Global Journal of Science Frontier Research
Biological Sciences
Volume 13 Issue 2 Version 1.0 Year 2013
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

The Study on the Histopathological Changes of Stomach of *Channa punctatus (Bloch)*. By used Pesticide Endosulfan

By Kishor Haloi, Monikankana Kalita & Ramesh Nath
Institute of Advanced Study in Science and Technology, Guwahati

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GJSFR-C Classification : *FOR Code: 069999*

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Kishor Haloi [α], Monikankana Kalita [σ] & Ramesh Nath [ρ]

Abstract - The aquatic ecosystem is faced with the threat of biodiversity loss due to indiscriminate use of pesticides. Other than targeted pests, pesticides affect a wide range of non target organisms, such as invertebrates and fish inhabiting aquatic environment. The present study deals with the impact of endosulfan on histopathology of stomach of *Channa punctatus* Bloch. In the laboratory condition fishes are divided into control and experimental groups. The LC50 value of endosulfan for *Channa punctatus* Bloch. is calculated which is 0.0004ppm. For the experiment two concentrations are selected 0.0002ppm and 0.0004ppm and fishes are exposed for 24hrs, 48hrs, 72hrs, 96hrs. Fishes showed severe histological changes in Stomach. The degenerative changes included fused microvilli, the outer membrane of microvilli are broken, hemorrhage in the sub mucosa region, cells swelling, vacuoles are recorded in an increasing order towards the higher tested doses. This type of work can be helpful in understanding hazards of pesticides and pollution of pesticide by anthropogenic activity.

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I. Introduction

Fishes are much vulnerable to their toxic substances and bioaccumulation cause serious risk to life. Such toxic substances enter to human through food chain, as fishes constitute an important part of animal protein in rural and urban areas. Alteration in the chemical composition of a natural aquatic environment, due to contact with hazardous substances like heavy metals, pesticides, and effluents from industries usually affect the behaviors, biochemistry, and physiology of the fauna including fish (M. Z. Vosyliene and N. Kazlauskiene, 1999). Fishes are one of the most precious natural resources on earth, and it creates a wide range of benefits to humans, including fisheries, wildlife, agriculture, urban, industrial, and social development .However, the unregulated release of agricultural chemicals especially pesticides into water bodies have caused environmental problems to all classes of organisms in the aquatic habitat. The aquatic ecosystem is faced with the threat of biodiversity loss due to indiscriminate use of pesticides. Other than targeted pests, pesticides affect a wide range of non target organisms, such as invertebrates and fish inhabiting aquatic environment (Pandey,1988). Agricultural runoff of rain and irrigation water introduces pesticides into the aquatic environment, where it poses significant toxicological risks to resident organisms (Kumari and Kumar, 1997). Pesticide pollution severely affects aquatic organisms and, in turn, the entire food chain including human beings (Dutta, Mawell 2003). Their availability and selectivity to toxic substances are main criteria for selection as an experimental animal. The present piece of work includes a detailed account of sub lethal effects of insecticides on the stomach and intestine of fresh water, air breathing, Indian murrel, *Channa punctatus* (Bloch), a commercially important fish, which is easily found in muddy shallow water, a good food fish of high nutrition as well as medicinal value. Since the intestinal tract is the first organ to come into contact with food-borne contaminants, ultra structural changes of these organs were chosen as criteria for the sub lethal action of endosulfan was selected for the study. Thus, the objective of this study was to investigate the lethal and sub lethal effects of endosulfan to *Channa punctatus* using mortality, behavioral and histopathological changes of Stomach. Changes in these parameters are being investigated as potential diagnostic tools in assessing the effects of endosulfan on fish with a view of setting up standards for safe disposal of wastes.

II. Materials and Methods

a) *Channa punctatus (Bloch)*

Fishes are collected from local market and weights were taken on electric balance. The length and breadth were taken. The length ranges from 12-14 cm. Weight varies from 18 to 20g.The fishes were washed in 0.2% K_2MnO_4. The fishes were just dipped on 0.1% K_2MnO_4 for few seconds and taken out and again washed in normal water (Joshi et al., 2002). Before this the two aquariums are washed thoroughly in running tap water and filled with water. Stones are placed on the bottom of the aquarium and aquatic plants were placed. O_2-dissolver is also introduced and methylene blue is added and kept the aquarium to settle it for two days. Now ten (10) fishes were introduced in each of the aquarium and kept there for 10 days for acclimatization.

