Toxicity of Sumithion in Channa Punctatus: Biochemical and Hematological Studies

Mishra B. P[1,*], MPS Marwaha[2], Bhupinder Kaur Anand[3], Lingidi Jhansi Lakshmi[4], Badade Z. G[5]

[1]Associate Professor, [4]Tutor, Department of Biochemistry, Mayo Institute of Medical Sciences, Uttar Pradesh,
[2]Wg Cdr, Aeropace Medicine Specialist, Airfare Station, Ambala, Haryana,
[3]Professor, Department of Community Medicine, Career Institute of Medical Sciences, Lucknow, Uttar Pradesh,
[5]Professor, Department of Biochemistry, M.G.M. Medical College, Navi Mumbai, India

***Corresponding Author:**
Email: bpmishra_72@yahoo.com, bpmishra_72@rediffmail.com

ABSTRACT

Introduction: *Owing to their easy availability and accessibility, organophosphate and carbamates are the most commonly used pesticides worldwide in the pest control of crops. Acute toxicity and clinical manifestations of organophosphate pesticide are caused by excessive synaptic accumulation of acetylcholine (AChE).*

Objective: *To analyze the biochemical parameters of serum cholesterol and hematological parameter of absolute erythrocyte count in fish Channa Punctatus exposed under organophosphate pesticide sumithion.*

Materials and Methods: *This research study has been done in fish Channa Punctatus, which was exposed 24 to 96 hours to four different concentration of sumithion pesticide. Serum cholesterol was determined as biochemical biomarkers while absolute erythrocyte counts as hematological biomarker. Serum cholesterol was determined by zlatkis method (Zlatkis A; Zak B. and Boyale A. J. 1953)[15]. Absolute erythrocyte count was determined by electrical conductivity method of cell counting.*

Result: *There was significant increase in serum cholesterol in Sumithion pesticide exposed, fish Channa Punctatus, comparative to normal control subject. The maximum rise of 18.93% (80.74 ± 78.56) in serum cholesterol was observed after exposure of 96 hrs at 0.40mg/l Sumithion pesticide. Toxicity of Sumithion resulted in decrease of absolute erythrocyte counts at all concentration of time intervals. Decrease was more pronounced with increasing time of exposures. The lowest erythrocyte count was 57.24% below control at the highest concentration of 0.73mg/L within 24hrs.*

Conclusion: *Results of our study indicates that hypercholesterolemia & decreased level of erythrocyte count in fish channa punctatus exposed under sumithion pesticide are biochemical & hematological biomarker for human beings also. Therefore it is advised to pesticide workers to take all precautions regarding protection from pesticide exposure and proper use of prophylactic supplementation for their healthy life.*

Key Words: *OPP (Organophosphate Pesticide), Sumithion, Serum cholesterol, AEC (absolute erythrocyte count).*

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INTRODUCTION

The wide spread use of pesticides in agriculture and forestry conservation program has prompted the need for evaluation of the hazards of such materials to wildlife. Recent reports has emphasized that the probability of exposure exists within the indoor living space, as well as in the agriculture and industrial work place (russel & over street, 1987)[1]. Moreover, Reinert (1984)[2] reported that the indoor use of pesticides may create a different and more direct exposure situation. Owing to the extensive use of these chemicals, they are responsible for numerous cases of poisoning in human and non-target wild life. Organophosphate pesticides are most commonly used worldwide in the pest control of crops[3]. In agriculture organophosphates pesticides are used to increase the yield of agriculture products and destruction of organism interfering with human food[4]. Animals exposed to these pesticides exhibited alterations in their biochemical and physiological activities. Pesticides / insecticides were found to affect protein, triglycerides and cholesterol contents in several animal tissues[5,6,7]. Many investigators observed alterations in the blood indices (RBC, WBC, Hb content & Hematocrit value) in different animals treated with organophosphate, organochlorine and carbamate insecticides[8, 9, 10, 11].

The toxic mechanism of organophosphate (OP) compounds is based on the irreversible inhibition of acetyl cholinesterase (AchE) due to phosphorylation of the active site of the enzyme. This leads to accumulation of acetyl choline and subsequent over activation of cholinergic receptors at the neuromuscular junctions and in the autonomic and central nervous system. The rate and the degree of acetyl cholinesterase (AchE) inhibition differ according to the structure of OP pesticides and nature of their metabolite[12], It is reported that OP pesticides, besides their inhibitory effect on AchE, also responsible for characteristic changes of oxidative stress (MDA) and antioxidant imbalance[13]. Sumithion pesticide was selected for this study, having active ingredients O, O – Dimethyl O – (3 – methyl – 4 –

nitrophenyl) phosphorothionate, widely used throughout the country (India).

