Histopathological Changes in the Gills and Brain of *Cyprinus carpio* Exposed to copper sulfate

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ABSTRACT:

Copper sulfate is a chemical which is used widely in the agricultural sector and in fishery sector but at high concentration it affect the aquatic life. To check the toxicity of copper on fishes this experiment is conducted. Two aquariums were used for the experiment, one is for control and the other is for experimental purpose but three aquariums with 10 fishes each were used to determine LC50 and was calculated by using probit analysis method which was 23.4ppm. Graph on dose response relationship was also constructed which was sigmoid shaped. Correlation between mortality percentage and dose was also calculated which showed perfect positive correlation. Hardness and alkalinity was also measured daily and average was 188.4058ppm and 135ppm respectively. Temperature was kept constant at 25 ^{o}C. For the experiment two aquariums with 10 fishes each were setup. In the experimental group fishes were treated with 2.3ppm copper sulfate dose for 15 days. 30% water was changed after every 48 hours. After 15 days slides of gills and brain of both experimental and control group were prepared and comparative study was done to check the effects. The normal structure of gills were highly altered. Hyperplasia , fusion of two secondary lamella, change in axis of secondary lamella, swelling of cells in primary lamella and basement membrane, buldging of the tips of secondary lamella were reported. In brain optic lobes showed some adverse effect such as Vacuolization, Spongiosis, and proliferation of the optic tectum occurred. Cells of optic tectum proliferated into the granular layer of valvula cerebellii. No histopathological changes observed in cerebellum and medulla ablongata.

KEYWORDS; LC50,Hyperplasia, Spongiosis, Vacuolization, Proliferation, Optic tectum, Valvula cerebelli.

INTRODUCTION:

Toxicity is the degree to which a chemical substance or a particular mixture of substance can damage an organism .Toxicity can refer to the effect on whole organism. The symbol for toxicity is a skull with two bones beneath it in cross position .Toxicity and toxicology is two different things .Toxicity is the degree to which a toxic substance can affect an organism and toxicology is a branch of science which deals with the study of the adverse effect of toxicant on organisms. So the central point of Toxicology is that the effect of a toxicant is dose dependent. Even water in high dose lead to water intoxication when taken in too high a dose, where as for very toxic substances such as snake venom when used in very minute dose cannot affect at all. Toxicity is also species specific .The affect of a particular toxicant can vary with species and among the strains of species too.

In this study, 96 hour Lc50 values of copper sulfate a highly toxicant heavy metal, on grey mullet, *Mugil cephalus* of average weight 7.42 g ; mean length 6.51cm was determined. At the first, for range finding test fish were exposed to copper sulfate at several selected concentration 1, 5, 10, 15, 20, and 30 mg/l, then fish exposed to five concentrations control 35, 40, 45, 50, 55, and 60 ppm of copper sulfate for Lc50 – 96 hour. Experiment was carried out in triplicate and 21 fish per each treatment. Behavioural changes increased with

increased concentration. The result obtained in this study clearly revealed the fact that it is necessary to control the use of heavy metals such as copper.

Azadeh Atabati *et al. (2015)* studied the effect of copper sulphate on the gill histopathology of grass carp. The toxic impact of copper sulfate in lethal and sub-lethal concentration was investigated on gills of grass carp , *Ctenopharynogodon idell.* In histopathological studies of the gill tissues, hyperplasia was clearly obvious in treatment specimens. In all of the treated groups, heavy gill mucus response was observed, which indicate a direct relation with high concentration. Also in histological study of the gills, epithelial cells faced to hyperplasia, which increased with high copper densities. Primary lamellae cells wrinkling and also changing in formation were observed in chloride cells. This lesion enhanced in higher densities and in concentration of 2.5 and 5 mg/l, primary and secondary lamellar epithelial cells were degenerated.

The main purpose of this study was to determine the effect of copper sulfate on the gills and brain of *Cyprinus carpio* at sublethal concentration.

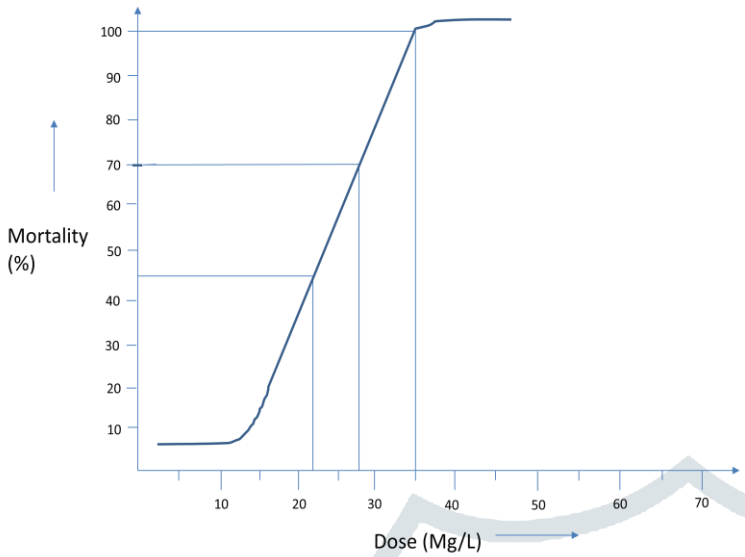
MATERIALS AND METHOD:

