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EFFECT OF DIFFERENT SHORT TERM EXPOSURES TO HEAVY METAL COPPER ON THE TESTICULAR STRUCTURE AND SPERMATOGENESIS OF THE FISH CYPRINION WATSONI

BY

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Islamabad
2005

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy

In

BIOLOGY

(Reproductive Physiology)

By

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Islamabad
2005

In the name of Allah
The most Beneficent and merciful
Who
Gave me the courage to complete
This piece of work

CERTIFICATE

This thesis, submitted by ***Mr.Shakeel Ahmed*** is accepted in its present form by the Department of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Master of Philosophy in Biology (Reproductive Physiology).

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Dated: 22-9-2005

This research work with all respect and honor is dedicated to,

The Chief Executive,

Lt. General (R) Nazar Hussain HI(M),T.Bt.

Cadets and Staff of Cadet College Kallar Kahar.

CONTENTS

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LIST OF ABBREVIATIONS

ppm	Parts per million
hr	Hours
GSI	Gonadosomatic index.
K	Faulton's condition factor
C^o	Centigrade
u	Micrometer
H and E	Hematoxylin and Eosine

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ABSTRACT

Copper is present in industrial wastes, which are coming with the effluent of industries. Copper is an essential element but in industrial wastes its concentration is very high which may be toxic to fish and other aquatic animals. In the present study effect of copper was observed on the testes of fish *Cyprinion watsoni*. The fish was exposed to constant dose of copper (0.08ppm) at different exposure periods. Fish were taken out after 7, 14, 21 and 28 days of copper treatment and different parameters were recorded, which are explained below. Neither fish length nor body weight affected ($P>0.05$) after 7, 14,21 and 28 days of copper treatment. Testicular weight (right, left testis and total) was not affected ($P>0.05$) among all the groups. Copper treatment caused no significant effect ($P>0.05$) on fish testicular length (right and left) in Group 1 (7 days) and group 2 (14 days) but caused significant decrease ($p< 0.05$) in case of Group 3 (21 days) and group 4 (28 days). No change ($p>0.05$) was observed in testicular breadth and on the condition factor ($p>0.05$) in all groups. Gonadosomatic index (GSI) of fish was not significantly decreased ($P>0.05$) in Group 1 (7 days) and Group 2 (14 days) but it decreased significantly in treated groups (Group 3 and Group 4). Number of spermatogonia type A and Type B decreased significantly among all the treated groups but there was no significant effect on the cells and their nuclear diameter. Inter lobular spaces increased significantly in all the treated groups except Group 1 (7 days). As far as testicular histology is concerned disorganization and loose arrangement of lobules occurred. Thickening of lobular wall was also observed in some treated groups. Spermatocytes and spermatids clumpings observed in treated groups. Therefore it is concluded from present observations that although copper is essential element but its high concentrations can cause certain reproductive abnormalities among fish and other aquatic animals which ultimately can effect the human bodies.

INTRODUCTION

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Introduction

Toxicologists deal with different chemicals, feed additives, environmental contaminants, pesticides and natural toxins of plants and animals origin, which may adversely affect the health of animals (Gary, 1996).

Water Pollution

Fresh water is required not only for drinking and other domestic purposes but also for crop irrigation, industrial use and energy production. Surface water from rivers, lakes and underground rivers called aquifers is there to meet these requirements. Besides excessive use, pollution of surface water, ground water and the oceans is one major reason for why we are running out of fresh water. When toxic substances enter lakes, streams, rivers, oceans and other water bodies, they either get dissolved, lie suspended in water or get deposited in the bed. This results in the pollution of water whereby the quality of water deteriorates, affecting the water ecosystem. Pollutants can also seep further down and affect the ground water deposits. Solid wastes from different sources including household trash, sewage sludge, agricultural residues, mining refuse and industrial wastes are polluting our fresh water resources (Mader, 2000)

Routine application of fertilizers and pesticides for agriculture and indiscriminate disposal of industrial and domestic wastes are increasingly being recognized as significant sources of water pollution (Gottrfired, 1993).

Wastewater from manufacturing or chemical processes in industry contributes to water pollution. Industrial wastes can include heavy metals such as copper, lead, zinc, mercury, chromium, tin and organochlorides like pesticides. These materials are not degraded readily under natural conditions or in the sewage treatment plants. Infact most

industrial plants do not have adequate effluent treatment facilities and industries such as sugar mills, distilleries, leather processing industries and thermal power stations release their wastes in different water bodies causing great threat to the ecosystem. Accumulation of these effluents directly in the mud of delta and estuaries of rivers cause severe environmental problems (Hassanein, 1999). Infact some organisms accumulate deadly toxic chemicals in their bodies, a process called biological concentration. Oysters, for example, accumulate heavy metals like mercury in their bodies, thus these organisms might be highly toxic while living in waters with relatively low concentration of this metal and later when these organisms are consumed by predators, these metals accumulate in their bodies causing structural, physiological and behavioral changes (Seeley, 1992).

Polluted water is unsuitable for drinking, recreation, agriculture and industry. It diminishes the aesthetic quality of lakes and rivers. More seriously, polluted waters destroy aquatic life and reduce its reproductive ability. The effects of water pollution are therefore not only devastating to people but also to animals, fish and birds (Ahmad, 2001).

Acid rain is another source of water pollution in industrial areas. It mixes with surface water, acidifies lakes and streams thereby killing fish and other aquatic life. It seeps into ground water causing heavy metals to leach out of the soil. These metals enter the ground and surface water, creating health problems for humans and aquatic organisms (Alters, 1996).

Most mineral elements found in the body, whether essential or non-essential, have high chemical and biological reactivity. Such as, they can be potentially toxic depending

upon the dose and other conditions. Indeed, many essential minerals are required only in trace amounts for their specific physiological functions. Above certain levels, these trace elements become potentially toxic. These elements become contaminants when they are found in foodstuff or in drinking water above nutritionally desirable levels (Bodamer, 1990).

Heavy Metals and Water Pollution

Heavy metal pollution of aquatic resources is the direct result of negligence in disposal of industrial wastes. Animals' life in fresh water often become target of chemicals released from factories and agricultural cut off (Azad et al., 1984).

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic at low concentrations. Examples of heavy metals include Mercury(Hg),Copper(Cu),Cadmium(Cd),Arsenic(As),Chromium(Cr),Thallium(Tl) and Lead(Pb). Among the most harmful metallic pollutants are mercury, lead, zinc, cadmium and copper. The seriousness and persistence of heavy metals in water are compounded by the fact that generally they are water soluble, nondegradable and vigorous oxidizing agents, which bind strongly to many biochemicals especially polypeptides and protein (Gurd and Wilcox, 1956).

The health of fish may be affected, either directly through uptake from the water, or indirectly through their diet of vegetation, invertebrates or smaller fish (Kime *et al.*, 1996). Metals released into aquatic ecosystems, are responsible for several fish physiological irregularities (Sehgal and Saxena, 1986). They can also disturb the ionoregulatory mechanism in aquatic organisms (Hansen *et al.*, 1996). All of these effects of heavy metals usually affect fish negatively leading to stress and eventually, in most cases, death.

Heavy metals residues render the protein in fish muscle unfit for human consumption. The primary effects of such pollutants are on the fish themselves. They also

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disturb the quality and quantity of food available to the fish in the aquatic environment (Jackim et al., 1970).

Heavy metals are continuously released into the aquatic environment from natural resources such as volcanic activity or weathering of rocks. Moreover, industrial processes and some agricultural uses (e.g. $CuSO_4$ is used to control aquatic vegetation) have greatly increased the mobilization of many metals in fresh water (Dunnick and Fowler, 1988). Therefore, in recent years concern has increased over heavy metal pollution.

In natural water, metal ions occur as free aqueous ions, complexes with organic/inorganic ligands or sorbed onto the surfaces of particles (Brezonik *et al.*, 1991), and most metals are taken up in the ionic form (Kotze *et al.*, 1999). Free metal ions cause more serious damaging effects on aquatic organisms than their more complex forms (James *et al.*, 1998). The duration of exposure of a specific concentration of toxin can influence rather it will kill an aquatic organism (Skidmore, 1964).

Copper

Copper compounds are also used to control algae, kill slugs and snails in irrigation water systems and municipal water treatment systems. The United States Environmental Protection Agency (USEPA) classifies copper sulphate as a pesticide.

Although copper is important, it is toxic when concentrations exceed than that of natural (<0.05 mol/L) concentrations (Stouthart *et al.*, 1996). At concentrations even found in natural waters, the ionic form of copper is very poisonous towards photosynthesis and growth of unicellular algae (Steemann Nielsen *et al.*, 1970).

Copper is one of the world's most widely used metals (DWAF, 1996), with the electrical industry probably making use of it, the most. It reaches aquatic systems through anthropogenic sources such as industrial, mining, plating operations, usage of copper salts to control aquatic vegetation or influxes of copper containing fertilizers (Nussey, 1998). Copper concentrations in locations receiving anthropogenic inputs such

as mine tailing discharges can vary anywhere from natural background to 100 µg/L (Hem 1989; Lopez and Lee 1977) and have in some cases been reported in the 200,000 µg/L range in mining areas (Robins et al. 1997). Mining leather and leather products, fabricated metal products and electric equipment are a few of the industries with copper-bearing discharges that contribute to anthropogenic inputs of copper to surface waters (Patterson et al. 1998).

Fish and its importance

Fish occupy a significant position in the socio-economic fabric of south Asian countries by providing the populations not only with the nutritious food but also with income and employment opportunities. Of the 21,723 fish species known to science, over 40 % live in the fresh waters and majority of them live in tropics between latitude 2 3.5° N and 23.5° S. No where in the world is a geographic region so blessed as Indian subcontinent (India, Nepal, Burma, Sri Lanka and Bangladesh) in respect with the diversity of fish wild life that dwells the inland waters (Talwar and Jhingran, 1992).

Cyprinion watsoni belongs to cyprinide group found in the streams in hilly areas of Northern Pakistan and extends up to Afghanistan, Iran, some parts of Syria and eastern corner of Arabian Peninsula (Jaya Rao, 1981).

This is a small (Maximum size 12 cm)cyprinid fish which is commonly found in the hilly stream of Islamabad(33.3° N,73° E).where the surface water temperature ranges between 14°C in the coldest month and 29°C in the warmest months. The reproductive cycle of this species comprises a spawning season between March and May(Spring to early Summer) followed by post spawning period extending between June and August .This is followed by a quiescent period lasting from September to November .Gonadal recrudescence begins in December (preparatory period) when the gonads show first signs of proliferative activity. The fish enter a pre-spawning phase during January to March when gametoganic progress becomes pronounced and the fish ultimately reach the final stage

of preparedness to start spawning in March/April and May/June (Shaikh and Jalali., 1986).

Effect of Copper on Fish

Copper, a common toxin in water, has an unclear mode of action on aquatic organisms, but toxicity is largely attributable to Cu^{2+} (EIFAC, 1978), that forms complexes with other ions (Nussey, 1998). Changes in the amount of free Cu^{2+} in solution affect the amount of copper that is bioavailability and hence its toxicity (Welsh *et al.*, 1993). A reduction in water dissolved oxygen, hardness, temperature, pH and chelating agents can increase the toxicity Cu^{2+} (Nussey, 1998). Organic and inorganic substances can easily complex the cupric form of copper, which is the most common specification of this metal and then adsorbed on to particular matter. Therefore, the free ion is rarely found except in pure acidic soft water (EIFAC, 1978). The chemical specification of copper strongly depends on the pH of water (Stouthart *et al.*, 1996). Copper in water precipitates at high pH (alkaline) and is thus not toxic, whilst at low pH (acidic) it is mobile, soluble and toxic (Nussey, 1998).The main difference in copper toxicity between mammals and fish concerns environmental uptake, occurring almost exclusively through the gills in fish. This organ is the principal site of toxic insult and important in the start of compensatory responses (Pelgrom *et al.*, 1995).

In polluted water, copper is often present with zinc. Toxic effects of copper on fish are also influenced by the chemical nature of water, such as calcium hardness (Tabata, 1969, Albaster and Lloyd, 1982) temperature and pH (Laws, 1981).

Copper can affect the life of fish when copper sulphate is used as algaecide and molluscicide in fishponds (Lioyd, 1992). Other copper salts like subacetate, oxychloride, chloride and oxide etc have fungicidal properties. Inspite of all these values, copper salts are toxic in nature and cause poisoning (Clark et al., 1981).

Copper's toxic effects in fish include; changes in biochemistry, anatomy, physiology, histology and behaviour. The lowest treatment of copper (0.03 mg Cu/L) cause little change in fish behavior, which may be the avoidance behavior of animals to pollutants in the receiving water. A significant response to 0.06 mg Cu/L when noted was the swimming activity and breathing rate of the fish increased. In the highest treatment (0.12 mg Cu/L) fish became lethargic and lost equilibrium.