The present research work was conducted to study the biochemical (serum cholesterol) and hematological (absolute erythrocyte count) effect of the organophosphate compound Sumithion, in fish channa punctatus.

MATERIALS AND METHODS

The fishes collected from river Gomti, at Lucknow were brought to the biochemical laboratory in the plastic bags in natural water, washed three times in tap water and treated with 2% $KMnO_4$ to remove external parasitic infections, normal and healthy fishes were selected for the biochemical and hematological experiment. The fishes of uniform weight (85±98 gm) and length (13.8±17.6 cm) were taken for the experiment. They were transferred to large glass aquaria and acclimatized for 96 hours. Water characteristics- temperature (^{0}C), pH, alkalinity (mg/l), hardness (mg/l) and dissolved oxygen (mg/l) were analyzed by using standard method (APHA et al; 1991)**[14].**

A. **Collection of Sample:** Blood was collected from caudal vessels, either by serving off the caudal end or directly from heart and ventral aorta. Anticoagulants, like EDTA, potassium citrate, potassium oxalate, and ammonium oxalate were used. The collected blood was transferred to clean dry test tube and allowed to clot, at 10 ^{0}C. Soon after contents of the test tube were centrifuged at 2000 rpm and serum transferred to another clean dry test tube and stored in refrigeration at 2-8 ^{0}C.

B. **Serum Cholesterol Estimation:** Sample was placed for the estimation of serum cholesterol by the modified method of Zlatkis, A; et al (1953). 0.1 ml serum was taken in large glass stoppered test tube having 5 ml glacial acetic acid contents were filtered and 0.5 ml filtrate was taken in another glass stoppered test tube and the volume was made up to 8.0 ml with glacial acetic acid. To this 2.0 ml color reagent (1.0 ml -10 % $FeCl_3$ + 99.0 ml concentrated sulfuric acid) was thoroughly mixed by brisk circular motion of the test tube. Simultaneously, a blank was prepared by using glacial acetic acid in place of filtrate. The test tube were kept in dark for color development and heat loss. Optical density was determined at 540 nm. Standard curve was plotted for gradually increasing volume of standard cholesterol solution (25 mg/dl). Cholesterol level was calculated as cholesterol mg/dl of serum**[15]**.

C. **Absolute Erythrocyte Count:** Absolute erythrocyte counts were counted by the electrical conductivity method of cell counting. Count was performed on dilution of whole blood in buffered saline dilutants which had controlled chemical and electrical characteristics. A 1:250 dilution of the whole blood was used for absolute erythrocyte count. The transducer was adjusted at the factory show that 0.3125 milliliter of sample was counted. The displayed readings for erythrocyte count were in millions of cells per cubic millimeter of whole blood.

RESULTS AND OBSERVATIONS

The summary of results is given in table no 2. Decrease in absolute erythrocyte count at all concentrations of time intervals is because of sumithion toxicity in fish Channa Punctatus. The decreased count of absolute erythrocyte was more pronounced with increasing time of exposures. The lowest erythrocyte count was reported i.e., 57.24% (below control range) in fish whenever it was exposed for 24 hours at highest concentration of sumithion pesticide i.e., 0.73 mg/L. Fishes which were exposed at 0.40 mg/L sumithion concentration, deceased erythrocyte counts reported as 0.71%, 11.40%, 24.22% and 33.97% below control range, after 24, 48, 72 & 96 hours of exposures respectively. Those fishes which were exposed at 0.50 mg/L sumithion concentration, gradually decreased erythrocyte count were reported with increasing time intervals. The decreased erythrocyte count was 8.79%, 24.47% and 30.40% below control range, within 24, 48 and 72 hours of exposure respectively. Fishes which were treated by the sumithion concentration of 0.65 mg/L, their decreased erythrocyte count were reported 26.37% & 42.99% below control after 24 and 48 hours of exposures respectively. At highest concentration of 0.73mg/L, 50% of fishes did not survive after 24 hours and erythrocyte count was 57.24% below control.

