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ADVERSE EFFECT OF CADMIUM CHLORIDE ON INTERRENAL AND CHROMAFFIN CELLS OF *HETEROPNEUSTES FOSSILIS* AND RECOVERY OF DAMAGED TISSUE BY HERBAL COMPOUND MULETHI

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ABSTRACT
The present study was conducted to investigate the effect of cadmium chloride on head-kidney of fresh water fish *Heteropneustes fossilis*. The fish were exposed to 0.5 ppm of cadmium chloride for 21 days. The most remarkable changes in head kidney due to cadmium chloride were lysed condition of interrenal and chromaffin cells. The traces of cytoplasm had dark brown to black coloured cytoplasm. Mostly the cells had deformed and necrotic cytoplasm. Their size were significant at ($P< 0.01$ and 0.001) increased after cadmium chloride. All these changes will be recovered by the administration of herbal compound i.e. Mulethi. The damaged tissues were recovered in already treated group.

KEYWORDS: Cadmium chloride, Chromaffin cells**,** *Heteropneustes fossilis*, Histopathology, Interrenal cells, Mulethi.

INTRODUCTION
Several heavy metals in the industrial waste streams, waste constitutes the major source of metal pollution in natural water.[1] The consumption of fish is the most significant source of contamination of heavy metal exposure in animals.[2] Fish are reported to be used as biological indicators to assess water pollution.[3] Cadmium is highly toxic element to all fish.[4] The Kidney of the most permeable region of the body of the fish is composed of three distinct system endocrine, haemopoietic, excretory and cortex. The anterior kidney (head-kidney) is integrated into the endocrine system of the fish. The head kidney of teleosts consist of two tissues i.e. interrenal and chromaffin tissues. The present study was undertaken to observe the deformities produced by safe dose of cadmium chloride 0.5ppm on interrenal and chromaffin cells of fish, *H. fossilis* and recovery of damaged tissue by herbal compound Mulethi.

MATERIAL AND METHODS
Experimental Animal: *Heteropneustes fossilis* is a bottom dweller, omnivore fresh water catfish. It is commonly known as "singhi".

Metal used: Cadmium was used for present study in the form of cadmium chloride ($CdCl_2$). The dose of cadmium chloride was determined after calculating LC_{50} value. It was found to be 0.5g/l. The 0.5 ppm is safe dose given to *H. fossilis.*

Recovery agent: Mulethi (Glycorrhiza glabra) (powdered form) was used as recovering agent for present investigation.

METHODOLOGY
Living, healthy, mature male and female fish, *H. fossilis* were used as the test specimen. The fishes were acclimated to standard laboratory conditions for a period of 10 days prior to the experiment. The fishes were treated with 0.01% of $KMnO_4$ solution to remove any dermal infection. The average weight and length of fishes 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 chopped prawn food.

Experimental design: 108 fishes were divided into three groups for maximum 21 days.

- **Control Group:** 36 Fishes of this group were fed on plain food and keep only in stored tap water (without administration of $CdCl_2$**)**.
- **Treated Group:** 36 Fishes of this group were treated with $CdCl_2$ (0.5ppm) solution up to 21days and fed on plain food.
- **Recovery Group:** 36 Fishes of this group were treated with $CdCl_2$ (0.5ppm) solution up to 21days then Mulethi with food was given up to these treated fishes up to 21 days.

Histopathological and Histochemical study

For Histological study kidneys was taken from control treated and recovery group of fishes. After a weak of acclimation, specimen of each species were anaesthetized with dichloro-ether. Fishes were decapitated out and Kidney immediately dissected out and fixed in aqueous Bouin's solution and 10% formalin for 24 hours. After fixation, the organs were washed with water and dehydrated with graded series of alcohols, cleaned in xylene and finally embedded in paraffin wax. Sections of head kidney were cut at 5-6 µm. The kidneys were stained with Hematoxylin and eosin method**s** and Masson's Trichrome were used histological observation and Periodic acid Schiff's **(**PAS**)** and Sudan Black B methods were used for histochemical observation and mount in DPX. All the data and results for final observation were processed in the form of microphotographs. The diameter of interrenal and chromaffin cells were recorded and difference if any were compared by statistical analysis was using student't' test.[5]

