E-ISSN: 2278-3229

IJGHC; December 2012 – February 2013; Vol.2, No.1, 130-138.

International Journal of Green and Herbal Chemistry

Available online at www.ijghc.com

Green Chemistry

Research Article

Ameliorative Effect of Spirulina on the Histology of Ovary of Mercuric Chloride Effected Fish, *Clarias gariepinus.*

Ajay Jaat, J.D.Saroch, Rekha Shrivastav, T. A. Qureshi, Susan Manohar, Pradeep Shrivastava, K. Borana.

Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal, M.P. India

Received: 16 December 2012**; Revised** 10 January 2013**; Accepted:** 22 January 2013

Abstract: Fishes are used in studies on the possible toxic effects of heavy metals even if they are susceptible to the accumulation of persistent pollution due also to their presence in an aquatic habitat. Mercury is a heavy metal, a significant environmental pollutant of industrial discharge containing toxic substances contribute tremendously to the pollution of aquatic ecosystem. The aim of this study was to analyze the cytotoxicity of Mercuric chloride and the ameliorative effect of 5 % spirulina on the histology of ovary in the freshwater catfish, *Clarias gariepinus.* Control, 0.5 mg/l mercuric chloride and 5% Spirulina fed fishes exposed to 0.5mg/l mercuric chloride were used in the basic test for a period of 60 days. The fishes were sacrificed after an interval of 15, 30, 45 and 60 days respectively. The ovaries were analysed by histological method. The tissues of the ovary appear slightly follicular damaged in a few areas around the oocyte after 15, 30, 45, and 60 days of mercury chloride treatment. Pre-vitellogenic and vitellogenic oocytes were less in number. The ameliorative effect of 5 % Spirulina fed treated fishes showed Pre-vitellogenic and vitellogenic oocytes. Loosening of tissue was also recovered which earlier led to necrosis when compared to the ovary of the fish treated with $HgCl_2$ solely at the same duration.

Keywords: Ovary of *Clarias gariepinus*, histological analysis, 0.5mg/l mercuric chloride, 5% Spirulina.

INTRODUCTION

The aquatic environment is an extremely diverse and highly variable system, crucial for the continued survival of life on planet earth[1]. As we all know water is the prime necessity of life. It directly influences all the aspects of life. About 70% of earth surface is covered by water. It is most common fluid in nature. General survey revealed that about 15 crore cubic km of water is found on the average on the layers of earth, but instead of such abundance only 0.03% of world's total water runs through rivers, streams, lakes

and ponds, which is available to man for various activities. Water pollution is generally associated with heavy industrialization and dense population and is generally one of the major ecological problems, industries are responsible for adding pollutants in our environment as a number of industrial effluents and emissions especially toxic substances and gases are spread into the environment. These major pollutants prevailing in environment are heavy metals, pesticides, synthetic polymers, aliphatic hydrocarbons, petroleum products etc. Industrial discharges containing toxic and hazardous substances including heavy metals, contribute tremendously to the pollution of aquatic ecosystem[2,3]. Heavy metals cause serious impairment in metabolic, physiological and structural systems[4]. Their introduction into aquatic environment is caused by direct or indirect agricultural and industrial discharges. The term heavy metal widely used in scientific literature with reference to several elements beginning with beryllium and going upto actinides[5]. The heavy metals are normally regarded as the ones having an atomic no. of 22 to 92 in all groups from period 3 to 7 in the periodic table[6], including iron, copper, cobalt, chromium, nickel, lead, cadmium, mercury, etc. Heavy metals may affect organisms directly by accumulating in their body or indirectly by transferring to the next tropic level of the food chain[7].

Toxicology literally means "study of poisons". The word is derived from the Greek word toxicon meaning arrow or poison and logos meaning study. "Toxicology is the study of the adverse physicochemical effect of chemical, physical or biological agent on living organisms and the ecosystem, including the prevention and amelioration of such as adverse effects". Example of such agents includes cyanide (chemical), radiation (physical) and snake venom (biological).

