1. INTRODUCTION

Nepal has the large network of snow fed rivers, lakes and torrential mountain streams which are fresh water resources. Hydrosphere of Nepal comprises about 7,26,380 hectares (Shrestha,1995). The aquatic environment of Nepal can be broadly divided into two headings: (a) lotic or running water such as river and streams, and (b) lentic or standing water such as lakes and swamps. Nepal falls in between the major Zoogeographic regions: Palearctic in the north and Oriental in the south. Basically, fish fauna of the highland river system is Palearctic while that of the mid-land is strictly Indo-Chinese (sub region of Oriental) and the fish fauna in the water mass of lowland is that of Indian (sub region of Oriental) sub region. Nepal's food fishes are carp, catfish, feather backs, minnows, eels, etc.

Agave americana is a large, rhizomatous succulent that grows in a wide range of conditions and rocky slopes. *A. americana* is tolerant of wind, salt, high temperatures, and extreme drought. It can grow in shallow, very dry and low fertility soil. It is grown for many reasons- ornamental, medicinal and agricultural. In South Australia *Agave americana* mainly invades disturbed sites, road sides and coastal vegetation. It may also harbour introduced animal species, such as rabbits, making feral animal control more difficult.

As originally described by Gentry (1970), Family Agavaceae consists of 18 genera and a little over 400 species, many of them native to western a North America. One of the most familiar species is *Agave americana*, a native of tropical American. Common names includes century plant, Maguey (in Mexico), or American Aloe (it is not, however, closely related to the genus Aloe). This plant is locally known as 'Ketuke' in Nepal. The name "Century Plant" refers to the long time the plant takes to flower, although the number of years before flowering occurs depend on the vigor of individual, the richness of the soil and the climate; during these years the plant is storing in its fleshy leaves the nourishment required for the effort of flowering. Agaves can cause severe dermatitis (Shrestha, 1991). The juice of the more virulent agaves has been used as fish poison and arrow poison (Dimmitt,2000). *A. americana* is used in Mexico, Brazil, India and China as

a traditional treatment, as it has anti-inflammatory, anti-bacterial and anti-fungal properties and can be used as a diuretic (Boscolo *et al*. 2010; Jin *et al.* 2004; Parmar *et al*. 1992; Peana *et al.* 1997; Rivera *et al*. 2010).*A. americana* appears in the FDA Poisonous Plant Database (McGuffin *et al.* 2000). *Agave americana* has several uses: ornamental, medicinal, as a vertebrate poison, agricultural, fodder, erosion control (USDA-ARS, 2010). *A. americana* is grown as an ornamental on all continents, except Antarctica (Nobel,1990). The plant dies after fruiting (Badano & Pugnaire 2004).

Great attempts have been made to understand the general toxicology. Due to alarming increase in population special emphasis is being given to the histopathological evidences of damaged tissue or changes occurring in animals exposed to toxic chemicals. Such studies have also offered opportunity to locate the effects of toxic chemicals on various organs and organ system of animals, reproductive system being one of them. The present paper records the effect of *Agave americana* on stomach and intestine of the fish (*Clarias batrachus*).

In mid 70's toxicologist took considerable interest to study the consequences of insecticides on the blood of fishes. Blood is the primary target of pesticidal action and since circulatory system is intimately related to the respiratory system, any change in one will affect the other. The change in fish blood exposed to varying degrees of environmental stressors/pesticides have been recorded in the publication of Mishra and Srivastava (1984), Kumar and Banerjee (1993) and others.

The fresh water fish *Clarias batrachus* (Linnaeus) of the family claridae locally known as Mangur is one of the stronger fish having accessory respiratory organs. Description of the specimen fish: *Clarias batrachus*

Local name	:	“Mungri” or “Mangur”
Classification	:	Leo. S. Berg (1947)
Phylum	:	Chordata
Division	:	Gnathostomata
Super Class	:	Pisces
Class	:	Osteicthyes

Sub Class	:	Actinopterygii
Order	:	Osterophyss
Sub Order	:	Siluroidea
Family	:	Claridae
Genus	:	*Clarias*
Species	:	*batrachus*

Clarias batrachus is omnivorous (Mills,1993) species that feed on a varied mixture of dried pellets, meaty frozen foods, vegetable matter and almost anything offered. It is highly predatory. It is tolerant of a very wide range of water chemistry and temperature.

It can also survive in oxygen-depleted conditions due to its ability to breathe atmospheric air. Walking catfish thrive in stagnant, frequently hypoxic waters, and are often found in muddy ponds, canals, ditches and similar habitats. The species spends most of its time on, or right above, the bottom surface, with occasional trips to the surface to gulp air

The maximum length of *Clarias batrachus* may be 47.0 cm and the weight 1,190 gm. They are potamodromous and lives in freshwater; brackish water; at the depth of 1 to several meters of range. They are found in tropical places at 29 °N- 7°S at the temperature of 10°C-28°C.

The walking catfish, *(Clarias batrachus)*, is a species of freshwater air breathing catfish found primarily in Southeast Asia, so named for its ability to "walk" across dry land, to find food or suitable environments.

This fish normally lives in slow-moving and often stagnant waters in ponds, swamps, streams and rivers and, flooded rice paddies or temporary pools which may dry up. This catfish has long-based dorsal and anal fins as well as several pairs of sensory barbells. The skin is scale less but covered with mucus, which protects the fish when it is out of water.

This fish needs to be handled carefully when fishing it out due to its hidden embedded sting or thorn-like defensive mechanism hidden behind its fins (including the middle ones before the tail fin, just like the majority of all catfish).

Histology – the study of microanatomy of specific tissues, have been employed as diagnostic tool within medical and veterinary science. The first cellular investigation was carried out in the mid 19th century. Since then considerable development have taken place in all aspect of cellular biology with the result that today many novel and sophisticated histological techniques only devised for the mammalian histology now available to the fish histopathology.

Histology of fish plays an important role in accurate diagnosis of diseases of fish as the state of fish health cannot be ascertained by the external appearance of the fish alone. Histopathology is one of the useful tools along with microbiology, virology, and other branches of science in disease diagnosis.

Histology encompasses the scientific area concerned with structure of tissues and histopathology. The relevant branch of histopathological and histochemical analysis can therefore provide information on the process and changes occurring in tissues and many cases form the basis for disease diagnosis and prognosis.