Calculation of LC50:

For the calculation of 96 h LC 50, 18 fishes of *Cyprinus carpio* were purchased from ocean fish world, located at Hoshangabad road Bhopal. All fishes were bathed in Potassium permanganate solution for 30 minutes and then transferred in to three aquariums which were aerated for 24 hours in the ratio of 1:1:1. Fishes were acclimatized for 3 weeks and were feed two times within 24 hours daily, in the ratio of 5:1. Five pellets of food were given to each fish in the morning and evening time. After the acclimatization period all three aquaria were contaminated by dissolving copper sulfate in it in different ratios. After this period fishes were contaminated with copper sulfate in the ratio of 22 mg/l, 28 mg/l, and 34 mg/l in aquarium 1^{st}, 2^{nd}, and 3^{rd} respectively. 2 fishes died in 1^{st} aquarium within 24 hours of duration and 1 fish died after 72 hour. In total 3 fishes were died in aquarium 1^{st} within 96 hours and no fishes died after 96 hours. In 2^{nd} aquarium 3 fishes died within 24 hours and 1 died after 72 hours. In total 4 fishes died in aquarium 2^{nd} within 96 h. In 3^{rd} aquarium all fishes were died within 24 hours of time. From the above data by using Probit analysis LC50 was calculated which was 23.44.

Concentration(ppm)	log10(**concentration**)	% **Dead**	**Probit**	
22	1.342422681	45	4.87	
28	1.447158031	70	5.52	
34	1.531478917	100	8.09	
	Intercept	-17.7526		
	X Variable 1	16.6019		
	y=ax+c			
	5=16.60x-17.75			
	5+17.75=16.60x			
	22.75/16.60=x			
	x=1.37			
	LC50=antilog1.37			
	LC50=23.44			

GRAPH ON DOSE-RESPONSE REALATIONSHIP:



- **STATISTICAL ANALYSIS OF DOSE –RESPONSE RELATIONSHIP**

The relation between dose and response was analyzed by using statistics. Correlation was calculated between dose and mortality.

Correlation coefficient was calculated by using Pearson's correlation coefficient method which is described below:

$$\text{Correlation coefficient}(r) = \frac{\sum(x - \bar{x})(y - \bar{y}) / (\text{s.d of x})(\text{s.d of y})}{N}$$

X = concentration of dose in mg

$\bar{X}$ = mean of X

Y = number of fishes died

$\bar{Y}$ = mean of Y

S.d = standard deviation

N = number of observation

When X was 22 mg/l two fishes died, similarly when X was 28 mg/l 4 fishes die and all 6 were died when X was 34 mg/l.

X	Y	$(x - \bar{x})$	$(y - \bar{y})$	s.d of x	s.d of y	$x-\bar{x}/s.d\ x$	$\frac{y-\bar{y}}{s.d\ of\ y}$
22	2	-6	-2	4.8	1.61	-1.30	-1.24
28	4	0	0	4.8	1.61	0	0
34	6	6	2	4.8	1.61	1.30	1.24

By computing all the above values the correlation coefficient was calculated.

Correlation coefficient (r) = +1

r = +1, indicates that the correlation between dose and mortality is perfect and positive. Which means more if we increase the concentration of dose more will be the mortality .

PHYSICO-CHEMICAL PARAMETERS:

Temperature was kept constant at 25 ^{0}C by using digital thermometer but alkalinity and hardness were measured.

Hardness of water: 10ml of standard water was collected in a conical flask and its pH was raised by adding appropriate volume of buffer. Few drops of Erichrome Black-T (EBT). After that indicator was added to it and titrated against EDTA to reach the end point of blue colour. After this repeat this process with water sample from aquarium. To calculate the permanent hardness half of the quantity of sample were boiled off and then calculate the permanent hardness.

Below table give the complete calculation of hardness of water.