Acute copper poisoning is rare but cases have been recorded when animals have been mistakenly given a too large therapeutic dose and occasionally when they have obtained access to food contaminated with copper salts. At the cellular level, copper inhibits the sodium/potassium-ATPase, causes lipid peroxidation, and can produce morphological damage, leading to disturbances in sodium the vertebral column occurred. (Muth, 1952).

Copper exposures cause reduced growth, often with impacts to specific growth rates most evident during initial exposure times (Marr *et al.*, 1996). It also interferes in bronchial ion transport and affects various blood parameters such as plasma ion concentrations, hematological parameters, and enzyme activities in blood and liver (Stagg and Shuttleworth, 1982). It may also cause immunosuppression, vertebral deformities and neurological disorders (Stouthart *et al.*, 1996).

Copper has been shown to affect swimming performance growth, and reproductive success in a variety of teleosts (Stagg and Shuttleworth, 1982). Handy *et al.* (1999) noted that copper affected routine swimming activity of rainbow trout. It is possible that locomotory activity of contaminated fish reduced as a type of metabolic 'sparing effect' to enable copper detoxification without associated feeding efficiency and growth rate parameter reductions.

Copper adversely affects fry survival, fry growth and reproduction of blunt nose minnows, *Pimephales notatus,* according to Horning and Neiheisel (1979). They found that the size of adult fish was affected negatively at higher concentrations, with

lower concentrations resulting in increased size. The copper was also found to affect sexual maturation in males with effects such as immaturity and a reduction in spermatozoa in the testes, with mature males full of motile sperm. Eggs present in females were either poorly developed, or in the process of being resorbed in exposure ranges from 0.018 to 0.119 mg Cu/L.

Marr *et al.* (1996) observed significant accumulation of copper in rainbow trout fry, and this accumulation was both dose- and time-dependent. Their experiments also demonstrated a clear relationship between copper residues and growth responses. The exposure of copper ions in distilled water elicited remarkable malformation in the nervous system of *B. rerio*, from the rhombencephalic region to the anterior margin of the dorsal fin (Stouthart *et al.*1996). It was noted that the rhombencephalic region appeared helical. It was not determined whether this induced spirality of the nervous system occurred primarily as an effect of copper ions on the nervous system or due to secondary action by interference with the notochord. Results of Stouthart *et al.* (1996) clearly show that a reduction in water pH causes an increase in copper toxicity towards the early life stages of the common carp (*Cyprinus carpio*).

Copper might also affect the gas exchange systems for fine regulation of buoyancy in fish. Stouthart *et al.* (1996) also reported that premature hatching, a concentration-dependent increase of larval mortality, and larval deformation was observed in the carp.

Lewis and Lewis (1971) noticed that golden shiners, *Notemigonus crysoleucas,* which were exposed to 5 mg/l copper sulphate, became restless and lost their 'ability' to school. Later they became very sluggish, completely lost their equilibrium, and died eventually after 46 hours. There was a coagulated layer of mucous present on the gill chambers and bodies, and a noteworthy decrease in the osmolality of the blood. Schreck and Lorz (1978) found that copper exposure to Coho Salmon (*Oncorhynchus kisutch*), was followed by a large, dose-dependent cortisol response.

Reproductive toxicity is defined as adverse effect on male and female reproductive system that results from exposure to chemical substances. Reproductive toxicity may be expressed as alteration in sexual behavior, decrease in fertility or loss of fetus during pregnancy. A reproductive toxicant may interfere in sexual functioning or reproductive ability if individuals are exposed from puberty throughout adulthood. (Ishaq, 2001).

Over the past 20 years artificial chemicals and substances are causing 8% fish kill. Fish population can display the effect of exposure of the reduced viability sperm, egg and larval increased incidence of abnormalities and reduced life expectancy. Reproductive dysfunction in aquatic species as a result of contaminant exposure is a considerable current interest (Collier, 1992).

The purpose of the present investigation was to study the histomorphological aspects of testicular tissues of Cyprinion watsoni.Gonadosomatic index (GSI) and Faulton's condition factor (K) were calculated to know whether testes are the most probable target organs.

MATERIALS AND METHODS

Material And Method

Procedure and Maintenance of Fish

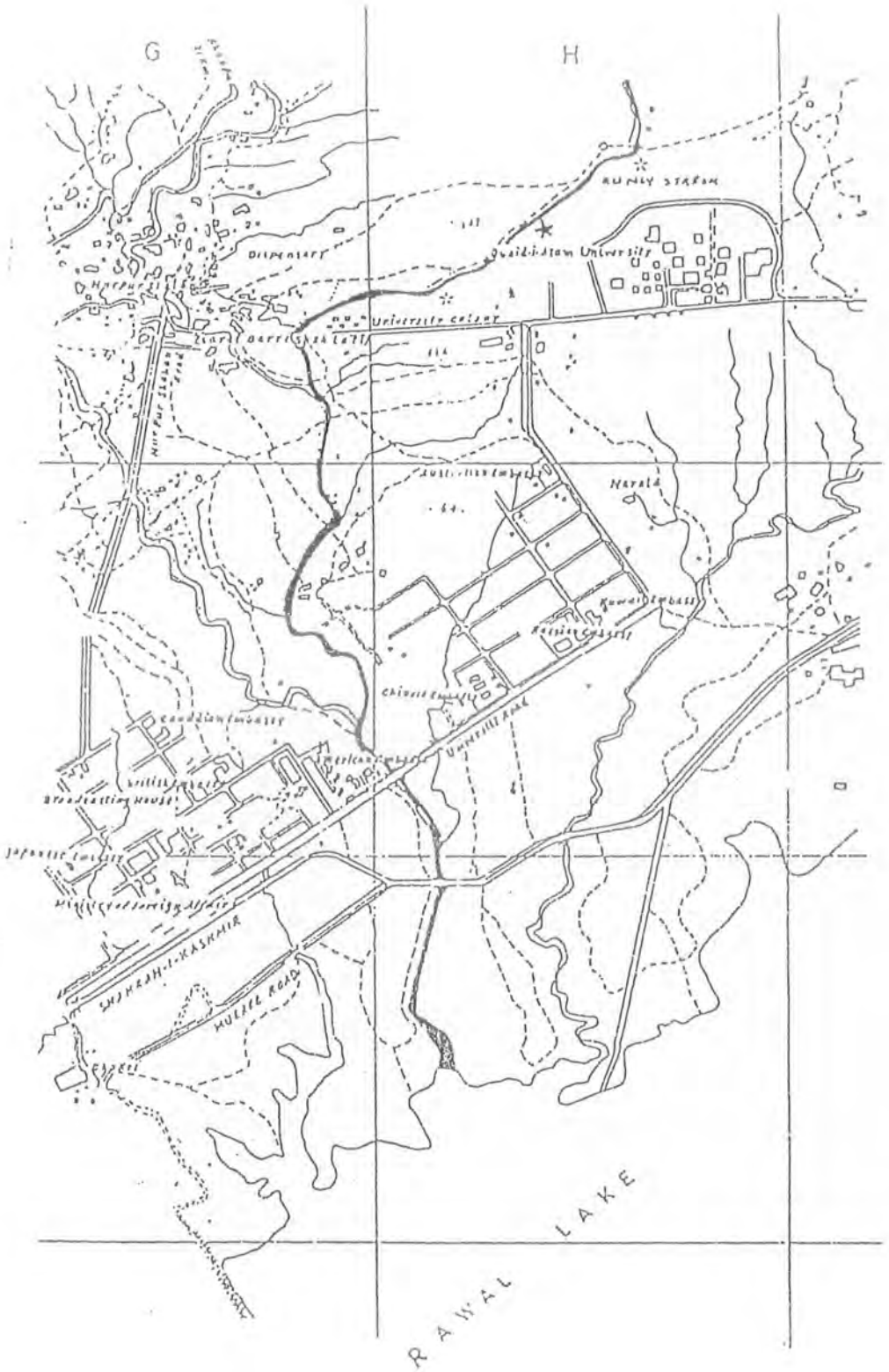
The fish of the medium size were collected in the start of July 2004. The size of fish used for present study ranged from 6-9 cm.

Live specimens of *Cyprinion watsoni* were collected with cast and nets from Ramly stream. The fish were transported to the experimental fish laboratory of the Department of Biological Sciences Quaid-e-Azam University Islamabad and kept in stocking glass aquaria that have the total capacity of 90 litres, containing 70 litres of water. They were allowed to acclimatize to the ambient environmental conditions for at least two weeks prior to the start of experiment.

The fish were fed daily on tropical fish food and were maintained in a photoperiod of 12 light and 12 dark using fluorescent tubes light and automatic timer clock, placed 10 inches above the water surface. The water was changed after alternate day. The fish were maintained at room temprature.

Preparation of Copper Solution, Route of administration and Dose.

The desired concentrations of copper were achieved using copper sulphate ($CuSO_4.5H_2O$). The copper sulphate for research purpose was taken from store of Department of Biological sciences Quaid-e-Azam University Islamabad. 0.08 ppm(parts per million) concentration of copper was selected. Copper solution was administrated through water of aquaria by dissolving calculated amount of the solution in the water of aquaria .Two aquaria were administrated with 0.08 ppm and other two aquaria were controlled.



Part map of Islamabad showing Ramly stream (Solid line) and sampling sites (*)

Experimental Design:

Before the start of experiment, the fish were stunned by placing them in petri dish containing ice flakes. The fish lengths were than measured from the tip of the snout to the tail with the help of vernier caliper. The fish were than weighed on Metter,s balance to the nearest grams and were grouped in different experimental aquaria.

In order to investigate the effect of copper on testicular histomorphology of *Cyprinion watsoni*, 0.08 ppm concentrations of copper was selected. Before treatment, the fish having length 6-9 cm were divided into four groups containing 24 animals each, placed in individual glass aquaria. Two groups (n=48) were maintained in separate glass aquaria as a control group and two groups (n=48) were maintained in separate glass aquaria as treated group. The desired concentrations of copper were achieved using copper sulphate ($CuSO_4$. $5H_2O$). The aquaria were cleaned and the test concentration restored after every alternate day. The duration of the experiment was 28 days.

Dissections were made after every seven days. After 7 days 12 fish were taken from each control and treated groups. Before dissections the weight of fish was measured to nearest grams and body length in centimeters. Then the testes of fish were dissected out, weighed to nearest (mg) and their length and breadth in (cm) was measured. Testes were immersed in fixative sera. Same process was repeated after 14,21 and 28 days respectively.

Histology and Cytometry:

Two stains were used in staining slides Hematoxylin and Eosin, the procedure to prepare these stains is as follows:

Ehrlich's Alum Hematoxylin:

Hematoxylin	6g	dye
Ethanol 95%	300 ml	Solvent

Potassium Alum	excess (50 g)	mordant
Distilled water	300 ml	solvent
Glycerol	300 ml	Stabilizer
Glacial Acetic Acid	30 ml	Acidifier

Procedure:

Dissolved the Hematoxylin in the ethanol mixed with acetic acid. Dissolved the alum in the water mixed with glycerol in an oversized container. Added the Hematoxylin solution to the alum solution. Plugged the container loosely with cotton wool. Ripened by leaving in a warm; sunlit place for several weeks. When sufficiently ripened, store tightly stoppered in a cool, dark place. The solution was stable for years.

Eosin Preparation:

Ethanol 70%	100 ml
Eosin	1g

Took 100 ml of ethanol (70%) and added 1g Eosin dye in it and mixed thoroughly and then used to stain the slides.

The process followed during histology is mentioned below. The testes fixed in fixative sera for 4-5 hours before further processing. The composition of sera was:

Absolute Alcohol = 60 ml

Formaldehyde =30 ml

Glacial acetic Acid =10 ml

After fixation testes were dehydrated in ascending grades of alcohol in a following manner.

80 %	over night
90 %	for 2 hours
100 %	for 5 hours

The testes were then transferred to cedar wood oil and left in it until they became transparent. After this the testes were embedded in paraplast by the following method.