The stomach of *Clarias batrachus* includes cardiac, fundus and pyloric region. The mucosa of cardiac and fundus are formed by a single layer of columnar epithelium with folds. The columnar epithelial cell contains a layer of cells with pepsinogen granules. The stomach of *Clarias batrachus* has thicker muscular wall and is lined by columnar epithelium raised into several primary and secondary folds. Numerous gastric glands of simple tubular type are present below the epithelium and open into the lumen of the stomach. A thin muscularis mucosa present below the gastric glands. The sub mucosa lamina proparia are highly vascular. The muscularis consists of an inner layer of circular muscle fibres and outer thin layer of longitudinal muscle fibres. The external covering is serosa.

The intestine has thin wall and its mucous membrane is thrown into prominent folds forming villi. In the proximal wider part of the intestine, the villi are numerous and are fused with each other. Histologically, the proximal and distal parts of the intestine do not differ from each other. The mucosa is composed of columnar epithelium, consisting of absorptive and mucus secreting cells. The submucosa is vascular and extends into the villi as lamina proparia. The muscularis is formed of inner circular and outer longitudinal muscle fibres. The serosa has blood capillaries in it.

1.1 OBJECTIVES OF THE STUDY

The objectives of the study are:

General objective

- Effect of *Agave americana* on the stomach and intestine of *Clarias batrachus.*

Specific Objectives

- Histopathological and histological study of stomach and intestine of *Clarias batrachus.*
- Effect of *Agave americana* on behaviour of *Clarias batrachus.*

1.2 STUDY AREA

Use of toxic herbs to kill fishes is common in Nepal. Generally toxic herbs are used to collect fishes from rivers. But the *Clarias batrachus* was purchased from fish market. Experiment was carried out in the Central Department of Zoology. *Agave americana* was procured locally.

The research work was carried out from the date July 2011 to September 2011 under the supervision of Dr. Archana Prasad (Thesis supervisor), Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal. The Kathmandu valley is situated from 85º 33' east to 85º 20' west and 27º 42' north to27º 7' south. Daily observation was kept under notice with the help of record book.

2. LITERATURE REVIEW

Studies on the fish started in 16th century. During those days only taxonomy and morphology were studied. Later on due to development of new scientific techniques and equipments different works were done in different fields. Scientists have given exhaustive historical review on different group of fish. In 18th and 19th century much progress was done on study of histomorphology and feeding habit of fishes. Curier (1800-1828) and Gunter (1880) contributed a various aspects of fishes. In 20th century detailed studies have been made on alimentary canal and its associated structures.

Several workers have worked on the effect of organophosphorus pesticides on different organs of *Clarias batrachus*. Use of the fish poison is very old practice in the history of human kind. In 1212 A.D. King FrederickII prohibited the use of certain plant pesticides and by the 15th century similar laws had been described in European country as well.

One specialized form of fishing which had advantages over many other forms (under proper conditions) was the use of poisons, a practice still in use today. In northwest Guyana for example, up to 16% of the village fishers still prefer to fish with poisons despite the superiority of modern netting materials (Van Andel,2000).

Fish-poisons (also known as piscicide or ichthyotoxins) were very commonly use throughout American history (Béarez,1997; Van Andel,2000) and are particularly interesting because they are used for an area effect rather than against an individual target. A multitude of plant species are known to possess chemicals toxic to fish, and evidence suggests that certain plant species have different effects depending on which variety of fish are targeted (Van Andel,2000). A general rule is that fish-poisons are only effective on relatively small fish. Two main molecular groups of fish poisons in plants, the rotenone and the saponins, as well as a third group of plants which liberate cyanide in the water, account for nearly all varieties of fish poisons (Béarez,1997) although plants with sufficient levels of ichthyocthereol, triterpene and other ichthyotoxins are also used. The rotenone and saponins are used in such small doses that they are harmful to fish, but not to humans who eat them. Fish killed with triterpenes need to be cleaned and gutted immediately to avoid human consumption of this toxin (Van Andel,2000).

While all three categories of fish-poisons are found in a diversity of plants, and although their effect on different species varies, they are each used in a similar manner. The active ingredient is released by mashing the appropriate plant parts, which are then introduced to the water environment. Poisoning was generally done in stagnant pools or slow-flowing streams and rivers, but has also been used by Californian Indians in saltwater environments for octopus and low-tide shellfish fishing (Heizer,1953), as well as for catching fish trapped in inter-tidal pools (Béarez,1998).

Saponin: The most common use of fishing poisons documented is plants containing Saponin, a glucosidal poison. This chemical is usually active in the stem or wood and is diversely distributed among several plant families (*Amaryllidaceae, Convolvulaceae, Dioscoreaceae, Lamiaceae, Lecythidaceae, Liliaceae, Papilionaceae, Sapindaceae, Scrophulariaceae, Solanaceae, Verbenaceae,* and others).Plants containing Saponin are also commonly used as soap substitutes because they can often be worked into a lather. Likely, Saponin plants were primarily used for washing or cleaning and secondarily used as a poison after their effect on fish in washing-streams was discovered. Saponin normally breaks down in the digestive system and must enter the bloodstream to be toxic (Elpel,2000), but fish assimilate Saponin directly into their bloodstream via their gills. Fish poisoned by Saponin become stupefied and float to the surface where they can easily be collected.

Rotenones: The second group of fish poisons, the rotenone (a flavonoid), are found almost exclusively among legumes (*Papilionaceae, Mimosaceae, Cesalpiniaceae*), and more specifically in the family *Fabaceae*. Rotenone was first isolated in 1929 in the roots of its Peruvian namesake, the plant Rotenone (*Lonchocarpus* sp., locally known as *barbasco* or *cube*). Two species of this genus, *L. utilis* and *L. urucu*, quickly became an export product as an insecticide due to their relatively high (5-12%) rotenone content. Two related species from Guyana, *L. martynii* and *L. chrysophyllus* contain only 2.4% rotenone and are not considered commercially competitive (Van Andel,2000). When rotenone is introduced to the water by crushing or mashing the appropriate plant parts (usually the roots) fish respiration is damaged and they are forced to gulp air at the water surface where they are vulnerable.

Triterpenes and other poisons: Northern Guyanan natives effectively use two plants containing triterpene, *Euphorbia cotinifolia* L. and *Phyllanthus brasiliensis* (Aubl.) prior to poison fish. A large basketful of the leaves and stem of these plants will poison small fish in a stream (Van Andel,2000). *E. cotinifolia* may be the most toxic of all fish-poisons; this plant's latex causes blistering if it contacts human skin and blindness if it comes in contact with the eyes. Fish killed using triterpene must be immediately gutted and cleaned to prevent human consumption of this toxin.

There are many additional plant species with ichthyotoxic properties that are less frequently used, and subsequently less studied in the literature. Some other fish-poison plants in Guyana that have been mentioned (Fanshawe,1948,1953; Killip and Smith,1935) include *Mora excels, Bauhinia* spp.,*Alexa imperatricis, Clathrotropis brachypetala, Gustavia augusta, Macrolobium acaciifolium, Paullinia pinnata, Pentaclethra macroloba,* and *Ryania pyrifera*.

