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Toxic impact of endosulfan on liver and kidney functions in *Clarias batrachus*: A biochemical study

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Abstract- Intense anthropogenic activities in terms of non-judicious use of agrochemicals have posed serious threats to the aquatic organism. Fishes being at apex of the aqueous ecosystem get easily victimized to their deleterious impact. In the present study, *Clarias batrachus* commonly called as "Mangur" has been used as experimental model for assessment of toxic impact of endosulfan on liver and kidney functions. Fishes have been collected from local pond in pre-spawning season having 5"-6" length and 100-110 gm weight. They were exposed to mild, moderate and high level of endosulfan concentration based on LC_{50} and impact was assessed after 4, 8 and 12 days through measurement of SGPT, SGOT and Urea level in blood serum. Due to its cyto-biochemical impact on kidney and liver, endosulfan lowers the clearance of urea through the urine and raises its level in blood serum while due to disruption of hepatic tissues, blood serum level of SGPT and SGOT also raised significantly. This study confirms the disruptive effects of endosulfan on kidney and liver functions.

Keywords : *Clarias batrachus*, endosulfan, Serum Urea, SGPT, SGOT, nephrotoxicity, hepatotoxicity

INTRODUCTION

Endosulfan is a chlorinated hydrocarbon insecticide which acts as a poison for not only pests/insects but other livelihood too. The lipophilic nature, hydrophobicity and low chemical and biological degradation rate of endosulfan led to its accumulation in adipose 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.

Urea is produced in the liver as a waste product in the urea cycle from catabolism of proteins in humans. Consequently the circulation levels of urea depend upon protein intake, protein metabolism and kidney function. Blood Urea Nitrogen (BUN) varies directly with protein intake and inversely with the rate of excretion of urea.

Blood Urea Nitrogen (BUN) levels are elevated due to renal insufficiency [acute and chronic nephritis, acute renal failure (tubular necrosis)], urinary tract obstruction, increased nitrogen metabolism associated with diminished renal blood flow or impaired renal function: dehydration

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from any cause, gastrointestinal bleeding, with a combination of increased protein absorption from digestion of blood, plus decreased renal blood flow.

SGPT is found in a variety of tissues but is mainly found in the liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic disease. Slight elevation of the enzymes is also seen in myocardial infection.

SGOT is an enzyme found mainly in heart muscles, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood stream. Elevated levels are found in myocardial infection, Cardiac operation, hepatitis, cirrhosis, acute pancreatitis, acute renal diseases and primary muscle diseases. Decreased level may be found in pregnancy, Beri-beri and diabetic ketoacidosis.

In the present study, the biochemical alteration in serum level of urea, SGPT and SGOT has been enumerated as indicator of renal and hepatic malfunctioning infresh water air breathing fish *Clarias batrachus* due to exposure of different concentration of endosulfan.

MATERIALS & METHODS

Experimental animal: *Clarias batrachus*, ranging from 50-80 gm and size between 18-20 cms were collected from Phulwarisharif Fish Farm, Patna 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 by puncturing the caudal vein in a heparinized tubes. After separation of the serum, it was finally stored at 4° C for colorimetric assessment of serum urea, SGPT and SGOT.

Biochemical tests: Kit and chemicals used for estimation of serum urea, SGPT and SGOT were of reagent grade and purchased from local Mercks India distributor. 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

Due to its cyto-biochemical impact on kidney, endosulfan lowers the clearance of urea through the urine and raises its level in blood. At 4 ppb endosulfan concentration, serum urea level showed elevation by 25% and 50% after 8 days and 12 days respectively whereas at 8 ppb concentration of endosulfan, an increase of 25% after 4 days and 37.5% in 12 days was reported. At the highest concentration of endosulfan i.e. 10 ppb, blood urea level increase by 37.5% in 4 days and 75% in 8 days. It has also been observed that endosulfan amplifies the blood urea level upto double the level in control after 12 days of 10 ppb exposure.

Joshi & Pandharikar[2] studied the hematological indices of *Clarias batrachus* after exposure of cadmium @ 2.5 ppm for 10, 20 & 30 days. They reported similar kind of increasing trend in the serum urea level from 16.017±0.211 mg/dl to 16.552±0.017 mg/dl in 10 days, 17.417±0.107 mg/dl in 20 days and 18.458±1.10 in 30 days exposure of Cadmium. High serum urea content in the present investigation may be correlated in the renal disorder generated by endosulfan exposure.

Lipika Patnaik[3] has shown a similar significant increase in serum urea content from 16.50±3.08 mg/dl to 18.68±3.75 mg/dl and 16.50±3.00 mg/dl to 16.77±4.65 mg/dl after sevin exposure @ 12.6 mg/L and 14.6 mg/L respectively in *Clarias batrachus*.

