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Biochemical changes in Kidney and Liver of *Clarias batrachus* induced by cypermethrin

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Abstract
The objective of this study was to observe the effect of Synthetic Pyrethroid (Cypermethrin) on the biochemistry of kidney and liver of *Clarias batrachus.* The biochemical parameters studies were RNA, Total protein, acid phosphatase, alkaline phosphatase, SGPT, SGOT and blood glucose. In kidney the RNA and protein content decreased up to 65.37, 72.22 percent and ACP, ALP increased upto 48.76 and 33.17 percent respectively. In liver the RNA and protein content decreased up to 92.55, 86.53 percent and ACP, ALP increased upto 25.82 and 11.60 percent respectively. The SGPT, SGOT and BLOOD GLUCOSE also increased up to 55.28, 12.70 and 46.33 percent. Thus on the basis of obtain result in the present investigation it can be concluded that 96 hrs. exposure of 80 ppm of Cypermethrin aqueous solution has toxic effect and alter the biochemistry of kidney and liver. Therefore, it is recommended to the user of this pesticide that should be careful about the dose they are using.

Keywords: biochemistry, cypermethrin, *Clarias batrachus*, synthetic pyrethroid, toxic, kidney, liver

1. Introduction
The pesticides are complex chemical compounds which are extensively used to kill the pest in agriculture and forestry in order to enhance the productivity level. The term 'pesticides' encompasses all chemical materials used for the control of pests. The pesticides chlorinated hydrocarbons and organophosphate are generally used in agriculture crop protection programmed. These pesticides are most toxic and stable chemicals which are not metabolized, degraded or excreted to any degree. The mutagenic and carcinogenic action of certain herbicides, insecticides and fungicides on experimental animals is well known and several studies have shown that chronic exposure to low levels of pesticides can also cause mutations and/ or carcinogenicity (Bull *et al.*, 2006; Karabay and Gunnehir, 2005) [4, 9].

Many new broad-spectrum pesticides have been developed in current decade that have the potential for widespread use in the environment. According to Environment Agency (1997) [7], the Large-scale usages of pesticides (by methods such as forest spraying, orchard, crop dusting or mosquito control) entered them into the aquatic environment.

Cypermethrin is used extensively in households, industrial and agriculture fields (Kakko *et al.*, 2003) [3] for control of several insect pests which is a synthetic pyrethroid broad-spectrum insecticide, (Yilmaz *et al.*, 2004) [18].

Cypermethrin affects the several target aquatic organisms and thus adversely affects fish, hematology due to its indiscriminate use and through agriculture run-off their entrance into natural water bodies (Adhikari *et al.*, 2004) [1], metabolism (Polat *et al.*, 2002) [15], meat quality and fish population (Cullen and Connell, 1992) [5]. According to Saha and Kaviraj (2008) [16] cypermethrin is considered as immobile and not expected to biomagnifies through food chain. The signal word "Danger" or "Caution" on the product label must bear the pesticide containing cypermethrin, depending on the content of the particular formulation (Meister; 1992) [13].

Therefore, the present experimental program was undertaken to study the Biochemical changes in kidney and Liver of *Clarias batrachus* induced by cypermethrin.

2. Material and methodology
Experimental animal
Healthy *Clarias batrachus* were used as an experimental animal and it is was collected from local fish market & acclimatized to the laboratory for one week during which they were regularly feed with prawn powder & soya meal.

Test chemical
Cypermethrin was used as a test chemical. Test fishes were exposed to sub-lethal doses (80ul/l) for maximum 96 hrs.

Experimental design
In the present investigation experimental fishes were divided into two groups.

1. Control group: - In this group 10 fishes were kept and exposed to normal water.
2. Experimental group: - In this group 40 fishes were exposed to 80 µl concentration of cypermethrin solution.

Experimental duration
In both control and experimental group fishes were exposed to maximum 96 hrs.

