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Amelioration of Arsenic Trioxide-Induced Histopathological Alterations in Kidney Tissue by Aspirin in *Cyprinus carpio*

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Abstract: This study investigated the potential ameliorative effects of aspirin against arsenic trioxide-induced kidney toxicity in fish *Cyprinus carpio*. Arsenic trioxide exposure to fish at different concentrations (0.9 mg/kg, 1.3 mg/kg and 1.8 mg/kg) resulted in histopathological renal damage such as glomerular contraction, tubular degeneration and tissue disintegration. However, simultaneous administration of aspirin (50 mg/kg) showcased few ameliorative effects. Fish treated with aspirin exhibited normal renal tissues with well-developed glomeruli and tubular networks. Aspirin administration after arsenic trioxide exposure demonstrated improvements, including reduced glomerular contraction, diminished tubular degeneration and minimized interstitial tissue damage. This study provides valuable insights into the potential therapeutic benefits of aspirin in mitigating arsenic trioxide-induced kidney toxicity.

Keywords: Arsenic trioxide, Aspirin, *Cyprinus carpio*, Kidney, Histopathology, Amelioration

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Introduction

Preserving our planet's water bodies and reducing exposure to harmful substances are imperative for securing a healthier and safer future for aquatic ecosystem. Among the most pressing environmental concerns is heavy metal toxicity, which causes a significant threat to aquatic life. Heavy metals, originating from industrial discharges, agricultural runoff and natural sources, accumulate in water bodies, affecting the delicate balance of aquatic ecosystem. They disrupt various physiological processes in aquatic organism leading to impaired growth, reproductive disorders and compromised immune system. Fish in particular serve as an important bioindicator of water quality, reflecting the extent of heavy metal contamination as they consume contaminated prey and absorb metals through their gills. These toxic substances bioaccumulate within their

tissue, further magnifying the ecological impact.

Arsenic is a naturally occurring metalloid that exists in various forms, including inorganic arsenic compounds and organic arsenic species, each with distinctive toxicological properties. Its levels are typically higher in the aquatic environment compared to most terrestrial area due to its relatively high-water solubility, which facilitates its release from arsenic-bearing rocks and subsequent washout into water bodies (Edmonds and Francesconi, 1993). It exists as arsenite (As^{3+}) or arsenate (As^{5+}), interconverting through redox and methylation reactions, influencing its bioavailability (Bears *et al*., 2006). Inside the cell, these two forms exhibit distinct reactivity: arsenite binds with sulfhydryl groups of protein, while arsenate disrupts the phosphorylation processes (Andrew *et al*., 2003). Its toxicity may be associated with an overproduction of reactive oxygen species, including superoxide, peroxyl-radicals and hydrogen peroxide, leading to oxidative stress (Kitchin and Ahmed, 2003).

Fish are commonly regarded as the preferred organisms for evaluating the impacts of environmental pollution on aquatic ecosystems. Blood parameters and body indices of fish have been employed as indicators of environmental risk, providing valuable insights into the effects of pollutants on aquatic ecosystems (Yang and Baumann, 2006). Arsenic suppresses the antibody production and can adversely affect the fish immune system by, leading to compromised immune responses (Ghosh *et al*., 2007). According to Bears *et al*. (2006), fish play a crucial role as indicators of arsenic toxicity, because of their constant exposure to arsenic through gill respiration and arsenic-contaminated food consumption. Heavy metals undergo metabolic activation and also trigger cellular changes in affected fish. Bioaccumulation, infection, diseases and parasites, stimulating necrotic alterations in the fish with an inflammatory defensive reaction causing tissue lesions and apoptosis (Roganovic–Zafirova *et al*., 2003).

