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Mercury and Selenium Induced Changes in Kidney Biochemistry of *Clarias batrachus*

R. Tiwari, Nageshwar Wast, Anis Siddiqui, S. Gaherwal and M.M. Prakash

Department of Zoology, Govt. Holkar Science College, Indore, India

Abstract: The aim of this study was to assess the short term toxic effect of Mercuric chloride (0.01 ppm) and Selenium (0.09 ppm) on RNA, protein, Alkaline phosphatase, Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) content in kidney of *Clarias batrachus* up to 96 hours. Fish exposed to mercuric chloride showed a decrease in RNA, protein, alkaline phosphatase, GOT and GPT content while those exposed to selenium showed an increase in RNA, protein and alkaline phosphatase but decrease in GOT and GPT content were noted after 96 hours. These results suggest that Mercuric chloride is an effective toxicant and may alter the kidney physiology of *Clarias batrachus* but Selenium normalize these parameters.

Key words: Mercuric Chloride · Selenium · Kidney Biochemistry · *Clarias Batrachus*

INTRODUCTION

Mercury is a mutagen and carcinogen, their toxicity depends upon the form of mercury, dose and route of ingestion and with the exposed organism's species, sex, age and general condition [1, 2]. Mercury has a high potential for bioaccumulation and biomagnifications, their biomagnified concentration reported in fish up to 100000 times the ambient water concentration [1, 3]. Mercury (II) or mercuric salts are much more common in the environment as compared to mercury (I) or mercurous salts. If these salts are soluble in water is considered toxic. Organomercury compounds, such as methyl or butyl mercury chloride are more toxic to aquatic plants than inorganic forms [4]. Inorganic mercury in aquatic systems can be converted to methylmercury by microorganisms [5-7].

Mercuric is released into the environment by several sources such as mining, sewage disposal, research laboratories, agriculture, fungicides and industrial operation where it is found in electrical equipment, paints and disinfects and is black listed by environmentalists [8, 9]. The toxicity of fish at the level of cyto-genetic mechanism by mercuric pollution at different lakes in Egypt was described by Siegel *et al*. [10] and Adel [11] and Mustafa *et al*. [12].

Selenium is an essential element but considered toxic depending on the ingested dose. If selenium level is less than 1 mg kg^{-1} in diet can result in deficiency but higher than 5 mg kg^{-1} can cause toxic effects [13, 14]. Se pollution occurs to the aquatic systems by high-salt drainage from agricultural irrigation [15], mining of high Se containing ores and coal power plant [16, 17]. Since a lot of literature have reported interactions or relationships between Se and Hg in aquatic species [18], so the aim of this study was to observed the short term toxic effect of Mercuric chloride and Selenium on RNA, protein, Alkaline phosphatase, Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) content in kidney of *Clarias batrachus* up to 96 hours.

MATERIALS AND METHODS

Healthy catfishes (*Clarias batrachus*) were collected from fish market and acclimatized to the lab condition for 7 days, during which they were regularly fed with soya meal and prawn powder.

Test Chemical: Mercuric c hloride and selenium powder (Ranbaxy fine chemical Ltd.) were used as test chemicals.

Experimental Design: In present study, experimental fishes were divided into two major groups-

- Control group
- Experimental group

Control Group: In this group, the fishes were kept in distilled water. The experimental water was dechlorinated with pH 7 and temperature 25-30°C.

Corresponding Author: Nageshwar Wast, Department of Zoology, Govt. Holkar Science College, Indore, India.

Experimental Groups: In this group experimental fishes (40) were divided into two sets.

Set A: 20 fish were exposed to sublethal dose of $HgCl_2$ (0.01 mg/l).

Set B: 20 fishes were exposed to sublethal dose of Selenium (0.09 mg/l).

Autopsy: Fishes of control and treated groups were sacrificed at 0, 6, 24, 48, 72, 96 hours.

Biochemical Analysis: The kidney were removed, blotted, weighed and processed for various biochemical tests.

Following standard biochemical methods were used, which are described in the manual of biochemical tests and experiments [19].

- Extraction and Estimation of RNA by Orcinol method.
- Estimation of total protein by Biuret method.
- Determination of alkaline phosphatase by King and King Method
- Estimation of glutamate oxaloacetate transaminase enzyme by Reitman and Frankles' method
- Estimation of glutamate pyruvate transaminase by Reitman and Frankles' Method.