RESULTS AND DISCUSSION

Control group: Histological studies revealed that the head kidney section of control group showed normal histoarchiteceture. The head kidney of *H. fossilis* is composed of interrenal and chromaffin cells. In 7, 14 and 21 days duration in control, the interrenal and chromaffin cells. Present their normal histological feature and arranged around the post cardinal vein. The interrenal cells were oval shaped had eosinophilic homogenously distributed cytoplasm with proper nuclear arrangement. The chromaffin cells were appeared with their granular cytoplasm and centrally placed nucleus. The chromaffin cells were located close to the endothelial lining of the blood vessels and also dispersed between haemopoietic tissue and interrenal cells (Fig. 1, 3, 5). In Masson's Trichrome stain these cells occupy red colored cytoplasm with centrally placed nuclei (Fig. 2, 4, 6) interrenal and chromaffin cells showed strong reaction with Schiff's reagent and Sudan dye (Table-3).

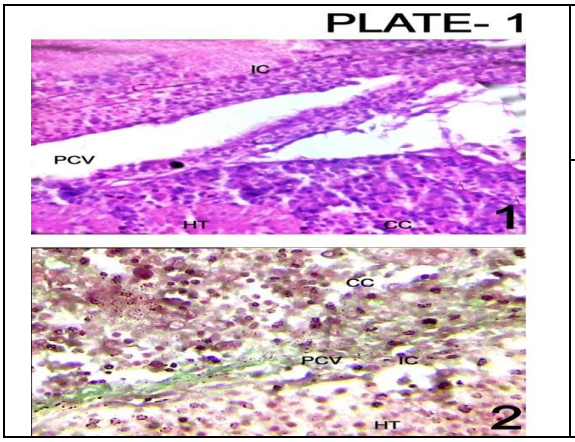


Fig. 1 Control group: Showing interrenal, chromaffin cells and haemopoietic tissue around the post cardinal vein. The cells with prominent nuclei and basophilic cytoplasm were visible (HE)

Fig. 2 Control group: Showing interrenal cells with red coloured cytoplasm and chromaffin cells exhibited basophilic condition of cytoplasm. Green colour fluid deposition were visible (Masson's Trichrome)

Plate 1: Photomicrograph of interrenal and chromaffin cells of control fish *H. fossilis* (7 Days) X 400

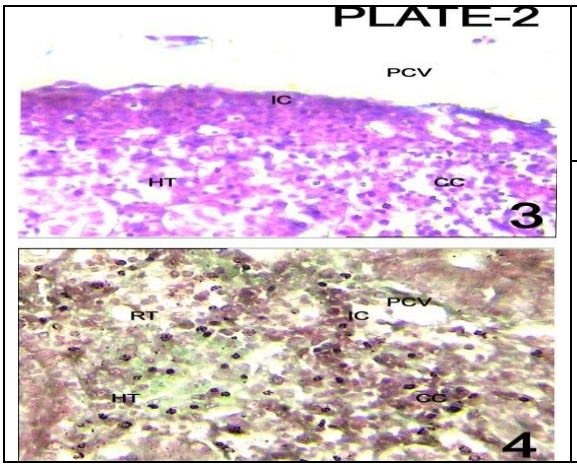


Fig. 3 Control group: Showing interrenal cells, cluster of chromaffin cells and haemopoietic tissue around the post cardinal vein. The cells were prominent nuclei and basophilic cytoplasm (HE)

Fig.4 Control group: Showing interrenal and chromaffin cells arranged along with lining of blood vessels. green colourd fluid were present (Masson's Trichrome)

Plate 2: Photomicrograph of interrenal and chromaffin cells of control fish *H. fossilis* (14 Days)X 400

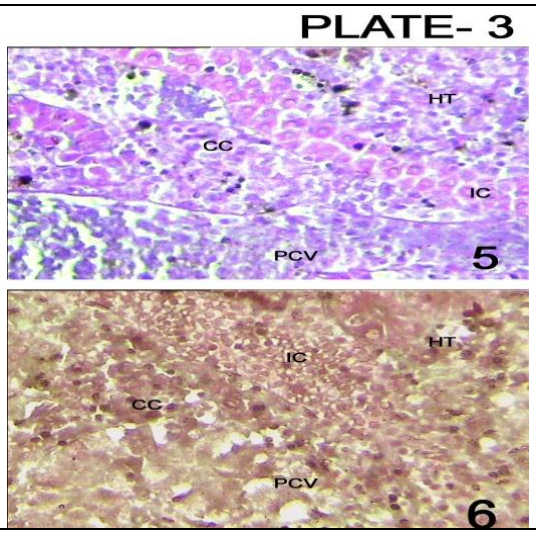


Fig. 5 Control group: Exhibited oval shaped interrenal cells with evenly distributed cytoplasm with proper nuclear position and cluster of chromaffin cells (HE)

