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Research Paper

Effect of cadmium chloride on kidney of *Heteropneustes fossilis* and their recovery by herbal compound mulethi

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Abstract

The present study was conducted to investigate the effect of cadmium chloride on kidney of fresh water fish *Heteropneustes fossilis*. The fish were exposed to 0.5 ppm of cadmium chloride (safe dose) for 7, 14 and 21 days. The most remarkable changes in kidney due to cadmium chloride were loosening of haemopoietic tissue; hemorrhagic conditions and degeneration of haemopoietic tissue. Renal tubules have lost their original appearance, wide spaces and oedema were visible in renal tubules, narrowing of tubular lumen, vacuolated cytoplasm and damaged glomeruli. The size of haemopoietic tissue and glomerulus was significantly ($p<0.001$) increased after treatment and the size of renal tubules significantly ($p<0.05$) reduced after treatment. All these changes will be recovered by herbal compound, mulethi. The damaged tissues of haemopoietic, renal tubules and glomerulus were recovered in already treated group.

Keywords: Cadmium chloride, Glomerulus, Histopathology, Haemopoietic tissue, Kidney, Mulethi, Renal tubule.

Introduction

Heavy metals have been recognized as strong biological poisons because of their persistent nature, toxicity and tendency to accumulate in organisms and undergo food chain amplification[1,2]. Heavy metals in aquatic organisms pose a long lasting effect on biogeochemical cycling in the ecosphere. Fish are often at the top of the food chain and have the tendency to concentrate heavy metals from water[3]. The heavy metals usually affect fish negatively, leading to stress, in most cases, death occurs. Cadmium is considered as major aquatic pollutant in many part of the world. The cadmium related contamination of aquatic habitat has greatly increased cadmium deposit in tissue of aquatic organisms[4,5]. Accumulation of cadmium in living organism is major ecological concern. In fish cadmium causes number of structural and morphological changes in various organs. The highest cadmium levels were detected in the kidney and liver[6]. The kidney is the most permeable region of the body of the fish is composed of three distinct system endocrine, haemopoietic and excretory organ of fish. Cadmium causes kidney tubules damage in fish[7]. The present study was undertaken to observe the deformities produced by safe dose of cadmium chloride on kidney of fish, *Heteropneustes fossilis* and recovery of damaged tissue by herbal compound mulethi.

Materials and Methods

Experimental Animal: *Heteropneustes fossilis*
Metal used: Cadmium was used for present study in the form of cadmium chloride ($CdCl_2$). The dose of cadmium chloride was decided after calculating by LC 50 value. It was found to be 1g/l. The 0.5ppm is safe dose given to fishes.

Recovery agent: Mulethi (powder from) were used recovery agent present investigation. The fish were acclimated to standard laboratory conditions for a period of 10 days prior to the experiment. The fish were treated with 0.01% of KMno4 solution to remove any dermal infection. The average weight and length of fish were 25±5 gm and 12±5 cm respectively. Fishes of all experimental groups were fed with dried and chopped prawn, twice a day. The daily dose of food for fish was 30 mg/fish/day. The water was changed on every third day of all aquaria. After changing the water $CdCl_2$ was added in treated, recovery group of aquarium water. Water was aerated by an aquarium pump for 30 minutes daily. The daily dose of recovery agent for fish was ¼ part of their food. 108 fishes were divided into three groups for maximum 21 days:

Control Group- 36 Fish of this group was fed on plain food and keep only in stored tap water (without administration of $CdCl_2$).

Treated Group- 36 Fish of this group was treated with $CdCl_2$ (0.5ppm) solution up to 21 days and fed on plain food.

Recovery Group-36 Fish of this group was treated with $CdCl_2$ (0.5ppm) solution up to 21 days then mulethi with food was given up to these treated fishes up to 21 days.

The fish were decapitated on 7, 14 and 21 days. The kidney was fixed in aqueous Bouin's solution and sectioned following the routine method. Fixed tissues were embedded in paraffin wax. Sections were cut at 5 µ thickness with help of microtome and stained with haematoxylin and eosin and mount in DPX. All the data and results for final observation were processed in the form of microphotographs and table. The diameter of kidney cells, renal tubules glomerulus and haemopoietic cells were recorded and difference if any were compared by statistical analysis using student 't' test[7].

