IJPSR (2016), Vol. 7, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 January, 2016; received in revised form, 13 February, 2016; accepted, 08 May, 2016; published 01 June, 2016

POSSIBLE RETRIEVAL OF ORGANOCHLORINE INDUCED RENAL TOXICITY IN FISH BY AQUEOUS ROOT EXTRACT OF *WITHANIA SOMNIFERA*: *IN VIVO* STUDY

G. B. Chand* and Prakash Singh

Aquatic Toxicology Laboratory, Post-Graduate Department of Zoology, Patna University, Patna, Bihar, India

Key words:

Clarias batrachus, LM, Organochlorine, Renal tissues, Serum total protein, TEM, *Withania somnifera*

Correspondence to Author:
Dr. G. B. Chand

Assistant Professor
Aquatic Toxicology Lab
P G Department of Zoology
Patna University, Patna - 800005, Bihar, India.

E mail: gbchand@rediffmail.com

ABSTRACT: The present study was aimed to assess the reno-protective impact of aqueous extract of root of *Withania somnifera* (WSR) commonly known as Ashwagandha against organochlorine induced renal toxicity in fish *Clarias batrachus* (Linn.).Fishes were exposed to 4ppb concentration of commercial brand Endocel, an organochlorine pesticide for one week and two week to prepare the toxic model. These toxic groups of fishes were further treated with aqueous root extract of *Withania somnifera* (WSR)@100 mg /kg body wt for four weeks. After schedule exposure, blood serum was extracted and analyzed for total protein (TP) content. Renal tissues were processed for light microscopy (LM) and transmission electron microscopy (TEM). Endocel lowered TP at almost every exposure level. At LM level WSR extract treatment showed restoration of normal luminal characteristics of PCT & DCT and renal corpuscles but it failed to minimize abortive glomerulus, necrotic renal tubules with hypertrophied area, haemorrhagical clots & chronic venous congestion. At TEM level, WSR extract showed maximum retrieval in the cytoarchitecture of cuboidal epithelial cells of renal tubules and renal corpuscles. Concomitant treatment of endocel and WSR showed a very little sign of anomalies in renal tissues. A non-significant change was marked in control group when treated only with WSR extract. The serum level of TP showed a significant decline in organochlorine treated fish when compared with control. They showed a significant recovery after WSR treatment for four weeks. Concomitant treatment of endocel and WSR to the experimental group showed non-significant (at $P<0.05$) changes in TP. The WSR treatment alone to control group didn't show any significant correction in lowered TP. A perfect correlation between biochemical and histopathological finding signifies the excellent restorative power of WSR extract against organochlorine induced renal toxicity in fish.

INTRODUCTION: Agrochemicals are specified group of pesticides used to control weeds, pests or diseases of crops. In aquatic organisms, the xenobiotics percolate upto cellular level through cell membrane and interact with cellular micromolecules to inhibit essential cellular metabolism.[1]

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(6).2365-78

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (6).2365-78

After binding with various cellular receptors either on cell surfaces or within cytoplasm, nucleus and any other cellular organelle, they may include abnormal cellular processes that have toxic or adverse effects on the cell and gene expression. [2] Fishes take up most of the xenobiotics from the surrounding water by passive diffusion through gills, epithelial tissues or gastro-intestinal tract. The contamination of water bodies adversely affects the life of fish by altering their reproduction, growth and nutritional values, cellular morphology and physiology. [3, 4, 5] Endosulfan is a chlorinated cyclodine insecticide used against a large variety of pests. Agricultural run-off, irrigation water and

wetland applications are major sources of this contaminant to the aquatic environment [6]. Due to lipophilic nature, hydrophobicity and low chemical and biological degradation rates, it is accumulated in biological tissues and undergoes biomagnifications. The technical grade of endosulfan contains two diasteriomers– α and β endosulfan in the ratio of 7:3 having different physiochemical properties [7]. In an organism, the endosulfan isomers are transformed by chemical or biological system and excreted as oxidative and hydrolysis products like endosulfan sulphate, alchohol, ether, lactone and endosulfan hydroxyl ether. These metabolites have been reported in the kidney of fish [8]. The toxicity of endosulfan to the fish is primarily mediated by inhibition of important ion-transport protein in a variety of tissues [9]. Pollutant related histopathological alterations in the kidney of fish have been reported by several workers.[10, 11, 12, 13]

Since last two decades, the bioremediation has emerged as one of the major strategic attributes in restoring the health status of aquatic animals.[14, 15, 16, 17, 18, 19, 20, 21] Herbal antioxidants have immense potential to scavenge free radical generated in the living body either due to xenobiotic exposure or any microbial invasion. Many synthetic antioxidants have been reported to be effective in this context but majority of them are implicated by toxic hazardous mutagenic impact. These limitations of synthetic drugs have opened a vast avenue for the use of natural herbal antioxidants as an antidote to xenobiotic induced toxicity stress in animals.

In the present study an attempt has been made to assess the ameliorative impact of aqueous extracts of root of *Withania somnifera* against endosulfan induced renal toxicity in the fish *Clarias batrachus(Linn.)*.

MATERIAL AND METHODS:

Healthy fresh water air breathing fish *Clarias batrachus* weighing 50±10g and 16 ±2 Cm length were collected from NMCH fish pond, Patna Bihar during spawning season. They were disinfected with 0.01% $KMnO_4$ and acclimated under ideal laboratory condition for 15 days. Fishes were fed *ad libitum* @3-4% of their body weight daily. They were alternatively fed with goat liver.

Pesticide used:

In experimental protocol commercial brand "Endocel (EC35%)" manufactured by Excel Industries Ltd, Bhawnagar, Gujrat was used to prepare toxic model. The 96 hour LC_{50} of endosulfan for fish was calculated by standard probit analysis method [22] and confirmed by pilot test as 20 ppb. The fishes were exposed to 4ppb of endosulfan for one and two week. For dose preparation the stock solution was prepared by analytical method. A prior permission from the ethical committee was obtained before the conduction of the experiments.

Medicinal Plant Used:

Root of *Withania somnifera* was procured from Dabur Herbal Store, Haridwar and the roots were identified and authenticated by CDRI, Lucknow. A voucher specimen of rhizome was retained in our laboratory.

Preparation of Plant Extract:

Lyophilized aqueous extract of WSR was prepared as per standard method.[23] The roots were weighed, washed and thoroughly grinded to a pest in mortar pistel and then homogenized in Potter Elvehjem homogenizer. It is dried in incubator at 40°C for two days afterwards dissolved in hot distilled water. The suspension was filtered under suction and the filtrate was freeze dried using "Labcono Freez Drier Model 75018" yielding brown residue.

Administration of Plant Extract:

The NOEL (No observed effect level) and MPD (Maximum permissible dose) were calculated by Probate analysis and Pilot test and a dose of 100mg/kg. b. wt. was selected for its administration to different group of fish for four weeks. The lyophilized powder is dissolved in distilled water and applied to the fish orally by gastric intubation method daily.

Collection of Blood Sample:

On determination of exposure day, blood sample were collected in a heparinized glass cultured tube syringe from caudal vein, the serum was separated

by centrifuging at 5000 rev./min. for 10 minutes at 4°C and stored for serum total protein assessment.

Determination of Serum protein:
Serum total protein was assessed by standard method using colorimeter.

Statistical Analysis:
For each biological analysis six observations were taken at random. Arithmetical mean & standard deviation were calculated and subjected to Student's 't' test for the difference between two mean independent samples. It is further confirmed by one way ANOVA test. The values at $p<0.05$ & $p<0.01$ were considered significant. The statistical analysis were done using sigma plot 12.0 version.