Fig. 6 Control group: Showing interrenal and chromaffin cells with proper cellular organization (Masson's Trichrome)

Plate 3: Photomicrograph of interrenal and chromaffin cells of control fish *H. fossilis* (21 Days) X 400

Treated group (7 Days)

In this duration cadmium chloride treated group exhibited histological abnormalities like cellular and nuclear hypertrophy and degeneration of cytoplasm were noted in interrenal and chromaffin cells. The cell boundaries were compact. The cell lost original appearance and become thick and necrotic. The cellular arrangement was also lost due to stress (Fig. 7). In Masson's Trichrome stain the heavy deposition of blue colour fluid were visible interrenal and chromaffin cells due to collagen deposition. The cytoplasm of cell occupy dark brown colour (Fig. 8). The PAS and Sudan Black B exhibited moderate reaction (Table-3).

Treated group (14 Days)

In this duration cadmium chloride treated group, the interrenal cells become swollen, enlarged and cytoplasm had vacuolized texture. The chromaffin cells exhibited shrinkage and disruption of tissue integrity. Only remains of cytoplasm and nuclear content were visible. The tissue destruction among cells was remarkable. Most of cells lost their staining affinity. Due to stress the cells lost their integrity and loosely arranged. The wide or empty spaces exhibited among cells (Fig. 9). In Masson's Trichrome most of the interrenal and chromaffin cells exhibited hypertrophied nature and present necrotic condition. The loosing of cells and pyknotic eccentric nuclei were quite prominent and arranged along with blue coloured collagen deposition on post cardinal vein (Fig. 10). The interrenal and chromaffin cells gave negative reaction with PAS and Sudan Black B test (Table-3).

Treated group (21 Days)

The fish exposed to cadmium chloride the arrangement pattern of interrenal and chromaffin cells in chords was distorted. In 21 days exposure all the cells of interrenal and chromaffin were in lysed condition, cells lost their boundaries, cytoplasm and nuclear content. The traces of cytoplasm occupy dark brown to black colour. Due to stress nuclei become pyknotic (Fig.11). In Masson's Trichrome stain atrophied nature of the cells exhibited. The wide spaces created due to loss of cells and tissues and the cells with heavy deposition of green coloured collagen (Fig. 12). The interrenal and chromaffin cells gave negative reaction with PAS and Sudan Black B test (Table-3). The diameter of interrenal and chromaffin cells were significantly (P<0.01 and 0.001) increased (Table-1).

Table: 1 Diameter of interrenal and chromaffin cells of *Heteropneustes fossilis* in control and experimental group (7, 14 and 21 Days).

No	Parameter	7days		14days		21days	
		Control	Treated	Control	Treated	Control	Treated
1	Interrenal cells(µ)	0.030±0.004	**0.055±0.009	0.031±0.005	**0.057±0.008	0.032±0.006	***0.0590±0.01
2	Chromaffin cells (µ)	0.525±0.009	**0.634±0.04	0.530±0.01	***0.699±0.048	0.545±0.009	***0.709±0.044

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

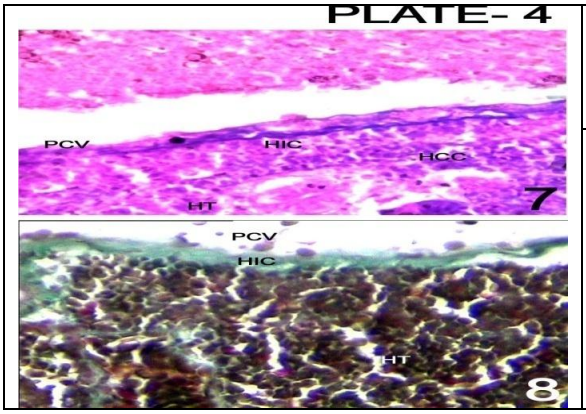


Fig. 7 Treated group: Showing cellular and nuclear hypertrophy in interrenal and chromaffin cells. Small clusters of lightly stained chromaffin cells with vacuolated cytoplasm were visible (HE)

Fig. 8 Treated group: Showing heavy deposition of blue coloured endogenous pigment around interrenal and chromaffin cells (Masson's Trichrome)

Plate 4: Photomicrograph of interrenal and chromaffin cells of treated of *H. fossilis* (7 Days) X400