RESULTS

The results obtained from this study are summarized in Tables 1 to 10 and presented by Figs. 1 to 10. The total content of RNA, protein, alkaline phosphatase, GOT, GPT in kidney of *Clarias batrachus* were found to be decrease after the exposure to 0.01 ppm mercuric chloride up to 96 hours but on exposure of 0.09 ppm concentration of selenium, the RNA, protein, alkaline phosphatase content were found to be increase with fluctuation whereas decreases in GOT and GPT content were observed.

The normal value of RNA content in the control animal was 1.64 mg/g weight of kidney which were reduced to 19.51%, 50.60%, 79.26%, 82.31% and 89.50% respectively on 6, 24, 48, 72, 96 hours of exposure to 0.01 ppm of mercuric chloride (Table 1 and Fig. 1) but these values were slightly increase (0.60%) after 96 hours of exposure to 0.09 ppm of selenium (Table 6 and Fig. 6).

The total protein content in control animal was 1.5 mg/g weight of kidney which were reduced to 93.3% after exposure to 0.01 ppm of mercuric chloride up to 96 hours (Table 2 and Fig. 2) but these values were increased up to 313.33% with slight fluctuation after 96 hours of exposure of 0.09 ppm of selenium (Table 7 and Fig. 7).

Table 1: RNA changes in the kidney of *Clarias batrachus* exposed to 0.01 ppm of Mercuric chloride

		RNA content in µg/mg wt. of tissue				
S. No.	Exposure duration in hrs	Control (Mean± SD)	Experimental ((Mean± SD)	Difference	% Alter	t-value
1.	0	1.64 ± 0.04	1.64 ± 0.03	0	0	0.000
2.	6	1.64 ± 0.04	1.32 ± 0.02	0.32	19.51	16.000
3.	24	1.64 ± 0.04	0.81 ± 0.03	0.83	50.60	20.537
4.	48	1.64 ± 0.04	0.34 ± 0.03	1.3	79.26	225.166
5.	72	1.64 ± 0.04	0.29 ± 0.045	1.35	82.31	233.826
6.	96	1.64 ± 0.04	0.18 ± 0.02	1.46	89.50	121.756

Table 2: Total protein changes in the kidney of *Clarias batrachus* exposed to 0.01 ppm of Mercuric chloride

		Protein content in µg/mg wt. of tissue				
S. No.	Exposure duration in hrs	Control(Mean± SD)	Experimental (Mean± SD)	Difference	% Alter	t-value
1.	0	1.5±0.1	1.5±0.0	0	0	1.0000
2.	6	1.5±0.1	0.6±0.1	0.9	60	9.0000
3.	24	1.5±0.1	0.4±0.1	1.1	73.3	11.0000
4.	48	1.5±0.1	0.3±0.0	1.2	80	20.7846
5.	72	1.5±0.1	0.2±0.1	1.3	86.6	22.5167
6.	96	1.5±0.1	0.1±0.0	1.4	93.3	24.2487

Table 3: Alkaline phosphatase changes in the kidney of *Clarias batrachus* exposed to 0.01 ppm of Mercuric chloride

S. No.	Exposure duration in hrs	Alkaline phosphatase content in µg/mg wt. of tissue		Difference	% Alter	t- value
		Control (Mean± SD)	Experimental (Mean± SD)			
1.	0	188 ± 4.58	188 ±4.58	0	0	0.000
2.	6	188 ± 4.58	181 ±3.06	7	3.72	1.734
3.	24	188 ± 4.58	50 ± 3.61	138	73.40	119.511
4.	48	188 ± 4.58	32 ± 4.04	156	82.97	467.000
5.	72	188 ± 4.58	30 ± 2.08	158	80.04	89.388
6.	96	188 ± 4.58	32 ± 3.00	156	82.97	156.000