Autopsy
Fishes of control and experimental groups were sacrificed at 0 hrs. 24 hrs, 48hrs, 72 hrs and 96 hrs. The kidney and Liver was blotted weighted and Blood collected by cardiac puncture of *Clarias batrachus* then processed for various biochemical tests.

Biochemical analysis

Following standard biochemical methods were used, which are described in Manual of biochemical tests and experiments (2007)

1) Extraction and estimation of RNA by Orcinol method.
2) Extraction and estimation of Total protein by Biuret method.
3) Determination of alkaline phosphatase (ALP) by King and King Method.
4) Determination of acid phosphatase (ACP) by King and King Method.
5) Determination of Serum Glutamate Oxaloacetate Transaminase (Manual of biochemical tests and experiments 2007)
6) Determination of Serum Glutamate Pyruvate Transaminase (Manual of biochemical tests and experiments 2007)
7) Estimation of Total Blood Glucose (Folin and Wu method)

3. Results and Discussion

3.1 RNA in kidney

RNA of the Kidney of experimental fishes was observed in decreasing manner as the duration of exposure of experimental solution increases. The decreases in RNA content after 96 hrs. Exposure of Cypermethrin (80ul/l) was 65.37 percent. The decrease in RNA concentration after 24, 48 and 72 hrs. Were 11.27, 36.68 and 56.35 percent respectively.

Table 1: RNA in the Kidney of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration.

S. No.	Exposure Duration in Hrs	RNA in mg/g wt. of tissue		Difference	% Alter
		Control	Experimental		
1	0	44.24	44.24	0	0
2	24	44.24	39.25	4.99	-11.27
3	48	44.24	28.01	16.23	-36.68
4	72	44.24	19.31	24.93	-56.35
5	96	44.24	15.32	28.92	-65.37

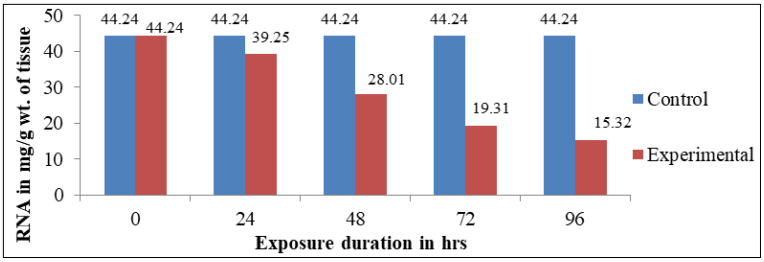

Fig 1: Showing the effect of cypermethrin in the RNA content of kidney of *C. batrachus*

3.2 Total Protein in kidney (TP)

The quantity of total protein in kidney of experimental fishes was found decreased in Cypermethrin treated group. The decrease in total protein concentration in Kidney of experimental fishes after 96 hrs. Was 72.22 percent. The decrease in protein value of Kidney after 24, 48 and 72 hrs. Were 19.44, 44.40 and 63.88 percent respectively.

Table 2: Total Protein in the Kidney of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	Protein in mg/g wt. of tissue		Difference	% Alter
		Control	Experimental		
1	0	3.60	3.60	0	0
2	24	3.60	2.90	0.7	-19.44
3	48	3.60	2.00	1.6	-44.4
4	72	3.60	1.30	2.3	-63.88
5	96	3.60	1.00	2.6	-72.22

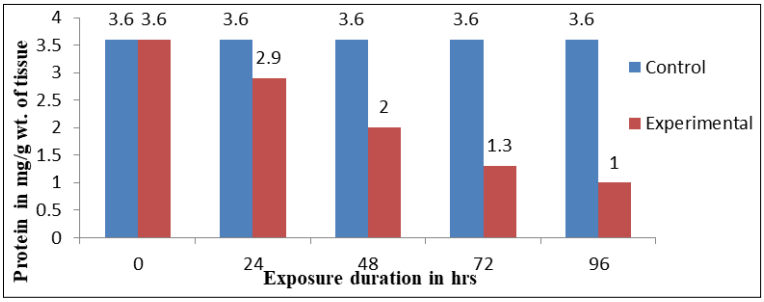

Fig 2: Showing the effect of cypermethrin in the Total Protein of kidney of *C. batrachus*

3.3 Alkaline Phosphatase in kidney (ALP)

The alkaline phosphatase activity was found increased as the duration of exposure of Cypermethrin increased. The increased in alkaline phosphatase after 24, 48, 72 and 96 hrs. of exposure of Cypermethrin was 8.09, 19.06, 25.00 and 33.13 percent respectively.