Benzol I	=	10 minutes (At room temperature)
Benzol II	=	10 minutes (At room temperature)
Benzol +Paraplast	=	20 minutes at 60 °C
Parapalst I	=	12 hours at 60 °C
Paraplast II	=	12 hours at 60 °C
Paraplast III	=	12 hours at 60 °C

After this process testes were ready to make blocks. The sections were cut out of paraffin block at the thickness of 6μm by using Reichert Microtome. Sections were affixed to pre cleaned albumenized glass slides and stretched at 60 °C on Fisher slide warmer and then transferred to paraffin oven for 12 hours for complete deparafinization. The slides were then transferred to xylene for half an hour to remove any remaining wax. The slides were then dehydrated in the descending grades of alcohol, washed in tap water and stained in haematoxylin and dehydrated in the ascending grades and counter stained with eosin). The slides were hydrated, dehydrated in the following ways:

Xylene I	=	15 minutes
Xylene II	=	15 minutes
100 % alcohol	=	2-5 minutes
90 % Alcohol	=	2-5 minutes
70 % Alcohol	=	2-5 minutes
50 % Alcohol	=	2-5 minutes

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30 % Alcohol	=	2-5 minutes
Tap water	=	2-3 dips
Hematoxylin	=	2-3 dips
Tap water	=	10 minutes
30 % Alcohol	=	2-5 minutes
50 %Alcohol	=	2-5 minute
70% Alcohol	=	2-5 minutes
90 % Alcohol	=	2-5 minutes
Eosin	=	2-3 dips
90% Alcohol	=	1 dip
100 %Alcohol=	=	3-5 minutes
Xylene	=	10 minutes

Now the slides were mounted with Canada balsam. Microscopic examination of slides was carried out under a Nikon optiphot research microscope equipped with an automatic microphotographic system.

Histological details and morphometric data, in combination with macroscopic features of the testes were used to determine any change caused by the toxicant in treated groups with reference to control group.

Record of body weight and testicular weight were used to determine gonadosomatic index (GSI) which was calculated according to the formula.

$$GSI = \frac{\text{Weight of the gonad (g)}}{\text{Weight of the fish (g)}} \times 100$$

Condition Factor:

Condition factor was calculated according to formula.

$$K = \frac{\text{Body weight in (g)}}{\text{Length (cm)}^3}$$

After this student "t" test was applied to analyze the data.

RESULTS

Results

Overall Behavior and Mortality:

During the entire experimental period overall behavior of the fish was observed. After first exposure of fish to 0.08 Cu, uneasiness was observed while in control group fish were more comfortable. In treated groups fish move to bottom of aquaria and gathered there in the form of cluster. In the bottom the movement of fish was very fast and circular, sometime striking with the walls of aquaria. Abundant mucus secretion observed in treated group as compared to control group. With the passage of time fish tend to recover from disturbed state and the frequency of abnormal behavior decreased. Few mortalities occurred on 27^{th} day of experiment.

Body weight (mg):

Mean body weight of control and Cu treated (0.08 ppm) groups are given in table 1 and Fig.1. Among all the treated groups the mean values decreased as compared to control groups but this decrease was non significant ($p > 0.05$).

Standard body length (cm):

Measurement of the total length of both control and treated groups remained unaltered. Little increase was found in all treated groups as compared to control group but these results were non significant ($p > 0.05$). Mean values of all control and treated groups are given in table 1 and fig.2. This result showed that over all growth of fish was affected by toxicant.

Condition factor (K):

Mean values of condition factor of all the control and treated groups are given in table 2 and Fig.3. The condition factor of all the control groups and treated groups showed non significant ($p > 0.05$) difference.

Gonadosomatic Index(GSI):

Effect of copper on the GSI of treated and control groups were compared. A non significant ($p > 0.05$) difference was noted in Group 1 (7 days) and Group 2 (14 days). In case of Group 3 (21 days) and Group 4 (28 days) GSI values for treated groups decreased significantly ($p<0.05$) as compared to their control groups. Mean values of GSI of all the control and treated groups are given in table 2 and Fig.4.

Table:1

Effect of copper (0.08 ppm) on body length and body weight of *Cyprinion watsoni:*

Groups	Body Weight(g)	Body Length(cm)
Group:1(7 days) Control (n=5)	9.36±0.23	7.33±0.35
Group:1(7days) Treated (n=6)	9.22±0.72	7.52±0.49
Group:2(14days) Control (n=4)	7.49±0.24	7.37±0.48
Group:2(14days) Treated (n=5)	7.37±0.20	7.96±0.39
Group:3(21days) Control (n=4)	10.40±0.67	8.03±0.23
Group:3(21days) Treated (n=4)	10.41±0.67	8.33±0.22
Group:4(28days) Control (n=3)	9.24±1.35	7.72±0.61
Group:4(28days) Treated (n=5)	8.54±0.33	7.86±0.19

Value(Mean±S.E) Student "t"test.
P>0.05 Treated compared with control.

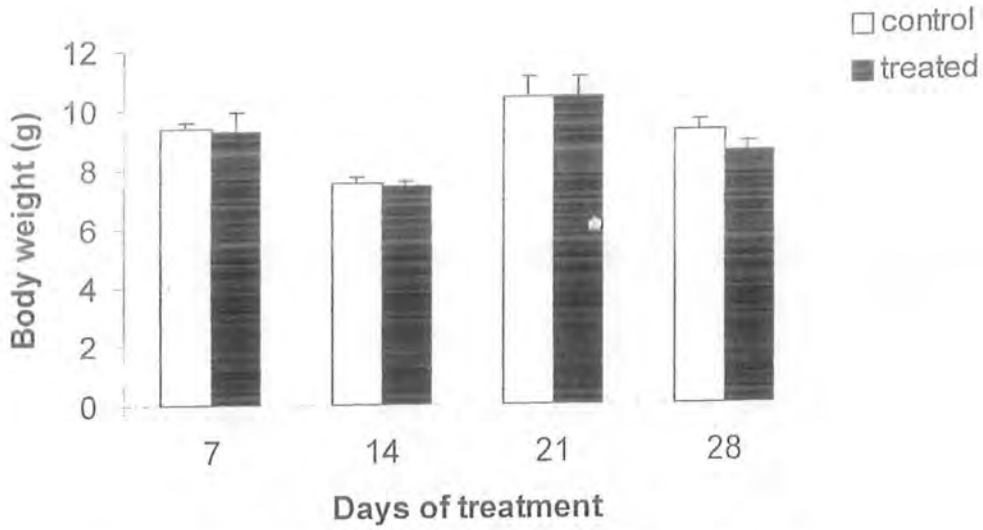


Fig:1 Effect of copper (0.08 ppm) on the body weight of fish (*Cyprinion watsoni*).
Values (Mean±S.E), student "t" test
P > 0.05 Treated compared with control.

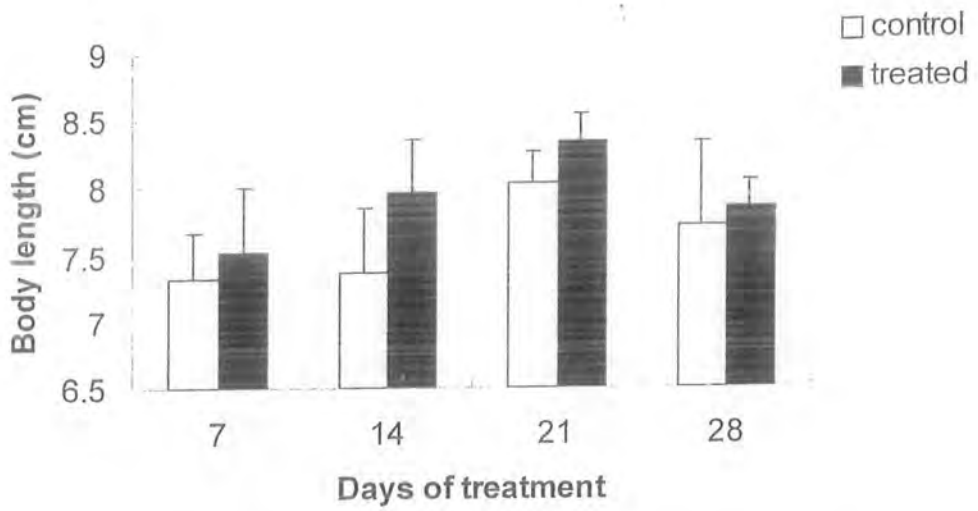


Fig:2 Effect of copper (0.08 ppm) on the body length of fish (*Cyprinion watsoni*).
Values (Mean±S.E), student "t" test
P > 0.05 Treated compared with control.

Testicular weight (mg):

Mean values of testicular weight of both control and treated groups are given in table 3 and Fig.5. In treated groups testicular weight decreased as compared to control groups and was non significant ($p > 0.05$).

Testicular length (cm):

Mean testicular lengths of both right and left testes of control and treated was compared. In case of group 1(7 days) and group 2 (14 days) non significant ($p > 0.05$) difference was observed but in group 3 (21 days) and group 4 (28 days) treated fish showed a significant ($p < 0.05$) decrease in testicular length as compared to control groups. Mean values of testicular lengths of both control and treated groups are given in table 4 and Fig.6.

Testicular breadth (cm):

Mean breadth of right and left testes of both control and treated groups are given in table 4 and Fig.7. Among all four groups the breadth of both testes decreased but statistically this decrease was non significant ($p > 0.05$).

Gross Morphology of Testes:

The testes of *Cyprinion watsoni* are a paired elongated organs suspended by lengthwise mesenteries in the upper section of the body and are found alongside the swim bladder. The sperm ducts of both the testes unite to form a common duct that opens to the exterior by a gonopore. The size and the color of testes varied according to the stage of sexual maturity and ripness.

A thin layer of connective tissue surrounds the testes, the tunica albuginea which is continuous with the connective tissue fiber of the wall of seminiferous lobules. Seminiferous lobules are separated by the narrow spaces that are filled by the interstitial tissue containing Leydig cells, connective tissue and blood vessels. Spermatogonia type "A" lie on the inner side of the lobule wall. As the process of spermatogenesis progresses, each lobule consists of a number of cysts which possess spermatogonia type "B" and different stages of spermatogenic cells. Spermatogenesis takes place with in the cysts of the lobules and the spermatids get nutrition by attaching themselves to sertoli cells and the mature spermatozoa are released into the spermatic ducts.

The following main stages of spermatogenic cycle were observed in the fish *Cyprinion Watsoni* by microscopic studies.

Stage I:Spermatogonia type "A"(Stem spermatogonia):

These are large spherical cells possessing clear nucleus with distinct nuclear membrane to which chromatin granules are attached. The spermatogonia type "A" have slightly little eosinophilic cytoplasm which is not very conspicuous (Fig.11b).

Stage II: Spermatogonia type "B":

These cells are smaller than the spermatogonia type "A". The nuclei have clearly visible nuclear membrane to which chromatin granules are attached towards the inner periphery. They also possess a prominent nucleolus. Like the spermatogonia type "A" they also have very little amount of cytoplasm (Fig.11b).

Stage III: Primary spermatocytes:

Spermatogonia type "A" divide mitotically into spermatogonia type "B" which in turn divide mitotically and produce primary spermatocytes which are smaller than spermatogonia type "B". The nuclei are distinct with densely stained chromatin. The cytoplasm is scanty. These cells are most abundant during breeding season and are located within the cyst of lobules (Fig.11b).

Stage IV: Secondary spermatocytes:

Primary spermatocytes undergo meiotic division and give rise to secondary spermatocytes which are smaller than primary spermatocytes. The darkly stained chromatin of the nucleus is eccentrically placed .

Stage V: Spermatids:

Secondary spermatocytes are soon converted into round spermatids whose nuclear diameter is smaller than those of the secondary spermatocytes (Fig.11a,b).

Stage VI: Spermatozoa:

The spermatids give rise to sperms or spermatozoa, which have a small rounded head, a small neck with a clearly visible elongated tail.

Table:2

Effect of copper(0.08 ppm) on Gonadosomatic index (GSI) and Faulton's Condition factor (K) of *Cyprinion watsoni*

Groups	GSI	K
Group:1(7days) Control (n=5)	0.86±0.21	0.03±0.003
Group:1(7days) Treated (n=6)	0.83±0.18	0.02±0.003
Group:2(14days) Control (n=4)	1.26±0.03	0.02±0.003
Group:2(14days) Treated (n=5)	1.20±0.28	0.02±0.001
Group:3(21days) Control (n=4)	1.24±0.32	0.02±0.001
Group:3(21days) Treated (n=4)	0.81±0.06*	0.02±0.001
Group:4(28days) Control (n=3)	1.47±0.68	0.02±0.002
Group:4(28days) Treated (n=5)	0.85±0.19*	0.02±0.001

Value(Mean±S.E) Student "t"test.
P<0.05* Treated compared with control.

