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Toxic Impact of Endosulfan on Serum Creatinine Level in *Clarias batrachus*: *In vivo* study

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***Abstract*: Pesticides are being used to keep pests away from the crops. They kill the pests but also cause imbalance in ecosystem. Endosulfan was also used extensively in Indian agriculture as pesticide. The residues of endosulfan reach to 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, genotoxicity and various other biochemical alterations in fishes. Present study is designed to study the alterations in serum creatinine in the blood serum of *Clarias batrachus* on 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 caused in creatinine are highly significant ($p>0.001$). This may be due to deteriorating effects of endosulfan on glomerular fenestrae. The results indicate the nephrotoxic nature of endosulfan in fishes.**

***Keywords: Clarias batrachus*, Endosulfan, Creatinine, Nephrotoxicity.**

I. 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. It is reported to be teratogenic, carcinogenic, nephrotoxic causing severe glomerulonephritis, neurotoxic, hepatotoxic, gonadotoxic. Besides, endosulfan is known to damage the endocrine system, nervous system, circulatory, reproductive, respiratory and excretory systems and developing foetus.

Serum creatinine is an important indicator of renal health since it is an easily measured by-product of muscle metabolism and excreted almost unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine, and adenosine triphosphate (ATP).

Creatinine is removed from the blood chiefly by the kidneys, primarily by glomerular filtration, but also by proximal tubular secretion. Little or no tubular reabsorption of creatinine occurs. If the filtration in the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance, which correlates with the glomerular filtration rate (GFR). Blood creatinine levels may also be used alone to calculate the estimated GFR (eGFR).

In the present study, the biochemical alteration in serum creatinine content of fresh water air breathing fish *Clarias batrachus* has been enumerated due to exposure of environmentally relevant concentration of endosulfan which is primarily associated with acute renal failure and mortality in the fish.

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II. MATERIALS & METHODS

A. Experimental animal:

Same age groups of *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 with 0.1% $KMNO_4$ solution and were acclimated for 15 days in the laboratory condition. To maintain normal water temperature, cooler and exhaust were used around the aquaria. Continuous aeration was provided to maintain dissolved O_2 near saturation. After acclimation, the fishes were transferred to plexi glass aquaria of 50 litre capacity @ 20 fish each having dechlorinated aerated tap water and the experiment was conducted.

B. 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.

C. 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 9 test groups (E1 to E9) were kept in aquariums containing 4 ppb, 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.

D. Blood sampling:

Blood of individual fish of each group was taken in separate RIA tube. After separation of the serum in Remi's high speed centrifuge, it was finally stored at 4^oC for colorimetric assessment of serum creatinine.

E. Biochemical tests:

Kit and chemicals used for estimation of serum creatinine were of reagent grade and purchased from local Mercks India distributor. Procedure as made available within the kit was followed.

F. 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 creatinine level. Values at $p<0.05$, $p< 0.01$ and $p<0.001$ were considered to be significant.

III. RESULTS & DISCUSSION

By virtue of its relative independence from such factors as diet (protein intake), degree of hydration and protein metabolism, the serum creatinine is significantly more reliable screening test or index of renal function. An increase in serum creatinine may occur in any one of the condition in which blood urea is increased. Elevated level of creatinine reflect malfunction of kidney under stress, being functionally insignificant because of cellular damage and after pesticide exposure[2]. The normal serum creatinine level in the fish is obtained as 0.39 – 0.41 mg/dl (Table-1). Abdel-Tawwab *et al*.[3] reported serum creatinine level in *Cyprinus carpio* as 0.28 mg/dl. Mehdi Yousefian *et al*.[4] have reported serum creatinine level as 0.40±0.10 mg/dl in fries of *Rutelus frisii Kutum*. Joshi & Pandharikar[5] have reported serum creatinine level in *Clarias batrachus* 0.256 mg/dl while Lipika Patnaik[6] reported it as 1.11±0.29 mg/dl.

TABLE – 1: Showing fluctuation in serum Creatinine (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 creatinine
			Mean ± SE
Control	-	C	**0.40**±0.013
4	4	E1	**0.50**±0.019**(+25.0)
	8	E2	**0.45**±0.018*(+12.5)

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	12	E3	**0.65**±0.021***(+ 62.5)
8	4	E4	**0.50**±0.014***(+25.0)
	8	E5	**0.50**±0.015***(+25.0)
	12	E6	**0.70**±0.012***(+75.0)
10	4	E7	**0.60**±0.013***(+ 50.0)
	8	E8	**0.70**±0.013***(+75.0)
	12	E9	**0.90**±0.018***(+125.0)

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. Figures in parenthesis show percentage increase (+) over control group.

