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EFFECT OF CADMIUM CHLORIDE ON HISTOLOGICAL AND BIOCHEMICAL PARAMETERS IN OVARY OF FRESHWATER AFRICAN CATFISH, *Clarias gariepinus* (Burchell, 1822)

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Abstract:

The present study investigated the histopathological and biochemical changes that occur in the ovaries of the freshwater African catfish, *Clarias gariepinus* (Burchell, 1822), after exposure of $CdCl_2$. Fish were exposed to two different concentrations of $CdCl_2$, a low dose (10 mg/L), a high dose (20 mg/L) along with control. The present study showed according to biochemical analysis protein and carbohydrate concentration of ovaries decreased in a dose-dependent manner with notable decrease seen at higher cadmium doses. Histopathological analysis revealed damage to ovarian tissue including oocyte degeneration, atresia, and vacuolization that increased with dose of cadmium exposure. The findings suggested that cadmium chloride has deleterious effects on the reproductive function of *Clarias gariepinus*, possibly affecting the ability of the species to reproduce in contaminated aquatic environments by disrupting the histoarchitecture of ovaries and metabolic equilibrium.

Keywords: *Clarias gariepinus*, $CdCl_2$, dose-dependent, Ovary, Histopathology, Biochemical.

Introduction:

The environmental toxicants such as cadmium may produce a variety of clinical manifestations in man and animals. Several organ systems including the renal, hepatic, respiratory and vasculature systems may be affected by cadmium exposure (Adams *et al.*, 1969; Axelsson & Piscator, 1966; Martin Deva, 2008; Perry *et al.*, 1976). Cadmium is one of the naturally occurring heavy metals. However, it is often used in industry, and exerts toxic effects on human health. It is

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classified as a human carcinogen by the International Agency for Research on Cancer and belongs to the group I carcinogens (Skipper *et al.,* 2016). Acute intoxication of cadmium may lead to damage to liver, lung, and testis (Kasuya *et al.,* 1992) while chronic intoxication may result in obstruction of pulmonary disease, disturbance of metabolism, disregulation of blood pressure, obstruction of kidney function, structure of bones and immune system (Järup *et al.,* 1998; Jin *et al.,* 1998; Skipper *et al.,* 2016). The African catfish *Clarias gariepinus* is an economically important species, apart from being model organism in research. Due to exposure of heavy metal, the histopathological and biochemical conditions are altered drastically. Very few studies have been carried out on histopathological and biochemical effects of cadmium chloride on the ovary of fishes. Therefore, the present study has been undertaken with the aim to study the toxicity of Cadmium chloride on the biochemical and histological parameters of African catfish, *Clarias gariepinus.*

Materials and Methods:

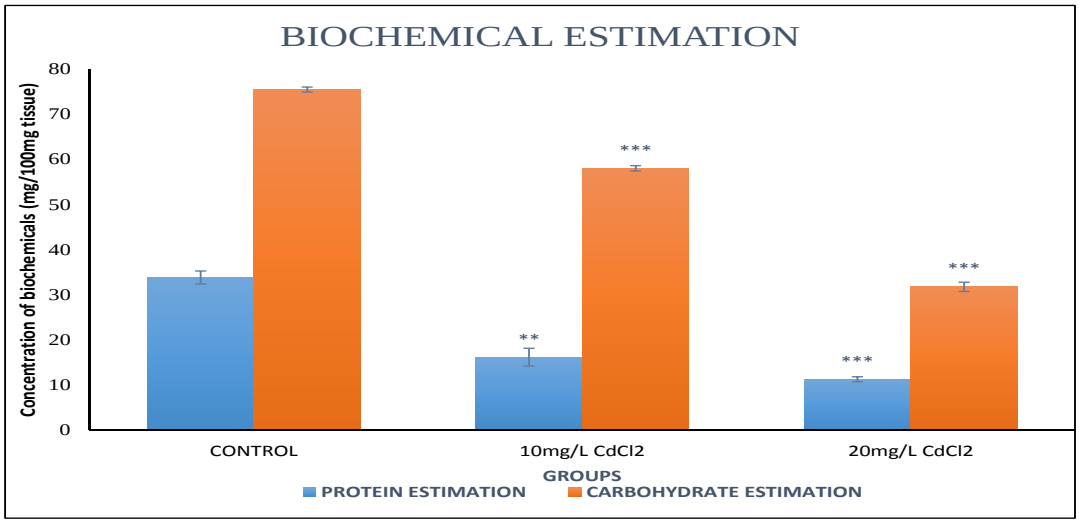
For the study, freshwater African catfish, *Clarias gariepinus* were procured from a nearby lake, with weights between 150 and 300 grams and lengths of 25 to 35 centimetres. They were housed in individual glass aquaria with 30 litres of tap water, maintained under controlled conditions of light intensity and temperature. The fishes were acclimatized for 15 days, with six fishes per aquarium. During the one-week acclimatization period, they were fed with commercial fish food. After acclimatization period, the fishes were carefully categorized into two groups namely control and experimental group. Experimental group subjected to two sublethal doses of cadmium chloride (10mg/L and 20mg/L) for exactly 15 days of duration. After completion of duration of exposure, ovary was dissected out and subjected for the biochemical and histological studies. Total protein concentration was estimated by the method of Lowry *et al.*, method (Lowry *et al.*,1951). The total carbohydrate concentration in ovary was estimated by Dubois *et al.,* method (DuBois *et al.*,1956) The ovary of experimental fishes was dissected, fixed, dehydrated, embedded in paraffin wax, and cut into 8-micron thick sections. These sections were stained using the HE double staining method and observed under the microscope for histological analysis. The observational data for all parameters were given as individual values and as mean ± standard error (SE) and differences were significant at $p<0.05$. Statistical analysis was done using the statistical program (GraphPad Software, 2024)