Histopathological Studies:
After each schedule exposure, the fishes were anesthetized with MS222 and renal tissues were dissected out, rinsed in NaCl (0.65%), cut into small pieces with sharp surgical blades, and were fixed and processed independently for LM and TEM studies. For light microscopy, tissues were fixed in aqueous Bouin's fixative, dehydrated through graded series of alcohol, stained in hematoxyline and eosin, cleared in xyline and mounted in DPX. Photography was done by Canon A450 digital camera.

For TEM studies, 1-2mm thick renal tissues were fixed in 2.5% gluteraldehyde in 0.1M phosphate buffer at 4ºc (pH 7.4) followed by its double fixation in 1% OsO_4 in 0.1M phosphate buffer, dehydrated through graded series of alcohol upto Amyl acetate, cleared in toluene and embedded in araldite mixture. Ultrathin gray sections (60-90 nm) were obtained through Leica Ultracut microtome, transferred to copper grid and stained in uranyl acetate and lead citrate. Finally processed tissues were viewed under 'MORGAGINI-268D Transmission Electron Microscope'. The entire processing and TEM photography were done at SAIF-EM unit, Dept. of Anatomy, AIIMS, New Delhi.

RESULTS AND DISCUSSION: Typical freshwater teleost kidney was highly glomerular and consisted of well-developed renal corpuscles having glomerular tuft made of glomerular capillaries, podocytes and mesengial cells. The renal epithelial cells of proximal convoluted tubule (PCT) were cuboidal with numerous closely packed tall microvilli on apical surface. Cells of distal convoluted tubules (DCT) were closely packed without distinct intercellular margin, having few microvilli at apical surface. The collecting tubule (CT) consisted of single cuboidal epithelial cells. Epithelial cells were of two categories; dark cells (DC) and light cells (LC). The interstitial cells were dispersed into renal corpuscles and tubules (**Plate-I**, **Fig. 1**). The transverse section of kidney of endosulfan treated fish for one week showed highly dilated glomerular capillaries due to constriction and necrosis of glomerular tuft, enlarged urinary space and infiltration of basophils and eosionophils in interlobular artery. Infiltration of eosionophils and lymphocytes were prominently marked in inter tubular space. Renal epithelial cells of PCT and DCT were inflamed. **(Plate-I, Fig.3**). After two week treatment, prominent necrosis in glomerulus, necrotic clumps of basophilic cells and deposition of edematous fluid in inter tubular space were marked (**Plate-I, Fig.4**).

Similar kind of dilation of lumen of kidney tubules, necrosis of tubules, shrinkage of glomerular tuft and vacuolation of blood cells in the glomerular tuft have been reported in *Heteropneustes fossilis* exposed to chloropyrifos [24]. Elsan treatment in *Channapunctatus* resulted in a significant decrease in the dimension of Bowman's capsule & glomerulus and irregular shape of tubules due to precipitation of cytoplasm and karyolysis [25]. Dilation of tubules and various other necrotic changes characterized by karyorvhexis and karyolysis at the nuclei of affected cells of *L.rohita* exposed to hexachloro-cyclohexane have been reported [26]. Similar kind of tubular necrosis, desquamation and vacuolization of tubular epithelial cells in kidney of fish exposed to lindane have been noticed [27]. Fish exposed to arsenic showed similar results [28]. The circulatory disorders recruit numerous macrophages and inflammatory cells which develop necrosis around the border of tissue. It is probably the main cause for change in shape of kidney [29].

At TEM level control fish kidney showed properly aligned cuboidal epithelial cells of DCT with

prominent nucleus having normal cyto-architecture, abundant tubular mitochondria and few microvilli in the lumen (**Plate-II, Fig:1** and **2**). The cells of PCT showed the presence of dense osmiophilic granules, secretory vesicles and abundant mitochondria (**Plate-II**, **Fig.3**). Endosulfan treated group of fishes showed heterochromatization of nucleus, irregular margin of inner and outer nuclear membrane, disintegration of chromatin material, intermingling of nucleoplasm and cytoplasm, extensive proliferations of RER, polymorphic mitochondria, increased vacuolationetc (**Plate-II, Fig. 5 and 6**).

Similar degenerative changes in the tubular epithelium as evidenced by presence of epithelial casts in the tubular lumen along with engorged blood vessels and dilation of inter-tubular capillaries and proximal renal tubules in gasoline and gasoline-menthol treated male rats were reported [30]. Ontogenic study of cisplatin (CP) induced nephrotoxicity in rats showed similar necrosis in renal tubules and tubular vacuolization[31]. The kidney of 5 weeks chloropyrifos exposed mice @ 8 mg/kg b.w. showed similar dilated Bowman's capsule, elongated glomerulus, dilated PCT, enucleating cuboidal epithelial cells of DCT [32].

Four fold increases of eosinophils, lymphocytic infiltration and abundance of plasma cells in the lumen of renal tubules as well as interstitial space is directly correlated to the stress response of fish to minimize the toxic impact of endosulfan on renal tissues. Eosinophils are specially designed granulocytes whose cytoplasm contains numerous electron dense granules and lysosome having peroxidase, histaminase, aryl sulfatase and other hydrolytic enzymes. Histaminase neutralizes the activity of histamine, being secreted by damaged renal tissues. Aryl sulfatase neutralizes the action of SRS (slow reactive substances). These enzymes are usually released at the site of allergic reaction thereby diminishing the effect of these vasoactive agents causing inflammatory responses. Besides they are also engaged in phagocytosis of antigen antibody complex. Likewise plasma cells as well lymphocytes are very much engaged in developing humoral and cell mediated immune response in fish to counter the toxic effect of endosulfan on renal tissues.

PLATE: I

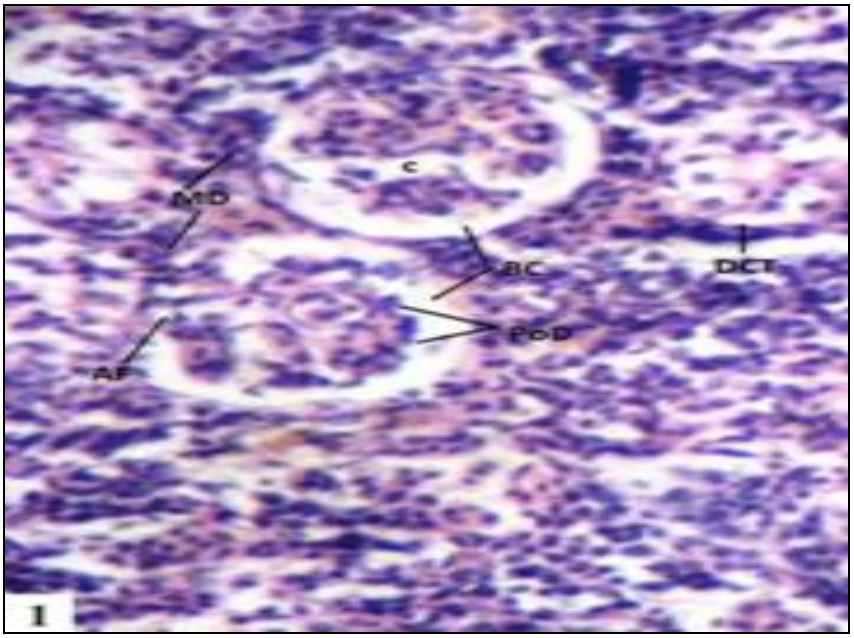


FIG. 1: KIDNEY OF CONTROL FISH SHOWING NORMAL RENAL CORPUSCLES (RC) WITH DISTINCT GLOMERULAR CAPILLARIES (C), PODOCYTES (PoD) AND MESANGIAL CELLS, URINARY SPACE BETWEEN PARIETAL AND VISCERAL LAYER. CUBOIDAL EPITHELIAL CELLS OF DISTAL CONVOLUTED TUBULE (DCT) ARE WELL ALIGNED ON THE BASAL LAMINA. (x 1200)

