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Histological Study of Effect of Heavy metal (ZnSO4) on Hypothalamo-Neurohypophysial System of Teleost

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ABSTRACT

This study evaluated the effect of $ZnSO_4$ on hypothalamic-pituitary-gonadal (HPG) axis of fresh water teleost male *Anabas testudineus*. The hypothalamus is projected ventrally as a round infundibulum which is connected with the hypophysis (pituitary gland) in the brain by an indistinct pituitary stalk; this concept is called as hypothalamo-neurohypophysial system or hypothalamic-pituitary-gonadal (HPG) axis.

For the study experiment were carried out by exposing the male *Anabas testudineus* to the test solutions containing various concentrations of heavy metal $ZnSO_4$ (zink sulphate) and 50% mortality was recorded at 96 hours exposure period. The LC_{50} value calculated was 3 mg/l. On the basis of lethal dose test animals were exposed to the sub-lethal dose for a long period of 30 days then histopathologically and quantitatively observed the effect of heavy metal on neurohypophysial complex of fish. Results indicated that the neurohypophysial complex of fresh water fish *Anabas testudineus* is sensitive to the heavy metal pollutant zink sulphate and affect the spawning of fish. Effect of Zink sulphate on GSI was found statistically significant ($P<0.05$) after 30 days exposure between control and pollutant group.

Key Words: Neurohypophysial complex, hypothalamic-pituitary-gonadal (HPG) axis, heavy metal Pollutant (HMP), Infundibulum, Histopathological.

INTRODUCTION

Fishes exhibit huge aquatic diversity in shape, size, and biology and in the habitats they occupy. They are the most primitive vertebrate from which all other vertebrates including man has evolved. They are important not only because of their economic value, but also because of sensitivity to ecological changes. So, fish assemblages have widely been used as biological indicators to estimate and evaluate the level of environmental aquatic toxicity, degradation and health of rivers and streams[1].

Analysis of most of the natural water bodies revealed a critical loss of biodiversity. The level of toxicity with heavy metals has been increasing in the last decades, partially because of increase in the use of such metals in a variety of agricultural and industrial processes; this has stimulated studies to identify the potential toxicity induced by such agents. In this regard, it has been reported that heavy metal like zink, copper, and cadmium can exert toxicity at specific experimental conditions, which may also depend on the evaluated organism and the associated environmental factors[2,3].

Metals and organic contaminants affect the spawning behavior and spawning duration of fish and reduce sperm motility[4]. It was found that the toxic effects of metal mixtures are the sum of the toxicities of each metal (additive effect), corresponding to the bioavailable form of the metals[5,6].These toxic substances may knock down immune system, reproductive system and endocrine system in animal and these effects can be at organ, tissue and cell level[7].

It was shown that accumulation of heavy metals results in inhibition of the secretion of noradrenalin and stimulation of the secretion of dopamine in the hypothalamus and the brain is also known to be a site of stress hormone synthesis[8]. These processes results in a disturbance of hormonal equilibrium of the HPG axis, which can unfavorably influence the efficiency of spawning in fish. It should be noted that the HPG axis functions as a dynamic system throughout each life-stages of an organism, early in development through gonad development and finally into adult life-stage[9,10] . The primary tissues involved in this hormonal cascade are the hypothalamus, pituitary gland, and gonads. The hypothalamus located at the base of the brain is sensitive to signals from sensory receptors and releases hormones in response to environmental cues, principal among these hormones are gonadotropic releasing hormones (GnRH), which travel from the hypothalamus to the pituitary gland. Certain cells of the pituitary receive GnRH and release it into the bloodstream. The pituitary is responsible for a wide variety of functions including growth and reproduction. The gonadotropic hormones travel to the gonads which synthesize steroids responsible for final maturation of the gametes[11].