Results and discussion:

Environmental toxicants like cadmium are harmful chemicals that have been found to cause damages to the tissues and organ systems including the ovary by triggering oxidative stress by producing free radicals (Opuwari and Henkel 2016). The concentration of protein in ovary of both experimental groups exposed to cadmium chloride for 15 days showed a marked decreased in the protein concentration as compared to the control group. The protein concentration of control group showed 33.84±1.4mg/dl whereas the protein concentration of fishes exposed to 10 mg/L and 20 mg/L Cadmium chloride showed 16.13±1.9mg/dl and 11.23±0.55mg/dl respectively. It may be due to the higher accumulation in the ovary that may alter the levels of various biochemical parameters thereby affecting the function of its vitellogenesis and development of maturity stages. Similar

results were reported by multiple researchers Verma and Tonk (1982); Smuckler *et al.*, (1962); Mustafa and Chandra (1972); Mohanty *et al.*, (2013); Martin and Arivoli (2008); Shobha *et al.*, (2007); Vutukuru and Basani (2013).

The concentration of carbohydrate in the ovary of female fishes from experiment group exposed to Cadmium chloride for 15 days showed a marked decrease in the carbohydrate concentration as compared to the control group. The carbohydrate concentration of female fishes from control group showed 75.46±0.54 mg/dl whereas the carbohydrate concentration of female exposed to 10 mg/L and 20 mg/L Cadmium chloride showed 58.0± 0.57mg/dl and 31.73 ± 1.04 mg/dl respectively. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant through glycolysis or Hexose Monophosphate



pathway. Decrease in glycogen levels is in corroboration with the reports of earlier workers. Roy *et al.*, (2012); De Bruin (1976); Mohamed (2008); Hadi *et al.*, (2009); Emad *et al.*, (2005), Vutukuru *et al.*, (2011).

Fig. 1. Showing effect of Cadmium chloride on protein and carbohydrate concentration in the ovary of African catfish *(C. gariepinus)*

Groups	Control	10mg/Lit	20mg/Lit
Protein Estimation	33.8±1.4	16.13±1.9**	11.23±0.55***
Carbohydrate Estimation	75.46±0.54	58±0.57***	31.73±1.04***

(Values are expressed in Mean ±SE) N = 06 for each group p<0.05*, p<0.001, p<0.0001***)**

Table 1. Showing the effects of Cadmium chloride on various biochemical parameters on ovary of the African catfish, *Clarias gariepinus*

After exposure to the Cadmium chloride, various abnormalities were recorded in the ovary of fish. Histopathological results from control group fishes showed various developmental stages such as chromatin nucleolar stage follicles, early perinuclear stage follicles, and secondary yolk stage follicles. The ovary dominated with the secondary yolk stage follicles. Histopathological examination revealed that the fish exposed to 10mg/L of cadmium chloride after 15 days showed changes in the normal shape of the ovarian follicles, enlargement of oocytes and degeneration of egg envelope starts, vacuolization of follicles, karyoplasmic retraction and degeneration of follicles. In 20mg/L concentration after, ovary wall got slightly thin and ruptured and shows yolk liquefication, cytoplasmic retraction, degeneration of vitelline membrane, disorganization in shape of the zona radiata and changes in normal shape of follicle. Similar results were obtained by multiple researchers (Babu and Nair, 1983; Dhawan and Kaur,1997; Kumar and Pant 1984; Lambert 1970; Uhrin, *et al.*, 2023; Ram, and Sathyanesan 1983; Sehgal and Saxena, 1986).

