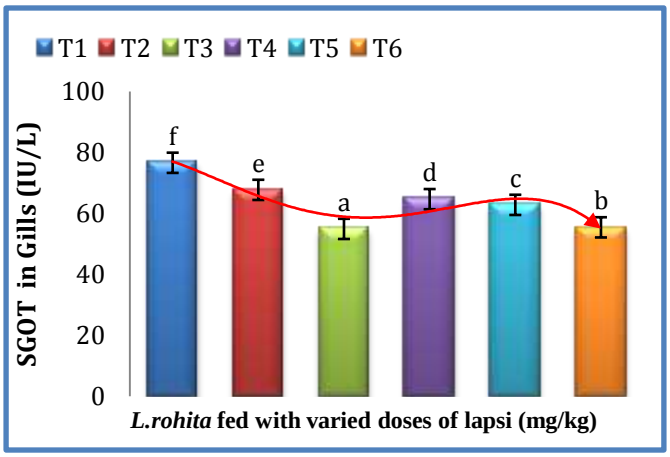

Figure 4.4.26 Serum glutamic oxaloacetic transaminase (SGOT) level in gills of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.27 Serum Glutamic Pyruvate Transaminase (SGPT) in Blood Serum

SGPT level was found significantly (P<0.05) high in blood serum of rohu fed with control diet T1 (83.78±1.31b IU/L) followed by T3 (73.13±1.35b IU/L), T4 (65.35±1.53b IU/L), T5 (54.92±1.56ab IU/L), T2 (51.20±2.61ab IU/L) and T6 (25.09±1.16a IU/L) diets fed rohu. SGPT level was 3.3 times higher in control diet (T1) fed rohu compared to rohu fed with T6 diet (Fig.4.4.27).

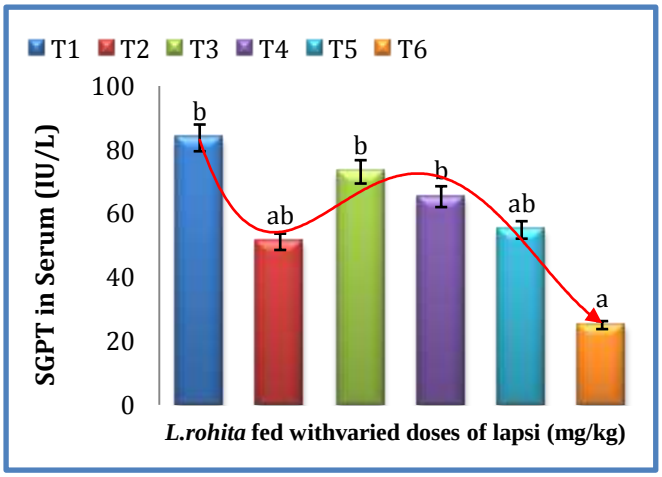

Figure 4.4.27 Serum glutamic pyruvate transaminase (SGPT) level in blood serum of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.28 Serum Glutamic Pyruvate Transaminase (SGPT) in Liver

SGPT level was found significantly (P<0.05) high in liver of rohu fed with control (T1) diet (172.15±1.42f IU/L) followed by T2 (164.39±1.49e IU/L), T5 (140.24± 1.76d IU/L), T3 (133.35±1.45c IU/L), T4 (125.44±1.64b IU/L) and T6 (117.83±1.42a IU/L) diets

fed rohu. SGPT level was 1.45 times higher in control (T1) diet fed rohu as compared to rohu fed with T6 diet (Fig.4.4.28).

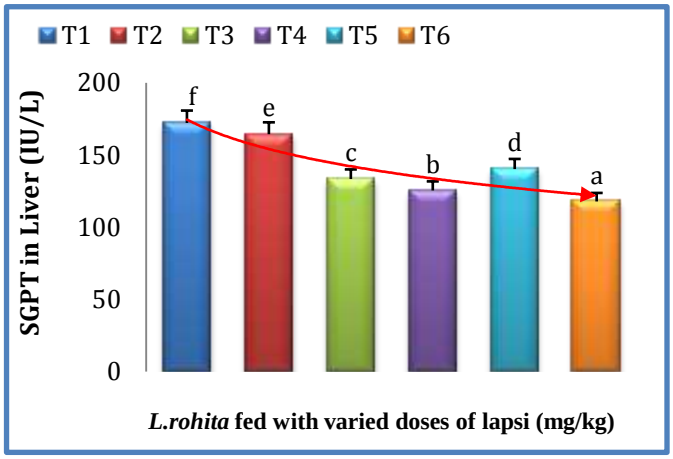

Figure 4.4.28 Serum glutamic pyruvate transaminase (SGPT) level in liver of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.29 Serum Glutamic Pyruvate Transaminase (SGPT) in Gills

SGPT level was found significantly (P<0.05) high in gills of rohu fed with control diet T1 (110.06±1.61f IU/L) followed by T2 (94.30±0.46e IU/L), T6 (89.87±0.39b IU/L), T3 (85.44±0.85c IU/L), T5 (80.52±0.63b IU/L) and T4 (71.72±1.27a IU/L) diets fed rohu. SGPT level in gills was 1.23 times higher in control (T1) diet fed rohu compared to rohu fed with T4 diet (Fig.4.4.29).

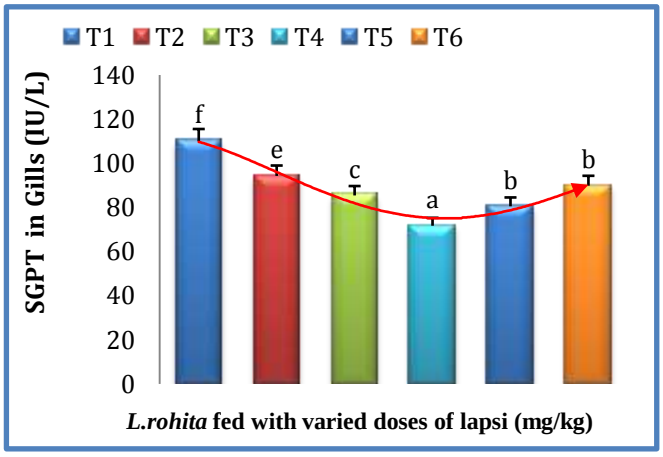

Figure 4.4.29 Serum glutamic pyruvate transaminase (SGPT) level in gills of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.30 Alkaline Phosphatase (ALP) in Blood Serum

ALP level was significantly (P<0.05) high (76.38±0.58f IU/L) in blood serum of rohu fed with diet T1 followed by T2 (65.77±1.24e IU/L), T3 (54.70±1.16 IU/L), T4 (47.40±1.52b IU/L), T5 (37.94±0.25d IU/L) and T6 (30.38±1.51a IU/L) diets fed rohu. ALP

level in blood serum was 2.5 times higher in control (T1) diet fed rohu compared to rohu fed with T6 diet (Fig.4.4.30).

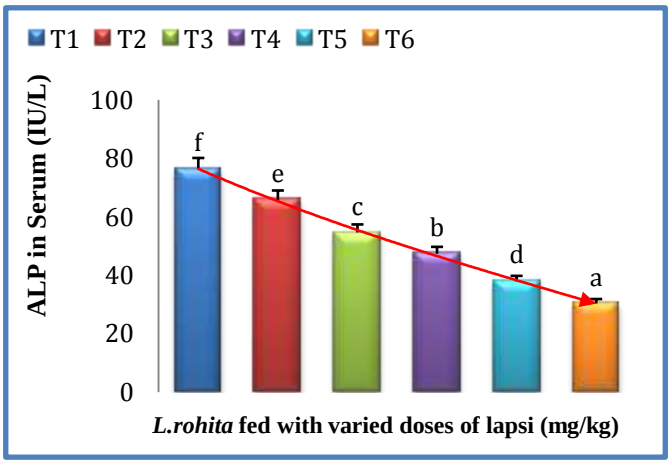

Figure 4.4.30 Alkaline phosphatase (ALP) level in blood serum of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.31 Alkaline Phosphatase (ALP) in Liver

ALP level was significantly (P<0.05) high (44.09±1.73f IU/L) in liver of rohu fed with diet T1 followed by T2 (38.18±0.81e IU/L), T5 (33.42 ±0.91c IU/L), T6 (30.94±0.33b IU/L), T3 (29.32±0.85d IU/L) and T4 (23.41±0.82a IU/L) diets fed rohu. ALP level in liver was 1.43 times higher in control (T1) diet fed rohu compared to T4 diet fed rohu (Fig.4.4.31).

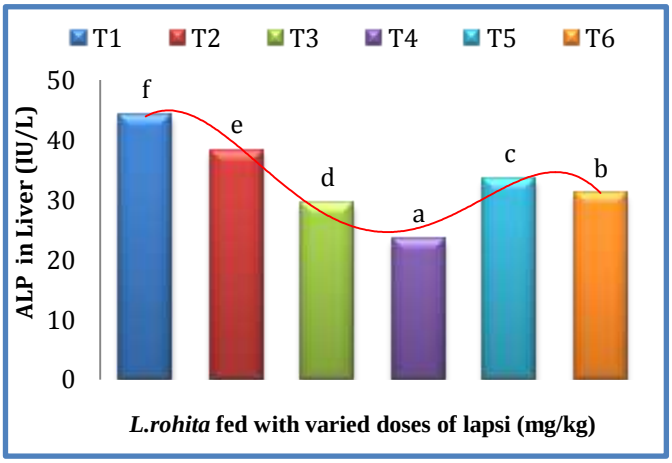

Figure 4.4.31 Alkaline phosphatase (ALP) level in liver of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.32 Alkaline Phosphatase (ALP) in Gills

ALP level was significantly (P<0.05) high (24.89±0.17f IU/L) in gills of rohu fed with diet T1 followed by T2 (22.49±0.35e IU/L), T5 (19.29±0.56 c IU/L), T3 (18.49±0.46 b IU/L), T6 (15.66±0.45d IU/L) and T4 (14.49±0.41a IU/L). ALP level in gills was 1.58 times higher in control (T1) diet fed rohu compared to rohu fed with T4 diet (Fig.4.4.32).

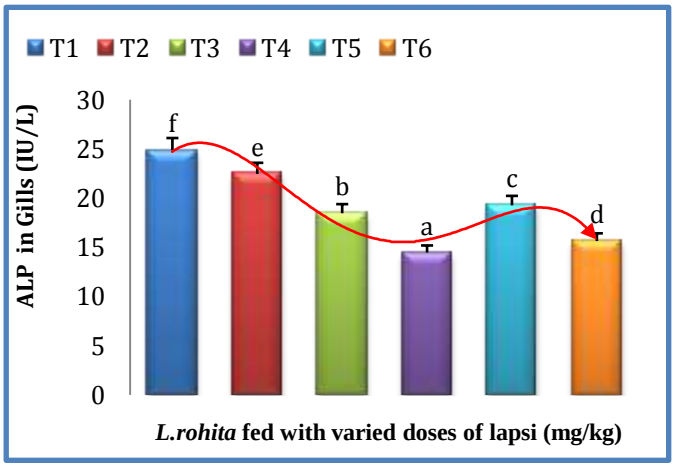

Figure 4.4.32 Alkaline phosphatase (ALP) level in gills of *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4. C-III BLOD PROFILE

a) Complete Blood Counts

4.4.33 Haemoglobin

Haemoglobin concentration in blood was significantly (P<0.05) higher in rohu fed with diet T4 (25.02±0.42e mg/dl) compared to other treated and control diets fed rohu. The concentration of haemoglobin in T5, T6, T3, T2 and T1 diets fed rohu were 23.04±0.36de, 20.71±0.47cd, 18.27±0.33bc, 15.58 ±0.15ab and 12.95±0.91a mg/dl respectively. The concentration of haemoglobin in T4 diet fed rohu was 1.9 times higher compared to rohu fed with control (T1) diet (Fig. 4.4.33).

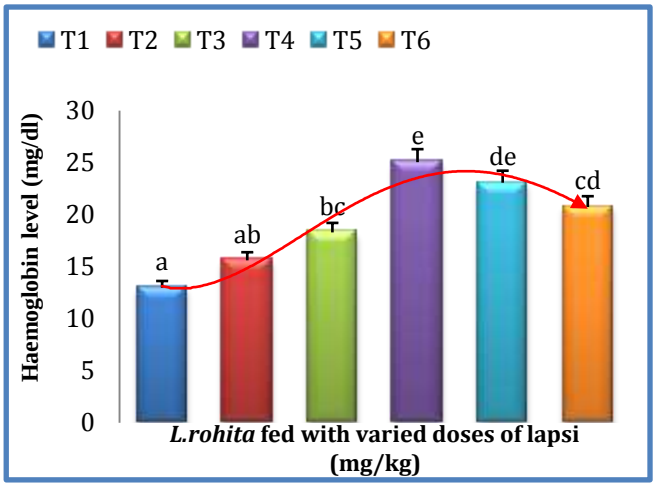

Figure 4.4.33 Haemoglobin level in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.34 Erythrocytes (RBCs)

Erythrocytes number in blood was significantly (P<0.05) higher in rohu fed with diet T6 (5.16 ±0.15e million/mm^3) compared to treated and control diets fed rohu. The number of erythrocytes in T5, T4, T3, T3 and T1 diets fed rohu were 4.75±0.21de, 4.41±0.16cd, 3.84± 0.32bc, 3.35 ±0.16b and 2.40 ±0.06a million/mm^3 (Fig.4.4.34).

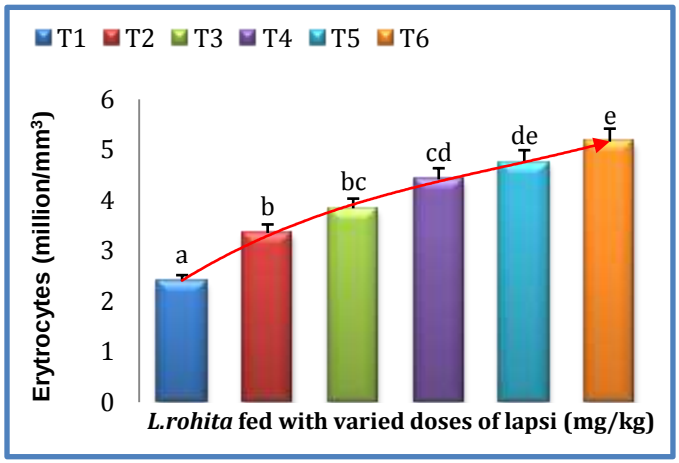

Figure 4.4.34 Erythrocytes in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.35 Leucocytes (WBCs)

Leucocytes number in blood was significantly (P<0.05) higher (45.95±0.70f 10^3/mm^3) in rohu fed with diet T1 compared to other treated and control diet fed rohu. The numbers of WBCs in T2, T6, T3, T5 and T4 diets fed rohu were 42.56±0.48e, 38.69±0.69d, 34.65± 0.04c, 31.42±0.06b, and 27.40±0.11a 10^3/mm^3 respectively. Leucocytes number in blood was 1.67 times higher in rohu fed with T1 diet fed compared to rohu fed with T4 diet (Fig. 4.4.35).

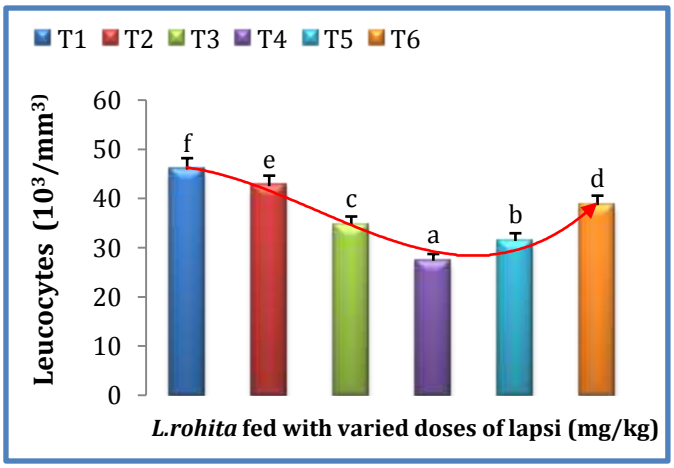

Figure 4.4.35 Leucocytes in *L. rohita* fed with diet containing six different doses of lapsi fruits

b) **Absolute Values**

4.4.36 Haematocrit (Hct) or Packed Cell Volume (PCV)

PCV level in blood was significantly (P<0.05) higher in rohu fed with diet T4 (55.18±0.51b %) compared to other treated and control diet fed rohu. PCV level in T5, T3, T6, T1 and T2 diets fed rohu were 47.04±0.27b, 46.49±0.13b, 45.62 ±0.27ab, 42.92±0.84ab and 41.05 ±0.91a % respectively. PCV level in blood was 1.31 times higher in rohu fed with T4 diet compared to rohu fed with control (T1) diet (Fig. 4.4.36).

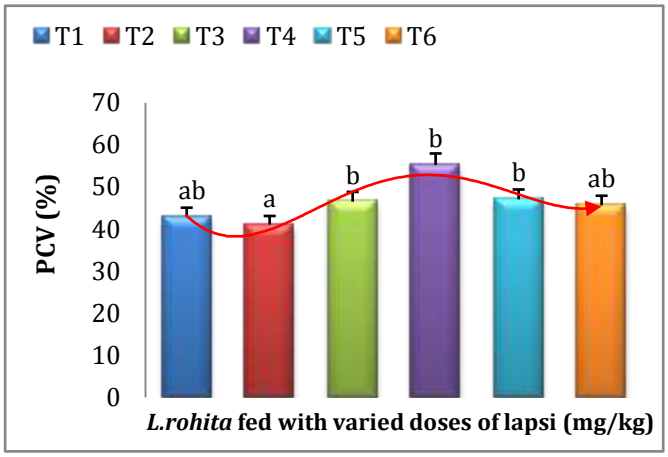

Figure 4.4.36 PCV level in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.37 Mean Corpuscular Volume (MCV)

Significantly (P<0.05) higher MCV level in the blood was recorded in rohu fed with diet T1 (179.24±1.62c µm³). MCV levels in rohu fed with diets T4, T2, T3, T5 and T6 diets were 125.08±1.53b, 123.15±1.79b, 121.96±1.57b, 99.38±1.75a and 88.57 ±1.06a µm³ respectively. MCV level in blood of T1 diet fed rohu was 2.1 times higher compared to rohu fed with T6 diet (Fig. 4.4.37).

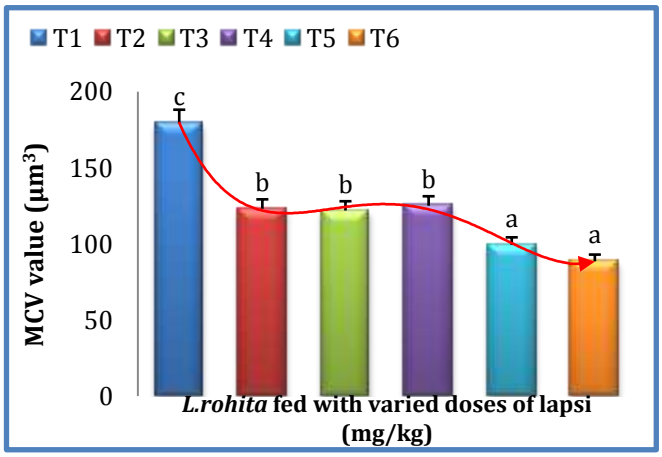

Figure 4.4.37 MCV level in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.38 Mean Corpuscular Haemoglobin (MCH)

Significantly (P<0.05) higher level of MCH (56.90±1.77b pg) was recorded in rohu fed with diet (T4). MCH levels in rohu fed with T1, T5, T3, T2 and T6 diets were 53.93±1.31ab, 48.73±1.78ab, 48.41±1.96ab, 46.96±1.35ab and 40.24±1.96a pg respectively (Fig.4.4.38).

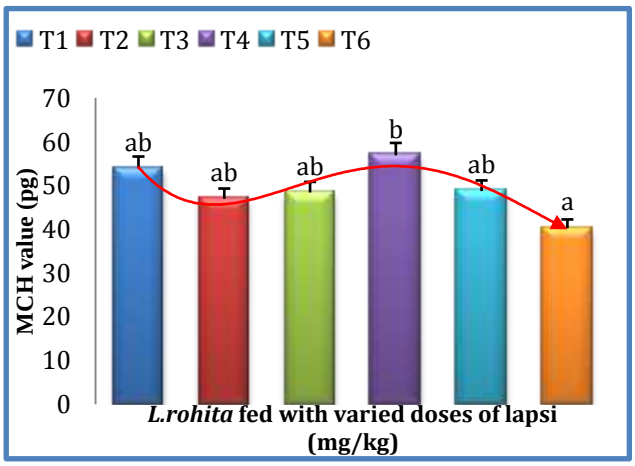

Figure 4.4.38 MCH level in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.39 Mean Corpuscular Haemoglobin Concentration (MCHC)

Significantly (P<0.05) higher percentage of MCHC was recorded in rohu fed with diet T5 (48.99±0.51c %) compared to other treated and control groups. The percentage of MCHC in rohu fed with diets T4, T6, T3 and T2 were 45.61±1.97bc, 45.38±0.76c, and 39.51±1.46b and 38.10±1.40ab % respectively. Lowest MCHC level was recorded in rohu fed with diet T1 (30.12±1.57a %). MCHC level in T5 diet fed rohu was 1.6 times higher compared to control (T1) diet fed rohu (Fig. 4.4.39).

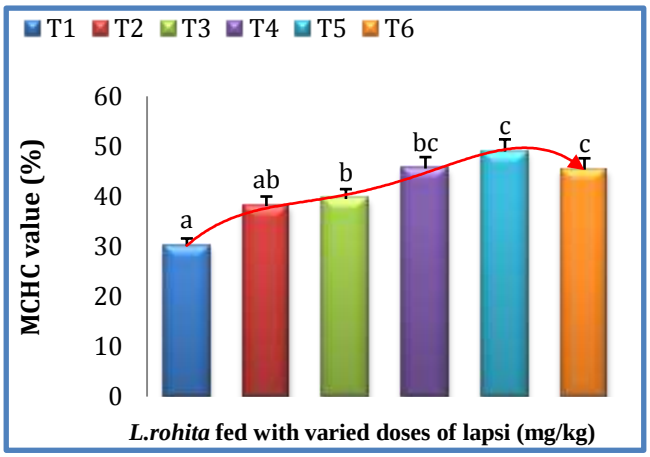

Figure 4.4.39 MCHC level in *L. rohita* fed with diet containing six different doses of lapsi fruits

c) Differential counts

4.4.40 Neutrophils (%)

Neutrophils % was significantly (P<0.05) higher (58.56±1.75c %) in the rohu fed with diet T3. Neutrophils % in T6, T4, T5, T1 and T2 diets fed rohu were 53.93±1.31b, 51.82±0.33b, 43.68±0.30a, 43.04±0.27a and 42.10±0.14a % respectively. Neutrophils % in T3 diet fed rohu was 1.3 times higher compared to rohu fed with control (T1) diet (Fig.4.4.40).

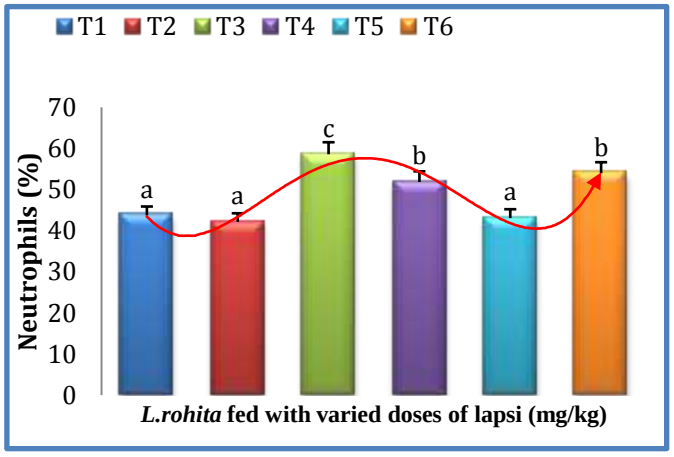

Figure 4.4.40 Neutrophils (%) in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.41 Lymphocytes (%)

Lymphocytes % was significantly (P<0.05) higher (45.95±0.13f %) in rohu fed with diet T4. Lymphocytes % in rohu fed with T5, T6, T3, T2, and T1 diets were 42.57±0.49e,

38.69±0.68d, 26.77±0.26c, 23.91±0.55b and 16.29±0.54a % respectively. Lymphocytes % in T4 diet fed rohu was 2.8 times higher compared to rohu fed with control (T1) diet (Fig. 4.4.41).