Table 3: Alkaline Phosphatase in the Kidney of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	Alkaline phosphatase activity in KA units/100 ml.		Difference	% Alter
		Control	Experimental		
1	0	24.08	24.08	0	0
2	24	24.08	26.03	1.95	+8.09
3	48	24.08	28.67	4.59	+19.06
4	72	24.08	30.10	6.02	+25.00
5	96	24.08	32.06	7.98	+33.13

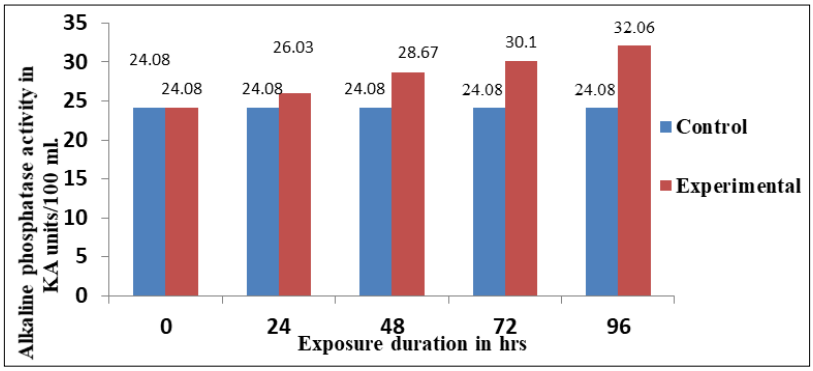

Fig 3: Showing the effect of cypermethrin in the Alkaline phosphatase activity of kidney of *C. batrachus*

3.4 Acid Phosphatase in kidney (ACP)

The Acid phosphatase activity was found increases as the duration of exposure of Cypermethrin increases. The total inhibition in acid phosphatase activity after 96 hrs. exposure of Cypermethrin was 48.76 percent. The inecrease in acid phosphatase after 24, 48, and 72 hrs. of exposure of Cypermethrin were 9.35, 18.89 and 41.83 percent respectively.

Table 4: Acid Phosphatase in the Kidney of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	Acid phosphatase activity in KA units/100 ml.		Difference	% Alter
		Control	Experimental		
1	0	21.06	21.06	0	0
2	24	21.06	23.03	1.97	+9.35
3	48	21.06	25.04	3.98	+18.89
4	72	21.06	29.87	8.81	+41.83
5	96	21.06	31.33	10.27	+48.76

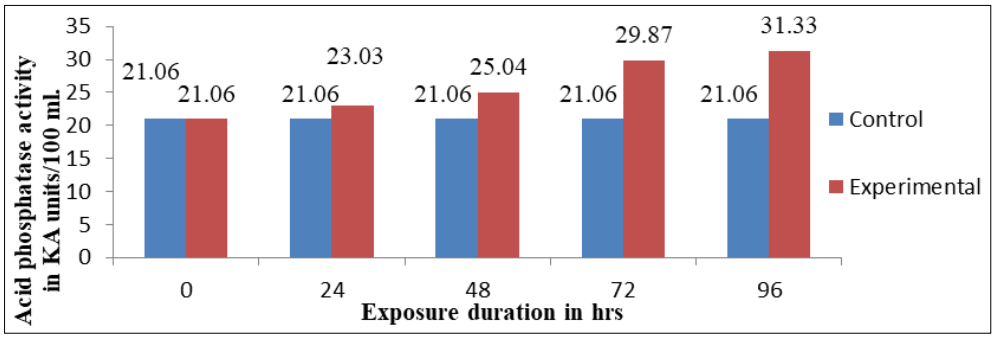

Fig 4: Showing the effect of cypermethrin in the Acid phosphatase activity of kidney of *C. batrachus*