Results and Discussion

Histological studies revealed that the kidney section of control group showed normal histoarchiteceture. The posterior kidney of *Heteropneustes fossilis* is composed of renal tubules (proximal and distal convoluted tubules and collecting duct), renal corpuscle of nephron, glomerulus and Bowman's capsule followed by tubular neck and glomerulus enclosed within Bowman's capsule . The renal tubules are numerous and they exhibited round or oval in shape with narrow lumen. Haemopoietic tissue is located in-between the renal tubules are composed of round to polygonal basophilic cell with spherical nuclei. Few scattered blood corpuscle are seen in haemopoietic tissue (Figure 1, 3 and 5).

In 7 days cadmium chloride treated group exhibited the degeneration of tubules and necrotic condition. The cells of renal tubules were fused and cytoplasm condensed in the central region. Renal tubules lost original appearance and degeneration of cytoplasm leading to wide spaces. The hypertrophy of glomerulus exhibited. The glomerulus in the Bowman's capsule exhibited shrunken or clumped condition and leading to wide space in the capsule. The deshaped glomerulus were also exhibited .The pyknotic nuclei in haemopoietic tissue were visible. The cytoplasm is not evenly distributed and it was shrunkened from periphery of the cells to load of cadmium chloride. Eccentric and pyknotic condition of nuclei exhibiting in almost all cells (Figure 1 and 2).

In 14 days duration the renal tubules undergo degeneration. The cellular structure becomes hypertrophied. The cells of renal tubules exhibited vacuolated condition, due to clumped cytoplasm in the central region. It gives blurred appearance. The cell boundaries were disturbed and become indistinct. Glomeruli in the Bowman's capsule were had only thick mass. Due to this empty Bowman's capsule were visible. The haemopoietic cells were in vacuolated stage (Figure 3 and 4).

In 21 days duration sever condition were visible. The renal tubules show wide spaces due to fusion of cells and clumped cytoplasm. The cytoplasm appears atrophied and granular texture. At places the oedema was visible in the renal tubules. The glomerulus exhibited atrophied condition. The atrophied and hemorrhagic condition was remarkable tissues. All the cellular structure including haemopoietic tissues were present in necrotic configuration due to loss of cytoplasm. Severe degeneration was seen in haemopoietic tissue (Figure 5 and 6). In all treated group the diameter of renal tubules along-with glomerulus and haemopoietic tissue exhibited significant ($P<0.05$, 0.01and0.001) reduction in their size (Table 1).

Table 1: Diameter of kidney cells *of Heteropneustes fossilis* in control and experimental group

	Parameter	7days (in µ)		14days (in µ)		21days (in µ)	
		Control	Treated	Control	Treated	Control	Treated
1	Renal tubules	15.5±0.92	*** 13.6±0.85	12.3±0.55	*10.83±0.54	13.0±0.26	** 7.4±0.15
2	Glomerulus	24.9±1.97	Ns 26.4±2.02	Ns 25±1.66	Ns 26.78±0.81	25.1±0.71	*** 32.1±1.73
3	Haemopoietic tissue	9.4±0.05	** 10.8±0.04	7.9 ±0.55	*** 12±0.73	7.6±0.64	*** 16.3±0.87