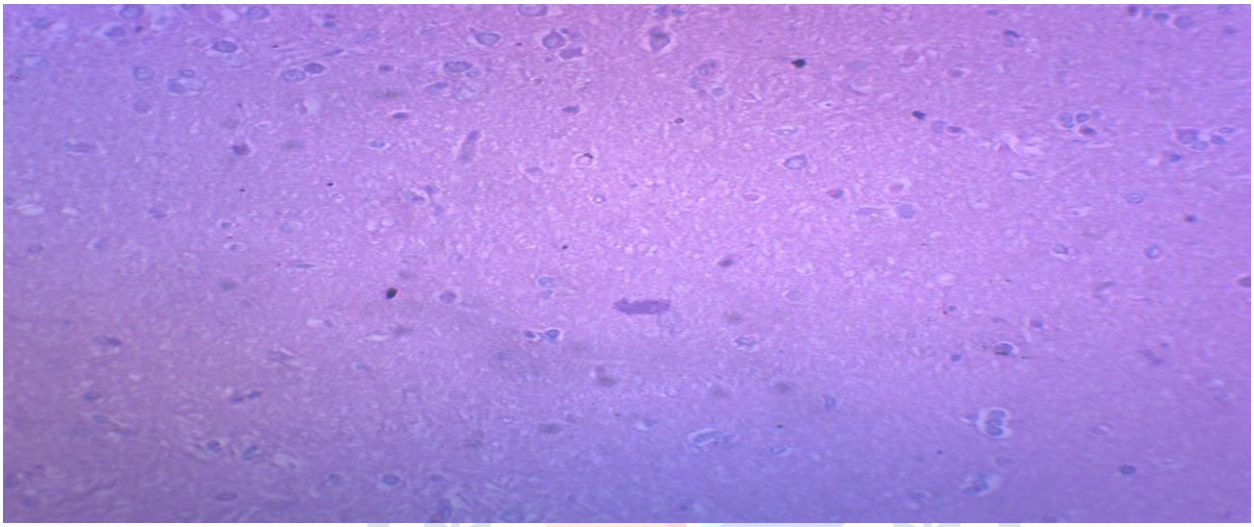
VOLUME OF WATER SAMPLE	TOTAL HARDNESS	PERMANENT HARDNESS	TEMPORARY HARDNESS
20ml	188.4058ppm	123.18841ppm	65.21739ppm

1. **Alkalinity:** Alkalinity by different ions in the water sample are shown in the tables below

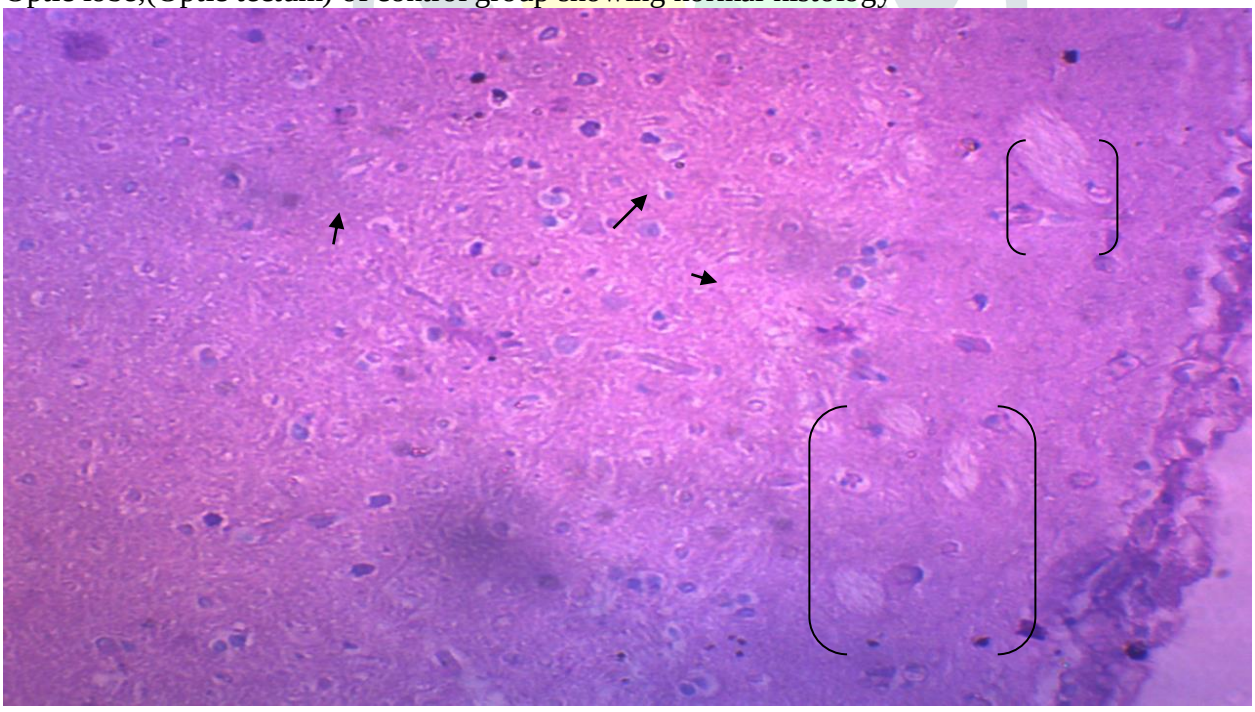
Due to $CO3^{--}$	20ppm
Due to $HCO3^{-}$	115ppm
Total Alkalinity	135ppm