The hypothalamus of teleost fishes comprises the Gomori-positive nucleus preopticus (NPO) and the Gomori-negative nucleus lateralis tuberis (NLT) [12]. The NPO, which is composed of the pars magnocellularis and the pars parvocellularis, is situated on both sides of the preoptic recess. The axons (Type A peptidergic fibres containing 100-200 nm granules) originating from the cells of the NPO form the preopticohypophysial tract which penetrates the pituitary, ramifies and terminates in the neurointermediate lobe[13-15]. These axonal fibres originated from the NPO cells forms tractus preopticohypophysis, which travels on the floor of hypothalamus and terminates in the neurohypophysis of the pituitary gland. The neurosecretory material is transported from NPO to the pituitary gland through this tract so tractus preopticohypophysis and neurohypophysis are important components of neurohypophysial compex[16, 17]. The gonadotrophs are also directly innervated by these fibres originating in the NPO. This implicates both, the NPO and the NLT in the regulation of gonadotropic functions of the pituitary[18]. The presence or absence of NLT varies in different teleost species. In *Anabas testudineus* NLT is absent[19].

MATERIAL AND METHODS

COLLECTION OF SPECIMENS

Collections from February to August were mainly used to fix the maturity of spawning period of test fish *Anabas testudineus* (weight: 40 grams and above) Mature males acquire glazy black colour during breeding season.

EXPERIMENTS

For the control and effect of heavy metal toxicant on the neurohypophysial complex and gonads, in the laboratory experiments were conducted in three phases.

PHASE I EXPERIMENT

In the first phase of experiment 10 male test fish as control were acclimatized in normal water fish tank in natural condition for one month (May-June). Dry prawn powder as fish food was provided and medium was changed daily. After acclimatization dissect out the animal in laboratory and following measurements were recorded during experimentation.

- ✓ Total length of each fish.
- ✓ Body weight of each fish.
- ✓ Gonad weight of each fish.
- ✓ The volume of gonad, measured by liquid displacement method.

PHASE II EXPERIMENT

In the second phase short term tests of acute toxicity were performed over a period of 96 hours using heavy metal toxicant zink sulphate. By exposing the test fish to test solutions (10 per test solution) of different concentration in the range of 1, 1.5, 2.0, 2.5 & 3 mg/l. Preliminary experiments were conducted to choose the concentration that resulted in the 50% mortality at 96 hours exposure period. The LC_{50} value calculated was 3 mg/l. The dose in which fishes were survived for a long period of a month in the fish tank is 2.5 mg/l, is taken as sub-lethal dose (had a pH 7.2±0.1, dissolved oxygen 8.0±0.3 mg/L).

PHASE III EXPERIMENT

In the third phase of experiment 10 test fish were acclimatized in separate fish tank, in the sub-lethal concentration (2.5 mg/l) of heavy metals $ZnSO_4$ for 30 days in spawning period. The solution of the toxicant was changed daily and fishes were aerated and feed properly. After 30 days sacrificed animals one by one and dissect out the fish for brain & testis after recording the necessary measurements (as recorded in ex. no.1).

Histopathological analysis

First Control fish then the fish exposed to sub-lethal dose of $ZnSO_4$ for 30 days, in the spawning period were sacrificed. Their brains and gonads were dissected out and fixed in aqueous bouin and hollande's modified, dehydrated in graded ethanol series, cleared in xylene and embedded in paraffin. Finally the paraffin blocks were prepared and slices were cut at 5µ to 6µ (micrometer thick) by means of rotary microtome (Leica RM 2145). Slides of these slices were dehydrated and stained with Aldehyde Fuchsin (AF) and Chrome Alum Haematoxylin Phloxine (CAHP) for brain sections, Mallories Triple Enrlich's Acid Alum Haematoxylin stains were used for gonads. The sections were examined and photographed using an Olympus BX 51 microscope.

Statistical analysis

The GSI for both groups (Control & HMT- $ZnSO_4$ treated group) were calculated according to the formula:

$$\text{GonoSomatic Index} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

Where, GW = Gonad weight of fish in milligram
BW = Body weight of fish in gram.

Means ± standard deviation (SD) were calculated for both groups. Statistical analysis (Two Way ANOVA) was done using the software Graph pad Prism 5, at a 5% significance level. The results of the experimental group (HMT-$ZnSO_4$ treated group) were compared with the control group to quantify the effect of the $ZnSO_4$ on the test animals.

RESULT AND DISCUSSION

HISTOPATHOLOGICAL RESULT OF NEUROHYPOPHYSIAL COMPLEX

The activity of the NPO have correlated with the annual gonadal cycle, the NPO is active during the spawning periods and inactive in the sexually quiescent period. Similarly, seasonal fluctuations in the quantity of neurosecretory material in NPO have been correlated with the gonadal activity[20, 21].

