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Altered serum protein level in fresh water teleost: a potential bioindicator of endosulfan induced nephrotoxicity

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Abstract- Persistent Organic Pollutants (POPs) are constantly being used as modern agrochemical in Indian agriculture despite banned globally and in many of the States of India.The residues of endosulfan, aPOP of organochlorine group, reach water bodies mostly through agricultural runoff and causes severe damage to aquatic fauna predominantly to fishes. They come in direct exposure to this toxic chemical, which has been implicated a gradual toxicity, neurotoxicity, hepatotoxicity, nephrotoxicity and various other biochemical alterations in fishes. The present study is designed to study the alterations in total protein and albumin in the blood serum of *Clarias batrachus* after exposure to various concentration of endosulfan in a dose as well as duration dependent manner. After statistical analysis of the data, it has been observed that alterations generated in total protein and albumin are highly significant ($p>0.001$). This may be due to deteriorating effects of endosulfan on glomerular fenestrae. The results indicate the nephrotoxic effect of endosulfan in fishes.

Keywords : *Clarias batrachus*, endosulfan, serum total protein, serum albumin, nephrotoxicity.

INTRODUCTION

Endosulfan is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup, which acts as a poison. The lipophilic nature, hydrophobicity and low chemical and biological degradation rate of organochlorine pesticides have led to their accumulation in biological tissues and subsequent magnification of concentration in organism progressing up in food chain. Exposure of endosulfan is implicated in several health anomalies in laboratory animals. Endosulfan is known to damage the endocrine system, nervous system, circulatory, reproductive, respiratory and excretory systems and developing foetus. Endosulfan ingestion is known to affect the kidneys and liver.

Serum total protein is estimated for monitoring gross change in protein levels marked in various pathological conditions. But total protein measurement is of little value without simultaneous measurement of albumin. Serum albumin is primarily synthesized in the liver and maintains the osmotic pressure in blood. It helps in transportation of drugs, hormones and enzymes. Elevated levels are rarely seen and are usually associated with dehydration. Decreased levels are seen in various diseases (hepatitis and liver cirrhosis), malnutrition, kidney disorders, increased fluid loss during extensive burns and decreased absorption in gastro intestinal diseases.

In the present study, the biochemical alteration in serum total protein and albumin has been enumerated as

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indicator of renal malfunctioning and mortality in fresh water air breathing fish *Clarias batrachus* due to exposure of environmentally relevant concentration of endosulfan.

MATERIALS & METHODS

Experimental animal: *Clarias batrachus*, ranging from 50-80 gm and size between 18-20 cms were collected during pre-spawning season (March-May). The fishes were brought to the laboratory, disinfected and were acclimated in the laboratory condition. After acclimation, the fishes were transferred to plexi glass aquaria of 50 litre capacity @ 20 fish each having dechlorinated aerated tap water.

Chemical used: In the present study, 'Endocel (EC 35%)' manufactured by 'Excel Industries Ltd, Gujarat' was used. The 96 hrs LC_{50} of endosulfan was calculated by standard APHA method[1] and confirmed by pilot test. The fish were exposed to non-lethal dose of 4 ppb, 8 ppb and 10 ppb for 4, 8 and 12 days.

Experimental design: Fishes were divided into 10 groups containing six fish each. First control group (C) was treated with normal saline (0.85% NaCl) while other 9test groups (E1 to E9) were kept in aquariums containing 4ppb, 8 ppb and 10 ppb endosulfan (35% EC) for 4, 8 and 12 days each. Everyday aquarium water was changed in morning time and stock solution of endosulfan (35% EC) was added to make the respective concentration.

Blood sampling: Blood of individual fish of each group was collected in heparinized tube by puncturing caudal vein. After separation of the serum, it was finally stored at 4^{o}C for colorimetric assessment of serum total protein and albumin.

Biochemical tests: Kit and chemicals used for estimation of serum total protein and albumin were of reagent grade and purchased from local Mercks India distributor.Standard procedure as made available within the kit was followed.

Statistical analysis: Data obtained after biochemical tests have been expressed as M ± SE (Mean ± Standard Error of Mean). Average percentage difference of test group from control group was also calculated. Two tailed unpaired Student's 't' test was performed to test the significance of alteration in serum total protein and serum albumin level. Values at $p<0.05$, $p< 0.01$ and $p<0.001$ were considered to be significant.

