RESEARCH ARTICLE

Effect of Cadmium Chloride on Histoarchitecture of Interrenal and Chromaffin cells of Fresh Water Fish *Heteropneustes fossilis* and Recovery of Damaged Tissue by Herbal Compound Ashwagandha

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ABSTRACT- The present study was conducted to investigate the effect of cadmium chloride on Histoarchiteceture of head kidney of fresh water fish *Heteropneustes fossilis*. The fishes 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. Most of cells are deformed and necrotic condition. Their size was significant at (P< 0.01 and 0.001) increased after cadmium chloride. All these changes will be recovered by herbal compound i.e. Ashwagandha. The damaged tissues were recovered in already treated group.

Key-words- Ashwagandha, Cadmium chloride, Chromaffin cells**,** *Heteropneustes fossilis*, Histopathology, Interrenal cells

INTRODUCTION

Today one of the serious problems in the world is the environmental pollution, which affects the health of aquatic ecosystem and physiological changes in aquatic animals. [1] Among pollutants, contaminants metals play an important role. [2] The heavy metal is a common aquatic pollutant and is known to be highly toxic to most organisms, even at small concentrations in natural waters. [3] In fish cadmium causes number of structural and morphological changes in various organs. 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 is integrated into the endocrine system of the fish.

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The head kidney of teleosts related to the endocrine, interrenal and chromaffin tissues. Cadmium causes damage in interrenal and chromaffin cells of fish and other organisms. The present study was undertaken to observe the deformities produced by safe dose of cadmium chloride on interrenal and chromaffin of fish, *H. fossilis* and recovery of damaged tissue by herbal compound Ashwagandha.

MATERIALS AND METHODS

The present retrospective study was undertaken in the Department of Zoology and Biotechnology, Vikram University, Ujjain (M.P). The present experiments were conducted during the period of March 2014 to May 2014.

Experimental Animal: *H. fossilis* is a bottom dweller, omnivore catfish and easily available from rivers. 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 decided after calculating by LC_{50} value. It was found to be 0.5 g/l. The 0.5 ppm was safe dose given to *H. fossilis*.

Recovery agent- Ashwagandha (powdered form) was used as recovering agent for the present investigation.

Methodology- Living, healthy, mature male and female fish, *H. fossilis* was 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 with 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 with 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 21 days and fed with plain food.

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

Histopathological and Histochemical study- For Histological study kidneys were 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 with 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 methods 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. [4]

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 (adrenaline and noradrenaline). Present their normal histological feature and arranged around the post cardinal vein. The interrenal cells were oval shaped cells 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, 5, 9). In Masson's Trichrome stain these cells occupy red colored cytoplasm with centrally placed nuclei (Fig. 2, 6, 10) interrenal and chromaffin cells showed strong reaction with Schiff's reagent (Fig. 3, 7, 11) and Sudan dye (Fig. 4, 8, 12).