Kumar *et al.*[4] reported very high level of urea in liver and kidney of *Channa punctatus* and *Clarias batrachus* exposed to a commercial grade γ -cyhalothrine.

Kumar *et al.*[5] have shown fourfold increase in serum urea level in 5 week chloropyrifos administered mice. The uric acid level has been shown increased by 3 folds whereas serum creatinine level has shown nearly 5 fold increase over the control.

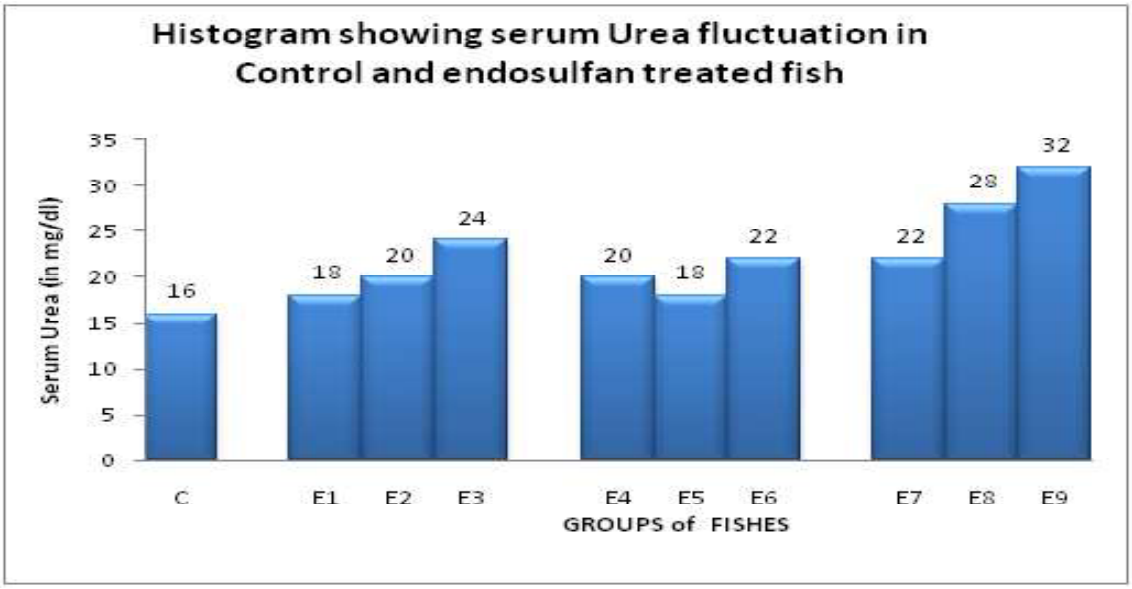
Pratheesh & Kutton[6] had shown that administration of cyclophosphamide leads to significant elevation of blood urea and SGPT activity in comparison with the control group.

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

Conc. of endosulfan used (in ppb)	Duration of endosulfan exposure (in days)	Code	Serum urea
			Mean ± SE
Control	-	**C**	**16**±0.577
4	4	**E1**	**18***±0.516
	8	**E2**	**20*****±0.577
	12	**E3**	**24*****±0.894
8	4	**E4**	**20*****±0.577
	8	**E5**	**18***±0.577
	12	**E6**	**22*****±0.577
10	4	**E7**	**22*****±0.577
	8	**E8**	**28*****±0.894
	12	**E9**	**32*****±0.973

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

Text Graph – 1



AST (SGOT) and ALT (SGPT) are sensitive indication of liver damage or injury from different types of disease. AST (SGOT) is normally, found in a diversity of tissues including heart muscles, kidney, and brain. It is released into serum when any one of heart tissue is damaged. SGPT is by contrast, normally found largely in liver. It is released into the blood serum as the result of liver injury. It, therefore, serves as a specific indicator of liver status. The degree of elevation roughly parallels the extent of liver damage. Both these enzymes may be elevated in a variety of infiltrative diseases of liver and biliary obstructions, including conditions such as gall stones, cholecystis, primary biliary cirrhosis, cancer and cholangitis etc., haemochromatosis, chemical injury (eg. Necrosis related to various toxins and pesticides) etc. is also responsible for high AST. On the other hand, uremia, vit. B6 deficiency, metronidazole,trifluroparazine etc. lowers serum AST.

At lower concentration of endosulfan (4 ppb) serum SGPT showed its peak elevation just within 4 days and then gradually declined up to 12 days treatment. The changes at all the three durations were significant at $P<0.05$. However, at 8 ppb endosulfan after 8 day treatment, SGPT showed non-significant at (at $P<0.05$) elevation. At higher sublethal exposure of endosulfan i.e. 10 ppb, it gets elevated just within 4 days and declined at higher durations. However, in all cases, it is higher than the normal, signifying toxic status of fish. It is further supported by histopathological anomalies and cellular necrosis in the hepatocytes.