Aspirin (acetylsalicylic acid) is a widely used class of non-steroidal anti-inflammatory drugs (NSAIDs) with cytoprotective properties, essential in modern medicine. Derived from willow bark, it dates back to ancient civilizations for pain relief and anti-inflammatory purposes. The mode of action of aspirin is primarily due to its ability to inhibit the activity of cyclooxygenase (COX) enzymes. Aspirin acts as an irreversible inhibitor of COX-1 and COX-2, which are important enzymes involved in the synthesis of prostaglandins and other inflammatory mediators (Vane, 1971). Through inhibition of these enzymes, aspirin diminishes the production of prostaglandins, which are responsible for promoting inflammation, pain and fever in the body. In addition to its anti-inflammatory effects, aspirin also exhibits antiplatelet activity by inhibiting COX-1 in platelets (Roth and Majerus, 1975). This antiplatelet action helps prevent blood clot formation and is widely used in preventing cardiovascular events, such as heart attacks and strokes.

In light of aspirin's pharmacological properties, the current investigation aims to assess its preventive efficacy against arsenic-induced histopathological alterations in the kidney of *Cyprinus carpio*. This study seeks to explore aspirin's potential cytoprotective role in mitigating the adverse effects of arsenic trioxide exposure on the vital organs in the fish.

Materials and Methods

Experimental animals

The experimental fish, *Cyprinus carpio* (100±20 g; 23±2 cm), were collected from Deoli fish farm in District Bilaspur, Himachal Pradesh, with permission from the Directorate of Fisheries. They were housed in a 150 L glass aquarium equipped with filters and aerators, maintaining a constant temperature of 22±2°C and natural photoperiod. To prevent parasitic infestation, the fish received a 3–4 min bath in a 0.1% $KMnO_4$ solution. Before the experiment, the fish were acclimatized to laboratory conditions in dechlorinated tap water for 15 days. The water's osmolarity was

maintained by changing only half of the volume every 24 h. They were fed with commercial feed equivalent to 1/10th of their body weight twice per day, with unconsumed feed and excreta removed daily. Food was withheld from the experimental fish 24 h prior to the start of the experiment.

Chemicals

The arsenic trioxide used for the study was sourced from CDH Fine chemicals (New Delhi, India). To prepare the stock solution, arsenic trioxide was dissolved in a 10% DMSO solution. The solution was renewed every 24 h.

The aspirin utilized for the study was obtained from CDH Fine chemicals (New Delhi, India). To create the stock solution, it was dissolved in a 10% DMSO solution. The solution was refreshed every 24 h.

Estimation of LD_{50}

The LD_{50} for arsenic trioxide has been calculated to be 13.74 mg/kg body weight, with lower and upper limits of 9.41 mg/kg and 18.47 mg/kg body weight, respectively. Probit analysis graph using IBM SPSS 21 was employed to determine these values. To conduct the experiment on the fishes, 1/10$_{th}$ of the LD_{50} dose (1.3 mg/kg body weight) along with its lower (0.9 mg/kg body weight) and upper (1.84 mg/kg body weight) limits were administered.

Experimental design

The fish were divided into following eight groups with six individuals in each:

Group 1: fish were employed as control.

Group 2: fish were administered with 0.9 mg/kg body weight of arsenic trioxide for 10 days.

Group 3: fish were exposed to 1.3 mg/kg body weight of arsenic trioxide for 10days.

Group 4: fish were administered with 1.8 mg/kg body weight of arsenic trioxide for 10days.

Group 5: fish were injected with 50 mg/kg body weight of aspirin alone for 10 days.

Group 6: fish treated with 0.9 mg/kg body weight of arsenic trioxide were administered with 50 mg/kg concentration of aspirin for 10 days.

Group 7: fish subjected to 1.3 mg/kg body weight of arsenic trioxide received an administration of 50 mg/kg concentration of aspirin for a period of 10 days.

Group 8: fish exposed to 1.8 mg/kg body weight of arsenic trioxide underwent a 10-day treatment with 50 mg/kg concentration of aspirin.