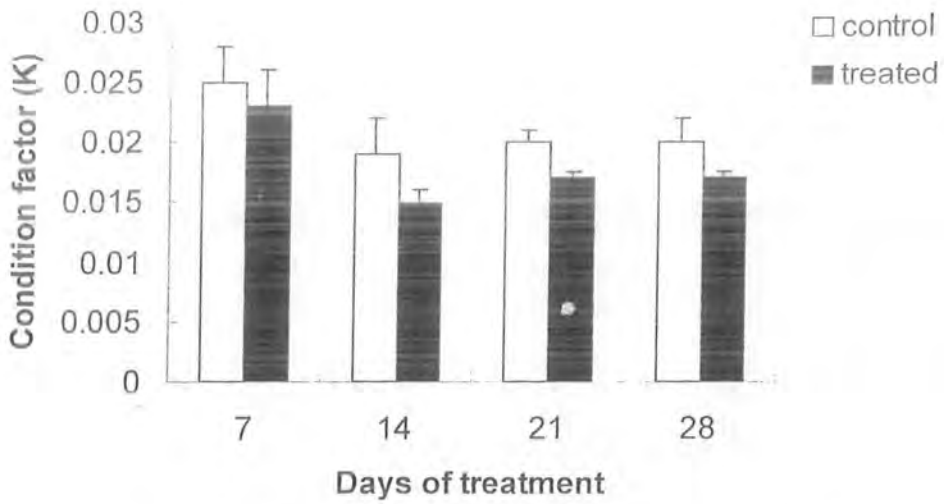


Fig:3 Effect of copper (0.08ppm) on the condition factor (K) of fish (*Cyprinion watsoni*).
Values (Mean±S.E), student "t" test
P > 0.05 Treated compared with control.

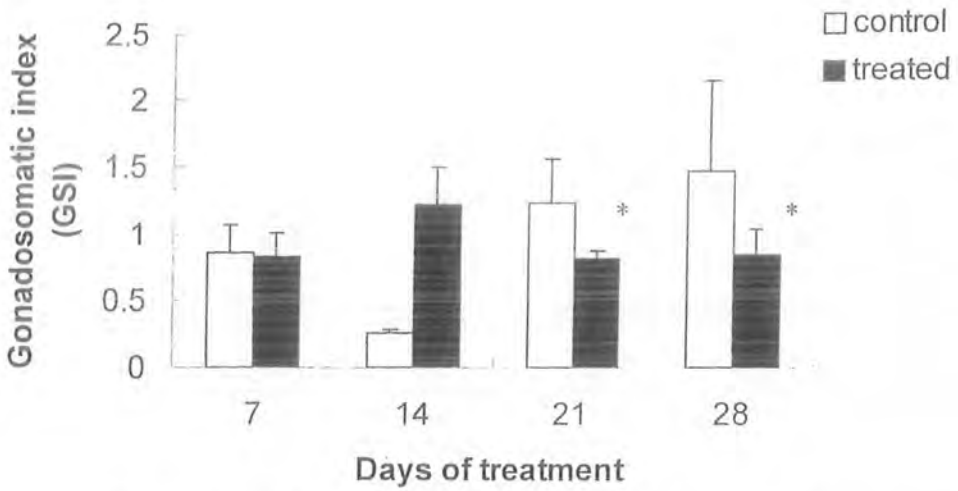


Fig:4 Effect of copper (0.08ppm) on the gonadosomatic index of fish (*Cyprinion watsoni*).
Values (Mean±S.E), student "t" test
P* < 0.05 Treated compared with control.

Table:3

Effect of copper(0.08ppm) on testicular weight (mg) of *Cyprinion Watsoni.*

Groups	Testicular weight(mg)		
	Right	Left	Total
Group:1(7days) Control (n=5)	38.48±9.48	43.98±12.34	82.46±21.76
Group:1(7days) Treated (n=6)	40.56±10.22	41.51±10.51	82.03±20.69
Group:2(14days) Control (n=4)	47.23±2.08	47.48±1.72	94.70±3.76
Group:2(14days) Treated (n=5)	46.32±12.11	45.62±11.81	91.94±23.89
Group:3(21days) Control (n=4)	62.02±16.77	65.33±15.86	127.35±32.58
Group:3(21days) Treated (n=4)	41.32±4.50	43.02±3.22	84.35±7.71
Group:4(28days) Control (n=3)	71.26±40.06	74.66±42.12	145.93±82.18
Group:4(28days) Treated (n=5)	35.06±7.46	36.48±7.30	71.54±14.76

Value(Mean±S.E) Student "t"test.
P>0.05 Treated compared with control.

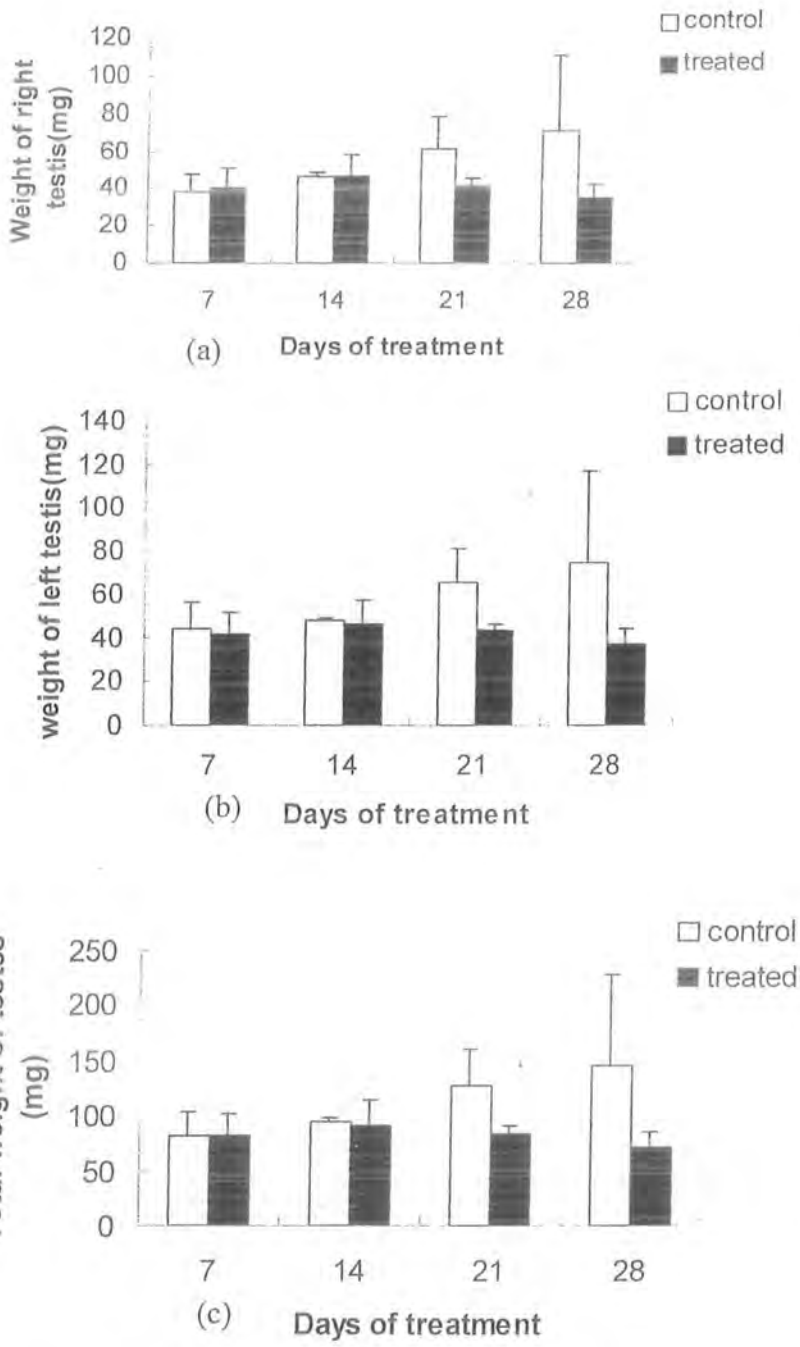


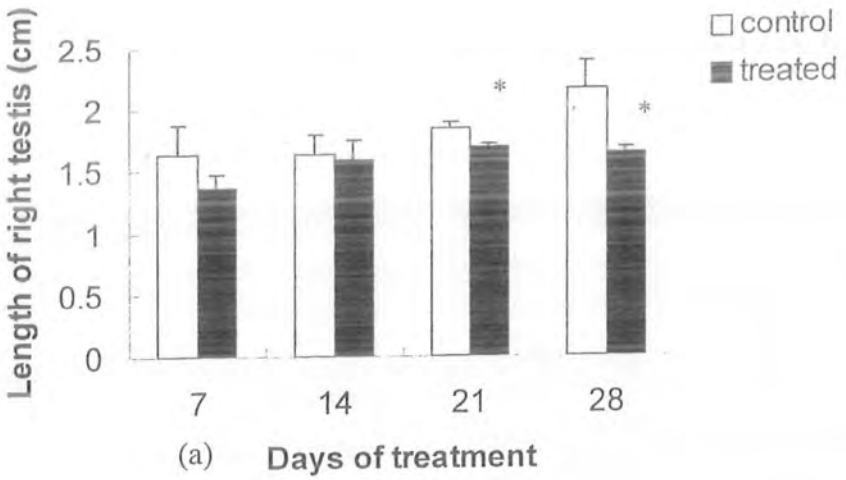
Fig:5 Effect of copper (0.08 ppm) on the testes of fish (Cyprinion watsoni). (a) weight of left testis, (b) weight of right testis, (c) total weight of testis.
Values (Mean±S.E),student "t" test
$P > 0.05$ Treated compared with control.

Table:4

Effect of copper(0.08ppm) on testicular length and breadth (cm) of *Cyprinion watsoni.*

Groups	Testicular Length (cm)		Testicular Breadth (cm)	
	Right	Left	Right	Left
Group:1(7days) Control (n=5)	1.63±0.23	1.65±.0.23	0.34±0.02	0.33±0.02
Group:1(7days) Treated (n=6)	1.36±0.12	1.37±0.11	0.35±0.02	0.34±0.02
Group:2(14days) Control (n=4)	1.63±0.17	1.62±0.17	0.38±0.02	0.38±0.03
Group:2(14days) Treated (n=5)	1.58±0.16	1.57±0.17	0.37±0.02	0.37±0.02
Group:3(21days) Control (n=4)	1.84±0.04	1.83±0.03	0.39±0.04	0.40±0.05
Group:3(21days) Treated (n=4)	1.69±0.02*	1.71±0.03*	0.34±0.03	0.35±0.03
Group:4(28days) Control (n=3)	2.16±0.21	2.19±0.20	0.39±0.06	0.39±0.05
Group:4(28days) Treated (n=5)	1.63±0.04*	1.63±0.05*	0.34±0.02	0.32±0.02

Value(Mean±S.E) Student "t" test
P<0.05* Treated compared with control.



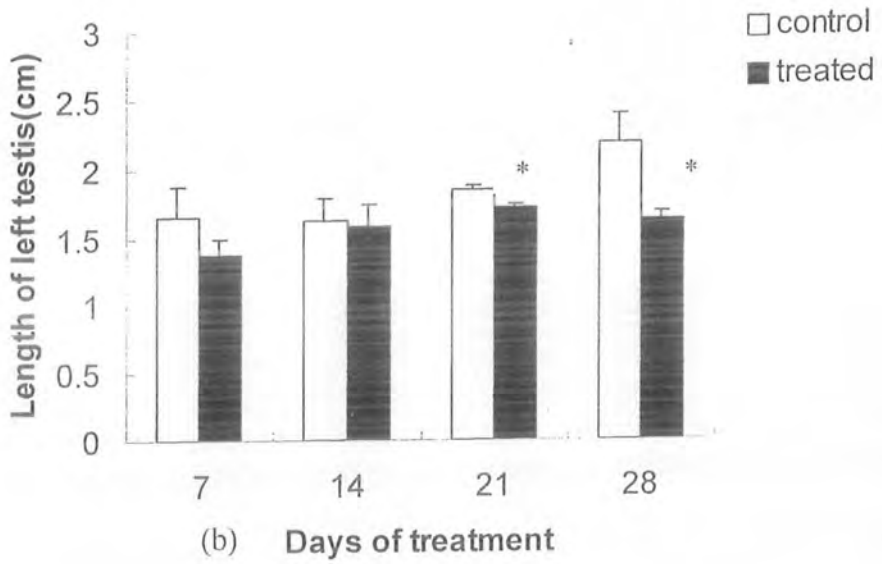


Fig:6 Effect of copper (0.08 ppm) on the testicular length (cm) of fish (Cyprinion watsoni). (a) right testis length, (b) left testicular length.
Values (Mean±S.E), student "t" test
P* < 0.05 Treated compared with control.