SGOT showed its elevation by 2.5 times, the normal value, immediately after 4 days treatment of ednosulfan (significant at $P<0.05$). Likewise, SGPT, it also declined in longer duration but in all the cases, the values were greater than the control. However, at 8 ppb, the changes incurred in SGOT at all the three duration were not significant at $P< 0.05$. This may be argued that at 8 ppb concentration, the fish showed maximum stress response.

At higher sublethal exposure of 10 ppb endosulfan SGOT showed the similar fluctuation as marked in 4 ppb but at 10 ppb 8 days treatment, fluctuation in SGOT was not significant.

Table – 2: Showing fluctuation in serum SGPT(in U/ml) in control and different group of endosulfan treated fishes

Conc. of endosulfan used (in ppb)	Duration of endosulfan exposure (in days)	Code	Serum SGPT
			Mean ± SE
Control	-	**C**	**30±3.033**
4	4	**E1**	72*±**4.733**
	8	**E2**	52*±**4.56**
	12	**E3**	43*±**4.899**
8	4	**E4**	30±**1.897**
	8	**E5**	38*±**2.828**
	12	**E6**	30±**3.521**
10	4	**E7**	56*±**5.656**
	8	**E8**	42*±**4.000**
	12	**E9**	40*±**3.577**

Text Graph – 2

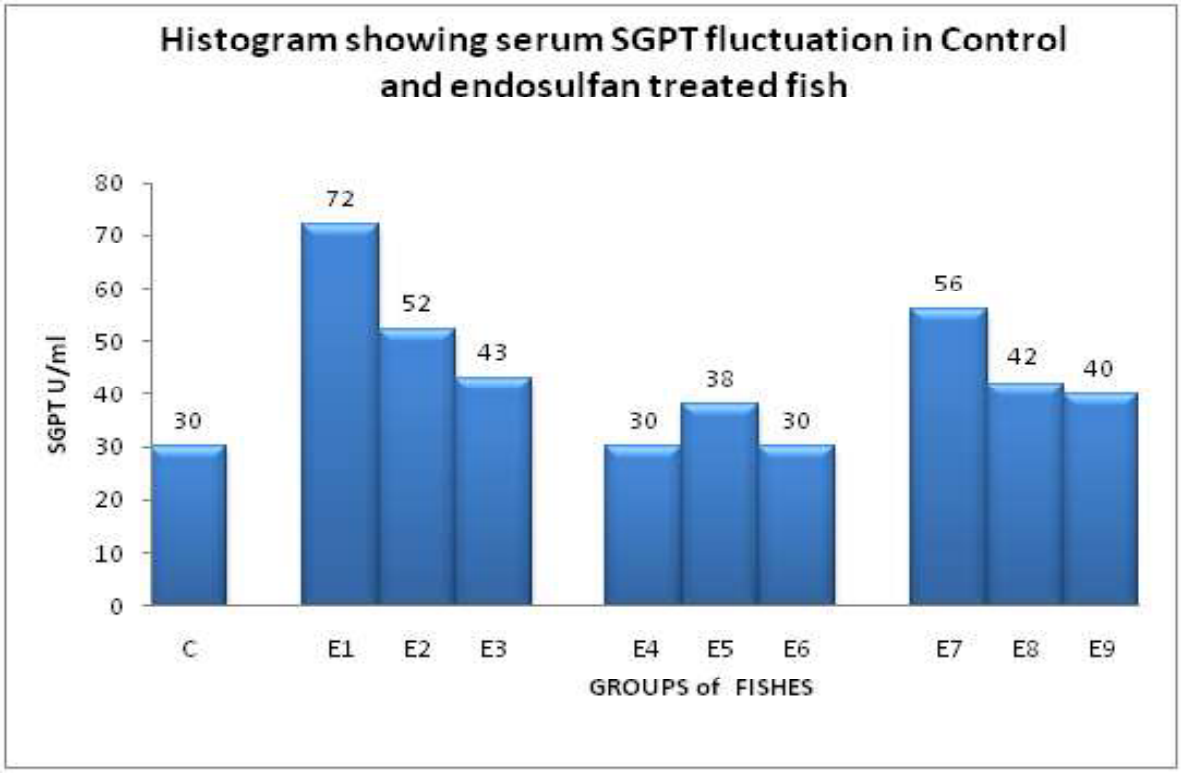
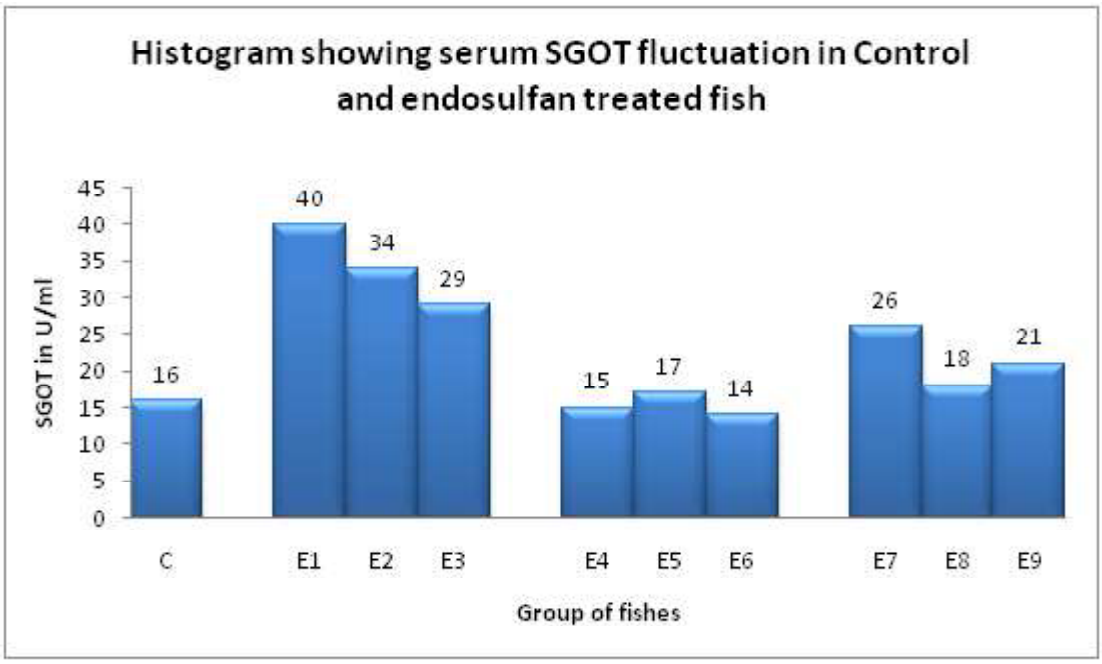