Histopathological studies

For histological studies kidney tissues were collected from control as well as treated fish after completion of 10 days. Histopathological alterations were assessed using light microscopy with H&E staining. Renal tissues of the fish were dissected immediately. The dissected tissues were fixed in 10% formalin fixative for 24 h. Excess fixative was removed thorough washing under running tap water. Subsequently, tissues were dehydrated in graded alcohol (30%, 50%, 70%, 90%, and 100%) for 30 min each. The tissues were cleared in xylene, embedded in paraffin wax (58-60°C), and cut into sections of 5-6 µm thickness using a rotary microtome and stained with Haematoxylin-eosin.

Results

Renal corpuscles in control fish display well-developed glomeruli and intricate tubular networks. The proximal segment exhibits tall columnar epithelial cells with basal nuclei and a prominent brush border, while the distal segment features large, clear columnar epithelial cells with central nuclei and a reduced brush border. The collecting duct, comprises columnar epithelial cells with basal nuclei and lacks a brush border.

Fish treated with 0.9 mg/kg arsenic trioxide for 10 days showed glomerular contraction, mild disruption of hematopoietic tissue, leading to expanded space between the glomerulus and Bowman's capsule, hypertrophy in proximal tissue and a loss of the normal rounded shape (Fig. 1 B).

Fish exposed to 1.3 mg/kg arsenic trioxide for

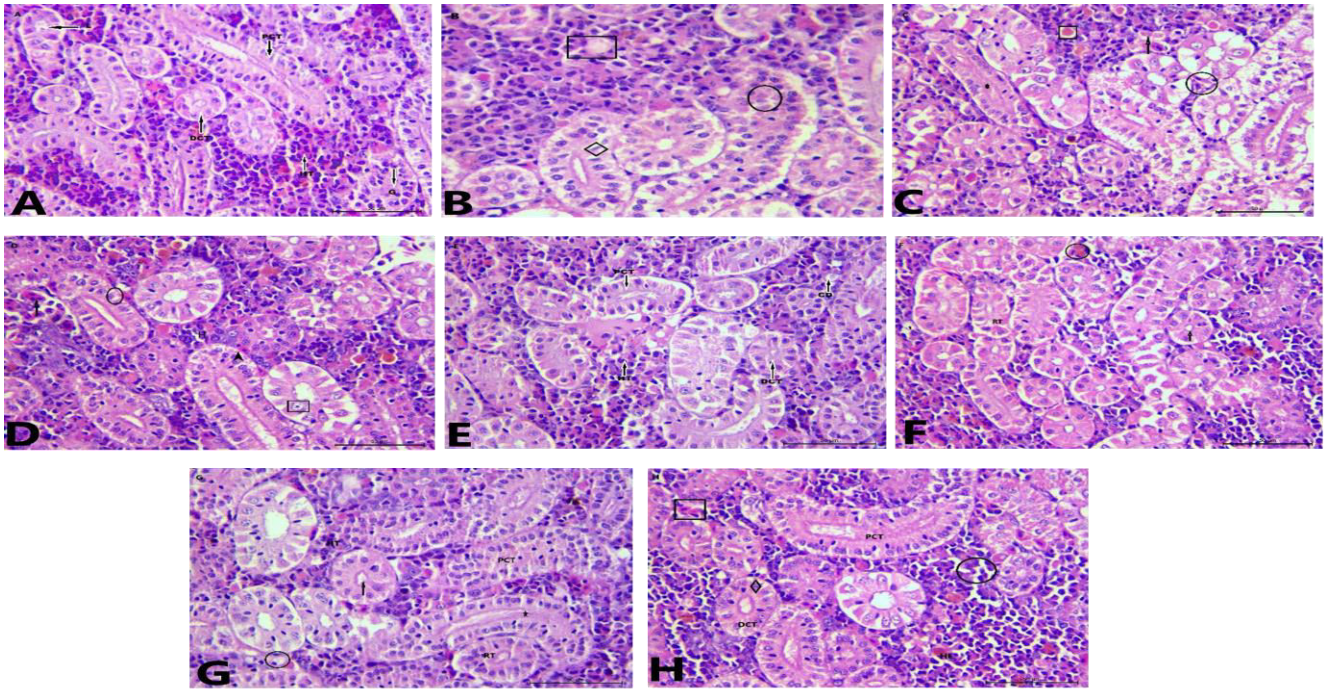

Fig. 1: Kidney *of Cyprinus carpio* exposed to arsenic trioxide and ameliorated by aspirin. **(A)** Kidney of control *Cyprinus carpio* showing normal glomerulus (G), proximal convoluted tubule (PCT), distal convoluted tubule (DCT), haematopoietic tissue (HT) and normal lumen of distal convoluted tubule(L); **(B)** Kidney of *Cyprinus carpio* exposed to 0.9 mg/kg of As_2O_3 for 10 days showing loss of round shape of nuclei (diamond), haematopoietic tissue disruption (rectangle) and hypertrophied proximal tubule (circle); **(C)** Kidney of *Cyprinus carpio* exposed to 1.3 mg/kg of As_2O_3 for 10 days showing obstructed proximal tubule lumen (star), crowding of tubules (circle), necrosis of haematopoietic tissue (arrow) and internal haemorrhage (square); **(D)** Kidney of *Cyprinus carpio* exposed to 1.8 mg/kg of As_2O_3 for 10 days