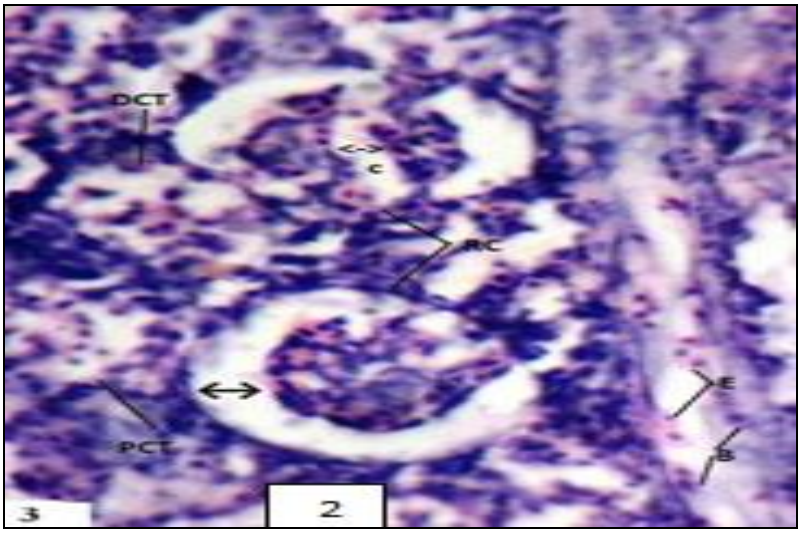


FIG.2: KIDNEY OF FISH AFTER ONE WEEK OF ENDOSULFAN EXPOSURE SHOWING TWO RENAL CORPUSCLES. THE GLOMERULAR CAPILLARY (c) AREA IS HIGHLY DILATED (DOUBLE ARROW) DUE TO CONSTRICTION AND NECROSIS OF GLOMERULAR TUFT. URINARY SPACE IS ALSO ENLARGED (LEFT RIGHT ARROW). MANY BASOPHILS (B) AND EOSINOPHILS (E) ARE MARKED IN INTER LOBULAR ARTERY. X1200

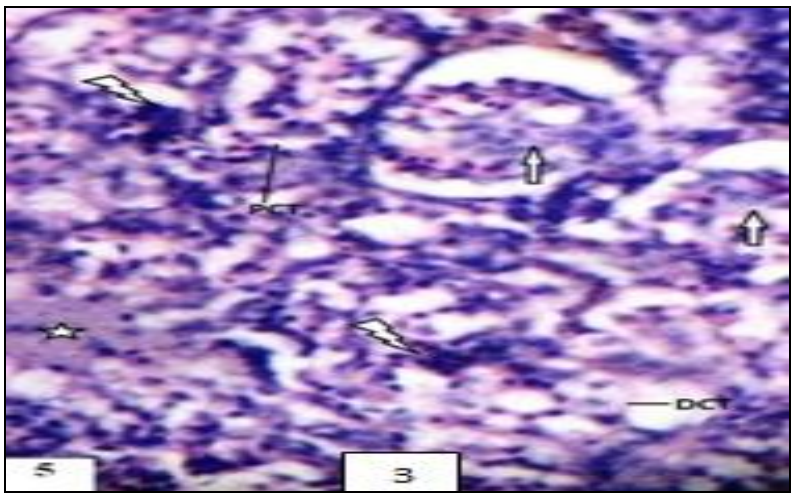


FIG.3: SECTION OF KIDNEY OF FISH AFTER TWO WEEKS OF ENDOSULFAN EXPOSURE SHOWING NECROSIS IN GLOMERULUS (ARROW). NECROTIC CLUMPS OF BASOPHILIC CELLS ARE ALSO PROMINENT (DOUBLE ARROW). DEPOSITION OF OEDEMATOUS FLUID (FIVE POINT STAR) IN INTER TUBULAR SPACE IS ALSO NOTICEABLE. X 1200

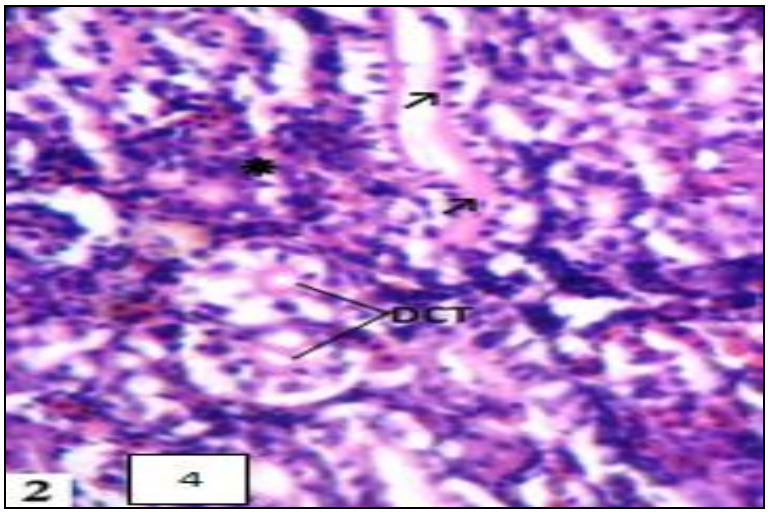


FIG. 4: AFTER 2 W TREATMENT OF WSR SHOWING CONTINUITY (ARROW) IN THE BRUSH BORDER OF THE CELLS OF PCT, CLEAR LUMEN OF DCT WITH DISTINCT RENAL CELLS. BUT EXCESSIVE INFILTERATION OF LYMPHOCYTES, EOSINOPHILS AND BASOPHILS (ASTERISK) ARE STILL MARKED.
X 1200

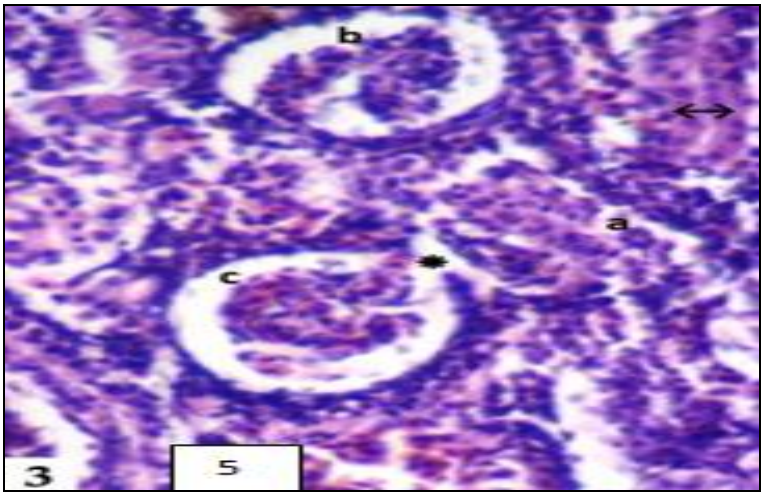


FIG.5: AFTER 4 W TREATMENT OF WSR SHOWING RESTORATION AT THE LEVEL OF RENAL CORPUSCLES AS MARKED BY (C) WITH DISTINCT PODOCYTES, URINARY SPACE AND GLOMERULAR CAPILLARIES WHILE IN A & B GLOMERULAR TUFT TOUCHES PERIETAL LAYER OF BOWMAN'S CAPSULE.