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

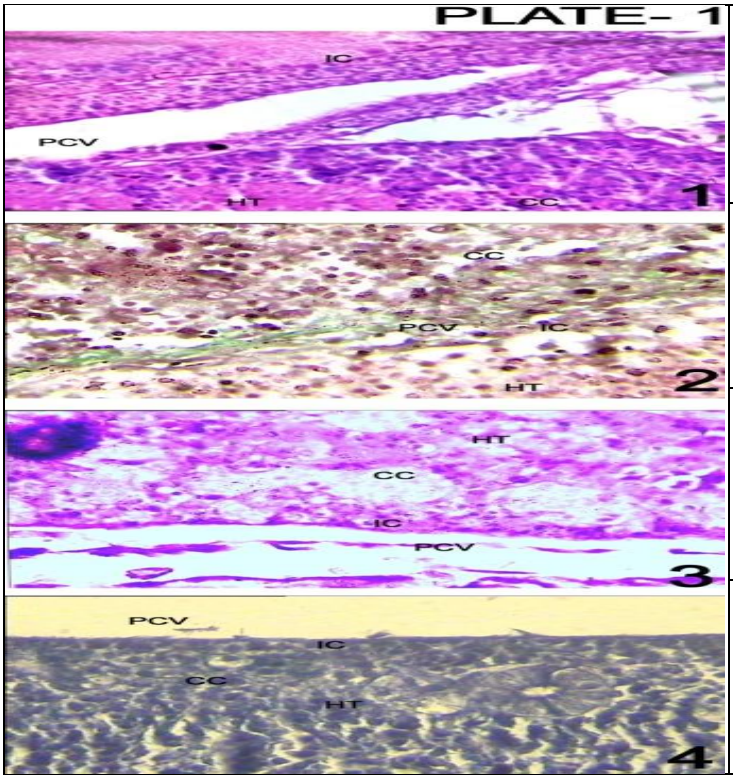
	**Fig. 1:** Control group: Showing interrenal cells, cluster of chromaffin cells and haemopoietic tissue around the post cardinal vein. The cells with prominent nuclei and basophilic cytoplasm were visible (HE)
	Fig. 2: Control group: Showing interrenal cells with red coloured cytoplasm and chromaffin cells exhibited basophilic condition of cytoplasm. Green colour fluid depositions were visible (Masson's Trichrome)
	Fig. 3: Control group: Showing strong positive reaction with Schiff's reagent in interrenal and chromaffin cells (PAS)
	Fig. 4: Control group: Showing strong positive reaction with Sudan Black B in interrenal and chromaffin cells

Plate 2: Photomicrograph of interrenal and chromaffin cells of control of *H. fossilis* (14 Days) X400

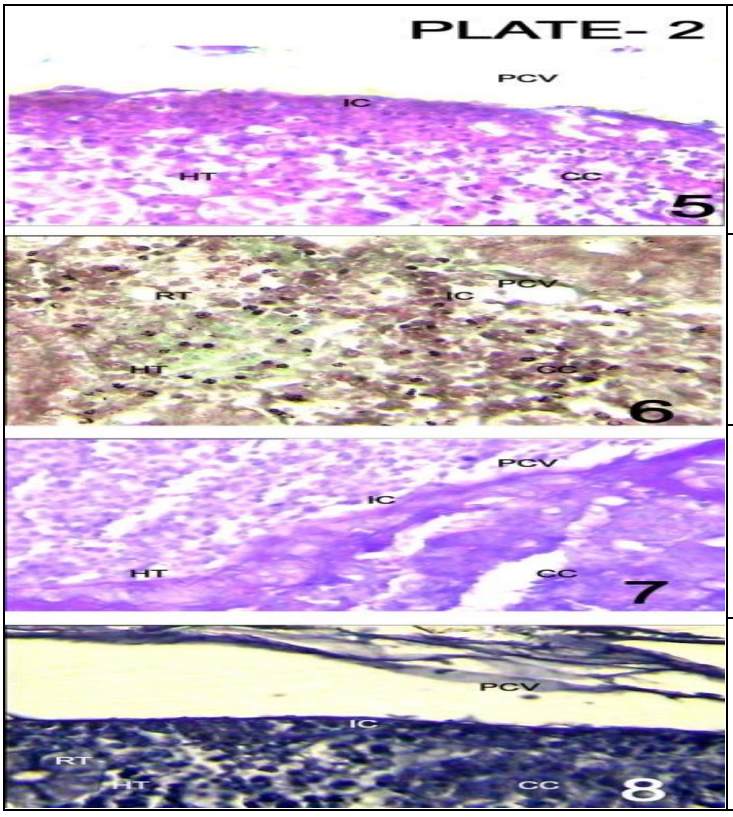
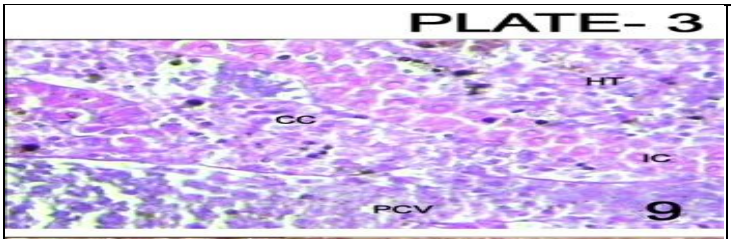
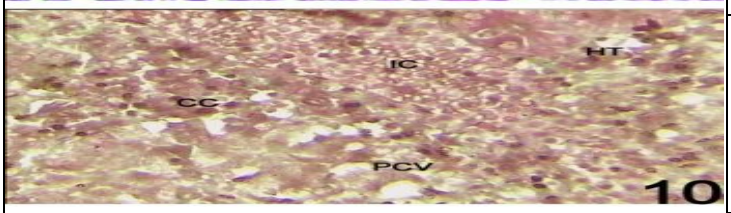
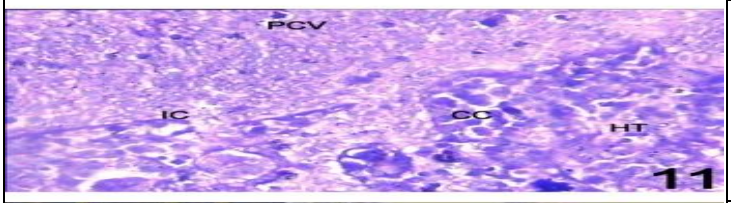
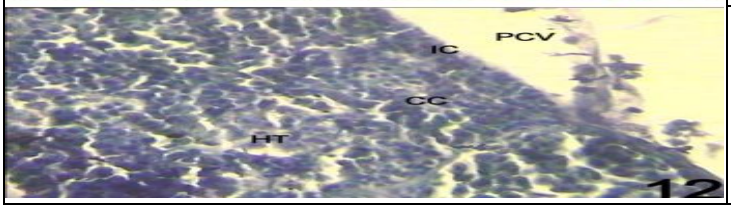
	**Fig. 5:** Control group: Showing interrenal cells, cluster of chromaffin cells and haemopoietic tissue around the post cardinal vein. The cells were prominent nuclei and basophilic cytoplasm (HE)
	Fig. 6: Control group: Showing interrenal and chromaffin cells arranged along with green colourd fluid (Masson's Trichrome)
	Fig. 7: Control group: Showing interrenal and chromaffin cells, exhibiting strong positive reaction with PAS showing presence of glycogen
	Fig. 8: Control group: Showing strong positive reaction with Sudan Black B exhibited presence of sudanophilic substance