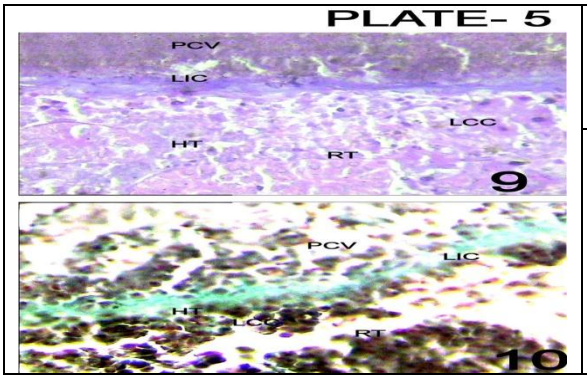


Fig. 9 Treated group: Showing swollen and vacuolized condition of interrenal cells with eccentric nuclei. The chromaffin cells exhibited shrinkage and disruption of tissue integrity (HE)

Fig. 10 Treated group: Showing deformed debris of the interrenal and chromaffin cells intermingled with blue colour collagen deposition (Masson's Trichrome)

Plate 5: Photomicrograph of interrenal and chromaffin cells of treated of *H. fossilis* (14 Days) X 400

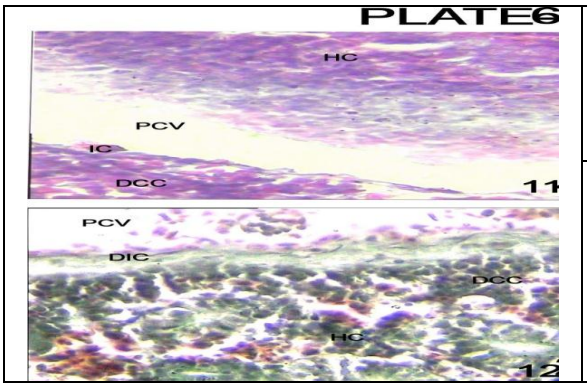


Fig. 11 Treated group: Demonstrating lysed condition of interrenal and chromaffin cells. The traces of cytoplasm had dark brown to black coloured cytoplasm. Most of cells are deformed and necrotic (HE)

Fig. 12 Treated group: Exhibiting lysed and deformed interrenal and chromaffin cells. The cells with heavy deposition of green coloured collagen (Masson's Trichrome)

Plate 6: Photomicrograph of interrenal and chromaffin cells of treated of *H. fossilis* (21Days) X 400

CC: Chromaffin Cells, HT: Haemopoietic Tissue, IC: Interrenal Cells, VIC: Vacuolized Condition of Interrenal Cells, PCV: Post Cardinal Vein, HCC: Hypertrophied Chromaffin Cells, HIC: Hypertrophied Interrenal Cells, DIC: Deformed Interrenal Cells, LCC: Lysed Condition of Chromaffin Cells

Similar findings were Donaldson,[6] has also reported in an increase in the diameter of the nuclei of interrenal cells of *Salmonids* exposed to a variety of contaminants and Norris etal[7] reported an increase in the diameter and area of interrenal cell nuclei was also seen in samples of *Salmo trutta* taken from water contaminated with heavy metals. The Bhattacharya[8] observed that an insecticide endosulfan exposed *O. mossambicus*, interrenal cells exhibited cytoplasmic vacuolization and cellular hypertrophy was more prevalent. These features have also been detected in *Polydon spathuls* after exposing to PCBs and chlordane.[9] The cellular atrophy observed in *Esox lucius* and *Perca flavescens* due to paper mill effluent and in *Oncorynchus mykiss* and *Oreochromis mossambicus* exposed and feed on PCBs compound.[10,11] Similar atrophied condition recorded in *A. altiparanae* after cadmium chloride treatment in interrenal.[12] In a study, Shrivastava and Ruhels[13] reported increased size of the chromaffin cells after prolong exposure of photoperiods to *H. fossilis* significantly increases ($P<0.01$) in the fish. But Bromage and Funchs[14] noticed that no changes in the size of chromaffin cells were observed in any stress related studies in fish.