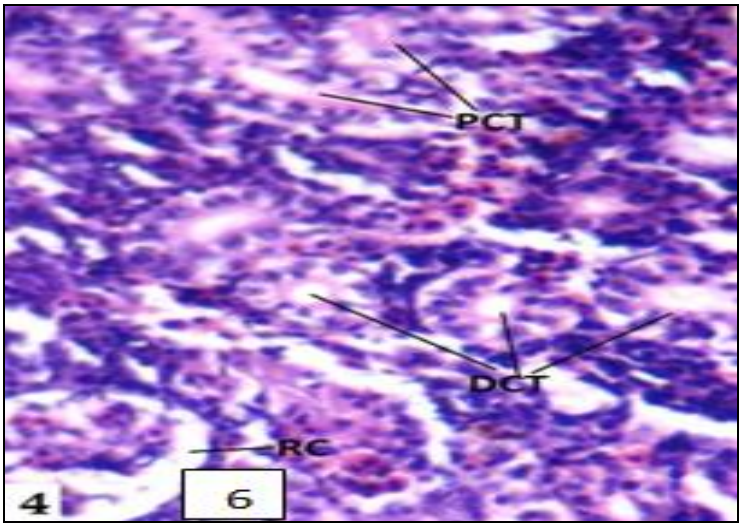


FIG. 6: AFTER 4 W TREATMENT OF WSR SHOWING RESTORATION AT THE LEVEL OF RENAL CORPUSCLES AS MARKED BY (C) WITH DISTINCT PODOCYTES, URINARY SPACE AND GLOMERULAR CAPILLARIES WHILE IN A & B GLOMERULAR TUFT TOUCHES PERIETAL LAYER OF BOWMAN'S CAPSULE.

PLATE: II
TRANSMISSION ELECTRON MICROGRAPHS OF CONTROL AND ENDOSULFAN TREATED GROUP OF FISH

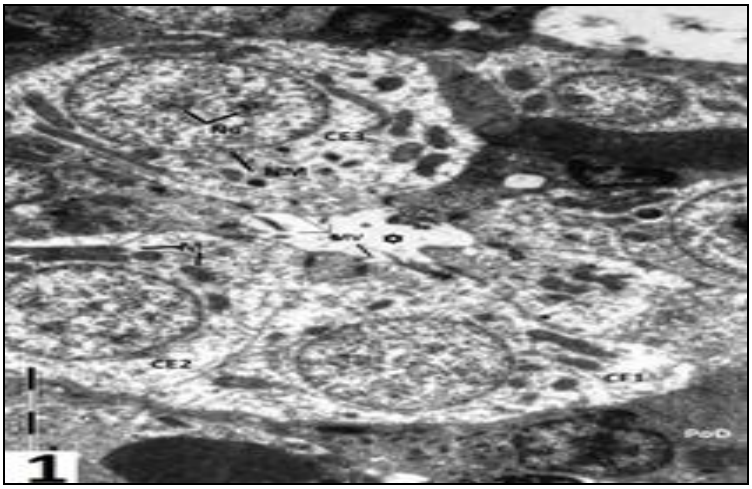


FIG. 1: (CONTROL KIDNEY) SHOWING CUBOIDAL EPITHELIAL CELLS OF DCT WITH ABUNDANT TUBULAR MITOCHONDRIA (TM), PROMINENT NUCLEUS (N) WITH MARKED NUCLEOLI (NU). FEW MICROVILI (Mv) ARE PRESENT IN THE LUMEN.

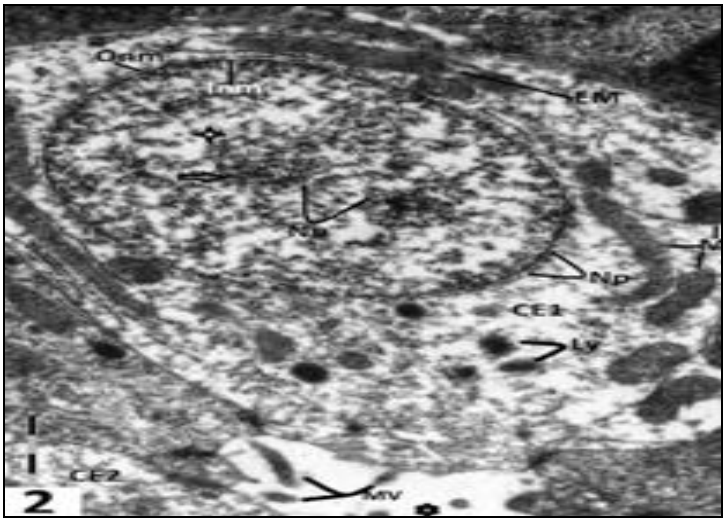


FIG. 2: (CONTROL KIDNEY) SHOWING CUBOIDAL EPITHELIAL CELLS OF DCT WITH ABUNDANT TUBULAR MITOCHONDRIA (TM), PROMINENT NUCLEUS (N) WITHMARKED NUCLEOLI (NU). FEW MICROVILI (Mv) ARE PRESENT IN THE LUMEN.

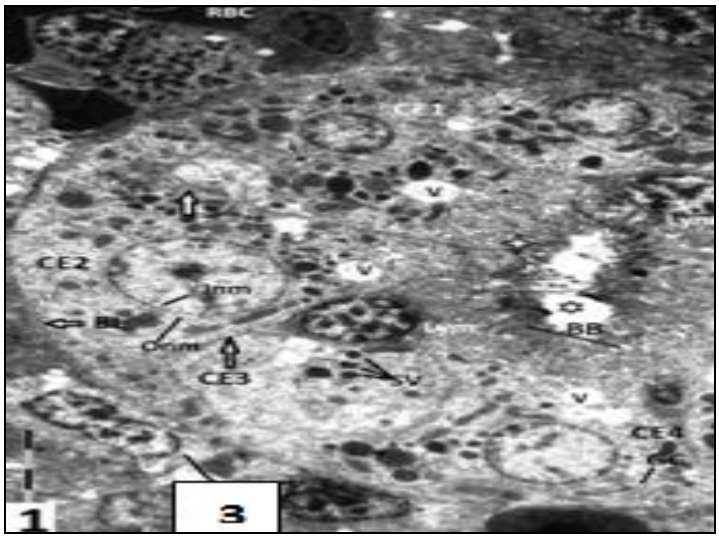


FIG.3: (CONTROL KIDNEY) SHOWING CUBOIDAL EPITHELIAL CELLS OF PCT. APICAL REGION OF THE CELLS CONTAINS ABUNDANT SECRETORY VESICLES (SV) AND NUMEROUS BRUSH BORDERS.

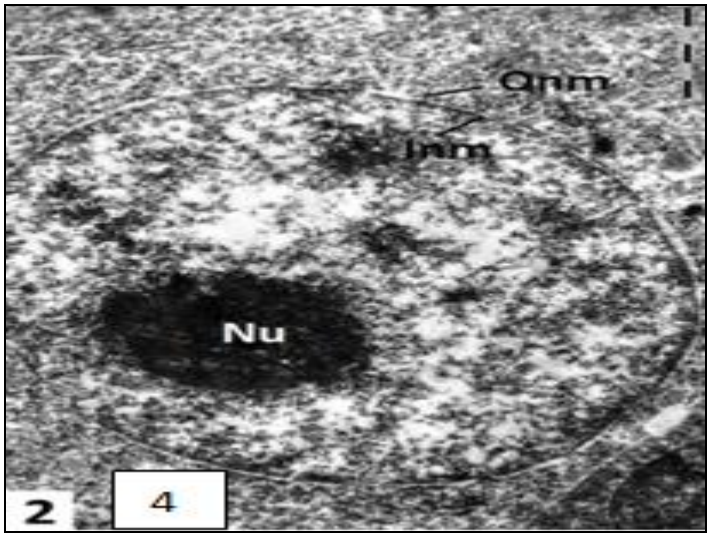


FIG.4: ONE WEEK ES TREATED GROUP OF FISH KIDNEY MARKING VERY FEW DEFORMITIES AS EVIDENCED BY NEARLY NORMAL ULTRASTRUCTURE OF NUCLEUS.