After calculating the LC50 value two aquariums were setup for the further experiment. One was used as control group and the other was used as experimental group. In the experimental group fishes were treated with 2.3ppm copper sulfate for 15 days and then dissected and slides of the organs were prepared. Brain and gills were removed and bathed with saline water and then kept in Bouin's solution for approximately 24 hours and then treated with a various grades of alcohol and then xylene. After this the organs were embedded in paraffin wax and the stained with haematoxylin-Eosin stain. After this the slides of control and experimental group were studied comparatively.

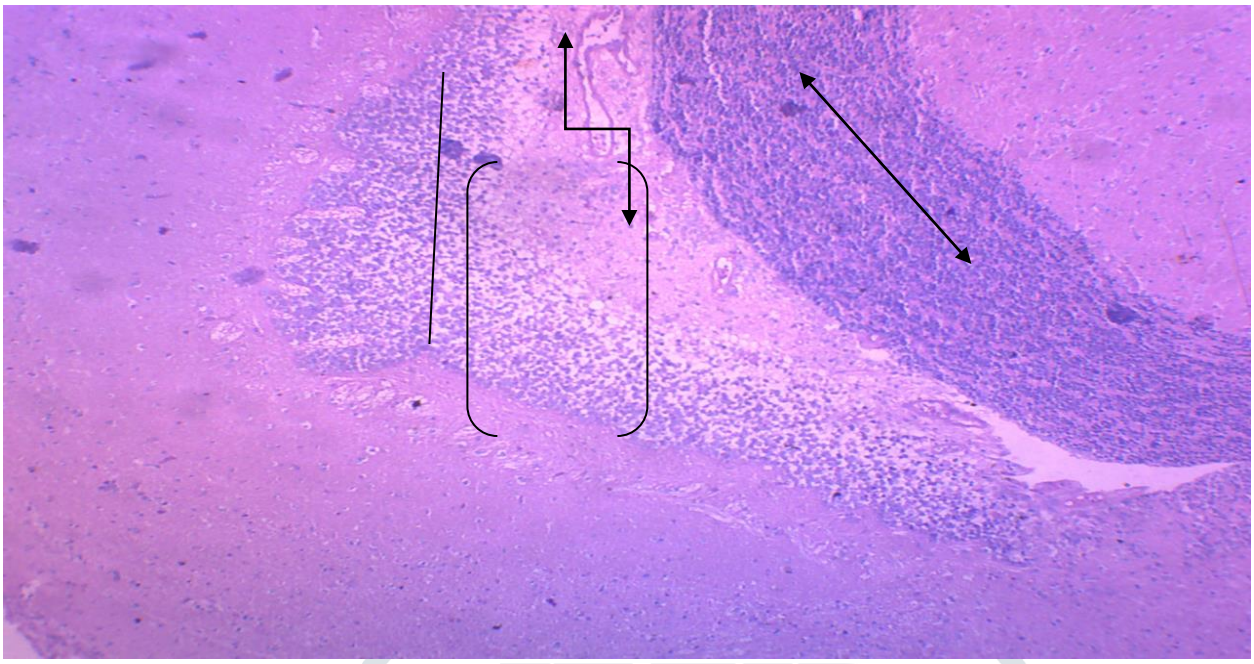
OBSERVATION AND RESULT:



