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Saiprasad Rangu
Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra, India

Digeshwar Bramhankar
Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra, India

Varsha Dhurvey
Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra, India

Aashikkumar Nagwanshi
Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra, India

Ankita Sharma
Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra, India

Pawan Chankapure
Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra, India

Corresponding Author:
Aashikkumar Nagwanshi
Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra, India

Cadmium induced nephrotoxicity in *Clarias gariepinus*: A histopathological and biochemical assessment

Saiprasad Rangu, Digeshwar Bramhankar, Varsha Dhurvey, Aashikkumar Nagwanshi, Ankita Sharma and Pawan Chankapure

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Abstract
The present study evaluates the histological and biochemical effects of cadmium chloride exposure on the kidneys of the African catfish (*Clarias gariepinus*). The aquatic biome, as well as the broader ecosphere, is adversely impacted by the heavy metal cadmium (Cd), particularly due to industrial discharges. In this study, freshwater African catfish (*Clarias gariepinus*) were exposed to increasing doses of cadmium chloride over a period of fifteen days. The impact of cadmium chloride on the kidneys was assessed using both histological and biochemical parameters. Biochemical analysis revealed decreased levels of proteins and carbohydrates, indicating metabolic disruption. Histopathological examination showed significant lesions in the glomeruli, vacuolization, tubular necrosis, and aggregation of inflammatory cells. These findings confirm that cadmium chloride induces nephrotoxicity.

Keywords: *Clarias gariepinus*, histology, biochemical, cadmium chloride, nephrotoxicity

Introduction
Urbanization, industrial activities, and the expansion of agriculture have significantly contributed to water pollution, which remains one of the most pressing ecological challenges today. The introduction of hazardous substances into aquatic environments particularly heavy metals poses serious risks to both aquatic organisms and human health [1]. Cadmium, in particular, is persistent in the environment and can biomagnify within the food web, endangering fish health and presenting a potential threat to humans through the consumption of contaminated fish. [2]. The African catfish (*Clarias gariepinus*) is currently the most widely cultivated and economically significant species in freshwater aquaculture. It also plays a vital ecological role in freshwater ecosystems. Due to its environmental sensitivity and ecological importance, *C. gariepinus* is a suitable model species for studying the effects of pollutants such as cadmium. As a keystone species in many aquatic ecosystems, its health reflects broader ecological impacts. Therefore, evaluating the nephrotoxic effects of cadmium on this species is essential for understanding the broader implications of heavy metal contamination. [3]. This study aims to investigate the histological and biochemical alterations in the kidneys of *Clarias gariepinus* following exposure to cadmium chloride ($CdCl_2$).

Materials and Methods
Experimental fish and design
Clarias gariepinus was selected for this study due to its availability, large size, air-breathing capability, and ease of handling under laboratory conditions. Specimens were collected from a nearby lake and acclimatized for one week in glass aquariums containing 30 liters of tap water. Each tank housed six fish (03 males and 03 females) and was maintained under standard laboratory conditions. The fish were fed blended goat liver every other day throughout the experiment. The experimental groups were exposed to sublethal doses of commercial cadmium chloride ($CdCl_2$) at concentrations of 10 mg/L and 20 mg/L for 15 days. The study was conducted in the Department of Zoology Laboratory to assess the impact of cadmium chloride on the histological and biochemical parameters of *Clarias gariepinus*.

Biochemical Techniques

The total protein content in the tissue was estimated by following Lowry *et al*., (1951) method [4]. The concentration of total carbohydrates in the tissue samples was assessed according to the Dubois *et al*., (1956) method [5].

Histological study

Fish used in the experiments had their kidneys dissected, preserved, dried, encased in paraffin wax, and then cut into slices that were 8 microns thick. For histological study, these sections were examined under a microscope after being stained using the HE double staining procedure.

Statistical Analysis

For each parameter, the observational data were presented as mean ± standard error (SE) and individual values; differences were considered significant at $p<0.05$. The online statistical program GraphPad Software, 2024 was used to perform statistical analysis.

Results

Biochemical parameters

Fish from the experimental groups exposed to cadmium chloride had significantly lower kidney protein concentrations in both male and female fishes when compared to the control group (Table 1 and Figure 1a). Fish in the experimental group exposed to $CdCl_2$ had a significantly lower kidney crbohydrate concentration than those in the control group (Table 1 and Figure 1b).