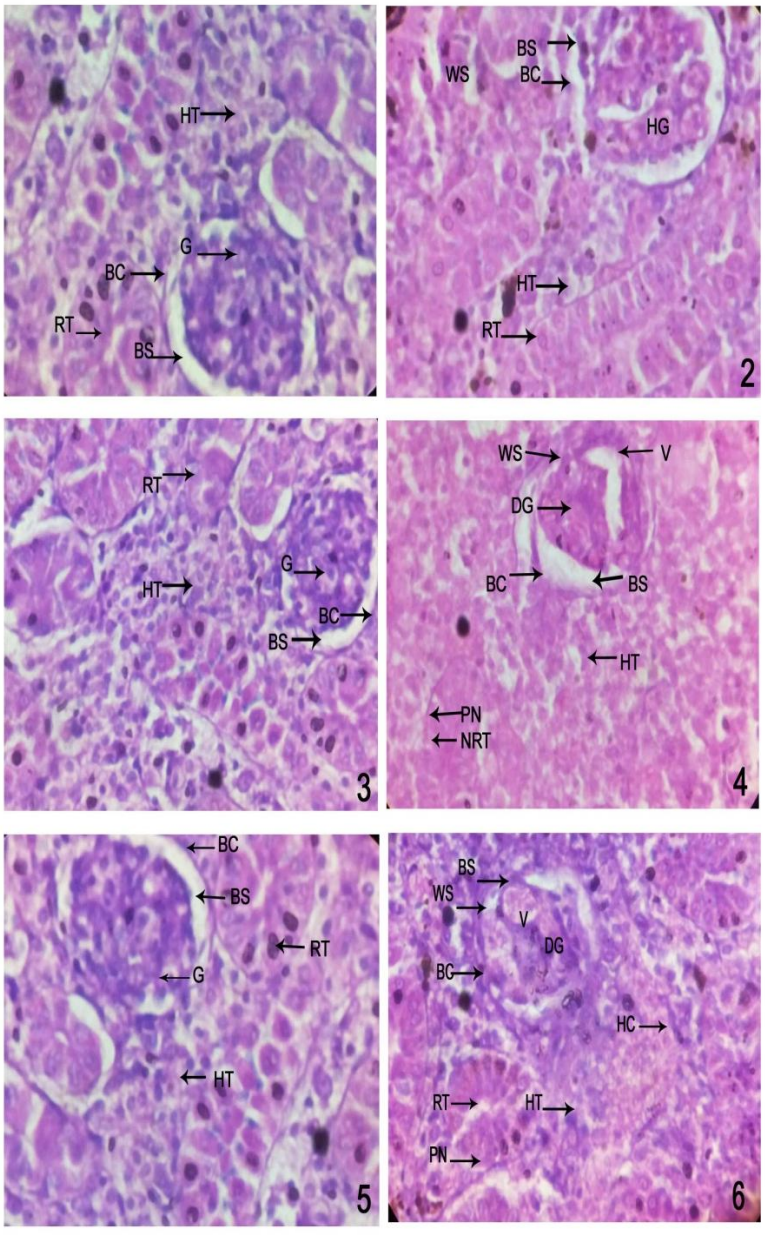

Plate 1: Photomicrographs of Kidney of Control and Treated, *H. fossilis* (HAE+EOSIN) X 100

Fig.1. Control group: 7days Showing general histology of kidney structure with well organized renal tubules, Bowman's capsule with glomerulus and haemopoietic tissue.

Fig.2. Treated group:7days Showing necrotic condition of kidney tubules, hypertrophy of glomerulus, wide spaces in the Bowman's capsule and pyknotic nuclei in haemopoietic tissue were visible.

Fig.3. Control group: 14 days showing general histology of kidney structure with well organized renal tubules, Bowman's capsule with glomerulus and haemopoietic tissue.
Fig.4.Treated group:14 days Showing hypertrophy and blurred cells of renal tubules ,disorganized glomerulus, wide spaces into the Bowman's capsule and haemopoietic cells were in vacuolated stage.
Fig.5. Control group:21 days showing general histology of kidney structure with well organized renal tubules, Bowman's capsule with glomerulus and haemopoietic tissue.
Fig.6. Treated group:21 days Showing wide spaces and oedema were visible in renal tubules, atrophied condition of glomerulus, hemorrhagic conditions were remarkable and degeneration of haemopoietic tissue.

There are few reports which show the damage of renal tubules, glomerulus and haemopoietic, tissue. Kidney severely affected by different toxic chemicals which is evident in form of pathological changes such as reduction of size and number of haemopoietic, vacuolization of tubular cells, dilation of epithelial cells linings[8,9].

Ghosh and Chkrabarti[10] was observed that a sub lethal concentration of cadmium chloride 57mg/liter for 30 days the kidney exhibited ruptured of tubular epithelium including degeneration of glomeruli extrusion of cellular material in to the tubular lumen and extensive loss of interstitial haemopoietic tissue. On other hand Rostami-Bashman[11] observed changes in kidney includes necrosis, hyaline degeneration of tubules after exposed to 10 mg/l of cadmium for 24h on *Cyprinuscarpio.*

While, Qureshi and Khaliq[12] was observed that the kidney of fishes exposed to 8 ppm cadmium chloride for 30 and 60 days exhibited several histological alterations like loosening, formation of clusters and lumps in haemopoietic tissue, deshaping of uriniferous tubules, narrowing of tubular lumen, vacuolization and degeneration of the cells of uriniferous tubules, wide space in renal corpuscles and shrinkage in glomeruli. Severity in alterations increased in proportion to increased dose and time period.