Many plants from different families have been applied for catching fish all over the world. Examples of these plants are the genera *Derris, Tephrosia and Lonchocarpus* of the family Leguminosae. The toxic parts of plants employed as fish poisons include roots, seeds, fruits, bark, latex or leaves. Some plants have been reported to have molluscicidal action hence; they may have high piscidal action. The study assessed the piscicidal activity of *Agave* on stomach and intestine of *Clarias batrachus.*

Great attempts have been made to study the general toxicity. Duodoroff *et al*.,(1953) studied the effect of acute toxicity of some organic insecticides to fishes. Ludemann Newmann (1960) reported the acute toxicity of some insecticides to a common carp (Cyprinus *carpio).*

According to Chiayvareesajja *et al*. (1997), air breathing species are more tolerant to piscicidal materials and therefore piscicidal activity should be tested in such species.

Plants are virtually inexhaustible source of structurally diverse biologically active substance (Istvan,2000). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscidal properties. Unlike synthetic chemical pesticides which leave harmful residues in the aquatic environment (Koesomadinata,1980; Cagauan and Arce,1992), botanical insecticides are believed to be more environments friendly

because they are easily biodegraded and leave no residues in the environment. Since some of these piscicidal compounds present in plants were also toxic to fishes, botanical pesticides can be used as piscicide.

The juice from many species of *Agave* can cause acute contact dermatitis. In may recur up to a year, thereafter, even though there no longer a visible rash. Irritation in part caused by calcium oxalate raphides (Dimmitt,2000). Dimmitt (2000) states the beneficial uses and toxicological property of family Agavaceae in the article "A natural history of the Sonoran Desert". He explains the edible and medicinal uses of *Agave.* Similarly "*Agave* Plant Health Benefit", a research paper by Sachelian (2008) explains the phytochemical analysis and anti allergic study of *Agave.*

The book ''Neurobehavioral Toxicity in fish" by Edward and Sondra (2010) focused on specific target organ or physiological system and describes how various agents disrupt the normal physiological system and processes. Two cases report about hallucinatory fish poisoning (Luc and Philip,2006) are published in ''Clinical Toxicology''. Daniel and William (2010), provides coverage of toxic effect in the central nervous, immune, neurobehavioural and reproductive system as well as describing general mechanism of toxicity in ''Target Organ Toxicity in Marine and Freshwater Teleosts Systems''. Duetsch (1988-2006), documented a concise overview of the contact poisonous plants in his research work ''Contact poisonous plants of the world''. The first part of the paper briefly introduces the active principles, effects, treatment and geographical distribution. The second part list about 35 important plant species and describes them in detail. Fafioye (2005) documented some Nigerian piscicidal plants with known active ingredients with the view of ensuring their further development and conservation in the research paper ''Plants with Piscicidal Activities in Southwestern Nigeria''. Ambedkar and Munian (2009) studied the piscicidal activity of Methanolic extreact of *Capparis stylosa* on the freshwater fish *Channa punctatus.* A list of poisonous plants is created by Triplett (1997) and Begley (2000) under the title of ''Poisonous plants can be a hazard to pets, including fish''.

Zaman and Sinha (1999) studied the toxic effect of cythion on freshwater air breathing teleost *Clarias batrachus*.Some of the herbal compounds have the toxic effect on liver of catfish (Kothari, Bhalerao and Sharma,1999).

Attempts has been study the histopathological changes induced by non lethal exposure of phosphamidon recovery in *Labeo rohita* fingerlings, Medda *et al.* (1992).

Singh (2007) extends the use notion for herbal fish stupefying plants. Bhattrai, Karki and Mandal (2008), reported the medicinal value of many plants including *Euphorbia royleana* in the ‘‘Medico-ethanobotanical study’’. The book ‘‘Fish Catching in the Himalayan Waters of Nepal’’ by Shrestha (1995, 2008) also reports about the herbal fish poison.

Seeds of the Indian fish berry *Anamirta cocculus* (Linn.) are a potential piscicidal agent used for catching fish from the wild by native people. Similarly, ‘‘Evaluation of the acute toxicity of the seeds of *A. cocculus* (Linn.) and its piscicidal effect on three species of freshwater fishes viz; *Clarias batrachus* (Linn.), *Channa striatus* (Bloch.) and *Mystus vittatus* (Bloch.)” done by Jothivel N. and Paul (2008).

The juice from many species of *Agave* can cause acute contact dermatitis. In human it may produce reddening and blistering lasting 1 to 2 weeks. Dimmit (2000) states the beneficial uses and toxicological property of family Agavaceae in the article “A Natural History of the Sonoran Desert”. He explains the edible and medicinal uses of the *Agave.* Similarly, “*Agave* Plant Health Benefit” a research paper by Sachelian (2008) explains the phytochemical analysis and anti-allergic study of *Agave.*

Pesticidal activity and phytochemical analysis of effective herbs of Nepal has been observed by Pandey (2010). Similarly Bhattrai (2010) observed the effect of ammonia on the stomach and intestine of *Clarias batrachus.*

Due to alarming increase in population special emphasis is being given to the histopathological evidences of damaged tissue or changes occurring in animals exposed to toxic chemicals. Such studies have been made to study the effect of water parameters on the stomach and intestine of fishes (Saxena and Garg,1978).

3. MATERIALS AND METHODS

3.1 Test plant

Locally available plant species *Agave americana* was used**.** Leaves were main part used. Fresh plant material was weighed using digital balance**.** The collected sample was cut into pieces and dried in aerated shade and stored at room temperature. The dried samples were powdered by blender. The fine powders were kept for experiment.

3.2 Experimental set up

Rectangular glass aquariums were used. The numbers of aquariums were five. Each aquarium was filled with 30l tap water.

3.3 Test fish species

Fish species *Clarias batrachus* were purchased from Kalimati Kathmandu, having the average weight of fish 250±5gm and 30 cm in length in the month of early July, 2011. The test species were acclimatized for fifteen days. Fishes were stocked in different aquarium for the addition of the plant extract. The water of the aquarium was changed every day after 24 hour at morning before feeding.