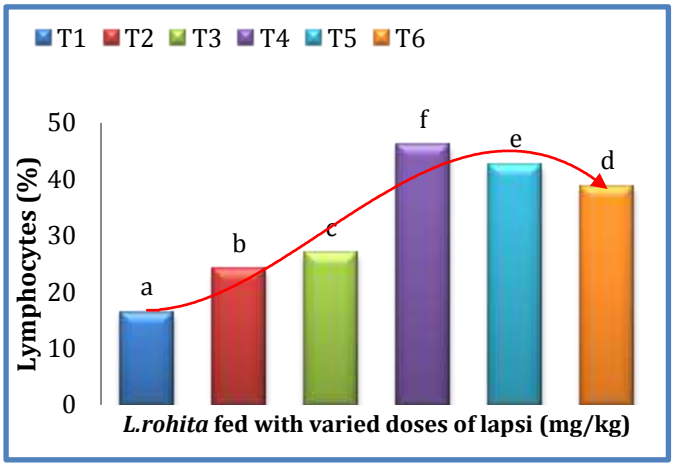

Figure 4.4.41 Lymphocytes (%) in *L.rohita* fed with diet containing six different doses of lapsi fruits

4.4.42 Monocytes (%)

Monocytes % was significantly (P<0.05) higher (6.07±0.10e %) in rohu fed with diet T4 (Fig.4.84). Monocytes % in rohu fed with T3, T2, T5, T1, and T6 diets were 4.55±0.10d, 2.02±0.47bc, 2.26±0.25c, 1.50±0.10ab and 1.12±0.10a % respectively. Lymphocyte % in T4 diet fed rohu was 5.2 times higher compared to rohu fed with control (T1) diet (Fig. 4.4.42).

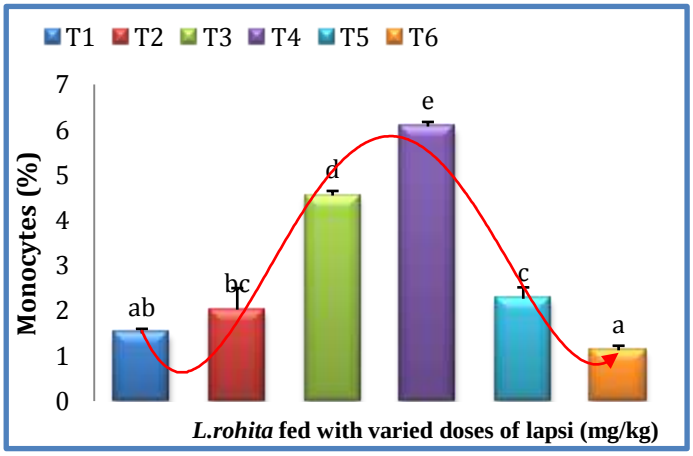

Figure 4.4.42 Monocytes (%) in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.43 Eosinophils (%)

Significantly (P<0.05) higher eosinophil level was recorded (Fig. 4.85) in rohu fed with T4 diet (0.75± 0.43b). Eosinophils % in rohu fed with diets T2, T3, T6, T1 and T5 were 0.67±0.09ab, 0.63±0.63ab, 0.62± 0.04ab, 0.56± 0.02a and 0.53± 0.26a respectively. Eosinophils % in T4 diet fed rohu was 1.3 times higher compared to rohu fed wityh control (T1) diet (Fig. 4.85).

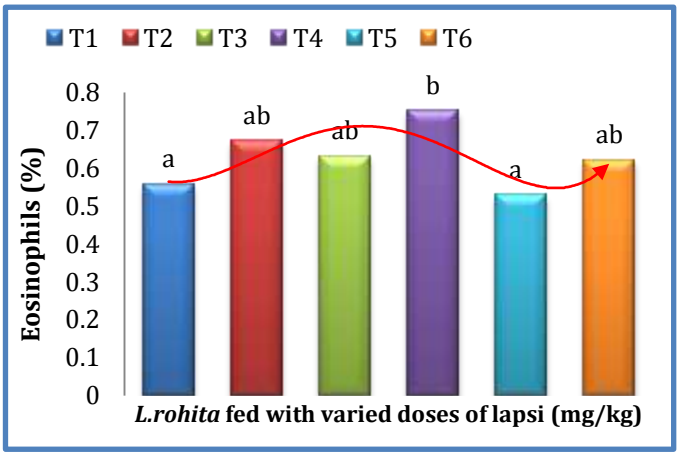

Figure 4.4.43 Eosinophils (%) in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.4.44 Basophils (%)

Significantly (P<0.05) higher basophils % (1.11±0.42b) was recorded in rohu fed with diet T4. In T5 diet fed rohu, basophils % was 0.97±0.47ab, followed by T3 (0.59±0.24ab), T6 (0.29±0.13b) and T2 (0.20±0.18ab) diets fed rohu. Lowest basophil % was found in rohu fed with control (T1) diet (0.06±0.02a). Basophils % in T4 diet fed rohu was 19.6 times higher compared to rohu fed with control (T1) diet (Fig. 4.4.44).

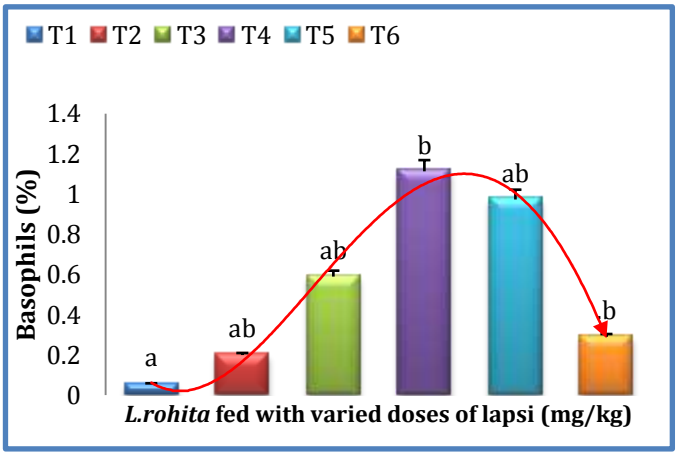

Figure 4.4.44 Basophils (%) in *L. rohita* fed with diet containing six different doses of lapsi fruits

4.5 Performance of rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 (Salmonidae) fed varied doses of ethanol extract of Lapsi *Choerospondias axillaris* fruit' pulp (LFP)

4.5 (A) Growth Performances

4.5.1 Survival rate and final weight gain

Cent per cent survival rate was observed in all the treated and control diet fed groups. At the start of the experiment the average weight of rainbow trout was 37.24±0.39 g.

After 90 days of culture, a direct relationship was found between final average weight gain of rainbow trout and diet containing different doses of LFP. Final average weight gain of rainbow trout fed with diet T4 was found significantly (P<0.05) high (138.19±0.75dc g) followed by groups fed with diets T6 (131.03±0.33abc g), T3 (130.83 ±0.29abc g), T5 (121.36±0.82ab g), T2 (112.46±0.77bc g) and T1 (105.76±0.51a g) (Fig. 4.5.1).

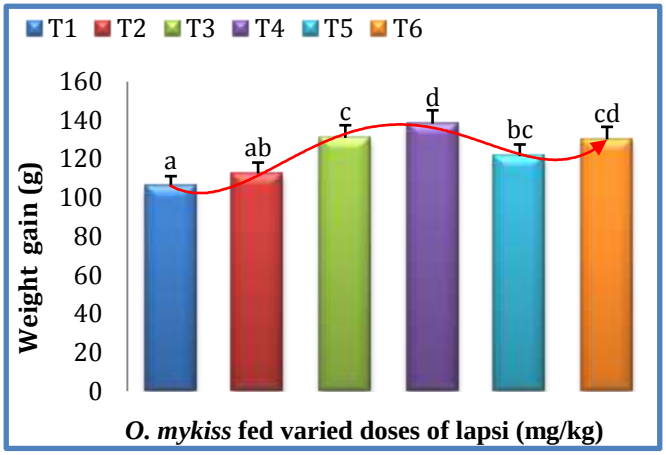

Figure 4.5.1 Final average weight gain of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.2 Final Weight Gain (%)

Final weight gain % was high in rainbow trout fed with T4 diet (371.39±0.11dc) followed by trout fed with T3 (351.37 ±0.73abc), T6 (349.93±0.77abc), T5 (325.58 ±0.84ab), T2 (301.58±0.06bc) and T1 (284.08 ±0.65a). Similarly, weight gain % was 30.67 % higher in trout fed with T4 diet compared to control (T1) group (Fig. 4.5.2).

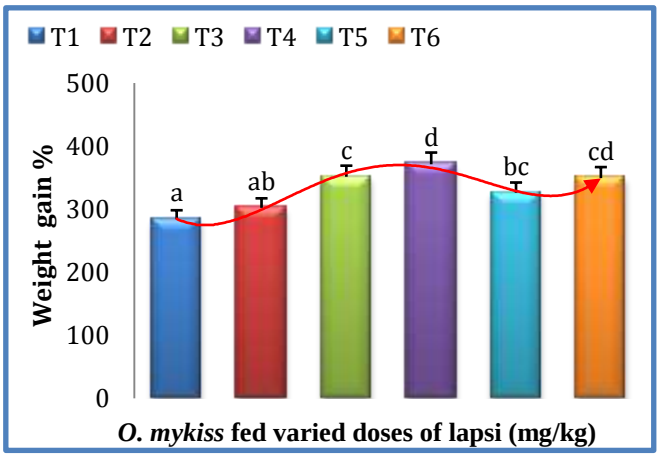

Figure 4.5.2 Weight gain percentages of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.3 Specific Growth Rate (SGR)

A direct relationship was found between SGR and LFP doses. SGR was significantly (P<0.05) high in trout fed with diet T4 (1.72±0.024dc) followed by trout fed with diets T3 (1.67 ±0.051abc), T6 (1.67±0.035abc), T5 (1.61±0.020ab), and T2 (1.54 ±0.059bc). Lowest SGR was found in trout of control group T1 (1.49±0.011a). SGR was 15.17 % higher in T4 diet fed rainbow trout compared to control group (Fig. 4.5.3).

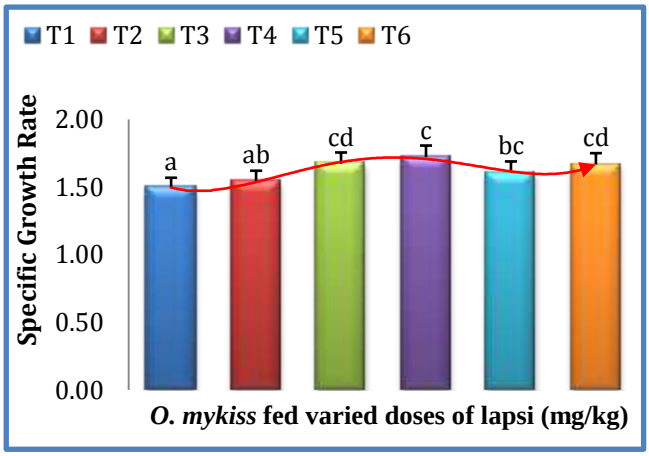

Figure 4.5.3 Specific growth rate of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.4 Feed Conversion Ratio (FCR)

An inverse relationship was found between FCR and LFP doses in diets. FCR level was high in trout fed with diet T1 (0.95±0.013c) followed by trout fed with diets T2 (0.91±0.060ab), T5 (0.83±0.023bc) T6 (0.78±0.036abc), T3 (0.77± 0.023abc) and T4 (0.73±0.023a). FCR in T4 fed trout was 30.56 % lower compared to the control diet fed trout (Fig. 4.5.4).

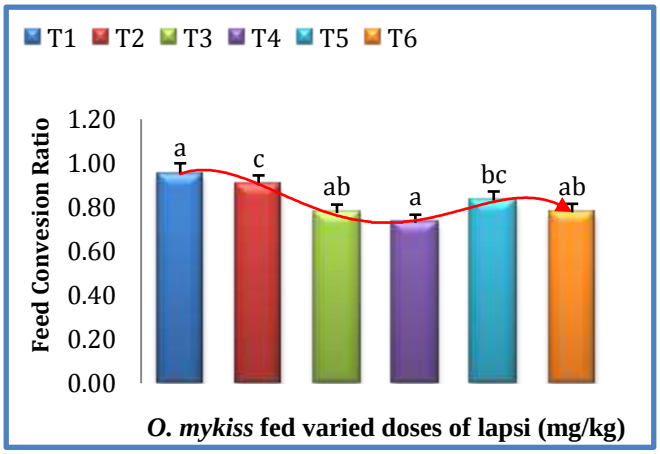

Figure 4.5.4 Feed conversion ratio of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5. (B) Biochemical Parameters

Vitamin C in blood serum, brain and liver of trout in treatment and control groups were recorded.

4.5.5 Vitamin C in Blood Serum

A direct relationship was found between LFP doses in diets and concentration of vitamin C in the blood serum (Fig. 4.5.5). Concentration of vitamin C in blood serum was

significantly (P<0.05) higher (76.34±3.31e µg/dl) in rainbow trout fed with T4 diet followed by trout fed with diets T5 (66.64±5.93d µg/dl), T3 (54.32± 5.16c µg/dl), T6 (51.37± 1.56c µg/dl) and T2 (41.98±1.35b µg/dl). The control diet fed trout had the least amount of vitamin C in blood serum (39.65±0.93a µg/dl). Vitamin C concentration was 92.5% higher in blood serum of trout fed with T4 diet compared to the control diet fed group.

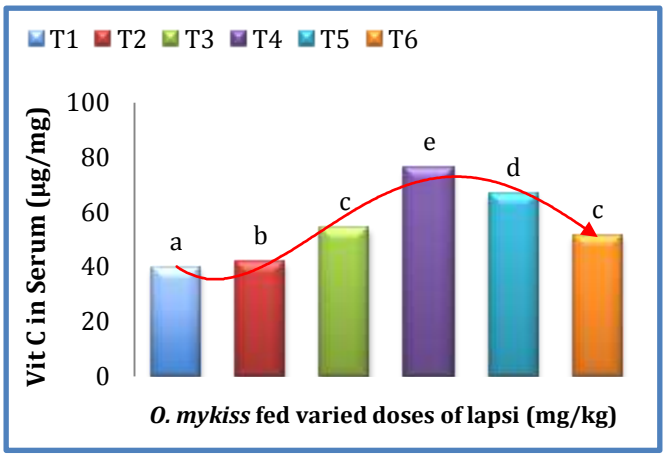

Figure 4.5.5 Vitamin C in blood serum of *O. mykiss* with diet containing six different doses of lapsi fruits

4.5.6 Vitamin C in Brain

Vitamin C concentration in brain was significantly (P<0.05) higher in rainbow trout fed with diet T4 (42.32±1.68c µg/mg) followed by trout fed with diets T3 (38.54±0.68d µg/mg), T6 (35.57±0.77c µg/mg), T5 (35.11 ±0.43c µg/mg) and T2 (29.82 ±1.18b µg/mg). The control diet (T1) fed group had the lowest vitamin C concentration in the brain (27.84±1.27a µg/mg). Vitamin C concentration was 51.95 % higher in trout fed with T4 diet compared to trout fed with control (T1) diet (Fig. 4.5.6).

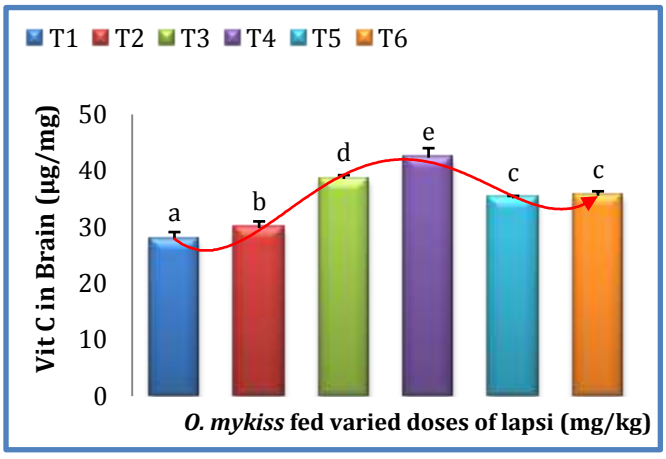

Figure 4.5.6 Vitamin C in brain of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.7 Vitamin C in Liver

Vitamin C concentration in liver was significantly (P<0.05) higher in rainbow trout fed with T4 diet (86.71±1.32e µg/mg) compared to trout fed with all other treatment and control diets. Concentration of vitamin C in rainbow trout fed with diets T5, T3, T6 and T2 were 81.6±1.04d, 79.95±1.32c, 78.45 ±1.32c and 76.35±1.53b µg/mg respectively. The lowest concentration of vitamin C in liver was recorded in trout fed with T1 diet (71.29±0.68a µg/mg). Vitamin C concentration was 21.63% higher in trout fed with T4 diet compared to trout fed with control (T1) diet (Fig. 4. 5.7).

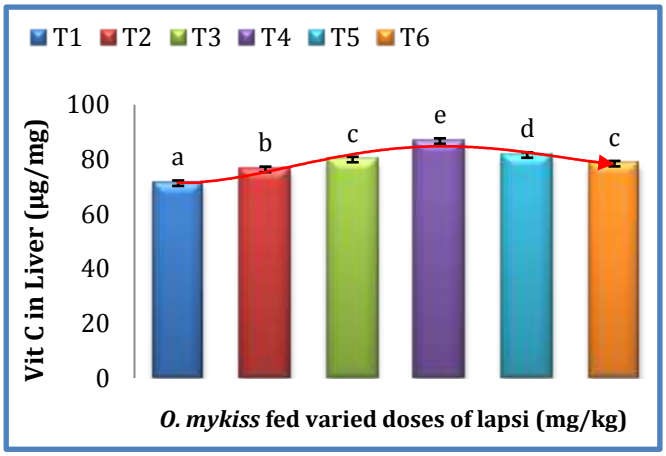

Figure 4.5.7 Concentration of vitamin C in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5. (C) Immuno-Haematological Parameters

4.5. C-I PROTEIN PROFILE

4.5.8 Total Serum Protein

A direct relationship was found between LFP doses and concentration of serum protein in the blood of rainbow trout. Concentration of serum protein was significantly (P<0.05) higher in the blood sample of rainbow trout fed with diet T4 (14.14±0.36e µg/dl) followed by trout fed with diets T5 (12.05±0.42d µg/dl), T6 (10.21 ±0.33c µg/dl), T3 (8.58±0.31bc µg/dl), and T2 (7.14±0.97b µg/dl). Lowest serum protein concentration (5.05±0.77a µg/dl) was found in trout fed with control diet (T1). Serum protein level was 4.34 times higher in trout fed with T4 diet compared to trout fed with control (T1) diet (Fig. 4.5.8).

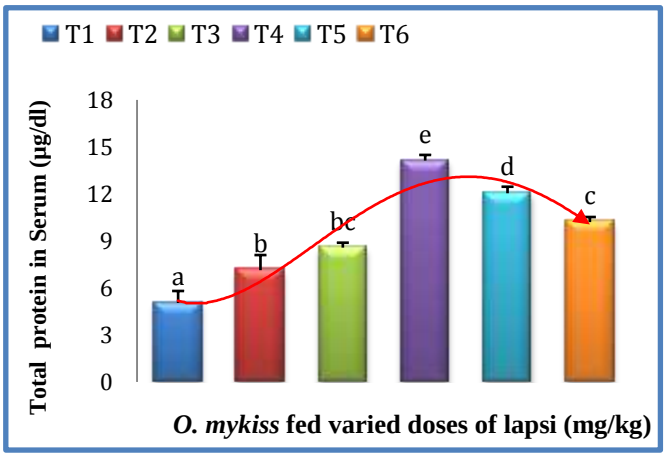

Figure 4.5.8 Concentration of Total serum protein in blood of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.9 Total Protein in Brain

The concentration of total protein was significantly (P<0.05) high in brain of trout fed with diet T4 (7.69±0.32d µg/mg) followed by trout fed with diets T6 (6.54±0.24cd µg/mg), T3 (6.03±0.32c µg/mg), T5 (5.57±0.46bc µg/mg) and T2 (4.38±0.32b µg/mg). Trout fed with control diet T1 had the lowest protein concentration (2.72±0.32a µg/mg). Total protein concentration in brain was 3.64 times higher in trout fed with T4 diet compared to trout fed with control diet T1 (Fig.4.5.9).

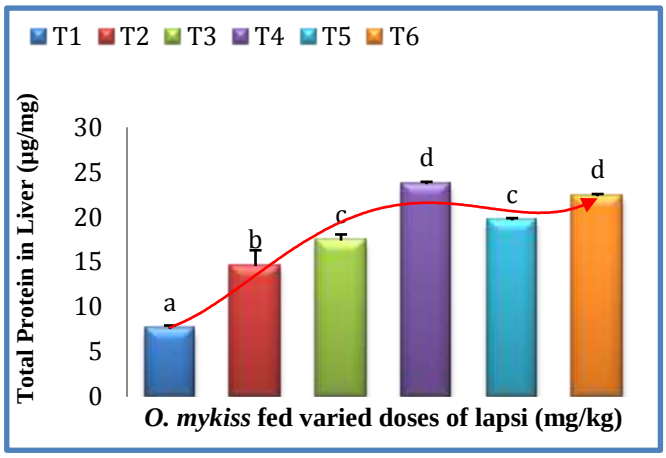

Figure 4.5.9 Concentration of Total protein in brain of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.10 Total Protein in Liver

A direct relationship was found between LFP doses and concentration of total protein in the liver. Concentration of total protein in liver was significantly (P<0.05) higher in rainbow trout fed with diet T4 (23.66±0.26d µg/mg) followed by trout fed with diets T6 (22.31±0.25d µg/mg), T5 (19.62 ±0.26c µg/mg), T3 (17.38±0.69c µg/mg) and T2 (14.55±1.75b µg/mg). Lowest liver protein concentration was measured in the control group (T1) fed trout (7.52±0.40a µg/mg). Total protein level was 2.3 times higher in trout fed with T4 diet compared to trout fed with control diet T1 (Fig.4.5.10).

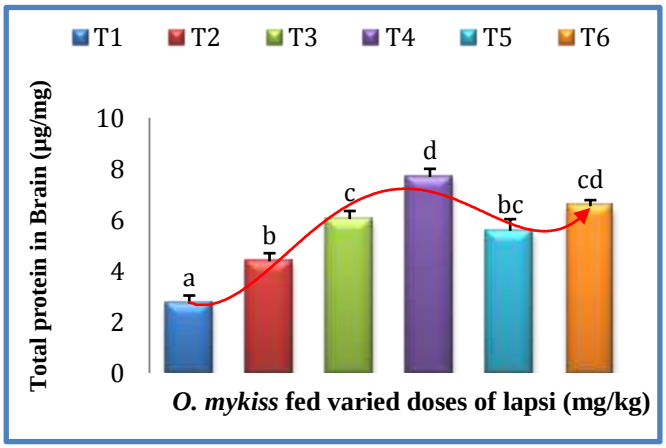

Figure 4.5.10 Concentration of Total protein in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.11 Total Protein in Muscles

Similar to brain and liver, concentration of total protein in muscles of rainbow trout was found to increase with increasing doses of LFP in the diet (Fig. 4.5.11). Concentration of total protein in muscles was significantly (P<0.05) high in trout fed with diet T4 (11.29 ±0.56d µg/mg) followed by trout fed with diets T3 (9.35±0.32c µg/mg), T5 (9.04±0.26c

µg/mg), T2 (8.39±0.56bc µg/mg) and T6 (7.02±0.46b µg/mg). Lowest muscle protein concentration (5.50±0.56a µg/mg) was measured in trout fed with control diet T1. Total protein concentration in muscles was 2.15 times higher in muscles of trout fed with T4 diet compared to trout fed with control (T1) diet.

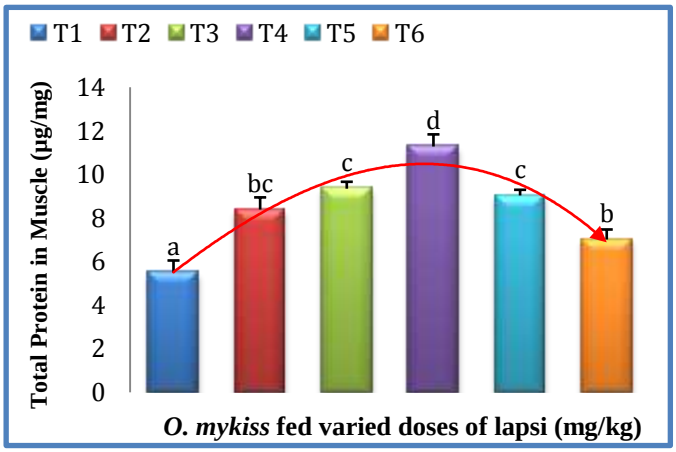

Figure 4.5.11 Concentration of Total protein in muscles of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.12 Albumin in Blood Serum

Concentration of albumin in blood serum was significantly (P<0.05) high in trout fed with diet T4 (6.43±0.13c µg/dl) followed by trout fed with diets T5 (5.55±0.07d µg/dl), T6 (4.57±0.27cd µg/dl), T3 (3.95±0.16bc µg/dl) and T2 (3.40 ±0.39b µg/dl). Lowest albumin concentration was recorded in trout fed with control (T1) diet (2.26±0.44a µg/dl). Serum albumin concentration was 3.64 times higher in trout fed with T4 diet compared to trout fed with control (T1) diet (Fig. 4.5.12).