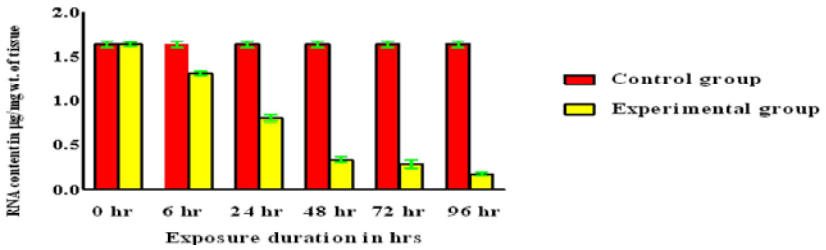

Fig. 1: RNA changes in kidney of *Clarias batrachus* exposed to 0.01 ppm of mercuric chloride

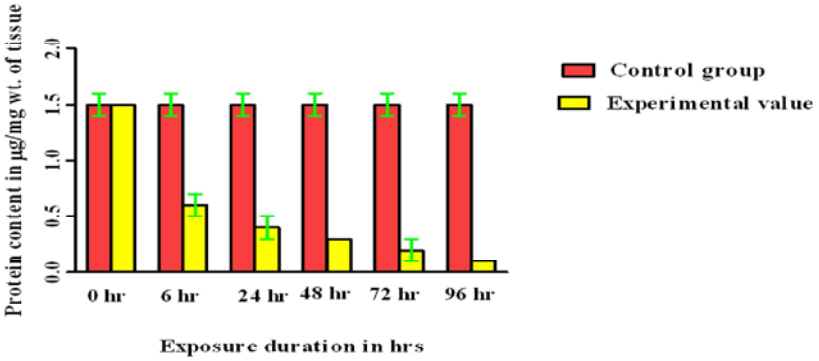

Fig. 2: protein changes in kidney of *Clarias batrachus* exposed to 0.01 ppm of mercuric chloride

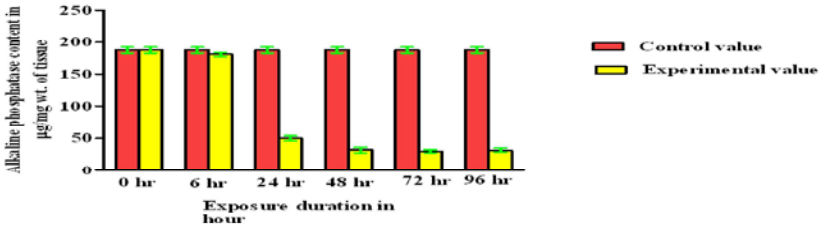

Fig. 3: Changes of alkaline phosphatase in kidney of *Clarias batrachus* exposed to 0.01 ppm of mercuric chloride

The control value of alkaline phosphates content was 188 µg/mg wt. o f tissue which were reduced into 3.72%, 73.40%, 82.97%, 80.04% and 82.97% respectively on 6, 24, 48, 72 and 96 hours of exposure to 0.01 ppm of mercuric chloride (Table 3 and Fig. 3) whereas these values increased to 19.68%, 69.68%, 73.93%, 84.57% and 31.91% respectively on 6, 24, 48, 72 and 96 hours of exposure of 0.09 ppm of selenium (Table 8 and Fig. 8).

The total content of GOT and GPT in the kidney of *Clarias batrachus* was decreased gradually after exposure of both, 0.01 ppm conc. of mercuric chloride and 0.09 ppm conc. of selenium. GOT values were reduced to 59.09 % (Table 4 and Fig. 4) and 43.18% (Table 9 and Fig. 9) respectively for 96 hours as compared to the control animal (88µg/mg wt. of tissue) while GPT values were reduced to 83.68 %

Table 4: GOT content in the kidney of *Clarias batrachus* exposed to 0.01 ppm of Mercuric chloride

		GOT content in µg/mg wt. of tissue				
S. No.	Exposure duration in hrs	Control (Mean± SD)	Experimental (Mean± SD)	Difference	% Alter	t- value
1.	0	88 ±2.65	88 ±2.00	0	0	0.0000
2.	6	88 ±2.65	76 ±1.00	12	13.63	12.0000
3.	24	88 ±2.65	60 ±2.08	28	31.81	21.2500
4.	48	88 ±2.65	54 ±1.73	34	38.63	58.8897
5.	72	88 ±2.65	37 ±2.08	51	57.95	152.000
6.	96	88 ±2.65	36 ±2.52	52	59.09	77.5000