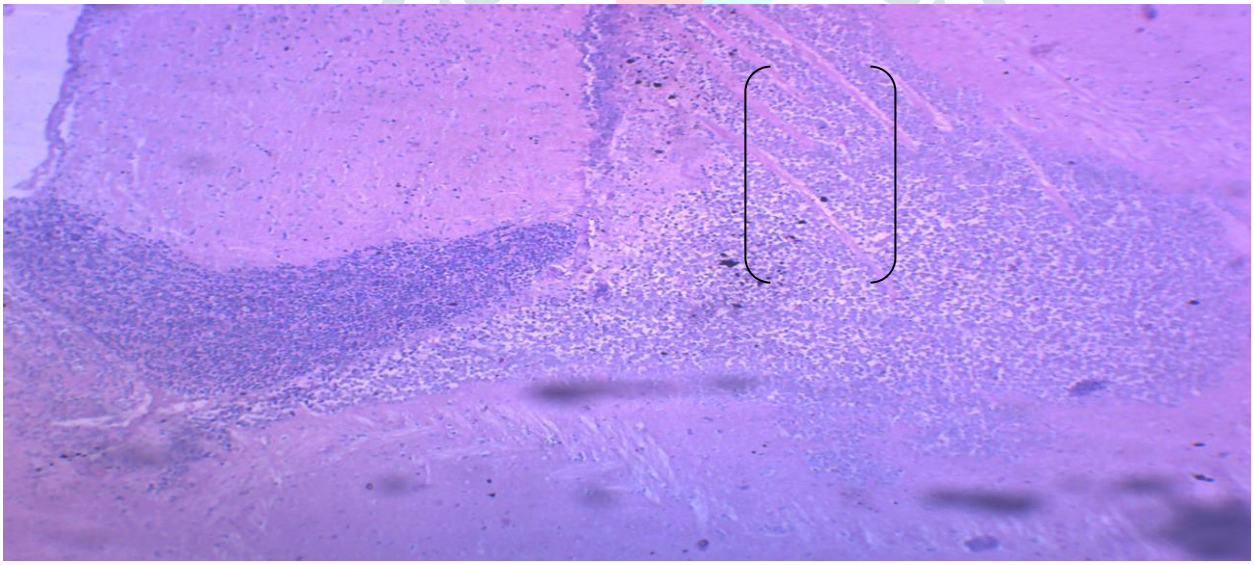
Optic lobe;(Optic tectum) of control group showing normal histology



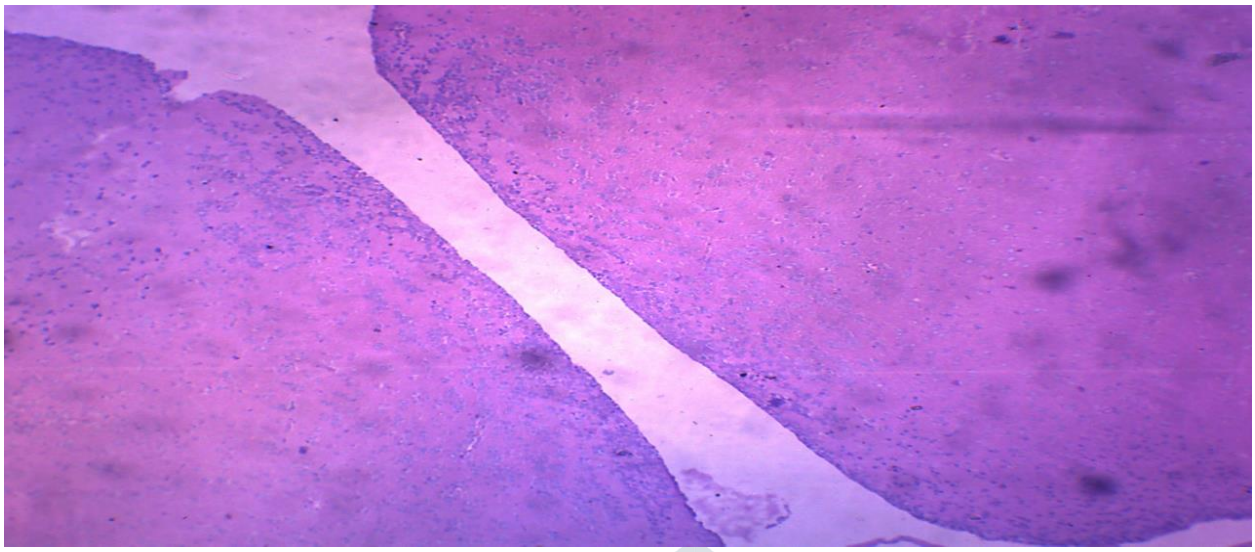
Optic lobe of Experimental group ; Spongiosis([ ]), Vacuolization(⟶),