In *Anabas testudineus* NPO of the control fish are spherical in shape. The cytoplasm is granular and deeply stained. The nucleus is situated near the periphery and contains one or two nucleoli, with chromatin material. The neurosecretory cells are more concentrated (granulation) and uniformly distributed with the cytoplasm. The staining intensity is also deep (figure 1). Tractus preoptico hypophysis and neurohypophysis also shows similar conditions (figure 2, 3) in control fish. The histological change in the NPO shows its involvement during reproductive cycle. The NPO cells of zink sulphate treated fish are highly affected. Most of the NPO cells are smaller in size with depleted neurosecretory materials (figure 4). The nucleus is also smaller in size and the nucleolus is rarely visible. Some of the NPO cells are hypertrophied and some are hypnotic condition. Tractus preopticohypophysis and neurohypophysis also shows further decrease in the quantity and intensity of neurosecretory material (figure 5, 6) due to which the spawning of fish is severely affected. Thus it can be inferred that greater the effect of heavy metal pollutants to the secretion of HPG axis neurohormones (FSH & LH), greater the effect to the spawning period & lesser the process of spermatogenesis[22].

HISTOPATHOLOGICAL RESULT OF GONAD

Fully mature gonads of control fish shows advance stages of spermatogenesis and mature sperms (figure 7). Pollutant treated fish indicates a sharp downward trend in the activity of gonads. Testis in fish represents the most dynamic organ having a high cell turnover during the reproductive period which makes it vulnerable to a wide variety of chemical toxicants due to the exposure of the different toxicants the changes may culminate in a partial or total arrest of spermatogenesis.

Testicular inflammation has been documented as one of the common responses on the aquatic animals exposed to environmental toxicants[23, 24].

Exposure dependent histological alterations in the testis were seen. Progressively there was an increase in the vacuolization, disorganization and distortion of seminiferous tubules (figure 8). At the 30th day of exposure condensation of spermatocytes besides inflammation and inter-tubular vacuolation was very much prominent[25].

STATISTICAL RESULT

According to the results obtained from the seasonal spawning phases of *Anabas testudineus* the graph has been made with respect of water temperature and gonad weight. The size of the spermatocyte increases with the temperature of the environment. Increase or decrease in gonad weight and volume correspond with the increase or decrease in water temperature (figure 9). In April, May and June the highest gonad weight and gonad volume was observed and higher the GSI of control group i.e. 0.036, 0.041 and 0.043 respectively. Thus the GSI increases with the maturation of fish and reaches to its maximum at the peak period of maturity that is why the GSI has been considered as reliable estimate for gonadal maturity and spawning of any species. Its abrupt decrease indicates beginning of spawning. Our study confirms the observations of earlier workers[26-31].

The reduction of GSI of test fish subjected to HMT-$ZnSO_4$ reflect the reduction of the gonad mass and gonad volume as compare to the control group in the month of April, May and June, the GSI is 0.020, 0.019, 0.017 respectively (figure 10,11,12). Similar findings were reported by earlier workers [32, 33].

Increase in GSI signified active spawning state in the gonads of control group, but these values are decreasing in toxicant ($ZnSO_4$) treated group. When compared the GSI of control group and toxicant group, the GSI of pollutant group were found to have decreased more than 50% ($p<0.05$) after 30 days of exposure of $ZnSO_4$. The Spawning phase between Control group and toxicant group shows statistically significant difference between them (0.037 ± 0.002, 0.017 ± 0.002, $P<0.05$)

Figures (1-8) of Control and Heavy Metal Toxicant (HMT) treated fish in spawning period

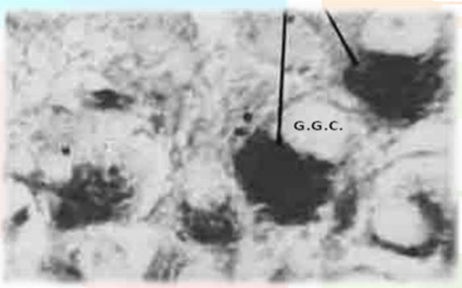

Figure 1- - VLS of brain with greater accumulation of neurosecretory material of NPO cells.