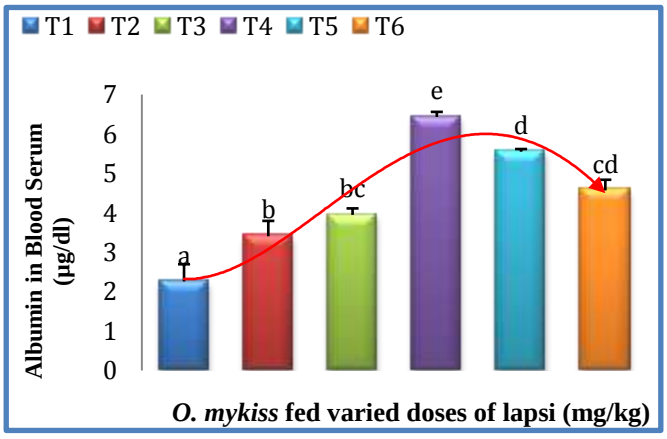

Figure 4.5.12 Concentration of Albumin in blood serum of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.13 Albumin in Brain

A direct relationship was found between LFP doses and concentration of albumin in the brain. (Fig.4.5.13). Concentration of albumin in the brain was significantly (P<0.05) higher (3.61±0.13d µg/mg) in rainbow trout fed with diet T4 followed by trout fed with diets

T6 (2.93 ±0.41cd µg/mg), T3 (2.85 ±0.08cd µg/mg), T5 (2.38±0.28bc µg/mg) and T2 (1.69±0.28ab µg/mg). Lowest albumin concentration was measured in the control group T1 (1.14±0.02a µg/mg). Albumin concentration in brain was 4.52 times higher in trout fed with T4 diet compared to trout fed with control (T1) diet.

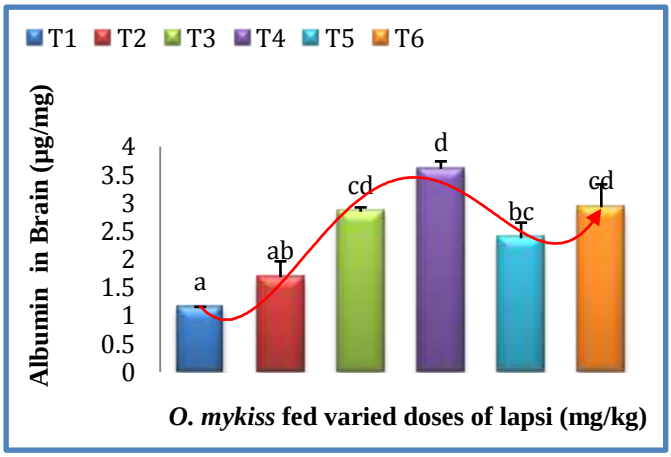

Figure 4.5.13 Concentration of albumin in brain of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.14 Albumin in Liver

Similarly, albumin concentration was high in liver of trout fed with diet T5 (9.34±0.36c g/mg) followed by trout fed with diets T4 (9.31±0.56c µg/mg), T6 (8.48±0.24c µg/mg), T3 (8.14±0.32c µg/mg), diet T2 (6.29±1.07b µg/mg). Liver albumin concentration was lowest in the control diet fed trout (3.34±0.16a µg/mg). Liver albumin concentration was 2.12 times higher in trout fed with T5 diet compared to trout fed with control (T1) diet (Fig.4.5.14).

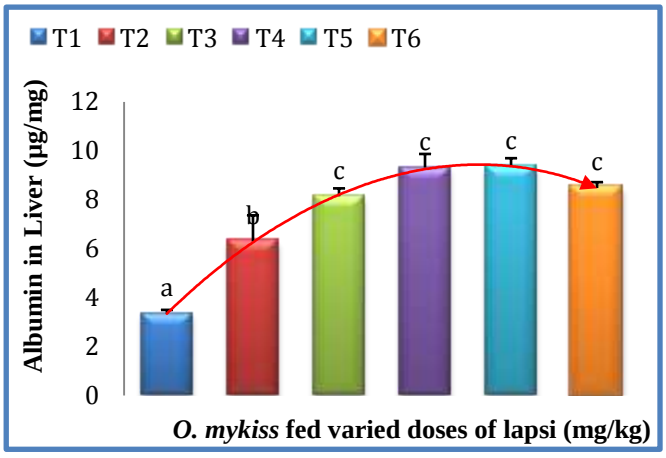

Figure 4.5.14 Concentration of albumin in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.15 Albumin in Muscles

Concentration of albumin was high in muscles of trout fed with diet T4 (5.34±0.13e µg/mg) followed by trout fed with diets T5 (4.34±0.10d µg/mg), diet T3 (3.88±0.36cd µg/mg), T2 (3.57±0.07bc µg/mg), T6 (3.21±0.28b µg/mg) and control diet T1 (2.44±0.13a

µg/mg). Albumin concentration was 2.55 times higher in trout fed with T4 diet compared to trout fed with control (T1) diet (Fig.4.5.15).

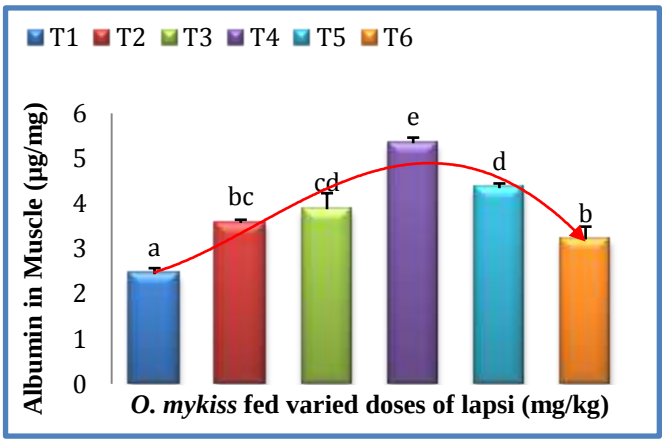

Figure 4.5.15 Concentration of albumin in muscles of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.16 Globulin in Blood Serum

A direct relationship was found between LFP doses and concentration of globulin in blood serum of trout. Concentration of globulin in blood serum was significantly (P<0.05) higher (7.70±0.31e µg/dl) in trout fed with diet T4 followed by trout fed with diets T5 (6.50±0.46d µg/dl), T6 (5.64±0.05c µg/dl), T3 (4.63±0.23bc µg/dl) and T2 (3.74±0.63ab µg/dl). Blood globulin concentration was measured lowest in control diet fed trout (2.79±0.34a µg/dl). Concentration of globulin in blood serum was 2.54 times higher in trout fed with T4 diet compared to trout fed with control (T1) diet (Fig.4.5.16).

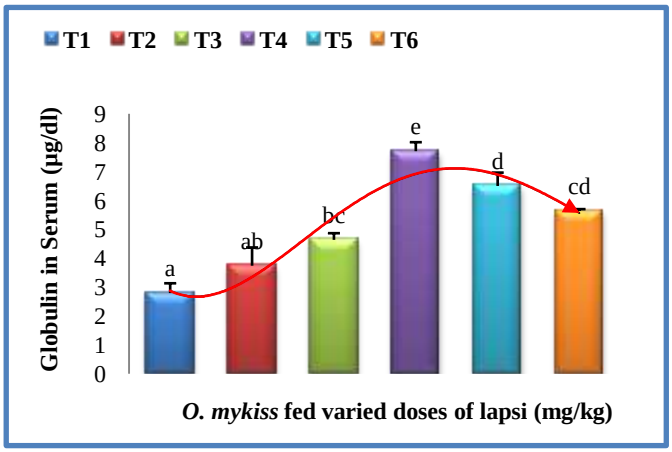

Figure 4.5.16 Concentration of globulin in blood serum of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.17 Globulin in Brain

A direct relationship was found between LFP doses and concentration of globulin in brain. Concentration of globulin in brain was significantly (P<0.05) higher (4.08±0.18d µg/mg) in trout fed with diet T4 followed by trout fed with diets T6 (3.62±0.24cd µg/mg), T5

(3.20±0.34bc µg/mg), T3 (3.19 ±0.25bc µg/mg) and T2 (2.69±0.04b µg/mg). Lowest brain globulin concentration was found in the control diet (T1) fed group (1.58±0.30a µg/mg). Globulin concentration in brain was 2.15 times higher in trout fed with T4 diet compared to trout fed with control (T1) diet trout (Fig.4.5.17).

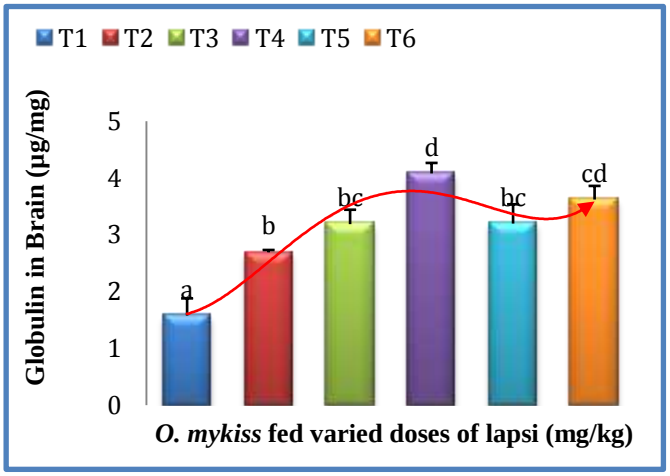

Figure 4.5.17 Concentration of globulin in brain of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.18 Globulin in Liver

Globulin concentration was high in liver of trout fed with diet T4 (14.35±0.78d µg/mg) followed by trout fed with diets T6 (13.83±0.06d µg/mg), T5 (10.28±0.36c µg/mg), T3 (9.24±0.42bc µg/mg) and diet T2 (8.25±0.69b µg/mg). Control diet fed trout had the lowest liver globulin (4.18±0.26a µg/mg). Globulin concentration in liver was 2.12 times higher in trout fed with T4 diet compared to trout fed with control (T1) diet (Fig.4.5.18).

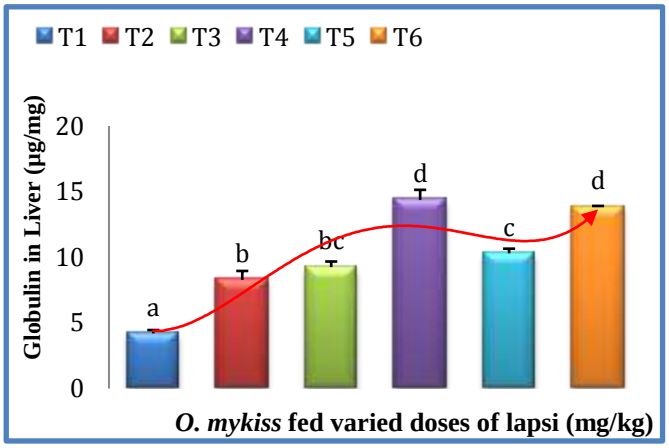

Figure 4.5.18 Concentration of globulin in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.19 Globulin in Muscles

In muscles globulin concentration was significantly ($P< 0.05$) high in trout fed with diet T4 (5.95±0.68e µg/mg) followed by trout fed with diets T3 (5.47±0.10c µg/mg), T2 (4.82±0.55bc µg/mg), diet T5 (4.70±0.16bc µg/mg), and T6 (3.81± 0.23ab µg/mg). Lowest

muscle globulin concentration was measured in the control group T1 (3.06±0.44a µg/mg). Globulin concentration was 1.64 times higher in muscles of trout fed with T4 diet compared to trout fed with control (T1) diet (Fig. 4.5.19).

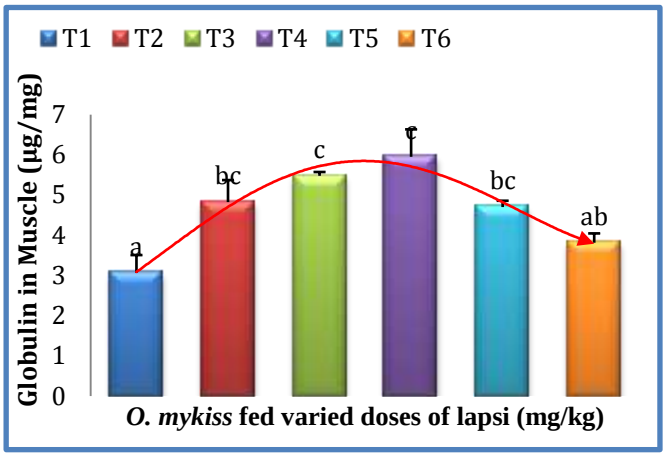

Figure 4.5.19 Concentration of globulin in muscles of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.20 Ratio of Albumin and Globulin (A/G) in Blood Serum

The A/G ratio in blood serum was higher in trout fed with diet T2 (0.94±0.12) followed by trout fed with diets T5 (0.86±0.07), T3 (0.85±0.05), T4 (0.84±0.03), T6 (0.81±0.04) and T1 (0.79±0.08). Lowest A/G ratio was found in T1 diet fed trout. A/G ratio was 18.9 % lower in control diet fed (T1) trout compared to trout fed with diet T2 (Fig. 4.5.20).

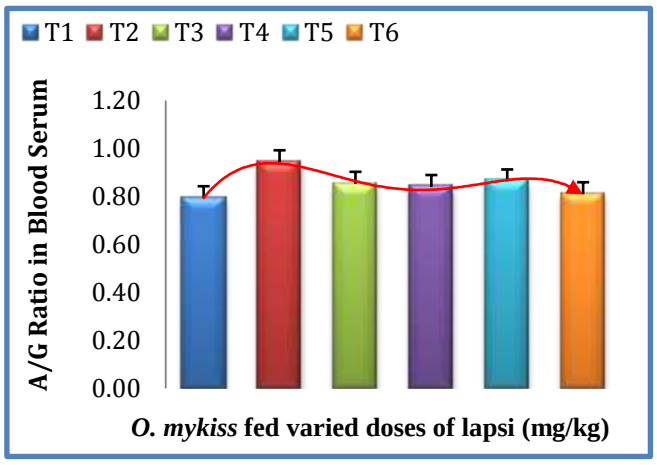

Figure 4.5.20 Ratio of albumin and globulin in blood serum of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.21 Ratio of Albumin and Globulin (A/G_) in Brain

The A/G ratio in brain was high in trout fed with diet T3 (0.90±0.05) followed by T4 (0.88±0.01), T6 (0.80±0.07), T1 (0.78±0.15), T5 (0.76±0.10) and T2 (0.62±0.10) (Fig. 4.5.21). Brain A/G ratio was lowest in T4 diet fed group. A/G ratio was 44.1% higher in trout fed with control (T1) diet compared to trout fed with T2 diet.

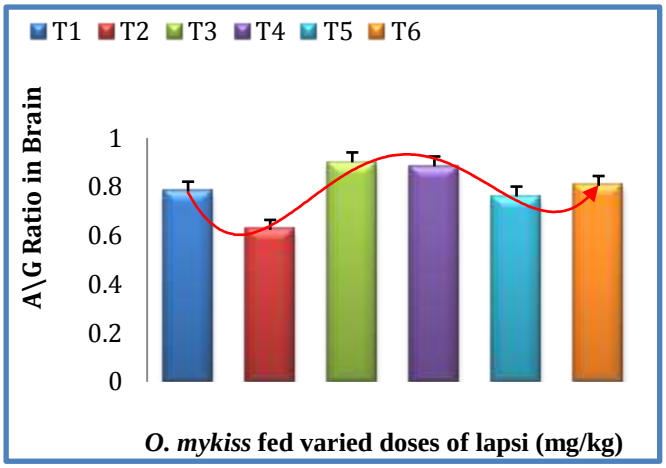

Figure 4.5.21 Ratio of albumin and globulin in brain of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.22 Ratio of Albumin and Globulin(A/G) in Liver

The A/G ratio in liver was highest in trout fed with diet T5 (0.91±0.06) and lowest in trout fed with diet T6 (0.61±0.02). The ratio was 0.88±0.03 in T3, 0.80±0.03 in T2, 0.75±0.07 in T3 and 0.75±0.07 in T5 (Fig. 4.108). A/G ratio was 48.37 % higher in T5 diet fed group compared to trout fed with control (T1) diet (Fig. 4.5.22).

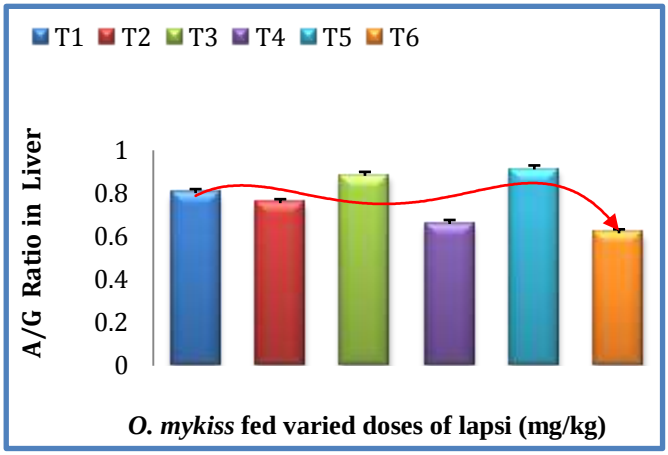

Figure 4.5.22 Ratio of albumin and globulin in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.23 Ratio of Albumin and Globulin *(A/G) in Muscles*

The A/G ratio in muscles was highest in trout fed with diet T4 (0.93±0.13) and lowest in trout fed with diet T3 (0.71±0.07). The ratio was 0.93 ±0.01 in T5, 0.84±0.06 in T6, 0.82±0.08 in T1 and 0.76±0.08 in T2. A//G ratio was 30.52% higher in T4 diet fed group compared to trout fed with control (T1).diet (Fig. 4.5.23).

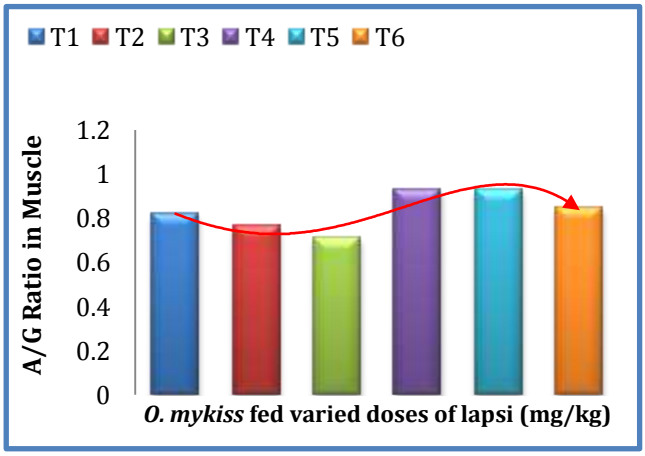

Figure 4.5.23 Ratio of albumin and globulin in muscles of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5. C-II ENZYME PROFILE

4.5.24 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Blood Serum

SGOT level was found significantly (P<0.05) high in blood serum of trout fed with control diet T1 (86.88±1.38e IU/L) followed by trout fed with diets T2 (77.53±0.86d IU/L), T3 (66.61 ±1.18c IU/L), T5 (61.35±1.71b), and T6 (55.42±1.93a IU/L). Lowest SGOT level was in trout fed diet T4 (55.45±1.63a IU/L). SGOT level was 56.7% higher in trout fed with control (T1) diet compared to trout fed with T6 diet (Fig.4.5.24).

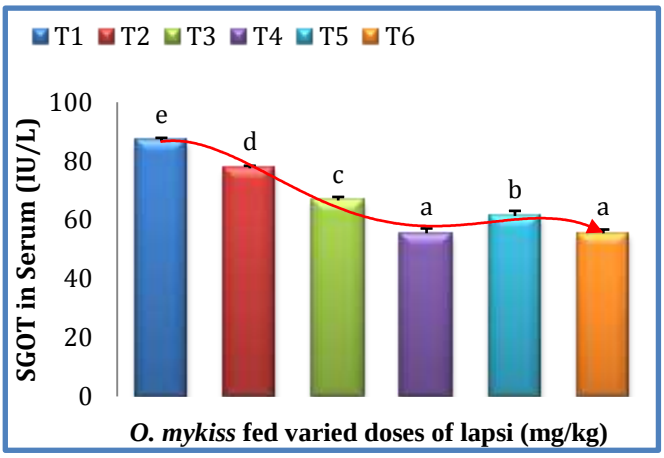

Figure 4.5.24 Serum glutamic oxaloacetic transaminase (SGOT) level in blood serum of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.25 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Liver

SGOT level was found significantly (P<0.05) high in liver of trout fed with control diet T1 (101.83±1.32d IU/L) followed by trout fed with diets T2 (98.06±0.82d IU/L), T3 (92.38±1.12c IU/L), T5 (84.82 ±1.29b IU/L), T6 (82.29±0.53ab) and T4 (79.30±1.46a IU/L). SGOT level in liver was 23.75 % higher in control (T1) diet fed trout compared to trout fed with T4 diet (Fig.4.5.25).

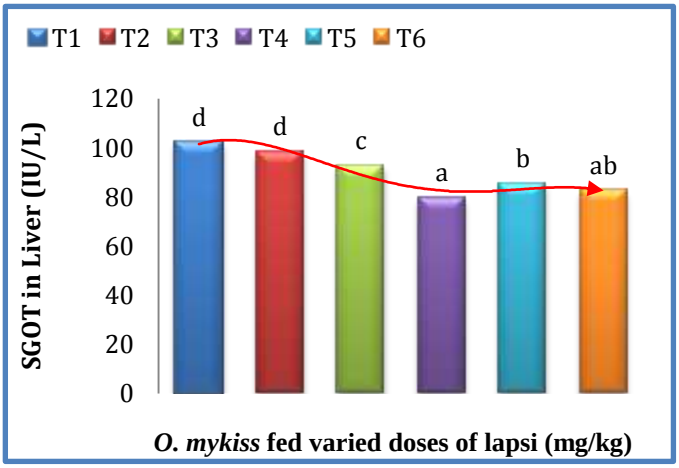

Figure 4.5.25 Serum glutamic oxaloacetic transaminase (SGOT) level in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.26 Serum Glutamic Oxaloacetic Transaminase (SGOT) in Gills

SGOT level was found significantly (P<0.05) high in gills of trout fed with control diet T1 (77.49±1.69c IU/L) followed by trout fed with diets T3 (77.01±0.48c IU/L), T2 (73.44±1.93c IU/L), T4 (58.82±1.84b IU/L), T5 (51.25 ±5.18ab) and T6 (47.83±4.47a IU/L).

SGOT level in gills was 62.21 % higher in control (T1) diet fed group compared to group fed with T6 diet (Fig.4.5.26).

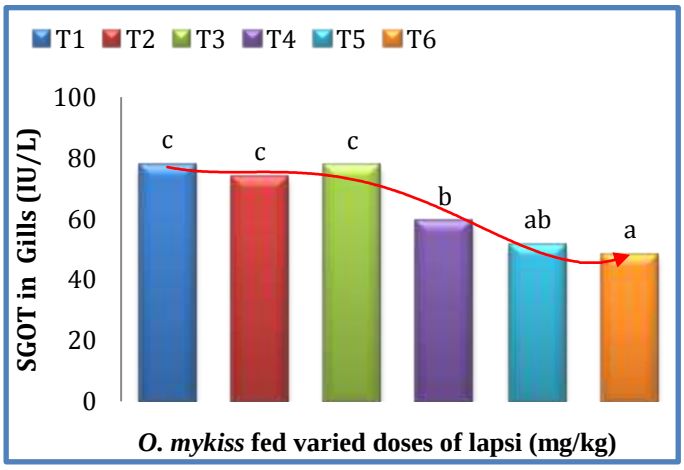

Figure 4.5.26 Serum glutamic oxaloacetic transaminase (SGOT) level in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.27 Serum Glutamic Pyruvate Transaminase (SGPT) in Blood Serum

SGPT level was found significantly (P<0.05) high in blood serum of trout fed with control diet T1 (117.23±1.53d IU/L) followed by trout fed with diets T2 (98.71±1.21c IU/L), T5 (89.59 ±1.27b IU/L), T6 (87.34±1.11b IU/L), T3 (85.41±1.65b IU/L) and T4 (69.77±1.29a IU/L). SGPT level was 68% higher in control diet (T1) fed group compared to group with T1 diet (Fig.4.5.27).

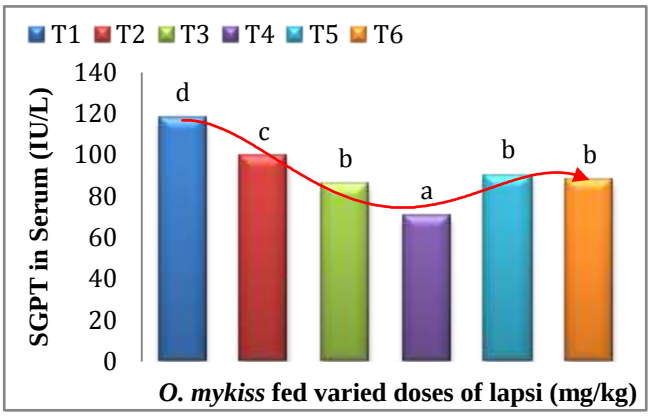

Figure 4.5.27 Serum glutamic pyruvate transaminase (SGPT) level in blood serum of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.28 Serum Glutamic Pyruvate Transaminase (SGPT) in Liver

SGPT level was found significantly (P<0.05) high in liver of trout fed with control diet T1 (172.15±1.42f IU/L) followed by trout fed with diets T2 (164.39±1.49e IU/L), T5 (140.24± 1.76d IU/L), T3 (133.35±1.45c IU/L), T6 (125.44±1.64b IU/L) and T4

(117.83±1.42a IU/L). SGPT level was 56.47 % higher in control (T1) diet fed trout compared to trout fed with T4 diet (Fig.4.5.28).