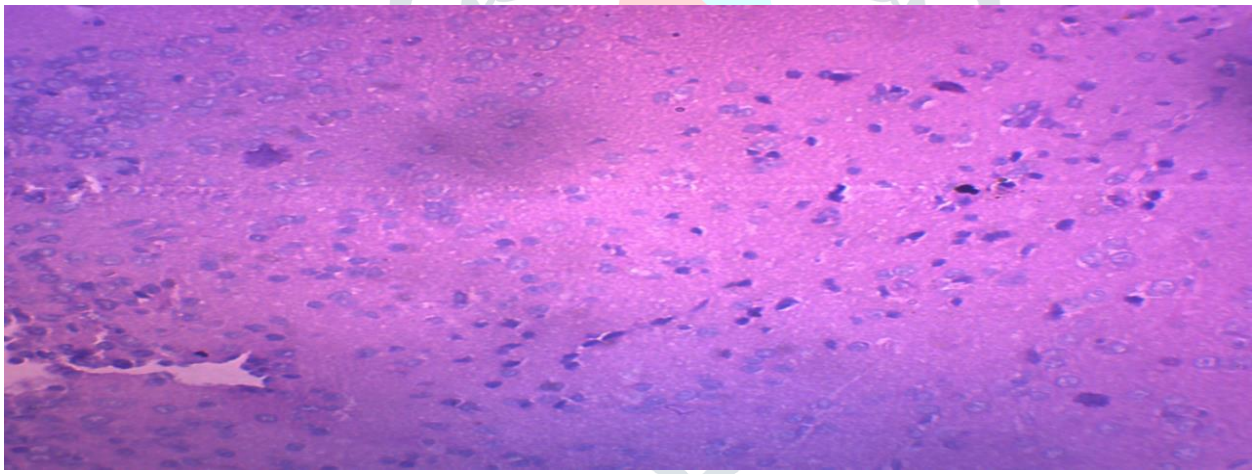
Optic lobe; Exp. Group Optic tectum( ), molecular layer( ), granular layer ( ), proliferation of optic tectum and molecular layer ( )



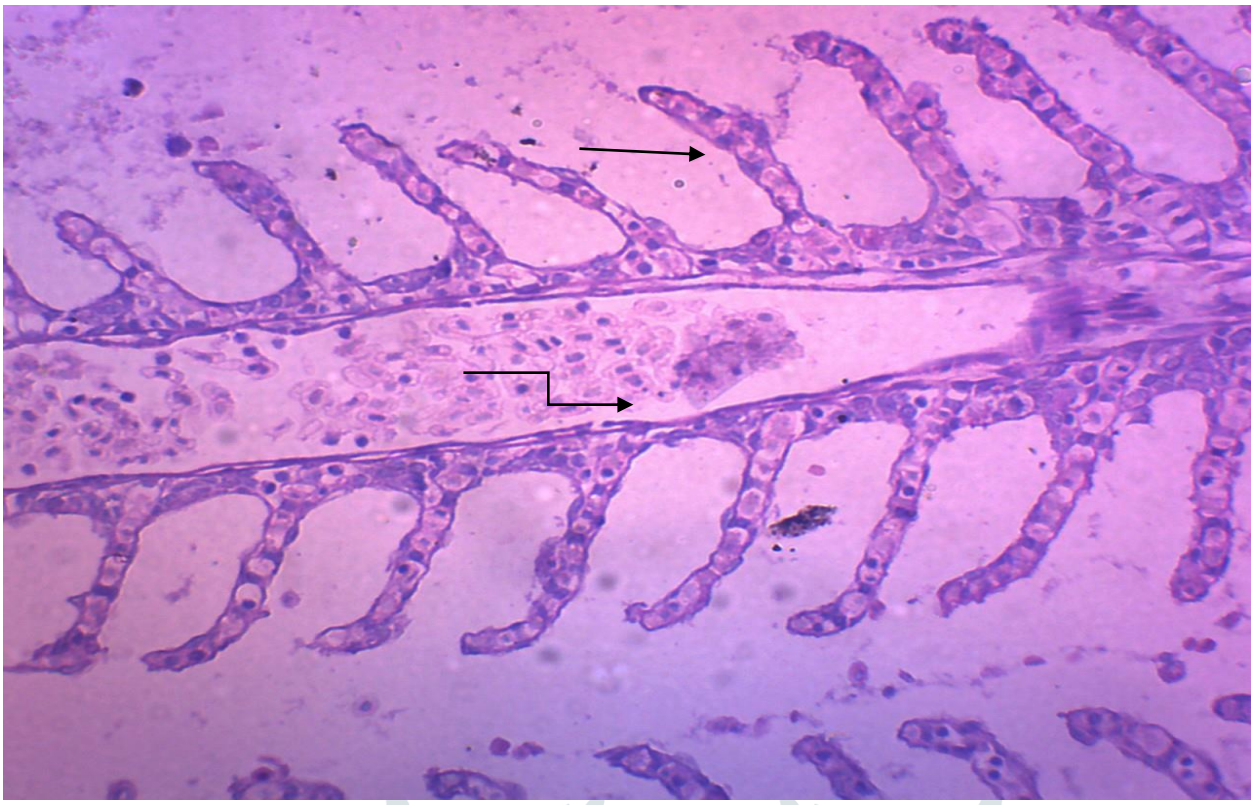
Optic Lobe; Experimental group; Optict tectum and Granular layer proliferation ( )