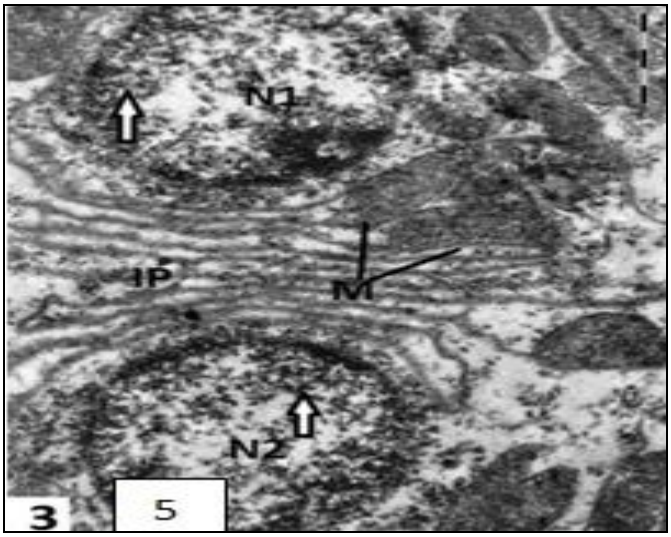


FIG.5: TWO WEEKS ES TREATED GROUP OF FISH KIDNEY SHOWING A PORTION OF CELLS OF PCT HAVING IRREGULAR MARGIN OF NUCLEAR MEMBRANE AND NUCLEAR LAMINA, DISINTEGRATION OF CHROMATIN MATERIAL AND MITOCHONDRIA

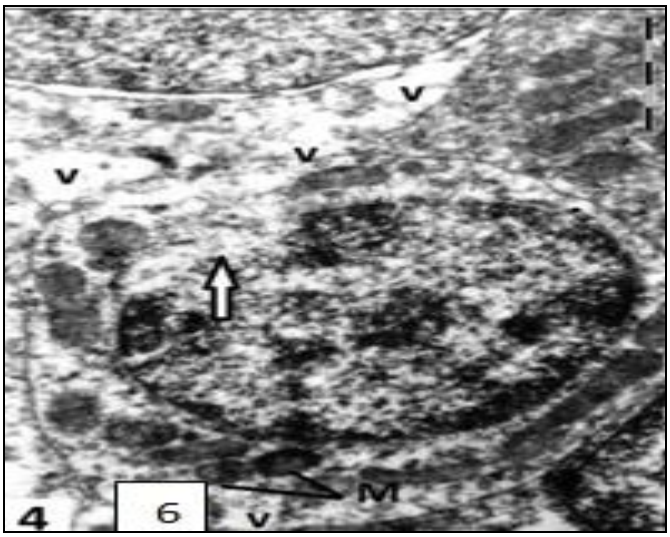


FIG. 6: TWO WEEKS ES TREATED GROUP OF FISH KIDNEY, SHOWING MORE PROMINENT NUCLEAR DISINTEGRATION AND INTERMINGLING OF NUCLEOPLASM WITH CYTOPLASM (ARROW). A SERIES OF DISINTEGRATING MITOCHONDRIA AND VACUOLES ARE MARKED

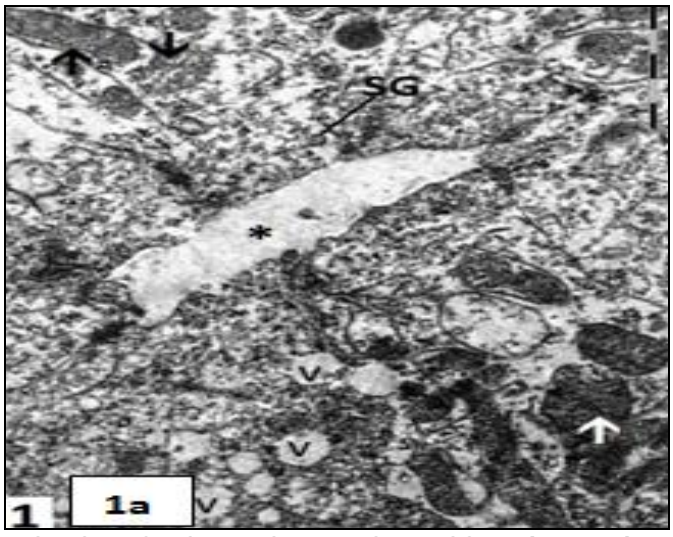


FIG.1a: TEM OF APICAL PORTION OF DCT OF TWO WEEKS ENDOSULFAN TREATED GROUP OF FISH SHOWING MARKED DEGENERACY AND VACUOLIZATION IN THE CYTOPLASM.

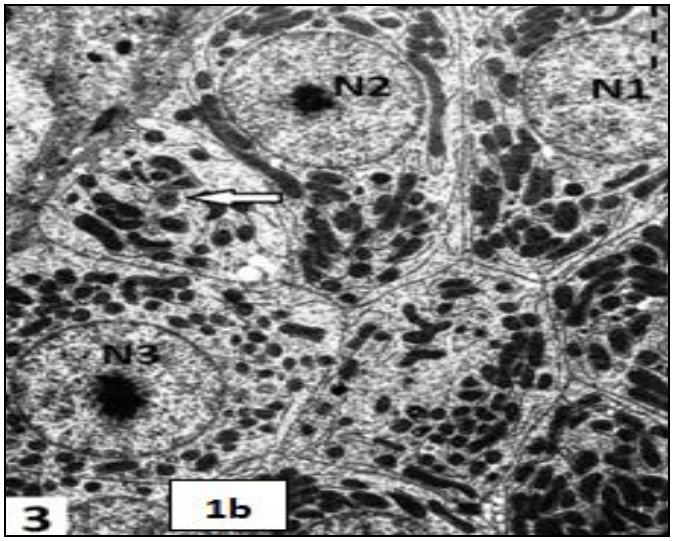


FIG. 1b:AFTER 4 WEEKS TREATMENT WITH WSR EXTRACT TO SAME GROUP SHOWING A CONSIDERABLE DEGREE OF RETRIEVAL IN THE NUCLEAR AND CYTOPLASMIC CYTOARCHITECTURE AS EVIDENCED BY PRESENCE OF ABUNDANT MITOCHONDRIA AND NORMAL NUCLEUS

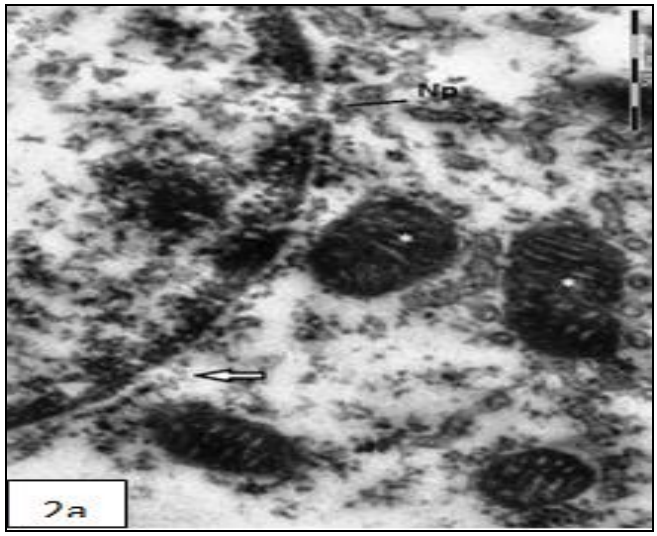


FIG.2a: TEM OF KIDNEY OF TWO WEEKS ENDOSULFAN TREATED GROUP OF FISH SHOWING DILATION OF NUCLEAR PORE (NP), DISCONTINUITY IN OUTER NUCLEAR MEMBRANE, SWOLLEN CRISTAE (ASTERISK) OF MITOCHONDRIA (M) AND LOSS OF OTHER CYTOPLASMIC ORGANELLES.