To observe the histological effect of *Agave americana* on the stomach and intestine of *Clarias batrachus*, the fishes were kept in three different aquarium to calculate LC_{50} the fishes were subjected to different doses and its 5o% mortality was observed. In the present study sub- lethal concentration i.e. 3gm/30 l was taken. Its effect on stomach and intestine was observed under light microscopy. The fishes were sacrificed after 24 hours, 48 hours, and 72 hours of treatment. Each aquarium was filled with 30 liters water with 3.0 gm, 4.5 gm and 6 gm of *Agave americana* on each. All the fish were fed with protein diet i.e. were liver of chicken and herbal extract i.e. powder of *Agave americana* weighing 3 gm was mixed well with the help of glass rod in the aquarium. The experiment was conducted for 19 days. The fish were sacrificed after treating them for 24

hours, 48 hours, 72 hours and 96 hours. Before scarifying the fishes, they were kept unfed for 24 hours.

The sample tissue i.e. stomach and intestine was collected from each hour treated fish and preserved in fixative to undergo the further observation. Meanwhile, investigation in their behaviour pattern of the remaining fishes were also observed and written in a record book to get the best output of the study.

3.4 Lethal concentration

Lethal concentration (LC_{50}) of the test plant was determined by plotting concentration of the plant against fish mortality within 24 hours after exposure to treatment. Interpolations between two concentrations where the mortality occurred less than and greater than 50% was done. LC_{50} or median lethal concentration is the concentration at which 50% of the test fish survived and 50% died. It is the basis of most toxicity and tolerance test. The LC_{50} was recorded as 0.15 gm/l. In the present investigation sub- lethal dose i.e. 0.1gm/l was taken.

3.5 METHODS FOR HISTOLOGICAL STUDY

3.5.1 *Fixation and microtomy*

Before undergoing fixation and microtomy objects to be studied under microscope needs certain preparation like fixative block preparation, sectioning, staining and mounting etc.
FIXATIVE: A fixative is a chemical which kills tissue with a minimum changes in volume, structure and chemical makeup. It hardens the tissue with post mortem changes in shape, size and also renders some substances insoluble. Bouin's fluid (alcoholic), the fixative was prepared by taking, following chemicals together in a clean dry tube.

(Saturated) Picric acid (1.4% aq.)	75ml
Commercial formalin	25ml
Glacial Acetic acid	05ml

After 24 hours of fixation in the above fixative, the tissue i.e. stomach and intestine was washed under running water for next 24 hours. Then, tissue were dehydrated in 50% alcohol (3 changes at one hour interval each) followed by 70% alcohol. Thereafter, tissues were further dehydrated (90% and absolute alcohol) and cleared in Xylene and finally embedded in paraffin wax (M.P. 58-60°C). The block ready for sectioning was finally adjusted on the microtome machine and the tissues were sectioned at the thickness of 7µ in transverse section.

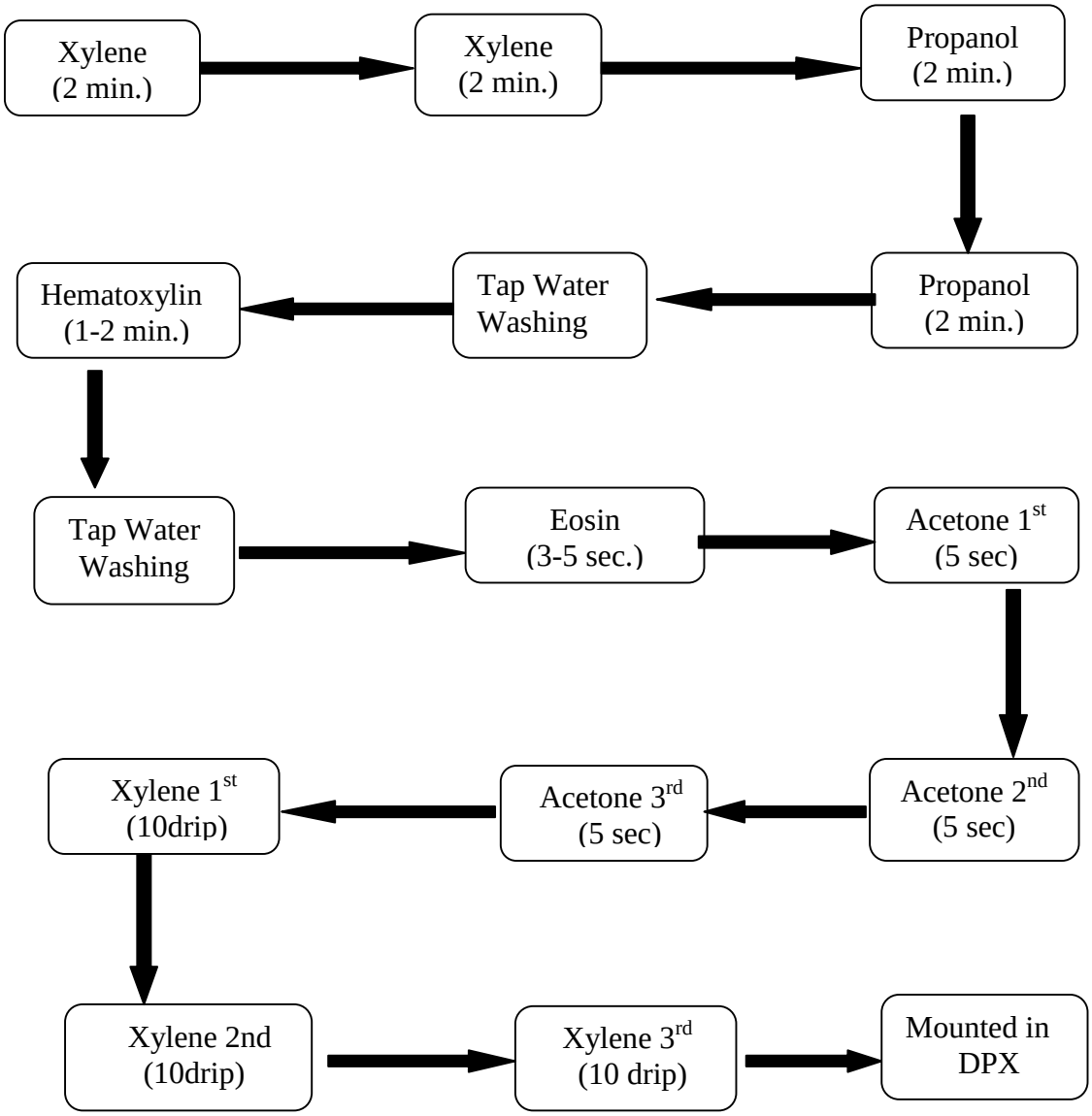
Staining: Sections of tissue are usually stained with two dyes to bring contrast between different histological structures. This makes detailed study easier. Staining with two dyes is known as double staining. The most common double staining practiced in a class work is with haematoxylin and eosin.