Author α σ : Institute of Advanced Study in Science and Technology, Paschim Boragaon, Guwahati-35. E-mail : kishorhaloi@yahoo.in
Author ρ: Department of Zoology, Dhing College.
E-mail : rameshnathdhing@gmail.com

Fishes were fed regularly with cut pieces of earth worm that were collected from neighboring areas.

Endosulfan (Thiodan Comercial Trade Product is used) is an organochlorine pesticide patented in the mid 1950s. Technical grade endosulfan is mainly composed of a mixture of two biologically active stereoisomer's named α-endosulfan and β-endosulfan in a ratio of 70 % to 30 % respectively. As minor impurities technical grade endosulfan may also contain up to 2 % endosulfan alcohol and 1 % endosulfan ether. Endosulfan was developed as a non-systemic insecticide with contact action, generally used in control of damage caused by aphids, ticks, mites and other insects on a broad range of crops and non crop vegetation such as cereals, maize, sorghum, oilseed crops, fruit, vegetables, olives, potatoes, cotton, tea, coffee, or ornamental plants. Endosulfan the commercial product Thiodan contains 39% of Endosulfan. To determined the LC_{50} value of Endosulfan on *Channa punctatus* prepared the following doses of Endosulfan .0001 ppm, .0002ppm,.0003ppm,. 0.000 4ppm,.0005ppm,.0006ppm. By the help of the formula- $N_1V_1=N_2V_2$ here, N= Concentration V= Volume. After 10 days, in both of the aquaria two separate doses of Endosulfan 0.0002 and 0.0004 ppm were applied. After the day of exposure, fish samples were collected from of the aquarium in the interval of 24 hrs –i.e. 24 hr, 48 hr, 72hr, 96 hr. Fishes were observed throughout the period of experiment and recorded. As per the plan of the study, after acclimatization in laboratory condition, fishes are divided into two groups- Group-A- Normal: One group of fish is used as normal or control group. Experimental Group :Group-B= One group of fish is treated with 0.0002 ppm of endosulfan. Group-C= One group of fish is treated with 0.0004 ppm of endosulfan. Histological study was carried out following the standard procedure of (Gurr et al., 1968). Intestinal tissues were dissected out very carefully to avoid any damage from the three groups of fishes (normal group A, treated group B and treated group C). the sections are cut in 5 μm using a microtome. Stained sections were Examined under Bright field of Fluorescent microscope.

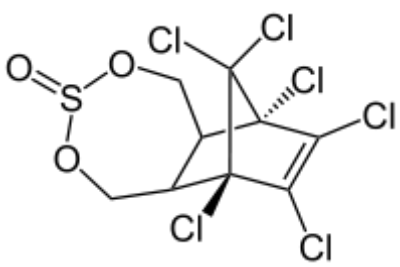

Figure1 : ENDOSULFAN(6,7,8,9,10,10hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide)

Concentration	No.of Exposed	No.of Survived	No.of Responding
0.0001 ppm	10	10	0
0.0002 ppm	10	8	2
0.0003 ppm	10	8	2
0.0004 ppm	10	5	5
0.0005 ppm	10	3	7
0.0006 ppm	10	1	9
1 ppm	10	0 (only after 1 hr)	0

Figure 2 : Survived and mortality of *Channa punctatus (Bloch.)* exposure with Endosulfan

III. Result

Normal fishes that were kept for acclimatization exhibit a drastic behavioral changes after exposure to Endosulfan. In fact, no morphological alteration like change in scales, gills or skin, fin etc change were noticed, but of course the body become much slimy due to excess secretion of mucous. The normal fishes weighs about 22- 24 grams which were found to be reduced in treated groups. The cause of reduction of weight was found to be the stress condition due to stopping of food supply during the period of the present study. In both of the concentration 0.0002 PPM and 0.0004 PPM fishes exhibit a highly uncomfortable state. They tried to jump out from the water for breathing and irritation. Fishes lost their balances, float on water. Histopathological Studies. Summary of histopathological changes observed in the stomach tissues of *Channa punctatus*.subjected to sub lethal concentrations of 0.0002 PPM and 0.0004 PPM of Endosulfan for 24, 48,72,96 hrs are presented in the Figures below.