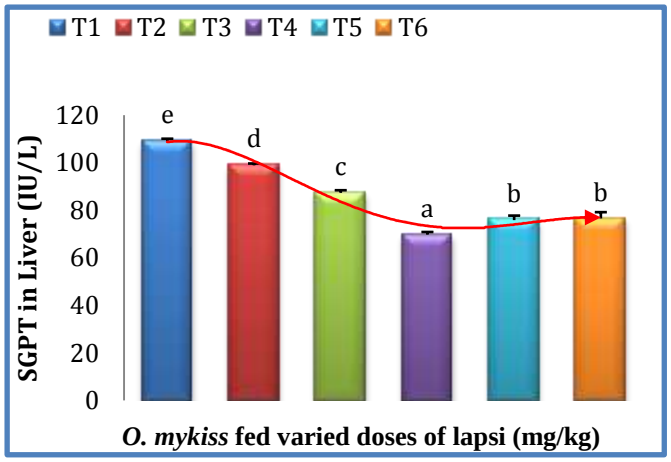

Figure 4.5.28 Serum glutamic pyruvate transaminase (SGPT) level in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.29 Serum Glutamic Pyruvate Transaminase (SGPT) in Gills

SGPT level was found significantly (P<0.05) high in gills of trout fed with control diet T1 (70.14±3.78d IU/L) followed by trout fed with diets T2 (68.13±0.87cd IU/L), T3 (63.60± 0.87bc IU/L), T5 (59.06± 0.17ab IU/L), T6 (56.98±0.36 a IU/L) and T4 (54.53±0.79a IU/L). SGPT level in gills was 28.63 % higher in trout fed with control (T1) diet compared to trout fed with T4 dirt (Fig.4.5.29).

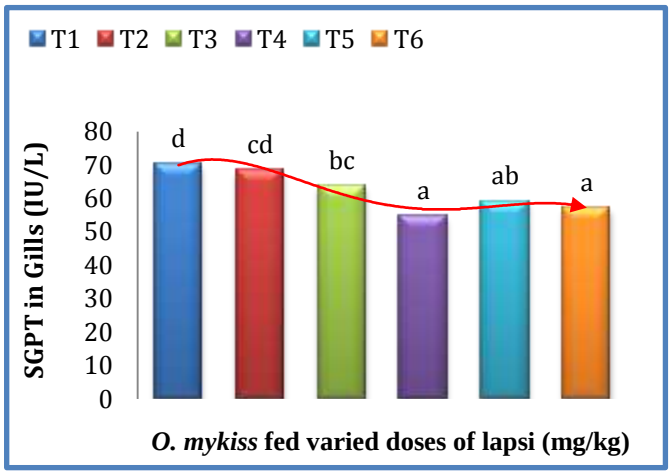

Figure 4.5.29 Serum glutamic pyruvate transaminase (SGPT) level in gills of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.30 Alkaline Phosphatase (ALP) in Blood Serum

ALP level was significantly (P<0.05) high (49.01±0.15c IU/L) in blood serum of trout fed with diet T2 followed by trout fed with diets T6 (48.94±0.32c IU/L), T1 (48.84±0.31c IU/L), T3 (38.21±1.33b IU/L), T5 (33.01±1.43b IU/L) and T4 (23.80±1.54a

IU/L). ALP level in blood serum was 2.05 times higher in trout fed with control diet (T2) compared to trout fed with T4 diet (Fig.4.5.30).

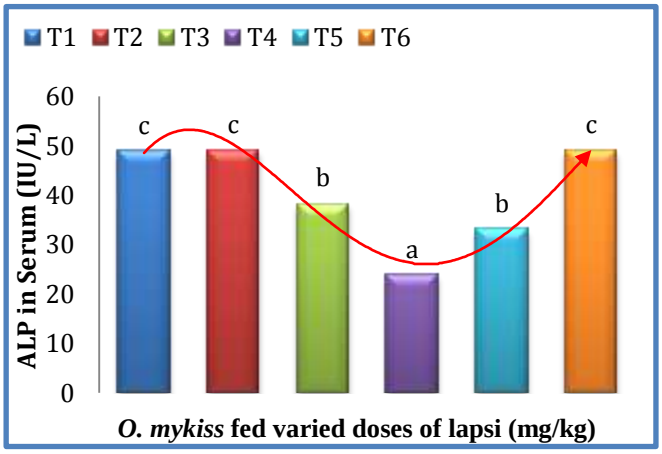

Figure 4.5.30 Alkaline phosphatase (ALP) level in blood serum of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.31 Alkaline Phosphatase (ALP) in Liver

ALP level was significantly (P<0.05) high (67.60±0.75d IU/L) in liver of rainbow trout fed with diet T1 followed by trout fed with diets (64.87±0.19cd IU/L), T3 (52.37 ±0.39bc IU/L), T5 (48.85±0.62ab IU/L), T4 (47.25±1.49ab IU/L) and T6 (35.43±10.61a IU/L). ALP level in liver was 90.79% higher in control diet (T1) fed trout compared to T4 diet fed trout (Fig.4.5.31).

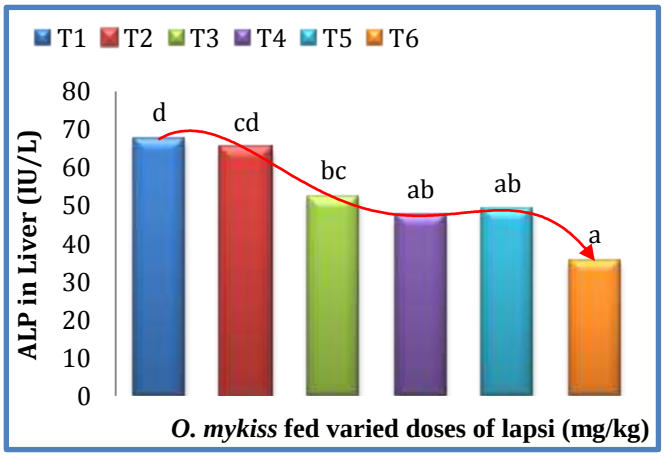

Figure 4.5.31 Alkaline phosphatase (ALP) level in liver of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.32 Alkaline Phosphatase (ALP) in Gills

ALP level was significantly (P<0.05) high (44.87±0.19e IU/L) in gills of rainbow trout fed with diet T1 followed by trout fed with diets T2 (42.89±0.51de IU/L), T5 (41.64±1.17cd IU/L) T6 (38.83 ±1.69bc IU/L), T3 (38.34±0.53b IU/L) and T4 (34.85±0.15aIU/L). ALP level in gills was 15.58 % higher in control diet (T1) fed trout compared to trout fed with T4 diet (Fig.4.5.32).

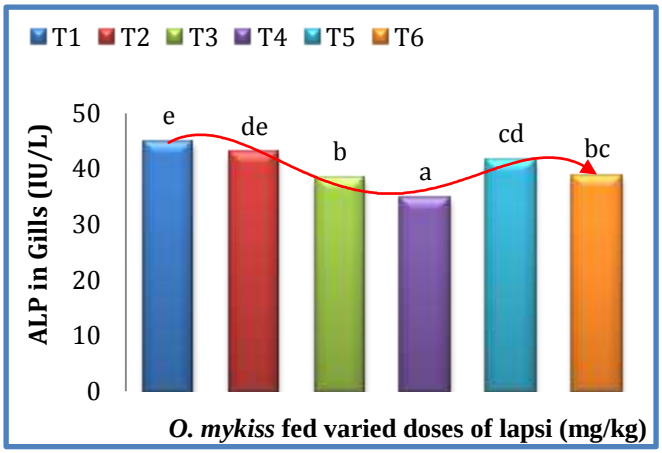

Figure 4.5.32 Alkaline phosphatase (ALP) level in gills of *O. mykiss* fed with diet containing six different doses of lapsi fruits

4. 5. C-III BLOOD PROFILE

a) Complete Blood Counts

4.5.33 Haemoglobin

Haemoglobin concentration in blood was significantly (P<0.05) higher in rainbow trout fed with diet T4 (16.91±0.98e mg/dl) compared to treated and control diets fed trout. Concentration of haemoglobin in T5, T6, T3, T2 and T1 diets fed trout were 16.24±0.36e, 13.55±0.38d, 10.94±0.77c, 8.40±1.06b and 3.52±0.74a mg/dl respectively. Concentration of haemoglobin in T4 diet fed trout was 4.8 times higher compared to trout fed with control (T1) diet (Fig.4.5.33).

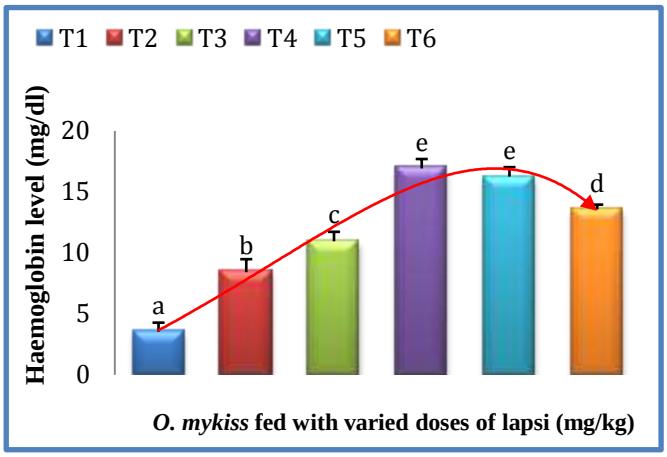

Figure 4.5.33 Haemoglobin level in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.34 Erythrocytes (RBC)

Erythrocytes number in blood was significantly (P<0.05) higher in trout fed with diet T5 (5.81±0.12d million/m^3) compared to trout fed with other treated and control diets. The number of erythrocytes in T4, T6, T2, T3 and T1 diets fed trout were 5.53±0.36cd, 4.88±0.22bcd, 4.59±0.50bc, 4.28±0.48b and 1.47 ±0.07a million/mm^3 respectively. The

number of erythrocytes in T5 diet fed trout was 3.9 times higher than in trout fed with control (T1) diet (Fig.4.5.34).

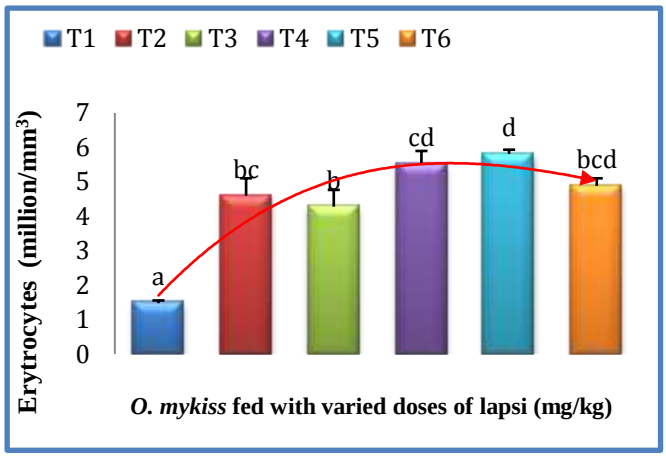

Figure 4.5.34 Erythrocytes in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.35 Leucocytes (WBC)

Leucocytes number in blood was significantly (P<0.05) higher (40.93±1.33c 10^3/mm^3) in trout fed with diet T4 compared to other treated and control diets fed groups. Leucocytes number in T5, T3, T2, T6 and T1 diets fed trout were 27.52± 1.86b, 25.90±1.21b, 19.60±1.21ab, 14.82±1.54a and 13.40±1.21a 10^3/mm^3 respectively. Leucocytes number in blood was 3.1 times higher in trout fed with T4 diet compared to trout fed control (T1) diet (Fig.4.5.35).

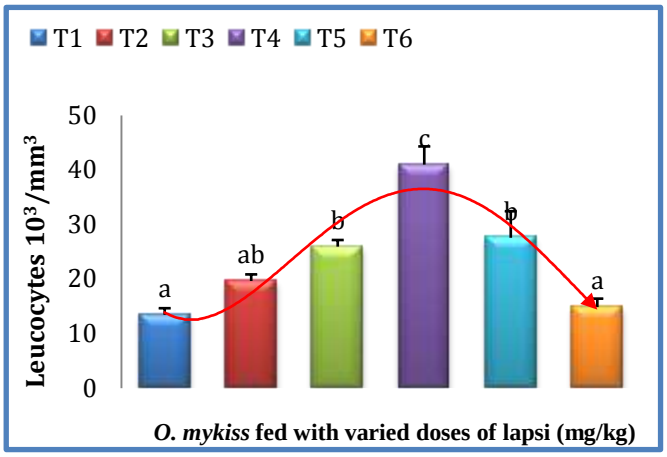

Figure 4.5.35 Leucocytes in *O. mykiss* fed with diet containing six different doses of lapsi fruits

b) **Absolute Values**

4.5.36 Haematocrit or Packed Cell Volume (PCV)

PCV level in blood was significantly (P<0.05) higher in trout fed with diet T4 (84.57±1.76d %) compared to other treated and control diets fed trout. PCV level in T5, T6, T2, T1 and T3 diets fed trout were 57.58±1.55c, 55.51±1.68c, 37.87±1.33b, 30.94±1.33ab

and 23.16±2.6a % respectively. PCV level in blood was 2.7 times higher in the trout fed with T4 diet fed trout compared to trout fed with control diet T1 (Fig.4.5.36).

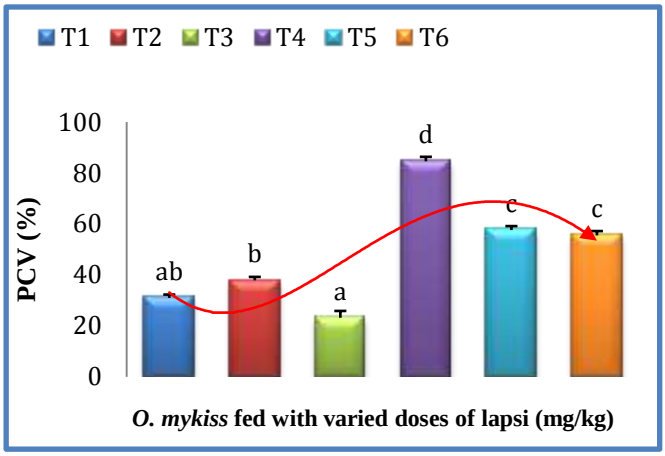

Figure 4.5.36 Packed Cell Volume level in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.37 Mean Corpuscular Volume (MCV)

Significantly (P<0.05) higher MCV level in the blood was recorded in rainbow trout fed with diet T4 (84.57±1.76d µm³). MCV levels were 57.58±1.55c, 55.51±1.68c, 37.87± 1.33b, 30.94± 1.33ab and 23.16± 2.68a µm³ in T5, T6, T2, T1 and T3 diets fed trout, respectively. MCV level in the blood of T4 diet fed trout was 2.7 times higher compared to trout fed with control (T1) diet (Fig.4.5.37).

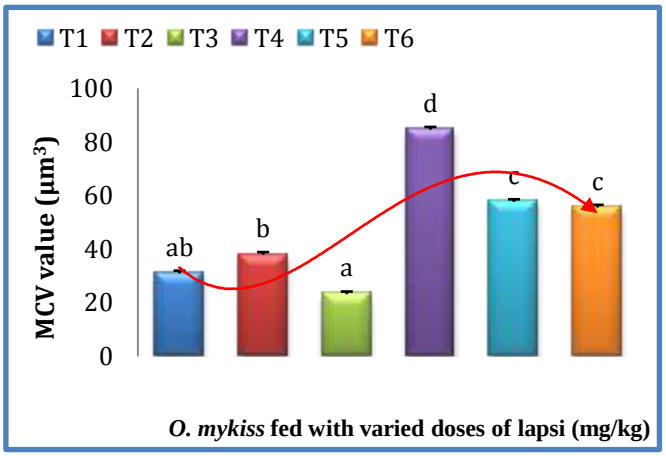

Figure 4.5.37 MCV level in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.38 Mean Corpuscular Haemoglobin (MCH)

Significantly (P<0.05) higher level of MCH (30.77±1.94b pg) was recorded in trout fed with diet (T4). MCH levels were 27.96±1.15b, 27.97±0.05b, 26.00±0.41b, 23.50±0.33ab and 18.23±0.71a pg in T5, T6, T3, T1 and T2 diets fed groups respectively. MCH level was 1.7 times higher in trout fed with T4 diet compared to trout fed with control (T1) diet (Fig.4.5.38).

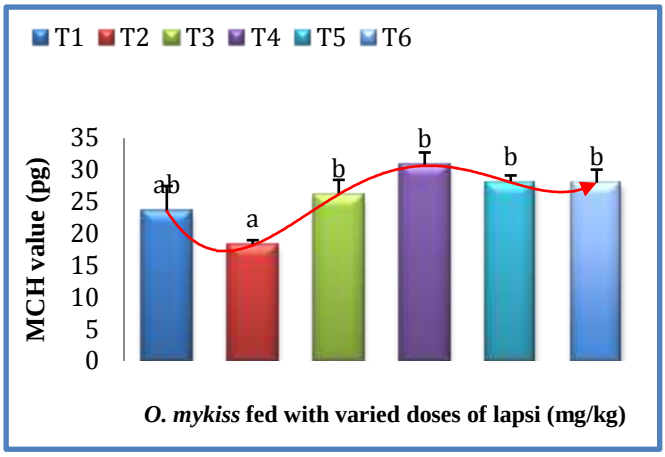

Figure 4.5.38 MCH level in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.39 Mean Corpuscular Haemoglobin Concentration (MCHC)

Significantly (P<0.05) higher MCHC (%) was recorded in trout fed with diet T3 (48.94±0.61c %) compared to other treated and control groups. MCHC (%) in trout fed with diets T5, T6, T2 and T4 were 28.49±0.27b, 25.41±0.18b, 22.05±0.11ab and 19.99±0.23ab %, respectively. Lowest MCHC (%) (11.27±0.20a %) was recorded in trout fed with diet T1. MCHC (%) in T3 diet fed group was 1.8 times higher compared to control (T1) diet fed trout (Fig.4.5.39).

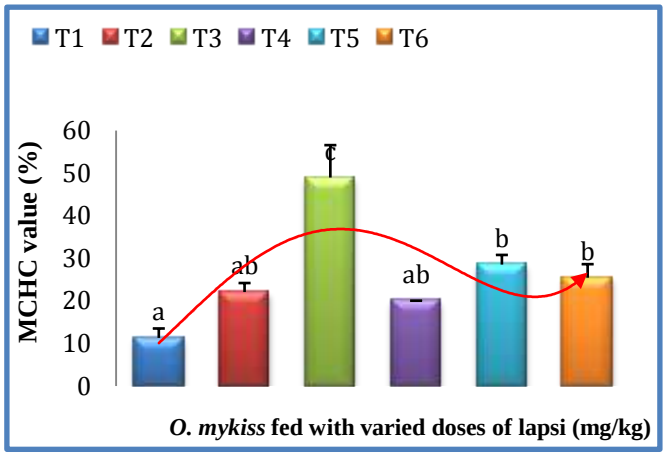

Figure 4.5.39 MCHC level in *O. mykiss* fed with diet containing six different doses of lapsi fruits

c) Differential counts

4.5.40 Neutrophils (%)

Neutrophils (%) was significantly (P<0.05) higher (51.97±0.27e %) in rainbow trout fed with T4 diet. Neutrophils (%) in T3, T6, T5, T2 and T1 diets fed fish were 49.67±0.13d, 47.36±0.44c, 45.05± 0.59b, 42.74±0.11a and 41.02±1.32a %, respectively. Neutrophils (%) in T4 diet fed trout was 1.3 times higher compared to trout fed with control (T1) diet (Fig.4.5.40).

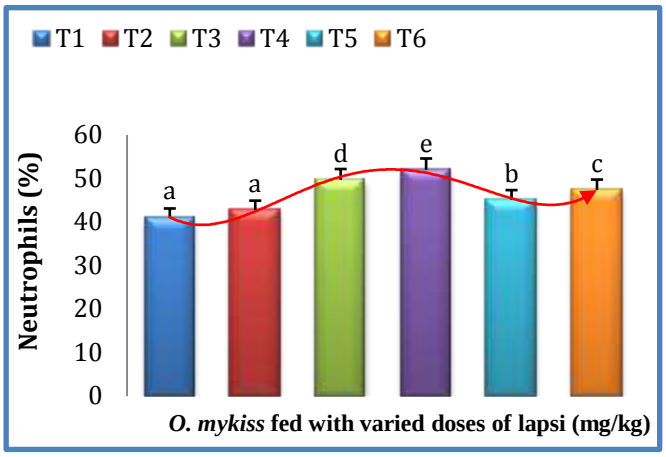

Figure 4.5.40 Neutrophils (%) in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.41 Lymphocytes (%)

Lymphocytes (%) was significantly (P<0.05) higher (47.95±5.91b %) in trout fed with diet T6. Lymphocytes (%) in T5, T4, T3, T1, and T2 diets fed groups were 45.97±3.35ab, 44.66±3.78ab, 37.34±1.29ab, 36.61± 1.13a and 35.36±1.03a% respectively. The lymphocytes (%) in T6 diet fed trout was 1.4 times higher compared to trout fed with control (T1) diet (Fig.4.5.41).

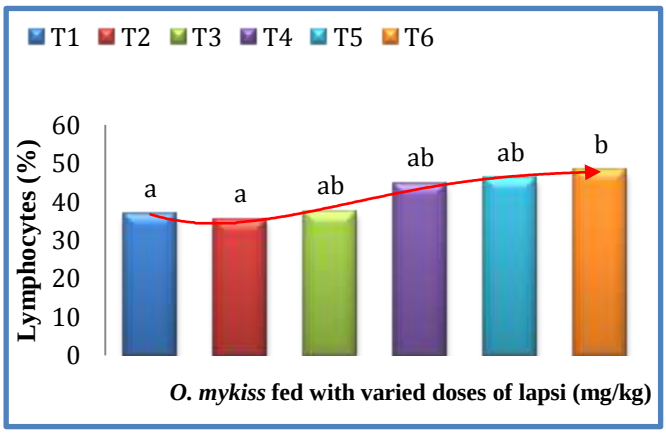

Figure 4.5.41 Lymphocytes (%) in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.42 Monocytes (%)

Monocytes (%) was significantly (P<0.05) higher (8.71± 0.61b %) in trout fed with diet T4. Monocytes (%) in T6, T2, T5, T3, and T1 diets fed fish were 6.82±1.25ab, 5.71±1.44ab, 4.71±1.02ab, 3.79±1.51a and 3.30±1.49a %, respectively. Monocytes (%) in T4 diet fed trout was 2.6 times higher compared to trout fed with control (T1) diet (Fig.4.5.42).

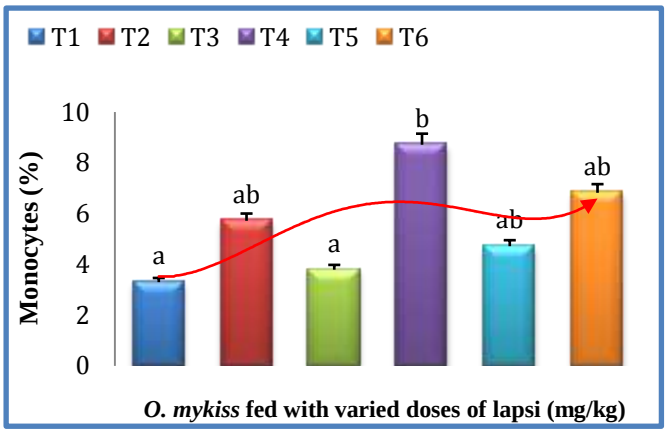

Figure 4.5.42 Monocytes (%) in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.43 Eosinophils (%)

Significantly (P<0.05) higher Eosinophils level was recorded in trout fed with T4 diet (1.87±0.25b). Eosinophils (%) in trout fed with diets T5, T3, T1, T2 and T6 were 1.80±0.13b, 1.38±0.15ab, and 1.30± 0.19ab, 0.90±0.19ab and 0.81±0.17a, respectively. Eosinophils (%) in T4 diet fed trout was 1.4 times higher compared to trout fed with control (T1) diet (Fig. 4.5.43).