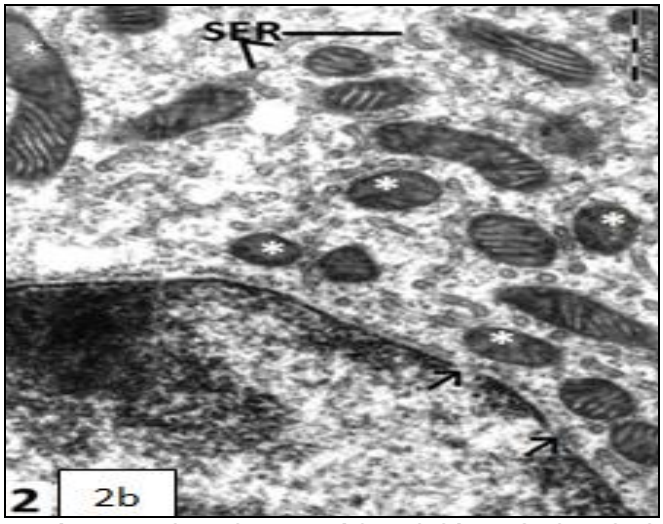


FIG.2b: AFTER FOUR WEEKS TREATMENT OF WSR EXTRACT TO SAME GROUP SHOWING MARKED CORRECTION AT THE LEVEL OF MITOCHONDRIA

Four weeks treatment of WSR extract to endosulfan treated group showed a considerable degree of retrieval in the nuclear and cytoplasmic cytoarchitecture (**Plate-III, Fig.1b** and **2b**). 'ROS' plays a pivotal role in apoptosis by initiating mitochondrial damage and activating sensitive signal pathway [33]. In the present study mitochondrial damage may be considered as initial sign of apoptosis caused by generated ROS due to endosulfan exposure. WSR extract is known to attenuate the elevation of apoptosis and restoration of normal level of Bcl-2 suggesting its antioxidant and antiapoptotic role [34]. Necrotic cell death induced by calcium overload is generally thought to be due to the activation of the cellular enzymes such as nuclease and lipase [35]. Increased intra cellular calcium is associated with mitochondrial calcium accumulation and activation of caspases, which initiates apoptotic cell death [36, 37]. WSR extract probably causes a marked reduction in the intracellular Ca++ level of renal epithelial cells, which in turn reverses the possibility of apoptosis.

Two weeks of self healing period shows a negligible improvement in the cytoplasmic contents of cuboidal epithelial cells of PCT. Even after four weeks of stipulated recovery period, the cytoarchitectural anomalies in the cuboidal epithelial cells of renal tubules worsen instead of any sign of their retrieval. Ultrastructural findings clearly reveal that at lower exposure level of endosulfan, self recovery of four weeks shows partial restoration due to stress response of fish but at higher exposure level, even self recovery of four weeks did not show any sign of retrieval rather further deterioration is marked. It clearly suggests that at higher exposure level, even the fish stress response fails.

When WSR extract was administered for two weeks in control fish, the nucleus and cytoplasmic features were found to be almost normal except congested lumen of DCT by clumps of microtubules. After four weeks treatment of WSR extract to the control fish, further rejuvenation in the cuboidal epithelial cells of DCT is marked with presence of intact nuclear membrane, clear amorphous and granular zone in nucleoli, uniform distribution of euchromatin and heterochromatin, polymorphic mitochondria, SER and abundance of secretory granules. It clearly suggests that WSR extract has immense potential to fortify renal physiology.

The kidney function tests (KFT) provide valuable information about the functional status of the kidney. It also provides necessary information about locations and extent of renal defects. Serum total protein is estimated for monitoring gross changes in protein levels marked in various pathological conditions.

The protein level may increase in several pathological conditions viz, cholelithiasis, liver cirrhosis, macroglobulinemia, multiple myeloma, pheochromocytoma, rheumatic fever and leishmaniasis, whereas it may decrease in several diseases like amyloidosis, analbuminemia, chronic lymphocytic leukemia, acute poststreptococcal glomerulonephritis, epidemic typhus, gastro-intestinal carcinoma, glomerulonephritis, hemolytic uremic syndrome, hepatolenticular degeneration, toxic hepatitis, nephrotic syndrome, chronic renal failure. A marked decline in total serum protein was found in all endosulfan treated groups.

TABLE 1: BIOCHEMICAL ANALYSIS OF SERUM TOTAL PROTEIN (G/DL) AFTER *WITHANIA SOMNIFERA* ROOT EXTRACT TREATMENT TO CONTROL AND PRE-ENDOSULFAN TREATED GROUP OF FISH.

Conc. of endosulfan used (in ppb)	Duration of endosulfan exposure (in days)	Endosulfan treated group		WSR extract treated group @ 100 mg/Kg.b.w.			
		Mean	SE	2 weeks		4 weeks	
				Mean	SE	Mean	SE
Control		4.95	±0.026	5.01 (+1.21)	±0.03	5.00 (+1.01)	±0.029
4	4	4.30	±0.028	4.60*** (+6.98)	±0.037	4.99*** (+16.05)	±0.067
	8	4.00	±0.053	4.35*** (+8.75)	±0.039	4.80*** (+20)	±0.053
	12	3.60	±0.043	4.20***	±0.056	4.50***	±0.113

				(+16.67)		(+25)	
8	4	4.32	±0.029	4.35	±0.026	4.85***	±0.043
				(+0.69)		(+12.27)	
	8	4.20	±0.047	4.40**	±0.034	4.75***	±0.058
				(+4.76)		(+13.1)	
	12	4.32	±0.033	4.65***	±0.061	4.95***	±0.043
				(+7.64)		(+14.58)	
10	4	3.62	±0.036	4.10	±0.041	4.19***	±0.045
				(+13.26)		(+15.75)	
	8	3.78	±0.031	4.01***	±0.042	4.25***	±0.063
				(+6.08)		(+12.43)	
	12	3.36	±0.024	3.75***	±0.047	3.90***	±0.053
				(+11.61)		(+16.07)	

Note: The values are expressed in Mean ± SEM of six replicates in each group. Two tailed unpaired 't' test was done between endosulfan treated group and control. Significant response have been marked as * = p<0.05, ** = p< 0.01 and *** = p<0.001. At other places where it has not been marked is considered as non significant (NS). Figures in parenthesis show percentage increase (+) over control group.

TABLE 2: BIOCHEMICAL ANALYSIS OF SERUM TOTAL PROTEIN (G/DL) IN ONLY *WITHANIA SOMNIFERA* ROOT (WSR) EXTRACT TREATED GROUP OF FISHES

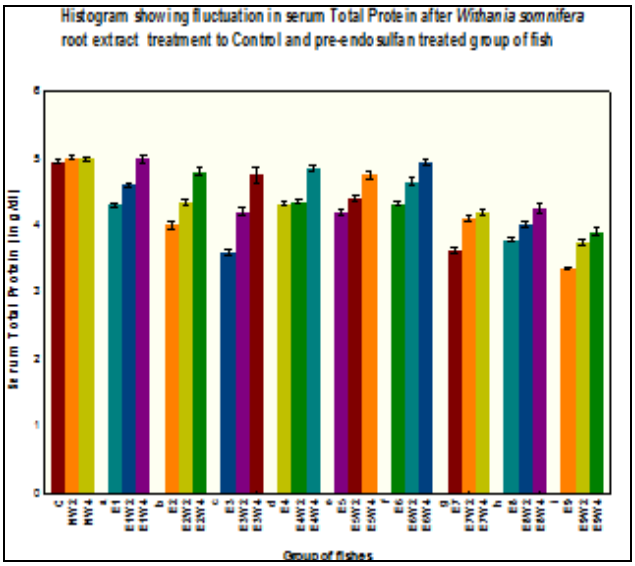
Treatment	Total Serum Protein (in g/dl)			
	2 weeks		4 weeks	
	Mean	SE	Mean	SE
Control group	4.95	±0.026	4.95	±0.026
Withania somnifera root (WSR) extract (100 mg/kg b.w.)	5.01 (+1.21)	±0.033	5.00 (+1.01)	±0.029