a) Control Stomach

The wall of the stomach was composed of mucosa, sub mucosa, muscularis and serosa. Intestinal mucosa was composed of the epithelial layer, the lamina propria and stratum compactum. Mucosal folds consisted of connective tissue cores covered by Intetinal epithelium. Columnar cells were the more numerous of the epithelial lining cells and closely resembled those of higher vertebrates. These tall and cylindrical cells had striated, free borders (brush border or microvilli) and contain oval nuclei which were situated either centrally or towards the bases of the cells. Stomach mucosa secreting cells or goblet cells were interspersed among the columnar cell being more numerous along the slides rather than on the crests or at bases of the mucosal folds.

b) Treated Stomach

i. *0.0002 PPM 24 Hrs Exposure with Endosulfan*

When the slides of .0002 PPM 24 Hrs were observed seen that fusion of stomach microvilli, aggregation of blood cells in the microvilli region as well as in the sub mucosa region. Formation vacuoles in sub

mucosa and microvilli region. Hemorrhage occurs in the sub mucosa region.

Irregularities of microvilli, aggregation of blood cells in the periphery of microvilli, vacuolation occurs in the microvilli region. The outer membrane of microvilli were breaken at some points. Vacuolation in sub mucosa layer.

iii. *0.0002 PPM 72 Hrs Exposure with Endosulfan*

Blood cells are aggregated in abundance in the sub mucosa and microvilli region. In flammation at the site of microvilli loss. Hypertrophy of the Intestinal microvilli. Large vacuoles were seen in the sub mucosa region.

iv. *0.0002 PPM 96 Hrs of Exposure with Endosulfan*

Fused microvilli were seen, vacuolation in the mucosa as well as in the sub mucosa. Appoptic cells are seen in the microvilli region.

v. *0.0004 PPM 24 Hrs Exposure with Endosulfan*

Aggregation of blood cells in the periphery of microvilli and sub mucosa region. Vacuolation seen in the sub mucosa region.

vi. *0.0004 PPM 48 Hrs Exposure with Endosulfan*

Large vacuolation seen in the sub mucosa. Cells swelling occurs, aggregation of blood cells, hemorrhage seen all over the sub mucosa region.

vii. *0.0004 PPM 72 Hrs Exposure with Endosulfan*

The breakage of the outer membrane of the microvilli. Large vacuoles are seen in the sub mucosa, Hemorrhage all over the surface, pigmentation in the different parts of the microvilli. Due to progressive cells loss narrower mucosa layer is seen compared to others.

viii. *0.0004 PPM 96 Hrs Exposure with Endosulfan*

Severe damage of microvilli occurs. The destruction of sub mucosa layer as well as vacuolation on it. Narrower mucosa layer, hemorrhage, inverted microvilli are seen.

IV. Discussion

Endosulfan induced pathogenicity in intestine and stomach may be due to the fact that organochloride in the presence of HCl secreted in the stomach forms organochloride acid, which has highly corrosive properties. This acid destroys mucous secreting cells of intestinal lining causing the observed abnormalities. Pathological gastrointestinal effects of endosulfan including damage to the mucosal lining, loss of microvilli, cracked clay appearance of duodenal mucosa and desquamated epithelial cells of gastric mucosa have also been observed by earlier workers. Hence causes irritation and destruction of the mucous membrane of the intestine, thereby hampering absorption. (Anderson, et al., 1969) It was suggested that lead increases the formation of gastric ulcers by interfering with the oxidative metabolism in the stomach that increased the incidence of gastric ulcer (Olaleye et al., 2007).