3.5 RNA in liver

RNA of the Liver of experimental fishes was observed in decreasing manner as the duration of exposure of experimental solution increases. The decreases in RNA content after 96 hrs. exposure of Cypermethrin (80ul/l) was 92.55 percent. The decrease in RNA content after 24, 48 and 72 hrs. were 32.27, 53.37 and 72.27 percent respectively.

Table 5: RNA in the Liver of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	RNA in mg/g wt. of tissue		Difference	% Alter
		Control	Experimental		
1	0	7.25	7.25	0	0
2	24	7.25	4.91	2.34	-32.27
3	48	7.25	3.38	3.87	-53.37
4	72	7.25	2.01	5.24	-72.27
5	96	7.25	0.54	6.71	-92.55

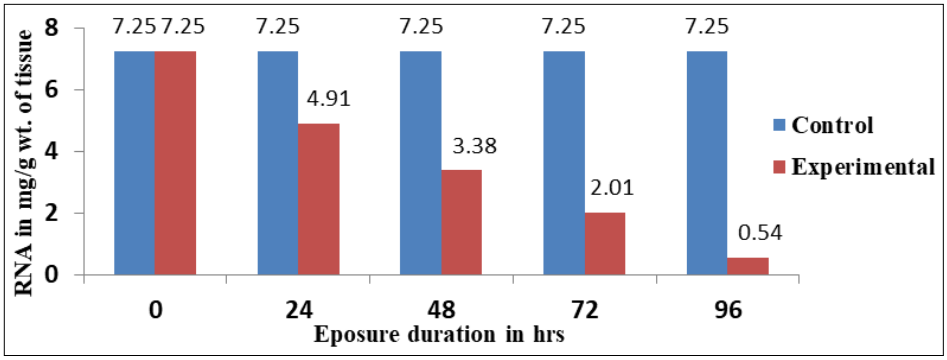

Fig 5: Showing the effect of cypermethrin in the RNA content of liver of *C. batrachus*

3.6 Total Protein in Liver (TP)

The quantity of total protein in Liver of experimental fishes was found decreased in Cypermethrin treated group. The decrease in total protein concentration in Liver of experimental fishes after 96 hrs. was 86.53 percent. The decrease in protein value of liver after 24, 48 and 72 hrs. were 7.05,61.50 and 83.01 percent respectively.

Table 6: Total Protein in the Liver of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	Protein in mg/g wt. of tissue		Difference	% Alter
		Control	Experimental		
1	0	3.12	3.12	0	0
2	24	3.12	2.90	0.22	+7.05
3	48	3.12	1.20	1.92	+61.50
4	72	3.12	0.53	2.59	+83.01
5	96	3.12	0.42	2.70	+86.53

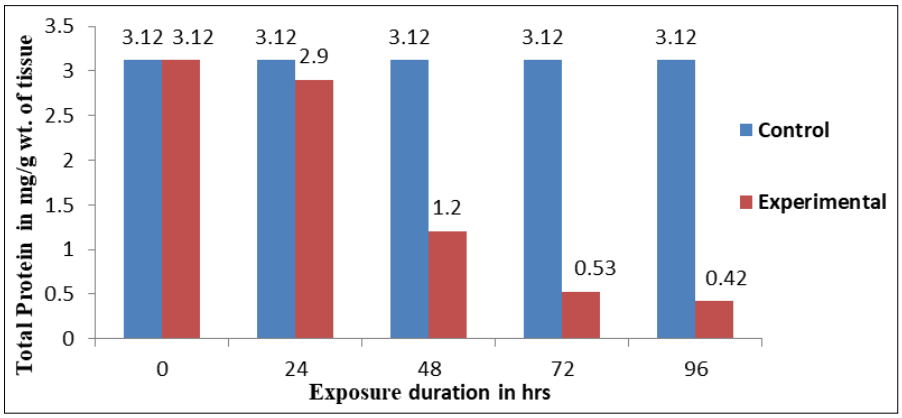

Fig 6: Showing the effect of cypermethrin in the Total Protein of liver of *C. batrachus*