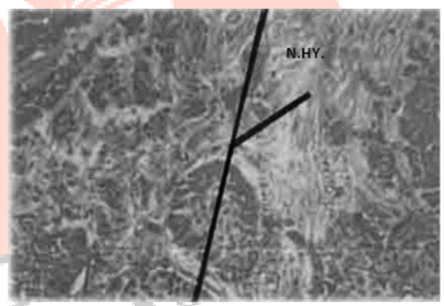

Figure 3 - VLS of brain with neurohypophysis

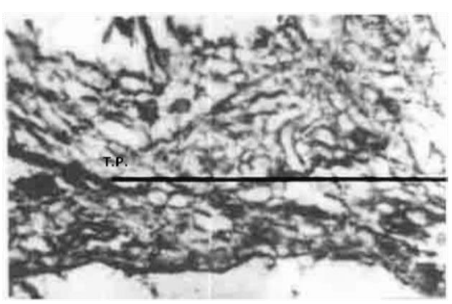

Figure 2 - VLS of brain with tractuspreoptico hypophysis.

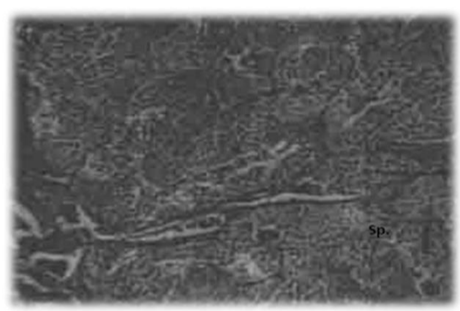

Figure 7 - T.S. of testis showing advance stages of spermatogenesis and spermatocytes

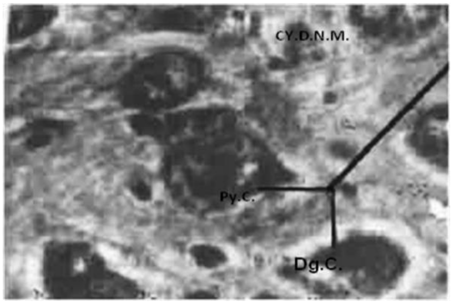

Figure 4 - VLS of HMT treated brain with depleted neurosecretory material of NPO cells.

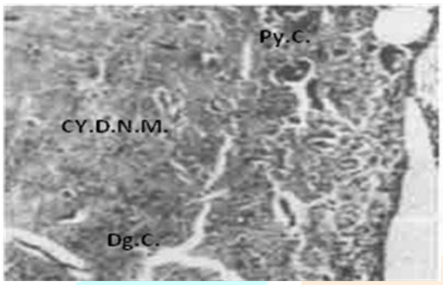

Figure 5 - VLS of HMT treated brain with tractuspreoptico hypophysis

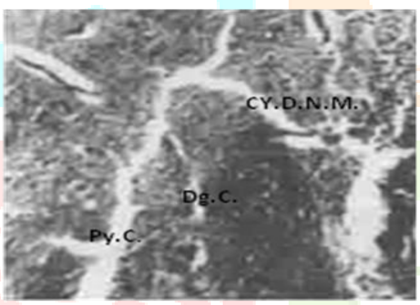

Figure 6- VLS of HMT treated brain with neurohypophysis

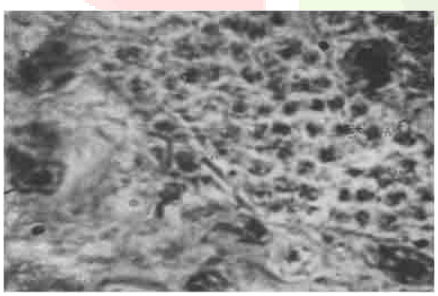

Figure 8 - T.S. of HMT treated testis showing spermatocytes besides inflammation and inter-tubular vacuolation