Table 5: GPT content in the kidney of *Clarias batrachus* exposed to 0.01 ppm of Mercuric chloride

		GPT content in µg/mg wt. of tissue				
S. No.	Exposure duration in hrs	Control (Mean± SD)	Experimental (Mean± SD)	Difference	% Alter	t-value
1.	0	239 ±1.73	239 ±2.65	0	0	0.0000
2.	6	239 ±1.73	151 ±2.08	88	36.82	99.4047
3.	24	239 ±1.73	54 ± 2.00	185	77.40	121.1109
4.	48	239 ±1.73	32 ±2.65	207	86.61	135.5133
5.	72	239 ±1.73	35 ±3.61	204	85.35	176.6692
6.	96	239 ±1.73	39 ±2.00	200	83.68	346.4102

Table 6: RNA changes in the kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

		RNA content in µg/mg wt. of tissue				
S. No.	Exposure duration in hrs	Control (Mean± SD)	Experimental (Mean± SD)	Difference	% Alter	t- value
1.	0	1.64 ±0.0300	1.64 ±0.0265	0	0	0.0000
2.	6	1.64 ±0.0300	1.64 ±0.0200	0	0	0.0000
3.	24	1.64 ±0.0300	1.65 ±0.0252	0.01	0.60	5.0000
4.	48	1.64 ±0.0300	1.64 ±0.0100	0	0	0.0000
5.	72	1.64 ±0.0300	1.64 ±0.0265	0	0	0.0000
6.	96	1.64 ±0.0300	1.65 ±0.0200	0.01	0.60	1.7321

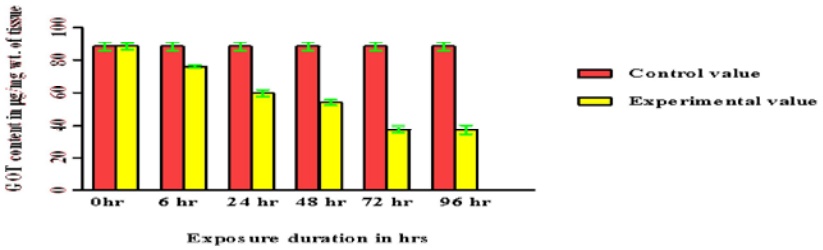

Fig. 4: Changes of GOT in kidney of *Clarias batrachus* exposed to 0.01 ppm of mercuric chloride

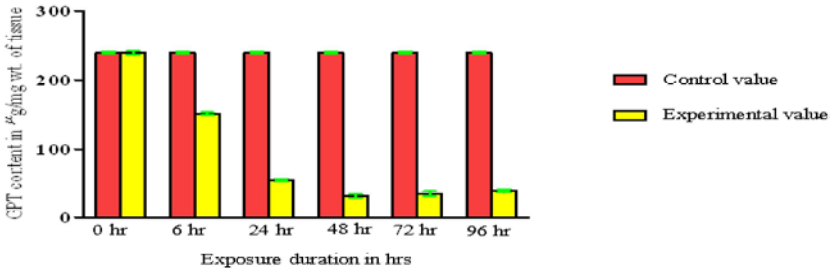

Fig. 5: Changes of GPT in kidney of *Clarias batrachus* exposed to 0.01 ppm of mercuric chloride

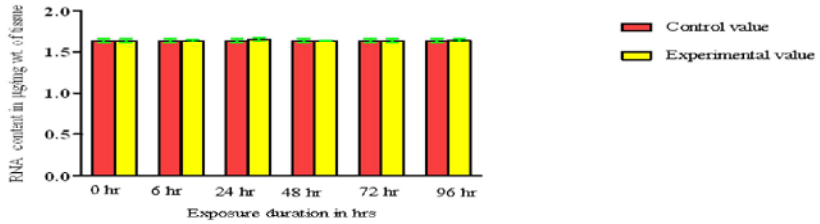

Fig. 6: Changes of RNA in kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

Table 7: Protein content in the kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