Plate 3: Photomicrograph of interrenal and chromaffin cells of control of *H. fossilis* (21 Days) X400

	**Fig. 9:** Control group: Exhibited oval shaped interrenal cells with evenly distributed cytoplasm with proper nuclear position and cluster of chromaffin cells (HE)
	**Fig. 10:** Control group: Showing interrenal and chromaffin cells with proper cellular organization (Masson's Trichrome)
	**Fig. 11:** Control group: Showing cytoplasm with magenta colour of interrenal and chromaffin cells exhibiting strong positive reaction with Schiff's reagent (PAS)
	**Fig. 12:** Control group: Exhibiting heavy accumulation of Sudan Black B granules presenting active condition of cell

Treated group (7 Days)- Seven days 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. 13). In Masson's Trichrome stain the heavy deposition of blue colour fluid were visible around interrenal cells and black colour fluid due to collagen deposition. The cytoplasm of cell occupy dark brown colour (Fig. 14). The PAS and Sudan Black B exhibited moderate reaction (Fig. 15, 16).

Treated group (14 Days)- In 14 days duration $CdCl_2$ treated group, the interrenal cells become swollen, enlarged and cytoplasm had vacuolized texture. The chromaffin cells exhibited shrinkage and disruption of tissue integrity. The only remains of cytoplasmic 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. 17). 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. 18). In PAS and Sudan Black B test gave negative reaction interrenal and chromaffin cells (Fig. 19, 20).