Mercury is emitted to the atmosphere by natural degassing of the earth's surface and by evaporation of mercury vapour previously deposited on the earth's surface. Mercury is emitted in the form of elemental vapour (Hg). Annual natural emissions are estimated to be between 2700 and 6000 tons[8], some of which originate from previous anthropogenic activity. Anthropogenic sources of mercury are numerous and worldwide. Mercury is produced by the mining and smelting of cinnabar ore. Mercury is the only common metal liquid at ordinary temperature. Its most important salts are mercury chloride (Corrosive sublimate, poison), mercurous chloride and mercury fulminate. Mercury has been recognized as environmental contaminant since Minamata disaster of Japan in the late 1950s, which was caused by consumption of fish living in the seawater that was severely polluted with mercury from local industrial discharge[9]. Mercury toxicity is affected by temperature, salinity, dissolved oxygen, and water hardness.

The name "Spirulina" is derived from the Latin word for "spiral" or "helix", denoting the physical configuration of the organism when it forms swirling, microscopic stands. Its length 350µ, width 3-6µ, In between two spiral distance 30-60µ, a single spiral width 24-45µ. Spirulina is microscopic blue- green multi- cellular algae, which cannot be seen by naked eyes. Spirulina is a spiral steam generally found in fresh water, brine, and brackish water. Spirulina consists of 60-70% protein in dry weight. The protein elements consist of 18 types of amino acid, several vitamins, such as vitamin A, B, E, H and minerals, fatty acid necessary to the body. Presently, it is very useful nutritious food for preventive and symptomatic treatment, such as antiviral activity, blood nourishment, enhancing immunity against bacteria or foreign substances for body and recovery during convalescent period. Spirulina is a rich source of protein (60-70%), vitamins, essential amino acids, minerals and essential fatty acids such as plasmatic acid, linolenic acid and linoleic acid. Therefore, it has been used as a nutrient for fish larvae[10,11] and as an ingredient in fish diet for juveniles and adult common carp[12-14]. Spirulina contain all the essential amino acids, rich in chlorophyll, beta-carotene and its cofactors, and other natural phytochemicals. Antioxidant defense system of fishes comprises of various enzymes (viz. superoxide dismutase, catalase, gluthathione peroxidase etc.) non-enzymatic molecules like minerals (Se, Mn, Cu and Zn) proteins and vitamins which operate to detoxify or scavenge these free radicals generated in the body. Spirulina, *Arthrospira platensis* is a freshwater blue-green filamentous alga, and it is receiving increasing attention for its bioactive

components such as vitamins, minerals, polyunsaturated fatty acids, carotenes and other pigments that have antioxidant[15-20].

MATERIAL AND METHODS

The present work was carried out at the laboratory of Department of Zoology & Applied Aquaculture and

Zoology, Barkatullah University, Bhopal (M.P), India. The fish, *Clarias gariepinus*, with average length of 12-15 cm and weight of 100 g were procured from local fish markets of Bhopal, M.P, India. The fishes were brought to laboratory and were kept in the glass aquarium to observe any visible pathological symptoms. The fishes were then treated with 0.1 KMnO4 solutions to obviate any dermal infection before introducing in the aquarium where they were acclimated to laboratory conditions for two weeks prior to exposure. During that period the fish were fed 4% of their body weight once daily (0900 hours) with laboratory formulated fish feed **(Table-1)**. Each aquarium was supplied with dechlorinated, well-aerated tap water, which was changed once daily. After the acclimation period, the fishes were randomly selected and stocked 20 fish per aquarium in 3 glass aquaria for the experimental runs. Mercuric Chloride (Ranbaxy, India) was used in original package form. By mixing with distilled water was used as stock-solution by adopting the dilution techniques[21]. Different test doses were prepared making dilution of the stock concentration. Fishes transferred to each aquarium and exposed to different concentrations of $HgCl_2$. In all cases, control groups of fish were maintained. Each experimental trial was carried out for a period of 96 hours.10 fishes were exposed to sublethal concentration. The mortality of the fish was recorded at logarithmic time intervals that is, after 24, 48, 72 and 96 hours of exposure. The test media was renewed daily during the experimental period. The physicochemical characteristics test of the water such as temperature, pH, alkalinity, hardness, oxygen concentration were conducted frequently following the standard procedures[21]. The effect of each concentration was tested at least in duplicate to verify reproducibility. The data obtained in course of the investigation were analyzed statistically to see whether there is any influence of different treatments (concentrations) on the mortality of fish. The median lethal concentration (LC_{50}) values and their 95% confidence limits for different exposure time were calculated by using the computer software "Probit Analysis", EPA version 1.5, USA**.** The LC_{50} value came out to be 1 mg/l.