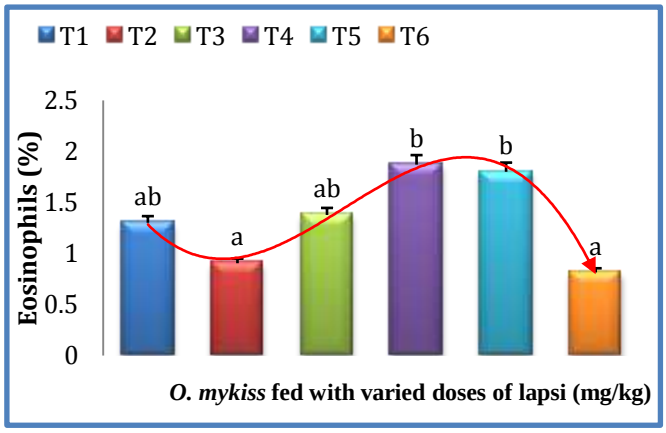

Figure 4.5.43 Eosinophils (%) in *O. mykiss* fed with diet containing six different doses of lapsi fruits

4.5.44 Basophils (%)

Significantly (P<0.05) higher basophils (%) (0.59±0.41b) was recorded in trout fed with diet (T4). In T6 diet fed trout, basophils (%) was 0.56±0.43b followed by trout fed with diets T5 (0.24± 0.55a), T3 (0.21±0.73a), T1 (0.19± 0.49a) and T2 (0.16±0.67a). Basophils (%) in T4 diet fed trout was 3.8 times higher compared to trout fed with control (T1) diet (Fig. 4.5.44).

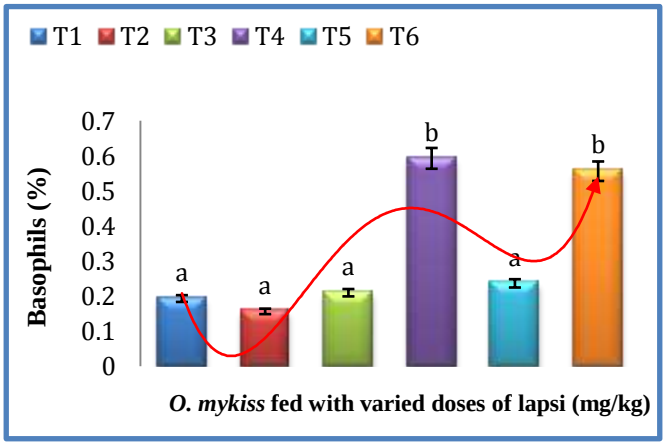

Figure 4.5.44 Basophils (%) in *O. mykiss* fed with diet containing six different doses of lapsi fruits

DISCUSSION

Three experiments were conducted to study the effect of dietary supplementation of lapsi *Choerospondias axillaris* (Roxb.) fruit extract on survival, growth, biochemical and immuno-haematological performances in common carp, rohu and rainbow trout.

4.6 Proximate Composition of Lapsi fruit incorporated diets

Fish feed is formulated to fulfill the requirements of nutrients and energy in fish. In this experiment the CP content of feed varied from 19.82±0.54 to 44.86±0.56 %; EE content from 9.7±0.04 to 10.14±0.02 %; total ash from 4.8±0.003 to 5.6±0.004%, carbohydrate from 34.95±0.72 and 60.2±0.35 % and moisture content from 5.82±0.004 to 6.7±0.002.

4.7 Antioxidant Properties of Lapsi (*Choerospondias axillaris*) Fruits

Antioxidants stop the activity of highly reactive free radicals and protect the cells which otherwise cause free radicals related diseases such as cancer, diabetes, cardiovascular, and neurodegenerative disorders (Kumar *et al.*, 2010; Li *et al*., 2013; Hwang, 2013). Free radicals are formed naturally in the body, and endogenous antioxidants interact with and neutralize them when overproduced, thus preventing them from causing damage (Singh *et al.*, 2009). However, under stress or pathological condition the internal (endogenous) antioxidant may not be sufficient to quench the damaging effect of free radicals. Therefore the body depends on external (exogenous) antioxidant, called dietary antioxidants. Fruits, vegetables, and grains are rich dietary antioxidants (Prakash and Gupta, 2009; Bouayed and Bohn, 2010).

Ascorbic acid (AA) is highly bioavailable and important water soluble antioxidant vitamin in cells. Lapsi fruits, rich in AA are consumed fresh, pickled or in processed forms. Ethanolic extract of LFP contains phytoconstituents which exhibit high scavenging activities compared to aqueous extracts and control (ascorbic acid). These in vitro assays indicate that lapsi fruit is a good source of natural antioxidant that prevents oxidative stresses *in vivo* (Labh *et al.,* 2015). Significant ($P < 0.05$) results for antioxidant properties of LFP were observed and as the concentrations of LFP were increased the antioxidant compounds such as phenolic, flavonoids, 2, 2 diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid (AA) also increased. No significant difference between the values of phenolic compounds in the LFP

was observed (Table 2), and these values are in accordance with those reported by Rocha *et al.* (2011).

4.8 Effect of lapsi (Choerospondias axillaris) on Survival and Growth of Fishes

After 90 days of feeding trials survival rate of 97% was observed in T4 and T5 diet fed common carp and 100 % in rohu and rainbow trout. Several studies reported that survival of infected fish increased after treatment with various immunostimulants (Sakai, 1999), vaccines (Bakopoulos *et al.*, 2003) and probiotics (Burnt *et al*., 2007). Feeding carp with chitosan and levamisole reduced mortality of common carp after challenge with *A. hydrophila* (Gopalakkanan and Arul, 2006). A similar result was reported after feeding large yellow croaker with glucan and challenging with *Vibrio harveyi (*Ai *et al*., 2007). Citarasu *et al.* (2002) developed an artemia-enriched herbal diet for *Penaeus monodon* with a combination of five herbs, which significantly increased the survival during stress conditions.

Food is a major limiting biotic factor affecting growth rate in fishes (Brett, 1979). Growth is greatly influenced by factors such as behavior of fish, quality of feed, daily ratio size, feed intake and water temperature. Most fish species can grow as long as they live in favorable conditions. They can survive starvation for days to months and lose very significant amount of weight. They resume their growth, without ill-effect, with the return of favorable conditions (Weatherley and Gill, 1987). Several herbs were tested for their growth promoting activities in aquatic animals (Sakai, 1999; Harikrishnan *et al.,* 2003). It was evident that feed incorporated with garlic peel could enhance fish immunity even at a lower dose of 5 mg kg^{-1} of feed. The results also suggested that inclusion of garlic peel in the diet would improve non-specific immunity of fishes and prevent bacterial infections in culture systems. Final weight gain was 28.34% higher in common carp fed with T4 diet compared to weight gain in control group. It was 53.78 % higher in rohu and 30.6 % higher in rainbow trout. Weight gain in rohu was 89.76 % and 75.75% higher than in common carp and rainbow trout respectively. This finding is similar to results of Shalaby *et al.* (2006) who showed significantly increased weight gain, feed efficiency, protein efficiency ratio (PER) and specific growth rate (SGR) in Nile tilapia fed with diet containing garlic powder. Similarly, Diab *et al.* (2002) recorded the highest growth performance in Nile tilapia fed diet containing 2.5% garlic. Metwally (2009) also reported the best growth in Nile tilapia fed with garlic powder incorporated diet. Other studies also recorded positive effects of garlic supplemented diets on the growth and feed utilization of fishes such as African catfish, *Clarias gariepinus* (Agbebi *et al*., 2013); rainbow

trout, *Oncorhynchus mykiss* (Gabor *et al*., 2012; Nya and Austin, 2009); Swordtail, *Xiphophorus helleri* (Kalyankar *et al.,* 2013) and Nile tilapia, *Oreochromis niloticus* (Shalaby *et al.*, 2006, Mesalhy *et al*., 2008, Metwally, 2009; Aly and Mohamed, 2010).

Several herbs such as garlic, onion, marjoram, caraway, basil, anise, fennel, licorice, black seed and fenugreek have been tested for growth promoting activities (Jayaprakas and Eupharsia 1997; Citarasu *et al*., 2002; Sivaram *et al*., 2004), feed conversion (Shalaby *et al.*, 2003; El-Darkar *et al*., 2004 a, b; Shalaby, 2004), and protein digestibility and energy retention improvement (El-Dakar *et al.*, 2004 a & b) in aquatic animals including fishes. In the present study better weight gain and SGR were observed in common carp, rohu and rainbow trout fed with LFP diets.

A significantly high ($P<0.05$) SGR was observed in T4 (400 mg kg^{-1}) diet fed fish, whereas lowest SGR was observed in control diet fed fish. Shalaby *et al.* (2006) reported significant SGR in Nile tilapia fed with diet containing garlic powder. Abou-Zeid (2002) also observed a positive improvement in biomass and SGR in the same species fed with garlic supplementation.

Feed conversion ratio (FCR) is an important indicator of fish feed quality and its utilization. Lower FCR indicates better utilization of the fish feed (Aibek, 2013). FCR was found to decrease with increased doses of LFP in diets. The lowest FCR was recorded in T4 diet fed common carp, rohu and rainbow trout. Decreasing FCR trend was observed in all three experiments. However, FCR level was found to decrease the most in rohu (53.75%) followed by trout (30.56%) and common carp (28.13%). FCR of T4 diet fed rohu was 88.67% higher than in common carp and 73.63% higher than in trout. The current FCR values coincided with ranges reported for Nile tilapia that ranged from 1.43 to 2.30 (Khattab, *et al*., 2000; El-Husseiny, *et al*., 2008). It is evident that lapsi supplemented diet promotes growth in common carp, rohu and rainbow trout. FCR values recorded in the experiments are comparable to findings of Jayaram (1998), Ramachandra Naik (1998), Srinivas (2000), and Hanumanthappa (1998).

4.9 Evaluation of Vitamin C in fish fed with lapsi supplemented diets

The increased growth rates and feed efficiency in several fish species fed with diets sufficient in AA are well documented (Dabrowski *et al*., 1990, 1996; Lee *et al*., 2001). Navarre and Halver (1989) reported that a higher weight gain was observed in rainbow trout fed with high dietary ascorbic acid (AA) (500 to 2000 mg kg^{-1} diet). The minimum requirement to support optimal growth was estimated to be between 10 and 25 mg AA kg^{-1} diet for channel catfish (Mustin and Lovell, 1992), rainbow trout (Cho and Cowey, 1993), hybrid striped bass (Sealey and Gatlin, 1999) and hybrid tilapia (Shiau and Hsu, 1999). Lee and Bai (1998) reported that Korean rockfish, *Sebastes schlegeli* (Hilgendorf), fed 1500 mg kg^{-1} diet showed the highest weight gain compared to fish fed 25 to 150 mg AA kg^{-1} diet. Gouillou-Coustans *et al.* (1998) reported maximum growth of common carp larvae fed with AA level of 90 mg kg^{-1} dry diet. Dabrowski *et al.* (2004) reported that dietary AA levels significantly affected growth rates and growth of fishes that were fed the high-ascorbate diet was greater than fishes that were fed the low ascorbate diets.

Lee and Dabrowski (2003) showed higher growth rate in fish that were fed AA supplemented diets than fish that were fed a diet devoid of AA. Tewary and Patra (2008) reported maximum growth in rohu fed with 1000 mg AA kg^{-1} diet and the lowest growth in control diet fed rohu. Japanese seabass needs adequate exogenous AA to maintain normal growth and physiological functions (Wang *et al*., 2003; Ai *et al.*, 2004). Ai *et al.* (2006) found that in common carp larvae 45 mg kg^{-1} diet was required to maximize body AA concentration. The blood serum concentrations of AA in common carp, rohu and rainbow trout were 64.25%, 61.27% and 92.5% respectively. The AA concentrations were higher in treated diet fed groups compared to control group. The concentration was 1.43 times higher in the blood serum of rainbow trout compared to common carp and rohu. However, brain AA concentration was 97.71%, 58.95% in and 57.9 % in common carp, rohu, and rainbow trout respectively. Similar results were recorded in liver AA concentrations. AA concentration was 72.34% higher in liver of common carp, 50.43% higher in rohu and 21.61% higher in rainbow trout that were fed with the T4 diet compared to control diet fed fishes. Mitra and Mukhopadhyay (2003) and Sahoo and Mukherjee (2003) also observed similar result in healthy and AFB1-treated fishes. The present study showed as dietary LFP increased, SGR increased and better growth was observed in fishes that were fed with diets 400 and 800 mg LFP kg^{-1}.

AA affects fish immune system in fishes, especially the non-specific pathway (Roberts *et a*l., 1995; Rougier *et al.*, 1994).The dose of AA used was based on earlier reports that high levels of AA stimulate the fish immune system and also act as an antioxidant vitamin; it has a high safety margin for dietary incorporation (Waagbo 1994; Sahoo *et al*. 1999). AA enhances some non-specific immune parameters such as bactericidal activity, phagocytic ratio, and respiratory burst activity, when compared to fishes fed with diets without AA supplementation. Ibrahem *et al.* (2010) suggested that AA could positively affect innate immunity and resistance of Nile Tilapia (*O. niloticus*. Lovell (1984) reviewed the use of AA in reducing signs of deficiencies in fishes. Lim and Lovell (1978) explained that supplementation of AA ranging from 25 to 50 mg kg^{-1} improved wound healing by three times in channel catfish.

Kumari and Sahoo (2006) recommended increasing the diet concentration of AA by ten times to stimulate the immunity against bacterial infection in Asian catfish *Clarias batrachus*. Such improvement was also observed in *O. niloticus* (Soliman *et al*. 1994). Labh and Chakrabarti (2011) also noted marked antioxidant and immunostimulants properties of AA (LATP-Ca) in fishes. Merchie *et al.'*s (1997) finding that 2000 mg AA kg^{-1} promoted resistance in shrimp under stressful conditions and to *Penaeus vannamei* infection. Ndong and Fall (2011) demonstrated that juvenile hybrid tilapia fed with garlic (0.5g kg^{-1}) enhanced total leucocytes, respiratory burst activity, phagocytic activity, phagocytic index and lysozyme activity. According to El-Sayed *et al.* (2006) herbal compounds such as phenolics, polyphenols, alkaloids, quinines, terphenoids, lectines and polypeptides have shown to be very effective alternatives to antibiotics and other synthetic compounds as growth promotion, immunostimulation, antistress, antibacterial, antifungal, antiviral, appetite stimulators and aphrodisiac.

LFP in fish diets may increase growth and dietary utilization, modulate the non-specific immune response and increase survivability against pathogenic agents. The results of this study indicate that LFP may be useful in field situations to counteract damage caused by pathogenic bacteria and other strains and thereby increase the general disease resistance of the fish.

4.10 Evaluation of Protein profiles in fish fed with lapsi supplemented diets

Dietary supplementation of LFP extracts in different concentrations had significant ($P<0.05$) higher protein profiles i.e. total protein, albumin and globulin levels in all groups when compared to control (T1) group. The serum proteins like albumin and globulin are good indicators for determining immune response in fish (Siwicki *et al.,* 1994). Globulins like gamma globulin are absolutely essential for maintaining a potential immune system. Serum albumin and globulin values in fish fed with garlic peel were higher than the control. Increase in serum protein, albumin and globulin levels are thought to be associated with a stronger innate immune response of fish (Wiegertjes *et al*., 1996).

This significant increase in total protein level suggests the stabilization of endoplasmic reticulum leading to protein synthesis. This may be due to the presence of AA and compounds such as flavonoids in LFP which act as antioxidant and immunostimulants. In agreement with present findings, Ajeel and Al-Faragi (2012) found that Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) or their mixture in the diet increased total plasma protein, albumin and globulin concentration significantly ($P<0.05$) and concluded that they protect the liver against deleterious agents and free radical- mediated toxic damages to the liver cells. The same result was also agreed by who found Dugenci *et al.* (2003) who observed the highest plasma protein in fishes fed with diet containing 1% ginger extract. Siwicki (1989) observed an increase in total protein content after feeding of b-glucan (0.2%) and chitosan (0.5%) in the diet.

Sivaram *et al.* (2004) used methanolic extracts of *O. sanctum*, *W. somnifera* and *Myristica fragrans* in juvenile Greasy grouper, *Epinephelus tauvina*, and found significantly improved immune parameters such as phagocytic activity, serum bactericidal activity, albumin–globulin (A/G) ratio and leukocrit against *Vibrio harveyi*. Higher concentration of protein in liver in the present study may be due to antioxidant property of LFP. Serum total protein and globulin are considered as good indicators for determining immune system activation (Siwicki *et al*., 1994). Certain herbal immunostimulants have been reported to increase total protein as well as total globulin in fish. Other reports indicated a lack of immunostimulant influence on serum proteins in such populations (Ispir and Mustafa, 2005; Misra, *et al.,* 2006). The increase in serum protein content might be in part due to an increase in the WBC, which is a major source of serum protein productions such as lysozyme, complement factors and bactericidal peptides (Misra *el al*. 2006).

4.11 Evaluation of enzyme profiles in fish fed with lapsi supplemented diets

Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs. Blood and tissues SGOT and SGPT levels have been measured to assess the toxic impact of aflatoxicosis and ochratoxicosis (Ellakany and Gaafar, 2002). An inverse relationship was found between the SGOT and SGPT levels and the dose of LFP in the diet of fishes regardless of species and weight.

The concentration of SGOT in blood serum was 55.7%, 23.7% and 62.7% higher in the control diet fed common carp, rohu and rainbow trout respectively compared to fishes that were fed with T4 diet. The concentration of SGOT was 2.6, 1.21 and 1.38 times higher in blood serum, liver and gills respectively of rohu fed with control diet compared to rohu fed with T4 diet. It showed that SGOT level was two times higher in blood serum of rohu fed with the control diet compared to the liver and gill. A similar result was observed in rainbow trout.

SGPT was five times higher in the blood serum of control diet fed common carp compared to T4 diet fed carp. In rohu and rainbow trout the SGPT concentration was 3.3 and 6.8 times respectively. In liver and gills of common carp the SGPT levels were 1.3 and 1.56 times higher in the control compared to T4 diet fed carp. SGPT levels in liver and gills were 1.45 and 1.23 times higher in the control diet fed rohu compared to the T4 diet fed rohu. In rainbow trout SGPT levels were 68.13%, 56.47% and 28.63% in blood serum, liver and gills respectively in control trout.

Alkaline phosphatase (ALP) level in blood serum, liver, and gills of common carp was 1.43, 1.16 and 1.77 times higher in control diet fed group compared to the T4 diet fed group. In rohu ALP level in blood serum, liver and gill was 2.5, 1.43 and 1.58 times higher in the control diet fed rohu compared to T4 diet fed group. Similarly ALP level was 20.05%, 90.79% and 15.58% in blood serum, liver and gills respectively in trout that were fed the control diet compared to trout that were fed the T4 diet.

As LFP doses in the diet increased, trends in the concentration of SGOT, SGPT and ALP in blood serum, liver and gills in common carp, rohu and trout decreased. In T4 diet fed

fishes, low concentrations of these enzymes were recorded compared to control and other treated diet fed fishes. The presence of SGOT and SGPT in blood plasma is an indication of tissue injury or organ dysfunction (Wells *et al.*, 1986) which is only released into the blood in pathological situations only. SGOT, SGPT and ALP levels were found higher in control diet fed fishes compared to fishes fed with diets containing LFP.

4.12 Immune response and blood profile of fish fed with lapsi supplemented diets

Haematological profiles or Haemato-immunological parameters such as hemoglobin concentration (Hb), number of erythrocytes, packed cell volume (PCV), and leucocytes were normal. Aditionally, differential leucocyte counts (lymphocytes, monocytes, neutrophils and basophils as leucocyte %), blood indices such as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were also found normal. The concentration of haemoglobin was 8.45, 1.91 and 4.8 times higher in T4 diet fed fishes than control diet fed fishes. Erythrocyte's number was 1.21, 2.15 and 3.91 times higher in T4 diet fed fish than control diet fed group PCV. in rohu and rainbow trout was 1.3 and 2.7 times higher in the T4 diet fed group respectively when compared to the control diet fed group. MCV were 1.3, 2.1 and 2.7 times higher in T4 diet fed fishes while MCHC were 1.6 and 1.8 times higher in rohu and rainbow trout that were fed T4 diet respectively, compared to the control diet fed fishes. Neutrophils were 1.3 times higher in all fishes fed with treated diets. Monocytes were 5.2 times higher in rohu and 2.6 times higher in rainbow trout fed with the T4 diet compared to the control diet fed fishes. Similarly, percent eosinophil was 1.3 times higher in T4 diet fed rohu compared to the control diet fed rohu. Bello *et al.* (2012) showed that incorporation of low level (0.5%) garlic peel in feed enhanced hematological parameters in *Clarias gariepinus* fingerlings and made them highly immunopotent and more resistant to infection by *A. hydrophila*.

Gabriel *et al.* (2009) reported increased haematological parameters such as leucocytes, erythrocytes, PCV, haemoglobin, MCH, MCV and MCHC in cat fish that were fed with aqueous leaf extracts of *Lepidagathis alopecuroides*. Binukumari and Subisha (2010) observed increased haemoglobin, erythrocytes and leucocytes in tilapia (*O. niloticus*) fingerlings exposed to lethal concentration of *Moringa oleifera* when compared to control. Christybapita *et al*. (2007) showed that *Eclipta alba* incorporated diet significantly enhanced the haematological parameters and reduced mortality rate against *A. hydrophila* in tilapia (*O.*

mossambicus). Abasali and Mohamad (2010) reported that diet with herbal immunostimulant significantly increased haemoglobin content in disease induced common carp. Rao *et al*. (2006) demonstrated increased production of haemoglobin and resistance to *A. hydrophila* in rohu fingerlings fed with diet containing *Achyranthus aspera.* Bello *et al*. (2014) reported increased levels of haematological parameters such as haemoglobin content, erythrocytes and leucocytes and PCV in Juvenile *Clarias gariepinus* fed with diets that contained onion bulb and walnut leaf residues. Results obtained showed that packed cell volume and haemoglobin content were significantly different (P<0.05) among the treated groups and boost immune response of cultured *Clarias gariepinus* juveniles. Similar to Bello *et al.* (2014), Shalaby *et al.*, (2006) reported a significant (P<0.05) increase in erythrocytes, heamoglobin content and PCV value in tilapia (*O. niloticus*) that were fed different levels of garlic (*Allium sativum*) and chloramphenicol.

The increase in total leucocytes of fishes fed with lapsi extract was high compared to the control group. High leucocyte count in fishes that were fed with LEP is similar to findings in *Paralicthys olivaceus* injected with *Hericium erinaceum* (Harikrishnan *et al*., 2003); in cultured fin and shell fish injected with *Punica granatum* (Harikrishnanv *et al*., 2010); in Jian carp injected with Chinese medicinal plants (Jian and Wu, 2004); in *O.mossambicus* administered with *O. sanctum* (Logammal *et al*., 2000) and in tilapia injected with 8 μg g^{-1} hot-water extract of *T.sinensis* (Wu *et al*., 2010). Subeenabegum and Vaseekaran (2017) reported significant increase in total leucocytes in fresh water fish *Channa striatus* that *were* fed with the aqueous methanolic extract of *Solanum trilobatum* and *Ocimum sanctum.* Thus, increased total leucocytes in fishes may be due to non-specific immune response (Manjrekar *et al*., 2007) or due to interdependent mechanism of an innate resistance and adaptive immunity (Mishra *et al*., 2009). Subeenabegum and Navaraj (2016) studied the individual and combined effect of *Solanum trilobatum* and *Ocimum sanctum* extracts against *A. hydrophila in Channa striatus*. Hematological parameters such as WBC, RBC and haemoglobin content were enhanced (P<0.05) in fish fed with herbal extracts compared to the control group. The MCV and MCH values of exposed fish showed a significant increase (P<0.05), while the MCHC values of both groups were not significantly different from each other.

The haematological study results revealed that the *Cyprinus carpio* fed with diets containing leaf extract of *Euphorbia hirta* significantly enhanced the haemoglobin content,

erythrocytes and leucocytes compared to control fish. It was also observed that erythrocytes, haemoglobin content and leucocytes were maximum in fish fed with diet that contain 50 g leaf extract of *Euphorbia hirta* (Pratheepa and Sukumaran, 2014). Harikrisnan *et al.* (2003) reported that mixed herbal extract supplementation diets for goldfish restored the altered haematological parameters and triggered the non-specific immune system (Ahilan *et al.,* 2010) against *A. hydrophila* infection. .

Prasad and Mukthiraj (2011) studied that increased levels of leucocyte, thrombocytes, Hb, PCV, MCHC could be due to the action of *A. paniculata* present in the diets. Adewoye (2010) observed increased level of leucocytes in *Clarias gariepinus* that received the *Tephrosia vogelii* extract compared to the control. Jeney and Anderson (1993) observed an increase in leucocytes after injection or bath administration of abarly extracted glucan in rainbow trout. They reported an initial decrease in leucocytes, 6 hours after immunostimulation followed by an increase at 12 hours and a greater increase at day 2 after injection and at day 3 after bath treatments. Furthermore, leucocyte count was always higher in fish that received immunostimulants. Muthu (2015) mentioned that leucocyte count increased in groups of common carps that were administered extracts from *Andrographis paniculata*. High concentration of plant extract of *A. paniculata* in feed showed maximum number of leucocytes.