Treated group (21 Days)- In 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. 21). In masson's trichrome stain atrophied nature of the cells exhibited. The wide spaces created due to loss of cells and tissue (Fig. 22). In PAS and Sudan Black B test gave negative reaction interrenal and chromaffin cells (Fig. 23, 24). 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, experimental (7, 14 and 21 Days)

S.No	Parameter	7days (µ)		14days (µ)		21days (µ)	
		Control	Treated	Control	Treated	Control	Treated
1	Interrenal cells	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	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)**

Plate 4: Photomicrograph of interrenal and chromaffin cells of treated of *H. fossilis* (7 Days) X 400

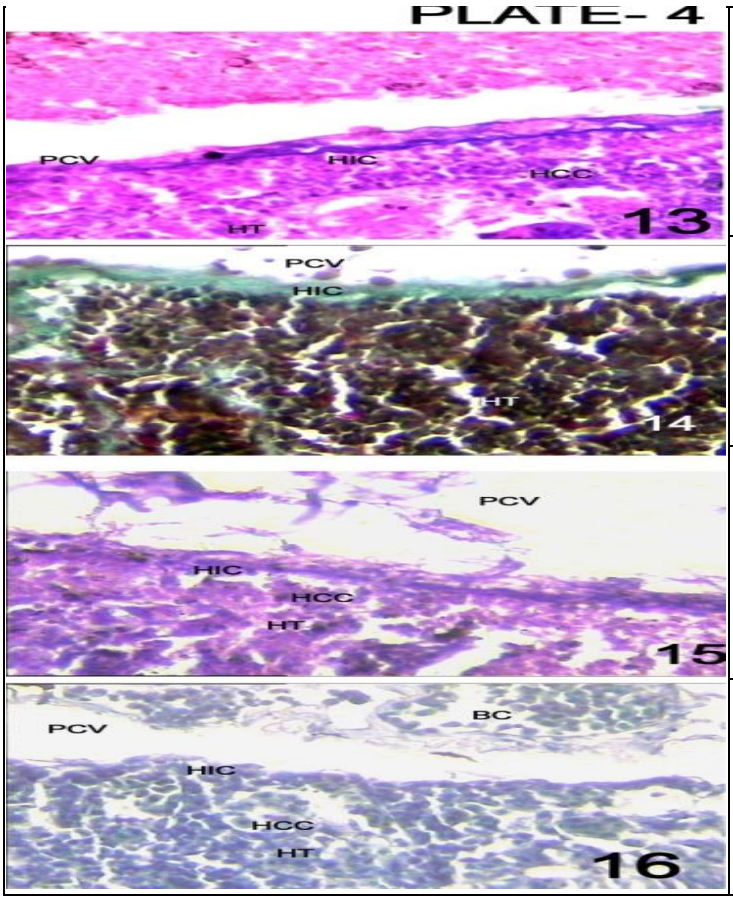


Fig. 13: Treated group: Showing cellular and nuclear hypertrophy in interrenal and chromaffin cells. Small clusters of lightly stained chromaffin cells with vacuolated cytoplasm were visible (HE)

Fig. 14: Treated group: Showing heavy deposition of blue coloured endogenous pigment around interrenal and chromaffin cells (Masson's Trichrome)

Fig. 15: Treated group: Showing moderate reaction with PAS in deformed interrenal and chromaffin cells (PAS)

Fig. 16: Treated group: Showing moderate reaction with Sudan Black B in interrenal and chromaffin cells