Table-1: Showing contents of the control and experimental diet

Preparation of Experimental Diets/100 gm					
Diets	Fishmeal	Wheat flour	Cod- liver oil	Vitamin Premix	Additive
Control	79.07%	8.93%	10.00%	2.00%	Nil
5% Spirulina	74.07%	8.93%	10.00%	2.00%	5 % Spirulina

The experiment was set for 60 days in aquaria of 200 litre capacity. The fishes were divided into three groups. Group first was kept as unexposed control; IInd Group was exposed to sublethal concentrations of 0.5 mg/l mercuric chloride and the IIIrd group was fed with 5% spirulina supplemented diet affected with 0.5 mg/l HgCl2. Histological studies were made after 15, 30,45 and 60 days of exposure. In acute and chronic studies, feeding was stopped one day before the experiment started and under chronic studies. Refeeding was done after one day of exposure. Water being changed after every fourth day in all aquaria.

Preparation of control and experimental diet: Artificial diet was prepared to feed the fingerlings with various feed ingredients. Fish meal, wheat flour were finally ground and proximate analysis was

performed using standard methods given in[22]. Based on this analysis, one practical diet was formulated containing 40% protein. The finely ground ingredients were thoroughly blend with cod liver oil using a food mixer for 15 minutes. Vitamin and mineral mixes were then added by continuous mixing. Distilled water was slowly added to the mixture to aim desired consistency for pelleting. This was then extruded in hand pelletizer using a 1mm die and dried for 4 - 6 hours below 45^0C and subsequently dried in hot air oven to reduce moisture. Dried feed was chopped into pellets in a blender passed through sieves to ensure a homogenous particle size (0.5 - 1.0 mm). The feed was then stored at room temperature in an air tight jar. In every feed experimented, same amount of fish-meal was removed as 5% of Spirulina was added. This was done due to the close range of protein level in the additives (60-65%).Dried feeds were kept in air tight jars for experimental use.

Histological analysis: To study the histopathological changes induced by mercuric chloride, the fishes were exposed to 0.5 mg/l $HgCl_2$ for 60 days. Fishes were sacrificed by decapitation after 15, 30, 45 and 60 days. Ovary was removed for histological studies.

Tissues were washed and cleaned in normal saline solution, about 4mm thick pieces of the tissues were fixed in bouins fluid for 24 hours followed by thorough washing in 70% alcohol and were dehydrated through alcohol series. The tissues were transferred in xylene and then in cedarwood oil for 36 hours after washing in xylene they were embedded in paraffin at 550C in incubator. Blocks were prepared and cut 6 micron thick. The ribbons were stretched on clean slide using mayor's albumin. They were stained with haematoxyline and eosin stain and analysed using a light microscope. Pictomicrography was performed thereafter to depict the results.

RESULTS

Group I (Control): T.S. of ovary of *Clarias gariepinus* of **Group 1(Control)** showed normal histopathological details and did not show any pathological lesions in any fish of the control group (Fig.1).

Group II (0.5 mg/l $HgCl_2$ treated): On day 15^{th} the Ovary sections of the fishes treated with 0.5 mg/l of $HgCl_2$ revealed damaged follicular lining & disrupted around the oocytes (Fig.2). After 30 days the ovary of exposed fishes showed more damaged follicular lining & disrupted around the oocytes. Damaged stroma was evident. The developing oocytes were represented by primary oocytes only. Pre-vitellogenic and vitellogenic oocytes were less in number (Fig.3). After 45 days the ovary of exposed fishes showed the ovigerous lamellae and follicular lining were freely floating near the oocytes. Damaged stroma was evident. The developing oocytes were represented by primary oocytes only. Pre-vitellogenic and vitellogenic oocytes were less in number, which may result in decrease in the vitellogenic activity and yolk formation (Fig.4). After 60 days the ovary of exposed fishes showed the developing oocytes were represented by few numbers of primary oocytes characterized by deshaped margins. Pre-vitellogenic and vitellogenic oocytes were less in number. Loosening of tissue with a sign of necrosis was seen (Fig.5).