The general staining paired with haematoxylin and eosin

Stain	Common Use	Nucleus	Cytoplasm	Red-Blood Cell (RBC)	Collagen Fibers	Specifically Stains
Haematoxylin	General staining when paired with eosin	Blue	-	-	-	Nucleic acids—blue ER—blue
Eosin	General staining when paired with eosin	-	Pink	Orange /Red	Pink	Elastic Fibers—pink Reticular fibers—pink

3.5.2 Staining Procedure

The slide with the paraffin sections of tissue were kept in Xylene for about 5 minutes. The paraffin dissolves. The slides were then transferred to absolute alcohol and then followed the double staining procedure as shown in the flow chart below.



3.5.3 Permanent Slide Preparation

Haematoxylin is in aqueous medium. Presuming eosin 70% alcohol the procedure to be followed is given below.

Time in each grade of alcohol is about two minutes with two changes in absolute alcohol. Time required for staining and differentiation is determined by trial. A small amount of DPX is put on the slide, depending upon the size of the cover slip to be used. The amount should be just enough to form a very thin film on the slide. The cover slip is put on it. If the air bubbles are locked between the cover slip and the slide, it may be removed by leaving the slide overnight on the hot plate.

The technique of micrograph was taken with a video camera connected to microscope in CDZ. Microtomy was carried at Central Veterinary Office (Pasu Chikitsa Nirdesnalaya) Tripureshwor.

4. RESULTS

4.1 Behavioural Changes

With the administration of the fine powder of *Agave americana*, fish species exhibited violent swimming activities. They often come to surface and exhibited increased gulping activity, indicating their respiratory distress and tried many times to jump out of the aquarium. Later on fish became lethargic and remained hung beneath the surface of water. They had also decreased their appetites and had become sluggish. None of the controlled fish showed any behavioural changes. The dead fish were exhibited vertical positioning with head above the water surface which was seen in 96 hours treatment. Finally they lost balance, settled at the bottom of aquarium and died. The fish was considered dead if it did not respond to prodding by a glass rod. No mortality was observed in controlled groups.

4.2 STOMACH

4.2.1 Stomach under control condition

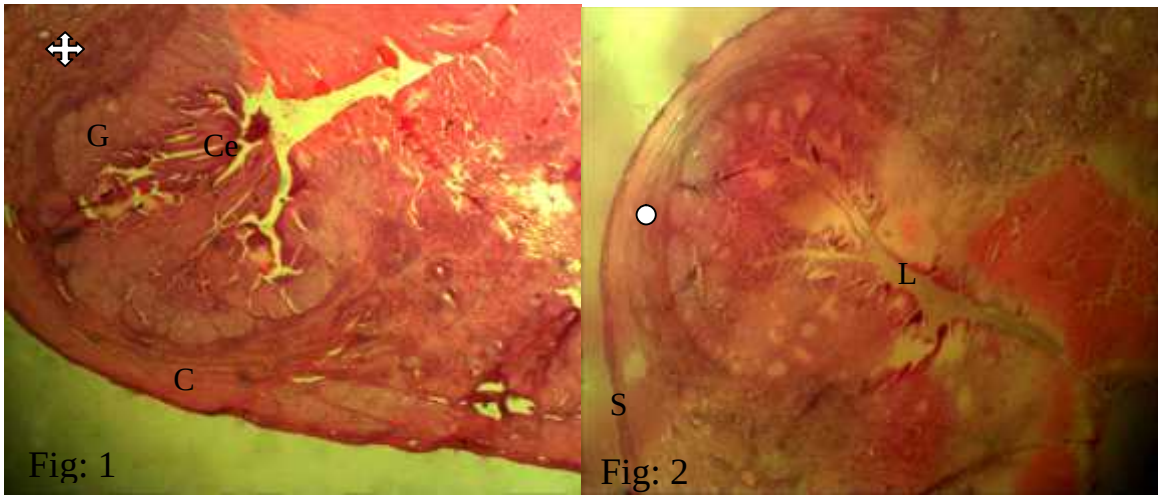


Fig 1& 2: Stomach under control condition with Serosa layer (S), Blood capillaries (C), Muscularis layer (○), Sub mucosa (✣), Gastric glands (G), Columnar epithelium (Ce), Muscularis mucosa (Mm) and Lumen (L).

Control condition of stomach shows well recognized forms of different layers; serosa is the outermost layer which consists of blood capillaries. The muscularis consists of an inner layer of circular muscle fibres and an outer layer of longitudinal muscle fibres. The submucosa is well vascularized. Gastric glands are present below the columnar epithelium (Ce) and open into lumen of stomach. A thin muscularis mucosa is present below the gastric gland. The wall of the stomach is lined by columnar epithelium raised into several primary and secondary folds.

4.2.2 Stomach treated for 24 hours

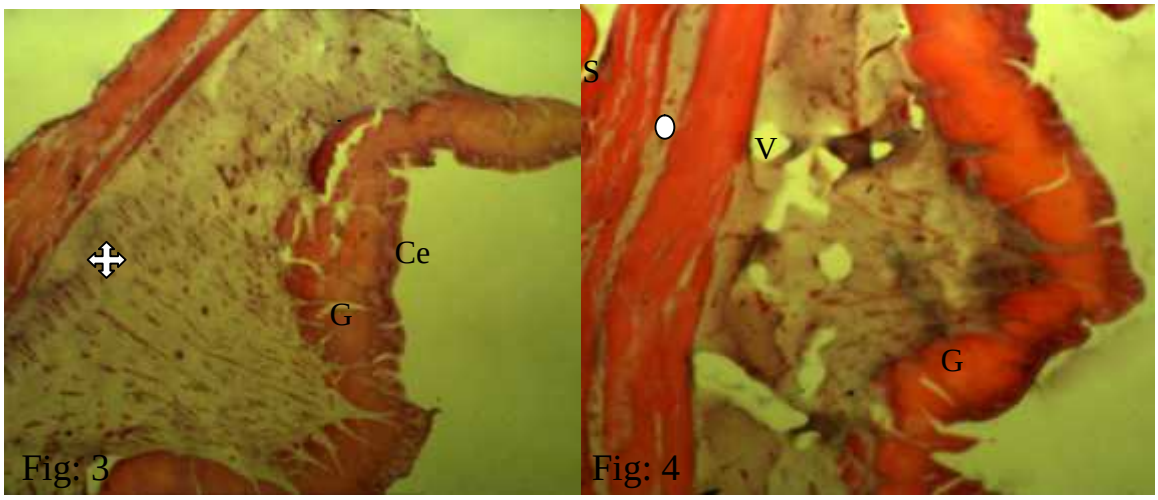


Fig 3& 4: Stomach treated for 24 hours with Serosa (S), Muscular layer (○) Sub mucosa, Gland cells (G), Vacuoles (V) and Columnar cells (Ce).

