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TOXIC EFFECTS OF PROLONGED ENDOSULFAN EXPOSURE ON SOME BLOOD PARAMETERS IN ALBINO RAT

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Abstract: Endosulfan is a worldwide used synthetic insecticide that has an important role on management of pests in agriculture. The present work was undertaken to determine the effect of endosulfan on the haematological and haemochemical parameters of albino rats. Rats were feed with 5 mg/kg body weight endosulfan in mixed food stuff for 42 days. The studies were conducted on sexually matured male rats covering five groups of animals with control. Total counts of erythrocytes and haemoglobin were decreased and leucocytes were increased in treated group. Differential counts of leucocytes showed significant increase in basophils and monocytes. The levels of serum glucose, urea, creatinine and bilirubin increased significantly, suggesting that the synthetic insecticide had remarkable toxic effects on the haematological and biochemical parameters in the experimental animals.

Key words: Endosulfan, prolonged exposure, haematology, haemochemical parameters, albino rat

mvivsk: ms‡kwIZ KxUbvkK wnmv‡e G‡Ûvmvjdvb weke¨vcx KwI‡Z ¶wZKi KxU-cZ½ e¨e¯vcbvq D‡jL‡hvM¨ fwgKv cvjb Ki‡Q| cix¶YwU‡Z †kZ B`‡ii i‡³ †ngv‡UvjwRK¨vj I †n‡gv‡KwgK¨vj ‰ewk‡ó¨ G‡Ûvmvjdv‡bi cfve †`Lv n‡q‡Q| cvPwU M"‡c wef³ cYeq¯‹ ci"l B`‡i 42 w`b chš 5 wgwjMvg/wK‡jvMvg kvixiK IRb gvÎvq Lv‡`¨i mv‡_ G‡Ûvmvjdvb LvIqv‡bv n‡q‡Q| wbqwšZ B`‡ii m‡½ cix¶Yxq B`‡ii Zjbvq †jwnZ i³ KwYKv I wn‡gv‡Mwe‡bi nvm I †kZ i³ KwYKvi ew× cwijw¶Z n‡q‡Q| †kZ i³ KwYKvmg‡ni ga¨Kvi †e‡mwdj I g‡bvmvB‡Ui ZvrchcY ew× n‡q‡Q| i‡³i wmiv‡g M‡KvR, BDwiqv, wµ‡qwUwbb Ges wewji"we‡bi gvÎv ew× N‡U‡Q| cix¶YwU †kZ B`‡i i‡³i Dci G‡Ûvmvjdv‡bi wel‡µqvi Dc¯wZi mv¶¨ †`q|

Introduction

Endosulfan is an organochlorinc (OC) insecticide of the cyclodine group with a mixture of two stereo isomers; α- and β-endosulfan (Hayes and Laws 1991) in the ratio of 70:30. It has widespread use in agriculture and forestry to control a wide variety of insect pests and on non-food crops such as cotton and tobacco. Endosulfan is used in India (Saiyed *et al*. 2003), Turkey (Oktay *et al*. 2003), Malaysia (Chan *et al*. 2004), Mexico (Castillo *et al.* 2002; Gonzalez-Farias *et al.* 2004**)** and many other developing countries. With its widespread use in agriculture, humans are most likely to be exposed to it by eating food contaminated with endosulfan. Humans may also be exposed to low levels of endosulfan by skin contact with contaminated soil or by smoking cigarettes made from tobacco that has endosulfan residues on it (Lonsway *et al.* 1997). Populations that are usually susceptible to endosulfan include the unborn and neonates, the elderly and people with liver, kidney, immunological, haematological or neurological disease (ATSDR 2000). Endosulfan is well absorbed through ingestion, inhalation and skin contact. The toxicity of endosulfan varies depending upon the route of administration, vehicle, and species and sex of animal (Dikshith *et al*. 1988).

Routes of exposure of endosulfan are inhalation, dermal and oral at the agricultural uses to farmer. Ingestion occurs through accidental or deliberate application or accidental ingestion of contaminated food stuffs. Symptoms of endosulfan poisoning have been in some people who were exposed to very large amounts of this pesticide during its manufacture. Symptoms of endosulfan poisoning have also been seen in people who intentionally or accidentally ate or drank large amounts of endosulfan. Results from animal studies showed that exposure to very large amounts of endosulfan for short periods of time can cause adverse nervous system effects such as hyperexcitability, tremors, and convulsions, and death (ATSDR 2000).

Other effects seen in animals after short-term, high-level exposures include harmful effects on the stomach, liver and kidney. Moreover, the liver and kidney are the most important organs in which chemicals are structurally altered and resulting metabolites are biologically toxic (Saha *et al*. 2006). After comparatively longer exposures, the ability of animals to fight infection is also impaired. The kidney, testes and possibly the liver are the organs in laboratory animals affected by long-term exposure to low levels of endosulfan.