RESULTS & DISCUSSION

Measurement of total protein levels alone may be misleading, and may be normal in the face of quite marked changes in the constituent proteins. For example, a fall in albumin may roughly be balanced by a rise in immunoglobulin levels. This is quite a common combination. Therefore, total protein level is always analyzed in combination with other tests.

In the present investigation, the total serum protein content in the fish was obtained as 4.92 - 4.98 g/dl. Similar kind of serum protein content of fish has been vividly reported by several workers. Abdel-Tawwab *et al.*[2] has recorded the serum total protein in *Cyprinus carpio* as 2.02 - 2.37 g/dl. In *Clarias gariepinus* the serum total protein has been recorded as 4.55±0.13 g/dl.[3] Joshi & Pandharikar[4] have reported total protein level in *Clarias batrachus* as 3.99 g/dl. Lipika Patnaik[5] has reported 5.97±0.56 g/dl of serum total protein in *Clarias batrachus*.

Here in the present investigation, endosulfan puts stress on fish and sets in a wave of biochemical imbalance showing a characteristic fluctuation in serum total protein and albumin.

At lower concentration of endosulfan (4 ppb) exposure, a characteristic decline of 13.13%, 19.19% and 27.27% were marked in serum total protein of fish after 4, 8 and 12 days exposure. However, at 8 ppb endosulfan exposure, the decline in serum total protein, although significant, was merely 13.13%, 15.15% and 12.72% respectively after 4, 8 and 12 days of exposure. At the highest dose of endosulfan i.e. 10 ppb, the significant decline in serum total protein was recorded as 26.87%, 23.64% and 32.12% respectively after 4, 8 and 12 days exposure in comparison to that of control fish. It is further supported by histopathological anomalies and cellular necrosis in the cuboidal epithelial cells of renal tubules and corpuscles.

Endosulfan also affects the concentration of albumin in the blood serum of fish in a duration dependent manner. At lower dose of endosulfan (4 ppb), a significant decline of 12.36% and 22.47% in serum albumin concentration has been observed after 8 and 12 days exposure, whereas at higher concentration of 8 ppb, serum albumin concentration declined by 10.67%. 26.40% and 35.39% respectively after 4, 8 and 12 days in comparison with that of control. However, at 10

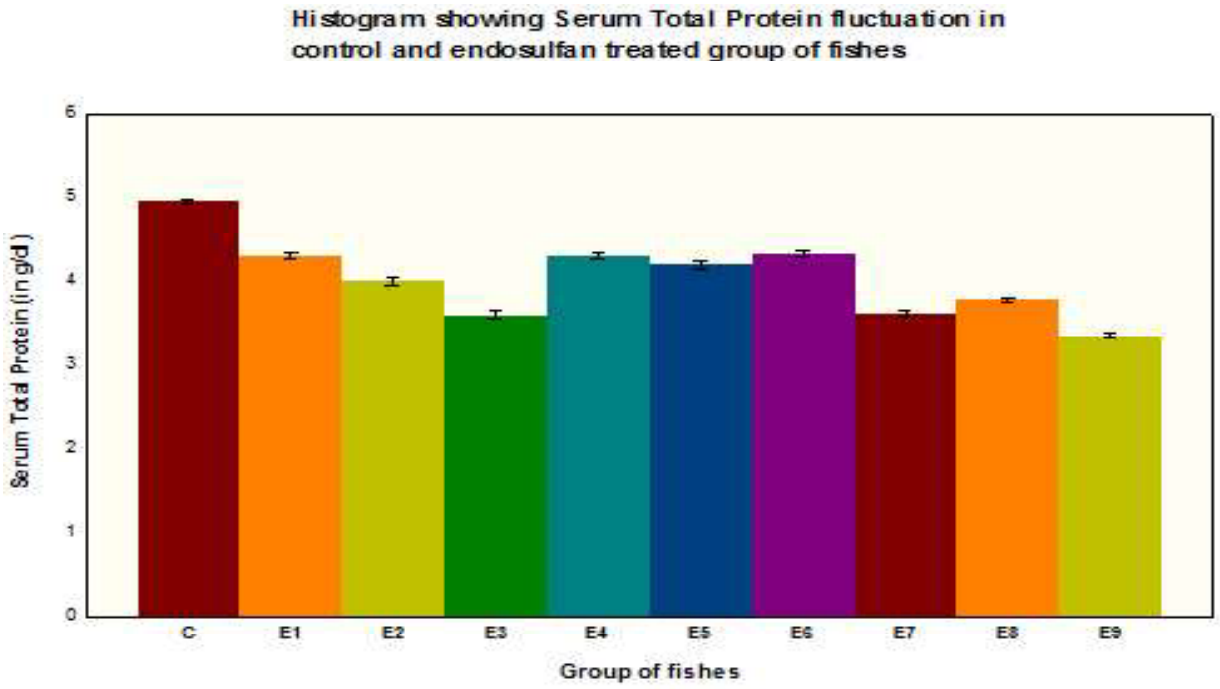
ppb endosulfan exposure duration dependent significant decline of 26.97%, 35.39% and 46.63% in serum albumin respectively after 4, 8 and 12 days have been recorded in comparison to that of control group.

