Toxicity analysis of histopathological alterations in liver and alimentary canal of *Channa punctatus* in response to clove oil as an anaesthetic agent

[1]Dr. Shanta P. Janyani, [2]Dr. A. K. Singh
[1]Associate Professor and I/C Principal, [2]Assistant Professor
[1,2] Department of Zoology, R. K. Talreja College of Arts, Science & Commerce, Ulhasnagar-3, India

Abstract: Histopathological technique is a very old technique of making sections of the tissues or organs, so that the structure of cells can be visualized under the microscope. Histopathological alterations in the structure of cells indicate the physiological state of the cell and hence help in the diagnosis of the disease.

With the advent of technology, camera microscope gives a clear image of the cells indicating their physiological state. Camera microscope enables us to capture live images, seen on computer screen & hence helps the investigator to analyse the data based on histopathological differences instantly.

A 40X magnification of camera microscope revealed huge differences in the histological structure of liver and alimentary canal of *Channa punctatus* in response to clove oil. *Channa punctatus* is an air-breathing fresh water fish, which can be acclimated in the laboratory conditions with ease.

Clove oil is an anesthetic agent used since immemorial times in medicine (dentistry). Clove oil seduces the fish and decreases its mobility to help in handling procedures during transportation.

On exposure to clove oil for 32 days, it was observed that the liver cells membrane got disintegrated gradually with increasing duration of clove oil. The alimentary canal also showed atrophy of the cells. The gastric glands chickened and lost their ability to secrete. The inner lining of the stomach and intestine became congruent indicating it's degradation & hence ability to lose function.

Hence, it was concluded that clove oil cannot be used as an anesthetic agent for fish during transportation.

Key words: *Channa punctatus*, clove oil, liver, intestine, histopathological alterations, degradation.

Introduction

Histopathological investigation analyses the structure of cells of various organs in living organisms. The alterations in structure indicate that the organ is undergoing degradation. The structure can be best studied by using camera microscope. Microscope cameras are fitted with a camera in an eyepiece and they connect to a monitor with a simple USB connection. The microscope camera uses software that can capture still images and video enabling measurements. They can also capture live images which can be viewed on a computer screen, hence are ideal for research science and educational professionals. The images can be saved in a hard drive. A magnification of 40X gives a better resolution of the image

With a rapid surge in human population and lack of food resources due to industrialization, deforestation and other factors, man has resorted to food resources of the sea. Various techniques of aquaculture are used, for breeding and producing better variety of animal resources from the sea, in many countries including India. Fishes form an important resource in the water bodies for man. Of all the living forms present in water, fishes form major population. Fishes are most affected by the quality of water. The natural occurring geological processes and biological interactions involving human activities in the field of technology have led to pollution of various water bodies. The fishes serve as biomarkers of environmental pollution. The fish *Channa punctatus* (Bloch), is a fresh water fish, available in local market in live condition. The fish can be easily acclimatized in the laboratory conditions. The fish is found in ponds, swamps, brackish water and even stagnant water in all parts of the world, chiefly Iran in the west, China in the east and parts of Siberia in

the far East. The fish is relished as food in Thailand, Cambodia, Vietnam and other South East Asian countries where they are extensively cultured.

The fish is elongated and covered with scales, the abdomen is rounded and the head is slightly depressed covered with large shield like scales above. Mouth is large and jaws are equal. Teeth are present on jaws and roof of the mouth. Eyes are lateral. The dorsal and anal fins are very long and without spines. The caudal fin is round in shape.

The fish can breathe air and can remain out of water for sufficiently longer time as it has an accessory respiratory organ. The accessory respiratory organ is present in the form of folded linings in the paired cavities on either side of the upper part of larynx. The fish is carnivorous and feeds on small fish, frogs, snakes, rodents, birds and insects. Fishes are known for having good quality proteins, fats and micronutrients (Stansby, 1962). The health benefits of fish include prevention against cardiovascular diseases and cancer of colon, breast and prostate (Rose and Connol, 1993, Marchioli 2001; Sidhu 2003). Clove oil is obtained from the leaves, stems and the flower buds of the *Eugenia caryophyllata* Clove oil is used in many industries like food, medicine, soap and detergents for various purposes. The use of clove oil in fish industry has been tried and studied extensively by various scientists and clove oil is used as anesthetic agent to immobilize the fish during transportation to facilitate various operations like handling, storage and also marketing (Kildea et al, 2004). Clove oil, when incorporated in water, easily penetrates the surface organs and affects the visual organs like liver and alimentary canal.