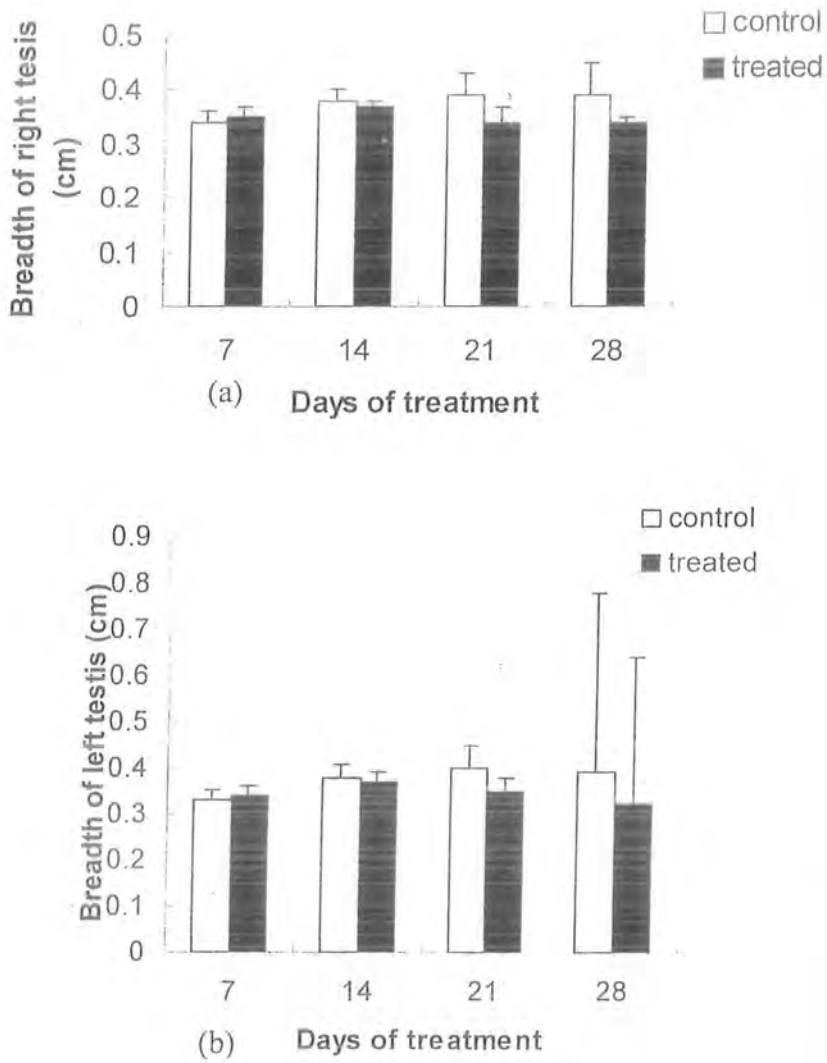


Fig:7 Effect of copper (0.08 ppm) on the testicular breadth of fish (*Cyprinion watsoni*). (a) Breadth of right testis (cm), (b) Breadth of left testis (cm).
Values (Mean±S.E), student "t" test
P > 0.05 Treated compared with control.

Morphometery of Spermatogonia type A, B and Interlobular space/cross section of control and copper (0.08 ppm) treated testes:

Number of spermatogonia type A/cyst:

The mean number of spermatogonia type A/cyst of control and treated groups are given in table 5 and fig.8 (a). In case of Group 1(7 days) the number of spermatogonia type A decreased in treated group and the decrease was significant ($p<0.05$). In case of Group 2(14days), Group 3(21 days) and Group 4(28 days) the number of spermatogonia also decreased in treated groups as compared to control groups and was highly significant ($p< 0.01$).

Number of spermatogonia type B/cyst:

Nnumber of spermatogonia type B of control and treated groups were also compared with each other.The number of B type spermatogonia decreased in case of treated groups as compared to control groups. In Group 1(7 days) and Group 2(14 days) this decrease was significant($p<0.01$). In Group 3(21 days) number of type B spermatogonia decreased significantly ($p<0.05$) but decrease was less as compared to Group 1 and Group 2. In Group 4(28 days) the decrease in number of spermatogonia was highly significant ($p< 0.001$). The mean number of type B spermatogonia are given in table 5 and fig.8 (b).

Inter Lobular Space:

Interlobular space of all the control and treated groups was also measured and compared. The mean values of interlobular space (μm) are given in table 5 and fig.10. Among all the groups inter lobular space increased. In case of Group 1 (7 days) this increase was non significant ($p> 0.05$). In Group 2(14 days) and Group 3(21 days) the interlobular space increased significantly ($p<0.01$).Group 4 (28 days) showed highly significant($p< 0.001$) increase in the size of interlobular space. The mean values of inter lobular spaces are given in table 5 and fig.8 (c).

Diameter (μm) of Spermatogonia type A:

Mean values of diameter of spermatogonia type A are given in table 6 and fig.9 (a).Among all the groups copper treated fish showed non significant($p>0.05$) difference in the diameters of spermatogonia type A.

Diameter(μm) of Spermatogonia type B:

Mean diameter of spermatogonia type B of control and copper treated groups were measured and compared. In case of all the groups non significant ($p> 0.05$) decrease was found. Mean values of diameter are given in table 6 and fig .9 (b).

Nuclear diameter (μm) of Spermatogonia type A:

The nuclear diameter (μm) of all the four groups was measured and compared with their respective control groups. Their values showed a non significant (p>

0.05) decrease in the nuclear diameters of spermatogonia type A. The mean values of nuclear diameter of spermatogonia type A are given in table 6 and fig.10 (a).

Nuclear diameter (μm) of Spermatogonia type B:

The nuclear diameters of all the control and treated groups are given in table 6 and fig.10 (b). A non significant ($p > 0.05$) increase or decrease was noted in case of all the control and copper treated groups.

Table:5

Effect of copper (0.08 ppm) on the number Spermatogonia type A, B and Inter Lobular (μm)Space per cyst of the testes of *Cyprinion watsoni.*

Groups	Spermatogonia type "A"	Spermatogonia type "B"	Interlobular space (μm)
Group:1(7 days) Control (n=5)	8.74±0.27	12.74±0.88	5.94±0.34
Group:1(7days) Treated (n=6)	7.55±0.25*	9.68±0.26**	6.94±0.36
Group:2(14days) Control (n=4)	8.63±0.14	13.28±0.17	4.59±0.12
Group:2(14days) Treated (n=5)	5.96±0.15**	9.46±0.19**	7.26±0.19**
Group:3(21days) Control (n=4)	8.97±0.29	12.38±0.56	4.51±0.17
Group:3(21days) Treated (n=4)	7.03±0.25**	9.90±0.49*	7.06±0.40**
Group:4(28days) Control (n=3)	7.70±0.17	11.36±0.43	4.80±0.15
Group:4(28days) Treated (n=5)	5.70±0.10**	9.02±0.15***	7.52±0.18***

Value(Mean±S.E) Student "t" test
P<0.05*, P<0.01**, P<0.001*** Treated compared with control.

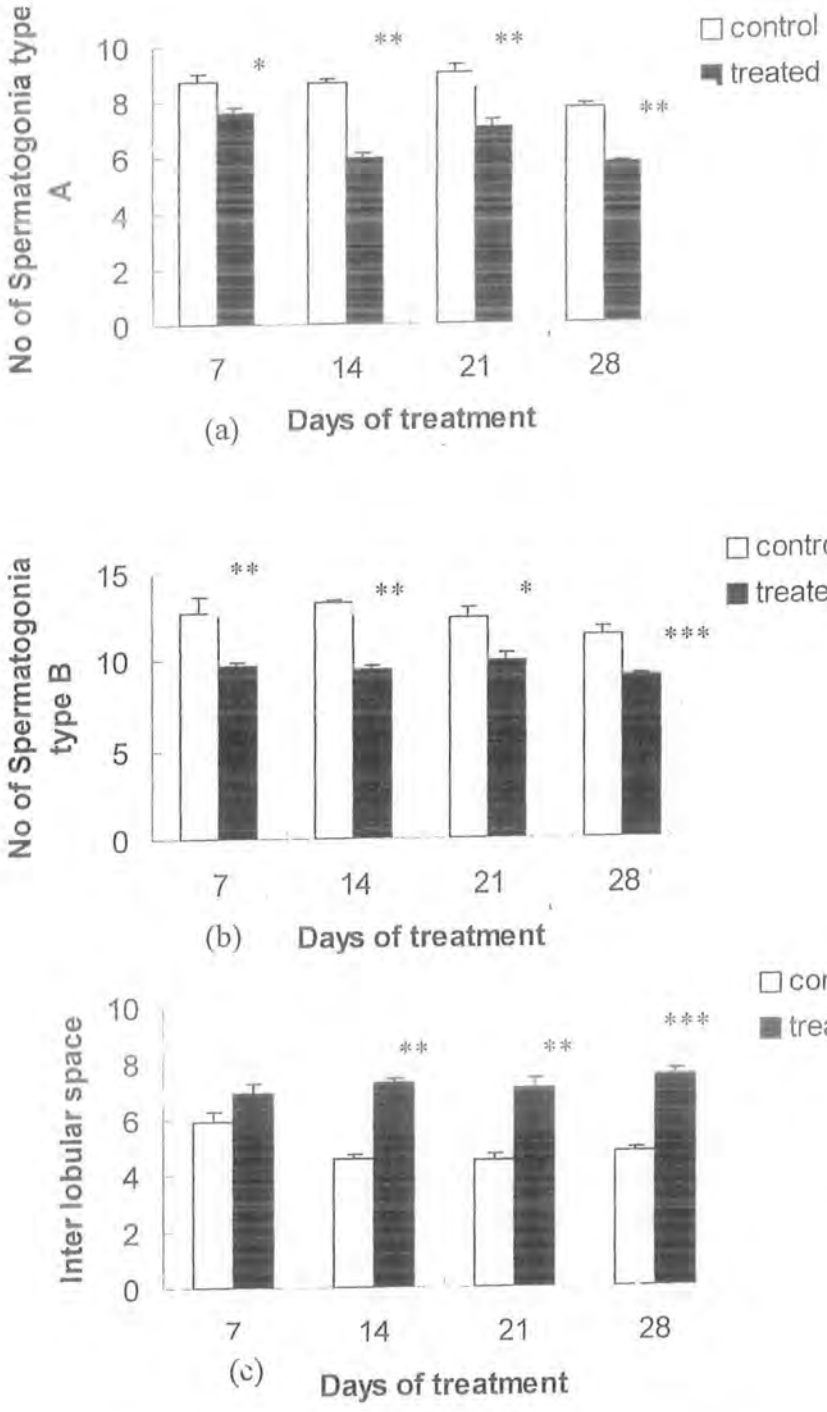


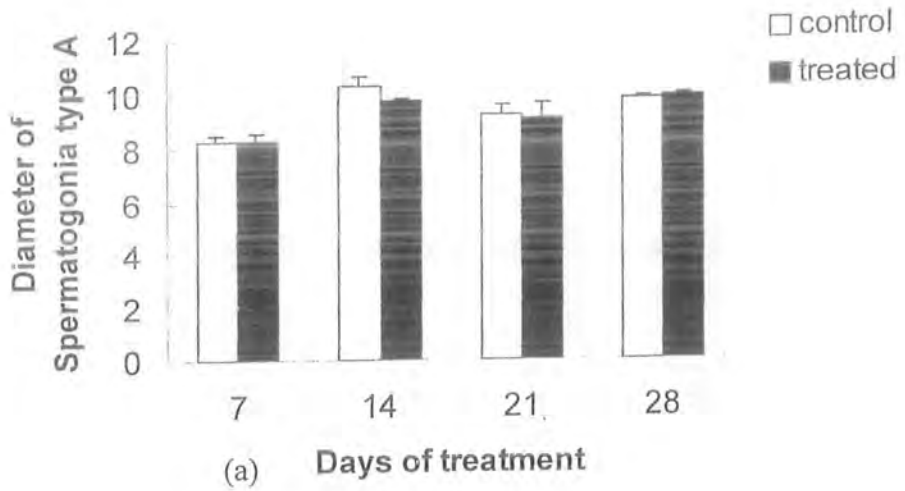
Fig:8 Effect of copper (0.08 ppm) on the Spermatogonia type A, type B and inter lobular space (μm). (a) No of Spermatogonia type A, (b) Spermatogonia type B, (c) Interlobular space ((μm).
Values (Mean±S.E),student "t" test
P*<0.05, p**< 0.01, p***< 0.001 Treated compared with control.

Table:6

Effect of copper (0.08 ppm) on the diameters (μm) and nuclear diameters (μm) of Spermatogonia type A and B per cyst of testes of *Cyprinion watsoni*.