3.7 Alkaline Phosphatase in Liver (ALP)

The alkaline phosphatase activity was found increased as the duration of exposure of Cypermethrin increased. The increased in alkaline phosphatase after 24, 48, 72 and 96 hrs. of exposure of Cypermethrin was 3.52, 6.66, 7.56 and 11.60 percent respectively.

Table 7: Alkaline Phosphatase in the Liver of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	Alkaline phosphatase activity in KA units/100 ml.		Difference	% Alter
		Control	Experimental		
1	0	45.8	45.80	0	0
2	24	45.8	46.67	1.59	+3.52
3	48	45.8	48.06	2.98	+6.66
4	72	45.8	48.49	3.41	+7.56
5	96	45.8	50.33	5.25	+11.6

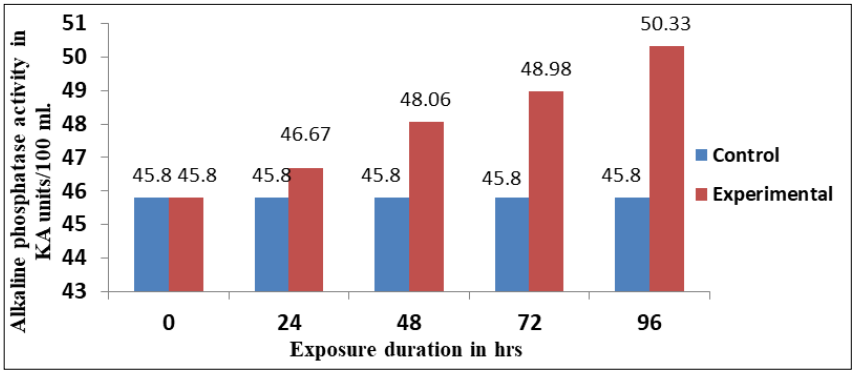

Fig 7: Alkaline Phosphatase in the Liver of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

3.8 Acid Phosphatase in Liver (ACP)

The Acid phosphatase activity was found increases as the duration of exposure of Cypermethrin increases. The total inhibition in acid phosphatase activity after 96 hrs. exposure of Cypermethrin was 25.82 percent. The increase in acid phosphatase after 24, 48, and 72 hrs. of exposure of Cypermethrin were 5.0, 11.80 and 15.10 , percent respectively.

Table 8: Acid Phosphatase in the Liver of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	Acid phosphatase activity in KA units/100 ml.		Difference	% Alter
		Control	Experimental		
1	0	19.4	19.40	0	0
2	24	19.4	20.01	0.97	+5
3	48	19.4	21.34	2.30	+11.8
4	72	19.4	22.33	2.93	+15.10
5	96	19.4	24.05	5.01	+25.82

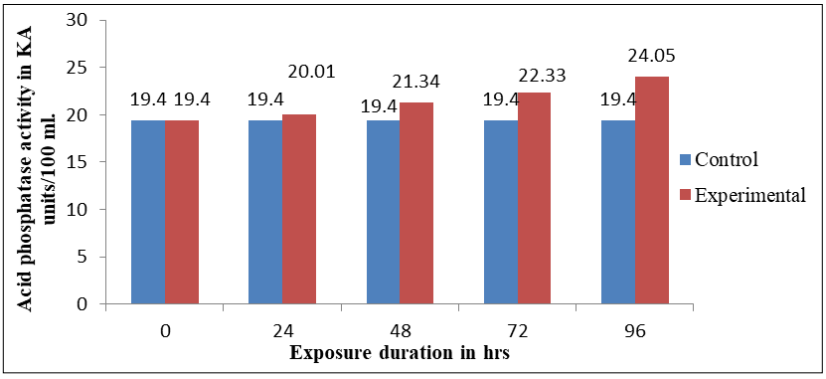

Fig 8: Acid Phosphatase in the Liver of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

3.9 SGPT activity

The SGPT activity was found increased as the duration of exposure of Cypermethrin increased. The increased in SGPT after 24, 48, 72 and 96 hrs. of exposure of Cypermethrin was 20.53, 30.79, 38.33 and 55.28 percent respectively.