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

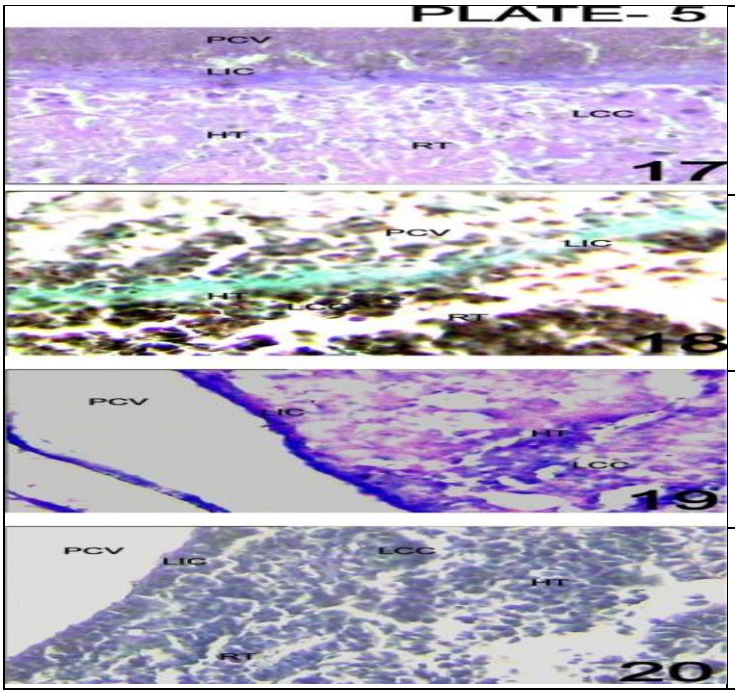


Fig. 17: Treated group: Showing swollen and vacuolized condition of interrenal cells with eccentric nuclei. The chromaffin cells exhibited shrinkage and disruption of tissue integrity (HE)

Fig. 18: Treated group: Showing deformed debris of the interrenal and chromaffin cells intermingled with blue colour collagen deposition (Masson's Trichrome)

Fig. 19: Treated group: Showing almost all the interrenal and chromaffin cells gave negative reaction with Schiff's reagent (PAS)

Fig. 20: Treated group: Showing almost all cells exhibited negative response with Sudan Black B stain

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

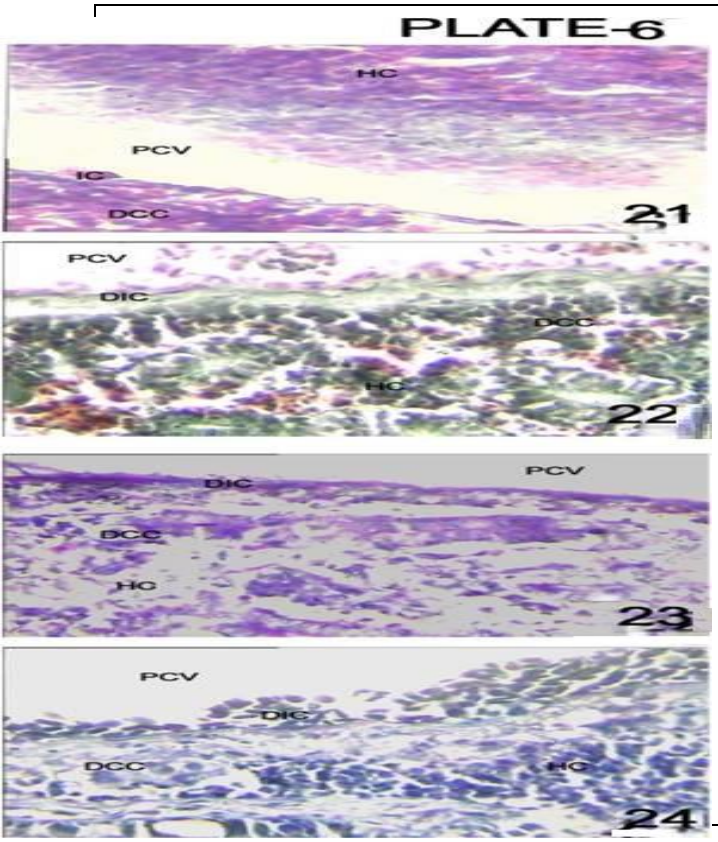


Fig. 21: Treated group: Demonstrating lysed condition of interrenal and chromaffin cells. The traces of cytoplasm had dark brown to black coloured cytoplasm. Most of cells are deformed and necrotic (HE)

Fig. 22: Treated group: Exhibiting lysed and deformed interrenal and chromaffin cells.The cells with heavy deposition of green coloured collagen (Masson's Trichrome)

Fig. 23: Treated group: Showing negative reaction in deformed interrenal and lysed chromaffin cells (PAS)

Fig. 24: Treated group: Demonstrating exhausted hypertrophied debris of interrenal and chromaffin cells showing negative reaction (Sudan Black B)

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 reported in *Salmonids* exposed to a variety of contaminants [5-6] in *Salmo trutta* with heavy metal contaminated water. While Bhattacharya [7] observed that an insecticide endosulfan exposed fish, interrenal cells exhibited cytoplasmic vacuolization and cellular hypertrophy was more prevalent. These features have also been detected in *Polydon spathuls* after exposing animals to PCBs and chlordane. [8] 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. [9-10] Similar atrophied condition recorded in *A. altiparanae* after cadmium chloride treatment with interrenal tissue, revealing endocrine dysfunction due to long term exposure to chemical stressor in the environment [11]. In a study, Shrivastava and Ruhels [12] reported increased size of the chromaffin cells after prolong exposure of photoperiods to *H. fossilis* the stress parameters like plasma glucose, plasma protein and cortisol level increased significantly ($P<0.01$) in the fish. But Bromage and Funchs [13] noticed that no changes in the size of chromaffin cells were observed in any stress related studies in fish.