Histological examination revealed great variability in the intestinal lesions severity existed among most fish caught including focal deformation with necrosis of mucosal epithelial layer of some villi, enlargement of the intestinal villi due to vacuolar degeneration or cloudy swelling of the mucosal epithelial cells Lymphocytic infiltration, dissociation and reduction of muscular bundles and serosal lysis were also detected. In some instance, the columnar epithelial layer in between the intestine villi carry long hair like extensions and lymphatic sinuses and heavily cellular infiltration were detected in the intestinal tissue underlying. This may be represent important link in the intestinal immune system which catch antigen and pass it into macrophage and lymphocyte underlying it to activate immune responses against antigen (Ali, et al., 2008). According to (Bhatnagar *et al., 2007)* the observed irritation and destruction of the mucosa membrane of the intestine, hampering absorption. The pathological alterations in the observed by many investigators about the effects of different toxicants on fish intestine. Epithelial degeneration, inflammatory cells infiltration in the sub mucosa as well as sub mucosal edema was seen in the intestine of tilapia fish exposed to carbofuran. The implication of this is that lead causes an increase in the formation of free radicals, which, if not mopped up by free radical scavengers, will expose the stomach to inflammation and gastric mucosal damage. These adverse effects of lead as well as its inhibition of enzyme activities (Dai et al., 2009; Abdallah et al., 2010) might be the main inducer of the obtained intestinal histopathological damage of the exposed mollies. The Intestine is the first organ which is come into contact with food- borne contaminants (Braunbeck and Appelbaum, 1999). Mandal and Kulshrestha 1980 describe the lesion formation in villi of *Clarias batrachus* after exposure to sumithion. Histological analysis of Intestine tissue of *Channa striatus* and *Heteropneustes fossilis* inhabiting the polluted water showed degenerative changes in the serosa, mucosa and sub mucosal layers, necrosis, proliferation and desquap-mation of the superficial parts of the villi (Kumari and Kumar, 1997). Braunbeck and Appelbaum 1999, have also found that in the intestine, exposure of Endosulfan is associated with changes in the epithelial lining, which indicates disturbance of intestinal absorption. (Cengiz et al., 2001) reported edema, degeneration, accumulation of lymphocytes and eosinophils were reported in the Intestine of *G. affinis* exposed to Deltamethtin (Cengiz and Unlu, 2006).

V. Conclusion

Pesticides are the most hazardous substance that not only affects the target organism but also the non-target organisms. This can be said that the toxic

chemicals enter the food chain and causes bio-magnification in different strata of food chain. Although the pesticides are frequently used in the paddy fields to yield a higher production of crops, perhaps it acts as a silent killer that have a detrimental effect on environment, damaging and causing that to non-target organism. This type of study can suggest that the use of pesticide in the paddy field should be in a control rate which does not affect the non-target organism. Government has formulated several action plans but the need of the hour is that the people realize by themselves about the negative effects of the pesticides.

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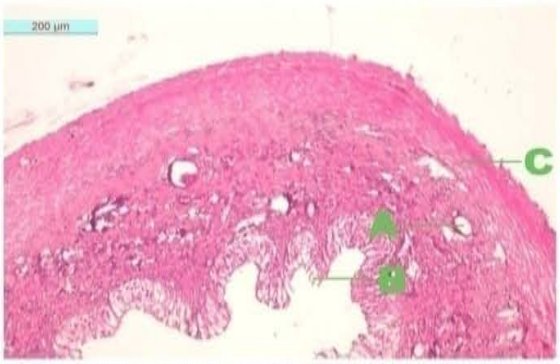

Fig- Treated Stomach 10x Resolution .0002 PPM 72 Hrs A- Vacuolation B- Breakage Microvilli C- Thinner Serosa, Sub Mucosa

Fig-Treated Stomach 40x Resolution .0002 PPM 72 Hrs A- Aggregation of Blood Cells B- Vacuolation By Necrosis C- Cells Swealling

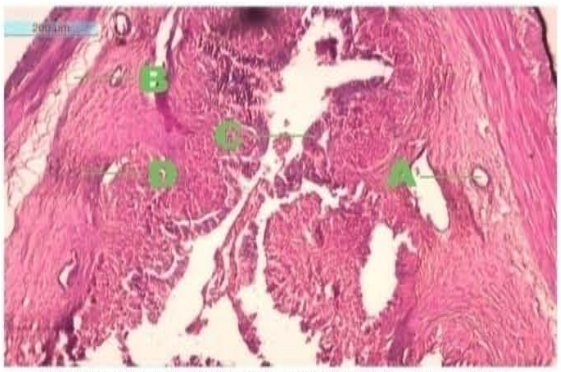

Fig- Treated Stomach 10x Resolution .0002 PPM 96 Hrs A- Vacuolation B- Damage Sub Mucosa C- Inverted Microvilli D- Shrinkage Tissue