Table 9: SGPT activity of *C.batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	SGPT activity in u moles /lit		Difference	% Alter
		Control	Experimental		
1	0	32.63	32.63	0	0
2	24	32.63	39.30	6.67	+20.53
3	48	32.63	42.68	10.05	+30.79
4	72	32.63	45.14	12.51	+38.33
5	96	32.63	50.67	18.04	+55.28

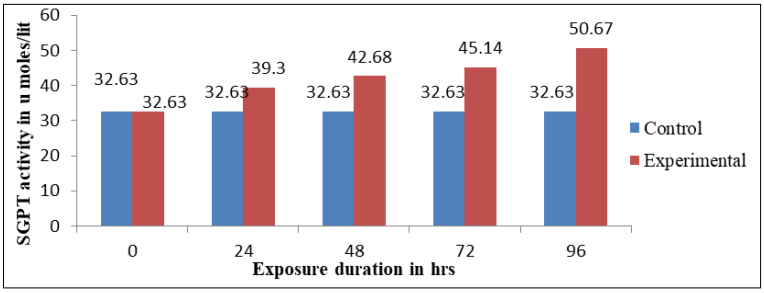

Fig 9: Showing the effect of cypermethrin in the SGPT activity of *C. batrachus*

3.10 SGOT activity

The SGOT activity was found increased as the duration of exposure of Cypermethrin increased. The increased in SGOT after 24, 48, 72 and 96 hrs. of exposure of Cypermethrin was 3.57, 6.48, 11.33 and 12.70 percent respectively.

Table 10: SGOT activity of *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	SGOT activity in u moles /lit		Difference	% Alter
		Control	Experimental		
1	0	150.30	150.30	0	0
2	24	150.30	155.67	5.37	+3.57
3	48	150.30	160.04	9.74	+6.48
4	72	150.30	167.33	17.03	+11.33
5	96	150.30	170.20	19.09	+12.70

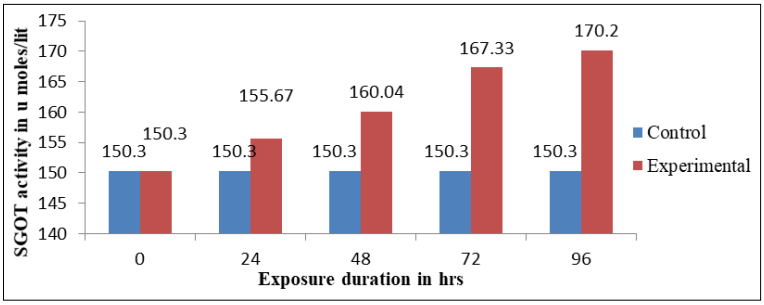

Fig 10: Showing the effect of cypermethrin in the SGOT activity of *C. batrachus*

3.11 Total Blood Glucose

The blood glucose level was found increased as the duration of exposure of Cypermethrin increased. The increased in level of blood glucose after 24, 48, 72 and 96 hrs. of exposure of Cypermethrin was 15.3, 39.67, 42.47 and 46.33 percent respectively.