Ashwagandha group (7 Days)- In 7 days duration administration of Ashwagandha showed few cells regeneration of the interrenal cells. The administration of Ashwagandha showed many interesting changes in the chromaffin tissue. The interrenal and chromaffin cells properly arranged on the wall of post cardinal vein. Histological abnormalities were slowly improved and regeneration of cytoplasm in the interrenal and chromaffin cells. The cells, which show regeneration in cytoplasmic content, exhibited improved staining capability. No vacuolated condition exhibited in the cells. Histological picture of chromaffin tissue exhibited a healthy appearance for the cells arranged in their cord or bunch and cells were getting characteristic feature. (Fig. 25). The cells occupy pink to red coloured cytoplasm with well visible nuclei. In Masson's Trichrome stain changing pattern of cytoplasm were visible (Fig.26). In PAS and Sudan black B test the cells gave moderate reaction (Fig. 27 and 28).

Ashwagandha group (14 Days)- The hypertrophied appearance reduced and cells arranged in proper position (Fig. 29). In Masson's Trichrome the cells were revealed better recovery. The cells distinctly showed the dividing stages and many new cells regenerated (Fig. 30). The cytoplasm of cell fully occupied space. The vascularization increased in the tissue. In PAS test the follicular cells of interrenal and chromaffin cells exhibited regeneration and they take the strong magenta colour (Fig. 31). In the Sudan, Black B the interrenal and chromaffin cells depicted moderate reaction with stain (Fig. 32).

Ashwagandha Recovery group (21 Days)- The eosinophilic activities were appeared in regenerating cells and reconstruction in their cytoplasmic contents (Fig. 33). All these cells were visible normal and nearly similar in appearance to control group. The histological profiles were almost identical to that of control group. In the Ashwagandha group the cytoplasm started to occupy its homogenous distribution instead of vacuolized condition. They were regenerated but hypertrophy in few cells was still persists. The cells were depicted normal texture of cytoplasm and position of nuclear material. In Masson's Trichrome the cells of interrenal take dark brown colour and the chromaffin cells also matched histologically with the cellular and nuclear material was almost similar to normal control and had similar patterns of staining capacity (Fig. 34). While PAS and Sudan Black B gave strong positive reaction in the interrenal and chromaffin cells (Fig. 35 and 36). The diameter of interrenal and chromaffin cells were significantly ($P<0.01$, $P<0.001$) reduced after the administration of herbal compound, Ashwagandha respectively (Table 2).

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

S.No	Parameter	7 days (µ)		14 days (µ)		21 days (µ)	
		Treated	Recovery	Treated	Recovery	Treated	Recovery
1	Interrenal cells	** 0.055±0.009	0.032±0.006	** 0.057±0.008	*** 0.0390±0.008	*** 0.0590±0.01	** 0.037±0.006
2	Chromaffin cells	** 0.634±0.04	0.545±0.009	*** 0.699±0.048	*** 0.639±0.050	*** 0.709±0.044	*** 0.629±0.049

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

There are no reports regarding the recovery pattern of interrenal and chromaffin tissue of fish. The present results were in good agreement with those observed in kidney exposed to vitamin E against lead in *Clarias gariepinus* induced oxidative stress. [14]

Above study suggests that exposure of fish to cadmium chloride poses great stress to the fish. The recovery by using Ashwagandha 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.