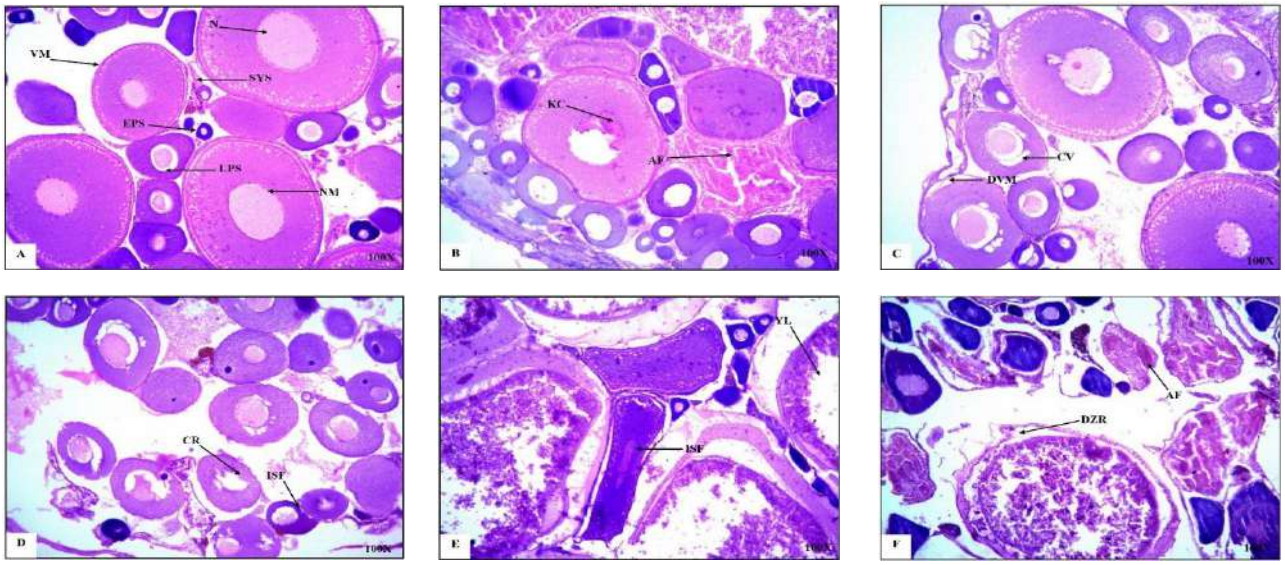


Fig. 2. Histological structures in the Ovary of Control (2A) fish shows Secondary Yolk Stage Follicles, Early Perinucleolus Stage, Vitelline Membrane, Nucleus, Late Perinuclear Stage, Nuclear Membrane. Histological structures in the Ovary of fish exposed to 10mg/lit (2B) Cadmium chloride shows Karyoplasmic Clumping, Atretic Follicles. Histological structures in the Ovary of fish exposed to 10mg/lit (2C) Cadmium chloride shows Degeneration of Vitelline Membrane, Cytoplasmic Vacuolization. Histological structures in the Ovary of fish exposed to 10mg/lit (2D) Cadmium chloride shows Cytoplasmic Retraction, Irregular Shape Follicle. Histological structures in the Ovary of fish exposed to 20mg/lit (2E) Cadmium chloride shows Yolk Liquefication, Irregular Shape Follicle. Histological structures in the Ovary of fish exposed to 20mg/lit (2F) Cadmium chloride shows Atretic follicle, Degeneration of Zona Radiata.

Abbreviations: SYS- Secondary Yolk Stage Follicles, **EPS-** Early Perinucleolus Stage, **VM-** Vitelline membrane, **N-** Nucleus, **LPS-** Late Perinuclear Stage, **NM-** Nuclear Membrane, **KC-** Karyoplasmic Clumping, **AF-** Atretic Follicles, **DVM-** Degeneration of Vitelline Membrane, **CV-** Cytoplasmic Vacuolization, **CR-** Cytoplasmic Retraction, **ISF-** Irregular shape follicle, **YL-** Yolk liquefication, **ISF-** Irregular Shape Follicle, **AF-**Atretic follicle, **DZR-** Degeneration of Zona Radiata.

This study demonstrates that 15 days of Cadmium chloride exposure at dosages of 10 mg/L and 20 mg/L causes significant biochemical and histological alterations to the ovaries of *Clarias gariepinus*. The findings reveal that ovarian tissue damage increases in a dose-dependent manner, while protein and carbohydrate levels fall significantly. These findings suggest that cadmium chloride poses a major threat to the reproductive health of *Clarias gariepinus*, emphasizing the importance of exercising caution in habitats contaminated with this heavy metal.

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S. L. Rangu was in responsible for planning the study, carrying out experiments, managing lab activities, analyzing data, and writing the report. Dr. (Mrs.) V. T. Dhurvey and Dr. A. M. Nagwanshi supervised the experimental procedures and contributed to the article. Dr. S. V. Gandhewar supported data collecting in Nagpur. Mr. S. S. Shahare, Ms. A. Sharma, and Mr. P. Chankapure were responsible for planning the study, guiding experimental operation, and contributing to the written content.

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