Fig: 3 & 4 showing the section of stomach treated for 24 hours; Serosa layer is detached from muscular layer and lining of the columnar cells are not continuous, Gastric gland started to enlarge. Vacuoles are also seen in the sub mucosa layer.

4.2.3 Stomach treated for 48hours

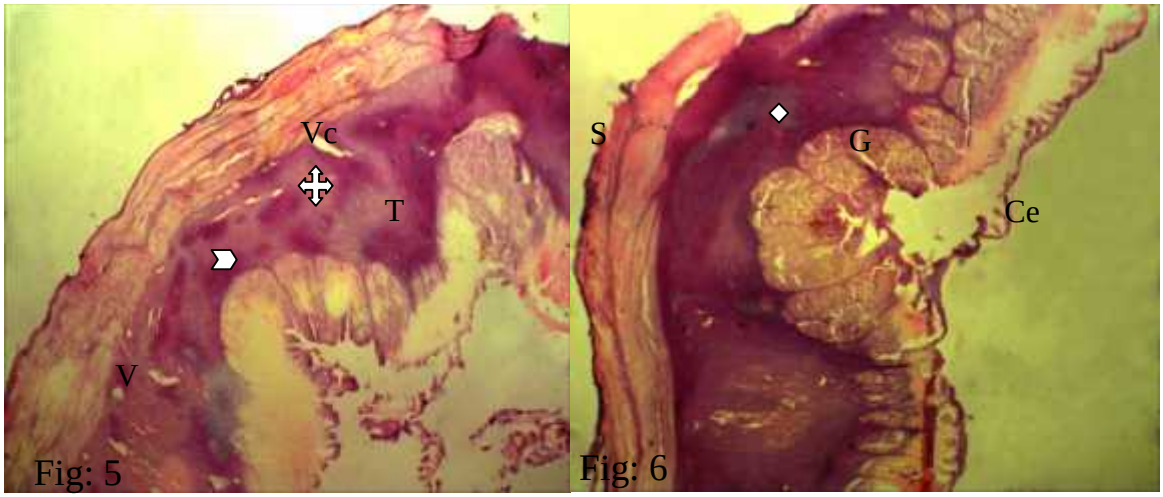


Fig: 5 and Fig: 6 with Serosa layer (S), Sub mucosa layer (✣), Leucocytes (◇), Gastric glands (G), Vacuoles (V) and vesicular cleft (Vc), Columnar cells (Ce), Deposition of toxicity (T), Sinus formation (⊃).

Stomach after the 48 hours exposure to *Agave americana* became shrunken. Serosa layer ruptured. Columnar epithelial cells become necrosed and accumulation of the toxicity within tissue spaces. The damage of epithelium of mucous fold and atrophy of mucosa may be associated with the hyper secretion of mucosa leading to erosion. Besides these, enlargement of glands, sinus formation and Vacuoles and Vesicular cleft can be seen in the sub mucosa layer.

4.2.4 Stomach treated for 72hours

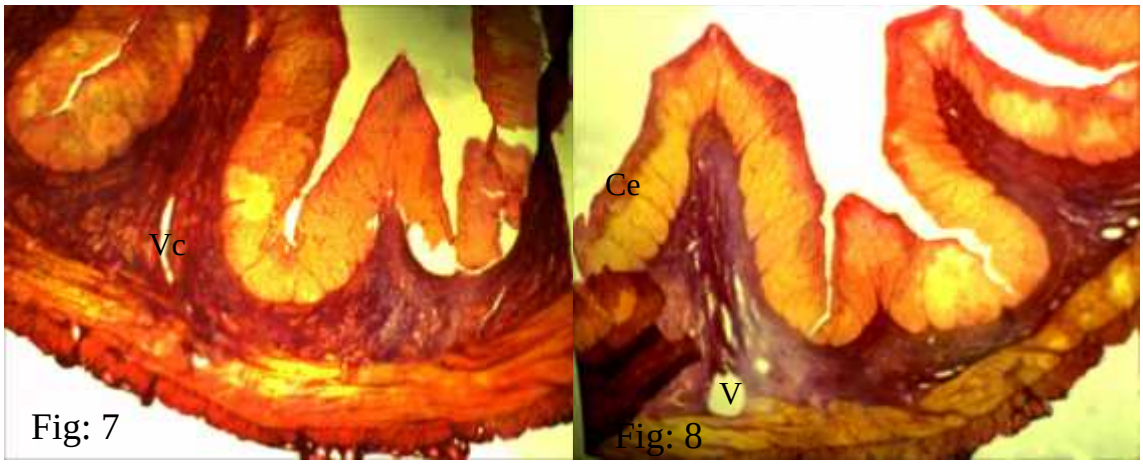


Fig: 7 and Fig: 8 Stomach treated for 72 hours showing Epithelial lining (Ce), Mucosa layer (M), Vacuoles (V) and Vesicular cleft (Vc).

Stomach of the *Agave americana* exposed fish after 72hour revealed following changes; shrinkage in the mucosa layer, the epithelium of the mucosal fold was almost damaged and epithelium lining is not continuous. Vacuoles and vesicular cleft are clearly visible. Serosa layer is not clearly visible.

In 96hours all the treated fishes were died due to effect of *Agave americana* on histology of fish.

4.3 INTESTINE

4.3.1 Intestine under controlled condition

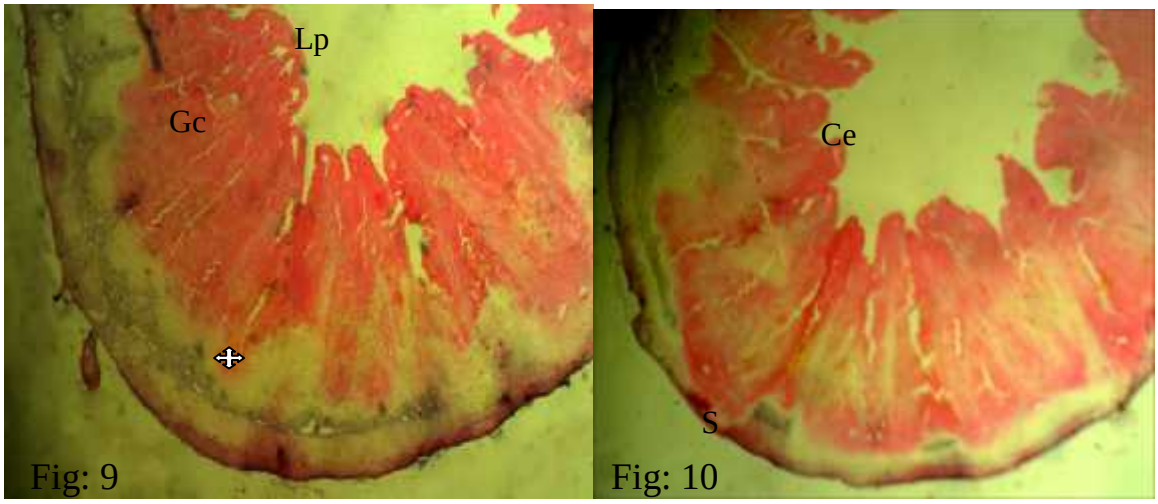


Fig 9 &10: Transverse section of intestine under control condition showing Serosa (S), Sub mucosa (✣), Lamina propria (Lp), Columnar cells (Ce) and Goblet cells (Gc).