showing vacuolation in proximal convoluted tubule (circle), pycnotic nuclei (square), ruptured basement membrane (pointed arrow) and disorganized connective tissue (arrow); **(E)** Kidney of *Cyprinus carpio* exposed to 50 mg/kg of aspirin for 10-day showing normal proximal convoluted tubule (PCT), distal convoluted tubule (DCT), haematopoietic tissue (HT) and collecting duct (CD); **(F)** Kidney of *Cyprinus carpio* exposed to 0.9 mg/kg of As_2O_3 and ameliorated with 50 mg/kg of aspirin for 10 days showing diminished lumen widening (arrow), reduced haematopoietic tissue degeneration and normal renal tubules (RT); **(G)** Kidney of *Cyprinus carpio* exposed to 1.3 mg/kg of As_2O_3 and ameliorated with 50 mg/kg of aspirin for 10 days showing reduction in vacuole size (star), contracted lumen of DCT (arrow) and reduced hyaline degeneration; **(H)** Kidney of *Cyprinus carpio* exposed to 1.8 mg/kg of As_2O_3 and ameliorated with 50 mg/kg of aspirin for 10 days showing infiltration of connective tissue (square), degenerated haematopoietic tissue (circle) and pyknotic nuclei (diamond).

10 days exhibited obstructed lumen in proximal convoluted tubules, necrosis of hematopoietic tissue. vacuolization in cytoplasmic tissue, internal haemorrhage, thinned lining of renal tubules and crowding with other tubules (Fig. 1C).

Fish exposed to 1.8 mg/kg arsenic trioxide for 10 days exhibited increased disintegration of glomerulus and its visceral layer, vacuolated proximal tubules with pycnotic nuclei, ruptured basement membranes, disorganised connective tissue infiltration of edematous fluid between tubules and hydropic swelling (Fig. 1D).

Cyprinus carpio administered with 50 mg/kg of aspirin for 15 days showed normal renal tissues with well-developed glomeruli and network of tubules (Fig. 1E).

After 0.9 mg/kg arsenic trioxide treatment, 10 days of aspirin amelioration showed improvement

in kidney tissues, reduced glomeruli contraction, diminished tubules degeneration and interstitial tissue damage (Fig. 1F).

Following 1.3 mg/kg arsenic trioxide treatment, aspirin administration produced cumulative effect on renal tissues reducing vacuole size, contracting lumen of distal convoluted tubules, restoration of lining thickness in renal tubules occurred with reduced hyaline degeneration and minimised blood infiltration (Fig. 1G).