Group III (0.5 mg/l $HgCl_2$ and treated with dietary 5% Spirulina supplemented feed): On day 15^{th} ovary of the fishes showed recovery in damaged follicular lining & disruption around the oocytes (Fig. 6). On day 30^{th} the ovary of Spirulina fed fishes showed recovery in the recovery in damaged follicular lining & recovery in disrupted oocytes. Damaged stroma was also recovered as compared to the ovary of the fish treated with $HgCl_2$ solely at the same duration (Fig. 7). On day 45^{th} the Spirulina fed treated fishes showed the ovigerous lamellae and follicular lining were freely floating near the oocytes. Recovery in damaged stroma was evident as compared to the ovary of the fish treated with $HgCl_2$ solely at the same duration (Fig. 8). On day 60^{th} the Spirulina fed treated fishes showed Pre-vitellogenic and vitellogenic oocytes. Loosening of tissue was also recovered which earlier led to necrosis when compared to the ovary of the fish treated with $HgCl_2$ solely at the same duration (Fig. 9).

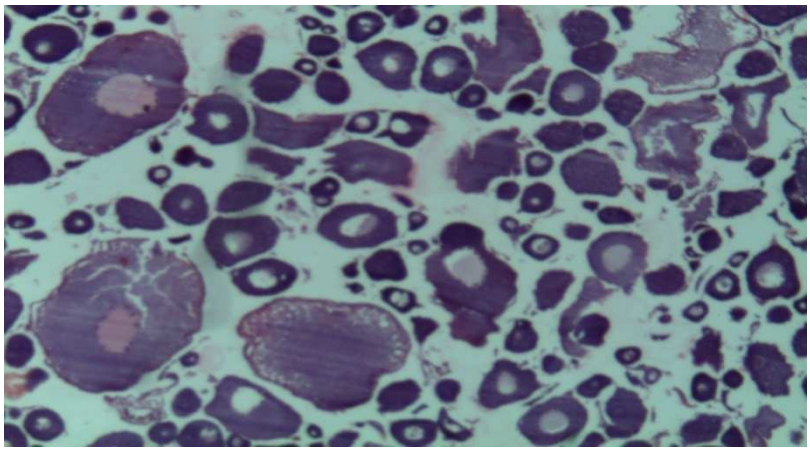

Fig.1 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group 1(Control) showing normal histopathological details and did not show any pathological lesions in any fish of the control group X 200.

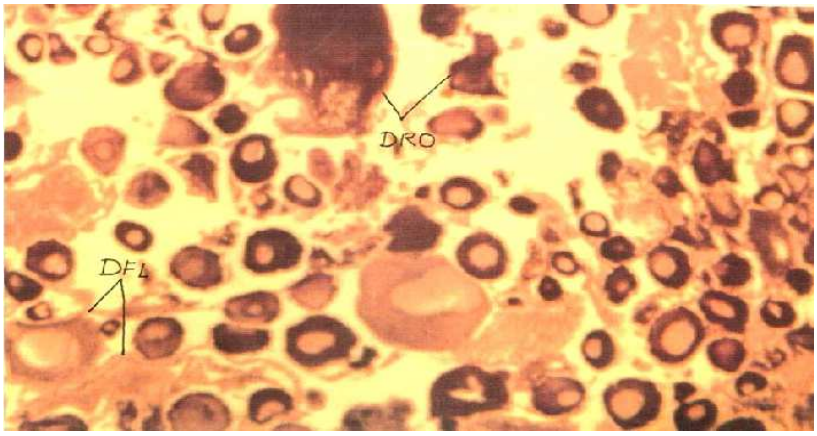

Fig.2 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group II (0.5 mg/l $HgCl_2$ treated) after 15 days showing damaged follicular lining & disrupted around the oocytes X 200.