A concentration of 1500 parts per million is safe for mammalian tissues, but higher concentration can cause significant damage to respiratory organs, liver, etc. (Chanseau et al, 2002).

Materials and methods:-

Animal procurement, maintenance and experimental design:-

The fish *Channa punctatus,* irrespective of sex, measuring 15±8cms body length and 40±5 g body weight were purchased from local fish market in Kalyan (Thane), Maharashtra. They were acclimatized in the laboratory conditions for 15 days by keeping them in glass aquaria of 20L capacity. The fish were fed daily with dried tubifex worms available in the commercial market as fish feed. The water in the tank was changed daily to remove the metabolic wastes and also dead fish, if any.

After acclimatization, the fishes were exposed to clove oil at a concentration of 0.5 (10% of LC50 for 48 hours). The fishes (N=4) were sacrificed by cervical dislocation, after 3 hrs, 6 hrs, 12 hrs, 24 hrs, 48 hrs, 96 hrs, 192 hrs, 384 & 768 hrs of exposure to clove oil. The recovery state was also studied, by withdrawal of clove oil exposure and exposing the fishes to tap water for 768 hours. The organs like liver and alimentary canal were dissected out and fixed in aqueous Bouins fixative for 18-24 hours at room temperature. The standard procedure for histopathological technique was carried out. Fixed tissues were dehydrated in alcohol series. The blocks were prepared by embedding in paraffin wax (Merck, melting point 58-60°c). The sections 6 µm thick were cut using a Rotary microtome. The tissues were then stained with Ehrlich's Haemotxylin – Eosin and observed under light microscope with camera.

Results & discussions:-

Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish.(Van der Oost, Beyer and Vermeulen, 2003).

Histopathological changes evaluate the health of fish exposed to various pollutants used in research and field studies. One of the important advantages of using histopathological biomarkers in environmental monitoring is that these biomarkers allow examining specific target organs like gills, liver and kidneys which are responsible for vital functions such as respiration, detoxification and excretion i.e. accumulation and transformation of xenobiotics in the fish. (Gernhofer, Pawet, Schramm, Muller and Triebskorn, 2001).

Also the alterations observed in these organs are easier to identify than functional ones.(Fanta, Rios, RomaoVianna and Freilberger, 2003), and hence serve as warning signs of damage to animal health.(Hinton and Lauren,1990).

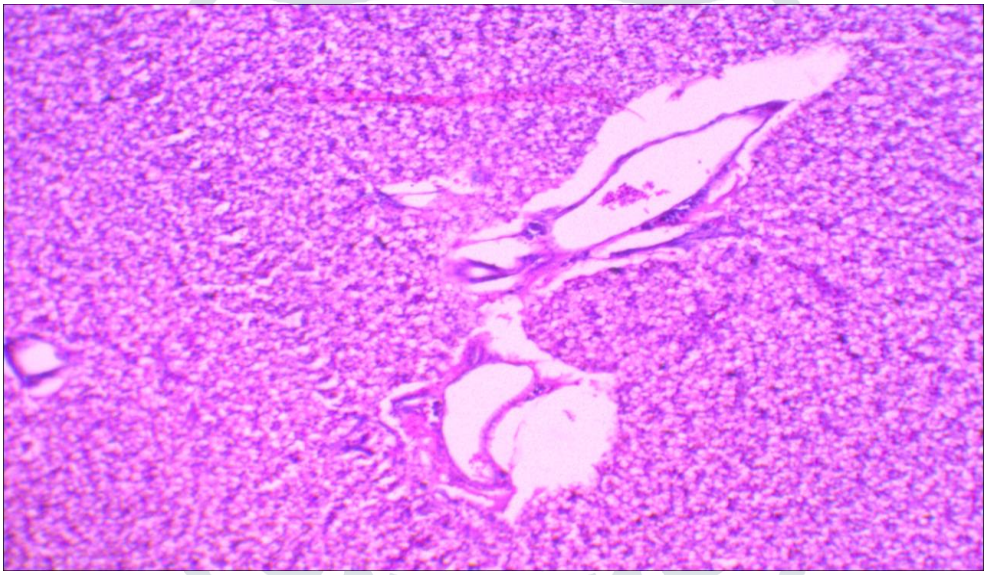
Gills and alimentary canal in fishes serve as entry points for pollutants to the internal body organs like liver and kidneys through the blood.(Takashima and Hibiya, 1995.).