Table – 1: Showing fluctuation in serum Total protein (in mg/dl) and serum albumin in control and different group of endosulfan treated fishes

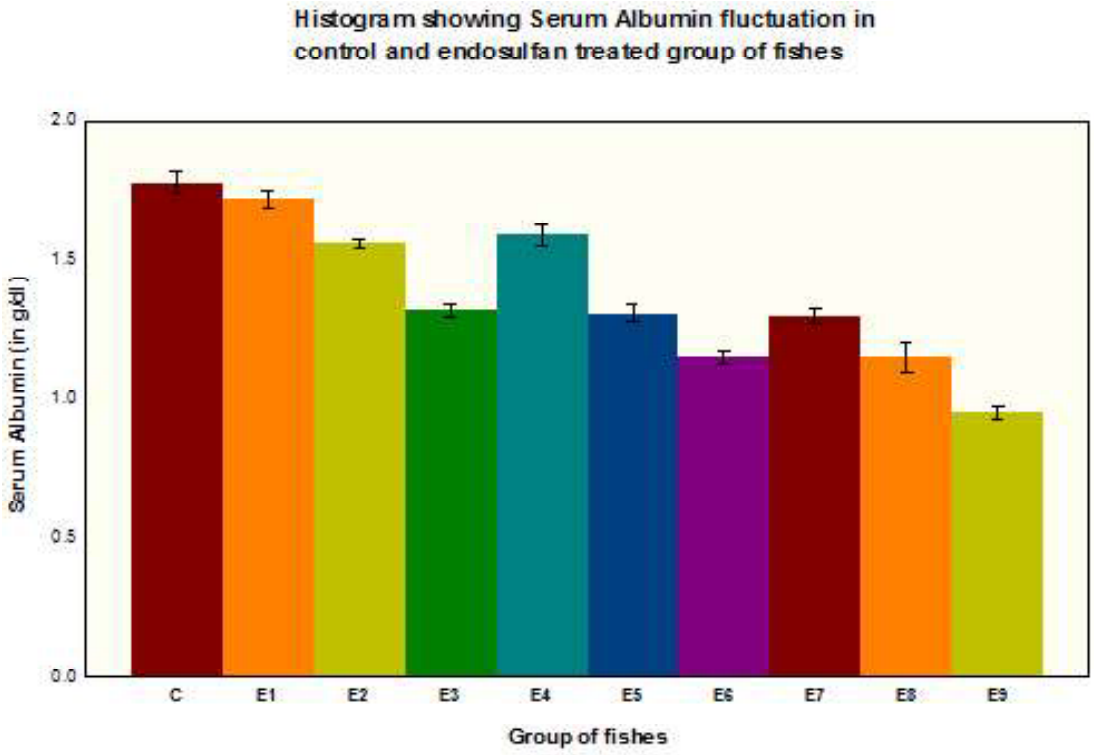
Conc. of endosulfan used (in ppb)	Duration of endosulfan exposure (in days)	Serum total protein	Serum albumin
		Mean ± SE	Mean ± SE
Control	-	**4.95**±0.026	**1.78**±0.040
4	4	**4.30*****±0.028(- 13.13)	**1.72**±0.033(-3.37)
	8	**4.00*****±0.053(-19.19)	**1.56*****±0.015(-12.36)
	12	**3.60*****±0.043(-27.27)	**1.32*****±0.021(-22.47)
8	4	**4.30*****±0.029(-13.13)	**1.59****±0.037(-10.67)
	8	**4.20*****±0.047(-15.15)	**1.31*****±0.032(-26.40)
	12	**4.32*****±0.033(-12.72)	**1.15*****±0.025(-35.39)
10	4	**3.62*****±0.036(-26.87)	**1.30*****±0.029(-26.97)
	8	**3.78*****±0.031(-23.64)	**1.15*****±0.052 (-35.39)
	12	**3.36*****±0.024(-32.12)	**0.95*****±0.026(-46.63)