		Protein content in µg/mg wt. of tissue				
S. No.	Exposure duration in hrs	Control (Mean± SD)	Experimental (Mean± SD)	Difference	% Alter	t- value
1.	0	1.50 ±0.2646	1.50 ±0.2000	0	0	0.0000
2.	6	1.50 ±0.2646	1.00 ±0.0000	0.50	33.33	3.2733
3.	24	1.50 ±0.2646	0.40 ±0.1000	1.10	73.33	11.0000
4.	48	1.50 ±0.2646	0.40 ±0.0000	1.10	73.33	7.2012
5.	72	1.50 ±0.2646	0.40 ±0.1732	1.10	73.33	4.3710
6.	96	1.50 ±0.2646	6.20 ±0.4000	4.70	313.33	47.0000

Table 8: Alkaline phosphatase content in the kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

		Alkaline phosphatase content in µg/mg wt. of tissue				
No.	Exposure duration in hrs	Control (Mean± SD)	Experimental (Mean± SD)	Difference	% Alter	t-value
1.	0	188 ±3.61	188 ±1.00	0	0	0.0000
2.	6	188 ±3.61	151 ±2.00	37	19.68	37.0000
3.	24	188 ±3.61	57 ± 2.65	131	69.68	226.8987
4.	48	188 ±3.61	49 ±1.73	139	73.93	45.4984
5.	72	188 ±3.61	29 ±1.00	159	84.57	63.1802
6.	96	188 ±3.61	248 ±2.52	60	31.91	90.5000

Table 9: GOT content in the kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

		GOT content in µg/mg wt. of tissue				
S. No.	Exposure duration in hrs	Control (Mean± SD)	Experimental (Mean± SD)	Difference	% Alter	t-value
1.	0	88 ±2.00	88 ±1.00	0	0	0.0000
2.	6	88 ±2.00	45 ±3.00	43	48.86	74.4782
3.	24	88 ±2.00	40 ±1.53	48	54.54	143.0000
4.	48	88 ±2.00	58 ±3.00	30	30.09	51.9615
5.	72	88 ±2.00	25 ±0.58	63	71.59	71.0573
6.	96	88 ±2.00	50 ±3.00	38	43.18	13.1636

Table 10: GPT content in the kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

		GPT content in µg/mg wt. of tissue				
S. No.	Exposure duration in hrs	Control (Mean± SD)	Experimental (Mean± SD)	Difference	% Alter	t-value
1.	0	239 ±2.00	239 ±1.73	0	0	0.0000
2.	6	239 ±2.00	122 ±3.51	117	48.95	133.0435
3.	24	239 ±2.00	122 ±1.73	117	48.95	202.6499
4.	48	239 ±2.00	110 ±2.65	129	53.97	223.4346
5.	72	239 ±2.00	96 ± 1.00	143	59.83	247.6833
6.	96	239 ±2.00	68 ±1.53	171	71.54	512.0000

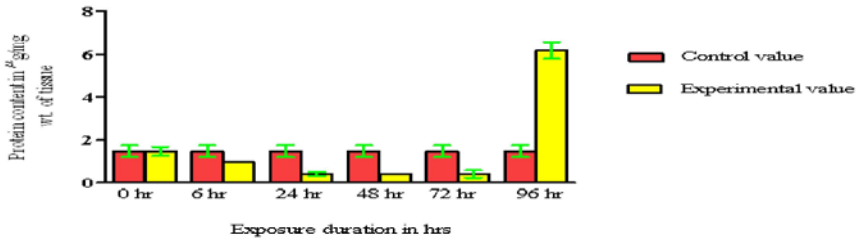

Fig. 7: Changes of Protein content in kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

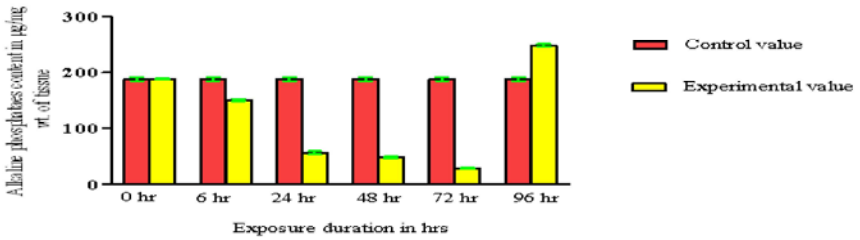

Fig. 8: Changes of Alkaline phosphatase in kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