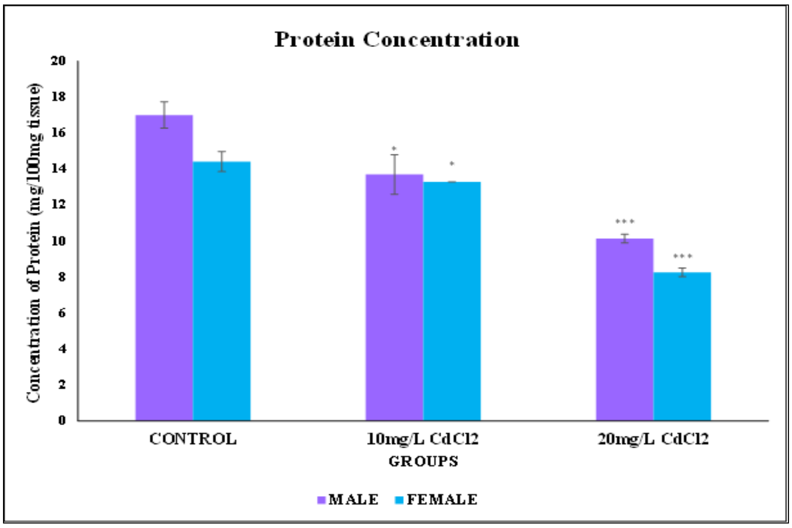

Fig 1a: Effect of $CdCl_2$ on protein concentration in kidney of African catfish, *Clarias gariepinus*

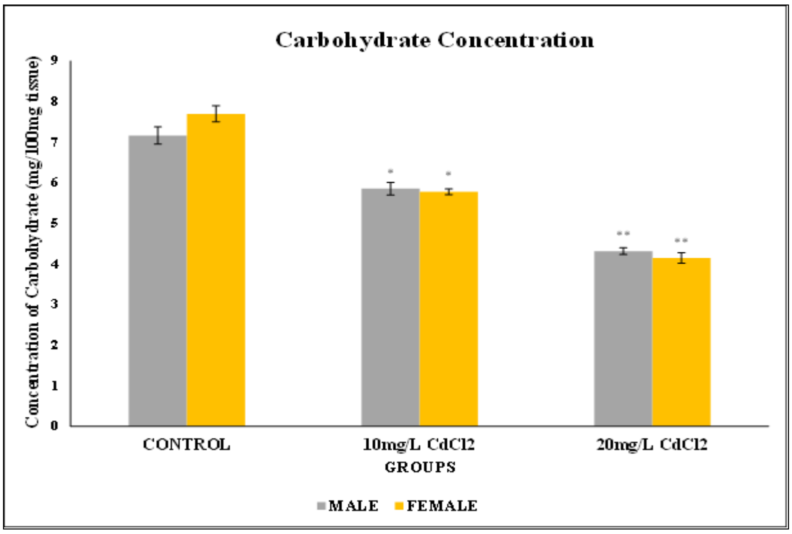

Fig 1b: Effect of $CdCl_2$ on carbohydrate concentration in kidney of African catfish, *Clarias gariepinus*

Table 1: Showing the effect of $CdCl_2$ on biochemical parameters in the kidney of African catfish, *Clarias gariepinus*

Groups	Sex	Protein	Carbohydrate
Control	Male	17.00±0.739	7.16±0.215
	Female	14.41±0.555	7.70±0.200
10mg/L $CdCl_2$	Male	13.69±1.1*	5.85±0.155*
	Female	13.29±0.0*	5.78±0.07*
20mg/L $CdCl_2$	Male	10.10±0.241***	4.31±0.085**
	Female	8.25±0.241***	4.15±0.13**

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

Histopathological study

The kidney of the *Clarias gariepinus* fish, control group displayed normal Glomerulus, Bowman space, hematopoietic tissue, renal corpuscle, collecting tubule, distal tubule, and proximal tubule. Histological analysis of the kidney of fish treated with 10 mg/L $CdCl_2$ for 15 days revealed toxic lesions, including degeneration of the urinary tubule, necrosis, and degeneration of the bowman space. Histological analysis of the kidney of fish treated with 20 mg/L $CdCl_2$ for 15 days revealed significant degeneration in proximal tubule and bowman space.

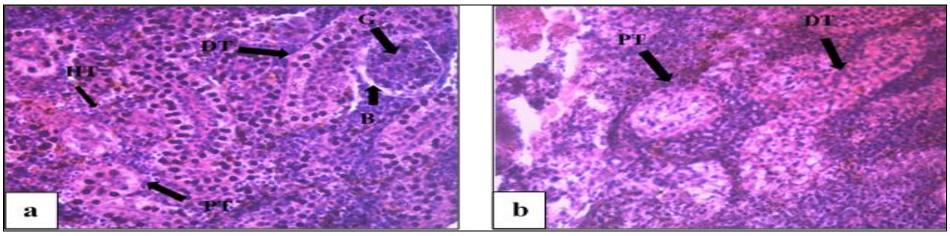

Fig 2: (a and b) Histological structures in kidney of African catfish, *Clarias gariepinus* in control group. (BS-Bowman space, G-Glomeruli, HT-Haemopoietic tissue, PT-proximal tubule, DT-Distal tubules).