Table 11: Blood glucose level in *C. batrachus* exposed to Cypermethrin (80 ul/l) for different duration

S. No.	Exposure Duration in Hrs	Blood glucose level in mg / 1lit		Difference	% Alter
		Control	Experimental		
1	0	70.40	70.40	0	0
2	24	70.40	81.20	10.8	+15.34
3	48	70.40	98.33	27.93	+39.67
4	72	70.40	100.30	29.90	+42.47
5	96	70.40	105.02	34.62	+46.33

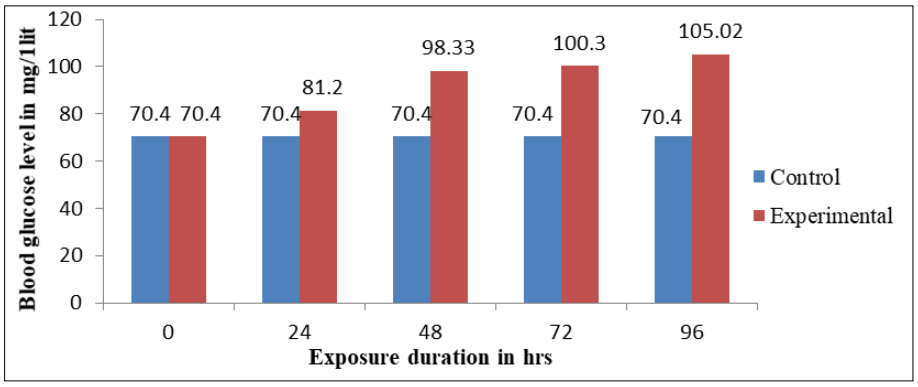

Fig 11: Showing the effect of cypermethrin in the blood glucose level of *C. batrachus*

In kidney the RNA and protein content decreased up to 65.37, 72.22 percent and ACP, ALP increased up to 48.76 and 33.17 percent respectively. In liver the RNA and protein content decreased up to 92.55, 86.53 percent and ACP, ALP increased up to 25.82 and 11.60 percent respectively. The SGPT, SGOT and BLOOD GLUCOSE also increased up to 55.28, 12.70 and 46.33 percent. Bulow *et al*. (1970) [3] and Brachet *et al.* (1955) [2] reported reduction in RNA content of brain, liver, and muscles. One of the specific reasons for these alteration in RNA contents of different organs might also be due to variation in RNA polymerase activity, as observed by Holbrook. (Holbrook *et al.*, 1980) [11]. The decrease in protein was significant in liver and kidney may be because these organs are more active and require large amount of energy. It also appears that vigorous struggling may enhance kidney activity which may probably contribute to protein degradation that is proteolysis. Decreased in protein level may be attributed to impaired synthetic machinery due to cypermethrin effect (David *et al*., 2004) [6]. ALP is a microsomal enzyme, which is involved in membrane transport because of its high concentration in vertebrate kidney and its action on a number of phosphomonoesters of organic materials such as glucose (Edquist *et al.*, 1992) [10]. The treatment of the fish with cypermethrin increase in the activities of transaminases (SGOT and SGPT), phosphatases (acid and alkaline) in the fish serum. Serum Glutamate Oxaloacetate Transaminase (SGOT) is a mitochondrial enzyme whereas SGPT is a cytosolic enzyme. Their rise in the serum level is the testimony of the hepatic insufficiency consequent upon the cellular damage observed in course of histological examination. This is in agreement with the findings of Patil and Radhakrishnamurty (1979) [14] who observed that these enzymes are enhanced when there is inflamation, degeneration and neoplastic lesions in liver. The increase in glucose level (hyperglycemia) in the blood of experimental fish can be assigned to intensive glycogenolysis and synthesis of glucose from extra hepatic tissue proteins and amino acids (Vinodhini and Narayana, 2009) [17]. It is also shown that the toxic stress could induce glycolysis resulting in higher glucose level in the blood of fish (Hog, 2014) [12]. These above observations are similar with our results.

In the present investigation cypermethrin (80ul/l) exposure for 96 hrs to *Clarias batrachus* was found toxic as it altered rather increased the ALP, ACP, SGPT, SGOT and blood glucose and decreased the studied biochemical parameter RNA and TP of kidney and liver and thus support the observation of previous authors. Therefore it is recommended to the user of this pyrethroid pesticide that they should be careful about the dose they are using.

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