Endosulfan treatment in the male rats showed changes in blood. Erythrocytes are clumped, deeply stained, deformed and have thickened walls. Ruptured neutrophils and many monocytes are also observed. In the females, multilobed as well as ruptured neutrophils and monocytes appear. The numbers of large and small lymphocytes become very high

(Sahai and Chaudhary 1995). Choudhary *et al.* (2003) studied on the oral administration of endosulfan for four weeks and showed toxic interference with the biochemistry and histology of rat liver and kidney. However, much works have been carried out on the effect of endosulfan on the liver and kidney. But there are no adequate reports on toxicological investigation of the pesticide outside its usual or traditional haematological and haemochemical studies. The present work was therefore undertaken to determine the toxic effects of the prolonged exposure of endosulfan on the haematological and haemochemical parameters in the albino rats.

Materials and Methods

Healthy and sexually mature albino rats were collected and reared in the Department of Zoology, Rajshahi University. Animals were reared in 20cm×30cm×25cm steel cages in the laboratory under constant conditions at room temperature before and throughout the experimental work. The experimental rats were exposed to endosulfan by 5 ml/kg body weight for different duration *i.e.* 15, 21, 28, 35 and 42 days. Some untreated rats were used as control. Both treated and control animals were sacrificed after certain intervals.

For treatment, each rat was placed in anesthetic jar containing cotton wool soaked in chloroform. Complete anesthesia was considered accomplished when the pedal movements and eyelid reflex disappeared and the animal becomes recumbent while still breathing. The belly of the rat was opened up and blood was collected by cardiac puncture. Eight haematological parameters *viz.* total counts (TC) of erythrocytes and leucocytes, haemoglobin content, and differential counts (DC) of neutrophils, lymphocytes, monocytes, eosinophils and basophils, and four haemochemical parameters *viz.* glucose, urea, creatinin and bilirubin levels in the blood sera, were studied. Standard pathological techniques and estimation toolkit were applied to collect experimental data. Microscopic observations were done by the Motic advanced system biological microscope with the help of Motic image J.01 software.

Results and Discussion

Haematological parameters: Total counts on erythrocytes, total and differential counts on leucocytes and level of haemoglobin determine the health situation of an animal. Treatment was accomplished through oral route by offering food stuffs mixed with endosulfan to albino rats. The results of the haematological parameters on the control and endosulfan treated lines are presented in Table 1. Data on the differential count of leucocytes are shown in Table 2.

It was observed that total count of erythrocytes significantly decreased in treatment groups after 35 days compared to their controls. Total count of leucocytes significantly increased in experimental groups after 28 days ($P<0.05$) and 35 days ($P<0.01$) compared to the controls. Hemoglobin content showed significant decrease in treated groups after 28 days compared to the control (Table 1). In differential counts of the leucocytes, lymphocytes increased significantly after 28 days of endosulfan exposure. While eosinophils increased significantly after 42 days of treatment (Table 2).

Siddiqui *et al.* (1987) reported that percentage of haemoglobin, erythrocyte and paked cell volume decreased after 24 hour of exposure with endosulfan. Saha *et al.* (2006) observed that total count of erythrocyte and leucocyte decreased after inhalation of chloropyriphos. Elevated leucocyte count was observed in additional case of fetal acute poisoning like $12000/mm^3$ to a reference range 5000-10000 (Lo *et al*. 1995).

Haemochemical parameters: The effect of endosulfan experiment on certain biochemical parameters of blood in the treated and control rats are shown in Table 3. It was observed that there was a change in creatinine, urea, bilirubin and glucose in the sera of treated animals compared to that of the control rats. The glucose, urea, bilirubin and creatinine levels in blood sera increased significantly after 35 days of treatment. Moreover, the glucose, urea, creatinine and bilirubin levels were significantly elevated after 42 days of treatment and the changes of the biochemical parameters after treatment of endosulfan were exposure time- and dose-dependent.

Sahai and Chaudhary (1995) observed some biochemical changes in albino rats after treatment with endosulfan and olive oil. Ashour *et al.* (2007) reported that serum urea, uric acid and creatinine levels increased significantly in lead loaded albino rat with some chelating agents and natural oil. Bhatia *et al.* (2002) evaluated the effects of five sub-lethal concentrations of endosulfan on serum glucose, cholesterol and protein of *Heteropneustes fossilis* after 5, 15 and 30 days of exposure. Noticeable differences were observed in the blood chemistry of the treated fish. Similar results were also reported by Couser (1988) and Choudhary *et al.* (2003) working with various insecticides.