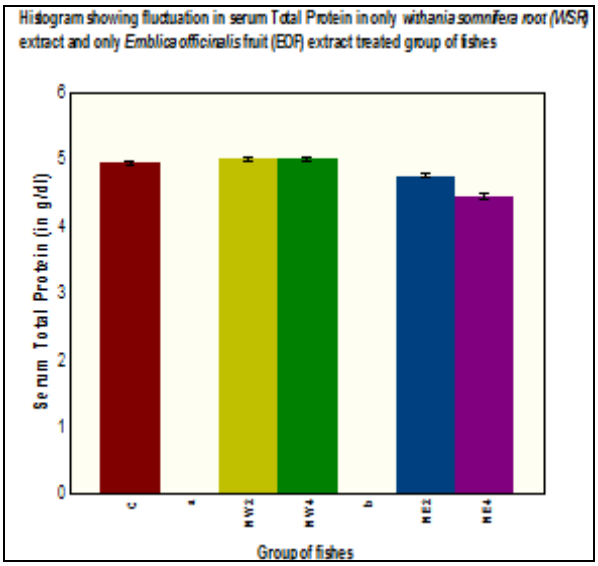
Note: The values are expressed in Mean ± SEM of six replicates in each group. Two tailed unpaired 't' test was done between endosulfan treated group and control. Significant response have been marked as * = p<0.05, ** = p< 0.01 and *** = p<0.001. At other places where it has not been marked is considered as non significant (NS). Figures in parenthesis show percentage decrease/increase (-/+) over control group.

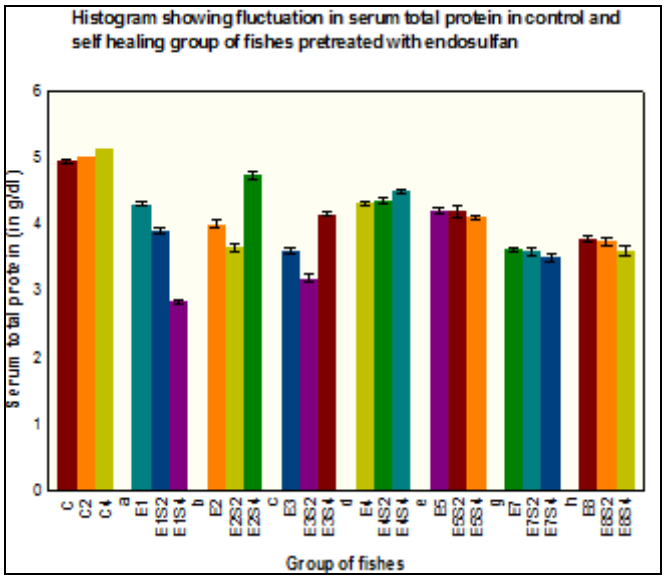
TABLE 3: BIOCHEMICAL ANALYSIS OF SERUM TOTAL PROTEIN (g/dl) IN SELF HEALING GROUP (SHG) OF FISHES PRETREATED WITH ENDOSULFAN.

Conc. of endosulfan used (in ppb)	Duration of endosulfan exposure (in days)	Endosulfan treated group		Self healing group (SHG)			
		Mean	SE	2 weeks		4 weeks	
				Mean	SE	Mean	SE
Control	-	4.95	±0.026	5.00 (+1.01)	-	5.15 (+4.04)	-
4	4	4.30	±0.028	3.90*** (-9.30)	±0.05	2.84*** (-33.95)	±0.024
	8	4.00	±0.053	3.66** (-8.50)	±0.06	4.73*** (+18.25)	±0.048
	12	3.60	±0.043	3.19*** (-11.39)	±0.05	4.15*** (+15.28)	±0.037
8	4	4.32	±0.029	4.35 (+0.70)	±0.04	4.49** (+3.94)	±0.036
	8	4.20	±0.047	4.20	±0.09	4.10 (-2.38)	±0.037
	12	4.32	±0.033	4.25 (-1.62)	±0.05	4.00*** (-7.41)	±0.023
10	4	3.62	±0.036	3.60 (-0.55)	±0.06	3.50 (-3.32)	±0.07
	8	3.78	±0.031	3.75 (-0.79)	±0.06	3.60* (-4.76)	±0.068
	12	3.36	±0.024	3.00 (-10.71)	±0.08	2.95* (-12.20)	±0.052

Note: The values are expressed in Mean ± SEM of six replicates in each group. Two tailed unpaired 't' test was done between endosulfan treated group and control. Significant response have been marked as * = p<0.05, ** = p< 0.01 and *** = p<0.001. At other places where it has not been marked is considered as non significant (NS). Figures in parenthesis show percentage decrease/increase (-/+) over control group.







Similar kind of reduction in total protein content in serum of *Channagachua* after administration of dichlorvos (DDVP) was reported [37]. Administration of zinc sulfate solution to the fish *Cyprinuscarpio* significantly deceased serum protein level [38]. This result might be due to breakdown of these molecules as energetic substrate to cope up zinc induced stress metabolically [39] or due to renal excretion, impaired protein synthesis and/or due to liver disorder [40]. A significant decline in serum total protein of *Clarias gariepinus* after administration of deltamethrin @ 0.75µg/l for two days was reported [41].

In the present study, the aqueous root extract of *Withania somnifera* (WSR) showed a profound impact on the altered KFT profile of the fish due to endosulfan exposure. When WSR extract was treated alone to the control group of fish, it showed non-significant increase in serum total protein by just 1.21% after 2 weeks while at the longer duration of WSR extract treatment, no significant changes has been observed in serum total protein.

Concomitant treatment of endocel and WSR to the experimental group showed non-significant (at $P<0.05$) changes in TP. Similar kind of dose dependent protection of WSR extract against bromobenzene induced nephrotoxicity in mice has been reported [42].

The data clearly represents that aqueous extract of *Withania somnifera* has immense restorative

potential in serum total protein of endosulfan treated group of fishes.

CONCLUSION: The finding of the present study affirms that WSR extract has immense potential to ameliorate the renal toxicity in fish caused by POP stress. Formulated supplementary feed with appropriate doses of WSR extracts can be given to affected group of fish as an antidote against xenobiotic stress. It will be a good strategy for their bio-conservation. Further studies are needed to isolate the pharmacologically active ingredients of these herbal extracts to know the molecular mechanism of their healing action.

ACKNOWLEDGMENT: Authors are thankful to the Head, Department of Zoology, Patna University, Patna for providing infrastructural facilities and SAIF-EM Unit, Dept. of Anatomy, AIIMS, New Delhi for excellent TEM facilities.