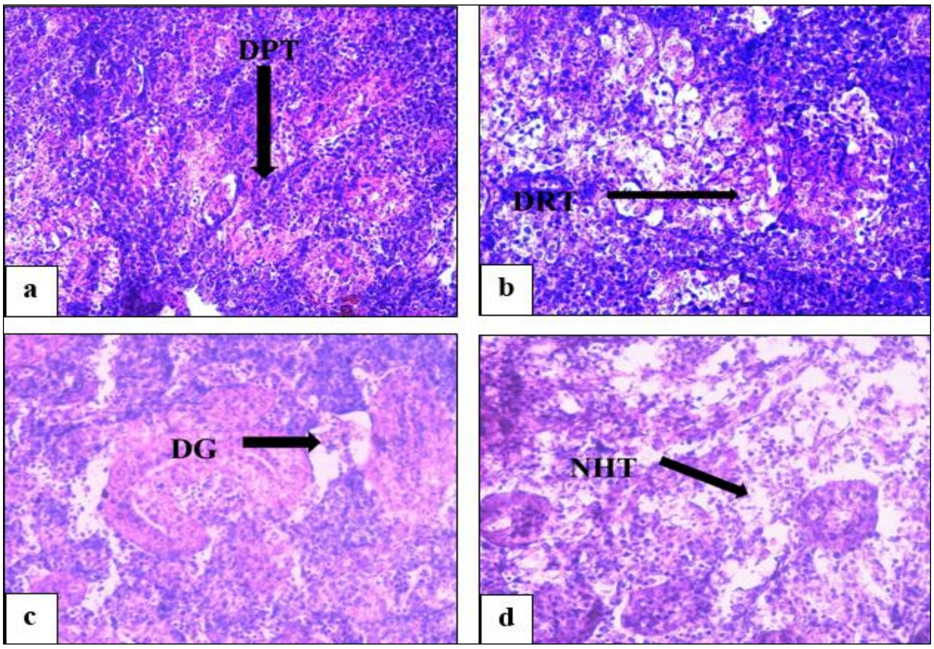

Fig 3: (a, b, c and b) Histological structures in kidney of African catfish, *Clarias gariepinus* in treated group (10 mg/L $CdCl_2$). (DPT-Damaged Uriniferous tubule, DRT-deformed renal tubules, DG-Degeneration Glomerulus, NHT-Necrosis Hematopoietic tissue)

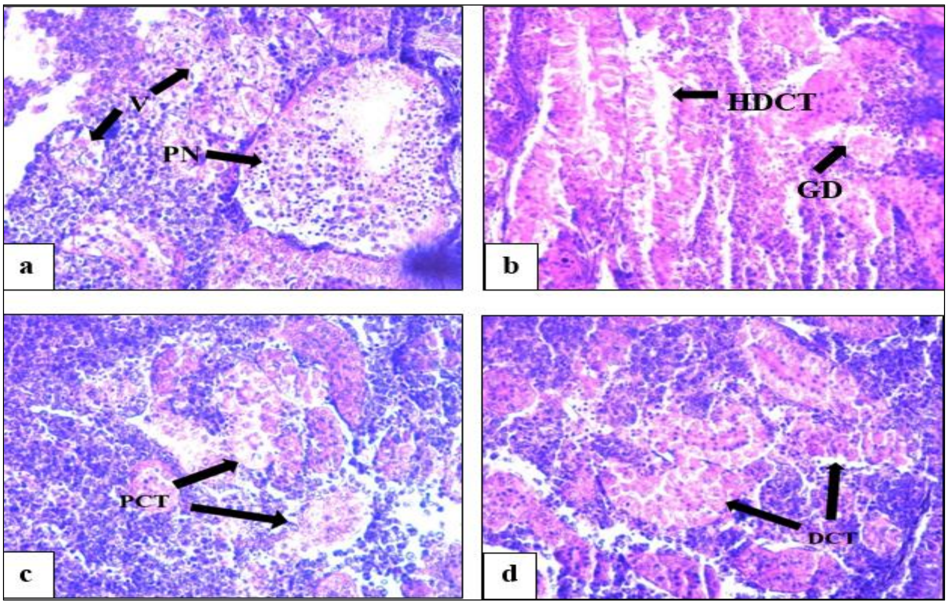

Fig 4: (a b c and b) Histological structures in kidney of African catfish, *Clarias gariepinus* in treated group (20 mg/L $CdCl_2$). (V-Vacuolation, PN-Pyknotic nuclei aggregation, HDCT-Highly damaged convoluted tubules, GD-Glomerulus degeneration, PCT-Proximal covalent tubule damage, DCT-Distal covalent tubule degeneration)