Serum leucocyte count, antibody level, lysozyme and bactericidal activity were significantly high in fishes treated with *Aloe vera* ($P<0.05$). No significant differences were seen in the erythrocyte count, PCV or complement activity among the groups. The relative percent survival (RPS) increased in fish that were fed with *Aloe vera*. The study reported that oral administration of *Aloe vera* enhanced some specific and non-specific immune responses in the common carp (Alishahi *et al.*, 2010). Gupta and Mishra (2014) observed significant increases in erythrocyte, haemoglobin and leucocyte in *C. gariepinus* that were fed with varied concentrations of aqueous and alcoholic extracts of leaf of *E. alba*. Significant differences were observed in PCV with alcoholic extract only. WBCs are the main components of the immune system. Ghareghani *et al.* (2014) showed that feeding rainbow trout (*Oncorhynchus mykiss*) with low (100 mg kg^{-1} feed) dose of *Peganum harmala* seed extract enhanced leucocyte and erythrocyte counts of rainbow trout compared to the control and can act as immunostimulants to enhance the immune response of cultured fish.

Das *et al*. (2009) evaluated the use of *Euglena viridis* in *Labeo rohita* diets and reported a significant (P<0.05) increase in leucocytes and erythrocytes in groups fed with treated diets containing different levels of *Euglena viridis* compared to the control. Onion bulb (*Allium cepa*) and walnut leaves (*Tetracarpidium conophorum*) have constituents such as alkaloids, flavonoids, tannin and thiosulfinates that may play a role in stimulating the immune system and in functions of the thymus, spleen and bone marrow, organs related to blood cell formation. The findings of the present study were similar to the results to Shalaby *et al.* (2006) who reported a significant (P<0.05) increase in erythrocyte count, heamoglobin content and hamatocrit value in *O. niloticus* fed with different levels of garlic (*Allium sativum*) and chloramphenicol. Ardo *et al*. (2008) also reported that feeding *O. niloticus* with two herbal extracts (*Astragalus membranaceus* and *Lonicera japonica*) alone or in combination significantly enhanced phagocytic cells. Pavaraj *et al*. (2011) reported that *O. sanctum* leaf extract enhanced phagocytic activity in *C. carpio* infected with *A. hydrophila*.

Aly *et al*. (2010) reported that garlic improved the immune response of *O. niloticus* through rapid increase in monocytes and enhanced phagocytic activity which afforded increased protection against immediate challenge infection. These findings are also similar to Gopalakannan and Arul (2006) who reported increase in leucocytes count after feeding the common carp with immunostimulants like chitin. Similar results have also been reported in tilapia following oral administration of *Rosmarinus officinalis* leaf powder (Abutbul *et al*., 2004), leaf aqueous extract (Christybapita *et al.*, 2007), and *Zataria multiflora* essential oil (Soltani *et al.*, 2009).

Leucocytes play an important role in non-specific (innate) immunity and their count can be considered as an indicator of the health status of a fish (Harikrishnan *et al.*, 2003). Choudhury *et al* (2005) found an increase in the Leucocyte count in *Labeo rohita* juveniles fed with immunostimulants like levamisole and ascorbic acid. The erythrocyte count was higher in garlic peel fed groups when compared to control group. The erythrocyte count increased with administration of garlic peel, which might indicate an immunostimulant effect. Similarly Duncan and Klesius (1996) reported significantly greater number of erythrocytes in channel catfish fed with a diet containing b-glucan. Duncan and Klesius (1996) reported that the number of erythrocytes in channel cat fish fed with a diet containing b-glucan was significantly (P<0.05) greater. It is known that the amount of leukocyte cells is normally lower in healthy fishes and can be used as a significant indicator for infectious diseases.

When infectious disease agents such as bacteria enter the fish body, the non-specific (cellular) defense system gets stimulated during the first stage of disease manifestation. In this situation, the leucocytes get increased (leucocytosis) initially to protect the fish body by phagocytosis and produce antibacterial chemicals to stop the agent from spreading. The significant increase in the total leucocytes count and the number of different leucocytes i.e. neutrophil, large lymphocytes, monocytes etc, observed in this present study signifies that the non-specific immunity of the fishes were stimulated to fight against the bacterial pathogen as the primary line of defense.

4.13 Water quality parameters

Water quality and aquatic productivity is a prerequisite for optimum growth and survival of fishes (Boyd, 1982). Water quality parameters such as temperature, pH, and dissolved oxygen were measured through the experimental period at fortnightly intervals. Water was replenished in all the aquaria to maintain its quality.

4.13.1 Temperature

Temperature is one of the most important environmental variables that influence the oxygen content of water. Every aquatic animal has its own tolerance limit for temperature. Increase in temperature accelerates growth up to a certain level, beyond which it is lethal. Optimum temperature for metabolism varies from species to species. It influences survival as well as growth of aquatic organisms depending on climate, sunlight and depth of water. Food intake increases with increasing temperature since higher energy is required for maintenance purposes (Cho and Slinger, 1980). Fish can tolerate temperature ranging from 18 °C to 32 °C, provided temperature fluctuation is not sudden and for longer duration (Faramanfarmain and Moore, 1980). For this study, the water temperature ranged from 26.3 °C to 28.3 °C in experiment 1, 25°C to 27.8°C in experiment 2 and 12.5 °C to 18 °C in experiment 3. These ranges were within the acceptable limits for growth of freshwater fish as reported by Ramachandra Naik *et al.* (2000), Srinivasa (2000) and Prakash (2004).

4.8.2 pH

The pH is an expression of hydrogen ion concentration in water and serves as an indicator of acidity and alkalinity. It is an important parameter to consider because it affects metabolism and other physiological processes of aquatic animals. If water is too acidic it decreases fish appetite, and eventually affect growth and immunity. According to Swingle (1961) and Banerjee (1967), near neutral to slightly alkaline pH range was mst favorable for fish ponds. Ideal range for culture of freshwater fish is between 7.0 and 8.5 (Hsieh *et al.,* 1989). In the present study, pH values were near neutral to alkaline and ranged from 7.2 to 7.9 in experiment 1, 6.8 to 7.6 in experiment 2 and 6.8 to 8.0 in experiment 3. These recorded values of pH during the course of experiments were within the desirable limits for fishes.

4.8.3 Dissolved oxygen

Dissolved oxygen in ponds is very crucial for aquatic life and survival of fishes (Boyd, 1982). For the best fish growth, the optimum level of dissolved oxygen is 5.65 ppm. Avault (1986) reported that dissolved oxygen levels down to 5 mg l^{-1} can be tolerated by fishes in ponds. Banerjee (1972) mentioned that oxygen concentration of above 5 mg l^{-1} is an indication of productivity and below that level the water is unproductive. In this study, the recorded dissolved oxygen ranged from 6.2 to 8.2 mg l^{-1} in experiment-1, 5.6 to 8.4 mg l^{-1} in experiment-2 and 6.4 to 7.8 mg l^{-1} in experiment 3. These recorded levels could be considered suitable for optimum growth of fish.

CHAPTER 5

5. CONCLUSION AND RECOMMENDRATION

5.1 Conclusion

The purpose of this study was to investigate the effect of Lapsi *Choerospondias axillaris* (Roxb) on fish growth, better-feed conversion ratio, and enhanced immunity of fishes. We found that lapsi fruits are rich in antioxidant compounds such as phenolic, flavonoids, DPPH and AA and might be useful for fish growth.

Fishes, common carp, rohu and rainbow trout, that were fed diet containing lapsi showed enhanced growth and normal blood profile. Thus, diet supplemented with lapsi fruits promoted fish growth regardless of species and weight groups. Fishes fed with lapsi-incorporated diets also showed weight gain. Higher weight gain was observed in fishes fed with 400 mg lapsi extract per kg of diet. Final weight gain was 28.34% higher in common carp fed with T4 diet compared to weight gain in control group. It was 53.78 % higher in rohu and 30.6 % higher in rainbow trout. Weight gain in rohu was 89.76 % and 75.75% higher than in common carp and rainbow trout respectively.

Vitamin C concentration increased in treated group of fishes compared to control. Immunostimulatory parameters such as protein and enzyme profiles exhibited good results. In the treatment group, total protein, albumin and globulin level increased while SGOT, SGPT and ALP profiles decreased. The most favorable results were found in 400 mg kg^{-1} lapsi extract fed groups. Similarly, in the treated groups, haematological parameters such as blood profile were found within normal range. Finally, lapsi-incorporated diet not only showed better growth, SGR and FCR for fishes, but also exhibited increased protein profile and decreased liver enzyme profile. Blood indices were found to be normal in all control and treated fish groups.

Hence, based on these results, we can conclude that lapsi fruits are useful for fish growth since it contains a rich amount of vitamin C. Lapsi is found to be a potential supplement for fish diets.

5.2 Recommendations

This section offers recommendations to fish farmers and future researchers based on the results of this study.

Recommendations for fish farmers

1. Fish farmers might consider using lapsi as a supplement to promote fish growth and to increase fish production.
2. Farmers must select good quality lapsi to prepare extracts for fish diets since lapsi quality varies by species.
3. Evaluation of antioxidant properties when selecting lapsi pulp can be beneficial.
4. To prepare fish feed, using quality ingredients such as fish meal powder and wheat flour are important.
5. Lapsi tree can be evaluated for fruit production.

Recommendations for future researchers

1. Future studies can investigate the quality of lapsi and its effect on fish growth.
2. Antibacterial, antifungal and antioxidant properties of lapsi collected from unknown area or newly cultivated area can also be studied.
3. We only studied three fish species to evaluate the effect of lapsi on fish growth and non-specific immunity. Future researchers might consider looking at other fish species.
4. A challenge test to study the effect of lapsi on stress in fishes needs to be done.
5. While we extracted lapsi fruit pulp using ethanol, future researchers can try to use methanol and other compounds.

CHAPTER 6

6. SUMMARY

In Nepal, aquaculture plays an important role in the country's economic development. Since carps are a major component of the freshwater aquaculture, promotion of carp and demand for healthy stockable carp seeds are of paramount significance. However, issues such as high fish mortality, stunted growth rate, skeletal deformities and frequent diseases are commonly seen in aquaculture. Knowledge of fish nutrition and feeding are essential for sustainable aquaculture.

Vitamin C (Ascorbic acid, AA) is an important antioxidant. In fishes, it is assumed that vitamin C is an essential nutrient for optimum growth and maintenance. In most bony fishes, vitamin C biosynthesis does not occur due to lack of L-gulonolactone oxidase (GLO). Major signs of ascorbate deficiency include reduced growth, scoliosis, lordosis, internal and fin haemorrhage, fin erosion and increased mortality. Another beneficial effect of vitamin C is to stimulate the non-specific immune response. Vitamin C deficiency has caused significant losses in fish farming, especially during the sensitive start period. Although few research suggest that the use of dietary vitamin C in fish improves their natural resistance to infections, the possible mechanism and doses are not well established.

Vitamin C-rich lapsi, *Choerospondias axillaris* (Roxb.) is native to Nepal and is also reportedly found in South-East Asian countries. Lapsi fruits containing vitamin C, phenol and flavonoid compounds are consumed to enhance immunity and to neutralize free radicals formed in the body. Therefore, this research aims to study the effect of lapsi-incorporated diets on growth, biochemical and immuno-haematological performance in fishes.

The entire study is described in seven chapters. Chapter one contains preface of nutrition and its place in aquaculture followed by rationale of study and the main objectives of research, chapter two portrays a brief review of literature, chapter three presents a detailed account of tools and procedures used during the study, chapter four explains experiment outcomes, chapter five provides a discussion of outcomes, chapter six includes summary, conclusion and recommendations, and chapter seven covers bibliography and appendices with supporting documents.

Methodology of this thesis is divided into three parts. The first part covers growth performances including total weight gain, weight gain percentages, specific growth rate, and feed conversion ratio and survival rate of fishes. The second part examines the biochemical parameters that include quantification of vitamin C in blood serum, brain and liver of fishes. Lastly, the third part evaluates the immuno-haematological part protein, enzyme and blood profiles. The protein profile includes total protein, albumin, globulin and the ratio of albumin and globulin in various tissues such as blood serum, brain, liver and muscles. The enzyme profile monitors SGOT, SGPT and ALP from blood serum, liver and gills. Finally, to understand the health status of fish, a complete blood profile has been evaluated along with blood indices and absolute values.

To begin the investigation, lapsi fruits were collected from local markets and sent to National Herbarium and Plant Laboratories, Godawari, for taxonomic identification. After identification, ethanol extract of lapsi fruits were prepared followed by analysis of the extract's antioxidant properties such as phenolic, flavonoids, 2,2, diphenyl-1 picryl-hydrazyl (DPPH) and ascorbic acid (AA). The study found that lapsi fruits are highly rich in antioxidant compounds. Vitamin C analysis was also done from the ethanol extract of lapsi fruits. According to previous literature, it is well known that lapsi fruits are very rich in essential amino acids, especially arginine, glutamine acid, glutamine, vitamin C and minerals such as potassium, calcium and magnesium.

Six practical diets (40% protein) were prepared for the experiment. Diet without any extract of lapsi fruit pulp (LFP) was used as control diet (0.0 mg kg^{-1}) and called T1. In the remaining five diets, 100, 200, 400, 800 and 1600 mg kg^{-1} of ethanol extract of lapsi fruits' pulp (LFP) were supplemented along with dry fish powder, wheat flour, cod liver oil and called T2, T3, T4, T5 and T6, respectively. Before the feeding trial, proximate analysis of fish feed was carried out using biochemical analysis. Dry matter, moisture, crude protein, ether extract, crude fiber, total ash, nitrogen-free extract and gross energy were tested using standard protocols. Three experiments were conducted for this study in three climatic conditions: first with common carp *Cyprinus carpio* in Kathmandu, second with indigenous major carp rohu *Labeo rohita* in Narayanghat, Chitwan, and the third with rainbow trout *Oncorhynchus mykiss*in Ranipauwa, Nuwakot.

Altogether eighteen glass aquaria (100 l) for experiment one and 18 cages (1m x 1m x 1m) of nylon net for experiments two and three were prepared. Fishes were divided in six groups with three replicates for the six treatments. Dechlorinated tap water was used during the experiment and replaced 2/3rd every alternate day in experiment1. During the acclimatized period, fingerlings were fed with diet without lapsi (control). After that, fingerlings were fed with test and control diet at the rate of 3% of their body weight daily at 9 a.m. and 4 p.m. Temperature, pH and dissolved oxygen were monitored. Faecal matter was siphoned daily in experiment 1. Initial weight and length were recorded at the start of experiment, and then to balance the feeding system, weight of whole body mass were taken every fifteen days. At the end of the experiment, sampling was done by measuring individual weights. Mortality was recorded to understand the survival rate during the entire experimental periods.

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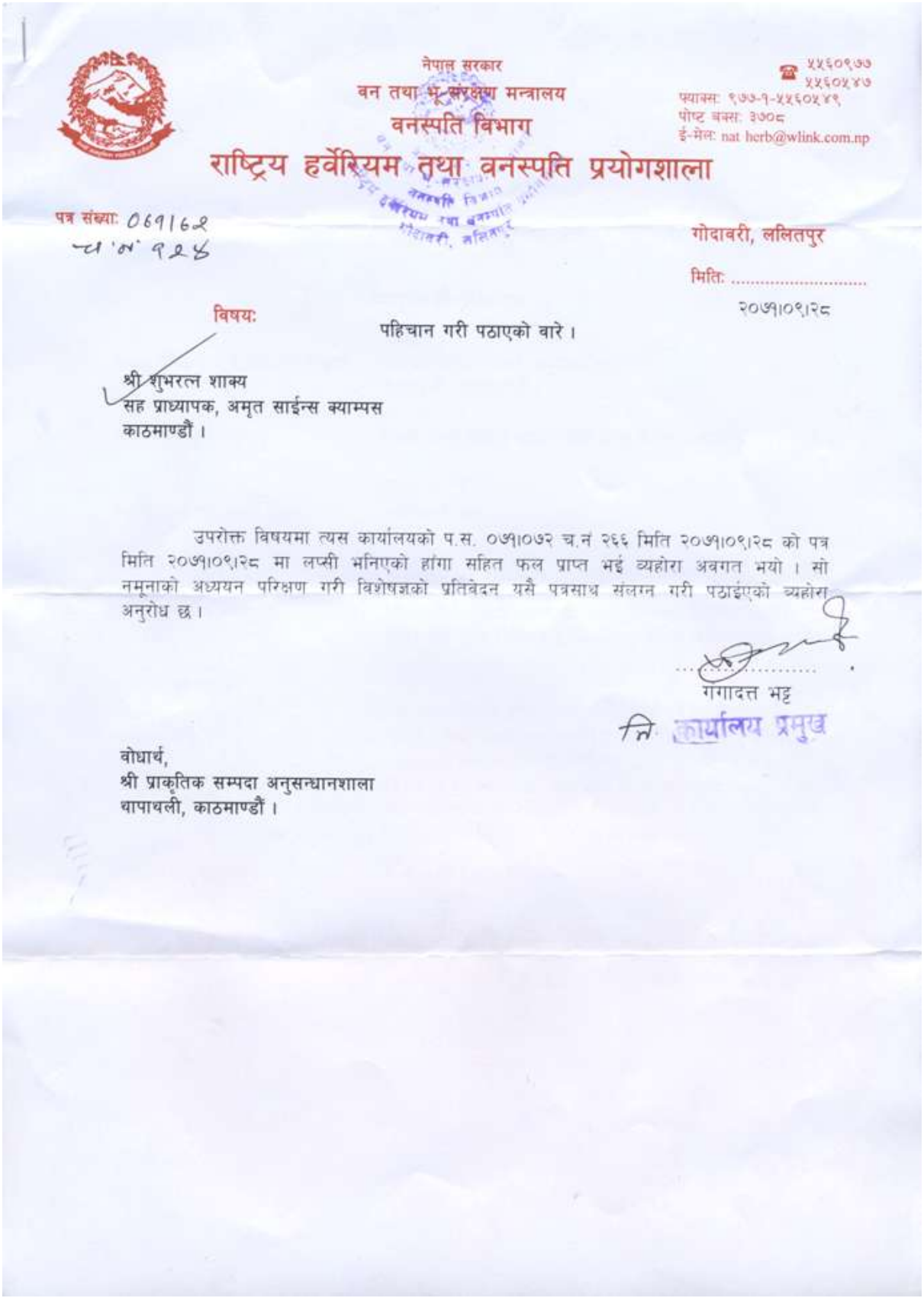
ANNEXURE-I

EXPERT REPORT



नेपाल सरकार
वन तथा भू-संरक्षण मन्त्रालय
वनस्पति विभाग
राष्ट्रिय हर्वेरियम तथा वनस्पति प्रयोगशाला

☎ ५५६०९७७
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पोष्ट बक्स: ३७०८
ई-मेल: nat herb@wlink.com.np

पत्र संख्या: ०६१।६२
च.नं. १२४

गोदावरी, ललितपुर

मिति: .....
२०७१।०९।२८

विषय: पहिचान गरी पठाएको बारे ।

श्री शुभरत्न शाक्य
सह प्राध्यापक, अमृत साईन्स क्याम्पस
काठमाण्डौं ।

उपरोक्त विषयमा त्यस कार्यालयको प.स. ०७१।०७२ च.नं २६६ मिति २०७१।०९।२८ को पत्र मिति २०७१।०९।२८ मा लप्सी भनिएको हांगा सहित फल प्राप्त भई व्यहोरा अवगत भयो । सो नमूनाको अध्ययन परिक्षण गरी विशेषज्ञको प्रतिवेदन यसै पत्रसाथ संलग्न गरी पठाईएको व्यहोरा अनुरोध छ ।

.....
गंगादत्त भट्ट
कार्यालय प्रमुख

बोधार्थ,
श्री प्राकृतिक सम्पदा अनुसन्धानशाला
थापाथली, काठमाण्डौं ।

विशेषज्ञको प्रतिवेदन

नमूना परिक्षण गर्न पठाउने निकाय	:	श्री प्राकृतिक सम्पदा अनुसन्धानशाला थापाथली, काठमाण्डौं ।
प्राप्त नमूनाको विवरण	:	लप्सी जस्तै देखिने फलसितको हांगा १, फल दाना-६
यस कार्यालयमा प्राप्त मिति	:	२०७१।०९।२८
पहिचान गरेको मिति	:	२०७१।०९।२८
परिक्षणका आधारहरु	:	Morphological comparison, उपलब्ध नमूनाहरु संग दाजेर हेर्दा तथा विभिन्न Literature हरुको अध्ययनबाट ।
पहिचान प्रतिवेदन (Result)	:	पहिचानका लागि प्राप्त नमूना बोलिचालीको भाषामा लप्सी भनिने *Anacardiaceae* परिवारको *Choerospondias axillaris* भएको प्रमाणित गरिन्छ । (This is to certified that the plant sample brought for identification at NHPL is found to be *Choerospondias axillaris* of Family Anacardiaceae)
परिक्षण गर्ने अधिकारी	:	स.अ.अ मित्रलाल पाठक

.....
मित्रलाल पाठक
स.अ.अ.

ANNEXURE-II

LAPSI FRUIT PULP POWDER PREPARATION AND ITS EXTRACT

Lapsi *Choerospondias axillaris* (Roxb.1832)Tree

Lapsi fruit

Dry Lapsi pulp

Dry Lapsi pulp in grinder

Grinded Lapsi pulp

Coarse Lapsi pulp

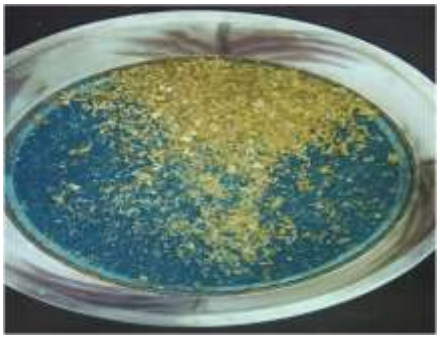
Powder Lapsi pulp

Ethanol Extract of Lapsi fruit pulp

ANNEXURE-III

FISH MEAL PREPARATION

Dry Fish



Fish Meal

WHEAT POWDER PREPARATION

Grinding Machine

Wheat Grain

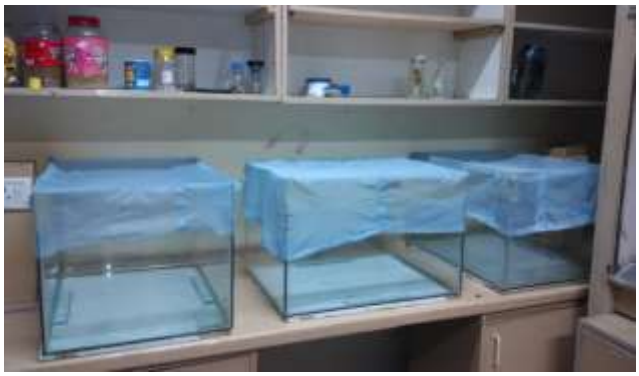
Wheat Powder

Feed Preparation - Six Treatments

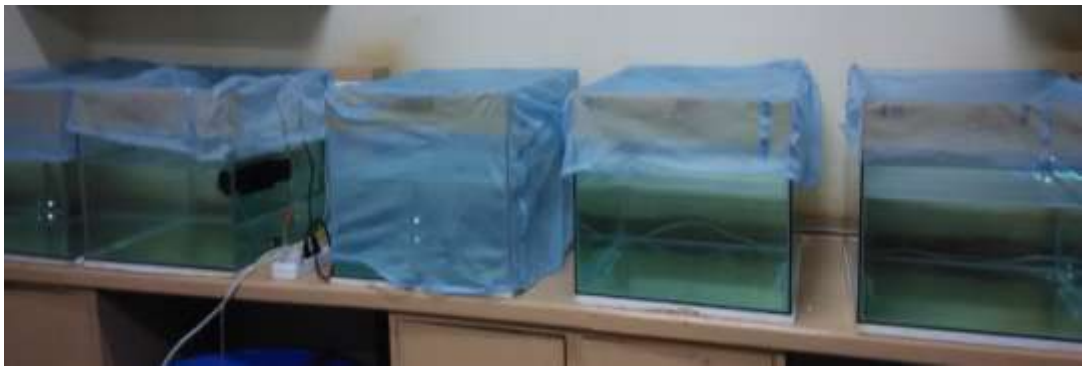
ANNEXURE-IV

PHOTOS FROM LAB CULTURE

Cleaning glass aquarium



Glass aquaria with cover



Water filled aquaria

Weighing machine

Water pump with aerator

DO meter, pH meter and thermometer for testing water

Siphoning

PHOTOS FROM FIELDS

Experimental pond

Happas with support of bamboo sticks erected in the pond

Weighing machine **Collection tissues from samples in the field**

Feeding the trout

Measuring length of the trout

Dissecting trout

Removing liver

Weighing brain

Withdrawing blood from the caudal vein

Blood of trout

ANNEXURE-V:

AWARDS AND PRESENTATIONS

National Seminar on Sustainable Fisheries and Aquaculture Development in Nepal: *Challenges and Opportunities*
(20 March, 2017)

Poster Award

Presented to

Mr. Subha Ratna Shakya

Department of Zoology, Amrit Science Campus

Tribhuvan University

For the poster entitled

Survival, growth and protein profile of Major carp *Labeo rohita* (H) cultured at Gunja Nagar, Chitwan and fed varied doses of Lapsi fruits (*Choerospondias axillaris*)

.....