Simultaneous exposure of fish to 1.8 mg/kg arsenic trioxide and 50 mg/kg aspirin resulted in average preservation of fish kidneys. Hematopoietic tissue showed degeneration, pycnotic nuclei were observed and connective tissue infiltration improved (Fig. 1H).

Discussion

The effectiveness of aspirin in ameliorating arsenic trioxide induced renal toxicity was assessed. Kidneys have ability to regulate blood composition, electrolyte balance and fluid volume in the body. Within the kidney functional units called nephrons are responsible for filtration. The kidney, a crucial organ for excretion and osmoregulation, is directly impacted by heavy metals through the circulation of blood (Newman and Maclean, 1974). Histopathological examination of kidney tissue from arsenic-exposed fish unequivocally confirmed arsenic trioxide as a potent renal toxicant. Chronic exposure to inorganic arsenic has been linked to the development of skin, lung, bladder and liver cancers (Aposhian *et al*., 2003). Persistent exposure to arsenic can result in alterations in gene expression and cellular damage in fish, potentially exerting enduring effects of their survival and reproductive success (Kobayashi *et al*., 2019). The pathological changes observed in the kidney, particularly the shrinkage of glomeruli and tubular necrosis, especially in the convoluted tubule, were consistent with the observations reported by Bhatnagar *et al*. (2007). In the current investigation, observations include the disintegration of kidney tubules, vacuolization, tissue breakage, aggregation of blood cells in specific areas and necrosis of the kidney tubules. Similar changes have also been observed by Jagadeesan (1994) in *Labeo rohita* treated with mercuric chloride. Disintegration, vacuolization, tissue breakage, aggregation of blood cells and necrosis of kidney tubules were observed in *Mystus vittatus* treated with copper (Rajamanickam, 1992). Similar findings were also reported from *Ameiurus nebulosus* and cod *Microgadus tomcod* from contaminated streams (Cormier *et al*., 1995). Omoniyi *et al*. (2002) and Shalaby (2009) reported similar results. The kidney plays a pivotal role in eliminating most toxicants and is considered a primary target organ for the impact of toxicity (Radhiah *et al*., 1987). In the present investigations, fishes treated with varying sublethal concentrations of arsenic trioxide exhibited notable effects, including vacuolization of tubular cells, pycnotic nuclei, shrinkage of glomeruli, enlargement in the lumen of the tubules and degeneration of renal tubules. Additionally, there were observed occurrences of disintegration of hematopoietic tissue and necrosis of kidney tubules. Similar findings have also been noticed by Soma Datta *et al*. (2009) in *Clarias batrachus* exposed to arsenic; Ravindrababu and Neeraja (2012) in *Dicentrachus labrax* exposed to ammonia and Prashanth (2011) in *Cirrhinus mrigala* exposed to cypermethrin. The current research also noted the ameliorative impact of aspirin on arsenic trioxide induced toxicity. Aspirin is commonly utilized by individuals, both healthy individuals and those at risk of cardiovascular disease, to reduce blood viscosity and benefit from its antithrombic effects (Durgun *et al*., 1998). During experimental observations, aspirin administration improved kidney tissues, reduced glomerular contraction, altered vacuole size, restored thickness lining, decreased hyaline degeneration, minimised edematous fluid infiltration preventing hydropic

swelling and also reduced necrosis.

Conclusion

In conclusion, this study establishes a direct link between toxic metal accumulation and histopathological abnormalities in fish renal tissues, suggesting predominantly harmful effects rather than adaptive responses. Being a vital food resource, fish expose a risk of transmitting metals to humans through the food chain, leading to potential health issues. Histopathology emerges as a valuable for environmental management, aiding in bio-monitoring aquatic ecosystems. Understanding histopathological changes in fish is a crucial for assessing human exposure risks to environmental pollutants, particularly through the consumption of aquatic- derived food

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Conflict of Interest

The authors declare no conflicts of interest.

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