Recovery group:
In 7 days duration mulethi administered group exhibited that cadmium chloride load still persists in the renal tubules, as exhibited with few degenerated and vacuolated cell. Few cell shows regeneration of cytoplasm in the cells. The compact mass of glomerulus in the Bowman's capsule lost its clumping nature and exhibited regenerating cells along with evenly spreaded cytoplasm and inside the Bowman's capsule wide spaces reduced as seen in simultaneous treated group. Numerous haemopoietic cells make their appearance with prominent nucleus (Figure 7).

Table: 2 Diameter of kidney cells of *Heteropneustes fossilis* in experimental and recovery group

	Parameter	7days (in µ)		14days (in µ)		21days (in µ)	
		Treated	Recovery	Treated	Recovery	Treated	Recovery
1	Renal tubules	3.6±0.85	** 11.5±0.88	10.83±0.54	*** 14. 0±0.47	7.4±0.15	*** 15.0±0.47
2	Glomerulus	26.4±2.02	*** 18.5±1.68	6.7±0.81	** 20.4±1.95	32.8±0.96	** 21.7±0.76
3	Haemopoietic tissue	0.8±0.04	** 10.4±0.06	12±0.73	** 11.5±0.04	16.3±0.87	*** 12.9±0.47

All values are expressed in Mean± SEM; Total no. of samples for each observation: 10
Significant level (** P< 0.01, *** P< 0.001)

While In 14 days administrated group exhibited recoupment in the cell of kidney. The cytoplasm was spreaded evenly and occupy whole cell. No vacuolated condition exhibited in the cells. The blurred appearances were lost. The reductions in vacuolated condition were quite prevalent. Similarly in glomerulus content the atrophied, thick conditions were lost and cells were occupying more space in the Bowman's capsule. The haemopoietic cells clearly visible with prominent nucleus (Figure 8).

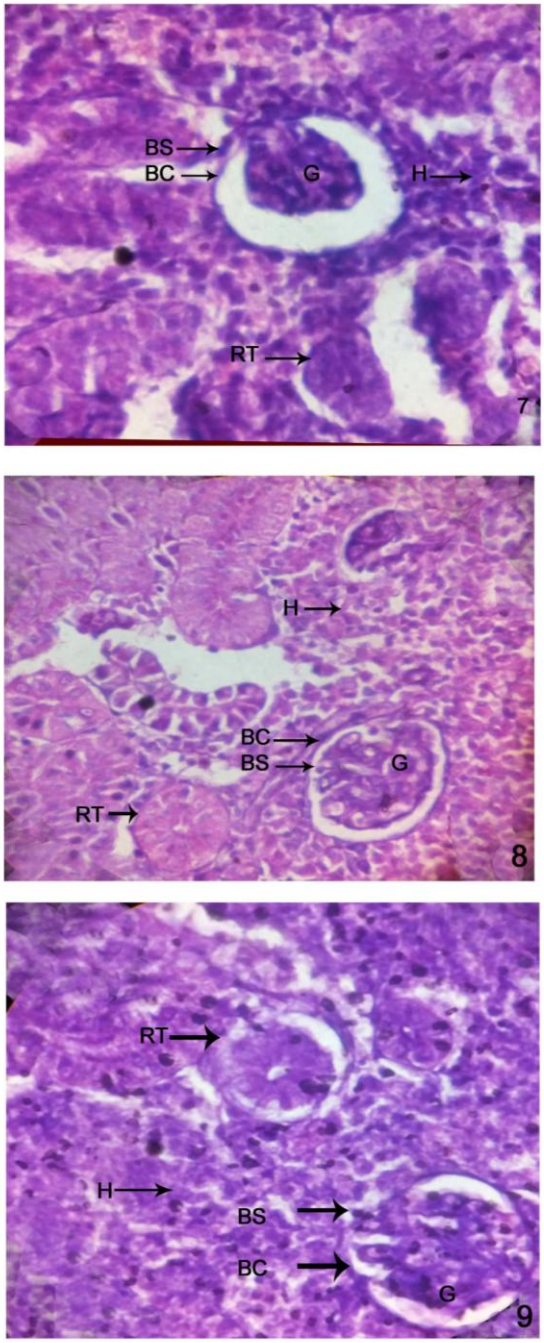

Plate 2: Photomicrographs of Kidney of Recovery of, *H. fossilis* (Hae+Eosin) X 100