Prof. Dr. Jivan Shrestha

Academician

Co-ordinator, Biological Sciences Sub-committee

Nepal Academy of Science and Technology

Khumaltar, Lalitpur

With my Supervisor Prof. Dr. S.N.Labh

Explaining about the poster

Receiving award for poster presentation from the Minister of Science and Technology-Prem Bahadur Singh


Organized by:
BSN
In Association with:
April 25
World DNA DAY
2017
Certificate of Excellence
This is to certify that Prof./Dr./Mr./Ms.
Shubha Ratna Shakya
A Professor/Scientist/Academician/Researcher/Entrepreneur/
Scholar/Student from
Amrit Science Campus, T.U
has participated in DNA day national symposium on
Bioscience and Biotechnology April 24 and 25, 2017 held at Nepal
Academy of Science and Technology (NAST) and awarded the
Third
prize in Oral/Poster presentation.
Mr. Prem Bahadur Singh
Chief Guest
Minister of Science and and Technology
Mr. Nabin Narayan Munankarmi
Chairperson
President, BSN
Prof. Dr. Jib Raj Pokharel
Special Guest
Vice-Chancellor, NAST
Mr. Anil Upreti
Chief Coordinator
Joint Secretary, BSN
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Receiving award for oral presentation from Vice Chancellor of NAST-Prof. Dr. Jib Raj Pokharel

ICAR-CIFRI Platinum Jubilee Year - 2017

29th All India Congress of Zoology

Certificate

Prof. Shubha Ratna Shakya, Kathmandu, Nepal.

Participated /Presented Paper/poster in the

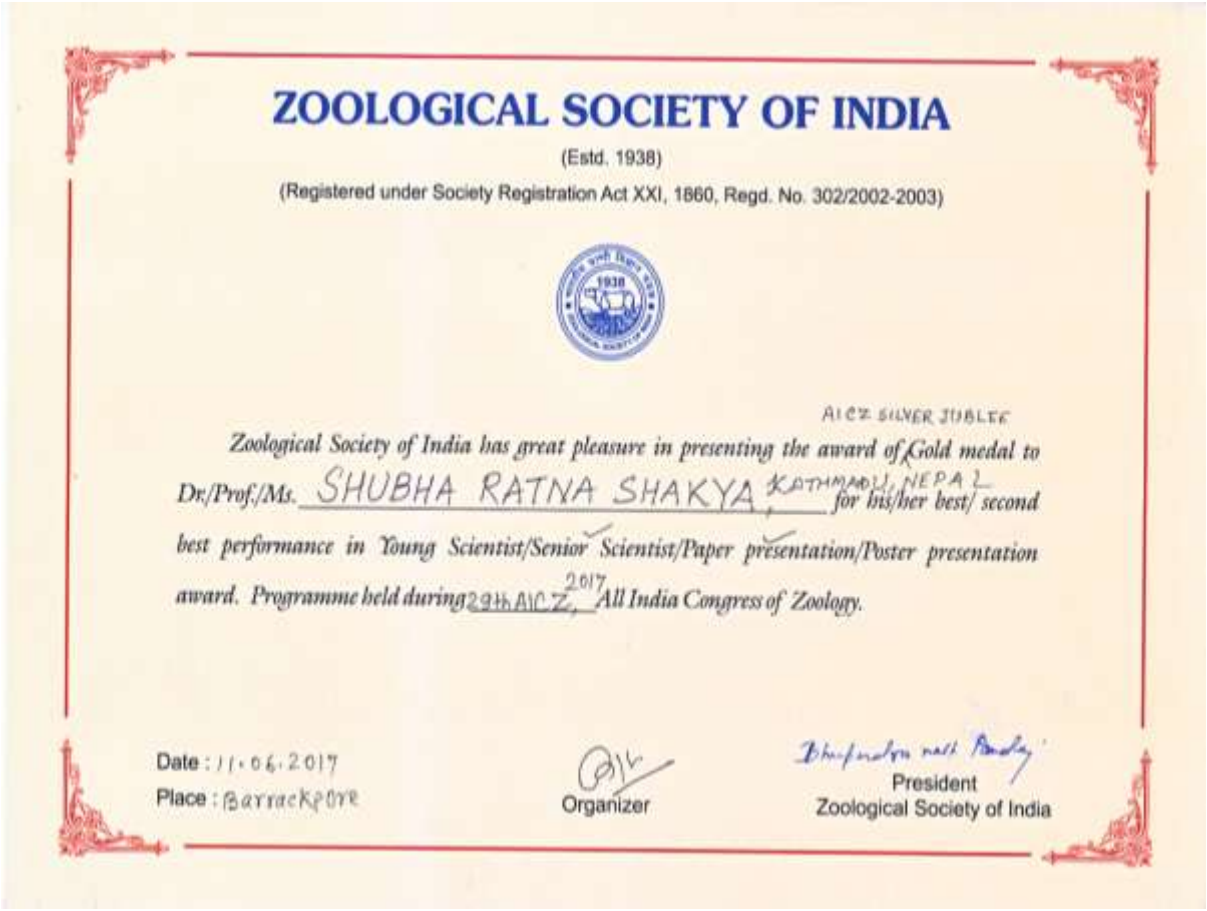
" International Symposium on Culture Based Fisheries in Inland Open Waters
&
Satellite Symposium on Fish Immunology "

at the ICAR-Central Inland Fisheries Research Institute, Barrackpore, India
during June 9-11, 2017

B. K. Das
Director, ICAR-CIFRI
& President, IFSI

Certificate

Oral Presentation, India



ZOOLOGICAL SOCIETY OF INDIA

(Estd. 1938)

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AICZ SILVER JUBLEE

Zoological Society of India has great pleasure in presenting the award of Gold medal to Dr./Prof./Ms. SHUBHA RATNA SHAKYA KATHMANDU, NEPAL *for his/her best/ second best performance in Young Scientist/Senior Scientist/Paper presentation/Poster presentation award. Programme held during* 29th AICZ, 2017 *All India Congress of Zoology.*

Date : 11.06.2017
Place : Barrackpore

Organizer

President
Zoological Society of India

Certifiate for best performance

Receiving Medal from Prof. Dr. Satyendra D Tripathi, Former Director & VC, ICAR-CIFE, Mumbai, India

Receiving Certificate from Prof. Dr. S. D. Tripathi, Prof. Dr. B. N. Pandey and Prof. Dr. B. K. Das, India

ANNEXURE-VI

PUBLICATIONS

WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 18, 1576-1585. Research Article ISSN 2277– 7105

BIOCHEMICAL ESTIMATIONS OF VITAMIN C IN BRAIN AND LIVER OF RAINBOW TROUT *ONCORHYNCHUS MYKISS* FED ETHANOL EXTRACT OF LAPSI FRUIT (EELF) *CHOEROSPONDIAS AXILLARIS* (ROXB.) IN RACEWAYS FISHERIES AT NUWAKOT, NEPAL

Shubha Ratna Shakya*[1] and Shyam Narayan Labh[2]

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[2]Department of Zoology, Amrit campus, Tribhuvan University, Kathmandu, Nepal.

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ABSTRACT

We found that vitamin C is an essential nutrient for cold water fish, the rainbow trout (*Oncorhynchus mykiss*). This was demonstrated by the absence of L-gulonolactone oxidase (GLO) activity, the enzyme responsible for the biosynthesis of vitamin C, in brain or liver of rainbow trout and by a feeding trial in which trout without vitamin C dietary supplementation developed poor growth rate. Vitamin C (Ascorbic acid or AA) is heat labile so directly cannot be given and it is found that lapsi (*Choerospondias axillaris*) contains rich amount of vitamin C in it. Thus, trout weighing 37.24±0.39 g were divided into six groups (T_1, T_2, T_3, T_4, T_5 and T_6), and each group was fed an ethanol extract of lapsi fruits (EELF) supplemented semi purified diet containing 0, 100, 200, 400, 800 and 1600 mg equivalent (EELF)/kg diet for 12 wk. The experiment was conducted in cold water raceways environment designed in recirculation system of water through nylon cages at SOSOD trout farm at Nuwakot, Nepal and vitamin C was supplemented in the diets as EELF which is found more stable to oxidation than AA. At the end of 90 days, trout fed no EELF had significantly lower weight gain than fish fed the EELF supplemented diets ($P < 0.05$). The concentration of AA in brain (51.95%) and in liver (21.63%) was higher in T4 diet group compared to trout fed with control.

KEYWORDS: Rainbow trout (*Oncorhynchus mykiss*), ascorbic acid deficiency, L-gulonolactone oxidase (GLO), lapsi (*Choerospondias axillaris*), ethanol extract.

The Journal of University Grants Commission, Vol. 6, No. 1, 2017

MEDICINAL USES OF GARLIC (*Allium sativum* L.) IN HUMAN HEALTH

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[2]Tsurumi University School of Dental Medicine, Japan

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Abstract: *Garlic (Allium sativum L., family Liliaceae) has acquired a reputation in different traditions as a prophylactic as well as therapeutic medicinal plant. The pungency of fresh garlic disappears soon after cooking or frying. With its sulfur containing compounds, high trace mineral content, and enzymes, garlic has shown anti-viral, anti-bacterial, anti-fungal and antioxidant properties. It has been used to treat infections, wounds, diarrhea, cancer, cardiovascular diseases (including atherosclerosis, strokes, hypertension, thrombosis and hyperlipidemias), diabetes, dermatological conditions, stress, and other diseases. This review focuses on health benefits of garlic in humans.*

Keywords: *Allicin, diabetes, lead poisoning, antibacterial, cardiovascular diseases*

Introduction

Garlic, *Allium sativum* L., commonly called lashun in Nepali and "laha" in Newari is used both as food and as medicine in different parts of the world. Its strong smell and delicious taste makes it a popular ingredient in cooking. The beneficial effects of garlic consumption in treating a wide variety of human diseases and disorders have been known for centuries. The health benefit of garlic is mainly due to the presence of organosulphurous compound called allicin (Queiroz *et al.*, 2009).

Ancient Chinese and Japanese used it to treat headache, flue, dysentery, colds, coughs and fever. Even today it is used in some Chinese herbal formulas for treating similar conditions. During World War I fresh garlic was applied to the wounds of injured soldiers to prevent infections (Palani *et al.*, 2014). In World War II it was again used extensively for its antibiotic properties (Kashyap *et al.*, 2006). Garlic is nicknamed as Russian penicillin for its widespread use as antimicrobial agent (Timbo *et al.*, 2006). With the development of effective antibiotics, its antibacterial effects are shifted in treating controlling cholesterol and atherosclerosis. Garlic has attracted particular attention of modern medicine because as it has been proven to be hypolipidemic (Sumiyoshi, 1997), antimicrobial (Kumar & Berwal, 1998), antihypertensive (Suetsuna, 1998), hepatoprotective, and insecticidal (Wang *et al.*, 1998) agent in various human and animal therapies. The use of garlic extracts reduces serum cholesterol levels and increases blood coagulation time (Bordia *et al.*, 1975). These effects of garlic are due to the presence of various organosulphurous compounds, including allicin (Augusti & Mathew, 1974).

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Effects of lapsi fruits (*Choerospondias axillaris* Roxburgh, 1832) on immunity and survival of juvenile tilapia (*Oreochromis niloticus* Linnaeus, 1758) infected with *Aeromonas hydrophila*

Shyam Narayan Labh, Shubha Ratna Shakya, Sanjay Kumar Gupta, Neeraj Kumar and Babia Labh Kayastha

Abstract
Lapsi fruit (a native from Nepal) is opulent source of essential amino acids, minerals and ascorbic acid and is commonly used for the treatment of cardiovascular diseases in Vietnam, Mongolia and China etc. The phytochemical constituents of lapsi fruit extracts (LFE) are phenol and flavonoid compounds which exhibit potent antioxidant activity to scavenge various free radicals and thus protect from toxic and harmful. A total of 252 fingerlings of *O. niloticus* (average weight 3.84 ± 0.17 g) were randomly distributed in four treatment groups T1 (Basal feed + 0% LFE) control, T2 (Basal feed + 0.1% LFE), T3 (Basal feed + 0.2% LFE) and T4 (Basal feed + 0.4% LFE) each in triplicate form. After 60 days of feeding trail highest ($p<0.05$) weight gain% (273.03%), protein efficiency ratio (1.82) and specific growth rate (2.19) and lowest feed conversion ratio (1.43) in the T3 group fed fish were recorded. Lowest ($p<0.05$) alanine aminotransferase and aspartate aminotransferase activity was found in the T3 group in both liver and muscle. Incorporation of 0.2% LFE in the feed increased the gill superoxide dismutase activity by 3-fold compared to control group (T1). Highest ($p<0.05$) respiratory burst activity (0.36), albumin (1.21) and globulin (3.48) contents were observed in the T3 group. When challenged with *A. hydrophila* after 60 days, maximum relative % of survival was noticed in T3 (83.34%) group. Overall, results indicated improvement in the growth, heamato-immunological responses and protected the animals against *Aeromonas hydrophila* infection at 0.2% LFE incorporation in *O. niloticus* while higher dose of LFE incorporation led to stress and immunosuppression.

Keywords: Lapsi, growth, tilapia, immunity, *Aeromonas hydrophila*, survival

Introduction
Aquaculture plays a significant role in eliminating hunger, malnutrition and promoting the socioeconomic status of the poorest of the poor among most of the developing countries. [1] Due to increasing global population and awareness about health benefits, demand for food fish has made tremendous progress over the last two decades. To encounter the demand of fish as the cheapest source of animal protein, aquaculturists are forced to practice 'high input high output' intensive culture. Nevertheless, intensification of aquaculture increases stress level in fish which impairs growth and immune responses against pathogens and eventually leads to outbreak various diseases, resulting into enormous economic losses to the poor farmers. [2, 3] To achieve the sustainable development of aquaculture, control of infectious diseases and maintenance of health of cultured fish is the utmost essential concern. Immune-protection by dietary manipulation has emerged as an important area of research, which is an ideal and sustainable means for enhancing the non-specific immunity of fish. Substances such as chemical agents, herbal extracts, and nutritional factors, stimulate the non-specific defense mechanisms and thus are proved to be efficient in resisting infectious diseases in fish [4] caused by various pathogens.

Aeromonas hydrophila, the main bacterial pathogen of freshwater aquaculture, causes haemorrhagic septicaemia, dropsy, tropical ulceration and ultimately leads to heavy mortality in cultured fishes. [5] Although a large number of costlier chemical formulations such as antibiotics, drugs, pesticides, vaccines, and chemotherapeutics have been attempted to cope up with this situations, but these are not so effective from ecologically safe and environmentally

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REVIEW ARTICLE

Effect of Herbs and Herbal Products Feed Supplements on Growth in Fishes: A Review

Shubha Ratna Shakya
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Abstract

The herbs and herbal products added to the feed cure many diseases, promote growth, reduce stress, improve immunity and prevent infections in fish under culture. The addition of herbs and herbal products in fish diet is cheaper and environmental friendly with low side effect to the fish and consumers. Hence, their use as drugs in disease management in aquaculture is gaining popular. They are better than various antibiotics and vaccines used in the treatment of diseases. The present review highlights the importance of herbs and herbal products supplementation in fish feed for better fish production.

Keywords: Aquaculture, growth promoter, herb, fishes

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Introduction

Fish of commercial importance are farmed in captivity under controlled conditions to fulfill the demand of white meat for human consumption. In commercial fish farming, the production is maximized by increasing the weight of individual fish [1, 2]. An artificial feed used in the aquaculture improves fish growth with maximum weight in short time [3]. New substances are added in fish feed to improve feed conversion efficiency that result in fish growth [4]. Many studies show that inclusion of herbs in fish diet has positive effect on growth and disease free fishes.

Excess use of various antibiotics, hormones and other synthetic drugs to control diseases and improve fish growth in aquaculture is the reason behind the emergence of drug resistant bacteria and production of toxic substances harmful to the environment and human health [5] and suppress immunity in the host [6]. Thus, their use has been criticized all over the world [7]. The herbs being cheaper, eco-friendly with minimum side effects are used as alternative to antibiotics in fish health management. World Health Organization (WHO) encourages supplemented diets incorporated with medicinal herbs or plants which minimizes the use of chemicals in fish diet [8]. In this context herbs and herbal products can be used in fish diet to increase feed consumption in fish under culture [9]. Thus, this review is an informative collection in relation to fish growth through herbal feed supplements which may be useful for aqua farmers.

Bioactive compounds present in various plants are used in animal nutrition to stimulate feed intake, improve secretion of digestive enzyme and activate immune responses. These plants are also known to possess antibacterial, antiviral and antioxidant properties [10]. In aquaculture practices many herbs and herbal products are included in the fish diet to cure diseases, promote growth, reduce stress, stimulate appetite, boost immunity and prevent infections in producing healthy fishes [11- 15].

The flavor imparted by herbs and herbal products added in fish diet changed the eating patterns, increased feed consumption and stimulated digestion by increasing the secretion of saliva, various digestive enzymes, bile, pancreatic enzymes activity and mucus in fishes [16,17].

Some herbal feed supplements used in Aquaculture

Various herbs such as *Hygrophila spinosa, Withania somnifera, Zingiber officinalis, Solanum trilobatum, Andrographis paniculata, Psoralea corylifolia, Eclipta erecta, Ocimum sacnctum, Picrorhiza kurooa, Phyllanthus niruri* and *Tinospora cordifoliaare* used to reduce stress, increase immunity and control bacterial activities. *Penaeus monodon* in culture fed

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Medicinal importance of fruits of indigenous lapsi *Choerospondias axillaris* (Roxb.) in Nepal

Shyam Narayan Labh and Shubha Ratna Shakya

Abstract
Choerospondias axillaris (Roxb.), a large, deciduous and subtropical fruit tree has been recognised as a potential agroforestry tree for income generation for subsistence farmers in Nepal. The tree, locally called *Lapsi*, produces fruits with high vitamin C content, which are consumed fresh, pickled and processed for preparing varieties of sweet and sour, tasty food products that are marketed locally and have potentials for exporting. A total of 301 Village Development Committees in 29 hill districts have reported cultivation and protection of Lapsi trees for some socio-economic purpose. Lapsi was grown from east to west Nepal from 850 m asl to up to 1900 m. Distribution of Lapsi has been found in much wider areas in the country than reported earlier. Over 40,000 trees are at fruit bearing stage and more than 450,000 new trees are planted in various districts of Nepal.

Keywords: *Choerospondias axillaris*, lapsi, vegetative propagation, fruit trees, terai, domestication, agroforestry.

1. Introduction

1.1 Background of the country

Nepal is a landlocked country situated in the central part of the Himalayas between China to the north and India to the south, the east and the west. The country has been geographically divided into three ecological belts: Mountains, Hills and Terai. All three ecological belts extend lengthwise from east to west across the country. The climate varies from alpine cold semi-desert type in the trans-Himalayan zone to tropical humid type in the tropical lowlands in the south. It has an area of 147,181 square kilometers, average length of 885 kms east to west, and average width of 193 kms north to south. The country has an immense variety of topography, ranging from lowland plains in the south with elevation as low as 90 meters to the Himalayan mountain range in the north with elevation up to 8848 meters at the Mount Everest. The climate varies from alpine cold semi-desert type in the trans-Himalayan zone to tropical humid type in the tropical lowlands in the south. Nepalese economy heavily relies on agriculture and contributes 33 percent to Gross Domestic Product (GDP) that generates direct employment to 67 percent population and 76.3 percent agricultural *households*, the major source of livelihoods of the Nepalese people [1]. There are altogether 75 districts and 5 development regions: Eastern, Central, Western, Mid-Western and Far Western and about 36% of the total population resides in the Central Development Region, which covers 19 districts including Kathmandu valley [2].

2. Distribution of lapsi in Nepal

Lapsi, *Choerospondias axillaris* is indigenous fruit tree of Nepal found growing within 900-2000 m above sea level in many parts of the country [4]. It is grown in 301 village Development committees of 29 hill districts of Nepal for some socio-economic purpose [4]. Lapsi trees are commonly found in places like Pharping, Machhaya gaon (Kirtipur), Phulbari, Panchkhal, Namobuddha, Kavre, Panauti and Dhulikhel of Kavrepalanchowk district as well as in Jiri, Charikot of Dholka district and Chautara of Sindhupalchowk district. The tree has long been cultivated in rural Nepal for its fruit. The lapsi tree is widely used for private planting in hills, as part of community forestry program [5]. In the natural forest lapsi trees are sparsely distributed. Over 40,000 trees at fruit bearing stage and more than 450,000 new trees were planted in these districts of the country. There is a tremendous opportunity for income and employment generation through proper management and use of Lapsi tree in Nepal [4].

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Review article

Medicinal importance of *Choerospondias axillaris* (Roxb.) Burtt & Hill fruits in Nepal

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Abstract: *Choerospondias axillaris*, a large, deciduous and subtropical fruit tree has been recognized as a potential agroforestry tree for income generation for subsistence farmers in Nepal. The tree, locally called "lapsi", . produces fruits with high vitamin C content, which are consumed fresh, pickled and processed for preparing varieties of sweet and sour, tasty food products that are marketed locally and have potentials for exporting. A total of 301 Village Development Committees in 29 hill districts have reported cultivation and protection of Lapsi trees for some socio-economic purpose. Lapsi was grown from east to west Nepal from 850 m to 1900 masl. Distribution of Lapsi has been found in much wider areas in the country than reported earlier. Over 40,000 trees are at fruit bearing stage and more than 450,000 new trees are planted in various districts of Nepal.

Keywords: *Choerospondias axillaris* - Lapsi - Vegetative propagation - Fruit tree - Agro forestry.

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INTRODUCTION

Nepal is a landlocked country situated in the central part of the Himalayas between China to the north and India to the south, the east and the west. The country has been geographically divided into three ecological belts: Mountains, Hills and Terai. All three ecological belts extend lengthwise from east to west across the country. The climate varies from alpine cold semi-desert type in the trans-Himalayan zone to tropical humid type in the tropical lowlands in the south. It has an area of 147,181 Km^2, average length of 885 km east to west, and average width of 193 km north to south. The country has an immense variety of topography, ranging from lowland plains in the south with elevation as low as 90 meters to the Himalayan mountain range in the north with elevation up to 8848 meters at the Mount Everest. The climate varies from alpine cold semi-desert type in the trans-Himalayan zone to tropical humid type in the tropical lowlands in the south. Nepalese economy heavily relies on agriculture and contributes 33 percent to Gross Domestic Product (GDP) that generates direct employment to 67 percent population and 76.3 percent agricultural households, the major source of livelihoods of the Nepalese people (LSS 2010–2011). There are altogether 75 districts and 5 development regions: Eastern, Central, Western, Mid-Western and Far Western and about 36% of the total population resides in the Central Development Region, which covers 19 districts including Kathmandu valley (Shakya 2011).

DISTRIBUTION

Lapsi, *Choerospondias axillaris* (Roxb.) Burtt & Hill is indigenous fruit tree of Nepal found growing within 900–2000 m above sea level in many parts of the country (Poudel *et al.* 2001). It is grown in 301 village Development committees of 29 hill districts of Nepal for some socio-economic purpose (Poudel *et al.* 2001). Lapsi trees are commonly found in places like Pharping, Machhaya gaon (Kirtipur), Phulbari, Panchkhal, Namobuddha, Kavre, Panauti and Dhulikhel of Kavrepalanchowk district as well as in Jiri, Charikot of Dholkha district and Chautara of Sindupalchowk district (Fig. 1).

The tree has long been cultivated in rural Nepal for its fruit. The lapsi tree is widely used for private

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Effects of lapsi *Choerospondias axillaris* (Roxb.) on survival, growth and hepatic enzyme activities in *Cyprinus carpio* fingerlings

Shyam Narayan Labh and Shubha Ratna Shakya

Abstract
Altogether, two hundred seventy fingerlings of *C. carpio* (4.71±0.012g) were randomly distributed in six treated groups in triplicates form. Carp were fed with basal diet containing 40% protein supplemented with ethanol extract of lapsi fruit at 0, 0.1, 0.2, 0.4, 0.8 and 1.6 g kg -1 @ 3% of their body weight twice daily for 70 days. Cent per cent survival rate were observed in T3 and T4 diet fed group while the survival rate was 91.11% in T1 control diet fed group. For growth profile final weight gain, final length gain, specific growth rate and feed conversion ratio were measured. Similarly, proximate analyses of all the treated diets were assayed with SGOT, SGPT and ALP. There was significant ($p<0.05$) differences in weight gain, length gain and specific growth rate in treated diet fed group to that of control diet fed group. Carp fed with T4 diet (0.4%) showed higher length gain, weight gain and SGR as compared to others while higher decreasing trend were observed in feed conversion ratio (FCR) of T4 diet. Growth rate was 87.89% higher in T4 diet fed group while it was only 53.68% in control diet fed group. Significant decreasing trend were observed in SGOT, SGPT and ALP in all the treated diet fed group but the lowest decreasing trend were in T4 diet fed group. It can be concluded that a minimum amount of 0.4 g lapsi fruit extracts kg^{-1} is sufficient to be added in diet for good serum enzymes levels and growth performances of common carp.