The intestine at control condition with the different layer is clearly defined. The outermost layer, serosa possesses the blood capillaries. The submucosa is vascular and extends into villi as lamina propria. The intestine had numerous elongated and deep folds lined by simple tall columnar cells and goblet cells.

4.3.2 *Intestine treated for 24 hours*

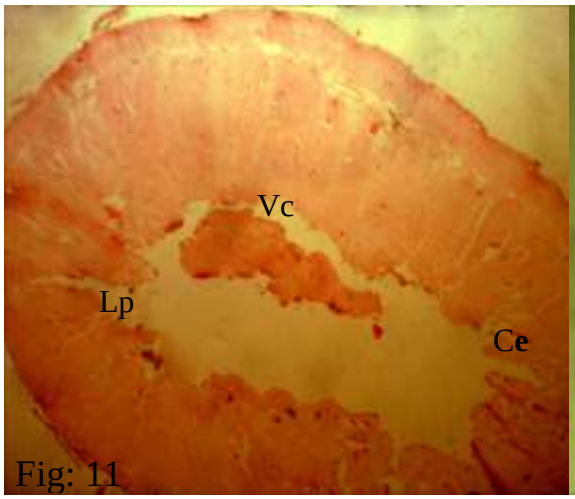


Fig: 11

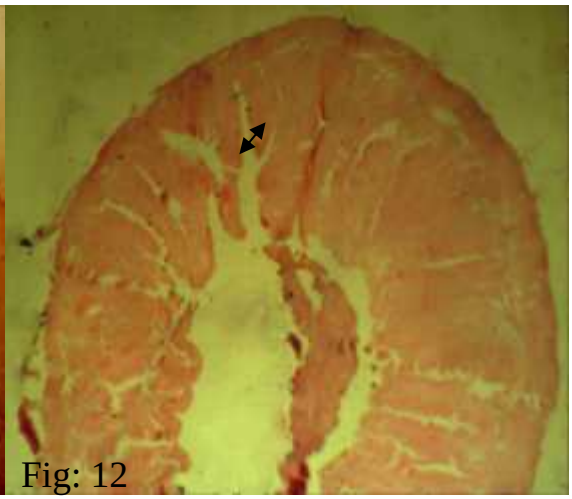


Fig: 12

Fig 11 & 12: Transverse section of intestine treated 24 hours showing Vesicular cleft (Vc) and Mucosa layer (↔).

After the treatment of *Agave americana* at the 24hours on the intestine of *Clarias batrachus*, showing the dissociation of mucosa layer. The vesicular cleft dividing the mucosa layer, lamina propria become reduced with leaving spaces indicates edema. Numerous blood vessels are also clearly seen.

4.3.3 Intestine treated for 48 hours

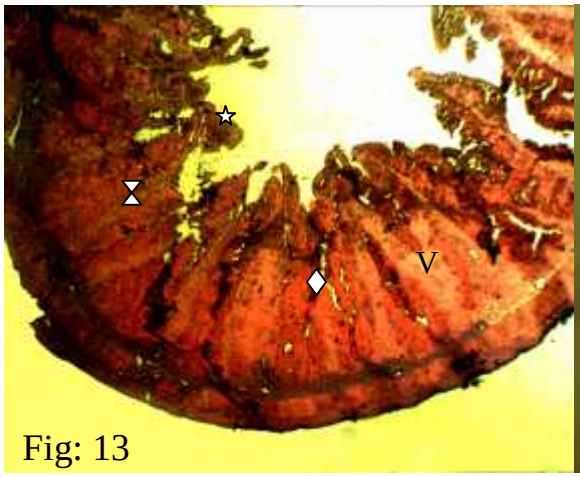


Fig: 13

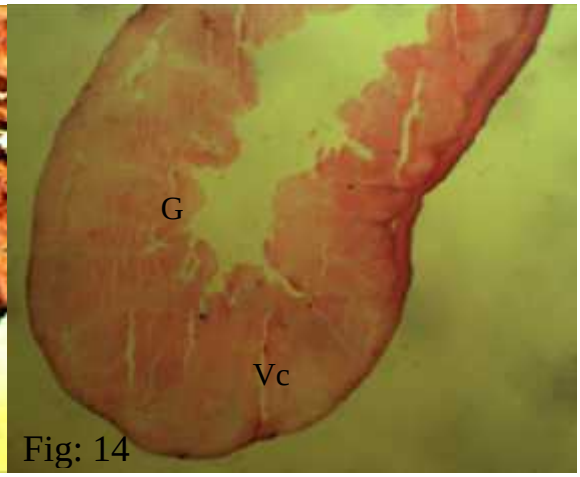


Fig: 14

Fig 13& 14: Intestine treated for 48 hours showing Serosa (S), Leucocytes (◊), Small vacuoles (V), Vesicular cleft (Vc), Villi (☆), Necrosis (⧖) and Glands (G).

Fig 13&14 showing the section of treated intestine of 48 hr., Rupture of the serosa layer. Leucocytes are prominent. Small vacuoles and vesicular cleft can be seen in the mucosa layer, villi get disorganized. Necrosis of intestine is another change during the 48hr. of treatment of *Agave americana*. Glands seem to be enlarged (more secretion of juices).