Groups	Diameter of Spermatogonia type "A"(μm)	Nuclear diameter of Spermatogonia Type "A" (μm)	Diameter of Spermatogonia type "B" (μm)	Nuclear diameter of Spermatogonia type "B" (μm)
Group:1(7days) Control (n=5)	8.25±0.25	5.78±0.20	5.30±0.36	3.50±0.16
Group:1(7days) Treated (n=6)	8.26±0.31	5.85±0.25	5.56±0.24	3.56±0.10
Group:2(14days) Control (n=4)	10.26±0.38	7.05±0.08	6.70±0.20	3.81±0.05
Group:2(14days) Treated (n=5)	9.74±0.12	6.82±0.11	6.67±0.13	3.70±0.02
Group:3(21days) Control (n=4)	9.24±0.40	6.58±0.38	6.09±0.23	3.75±0.07
Group:3(21days) Treated (n=4)	9.06±0.63	6.31±0.38	6.26±0.27	3.64±0.10
Group:4(28days) Control (n=3)	9.86±0.08	7.17±0.04	6.10±0.23	3.70±0.04
Group:4(28days) Treated (n=5	9.88±0.12	7.17±0.13	6.02±0.24	3.74±0.04

Value(Mean±S.E) Student "t"test.
P>0.05 Treated compared with control.



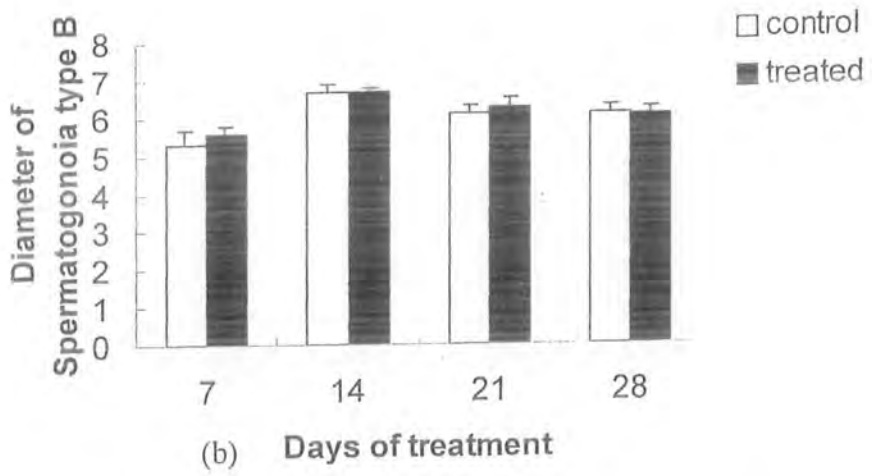


Fig:9 Effect of copper (0.08 ppm) on the diameter (μm) of Spermatogonia type A and type B of fish (*Cyprinion watsoni*). (a) Diameter of Spermatogonia (μm) type A, (b) Diameter of Spermatogonia (μm) type B.
Values (Mean ±S.E), student "t" test
$P > 0.05$ Treated compared with control.

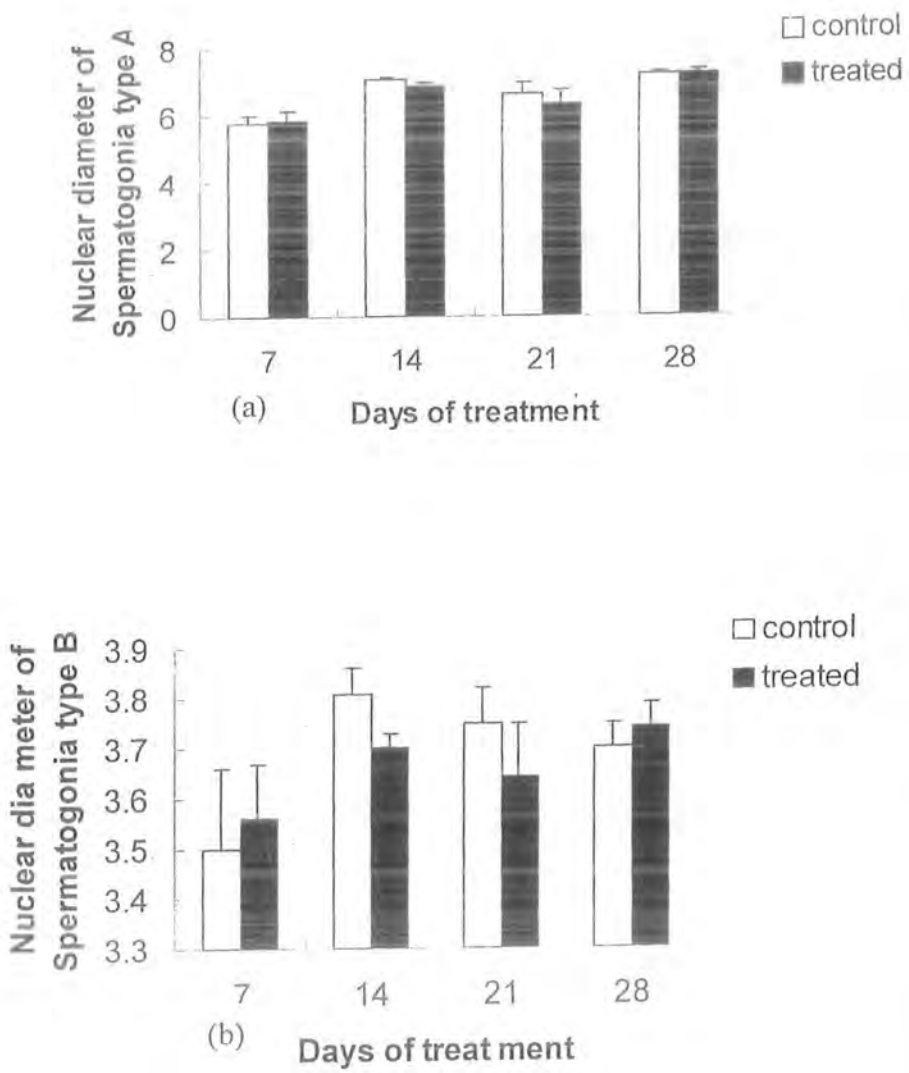


Fig:10 Effect of copper (0.08 ppm) on the nuclear diameter (μm) of Spermatogonia type A and type B.
(a)Nuclear diameter (μm) of Spermatogonia type A
(b) Nuclear diameter (μm) of Spermatogonia type B.
Values (Mean±S.E), student "t" test
$P > 0.05$ Treated compared with control.

Histomorphology

Group 1 (7 days treated with 0.08ppm copper):

Control:

Cross section of the testes of *Cyprinion watsoni* showed many spermatogenic lobules (Fig. 11a). The lobules occupied a larger area of the testis. The interlobular walls separated the lobules. The inter lobular walls were distended and thin. Testis was covered by tunica albugenia on its outer surface. Compactly arranged cysts were present in the lobules. The cyst containing spermatogonia were mostly visible in the peripheral area. There were two types of spermatogonia i.e spermatogonia type A and spermatogonia type B (Fig.11b). Each spermatogonium was a large and spherical compared with other cells and possessed a large and lightly stained nucleus with distinct nucleolus (Fig.11b). Other cyst contained primary and secondary spermatocytes. These spermatocytes were smaller than the spermatogonia, their nuclei were darkly stained and they have scanty cytoplasm (Fig.11b). Round spermatids were also observed in some cysts (Fig.11b). The interlobular space was filled with interstitium.The number of Spermatogonia type A and type B per cyst of lobule was normal. All the stages of spermatogenic cells were in normal state. In control testes the number of clumped cyst was very few, if any.

Treated:

Histomorphological examination of the testicular section of the treated group showed following changes:

The lobules changed their organization and were loosely arranged. The cysts were also arranged loosely as compared to control. The interlobular space increased but this increase was non significant (Fig.11c).The cysts containing spermatogonia type A and type B were fewer, loosely arranged and reduced in number respectively. The number of primary and secondary spermatocytes increased due to arrest of spermatogenic cycle. The primary and secondary spermatocytes showed clumping (Fig.11d). The cyst containing spermatids also increased due to arrest of spermatogenesis. The spermatids showed clumping like primary and secondary spermatocytes (Fig 11d).

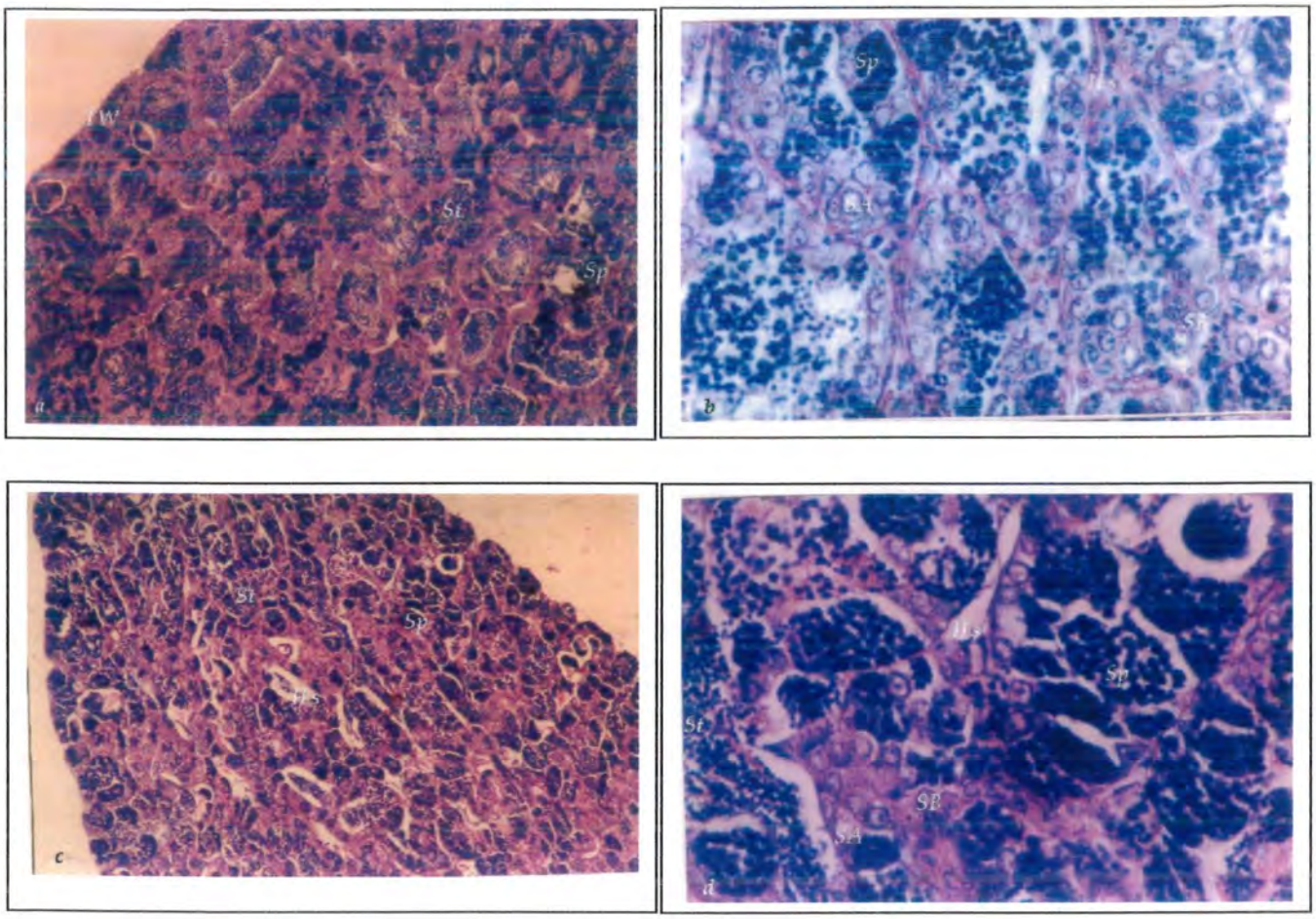


Fig:11 Photomicrograph of control and treated testes (7 days with 0.08ppm Cu)) of fish (Cyprinion watsoni). (a) control group with compact lobules of spermato gonia, spermatocytes (Sp) and spermatida (St) X210.33. (b) control group showing number of spermatogonia type A (SA) and type B (SB),spermatocytes (Sp) and spermatids (St) X841.34. (c) Testes treated with 0.08ppm Cu showing loose lobules, more inter lobular space (ILs) and spermatids (st) X210.33. (d) treated testes with 0.08ppm Cu showing decreased no of spermatogonia type A and typeB (SA & SB), spermatocytes clumping (Sp) and spermatids (St) X841.34.