Serum enzymes and level of serum bilirubin, urea and creatinine were evaluated to establish hepatic and renal

dysfunction (Saba *et al.* 2000). Long term exposure of endosulfan adversely affects kidney, liver and blood cells. It also affects the biochemical parameters by increasing glucose levels in the treated animals (Sanghi *et al.* 2003).

Conclusion

From the present investigation it can be inferred that endosulfan is capable of inducing significant toxic effects on blood parameters in albino rats.

Table 1. Effect of endosulfan on heamatological parameters of albino rats.

Days	Exposure	Erythrocytes ($x10^6/mm^3$)	Leucocytes ($x10^3/mm^3$)	Heamoglobin (gm/mm^3)
15	Treatment 1	6.313 ± 0.333	6.673 ±0.409	13.24 ± 0.096
	Control 1	6.62 ± 1.732	6.566 ± 0.358	13.42 ± 0.140
21	Treatment 2	5.966 ± 0.101	8.32 ± 0.531	12.84 ± 0.129
	Control 2	6.463 ± 0.301	6.436 ± 0.732	13.38 ± 0.126
28	Treatment 3	5.37 ± 0.253	9.746* ± 0.518	11.86* ± 0.486
	Control 3	6.65 ± 0.337	6.486 ± 0.329	13.33 ± 0.096
35	Treatment 4	5.033* ± 0.183	10.276** ± 0.462	11.09* ± 0.150
	Control 4	6.513 ± 0.355	6.423 ± 0.365	13.23 ± 0.174
42	Treatment 5	5.043* ± 0.326	10.156** ± 0.196	10.87* ± 0.070
	Control 5	6.85 ± 0.391	6.696 ± 0.105	13.24 ± 0.096

Values are Mean±SE; Comparisons between treated and control lines were made using Student's t-tests; *P<0.05,**P<0.01, ***P<0.001.

Table 2. Effect of endosulfan on differential count of leucocytes of albino rats.

Days	Exposure	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
15	Treatment 1	23.33±2.56	60.66±3.85	2.33±0.60	6.66±0.84	4.00±0.25
	Control 1	24.66±1.36	56.00±1.20	2.66±0.33	5.00±0.57	3.33±0.33
21	Treatment 2	20.00±0.98	64.00±2.52	3.00±0.95	6.66±0.25	3.66±0.57
	Control 2	25.66±3.40	54.33±1.95	2.66±10.33	5.00±0.57	3.66±0.46
28	Treatment 3	19.66±2.84	68.25±2.08	4.33±0.65	9.00±1.42	3.66±0.57
	Control 3	22.33±3.05	60.00±3.22	3.00±0.46	5.00±0.57	4.00±0.33
35	Treatment 4	19.00±3.77	73.58**±1.05	4.00±0.22	8.66±0.84	3.00±0.87
	Control 4	24.00±2.02	55.66±2.28	2.66±0.33	5.00±0.57	3.66±0.57
42	Treatment 5	19.33±2.45	76.58**±1.05	4.00±0.22	9.00*±1.42	3.00±0.87
	Control 5	23.33±2.48	59.85±2.15	2.66±0.33	5.00±0.57	4.00±0.25

Values are Mean±S. E.; *p<0.05,**p<0.01, ***p<0.001 Vs Control, Students *t*-test

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Table 3. Effect of endosulfan on glucose, urea, creatinine and bilirubin level in sera of Albino rats.

Days	Exposure	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Bilirubin (mg/dl)
15	Treatment 1	132.61 ± 1.283	32.486 ± 0.336	0.78 ± 0.06	0.328 ± 0.036
	Control 1	103.08 ± 8.21	31.7 ± 0.672	0.51 ± 0.21	0.286 ± 0.022
21	Treatment 2	134.326 ± 1.982	33.5 ± 0.753	1.08 ± 0.139	0.37 ± 0.02
	Control 2	111.29 ± 0.365	33.043 ± 0.924	0.74 ± 0.016	0.308 ± 0.022
28	Treatment 3	145.09 ± 1.96	36.536 ± 0.388	1.35 ± 0.259	0.59* ± 0.230
	Control 3	110.39 ± 0.792	33.48 ± 0.421	0.79 ± 0.02	0.286 ± 0.022
35	Treatment 4	149.995** ± 4.160	38.023*±0.416	1.71** ± 0.086	0.44* ± 0.11
	Control 4	110.205 ± 0.618	33.176 ± 0.735	0.88 ± 0.05	0.308 ± 0.022
42	Treatment 5	149.993** ± 2.943	38.936** ± 0.346	1.73** ± 0.096	1.014** ± 0.045
	Control 5	110.76 ± 0.91	33.65 ± 0.742	0.98 ± 0.87	0.308 ± 0.022

Values are Mean±S. E.; *p<0.05,**p<0.01, ***p<0.001 Vs Control, Students *t*-test

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