Fig. 7: 7days Mulethi recovery group: Showing regeneration of renal tubules. The glomerulus exhibited decreases of shrunken condition and reduction of wide spaces Bowman's capsule and haemopoietic tissue had prominent nuclei.
Fig. 8: 14days Mulethi recovery group: Showing reformed renal tubules, reduction in vacuolated condition, lost of atrophied condition in glomerulus, more spaces of Bowman's capsule, and clearly visible haemopoeitic cells.
Fig. 9: 21days Mulethi recovery group: Showing regenerated and normal condition of renal tubules, glomerulus with Bowman's capsule and haemopoietic tissue.

While 21 days mulethirecovery group exhibited that kidney of fish exposed a marked recovery in their histological architecture compared with treated group. The renal tubules exhibited compact arrangement. The oedema which was seen in renal cell got reduced and cell arranged properly. The shape becomes normal with evenly distributed eosinophilic cytoplasm and centrally situated nuclei

were clearly visible. The glomerulus and Bowman's space exhibited almost normal configuration in haemopoietic cells. The haemopoietic cells were rounded in shape and enlarged in size. The kidney exhibited better regeneration due to herbal compound. They start to regenerate its nuclear and cytoplasmic contents (Figure 9). In all recovery group the diameter of the cells renal tubules, glomerulus, Bowman's capsule and haemopoietic tissue reveal significant (P<0.01and0.001) improvement in shape and configuration (Table 2).

Abbreviation:

BC	**:**	**Bowman's capsule**
BS	**:**	**Bowman's Space**
DG	**:**	**Disorganized Glomerulus**
G	**:**	**Glomerulus**
HG	**:**	**HypertrophiedGlomerulus**
HT	**:**	**Haemopoietic Tissue**
HC	**:**	**Hemorrhagic Conditions**
NRT	**:**	**Necrotic Renal Tubules**
PN	**:**	**Pyknotic Nuclei**
RT	**:**	**Renal Tubules**
WS	**:**	**Wide Space**
V	**:**	**Vacuolization**

The degenerative changes may be due to altered metabolic activity or due to metal ion-renal tissue interaction as suggested by Gupta and Rajbanshi[13] and Sharma and Sharma[14] While, severe structural damage (Venkataramana and Radhakrishnaiah[15], AnithaKumari and Sree Ram Kumar[16] and histopathological change in kidney were accompanied with high zinc accumulation. The histopathological change shows sign of recovery in fish *C. punctatus* in pre-exposed fish transfer in plain water[17].

On other hand, Ravindrababu and Neerja[18] observed that the kidney of fish *Oreochromis mossambicus* exposed to ammonia for 7 and 14 days exhibited several histological alterations like vacuole formation, reduced lumen in the proximal tubules, and shrunkened glomeruli and damaged renal tubules. While in recovery experiment histopathological kidney showed reduced pathological conditions, shrunkened glomeruli have become enlarged and also renal cells recovered to normal levels, and damaged connective tissues were slowly regenerating in kidney in 14 days duration in *Oreochromis mossambicus*. Above study suggest that exposure of fish to cadmium chloride poses great stress to the fish. The recovery by using mulethi to pre-exposed fish revels that the fish slowly over comes to the stress of cadmium chloride with in 21days, and the damaged tissue becomes free of stress and start regeneration.

Conclusion

Herbal products have great importance in ancient traditional medicine systems. The plant material contains various bioactive components specially Alkaloids, tannins, phenols, flavonoids etc. which indicate that these extracts may be beneficial in treatment of damaged kidney of fish. In the present study herbal compound mulethi exhibited protective nature against cadmium chloride (even though of safe dose) and recovers the affected tissue of fish.

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