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

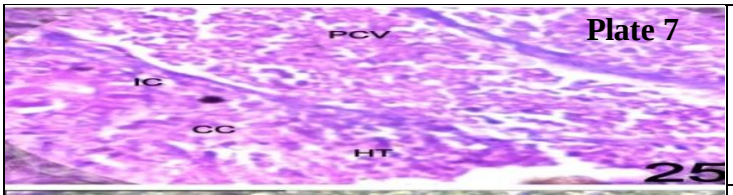
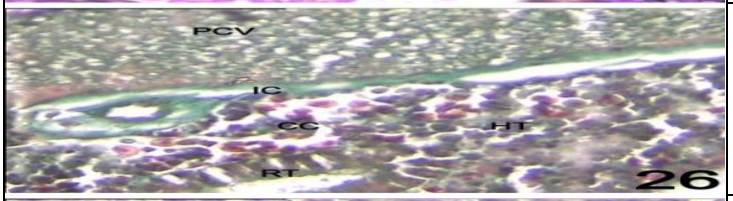
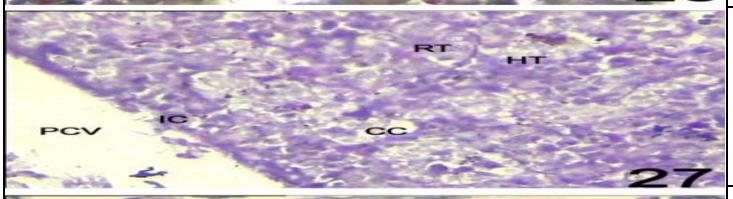
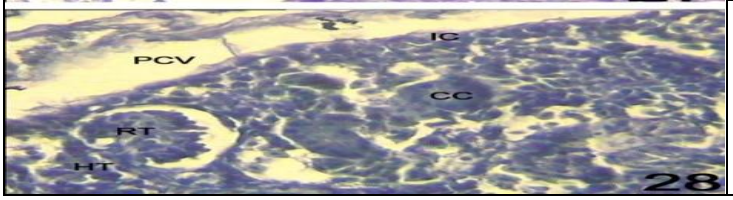
	**Fig. 25:** Ashwagandha group: Showing regeneration of interrenal and chromaffin cells. The hypertrophied appearance reduces and cells arranged in proper position (HE)
	**Fig. 26:** Ashwagandha group: Showing interrenal cells regenerate green colour and regenerate chromaffin cells had red colour (Masson's Trichrome)
	**Fig. 27:** Ashwagandha group: Showing moderate reaction in interrenal and chromaffin cells (PAS)
	**Fig. 28:** Ashwagandha group: Showing moderate reaction in interrenal and chromaffin cells (Sudan Black B)

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

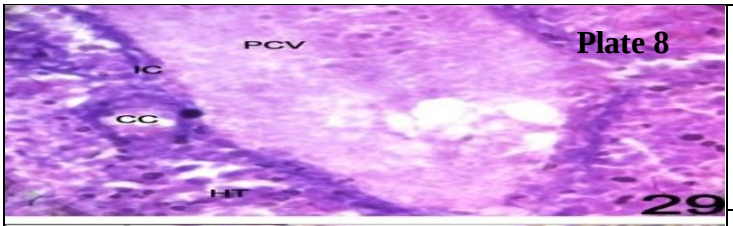
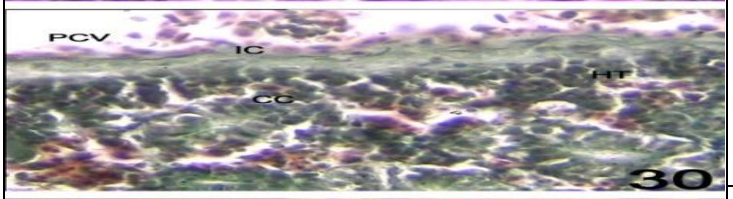
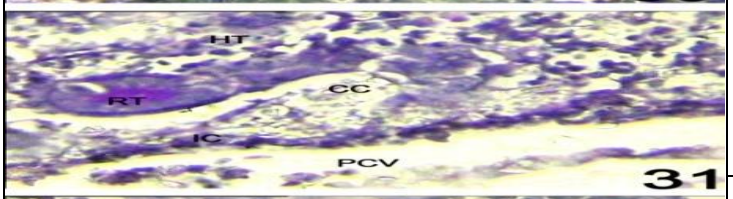
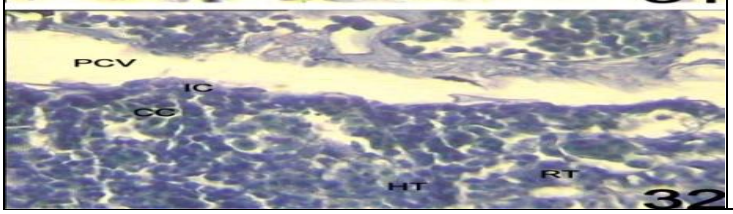
	**Fig. 29:** Ashwagandha group: Showing reformed Interrenal and chromaffin cells, decreased hypertrophied nature (HE)
	**Fig. 30:** Ashwagandha group: Showing interrenal cells around regenerated green colour layer and newly constructed chromaffin cells had dark brown colour (Masson's Trichrome)
	**Fig. 31:** Ashwagandha group: Showing positive reaction in interrenal and chromaffin cells (PAS)
	**Fig. 32:** Ashwagandha group: Showing the moderate reaction in interrenal and chromaffin cells (Sudan Black B)