Mulethi Recovery group (7 Days)

On the other hand the interrenal cells of 7 days in Mulethi group still hypertrophy visible but cytoplasm becomes dark red colour due to relief from stress instead

of black. In Mulethi administrated recovery group the chromaffin cells exhibited reduced hypertrophied nature. The cytoplasm of cells fully occupied and vacuolized nature were reduced. The cytoplasm of cell lost their dark colour and pink colour clearly seen. In this group the thickness of wall of interrenal cells were reduced and it was regenerated at some places. The interrenal and chromaffin cells were arranged compactly and intertubular spaces were reduced. Histological abnormalities were greatly improved and regenerated condition in the cells exhibited. The eosinophilic cytoplasm shows regeneration in their cytoplasmic content and improved their staining capability. No vacuolated condition exhibited in the cells. The fused and clumping nature reduced and regenerating condition prevailing with proper staining capability (Fig.13). In Masson's Trichrome the both cells had regenerated cytoplasm (Fig.14). While in PAS test the follicular cells of interrenal clearly showed regenerated condition and they take positive magenta colour and chromaffin gave moderate reaction. The Sudan Black B gave moderate reaction in interrenal and chromaffin cells.

Mulethi Recovery group (14 Days)

Mulethi administrated recovery group exhibiting reduction in of hypertrophied nature and cytoplasm of cells present activeness as reflected by their staining properties. The different types of interrenal cells were regenerated and showed increasing staining affinity when compared to corresponding treated group (Fig.15). In Mulethi group degrees of recoupment were well marked and better recovery exhibited in both stain. The defused chromaffin cells start to arrange in their cords. In Masson's Trichrome the interrenal cells had green in colour and chromaffin cells had red colour (Fig.16). The interrenal and chromaffin cells gave positive reaction in PAS and Sudan Black B test (Table-3).

Mulethi Recovery group (21Days): All these cells were visible normal and nearly similar to control group. In the Mulethi group the cytoplasm regenerates in the both cells. The interrenal cells of Mulethi recovery group exhibited reduced wide gapes and regenerated but hypertrophy in few cells was still persistent. The cells were in demonstrated normal texture of cytoplasm and position of nuclear material. The faster recovery was noted in chromaffin cells. The histological textures of the cells were almost similar. All these cells were arranged in cord with clearly visible cytoplasm and nuclear material. They exhibit good staining affinity (Fig.17). In Masson's Trichrome the cells take dark brown colour and the cytological structure of the cells clearly visible with prominent nuclei. They take bright colouration with stain and present activeness of the cells with reduced size. The green coloured fluids around cells were quite similar to simultaneous control. (Fig.18). The interrenal and chromaffin cells gave positive reaction with PAS and Sudan Black B test (Table-3). The diameter of interrenal and chromaffin cells was significantly ($P<0.01$, and $P<0.001$) reduced after the administration of herbal compound Mulethi (Table-2).

Table: 2 Diameter of interrenal and chromaffin cells of *Heteropneustes fossilis* in experimental and recovery group Mulethi (7, 14 and 21 Days).

No	Parameter	7days		14days		21days	
		Treated	Recovery	Treated	Recovery	Treated	Recovery
1	Interrenal cells(µ)	**0.055±0.009	**0.044±0.008	**0.057±0.008	***0.038±0.0081	***0.0590±0.01	***0.0368±0.008
2	Chromaffin cells(µ)	**0.634±0.04	**0.580±0.035	***0.699±0.048	***0.6190±0.051	***0.709±0.044	***0.628± 0.047

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

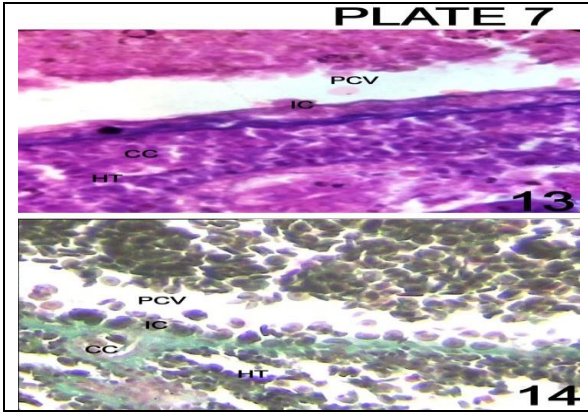


Fig.13 Mulethi group: Showing the hypertrophied nature and cytoplasm of cells present inactiveness. Defused interrenal and chromaffin cells start to arrange in their cord (HE).

Fig.14 Mulethi group: Showing interrenal and chromaffin cells are regenerated. (Masson's Trichrome).