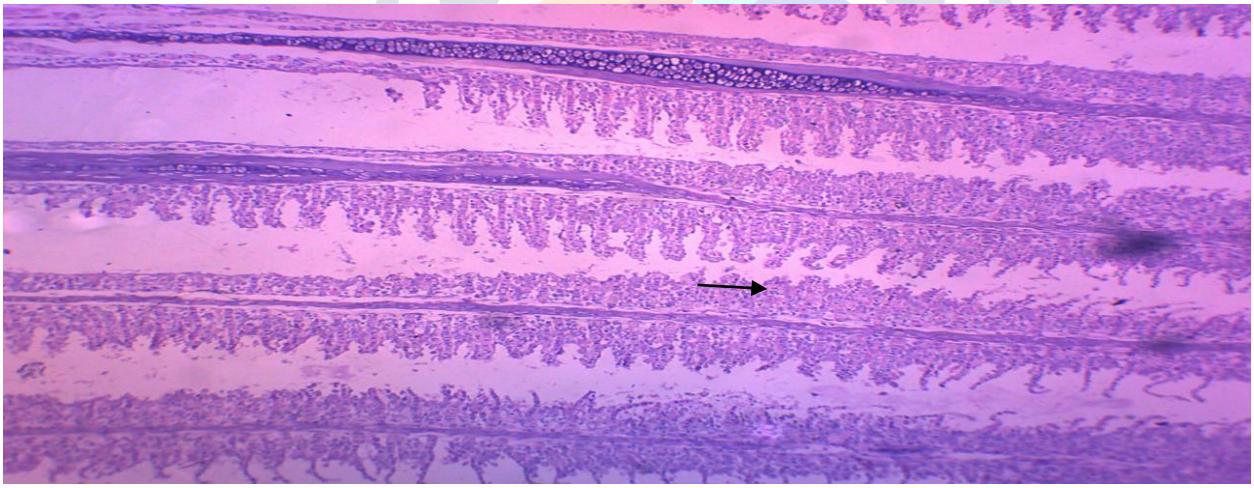
Medulla ablongata of control group, normal histology



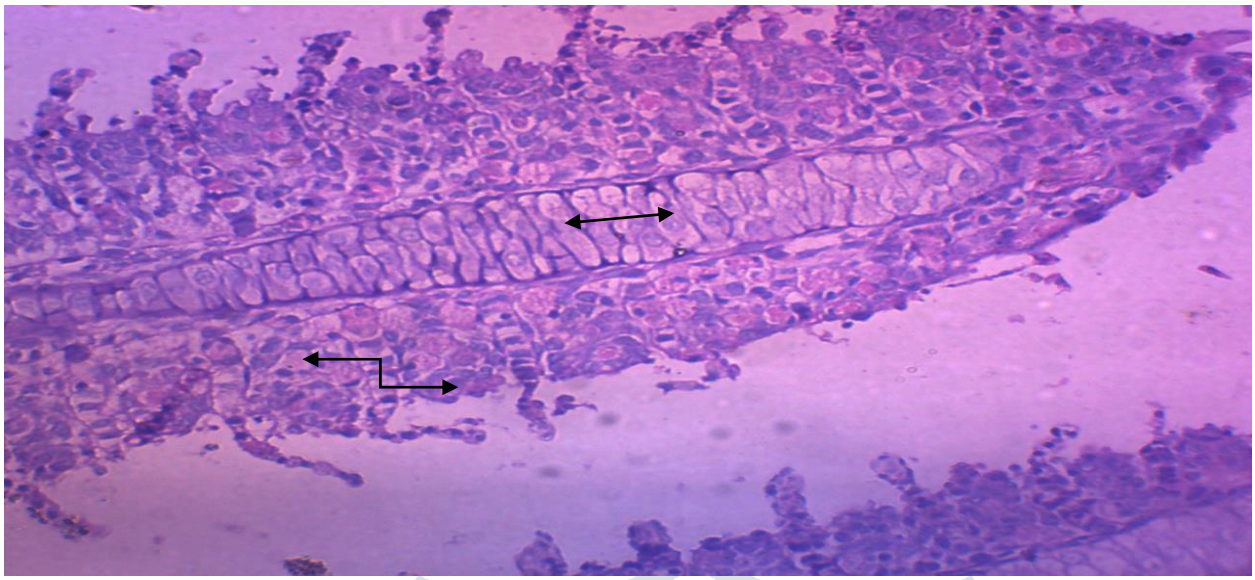
Medulla ablongata of experimental group, normal histology