Group 2 (14 days treated with 0.08ppm copper):

Control:

In case of Group 2(14 days treated with 0.08ppm copper) lobular size increased but all these lobules were compactly arranged. Spermatogonia type A and type B number remained unchanged as compared to control of Group 1(7 days treated with 0.08ppm copper). Interlobular walls were distended and thin.. There was also no change observed in the inter lobular space. Number of spermatid was less as compared to treated group because the spermatogenesis remained unchanged. Few clumpings of spermatocytes and spermatids were also seen in some cysts (Fig.12a).

Treated:

The lobules changed their organization and more loosely arranged (Fig.12b). Number of spermatogonia type A and type B decreased significantly (Table.5). More space was observed between spermatogonia and spermatids. Clumpings were also seen in both spermatogonia type A, type B, spermatocytes and spermatids. In case of spermatids clumpings were more towards the peripheral portion of the cyst (Fig.12b).Number of spermatids and spermatocytes increased due to the arrest of spermatogenic cycle. Interlobular walls increased between the lobules (Fig.12c).

Group 3(21 days treated with 0.08ppm copper):

Control:

Histomorphological study of control group showed no particular change as compared to the control of Group 1 and Group 2. Lobules were compactly arranged. Both Spermatogonia type A and type B were well organized around the peripheral portion of cyst. Interlobular spaces were also normal. Clumping was observed in case of spermatocytes but these were also very few. However in case of spermatids no clumping was found (Fig.13a).

Treated:

Cross section of the testis of *Cyprinion watsoni* of treated group showed following changes:

Interlobular walls were not distinct and lobules were disrupted (Fig.13b).Number of both spermatogonia type A and type B decreased as compared to its control group (Table.5) and this decrease was highly significant ($p< 0.01$). More clumpings were seen in case of spermatocytes as well as in spermatids. Interlobular spaces also increased significantly ($p<0.01$). Number of spermatocytes and spermatids were also observed more as compared to control. This increase was also due to the arrest spermatogenesis (Fig.13b).

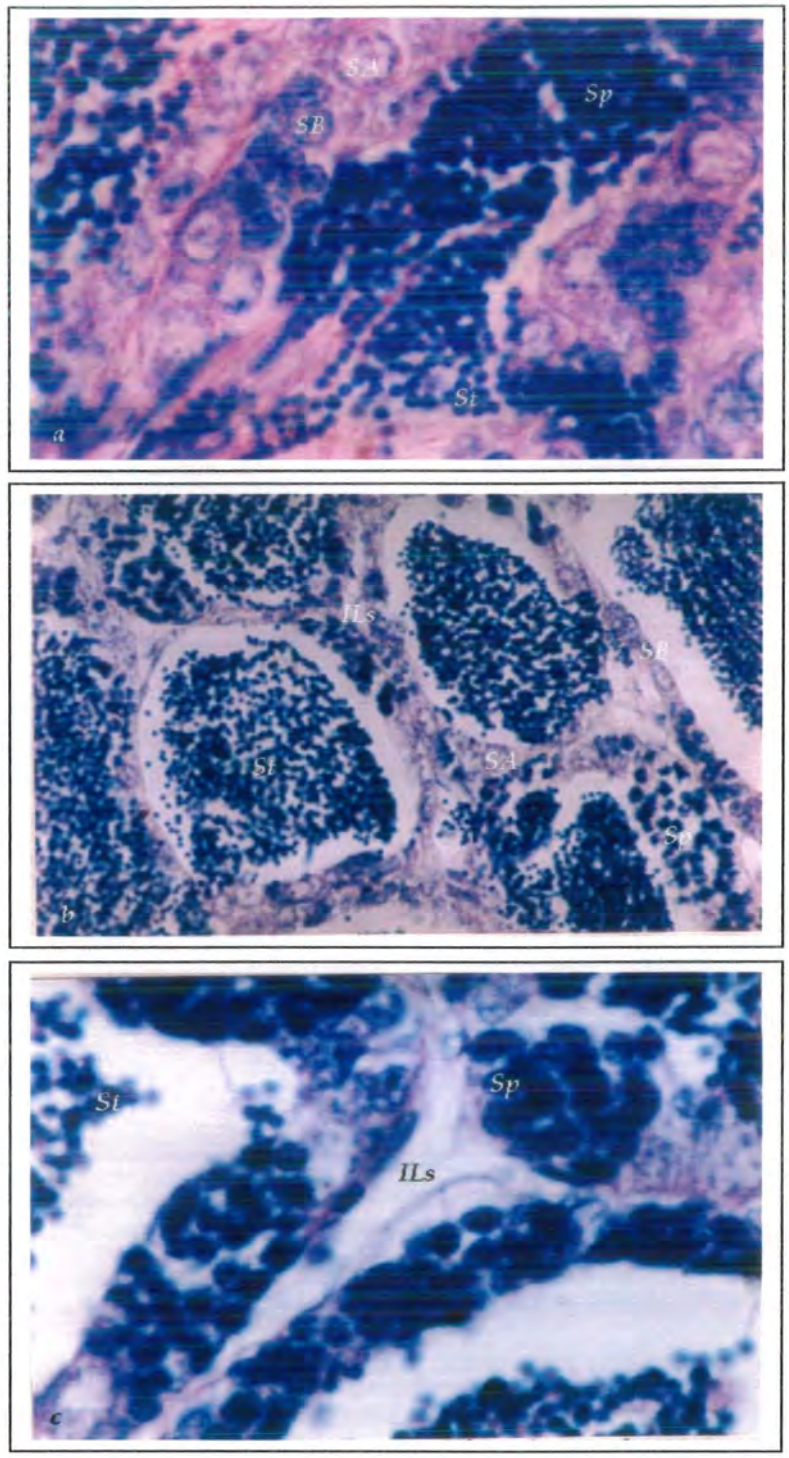


Fig:12 Photomicrograph of control and treated testes (14 days with 0.08ppm Cu)) of fish (*Cyprinion watsoni*). (a)control group showing normal no of spermatogonia type A (SA) and type B (SB), spermatocytes (Sp), spermatids (St) and normal interlobular space (ILs) X2103.35. (b) treated testes with 0.08ppm Cu showing loosly arranged lobules, decreased no both spermatogonia types (SA and SB) and more interlobular space (ILs) X841.34. (c) Treated testes with 0.08ppm Xu showing clumping of spermatocytes (Sp) and spermatids clumping (St) X2103.35.

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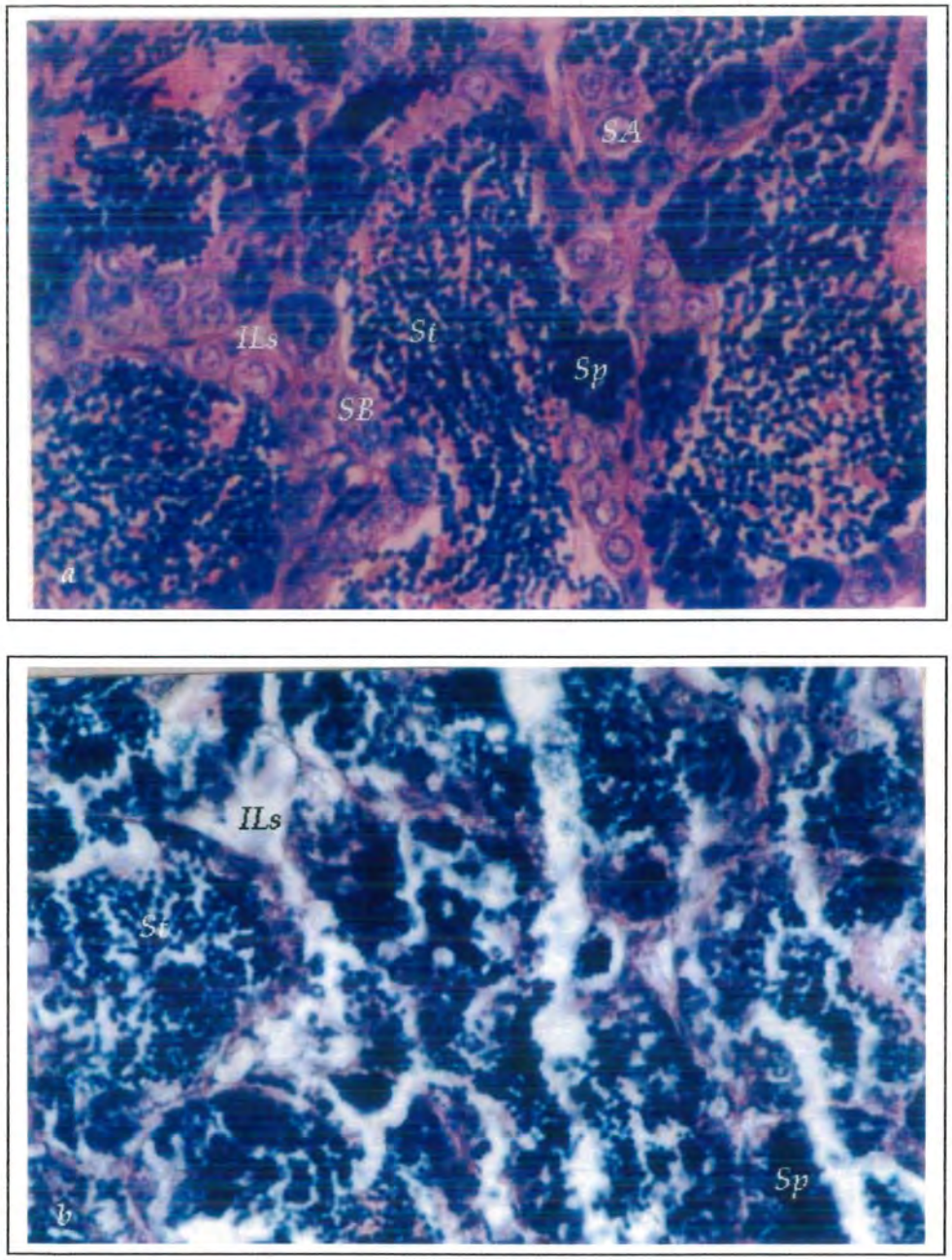

Fig:13 Photomicrograph of control and treated testes (21 days with 0.08ppm Cu) of fish (*Cyprinion watsoni*). (a) control group showing normal lobular morphology containing normal no of spermatogonia type A (SA) and type B (SB), spermatocytes (Sp), spermatids (St) and inter lobular space (ILs).X841.34. (b) Testes treated with 0.08ppm Cu showing disruption of cells, more interlobular space (ILs), clumping of spermatocytes (Sp) and spermatids (St) X 841.34

Group 4 (28 days treated with 0.08ppm copper):

Control:

Histomorphological examination of testes of 28 days contro group showed no any particular change. All the spermatogenic stages were normal.Spermatonia type A and type B were present more towards the peripheral region (Fig.14a) as in the case of other control groups. Interlobular spaces were also at normal. In case of some cysts few clumpings among spermatids were found but these were very few Fig.14a).

Treated:

In treated group of 28 days lobules increased in size and were loosely arranged. No any particular distinction was present between the lobules. Few spermatogonia type A and type B were observed in the peripheral portion of lobules (Fig.14b). Spermatocytes showed abundant clumping. Number of spermatids were abundant due to arrest of spermatogenesis. Interlobular space increased as compared to control group (Table.5,Fig.14b) and this increase was highly significant ($p < 0.001$).