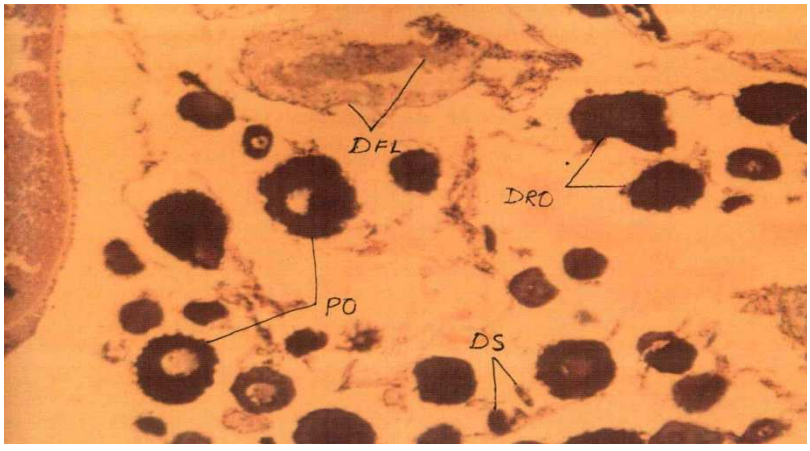

Fig.3 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group II (0.5 mg/l $HgCl_2$ treated) after 30 days showing more damaged follicular lining & disrupted around the oocytes. Damaged stroma was evident. The developing oocytes were represented by primary oocytes only. Pre-vitellogenic and vitellogenic oocytes were less in number X200.

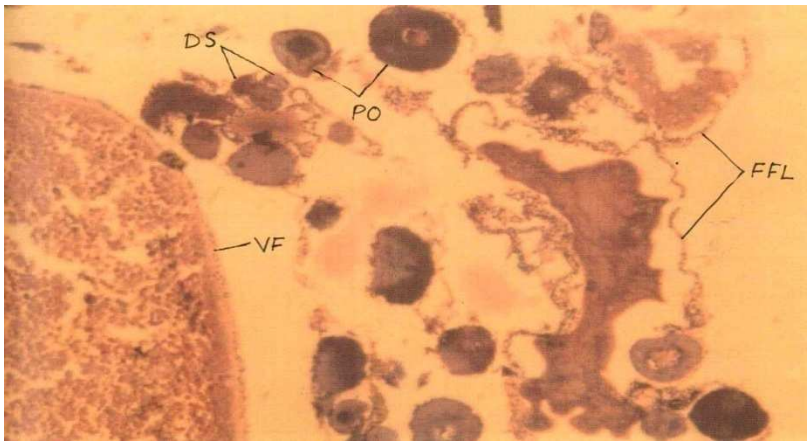

Fig.4 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group II (0.5 mg/l $HgCl_2$ treated) after 45 days showing the ovigerous lamellae and follicular lining were freely floating near the oocytes. Damaged stroma was evident. The developing oocytes were represented by primary oocytes only. Pre-vitellogenic and vitellogenic oocytes were less in number, which may result in decrease in the vitellogenic activity and yolk formation X200.

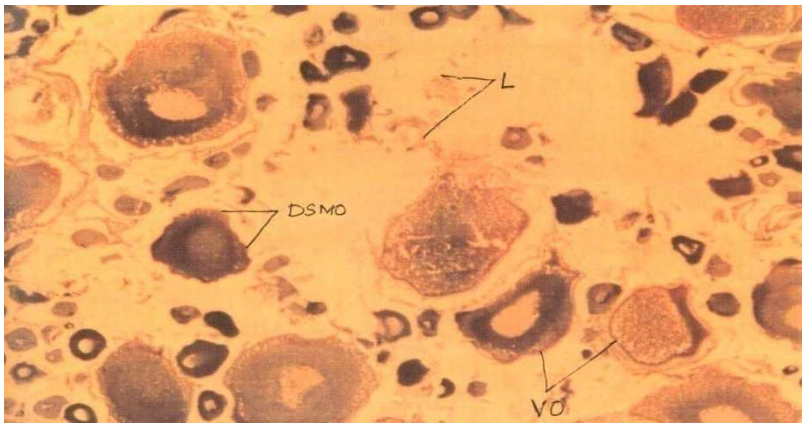

Fig.5 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group II (0.5 mg/l $HgCl_2$ treated) after 60 days showing the developing oocytes were represented by few numbers of primary oocytes characterized by deshaped margins. Pre-vitellogenic and vitellogenic oocytes were less in number. Loosening of tissue with a sign of necrosis was seen X200.