Plate 9: Photomicrograph of interrenal and chromaffin cells of Recovery of *H. fossilis* (14 Days) X 400

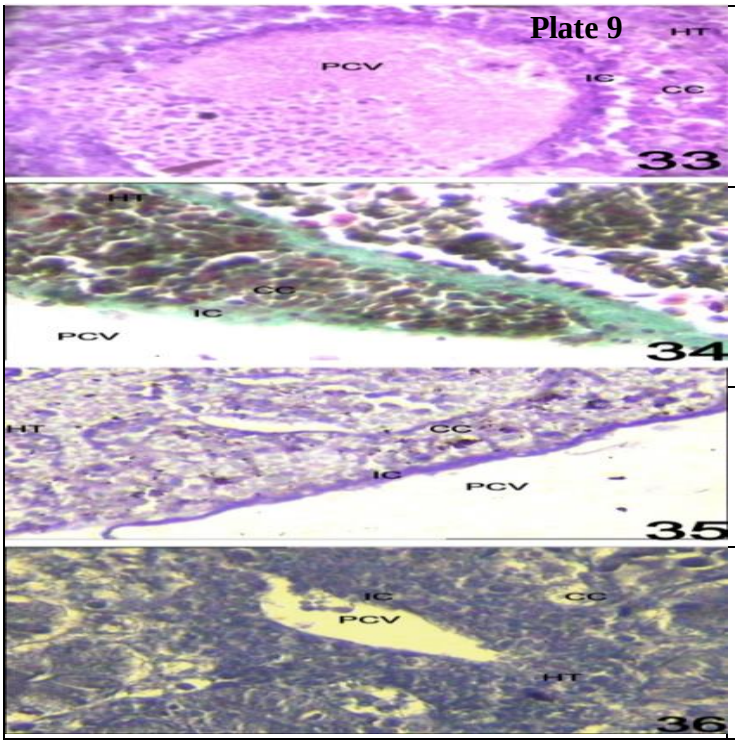


Fig. 33 Ashwagandha group: Showing regenerated and normal condition of Interrenal and chromaffin cells (HE)

Fig. 34 Ashwagandha group: Showing interrenal cells rapidly regenerated green colour and quickly regenerate chromaffin cells had red colour (Masson's Tri-chrome)

Fig. 35 Ashwagandha group: Showing strong positive re-action in interrenal and chromaffin cells (PAS)

Fig. 36 Ashwagandha group: Showing histochemical reaction for the strong positive reaction of interrenal and chromaffin cells (Sudan Black B)

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

CONCLUSIONS

Herbal products have great importance in ancient traditional medicine systems. The plant materials have various bioactive components, especially Alkaloids, tannins, phenols, flavonoids etc. In the present study, herbal compound Ashwagandha exhibited protective nature against cadmium chloride (even though of safe dose) recovers the affected tissue of fish. Ashwagandha has been effective in recovering the tissue damage, improved elimination of cadmium from kidney tissue and reduced oxidative damage as well. The results of the proposed study will add new information related to the effective role of Ashwagandha in fish body against cadmium chloride.

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