Liver is the most important affected organ due to its position, function and blood supply by pollutants in water. (Rodriguez and Fanta,1998).

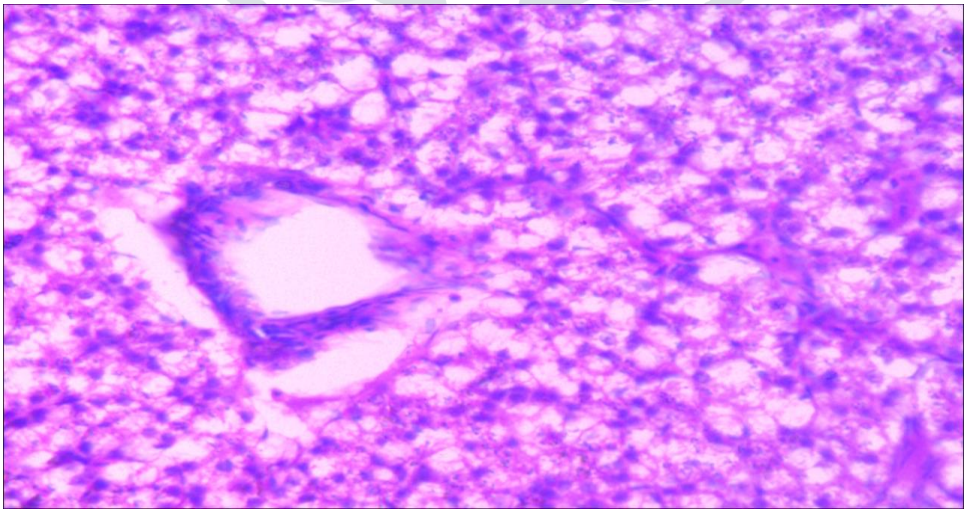
Hence this study was carried out to examine the effect of clove oil on histopathological aspects of liver and alimentary canal of fresh water fish *Channa punctatus*.

The liver is made up of cells called hepatocytes. These are not arranged in lobules as in humans but are arranged in branched laminae. They are joined together by interspersing connective tissue. The liver also shows sinusoids and the central vein.

On exposure to clove oil at a concentration of 0.5µl/lt., the liver showed alterations in its structure such as congestion in the capillaries and inflammatory processes. Vital functions like metabolism and breathing processes are affected as evident from its altered structure. The detoxification of the body, altering the homeostasis and hence osmoregulation is impaired. The structural changes also revealed vacuolar degeneration and necrosis of hepatocytes. Dilation of sinusoids was also observed.



T.S. of liver showing histopathological changes after 192 hours of exposure to clove oil