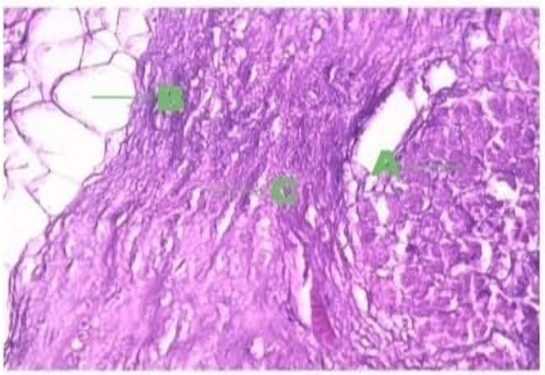

Fig- Treated Stomach 40x Resolution .0002 PPM 96 Hrs A- Cells Swealling B- Damage Sub Mucosa C- Shrinkage Tissue

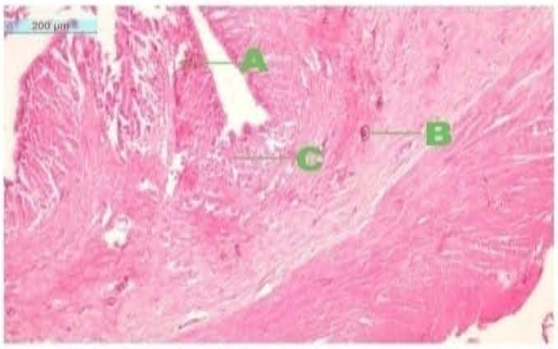

Fig-Treated Stomach 10x Resolution .0004 PPM 24 Hrs A- Breakage Microvilli B- Haemorhhage C- Necrosis

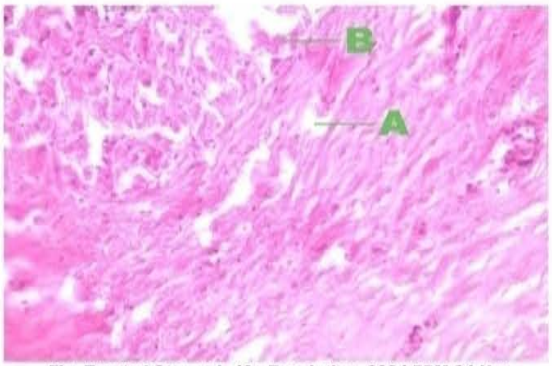

Fig- Treated Stomach 40x Resolution .0004 PPM 24 Hrs A- Vacuolation B- Cells Swealling

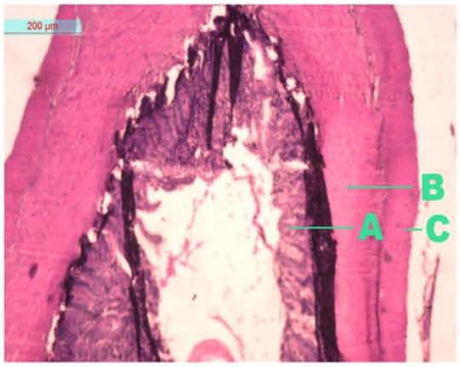

Fig-Control Stomach 10x Resolution
A- Microvilli B- Sub Mucosa C- Mucosa

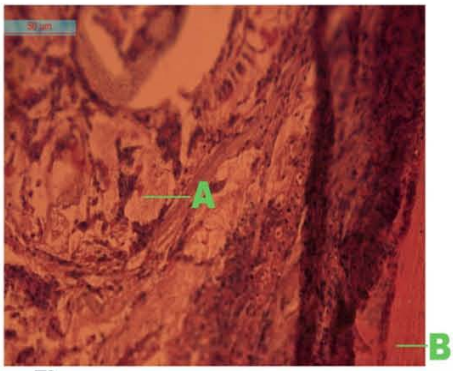

Fig-Control Stomach 10x Resolution
A- Normal Microvilli No Vacuolation
B- Normal Sub Mucosa