The results obtained on serum cholesterol levels of the fish channa punctatus, exposed for 24 to 96 hours to four different concentrations of Sumithion, have been summarized in table no 3. It was generally observed that the level of serum cholesterol was maximum after the longest interval of exposure at all concentrations used in this case. The increased cholesterol levels were reported as 8.54%, 4.50%, 2.97% and 18.93%, above control range after exposure for 24, 48, 72 and 96 hours respectively at 0.40 mg/L Sumithion concentration. At 0.50 mg/l Sumithion concentration, the cholesterol levels increased 5.56%, 5.91% and 17.26% after 24, 48, 72 hours of exposure respectively above control range. The increased cholesterol levels were reported as 10.20% and 8.80% above control range, whenever fishes were exposed at 0.65 mg/L concentration for duration of 24 and 48 hours of exposures respectively. At the highest concentration of sumithion i.e., 0.73 mg/L, it was reported that 50% fishes were died within 24 hours of duration and serum cholesterol was elevated 8.90% above control range.

Table 1: The water characteristic analyzed in month July at the beginning of the experiment

Water characteristic (July Month)	
Paramaters	**Values (mean ± S. D) Range in Parenthesis**
Temperature (° C)	25.92 ± 1.49 (24.00 – 27.30)
pH	7.22 ± 0.09 (7.10 – 7.30)
Alkalinity (mg/L)	118.50 ± 2.64 (115.0 – 121.00)
Hardness (mg/L)	114.50 ± 1.29 (113 – 116)
Dissolved oxygen (mg/L)	5.87 ± 0.17 (5.70 – 6.10)

Table 2: Effect of pesticide Sumithion on absolute Erythrocyte count in Fish *Channa Punctatus*

Pesticide conc. mg/L, no. of observation 10 in each case	**Absolute erythrocyte count (million/cumm) Mean ± S. D (range in parameters)**			
	Time of exposure in hours			
	24	48	72	96
	Control value 4.21 ± 0.08 (4.13 – 4.32)			
0.40	4.18 ± 0.07 (4.11 – 4.27)	3.73 ± 0.13 (3.52 – 3.85)	3.19 ± 0.07 (3.13 – 3.30)	2.78 ± 0.04 (2.72 – 2.83)
0.50	3.84 ± 0.09 (3.72 – 3.93)	3.18 ± 0.80 (3.10 – 3.17)	2.93 ± 0.06 (2.85 – 2.99)	
0.65	3.10 ± 0.04 (3.05 – 3.15)	2.40 ± 0.07 (2.30 – 2.48)		
0.73	1.80 ± 0.07 (1.70 – 1.88)			

Table 3: Effect of Sumithion pesticide on serum cholesterol levels of fish *Channa Punctatus*

Pesticide conc. mg/L , no. of observation 10 in each case	**Serum cholesterol mg/100ml (Mean ± S. D) range in parentheses**			
	Time of exposure in hours			
	24	48	72	96
	Control value 673.25 ± 0.08 (4.13 – 4.32)			
0.40	730.77 ± 89.60 (680.45 – 812.32)	703.56 ± 68.88 (613.88 – 712.75)	693.28 ± 65.35 (605.85 – 751.22)	800.74 ± 78.56 (702.18 – 885.23)
0.50	710.71 ± 104.49 (600.03 – 850.65)	713.09 ± 52.92 (660.17 – 785.23)	722.16 ± 74.83 (635.03 – 795.07)	
0.65	752.22 ± 77.47 (650.56 – 820.73)	732.68 ± 82.49 (630.72 – 810.31)		
0.73	733.17 ± 66.37 (660.11 – 795.18)			

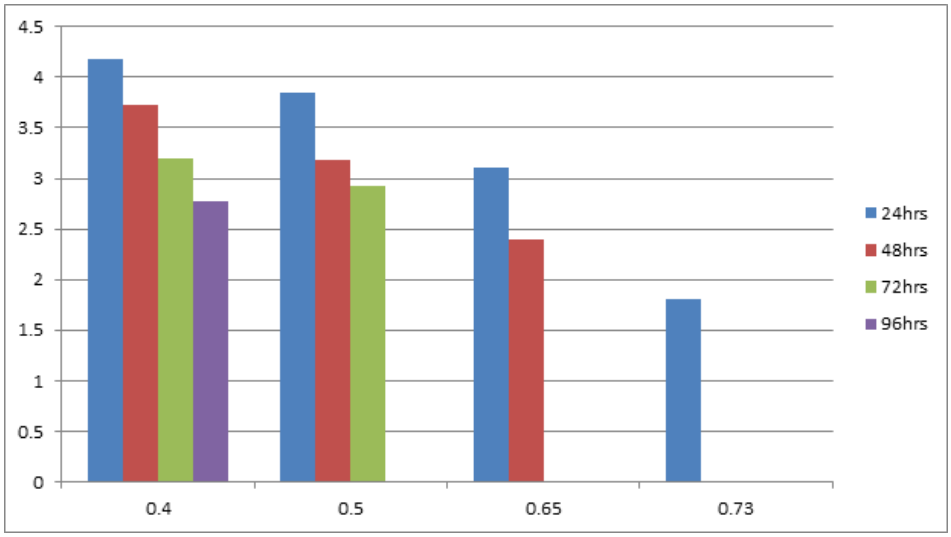

Fig. 1: Effect of pesticide Sumithion on absolute Erythrocyte count in Fish Channa Punctatus