Keywords: Carp growth, proximate analysis, survival, SGOT, SGPT and ALP

1. Introduction
Aquaculture is probably the fastest growing food-producing sector, now accounts for nearly 50 percent of the world's food fish. Aquaculture in Nepal is basically small but contributes 3% to the agricultural GDP. River is the major source of capture fishery covering 3, 95, 000 ha. of the surface of natural water resources. Around 75,000 people are engaged in aquaculture with net fish production of 64,900 Mt. (culture fisheries 43,400 Mt. and capture fisheries 21,500 Mt.) in the year 2014 [1] against 57,500 tonnes in fiscal 2012-13. The present annual fish production in Nepal is 69,500 Mt [2]. The country's fish production has not been able to meet local demand despite a rapid growth in fish farming however; around 80 percent of the domestic requirement of fish is fulfilled by local production while the rest is met by imports. In Nepal, many fishermen, their families and others are engaged in capture fisheries, which represent nearly 0.28% of the total population of Nepal. Lapsi *Choerospondias axillaris* (Roxb.) of family Anacardiaceae is a large, dioecious and deciduous fruit tree. The tree is native to Nepal [3] found growing in hills between 850-1900 m above the sea level and has also been reported from various countries like India, China, Thailand, Japan, Vietnam and Mongolia [4]. The fruits are rich in vitamin C content [5] and are used as a medicinal plant to enhance the immune system of the body [6]. The lapsi fruits contain phenolic and flavonoid compounds [7, 8]. The secondary products of medicinal plants and many edible plants contain phenolic compounds [9] which serve as antioxidants. So there are potential benefits of consuming phenolic rich foods [10]. Thus, keeping these things in mind an experiment was carried out in the wet laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur to understand the antioxidant activities of lapsi fruits on carp growth.

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Effects of dietary lapsi, *Choerospondias axillaris* (Roxburgh, 1832) fruit extract on haematological parameters in *Cyprinus Carpio* (Linnaeus, 1758) fingerlings

Shyam Narayan Labh and Shubha Ratna Shakya

Abstract
An indoor experiment was conducted to study the effects of Lapsi *Choerospondias axillaris* on some haematological parameters of common carp *Cyprinus carpio* fingerlings.
Two hundred seventy fingerlings (average weight 4.71±0.012g) were randomly distributed in six treatment groups in triplicates of control (T1), 0.1 g kg^{-1} (T2), 0.2 g kg^{-1} (T3), 0.4 g kg^{-1} (T4), 0.8 g kg^{-1} (T5) and 1.6 g kg^{-1} (T6) supplemented with ethanol extract of lapsi fruits in the diet containing 40% protein. Fingerlings were fed at 3% of body weight twice daily. Significant differences ($P<0.05$) were observed in haematological parameters of treated diets fed groups to that of control diet fed group. Haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), White blood cells (WBC) and other blood indices were observed to be significantly higher in the treated groups as compared to the control. It was concluded that a minimum amount 0.4% (0.4g kg^{-1}) of lapsi fruit extracts in fish feeds elicited more increase in haematological parameters of common carp. Inclusion of lapsi fruit extract at 0.4% concentration is therefore beneficial for use in aquaculture to enhance immunity in common carp.

Keywords: Aquaculture, haemoglobin, packed cell volume, ethanol extract

1. Introduction
Common carp, *Cyprinus carpio*, is one of the most important fish species in aquaculture [1]. Common carp is an economically significant fish species cultivated mainly in Asia and Europe. Global production of cultivated common carp was about 6.14% of the global aquaculture production [2]. It is a warm water freshwater fish species that is native to Asia. It is cultivated commercially [3] in other parts of the world, including Nepal, because of its fast growth rate, facile cultivation and high feed efficiency ratio [4]. China is by far the widest commercial manufacturer of common carp, which reports nearly 70% of the country's freshwater fish production [5]. In the last two decades the annual production of common carp raised exponentially and obtained more than 3 million tons in 2010 [6]. Currently, it represents 14% of the total world freshwater aquaculture production and is mostly cultivated in Asian countries, especially in China which accounts for 70% of the total global production [6].
Lapsi (*Choerospondias axillaris)* is one of the known medicinal plants rich in vitamin C content [7] and used as a medicinal plant to enhance the immune system of the body [8]. The constituents of lapsi fruits have been investigated chemically and shown to include phenolic compounds and flavonoid content [9]. The ability of phenolic compounds to serve as antioxidants has been recognized, leading to speculation about the potential benefits of ingesting phenolic rich foods [10]. It is assumed that the antioxidant activity of fruits of lapsi *Choerospondias axillaris* (Roxb.) may enhance the blood parameters of carp by improving immunity in the body so that carp can survive in adverse conditions and have capacity to fight against the diseases.
The haematological parameters are used as health indicators in aquatic medicine following different stress conditions [11, 12]. Haematological parameters are therefore ready tools used by fish biologists and researchers in many parts of the world. The knowledge of the haematological characteristics can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes [13]. These parameters are also closely related to the response of the animal to the environment, an indication that the environment where

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Fruits of lapsi *Choerospondias axillaris* enhances ascorbic acid level in brain and liver of common carp (*Cyprinus carpio* L) during intensive aquaculture

Shubha Ratna Shakya and Shyam Narayan Labh

Abstract
Teleost fish lack the enzyme for endogenous synthesis of ascorbic acid (AA), an essential micronutrient for fish and fruits of lapsi are rich in vitamin C. Thus, the aim of this study was to examine the effect of higher levels of dietary vitamin C on growth and protein levels in the brain and liver of common carp, *Cyprinus carpio* through lapsi fruits supplemented in the diets. Six groups of *C. carpio* were fed with experimental diets containing lapsi fruits supplemented at 0 mg kg^{-1} (T1), 100 mg kg^{-1} (T2), 200 mg kg^{-1} (T3), 400 mg kg^{-1} (T4), 800 mg kg^{-1} (T5) and 1600 mg kg^{-1} (T6) for 70 days. Growth parameters (WG, SGR and FCR) and concentrations of vitamin C in brain and liver were estimated. Fish fed with lapsi fruits supplemented diet showed higher weight gain and specific growth rate (SGR) up to 400 mg kg^{-1} compared with control fish. Concentrations of vitamin C was found higher in liver of T4 diet fed group as compared to brain. In both tissues (brain and liver) the lowest vitamin C concentrations were always in control T4 diet fed carp. Results from this study help to establish the beneficial effect of lapsi fruits on growth and immunmodulation in *C. carpio*.

Keywords: Growth, vitamin C, brain, liver, lapsi, *Choerospondias axillaris*, carp

1. Introduction
Vitamins are the important essential nutrients for most animal species. Vitamin deficiencies in fish under aquaculture are known to produce biochemical dysfunction, leading to tissue and cellular level clinical manifestations. Several morphological and functional abnormalities have been reported in various fish species deprived of vitamins. Vitamin C is synthesized in animals from either D-glucose or D-galactose as part of the glucuronic acid pathway [1]. Branching from L-gulonic acid, the biosynthetic pathway of vitamin C comprises three consecutive steps: first, the enzymatic lactonization of L-gulonic acid catalyzed by L-gulonolactone hydrolase [2]; second, the oxidation of L-gulonolactone catalyzed by L-gulonolactone oxidase (GLO); and third, the spontaneous isomerization of 2- keto-L-gulonolactone leading to vitamin C [3]. The general view is that the animals lacking GLO are not able to synthesize vitamin C and thus depend upon a dietary source of the vitamin [4]. Among the fishes analyzed to date, only those retaining numerous ancestral characters, such as lamprey, shark, ray, lungfish and sturgeon [5-10] have been shown to have GLO in the kidney, whereas teleost fish lack GLO activity [11].
Lapsi *Choerospondias axillaris* (Roxb.) [12] of family Anacardiaceae is a large, dioecious and deciduous fruit tree found growing in hills between 850-1900 m above the sea level in Nepal and has also been reported from various countries like India, China, Hong Kong, Thailand, Japan, Vietnam, Thailand, and Mongolia [13]. The fruits are rich in vitamin C content [14] and are used as a medicinal plant to enhance the immune system of the body [15]. Phenol and flavonoid compounds [16, 17] present in the fruit of lapsi serve as antioxidants. So there are potential benefits of consuming phenolic rich foods [18]. Thus, keeping these things in mind an experiment was conducted to understand the effects of lapsi fruits supplemented diets on carp growth and the concentrations of vitamin C (ascorbic acid) in some tissues (brain and liver) of common carp *C. carpio* fingerlings.

2. Materials and Methods
2.1 Preparation of ethanol extract of lapsi fruits
The ethanol extract of lapsi fruits was prepared as described by Labh *et al.*, [17] with slight modifications.

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Effects of dietary lapsi (*Choerospondias axillaris* Roxb.) on survival, growth and protein profile of common carp (*Cyprinus carpio* L) fingerlings

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Abstract
This study was conducted to evaluate the effects of dietary lapsi *Choerospondias axillaris* (Roxb.) on survival, growth and protein profile of *Cyprinus carpio* (L) fingerlings. Six practical diets were formulated to contain 0.0 (control), 100, 200, 400, 800, and 1600 mg ethanol extract of lapsi fruits equivalent kg ($^{-1}$) diet. Each diet was fed to triplicate groups of fingerlings of *C. carpio* (4.71± 0.012 g) in 100-L glass aquaria as T1, T2, T3, T4, T5 and T6 @ 3% of their body weight twice daily for 70 days. The results showed statistically significant (P<0.05) increased in weight gain, SGR, total protein and globulin which may be considered as a sign of improvement in immune system. It may be due to presence of antioxidant properties (vitamin C) in lapsi fruit extract which act as an antioxidant. Finally, it has been concluded that a mininum amount of 0.4 g lapsi fruit extracts kg^{-1} is sufficient to be added in diet for increment of good growth and serum protein in common carp.

Keywords: Carp, protein, growth, SGR, lapsi, *Choerospondias axillaris*

1. Introduction
Aquaculture is the fastest-growing food-production sector in the world, now providing almost half of the global fish supply. Increases in demand for fish indicate that aquaculture needs to expand, particularly in Asia. To meet this growing demand, World Fish uses its technical and scientific expertise in fisheries and aquaculture to promote evidence-based development solutions and increase aquaculture productivity, while minimizing impacts on the environment by developing technologies, improving resource management, securing access to essential inputs and improving connections to markets. Development of sustainable fish feeds represents a key component of future program. Aquaculture in Nepal is basically small and new that contributes 3% to the agricultural GDP. Rivers are the major source of capture fishery covering 395000 ha. of the surface natural water resources. Around 75000 people are engaged in aquaculture with net fish production of 64,900 Mt. (culture fisheries 43,400 Mt. and capture fisheries 21,500 Mt.) in the year 2014 [1] against 57,500 Mt. in fiscal 2012-13. The present annual fish production in Nepal is 69,500 Mt [2]. The country's fish production has not been able to meet local demand despite a rapid growth in fish farming however; around 80 percent of the domestic requirement of fish is fulfilled by local production while the rest is met by imports. In Nepal, many fishermen, their families and others are engaged in capture fisheries, which represent nearly 0.28% of the total population of Nepal.

Lapsi *Choerospondias axillaris* (Roxb.) [3] of family Anacardiaceae is a large, dioecious and deciduous fruit tree found growing in hills between 850-1900 m above the sea level in Nepal and has also been reported from various countries like India, China, Hong Kong, Thailand, Japan, Vietnam, Thailand, and Mongolia [4]. The fruits are rich in vitamin C content [5] and are used as a medicinal plant to enhance the immune system of the body [6]. Phenol and flavonoid compounds [7, 8] present in the fruit of lapsi serve as antioxidants. So there are potential benefits of consuming phenolic rich foods [9]. Thus, keeping these things in mind an experiment was carried out in the wet laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur (Nepal) to study the effect of lapsi extract supplemented diets on survival, growth and protein profile of common carp *Cyprinus carpio* fingerlings.

2. Materials and Methods
2.1 Preparation of lapsi fruits supplemented artificial diets
The crude extract of the pulp of lapsi fruits was prepared by using ethanol (70%) as described by Labh *et al*., [8].10 g of lapsi fruit powder was taken in conical flask and added 500 ml of 70 % ethanol. The flask was sealed by cotton plug and aluminum foil and then kept in orbital shaker for 48 hrs. The mixture was then filtered using Whatman filter paper No.1 and filtrate was centrifuged at 10,000 rpm for 5 minutes to collect the supernatant. The supernatant was concentrated at 70 °C using the water bath. Finally, a greasy substance (crude extract) of the lapsi fruit was obtained which was transferred to screw-cap bottle labeled and stored at 4° C until use. Altogether six treated diets T1, T2, T3, T4, T5 and T6 were prepared in which T1 was treated as control while rest of the diets were supplemented with 100, 200, 400, 800 and 1600 mg kg $^{-1}$ lapsi fruit extracts. Other standard ingredients were used during feed preparation (Table1).

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Medicinal uses of ginger (*Zingiber officinale* Roscoe) improves growth and enhances immunity in aquaculture

Shubha Ratna Shakya

Abstract
The medicinal plants are of great use in pharmaceutical, cosmetic, agricultural and food industry. The efficacy of some herbal products is beyond doubt, the most recent examples being *Silybum marianum* (Linn.) Gaertn (Silymarin), *Artemisia annua* Linn. (Artemesinin) and *Taxus baccata* Linn.(taxol). Randomized, controlled trials have proved the efficacy of some established remedies, for instance *Zingiber officinale* Roscoe, commonly known as ginger. Ginger contains natural organic materials beneficial to health and enhances resistance to infectious diseases by increasing non-specific and specific immune mechanisms. The rhizome of ginger has shown to be effective in the control of a range of bacterial, viral, fungal and parasitic diseases in humans, poultry and aquaculture owing to its antimicrobial, antioxidant, growth promoter and as immunostimulant properties to health. Hence, this review focuses on the use of ginger as growth promoter, antimicrobial agent, and antioxidant and as immunostimulant in aquaculture.

Keywords: Ginger, *Zingiber officinale*, gingerols, antioxidant, aquaculture.

1. Introduction
The world trend to improve food security and to use natural products will drive the chemically synthesized antibiotics and growth promoters out of use. Aquaculture is therefore an emerging industrial sector which requires continued research with scientific technical development and innovations (Ibrahem *et al.*, 2010) [24]. Extensive use of antibiotics in aquaculture leads to the emergence of antibiotic-resistant bacteria and generation of toxicants, which may cause risks to the environment (Esiobu *et al.*, 2002) [13], and immunosuppression in the host (Panigrahi & Azad, 2007) [38]. There are a large number of feed additives available to improve fish growth performance. Some of these additives used in feed mill are chemical products, especially hormones and antibiotics, which may cause unfavorable side effects. To alleviate these problems, increasing attention is being given to the use of natural alternative feed additives such as ginger for disease-control strategies in aquaculture. Ginger enhances resistance to infectious disease by increasing non-specific and specific immune mechanisms (Harikrishnan *et al.*, 2011) [21]. Ginger contains natural organic materials that facilitate growth, anti-stress, environmentally friendly and antimicrobial properties in fish (Maqsood *et al.*, 2011) [33].
Ginger as a natural antibiotic is the earliest known medicinal plant. It has shown to be effective in treating diseases in humans, poultry and aquaculture owing to its antimicrobial, antioxidant, growth promoter and immunostimulant properties. An optimized dose of ginger is recommended in the diet. Ginger (*Zingiber officinale* (L.) Roscoe) has been used as a spice for over 2000 years (Bartley & Jacobs, 2000) [6]. It is also called "*The Great Medicament*" in Ayurvedic medicines (Tan & Vanitha, 2004) [44] and is generally considered as a safe herbal medicine (Weidner and Sigwart, 2000) [46].
Ginger (*Zingiber officinale* Roscoe) is a creeping perennial underground rhizome belonging to family Zingiberaceae (Sharma,*et al.*, 2010) [41]. Nepal is the third biggest producer of ginger in the world (FAO, 2012). In the first year, a green, erect reed like stem about 60 cm high grows from this rhizome. The plant has narrow; lanceolate to linear-lanceolate, 15-30 cm long leaves which die of each year. The odour and taste are characteristic, aromatic and pungent. Ginger valued as a spice has been used through ages in almost all systems of medicine against many maladies. The plant is indigenous to Southeast Asia and is cultivated in a number of countries including Nepal. The smell and taste of the drug are typical and aromatic. The medicinal part

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Extract of Medicinal lapsi *Choerospondias axillaris* (Roxb.) exhibit antioxidant activities during *in vitro* studies

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Abstract
Lapsi *Choerospondias axillaris* (Roxb.) fruit has a soft whitish sour flesh and green to yellow skin used to make pickles, fruit tarts and spicy candy in Nepal. The present study evaluated the antioxidant activity of the fruit of lapsi Choerospondias *axillaris* (Roxb.) *In vitro* using two different solvents: aqueous and ethanol. The antioxidant properties of the lapsi fruit extracts was quantified by the DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) and the results showed that lapsi fruit possess a strong antioxidant power with higher percentage of inhibition of DPPH radical recorded in ethanolic extracts (98%), followed by ascorbic acid (95%) and aqueous extract (91%). The results clearly showed that lapsi fruits contain exploitable and potent antioxidant molecules, and could be promoted as a prospective dietary supplements and or Nutraceutical for both human and animal use.

Keywords: DPPH, Choerospondias axillaris, phenolic, antioxidant and lapsi fruit.

Introduction

Lapsi, *Choerospondias axillaris* (Roxb.) is a popular fruit tree of Nepal and many other Asian countries [1]. This deciduous tree can grow up to 20 meters tall and has smaller purple-brown branches. Lapsi fruit is about 3 centimeters long with green-yellow skin, and is incredibly sour if eaten raw. Inside the fruit is a large seed that is segmented so that it looks like a star [2]. To prepare the fruit, the skin is peeled, sometimes the seeds are also removed, and the fruit is mixed with salt, spices, or sugar to make various aachars. Lapsi *achar* can be intensely sweet, salty, sugary or spicy as depending on how it is prepared. The lapsi fruit is rich in Vitamin C [3] and known for its antibacterial, antimicrobial and antioxidant properties [4], [5].

Fruits are important sources of minerals, fibers and vitamins, which provide essential nutrients for the human health. Increased consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies [3]. The wild underutilized edible fruits can also play an important role as food supplement. Fruits offer protection against free radicals that damage lipids, proteins, and nucleic acids. Polyphenols, carotenoids (provitamin A), vitamins C and E present in fruits have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases [6]. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, singlet and triplet oxygen, or decomposing peroxides [7].

Antioxidants are chemicals that block the activity of other chemicals known as free radicals [8]. Free radicals are highly reactive and have the potential to cause damage to cells [9] that may lead to cancer. Free radicals are formed naturally in the body, and antioxidants interact with and neutralize them when overproduced, thus preventing them from causing damage [10]. These antioxidants are called endogenous antioxidants enzyme. However, under stress or pathological condition the endogenous antioxidant may not be sufficient to quench the damaging effect of free radicals, therefore the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs [11]. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants [12]. Some dietary antioxidants are also available [13] as dietary supplements.

In recent times natural antioxidants are gaining considerable interest among nutritionists, food manufacturers, and consumers because of their perceived safety, potential therapeutic value, and long shelf life. Plant foods are known to protect against degenerative diseases and ageing due to their antioxidant activity (AOA) attributed to their high polyphenolic content (PC) [14]. Epidemiological studies have shown that high consumption of fruits and vegetables is associated with a lower incidence of cancer, heart disease, inflammation, arthritis, immune

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Medicinal uses of garlic (*Allium sativum*) improves fish health and acts as an immunostimulant in aquaculture

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Abstract
Aquaculture is a source of livelihood for many economically under privileged people in the least developed countries including Nepal. In recent years, with intensification and rapid development of aquaculture enterprises, occurrence of infectious diseases has cause huge economic losses. The main disease causative agents are bacteria, virus and parasites. The excess use of antibiotics and various synthetic chemicals have resulted in drug residue and resistant pathogens in treated fish. Drug residue pollutes the environment and threatens humans consuming them. Antibiotics that accumulate in the environment and fish pose a potential threat to consumers and to the environment. Increased public awareness of the negative effects caused by overexposure to synthetic chemicals has led to search for ''green drugs'' such as organic and synthetic chemical-free food products. In this regard, garlic (*Allium sativum*) as a natural antibiotic is the earliest known medicinal plant, has shown to be effective for the treatment of many diseases in humans and animals owing to its antimicrobial, antioxidant, anti-cancer, and antihypertensive properties. In aquacultural operations, optimized dose of garlic is strongly recommended. Hence, this review focuses on the application of garlic in on growth performance, flesh quality, antimicrobial activity, as an immunostimulant and antiprotozoal agent in aquaculture.

Keywords: Garlic extract, aquaculture, growth, resistance, challenge, fish.

1. Introduction
Garlic, *Allium sativum* L. has been used for centuries in many societies against parasitic, fungal, bacterial and viral infections [1]. Garlic has been proven effective as a hypolipidemic [2], antimicrobial [3], antihypertensive [4], hepatoprotective, and insecticidal [5] agent in various human and animal therapies. The use of garlic extracts reduces serum cholesterol levels [6] and increases blood coagulation time [7]. In aquacultural operations, garlic promotes growth, enhances immunity, stimulates appetite, and strengthens the control of bacterial and fungal pathogens. Many reports have documented that garlic can effectively eliminate principal pathogenic bacteria such as *Pseudomonas fluorescens, Myxococcus piscicola, Vibrio anguillarum, Edwardsiella tarda, Aeromonas punctata, Fibrobacter intestinalis* and *Yersinia ruckeri* in freshwater fish. Garlic improves flesh quality in fishes. These effects of garlic are due to the presence of various organosulphur compounds, including allicin [8].
Garlic extracts and most commercial garlic food supplements in the form of tablets and capsules containing garlic powder are based on either the allicin content or the potential to produce allicin [9]. The content of allicin and other sulfurous chemicals in garlic varies significantly and depends on several factors. For medicinal applications, higher levels of allicin are favorable [10]. The utilization of garlic in aquaculture has developed alongside the application and popularization of Chinese herbs in aquaculture. Most aquatic garlic researches have involved fresh garlic extracts, with experimental subjects either fed a garlic-added feed or treated with a garlic juice immersion. Allicin is the most powerful component present in garlic that actively and directly kills parasites [11]. Freshly pressed garlic, liquid garlic products are made for aquarium use, and even most food items containing garlic can be effective. Some people consider garlic to be an immune system booster and compare it to Vitamin C in humans [12].

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Application of immunostimulants as an alternative to vaccines for health management in aquaculture

Shyam Narayan Labh and Shubha Ratna Shakya

Abstract
Aquaculture, one of the fastest growing food producing sectors, is gaining momentum in several parts of the world. Diseases in fish constitute one of the most important problems and challenges for aquaculturists. Hence, aquaculturists undertake good management practices to ensure the production of healthy fish. Intensification has become a common practice in both finfish and shellfish culture to optimize the returns. High stocking densities, artificial feeding and fertilization have become common husbandry practices in carp culture systems. Due to intensification of culture practices, diseases in rearing of fishes have become major threat to the sustainability of aquaculture industry. Use of synthetic chemicals and antibiotics has been partially successful in preventing or treating fish diseases. Vaccination is an important tool in preventing infectious disease in humans and animals and both passive and active vaccinations are extensively employed in fish. A vaccine targets the specific immune response. It requires primary challenge with antigen and is dependent upon the clonally derived lymphocytes subsets to be implemented. Vaccines against some specific pathogens have been developed recently against some particular diseases. Thus, an alternative approach to boost or stimulate the innate immune system of farmed fish is an application of immunostimulants. Immunostimulants are considered as attractive and promising agents for prevention of diseases in fish and shellfish. In recent years, the proven beneficial effects of immunostimulants in many living systems promote their application for disease management in aquaculture practices.

Keywords: Immunostimulants; Immune system; Aquaculture, Vaccine, fish

1. Introduction
Aquaculture has been growing rapidly for food production in the last few decades. Several commercial fish species have been cultured intensively in narrow or enclosed spaces such as ponds, cages or tanks under overcrowding or high density conditions, thereby causing adverse effect on their health with a potentially stressful environment and infectious diseases [1]. The outbreaks of infectious disease in cultured fish have emerged as constraints for the development of aquaculture. These occurrences have spread through the uncontrolled movement of live aquatic animals resulting in the transfer of pathogenic organisms among countries [2]. Antibiotics and chemotherapeutics have been used to prevent or control bacterial infections in aquaculture for about 20 years [3]. Unfortunately, antibiotics treatment is not successful and sustainable due to increase antibiotic-resistant in bacteria, negative effects on the indigenous microflora of juveniles or adult fish [4], accumulation of antibiotic residues in fish tissue and environment causing human and animal health issues. Vaccination is an effective prophylactic treatment for infectious diseases in fish culture, but it may be very expensive and stressful to the fishes. A single vaccine is effective against only one specific type of pathogen, but limits the effectiveness for wide range of pathogens due to the complex antigenic structure [5]. Therefore, eco-friendly disease-preventive alternative techniques have to be taken into account. One such promising alternative technique to strengthen fish immune systems is the application of immunostimulants in aquaculture [1].

1.1 Concept of Immunostimulant
Immunostimulants, also known as immunostimulators, are substances comprising of drugs and nutrients that activate the immune system by increasing activity of any of its components.