Figures (9-12) - Graphical representations

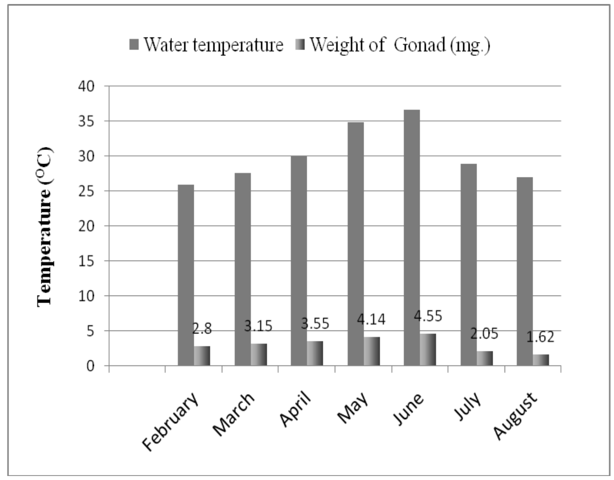

Figure 9 - showing the increase in gonad weight according to water temperature

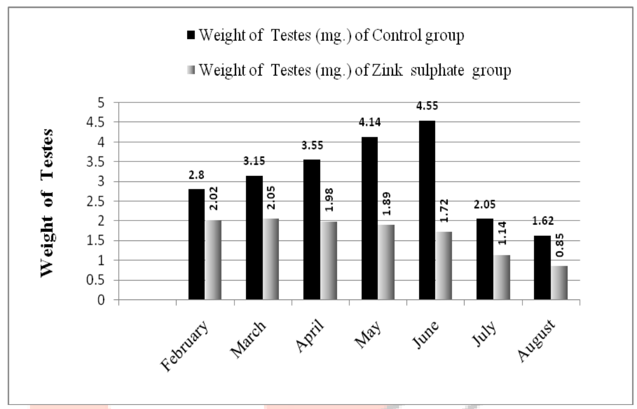

Figure 10 - showing the monthly comparison of gonad weight between Control group and $ZnSO_4$ treated group.

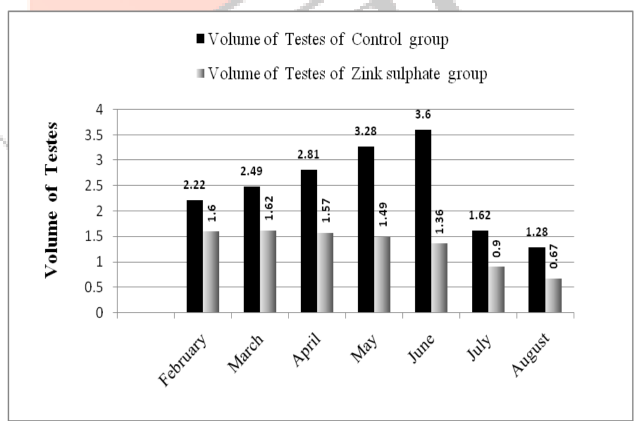

Figure 11 - showing the monthly comparison of gonad volume between Control group and $ZnSO_4$ treated group.

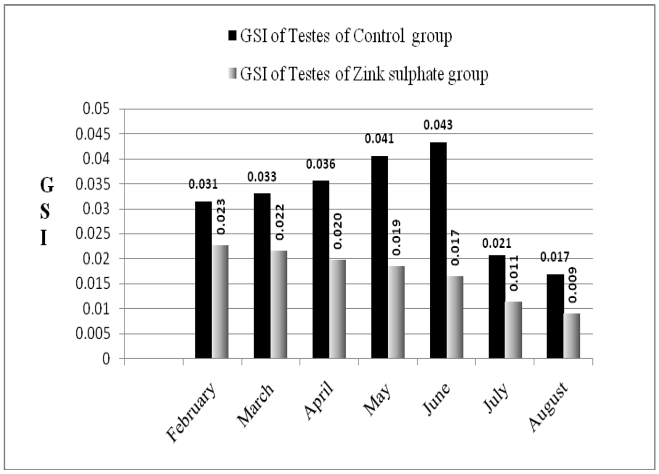

Figure 12 - showing the monthly comparison of GSI between Control group and $ZnSO_4$ treated group

CONCLUSION

The survival and existence of any species is ultimately determined by the ability to reproduce successfully in a fluctuating environment and show the diversity aspect. Maturation and spawning are very important biological processes for existence. Variation in these processes due to alteration in geographical locations and the environment are common, but heavy metal toxicity in natural water is the major source of contamination which have adverse effects on the HPG relationship of fish and disturbs the aquatic biodiversity which is responsible for maintaining and supporting overall environmental health. An understanding of these factors is essential for the existence, preservation, development and conservation of the species to maintain the Global biodiversity because fish are irreplaceable bio-indicators of the degree of damage to the water environment in terms of ecological aspect.

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