Table – 3: Showing fluctuation in serum SGOPT (in U/ml) in control and different group of endosulfan treated fishes

Conc. of endosulfan used (in ppb)	Duration of endosulfan exposure (in days)	Code	Serum SGOT
			Mean ± SE
Control	-	**C**	**16±1.673**
4	4	**E1**	40*±**4.56**
	8	**E2**	34*±**4.195**
	12	**E3**	29*±**2.366**
8	4	**E4**	15±**2.00**
	8	**E5**	17±**2.59**
	12	**E6**	14±**1.788**
10	4	**E7**	26*±**2.828**
	8	**E8**	18±**4.049**
	12	**E9**	21*±**1788**

Text Graph – 3



Parallel to this findings, similar kind of elevation in SGPT and SGOT, AlP in *Channa punctatus* after DDVP treatment have been reported by Koul *et al.*[7] He studied the sublethal effects of dichlorovos on various biochemical parameters of *Channa punctatus*.

Mukhopadhyay & Dehadrai[8] observed various biochemical changes such as GOT and GPT in air breathing catfish *Clarias batrachus* under sublethal malathion exposure.

SGPT and SGOT are also known to be increased in Raje syndrome.[9] Hepatotoxicity from lungs may cause high amino transferase activity with elevation of AST:ALT ratio.[10] Chand *et al*.[11] have shown biochemical imbalance and related hepatic failure in *C. batrachus* after rogor exposure.

Injury to liver, whether acute or chronic, eventually results in an increase in serum concentration of amino transferase AST and ALT. The tranaminases GOT and GPT are two key enzymes known for their role in the utilization of protein and carbohydrates. In the present investigation, both GOT and GPT are found to be increased after endosulfan. Similar activation of GPT and GOT have been observed in *Tilapia mossambica* by Rao & Rao[12]. Similarly in the murrel, *Channa striatus*, a 10 day exposure to OP., Malathione and Phosphamidon have increased GOT and GPT activities.[13] Holmberg *et al.*[14] found decreased activity of liver GPT and enlargement of the liver in fish chronically exposed to pentachlorophenol (PCP). The GPT activity in the muscle was depressed significantly in the eels exposed to PCP for 8 days and subsequent recovery in clean water.

In view of above, it can be assumed that endosulfan may lead to tissue disruption in kidney and liver of the fish resulting to significant dose and period dependent increase in serum urea, SGPT and SGOT which is associated with acute hepatic and renal failure in fish. The present study marks the extreme nephrotoxic and hepatotoxic potential of endosulfan in the aquatic organism.

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