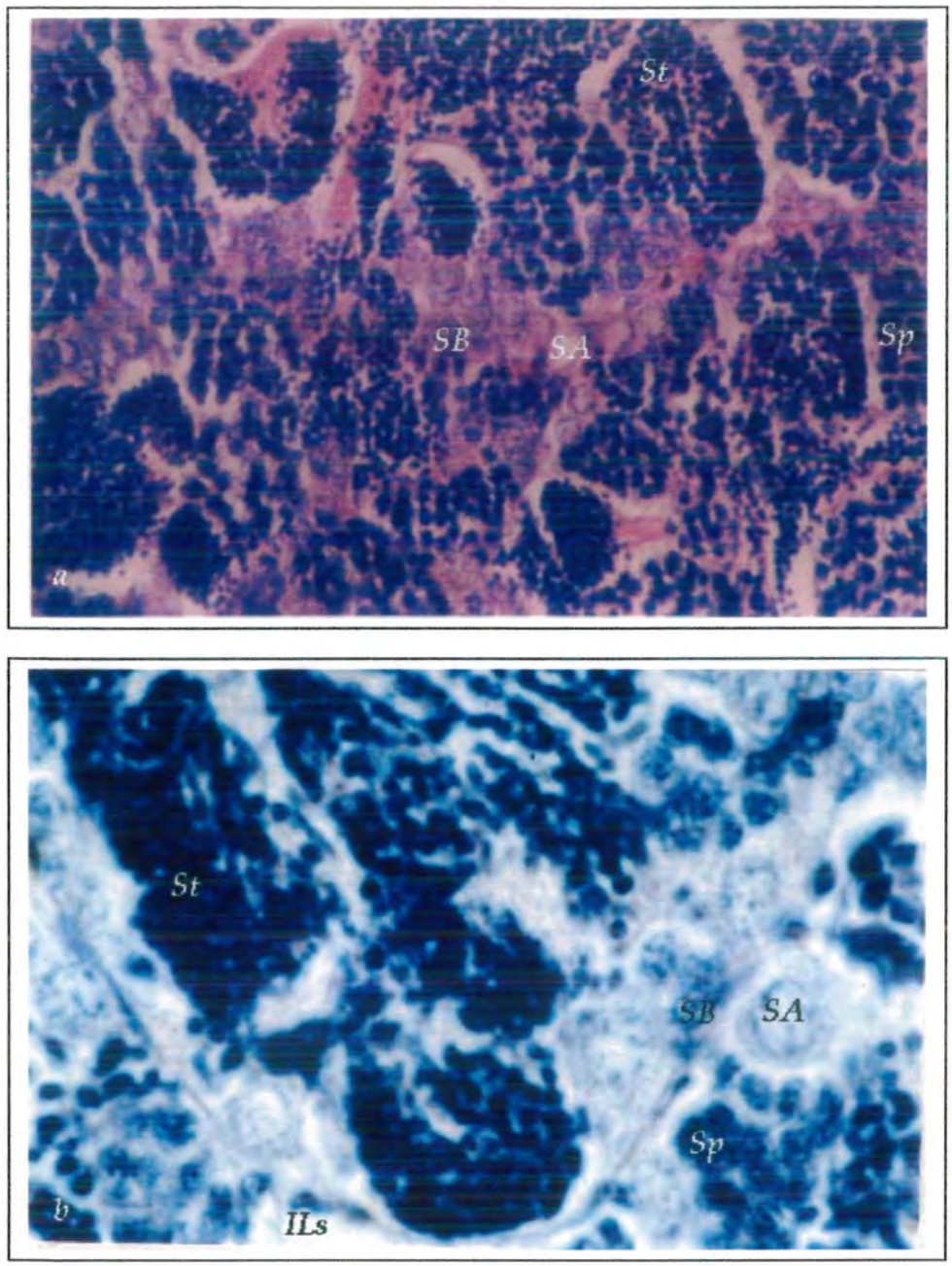

Fig:14 Photomicrograph of control and treated testes (28 days with 0.08ppm Cu) of fish (Cyprinion watsoni). (a) Control group showing normal morphology of spermatogonia type A (SA) and type B (SB), spermatocytes (Sp), spermatids (St) and interlobular space (ILs) X841.34. (b) Testes treated with 0.08ppm Cu showing decreased no of spermatogonia type A (SA) and type B (SB), spermatocytes clumping (Sp), spermatid clumping (St) and more interlobular space (ILs) X2103.35.

DISCUSSION

Discussion

The main objective of the present study conducted in the Department of Biological sciences at Quaid-I-Azam University Islamabad to evaluate the effect of copper on testicular histology and function as there is little information available on the effect of copper on the reproductive system male fish with special reference to *Cyprinion watsoni.*

Cyprinion watsoni was selected as a test organism due to its easy availability and maturation. Moreover these are smaller in size and can be maintained in experimental laboratories.

Water pollution episodes have focused the attention of many scientists and also of public on the environmental problems. Heavy metals discharged by a number of industries added into the aquatic ecosystem cause several irregularities in fish physiology (Doudoroff and Katz, 1950, Sanglang and Freeman 1974, Sehgal et al. 1984). In fish, the two metals (Cu and Zn) are always present in all the tissues but the concentrations vary, depending on the cellular ligands, which in turn reflect metabolites and the expressed proteins typical of each tissue. The exposure root is of major importance for the uptake xenobiotics in fish (Ekulend, 1989). In the present study copper was given in the form of copper sulphate dissolved in water.

Behavioural changes as shown by *O.mossambicu* due to exposure to copper, are typical sign of stress. Similar observations have been reported in a catfish. *Heterpneustes fossilis* after chromium chloride treatement (Dhakad et al. 1993). In an earlier study, Vogal (1959) has shown that gold fish exposed to 1 mg/L of copper develops severe neurotoxic effects. Jerky, uncoordinated movements of fish have been referred as analogous to, "Wilson's disease, an inborn metabolic disorder of humans (Baker, 1969), in which excessive assimilation of copper results into neurological disorders, hepatic necrosis and anemia. Recently such behavioural changes have been reported by Alkahem (1995) in *Clarias gariepnus* treated with another heavy metal compound, cadmium chloride.

In the present study fish also showed changed behavioural response. After first exposure of fish to copper uneasiness was observed while in control groups fish were more comfortable. Fish moved to bottom in the form of cluster. Fish movement was very fast and striking with the walls. Abundant mucus secretion occurred in treated group as compared to control group. Few mortalities also occurred during experiment. These results are in good agreement with the results of Vogal (1959).

When fish was treated with 0.08 ppm copper no profound change was observed in the body weight of fish. Body weight decreased but that decrease was non significant ($p > 0.05$) as previously described by Sehgal and Suxena (1986). Collier et al. (1992) has investigated that size of fish is an important reproductive success in fish. In the present study neither fish length nor fish weight showed any significant effect of copper.

Condition factor (K) is a generalized indicator of overall health of a fish and can reflect the integrated effect of nutritional status and metabolic stress (Adam and Mclean, 1985). In rainbow trout family the higher value of (K) might be due to higher fat contents in the body cavity and large fat deposits around the internal organs (Thegaard and Gall, 1979) and this might be due to lipid withdrawal from the body reserves for gametogenesis during breeding season. In the present study the condition factor showed a non significant difference among all the four groups when their control and treated values were compared. No significant intersite differences in either condition factor (K) or length-weight relationship in English Sole (*pleuronectes vetulus*) from contaminated sites were noticed by Jhonson et al. (1994).

The gonadosomatic index (GSI) has been used by some authors as an objective, sensitive and reliable indicator of gonadal state (Clemns and Reed, 1967). The simplest measure of gonadal dysfunction is to measure gonadosomatic index in control and treated fish (Kime, 1998). Zinc caused a significant decrease in GSI of male fish (*Labistes reticulates)*, which reflect the lowered gonadal activity (Sehgal and Suxena, 1986). Exposure of adult catfish (*Clarius batrachus*) to mercury for 45, 90, 180 days had inhibited testicular activity as shown by the arrest of steroidogenesis and significant change in GSI (Kirubagaran and Joy, 1992).

Exposure of *Cyprinus carpio* to 56 µg/L to 100 µg/L and *cirrhina marigula* to 560 to 1000 µg/L of zinc for 60 days during each of the pre spawning and breeding phase of reproduction revealed concentration dependent decline in gonadosomatic index (Dhawan and Kaur, 1997).

In male Tilapias (*Oreochromis nilotica*) the gonad index increased progressively till the middle of May, June and July. From August, the gonadal index decreases gradually through September and November. In November onwards the gonad index begins to increase again (Hamed et al. 1972).

In present studies gonadosomatic index decreased in all four groups. In case of Group 1 (7 days) and Group 2 (14 days) this decrease was non significant ($p > 0.05$) but in case of Group 3 (21 days) and Group 4 (28 days) this decrease was significant ($p < 0.05$). In first two groups this non significant decrease was due to very small dose of copper for 7 and 14 days show that decline in gonadosomatic index is concentration dependant. A large number of pollutants have been found to cause a decrease in gonadosomatic index but frequently it is not clear whether the primary dysfunction is at gonadal level itself or it is the result of deficiency of pituitary hormone secretions (Kime, 1998).

Reproductive potential in fish is controlled by hypothalamo-hypophyseal-gonadal axis, Peter et al.1986). Katti and sathyanasen (1986) reported degeneration of nucleus preopticus, neurons and inhibition of gonadal maturation and alteration in reproduction in *Clarius batrachus* following exposure to lead nitrate, possibly mediated through hypothalamo-hypophyseal-gonadal axis.Tulasi et al. (1989) reported accumulation of lead in brain of *Anabas testudineus*, which might have altered

hypothalamo-hypophyseal-gonadal function resulting in the altered reproductive potential.

Exposure of fish to heavy metals is known to elevate corticosteroids and this effect was found to be dose dependant (Schreck and Lorz, 1978). In teleosts, corticosteroids are known to be directly involved in reproduction and high levels of corticosteroids occur at spawning time in fish (Katz and Eckstein, 1974). So the decline in the reproductive potential in the present study may be attributed either to changes in pituitary mediated reproductive activity or due to altered levels of corticosteroids in response to copper accumulation. Ideally this measure should be combined with histological examination to determine whether a particular maturation stage is inhibited, e.g there may be a block to development of spermatogonia which may provide some information as to where the pollutant is acting (Kime, 1998).

In vitro and in vivo studies have shown that the fish treated with sub-lethal concentration of zinc sulphate for 20 days exhibited drastic changes in the testes. The elaborate vacuolization was observed in spermatocytes. The cysts of spermatogonia, spermatocytes, spermatids and sperms exhibited significant reduction in their counts in *Labistes reticulatus* (Sehgal and Sexena, 1986). Sub-lethal exposure of Zn to *Labistes reticulatus* inhibited the gonadal response in both female and male fish. Some scientists reported that pollutants disturb the normal histology and function of reproductive organs in fish, (Sehgal et al. 1984, Sexena and Grag, 1978).

In the study on comparative effects of two heavy metals copper and zinc on the testes of fish *Labistes reticulates* (Sehgal et al, 1984) noted that both pollutants produced deleterious effects on spermatogenesis and disturb the normal histology and function of reproductive organs. Exposure of catfish to mercury (Hg) for 45, 90 and 180 days had inhibited testicular activity as shown by degenerative changes in testicular cells (Kirubagaran and Joy, 1992).

In the present study histological observation of testes of copper treated fish showed a number of abnormalities, vacuolization, disruption of lobules decline of the number of spermatogonia, reduction in the length regression of spermatogenesis. These results are in good agreement with the investigation of Sehgaland Sexena (1986) and Kirubagaran and Joy (1992).

Computer assisted sperm analysis (CASA) has been used to analyse the effect of zinc on motility of fish sperm treated for 24 hours after partial dilution in extender. The progressive motility of catfish (*Clarias gariepinus*) sperm decreased after exposure to 2000 ppm zinc in extender for 24 hours (Kime et al 1996).

In present study, the clumping of spermatid was observed in case of all the groups treated with copper. However this clumping was abundant in case of Group 3 (21 days) and Group 4 (28 days). These results suggest that concentration of copper in the testes of *Cyprinion watsoni* as a result of bioaccumulation from water has decreased the motility of sperm during maturation or storage in the testes and these results are in good agreement with the investgation of Kime et al. (1996).

Copper appears to inhibit the release of mature sperms from the testes. Very few comparable studies are available on this aspect of toxicity. Kirubagaran and Joy (1992) have conducted a long term study (90 and 180 days) on the effect of mercury on testes of a catfish, *Clarias batrachus*. It has been demonstrated that mercury caused the arresting of spermatogenesis at spermatid stage. Similarly exposure of fish to lead (katti and Sathyanasen, 1985) and cadmium (Sanglang and Freeman, 1974; Kime, 1984) also resulted into inhibition of gametogenesis in fish. This effort may be mediated through the pituitary gonadal axis. In the present study in case of treated groups number of spermatids increased. This might be also due to the arrestement of spermatogenesis at spermatid stage. These results are similar to the results of Sanglang and Freeman, (1974); Kime, (1984).

Conclusion:

In the present study copper (0.08ppm) was given to *Cyprinion watsoni* for variable days which caused various histological changes and abnormalities in the testes. The testes of fish treated with copper (0.08ppm) showed, vacuolization in lobules and disorganization of spermatogenic elements. The number of spermatogonia was observed to be reduced where as the number of spermatocytes and spermatids increased. Varying testicular damage was also observed in the testes of fish treated with copper (0.08ppm) for 21 days. The length of testes treated with copper was reduced to a large extent. Disintegration of spermatogonia was also observed. In case of Group 3 (21 days) and Group 4 (28 days) there was also a significant decrease in GSI due to loss of motility of sperms. Clumping of sperm was also observed in treated groups.

Thus, it is evident that copper is a necessary element for normal growth and activity of fish but if its concentration is greater then it can cause certain problems on the normal physiology of gonads of fish and can produce deleterious effects in them. So there should be taken some protective measures not to release the industrial wastes in the water bodies, because these wastes contain the heavy metals which can effect the growth and the reproduction of aquatic organisms.

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