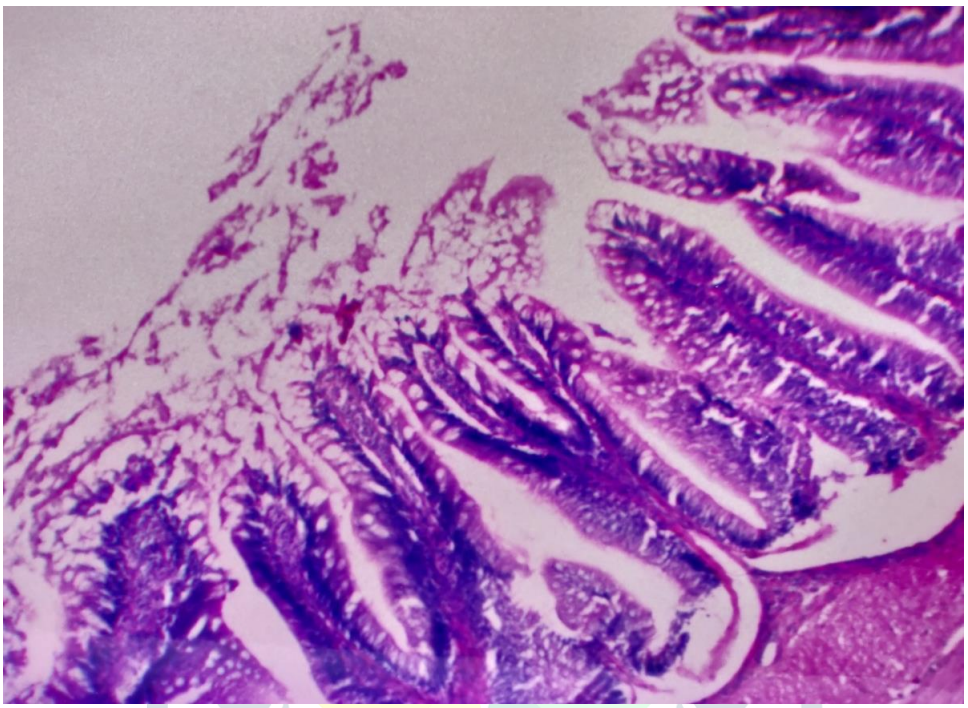


T.S. of liver showing histopathological changes after 768 hours of exposure to clove oil

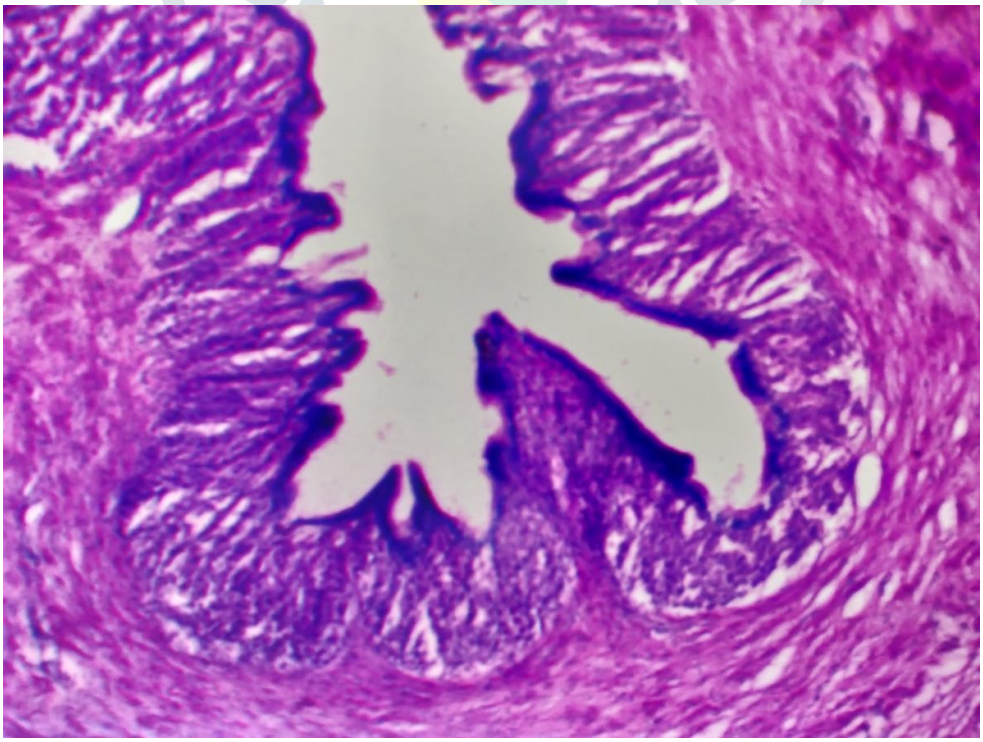
The histopathological alterations were observed in all parts of the alimentary canal. Stomach is one of the important part of alimentary canal as it is involved in the digestion of food materials for the growth and development of any living organism. Stomach and intestine are lined by four histological layers. The

mucosa of the intestine is made up of columnar epithelial cells. These cells are simple with a central nucleus and scattered mucous cells. The loose connective tissues of submucosa projects into the mucosal folds to form laminar propria. After exposure to clove oil, severe damage was observed in columnar epithelial cells. Distortion of laminae and detachment of epithelial layer with severe mucus secretion are other alterations observed.