Discussion
Aquatic organisms, such as fish, are often exposed to heavy metals due to a variety of stressors, both natural and anthropogenic, present in their environment. The contamination of fish with pollutants can have adverse effects on the exploitation of aquatic resources. Heavy metals are naturally occurring in the environment and are present in varying levels in all ground and surface waters [6, 7].
According to the study, the kidneys of the freshwater catfish *Clarias gariepinus* underwent significant histological alterations following exposure to cadmium chloride ($CdCl_2$). Observed changes included reduced Bowman's space, enucleation, eccentrically positioned vacuoles within cells, necrosis of renal tissues, glomerular alterations, and thickening of the basement membrane of the urinary tubules in fish exposed to 10 mg/L and 20 mg/L of $CdCl_2$ for 15 days. Similar histological disruptions have been reported by other researchers [8–12]. In contrast, the control group exhibited normal renal architecture, including normal Glomerulus, Bowman space, hematopoietic tissue, renal corpuscle, collecting tubule, distal tubule, and proximal tubule. However, in the experimental groups exposed to $CdCl_2$, significant pathological changes were noted: degeneration of Bowman's space, glomerular distortion, nuclear deterioration, loosening of hematopoietic tissues, cytoplasmic vacuolization, damage to uriniferous tubules, and shrinkage of the glomeruli with expanded Bowman's space. Comparable findings have been documented in *Channa punctatus*, where $CdCl_2$ exposure led to dilation, edema, hypertrophied nuclei in renal tubules, vacuolated glomeruli, and disorganized blood capillaries [13]. In the present histological analysis of *Clarias gariepinus* kidneys, increased tubular vacuolization and interstitial necrosis were prominent. The glomeruli appeared deformed and shrunken, accompanied by widened Bowman's space. Nuclear pyknosis and aggregation of necrotic cells were also evident. The Proximal Convoluted Tubules (PCT) displayed enhanced vacuolization and tubular dilation, while the Distal Convoluted Tubules (DCT) were similarly dilated and vacuolated [14].
Biochemically, exposure to sublethal concentrations of $CdCl_2$ led to a marked decrease in the protein content of kidney tissues in *Clarias gariepinus*, as determined by total protein and carbohydrate analysis. This reduction suggests tissue proteolysis, whereby proteins are degraded into amino acids that are subsequently utilized in the tricarboxylic acid (TCA) cycle to meet the organism's heightened energy demands under stress. The observed protein depletion is attributed to elevated protease activity in the renal tissues. Furthermore, cadmium-induced stress may trigger protein mobilization from kidney tissue to support metabolic energy requirements. Cadmium exposure has been widely documented to significantly alter the biochemical composition of fish. For example, in *Catla catla*, kidney protein content decreased after $CdCl_2$ exposure [15].
Similarly, in *Cirrhinus mrigala*, both sublethal and lethal concentrations of $CdCl_2$ resulted in a significant decline in total kidney protein, with statistically significant changes compared to controls [16]. In *Labeo rohita*, sublethal exposure to $CdCl_2$ led to reductions in both total protein and carbohydrate levels in renal tissues. The decline in carbohydrate content is indicative of their accelerated use to meet energy demands under toxic stress. This may be explained by increased glycogen transferase activity, which promotes rapid glycogenolysis [17]. The decrease in protein concentration has also been linked to a reduction in the specific growth rate (SGR) in cadmium-exposed fish, likely due to impaired protein synthesis and increased degradation. Prolonged exposure to cadmium leads to cellular damage, including glycocalyx degradation, further compromising physiological functions.

Conclusion
This study confirms that cadmium chloride exposure leads to pronounced nephrotoxicity in *Clarias gariepinus*, as evidenced by structural kidney damage such as glomerular shrinkage, vacuolization, and necrosis and significant reductions in protein and carbohydrate content. These changes reflect metabolic stress and impaired kidney function. The results emphasize the toxic impact of cadmium on aquatic organisms and reinforce the need for strict regulation of heavy metal discharge to protect freshwater ecosystems and public health.

Conflicts of Interest
The authors claim that none of the tasks as described in the study was subject to any known competing economic interests or personal relationships that could bias the outcome.

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