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.

Text Graph – 1



Text Graph – 2



Similar kind of reduction in total protein content in serum of the many fishes have been reported. Mastan & Ramayya[6] studies acute and chronic effects of dichlorvos (DDVP) on biochemical profile of *Channa gachua*. After 16 hours exposure of DDVP, total protein content recorded was 4.17 mg/dl, within 24 hours of exposure, it became 3.99 mg/dl and after 48 hours of exposure it became 4.11 mg/dl. In chronic exposure studies, after 15 days of DDVP exposure serum total protein was found as 4.61 mg/dl which declined to 4.51 mg/dl after 30 days of exposure. After 45 days it further declined to 4.45 mg/dl. Abdel-Tawwab *et al.*[2] reported that administration of zinc sulfate solution to the fish *Cyprinus carpio* significantly deceases serum protein level which was found to be dose and duration dependent This result might be due to breakdown of these molecules as energetic substrate to cope up zinc induced stress metabolically[7] or due to renal excretion, impaired protein synthesis and/or due to liver disorder[8]. This decrease may be due to the zinc exposure which causes significant alteration in the protein secondary structure by decreasing the α-helix and increasing the β-sheet content of the gill tissues of the *Lebeo rohita*[9]. Amin & Hashem[3] have shown a significant decline in serum total protein of *Clarias gariepinus* after administration of deltamethrin @ 0.75 µg/l for two days.

In contrary to my findings, Joshi & Pandharkar[4] have shown a significant increase in serum protein concentration in *Clarias batrachus* due to cadmium exposure.

Lipika Patnaik[5] has reported a decline in serum albumin of *Clarias batrachus* from 1.80±0.30 to 0.82±0.31 and 0.88±0.52 g/dl after exposure of Sevin @ 12.6 mg/L and 14.6 mg/L respectively.

Amin & Hashem[3] have shown a significant decline in serum albumin content of *Clarias gariepinus* from 1.83±0.05 g/dl to 1.46 ±0.08 g/dl after exposure of deltamethrin @ 0.75 mg/L.

The findings are in agreement with the Yousef *et al.*[10] and El-Demerdash *et al.*[11] The decrease in plasma protein could be attributed to changes in protein and free amino acid metabolism and their synthesis in liver. It can be further correlated to the damaging effects of endosulfan on hepatic tissues as confirmed by increasing in the activities of AST & ALT[12].

Adamu & Kori-Siakpere[13] have reported similar kind of significant decline of nearly 24% in serum albumin content of hybrid catfish of *C. batrachus and Heterobranchus bidorsialis*. The decrease in serum albumin may impede its function of transportation. The significant decrease in serum albumin may have resulted from the inhibitory effects of endosulfan on protein hydrolytic activities due to elevation of protease activity correspond to the value of total protein.

Fafioye *et al.*[14] have reported similar reduced albumin value in the kidney of *Orechromis niloticus* after exposure of sublethal concentration of aqueous extract of *Raphia vinifera*.

In view of above, it can be assumed that endosulfan may induce glomerulonephritis, and nephrotic syndromes leading to significant decrease in serum total protein and serum albumin which is associated with acute renal failure in fish. The present study marks the extreme nephrotoxic potential of endosulfan in the aquatic organism.

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