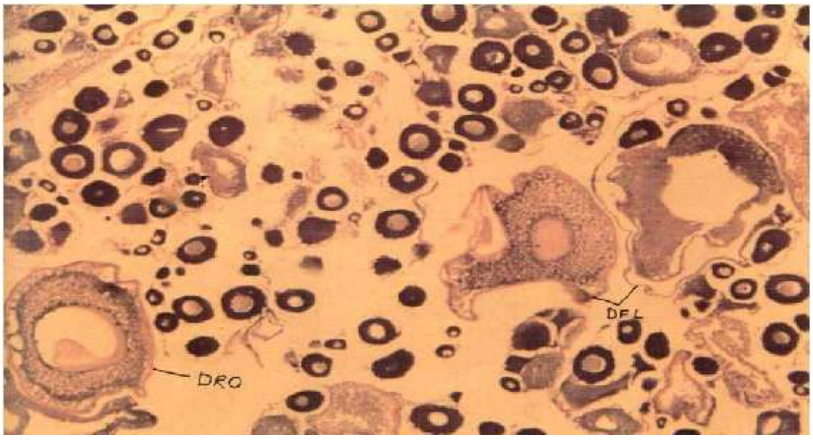

Fig.6 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group III (0.5 mg/l $HgCl_2$ and treated with dietary 5% Spirulina supplemented feed) after 15 days showing recovery in damaged follicular lining & disrupted around the oocytes X100.

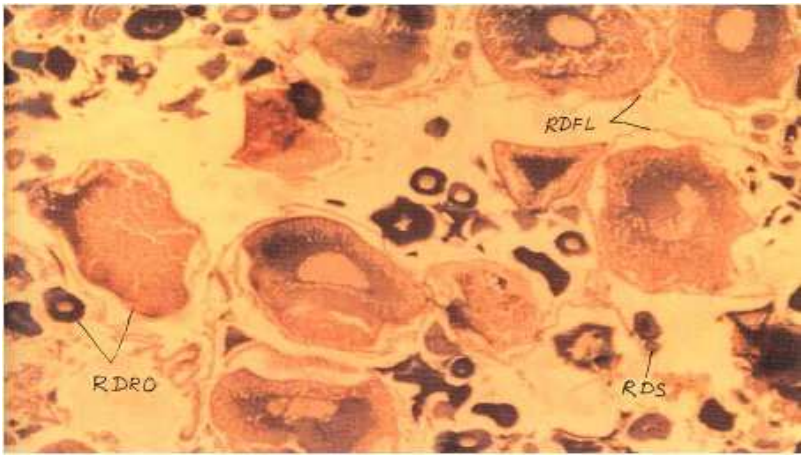

Fig.7 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group III (0.5 mg/l $HgCl_2$ and treated with dietary 5% Spirulina supplemented feed) after 30 days showing recovery in damaged follicular lining & recovery in disrupted oocytes. Damaged stroma was also recovered as compared to the ovary of the fish treated with $HgCl_2$ solely at the same duration X200.

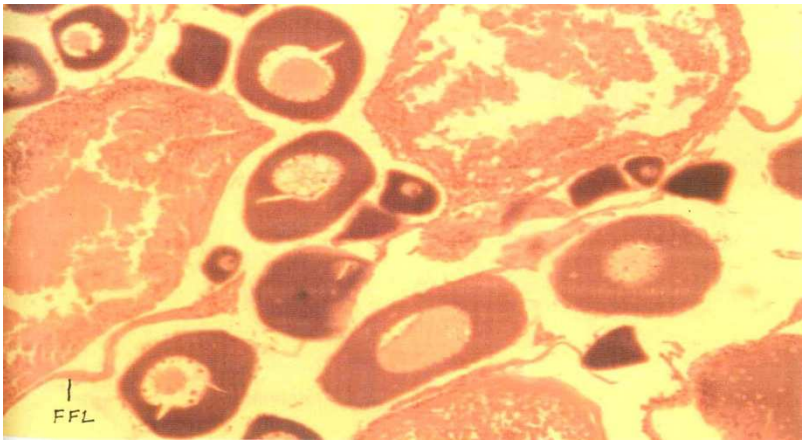

Fig.8 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group III (0.5 mg/l $HgCl_2$ and treated with dietary 5% Spirulina supplemented feed) after 45 days showing the ovigerous lamellae and follicular lining were freely floating near the oocytes. Recovery in damaged stroma was evident as compared to the ovary of the fish treated with $HgCl_2$ solely at the same duration X200.