Plate 7: Photomicrograph of interrenal and chromaffin cells of Recovery of H. fossilis (7 Days) X 400

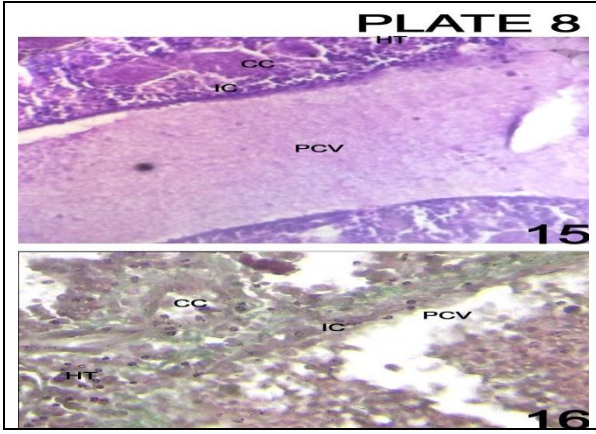
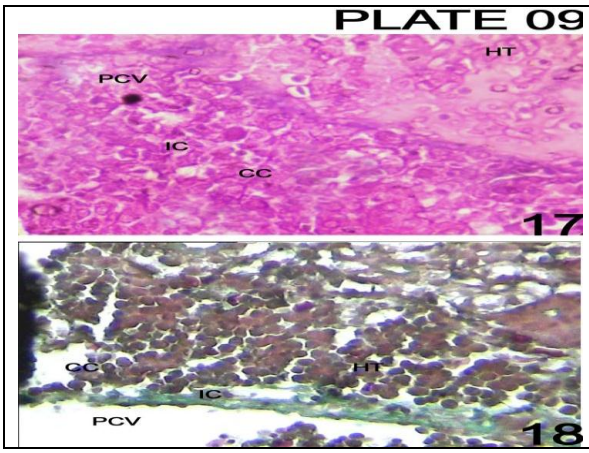


Fig.15 Mulethi group: Showing newly reformed Interrenal and chromaffin cells, nodular pattern still exhibited along-with hypertrophied nature (HE).

Fig.16 Mulethi group: Showing interrenal cells regenerated around post coordinal vein and chromaffin cells also regenerated and had red colour (Masson's Trichrome).

Plate 8 Photomicrograph of interrenal and chromaffin cells of Recovery of H. fossilis (14 Days) X 400



Fi.g17 Mulethi group: Showing newly reformed Interrenal and chromaffin cells (HE).

Fig. 18 Mulethi group: Showing interrenal and chromaffin cells regenerated. interrenal cells had green colour and chromaffin cells had red colour (Masson's Trichrome).

Plate 9: Photomicrograph of interrenal and chromaffin cells of Recovery of H. fossilis (21Days) X 400

CC: Chromaffin Cells, HT: Haemopoietic Tissue, IC: Interrenal Cells, PCV: Post Cardinal Vein.

Table: 3 Histochemical test of the Interrenal cells (IC) and Chromaffin cells (CC) of *Heteropneustes fossilis* in control, experimental and recovery groups (7, 14 and 21 Days).

SN	Days	Histoch.t	Control		Treated		Recovery (Mulethi)	
			IC	CC	IC	CC	IC	CC
1	7 Days	PAS	+++	+++	++	++	++	++
		Sudan	+++	+++	++	++	++	++
2	14 Days	PAS	+++	+++	-	-	+++	+++
		Sudan	+++	+++	-	-	+++	+++
3	21 Days	PAS	+++	+++	-	-	+++	+++
		Sudan	+++	+++	-	-	+++	+++

No reaction, (+) weak reaction, (++) moderate reaction, (+++) strong reaction

There are no reports regarding the recovery pattern of interrenal and chromaffin tissue of fish. Except Nagar et al[15] they found the deformed cells of interrenal and chromaffin cells of *H. fossilis* due to cadmium chloride were recorded after administration of herbal compound Ashwaghanha in 21days duration in *H. fossilis*. The recovery of Mulethi in pre-exposed fish exhibited faster recovery in 21 days of duration. The damaged tissues start regeneration in fish.

CONCLUSION

Herbal products have great importance in ancient traditional medicine systems. In the present study herbal compound Mulethi exhibited protective nature against cadmium chloride (even though of safe dose) recovers the affected tissue of fish. Mulethi were effective in recovering the tissue damage, improve elimination of cadmium from kidney tissue and reduced oxidative damage as well. The results of proposed study will add new information related to effective role of Mulethi in fish body against cadmium chloride.

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