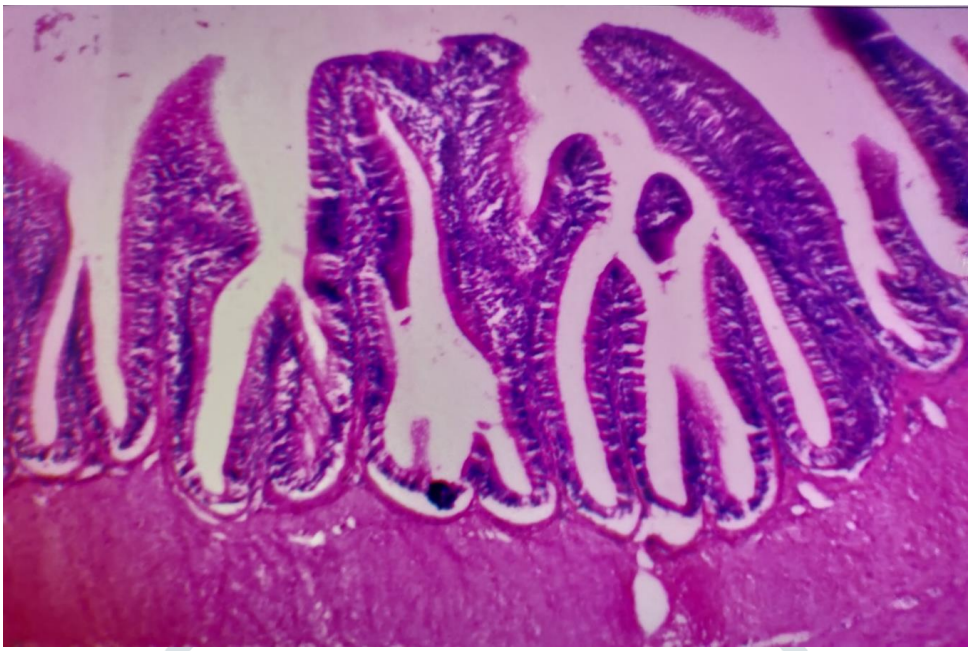
Sastry and Gupta also reported similar findings such as loss of structural integrity and vacuole formation. Ghanbahadur also reported similar findings in *Rasbora daniconius* after endosulfan exposure. Haque also observed damages in columnar epithelial cells resulting in fragmentation, extensive mucus secretion and loss of microridge structure in stomach of *Channa punctatus* in response to fluoride.



T.S. of alimentary canal showing histopathological structures in control fish



T.S. of alimentary canal showing histopathological changes after 384 hours of exposure to clove oil



T.S. of alimentary canal showing histopathological changes after 768 hours of exposure to clove oil

Conclusion: The histopathological alterations in liver and alimentary canal of *Channa punctatus*, in response to clove oil, indicate extensive degradation of cells due to stress induced by clove oil.

More research needs to be done in this aspect as it is vital to reduce extensive loss of fish resources during transportation for the development of the country.

REFERENCES:

[1] Chanseau, M. Bose, S.,Galiay E., Oule, G., (2002). The use of clove oil as an anaesthetic for Atlantic salmon smolts (Salmosalar) and comparison of its effects with those of 2-phenoxyethanol.Bull.Fr.PechePiscic, 365/366: 576-589.

[2] Fanta E. F., Rios S., Romao A. C., Vianna C. and Frieberger S. (2003). Histopathology of the fish Corydoruspaleatus contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicology and Environmental Safety, 54: 119-130.

[3] Gernhofer M., PawetM., Schramm M., Muller E., Triebscorn R., (2001). Ultrastructural biomarkers as tools to characterisethe health status of fish in contaminated streams. J. Aquarium. Ecosystem. Stress Recover, 8: 241-260.

[4] Marchioli R, SchweigerC., Tavazzi L.,Valagussa F.Lipids(2001); 36 Suppl.S:119-126.

[5] Rodriguez, E.L. and Fanta E.(1998). Liver histopathology of the fish Brachydaniorerio, acute exposure to sublethal levels of the organophosphate dimetoato 500. Revisits Brasileira de Zoologist, 15: 441-450.

[6] Sastry and Gupta (1991) Effect of nuvacron on the nutritive value of fresh water fish Channa punctatus, Journal of Environmental Biology 12-(3,): 243-248.

[7] Takashima F., Hibiya T (1995). An Atlas of Fish Histology, Normal and Pathological features : Gustav Fisher Overlay, Kodansha, Tokyo, Japan.

[8] Van dear Oost R., Beyer J., Vermeulen, N.P.E.(2003). Fish bioaccumulation and biomarkers in environmental monitoring risk assessment: A review. Environmental toxicology and pharmocology, 13: 57-149.