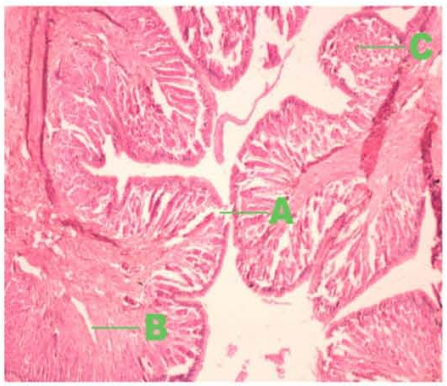

Fig-Treated Stomach .0002 PPM 10x Resolution 48 Hrs
A- Breakage Microvilli B- Vacuolation C- Necrosis

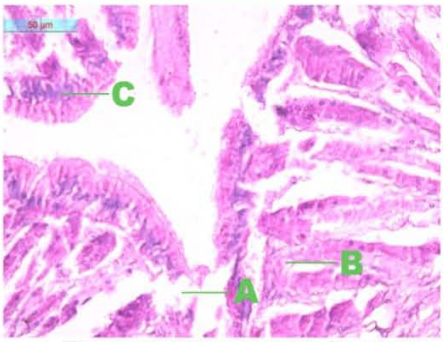

Fig-Treated Stomach 40x Resolution
.0002 PPM 48 Hrs A- Microvilli
Break B- Appoptic Cell
C- Aggregation of Blood Cells

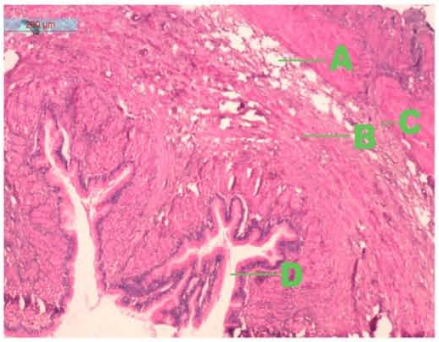

Fig-Treated Stomach 10x Resolution
.0004 PPM 48 Hrs A- Vacuolation on
Sub Mucosa B- Haemorhhage
C- Aggregation of appotic cells
D- Detouched Microvilli

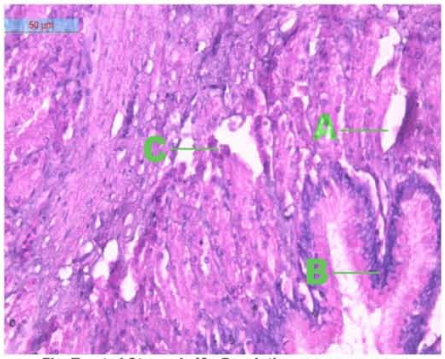

Fig- Treated Stomach 40x Resolution .0004 PPM 48 Hrs
A- Vacuolation B- Aggregation of Blood Cells C- Appoptic Cells

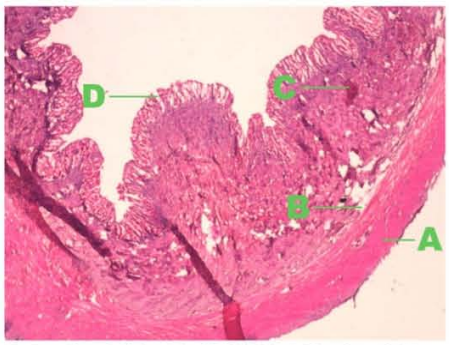

Fig- Treated Stomach 10x Resolution .0004 PPM 72 Hrs A- Thinner Serosa, Sub Mucosa
B- Damage Sub Mucosa C- Haemorhhage D- Breakage Microvilli

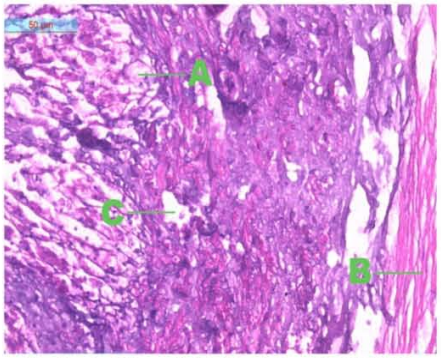

Fig- Treated Stomach 40x Resolution .0004 PPM 72 Hrs
A- Cells Swealling B- Sub Mucosa Damage C- Vacuolation