As endosulfan is a potent nephrotoxic compound, it causes disturbance in renal filtration process through various manners. Due to this effect of endosulfan, serum creatinine, which is chiefly filtered through the glomerulus and proximal tubules, gets disturbed and ultimately its concentration in the blood is enhanced. Even a lower dose of endosulfan (4 ppb) increased the serum creatinine level by 25% and 62.5% after 4 & 12 days of exposure respectively. Similarly, 8 ppb concentration of endosulfan causes increased level of serum creatinine by 25% after 4 days and 75% after 12 days. At 10 ppb concentration of endosulfan, serum creatinine level shoots up by 50%, 75% and 125% after 4, 8 and 12 days of exposures respectively (Fig-**1**).

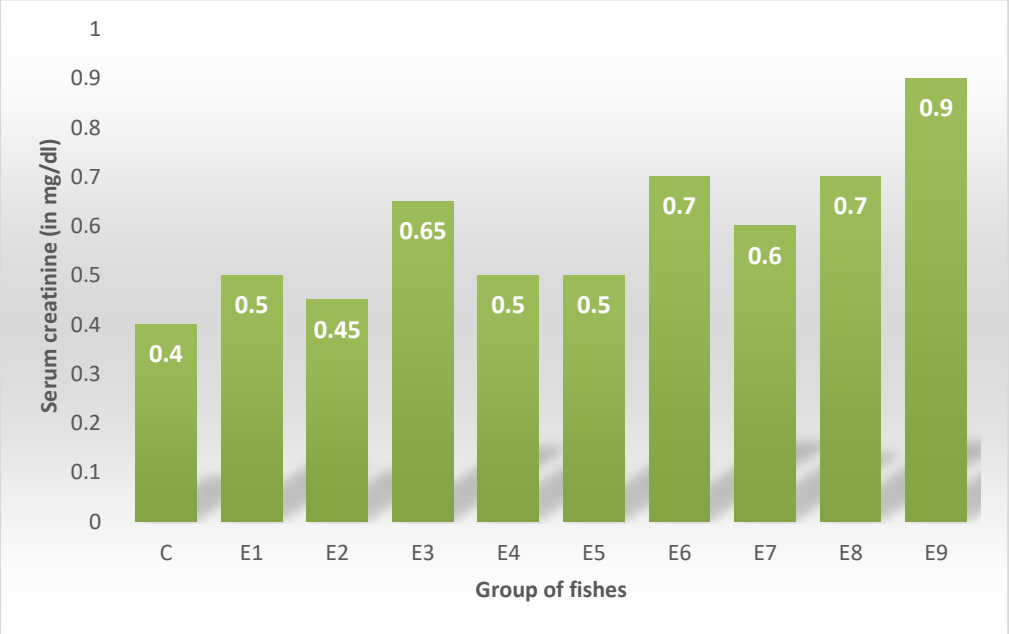

Fig.1: Histogram showing serum creatinine fluctuation in control and endosulfan treated group of fishes

Joshi & Verma[7] reported a significant rise in blood urea nitrogen, creatinine and bilurubin in gasoline and gasoline-menthol exposed rats as compared with the control.

Jayashree *et al*.[8] reported a significant increase in the concentration of serum urea, serum creatinine and blood urea nitrogen in the group of broiler chicken fed on deltamethrin @ 100 mg/kg containing feed for six weeks as compared to control.

Saad *et al*. reported a similar characteristic fluctuation in serum albumin, creatinine and urea of Wistar albino rats after one week of exposure of cisplatin (single intravenous dose of 6 mg/kg b.w.). He found raised level of serum creatinine from 0.673±0.066 mg/dl to 2.99±0.10 mg/dl.

El-Sayed *et al*.[10] have shown similar significant increase in urea and creatinine level in the serum of male albino rats exposed to dimethoate, carbofuran and carbendazine pesticides.

IV. CONCLUSION

In view of above, it can be assumed that endosulfan may induce glomerulonephritis, and nephrotic syndromes leading to significant rise in the level of serum creatinine 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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