REFERENCES:

1. Siroka Z and Drastichova J: Biochemical markers of aquatic environment contamination cytochromeP_{450} in fish. A review. Acta Vet Brno 2000; 73:123-132.
2. Kavlock RJ: Research needs for the risk assessment of health and environmental effects of endocrine disruption. A report the U.S.EPA sponsored workshop. Environ. Hlth. Perspect 1996; 104: 715-740.
3. De Vlaming V, Connor V, De Giorgio C, Bailey HC, Deanovic LA and Hinton DE: Application of whole effluent toxicity test procedures to ambient water quality assessment. Environ. Toxicol.Chem.2000; 19: 42-62.
4. Parma MJ, Loteste A, Campana M and Bacchetta C: Changes of haematological parameters in *Prochiloduslineatus* (Pisces, Prochilodontidac) exposed to sublethal concentration of cypermethrin. J. Environ. Biol.2007; 28: 147-149.
5. Srivastata RK, Yadav KK and Trivedi SP: Devicyprin induced gonadal impairment in a freshwater food fish, *Channapunctatus* (Bloch). J. Environ. Biol.2008; 29: 187-191.
6. Scott GI, Fulton MH, Moore DW, Wirth EF, Chandler GT, Key PB, Daugomah JW, Strozier ED, Devane J, Clarke JR, Lewis MA, Finley DB, Ellengerg W and Karnaky KJ: Assessment of risk reduction strategies for the management of agricultural non-point source pesticide runoff in estuarine ecosystem. Toxicol.Industr.Hlth.1999; 15: 200-213.
7. EFSA: European Food Safety Authority: Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to endosulfan as undesirable substance in animal feed. EFSA J 2005; 234: 1-29.
8. Rao DMR, Priyamavada DA & Murthy AS: Relative toxicity of endosulfan its isomer and formulated products to the freshwater fish *L. rohita*. J. Toxicol. & Environ. Health.1980; 6: 825-834.
9. Naqvi SM and Vaishnavi C: Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals. Biochem. Physiol.1993; 105C: 347-361.
10. Srivastava SK, Tiwari PR and Srivastav AK: Effects of chloropyrifos on the kidney of freshwater catfish *Heteropneustes fossilis.* Bull. Environ. Contam. Toxicol. 1990; 45: 748-751.
11. Banerjee S and Bhattacharya S: Histopathology of kidney of *Channapunctatus* exposed to chronic nonlethal level of Elsan, mercury and ammonia. Ecotoxicol. Environ. Saf.1994; 29: 265-75.
12. Vinodhini R & Narayanan M: Heavy metal induced histopathological alterations in selected organs of the *Cyprinuscarpio* L. (common carp). Int. J. Environ. Res.2009; 3(1): 95-100
13. Joshi GS and Verma RJ: Gasoline, methanol and gasoline: methanols blend exposure-induced nephropathy in male rats. Toxicology International2003; 10(1):61-66.
14. Ellis AE, Munro ALS and Robert RJ: Defense Mechanism in Fish. A study of phagocytic and the fate of interior peritoneal injected particulate material in the plaic (*Pleuronectesplatessa*) J of Fish Biol.1976; 8:67-78.
15. Hayes J: Keeping Fish healthy: Year Book of Agriculture, Animal Health, Live stocks and Pets, Hayes ed., US Government Printing office Washington DC, partVI:P309-370.
16. Kimble CE: Aquaculture: Public Health Regulatory and Management Aspect, Zircon Press, Silver Spring, Md. 1985:185.
17. Fujiki K, Matsuyama H and Yamo T :Protective effects of sodium alginates against bacterial infection in common carp *Cyprinuscarpio*, Ind J of Fish Disease.1994; 17(45):349-355.
18. Fukiji K, Shin D, Nakao M and Yano T: Protective effect of Kappa-Carragenan against bacterial infection in carp *Cyprinuscarpio*. J of Faculty of Agriculture, Kyushu University.1997; 42(1/2):113-119.
19. Du Aifang, Ye Junan, Yu Li An, Du AF, Ye JN and Yu LA: Immunopotentiation activities of garlic oil compound as a feed additive in *Panneuschinensis.* J. of Zhejiang Agriculture University. 1997, 23((3):317-320.
20. Anderson DP and Dixon OW: Fish Biologics: A Guide for the production and use of antisera, antigens and other reagents in fish disease serodiagnosis. US Fish and Wildlife Service, National Fish Health and Research Laboratory, Kearneysville W.Va., 1988; 255.
21. Panda S and Kar K: Betal leaf extract can be both antiperoxidative and peroxidative in nature. J. Current Science, 1998; 74(4): 284-285.
22. Finney DJ: Probit analysis: A statistical treatment of sigmoid curve. 3rd Edn., Cambridge University press. London, 1971, 568 p.
23. Prabhu MS, Patel K, Sharaswathi G and Srinivasan K: Effect of orally administered betal leaf (*Piper betle Linn.*) on digestive enzymes of pancreas and intestinal mucosa and on the bile production in rats. Indian J. of Exp. Biol.1995; 33: 752-756.
24. Das BK and Mukherjee SC: A histopathological study of carp *(Labeorohita)* exposed to

hexachlorocyclohexane. Veterinarski Archiv. 2000; 70: 69-180.
25. Oritz JB, De Canales MLG and Sarasquete C: Histopathological changes induced by lindane (γ-HCH) in various organs of fish. Sci. Mar. 2003; 67: 53-61.
26. Roy S and Bhattacharya S: Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of *Channapunctatus*. Ecotoxicol. Environ. Saf.2006; 65(2): 218-29.
27. Ali BH, Al-Moundhri M, Tageldin M, Al-Hsseini IS, Mansour MA, Nemmar A and Tanira MO: Ontogenic aspects of cisplatin-induced nephrotoxicity in rats. Food and Chemical Toxicology.2008; 46: 3355-3359.
28. Kumar R, Kumar A, Singh JK, Nath A and Ali M: Study of bioremedial impact of curcumin on chloropyrifos induced kidney damage in mice. Pharmacie Globale (IJCP).2011; 8(5): 1-4.
29. Zha J, Sun L, Zhou Y, Spear PA, Ma M and Wang Z: Assessment of 17α-ethinylestradiol effects and underlying mechanisms in a continuous, multigeneration exposure of the Chinese rare minnow (*Gobiocyprisrarus*). Toxicology and Applied Pharmacology.2008; 226: 298-308.
30. Kannan K and Jain SK: Oxidative stress and apoptosis. Pathophysiology, 2000; 7: 153 – 63.
31. Hamaza A, Amin A, Daoub S: The protective effect of a purified extract of *Withania sominifera* against doxorubicin-induced cardiac toxicity in rats. Cell Biol. Toxicol., 2008; 24: 63-73.
32. Waring P: Redox active Calcium ion Channels and Cell Death. Arc. Biochem.Biophs., 2005; 434: 42.
33. Mastan S and Ramayya P: Biochemical profile of Channagachua (Ham.) exposed to sub lethal doses of dichlorvos (DDVP). The International Journal of Toxicology, 2009; 8(1):1-8.
34. Abdel-Tawwab Mohsen, Mohammad NM, Mousaad Khaled M, Sharafeldin and Nahla EM Ismaiel: Changes in growth and biochemical status of common carp *Cyprinuscarpio L.* exposed to water-borne zinc toxicity for different period. International Aquatic Research, 2013; 5:11.
35. Vijayan MM, Pareira C, Grau, EG and Ewama GK: Metabolic responses associated with confinement stress in *Tilapia*: The role of cortisol. Comp. Biochem. Physiol.C.1997; 116:89-95.
36. Kori-Siakpere-O: Some alterations in biochemiocal parameters in *Clarias isheriensis*(Sydenham) exposed to sublethal concentration of water-borne lead. Biosci. Res. Comm. 1995; 8(2):93-98.
37. Amin KA and Hashem KS: Deltamethrin induced oxidative stress and biochemical changes in tissues and blood of cat fish (*Clarias gariepinus*): Antioxidant defense and role of α-tocopherol. BMC Veterinary Research, 2012; 8:45.
38. Vedi M, Rasool M and Sabina EP: Protective effect of administration of *Withania somnifera* against bromobenzene induced nephrotoxicity and mitochondrial oxidative stress in rats. Renal Fail. 2014; 36(7): 1095-1103.

How to cite this article:
Chand GB and Singh P: Possible Retrieval Oforganochlorine Induced Renal Toxicity in Fishby Aqueous Root Extract of *withania Somnifera*: *In vivo* Study. Int J Pharm Sci Res 2016; 7(6): 2365-78.doi: 10.13040/IJPSR.0975-8232.7(6).2365-78.