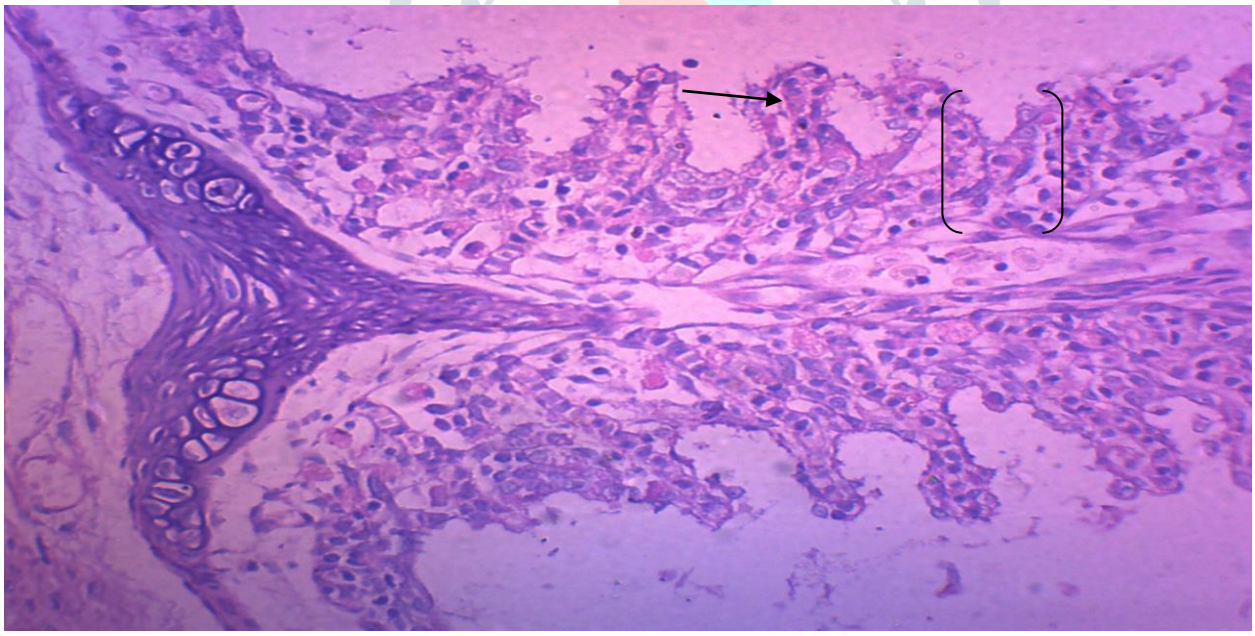
Controle group; normal secondary lamellae (→), primary gill lamella (↳)



Experimental group; Hyperplasia of secondary gill lamellae(→)



Experimental group; Enlargement of cells of primary gill lamella ( ), Hyperplasia of secondary gill lamellae ( )



Experimental group; fusion of secondary gill lamellae ( ); swollen tip of secondary gill lamella ( )

RESULT:

As the variations in normal structures of organs showed that copper sulfate effected brain and gills very hard. The normal structures of gills were highly altered but in the case of brain there are few points where copper sulfate effected most and that is the optic lobe of mid-brain. The primary gill lamella is not severley effected but the secondary gill lamella were effected at very high rate. The epithelium covering of secondary lamella were distorted and hyperplasia of it was observed and fusion of two secondary lamella also occured. Buldging of the tip of the secondary lamella was there. The change in axis of the secondry lamella and the swelling of the cells of the basement membrans occurred. In the primary lamella the cells between the epithelial covering showed swelling. In the tissues of brain the optic lobes showed some adverse effect. Vacuolization, spongiosis and proliferation of the optic tectum occurred. Cells of optic tectum proliferated into the granular layer of valvula cerebellii. No histopathological changes observed at 400x in cerebellum and medulla ablongata.

CONCLUSION:

16 fishes were acclimatized for three weeks and contaiminated with copper sulfate for 15 days and then dissected and histopathological changes on brain and gills were observed. Gills showed more adverse affect than brain. Primary structure and secondary structure of gills were alterd. In the secondary gill lamellae its fusion, swelling of tips, hyperplasia, cell necrosis and epithelial damaging were observed. While in brain optic lobe showed some histological changes such as vacuolization, proliferation and spongiosis. In this research significance of the toxicity of copper sulfate was analyzed. The results concluded that copper sulfate has more toxic significance to gills than brain but it was also concluded that copper has a role in neurodegeneration. From this research it can be easily concluded that copper play an important role in neurodegeneration and can cause many neurodegenerative problems if it is present in water. During the experiment behavioural changes were observed in experimental group. Such as fast swimming for very short period of time, avoiding feeding, laziness and slow movement.

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