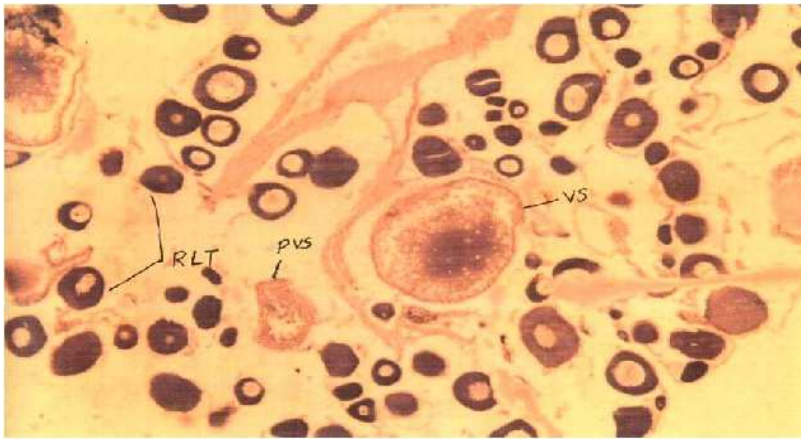

Fig.9 Pictomicrograph of T.S. of ovary of *Clarias gariepinus* of Group II (0.5 mg/l $HgCl_2$ treated) after 60 days showing Pre-vitellogenic and vitellogenic oocytes.. Loosening of tissue was also recovered which earlier led to necrosis when compared to the ovary of the fish treated with $HgCl_2$ solely at the same duration X200.

DISCUSSION

In the present study, the exposure of mercuric chloride in fish *Clarias gariepinus* resulted in marked degenerative changes in the ovary. These changes included damaged follicular lining & disrupted around the oocytes, Pre-vitellogenic and vitellogenic oocytes were less in number,follicular lining were freely floating near the oocytes. Damaged stroma was evident, Ovary of the fishes showed recovery in damaged cells when the fishes were fed with 5% Spirulina.

A significant atresia in the ovary with major damage to younger oocytes in *Puntius conchonius*, after exposure to zinc on gonads[23]. Partial lysis, swelling, atresia and change in nucleus after exposure for 20 days[24]. Carbaryl treatment arrested ovarian activities and caused increase in atrectic follicles in *Channa punctatus*[25]. The impact of carbofuran in the oocyte maturation of catfish, *Clarias gariepinus* and found the degeneration of follicular walls, connective tissue and vacuolization in the ooplasm[26]. Fish exposed to toxicologically safe concentration (0.5 ppm) of $HgCl_2$ showing normal behavior the ovaries showed inhibition of early vitellogenesis as they were full of concurrently degenerating oogonials and immature oocytes[27]. B-carotene of Spirulina reduced cell damage, thus playing the role in the repair of regeneration process of damaged cells[28].

The results of the study will be helpful to specify safe levels of concentrations of Mercuric chloride to the aquatic biodiversity especially the fish which is the main dietary content of the present human population. It will also bring in record the damages caused to different organs of the fish by different concentrations of this heavy metal and the recovery by using Spirulina in the feed of the fishes. On the basis of which it may be suggested that how far the use of fish will be injurious for human health toxicological significance and after recovery fish could serve as a very good model system to study its effects on tissues and cells.

ACKNOWLEDGEMENT

The authors are thankful to DST, New Delhi for providing funds for completion of the research work and Department of Zoology and Applied Aquaculture, Barkatullah University for providing lab facilities.

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Correspondence Author: J.D.Saroch, Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal-462026, M.P.