4.3.4 Intestine treated for 72 hours

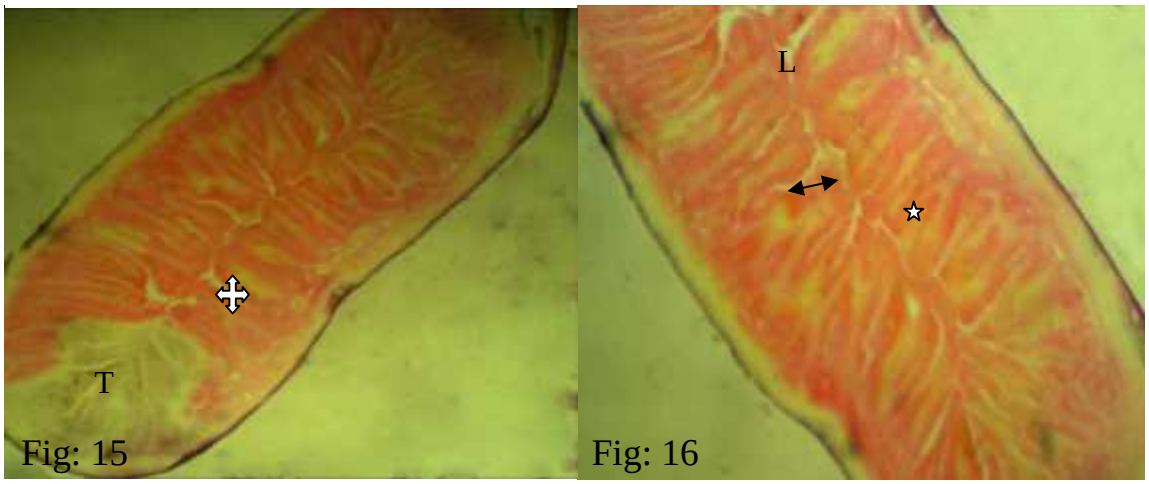


Fig: 15 Fig: 16

Fig 15 & 16: Intestine treated for 72 hours with Mucosa layer (↔), Villi (☆), Toxicity (T), Sub mucosa layer (✣) and Lumen (L).

After the 72hours of treatment, changes occurring in tissues are; mucous layer become proliferated, cloudy appearance is seen in between villi ,indicating the accumulation of toxicity, villi became greatly reduced, flattened, their tips were found ruptured at places leading to exudation of mucus in lumen. Hemorrhage and congestion in the sub mucosal layer is clearly seen. It seems to be the structure of mitochondria due to proliferation of cell.

In 96hours all the treated fishes were died due to effect of *Agave americana* on histology of fish.

5. DISCUSSION

With the administration of the fine powder of *Agave americana*, fish species exhibited violent swimming activities with indicating their respiratory distress and tried many times to jump out of the aquarium. Later on fish became lethargic and remained hung beneath the surface of water. They had also decreased their appetites and had become sluggish. Similar activities were also found by Jothivel and Paul (2008) after the evaluation of acute toxicity of the seeds of *Anamirta cocculus* and its piscicidal effect on three species of freshwater fish. Similarly, Jagadeesan and Vijayalakshmi (1999) reported absence of locomotor activity, increased opercular movements following four days treatment (96 hours) suggested their ability to withstand the toxic environment on studying alteration in the behaviour patterns in *Labeo rohita* fingerlings induced by mercury.

Stomach at control condition shows gastric glands which are present below the columnar epithelium and same result was found by Raji, and Norouzi, (2010) while studying in histological and histochemical study on the alimentary canal in Walking cat fish and Piranha.

In the present investigation stomach became shrunken, columnar epithelial cells become necrosed and accumulation of the toxicity within tissue spaces. The damage of epithelium of mucous fold and atrophy of mucosa may be associated with the hyper secretion of mucosa leading to erosion, the epithelium of the mucosal fold was almost damaged and epithelium lining is not continuous which is observed during the 48 and 72 hours of exposure to *Agave americana*. This result is matching with the investigation found in the book of Pandey (2004).

Hemorrhage and congestion in the sub mucosal layer were noted in the intestine of *Clarias batrachus* after the 72hours of treatment of *Agave americana*. Similar observations have also been made by Sri Lestari (1990) while studying on walking Catfish, *Clarias batrachus*.

The disorganization of villi at 48hours of treatment, reduction in their number, flattened and inflamed at the base was found after the 72 hours of treatment. Lamina propria

became reduced with leaving spaces indicating edema is clearly seen in the 24hour of treatment. Similar result was also found in the book of Pandey (2004) while studying on toxicological impact of house hold detergent, surf, on digestive tissue of *Clarias batrachus.*

Tembhre and Kumar (1994) investigated vacuolation of columnar epithelial cells, extremely reduced villi and exudation of mucus in lumen in the intestine of dimethoate treated fish *Cyprinus carpio* and similar result have also been found by Anithakumari and Kumar (1997) in *Channa punctatus* and influence of aquatic pollutants. But in our present investigation vacuolation is seen in mucosa layer of intestine.

Kaldate (2012) reported the villi are erupted at certain region of intestine which leads to intrusion of muscularis layer where the sub mucous membrane is totally shrinked, dilation of blood vessels, degeneration of intestinal folds, vacuolation of sub mucosal cells, degeneration of various layer of intestine.

Presence of small vacuoles and vesicular cleft in the mucosa layer of intestine of present investigation is similar with the result of Bhattarai (2010). Very few Vesicular cleft was seen as reported by Kuehnel (2003).

There are great differences in the histology of stomach and intestine among the different fish species, the wall of tract (stomach and intestine) of *Clarias batrachus*, as also occurs in other fish, is composed of the four layers described for vertebrates (Kumar and Tembhre 1996).

As regard the disorganization of the columnar epithelial cells, necrotic and edematous changes in the epithelial and mucus cell, these appear to be governed by Starling's law (Jha and Pandey, 1989), which states hydrostatic pressure in vascular system moves fluid out of system.

Damaged in the epithelial layer results matching in accordance with the studies carried out by (Gopal Krishnana 1968)

6. CONCLUSION

The effect of *Agave americana* showed damaged in the different tissue layers, accumulation of the toxicity within tissue spaces. The damage of epithelium of mucous fold and atrophy of mucosa may be associated with the hyper secretion of mucosa leading to erosion. Due to enlargement of gastric glands of stomach lead to over secretion of gastric juices.

Leucocytes are prominent. Villi became greatly reduced, flattened, their tips were found ruptured at places leading to exudation of mucus in lumen.

Chemical piscicides are also used for killing fishes but it leaves serious backlashes in the environment. Therefore alternative piscicide such as *Agave americana* that are not hazardous to the environment, with shorted residual effects and easy biodegradability are being appreciated but still its deposition can be seen in the tissues. Therefore, proper monitoring is necessary before its administration. It may be affecting the hormonal balance as observed in their behaviour. Hormonal alterations affect the physiology in long-term. *Clarias* being one of the most tolerant fish but still 0.15gm/l paralyzed the fishes i.e. it may be affecting the nervous system. In the practice unmonitored dose is administered which can cause serious issues to both large and small aquatic species which is still to be monitored.

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