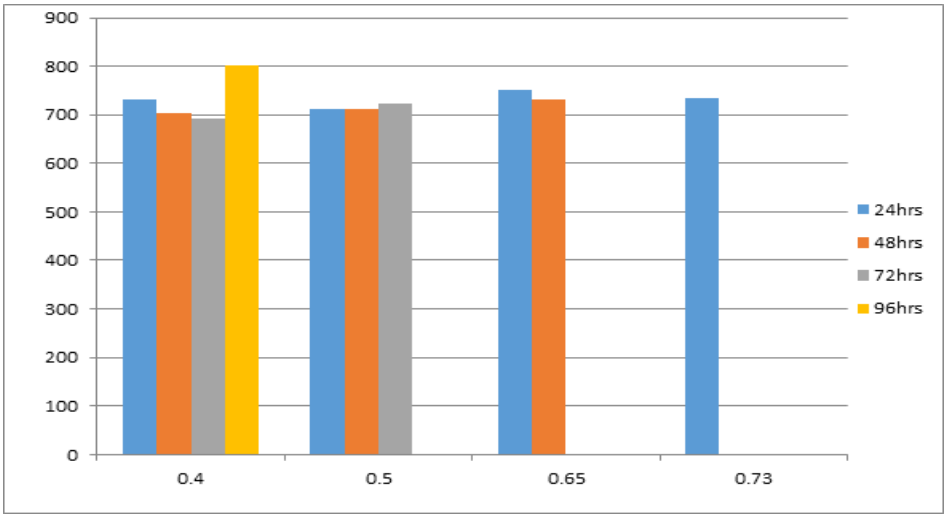

Fig. 2: **Effect of Sumithion pesticide on serum cholesterol levels of Fish Channa Punctatus**

DISCUSSION

In the present research we studied the hazardous effects of the organophosphate pesticide Sumithion. Organophosphate pesticides degrade the environment frivolously. The hematological and biochemical parameter can be used as indicators or monitors of prevailing aquatic pollution under natural ecological conditions and in experimentally created aquatic environment polluted with contaminants such as pesticides, insecticides, herbicides, toxic metals etc. In the present study it was concluded that the decrease of erythrocyte count in fish channa punctatus at all concentrations and time intervals is because of experimentally exposed toxicity of sumithion pesticide. The decrease in absolute erythrocyte count was more pronounced with increasing time of exposure. It shows the toxic severity of sumithion with regard to the time exposure. Similar finding had been observed by Koundinya and Ramamurthi (1979)[16], reported decline in RBC of Oreochromismossombica after exposure to sumithion. Reddy and Bashamohideen (1989)[17] also observed decreased RBC count in Cyprinuscarpio after 48 hour exposure to cypermethrin; and Santakumar*et al.* (1999)[18] in *Anabas* testudineus exposed to azodir. Similar results also reported by Mishra B P et al[19] 2000, in fresh water fishes due to effects of other organophosphate pesticides.

Hypercholesterolemia was observed due to toxicity of Sumithion in rainbow trout and it was due to cholinesterase inhibition at neuroeffector site in adrenal medulla which resulted in excess secretion of adrenaline, while dichloro diphenyl trichloroethane (DDT) cause pathological symptoms in liver, intestine and kidney of brown trout[20].

Similar result has been observed in this study, with sumithion pesticide in fish channa punctatus. The hypercholesterolemia in channa punctatus due to sumithion toxicity is neither genetic nor diet induced but environment induced, it appears that phosphate ion derived from pesticides in the medium, enter the fish body fluids and somehow cause partial or total breakdown of factors which inhibit the enzymes activity of HMG – CoA reductase, is principal enzyme for biosynthesis of cholesterol, its activation leads to excess synthesis of cholesterol (Talwar G. P. et al, 1989).

In contrast to these findings, some researchers observed hematological changes in mammalian animals treated with organophosphates, carbamates and organochlorine pesticides[8, 21, 9, 10, 11].

CONCLUSION AND RECOMMENDATION

In the present research we concluded that hypercholesterolemia and leucocytosis are the result of sumithion toxicity. Therefore it is recommended to pesticide workers to take all the precautions regarding protection from pesticide exposure and proper use of prophylactic supplements for their healthy life.

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