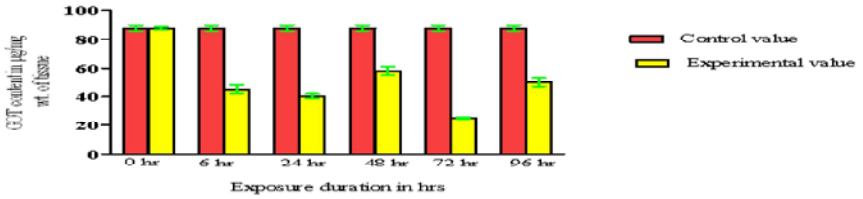

Fig 9: Changes of GOT in kidey of *Clarias batrachus* exposed to 0.09 ppm of selenium

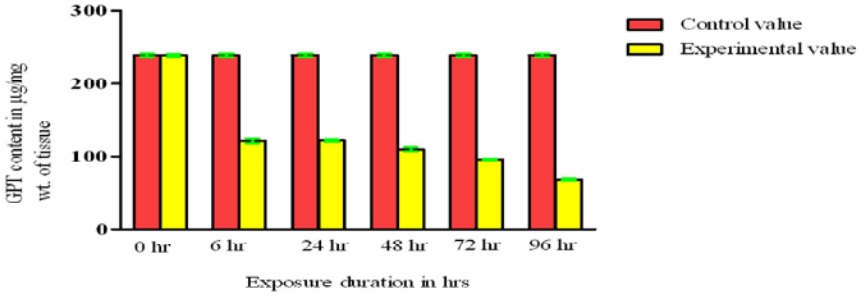

Fig. 10: GPT changes in kidney of *Clarias batrachus* exposed to 0.09 ppm of selenium

(Table 5 and Fig. 5) and 71.54 % (Table 10 and Fig. 10) respectively for 96 hours as compared to the control fish (239µg/mg wt. of tissue).

DISCUSSION

The protective effect of selenium and mercury toxicity has been already known [20 and 21] and both are correlated in tissues [22-25]. In animals of aquatic systems, such as crayfish [26], benthic organism oligochaeta [27] and largemouth bass [28].

Selenium concentrations in the food approaching natural background levels increase the elimination of methyl mercury from fish. Thus, selenium levels in a given aquatic food chain may affect mercury contamination along the food chain [29]. The variation of the molar ratios of selenium: mercury in freshwater fish from Tennessee at Poplar Creek that might affect the protectiveness of selenium against mercury toxicity [30].

According to Sormo [31], Hg in molar excess over Se was a stronger inducer of MT synthesis than tissue Hg levels in the trout, supporting the assumption that selenium has a prominent protective effect against Hg toxicity. Arribere [32] have studied the (Hg) and selenium (Se) contents in two native species, catfish (*Diplomystes viedmensis*) and creole perch (*Percichthys trucha*) and three introduced species, brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and brook trout

(*Salvelinus fontinalis*) and observed the Se concentrations in the liver of brown and rainbow trout, up to 279 μg^{-1} DW, are higher than those expected for nearly pristine lakes, exceeding 20 μg^{-1} DW, the threshold concentration associated with Se toxicity. These species show lower Hg contents in muscle, suggesting a possible detoxification of Hg by a Se-rich diet.

Analysis of whole body Se and Hg concentrations in 468 fish showed that 97.5% of the freshwater fish have sufficient Se to potentially protect them and their consumers against Hg toxicity suggests that Se in fish tissue (Se:Hg molar ratio) must be considered when assessing the potential toxic effects of Hg [33]. High glucose glucose concentrations were reported on exposure of mercury in fishes [34]. According to Arya [35], glucose, triglyceride, Serum SGPT, total protein and cholesterol levels were significantly altered in fish exposed to Hg or Cd salt alone. However, these parameters were normalized by combined exposure of Hg or Cd.

Since in our experiment, decrease in RNA, protein, alkaline phosphatase, GOT and GPT content were noted in kidney of *Clarias batrachus* after the exposure of 0.01 ppm mercuric chloride but increase in the RNA, protein, alkaline phosphatase content and decreases in GOT and GPT content were observed after exposure to 0.09 ppm concentration of selenium up to 96 hours suggest the protectiveness of selenium against mercury toxicity and support the finding of previous author.

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