

Clinicopathological Studies on Endosulfan-Induced Oxidative Stress and the Protective Role of Vitamin E

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Abstract: Endosulfan, an organochlorine acaricide and insecticide, has been used in agriculture on food and non-food crops for several years. The present study aimed to evaluate the clinicopathological changes associated with the oxidative stress induced by endosulfan in male rats and to evaluate the possible protective effect of vitamin E. The present experimental study was carried out on 40 albino rats (150-200 g) which were divided randomly into 4 groups; control group, endosulfan treated-group (2 mg/kgb.wt./day), vitamin E treated-group (200 mg/kgb.wt. twice a week) and endosulfan with vitamin E treated-group. Blood samples were weekly collected for studying changes in clinical hematology and serum biochemistry. At the end of experiment, antioxidant enzymes activities [superoxide dismutase (SOD), glutathione peroxidase (GPX) and Catalase (CAT)] in RBCs hemolysate and tissue homogenates (heart, liver, kidney and spleen) were studied. Results revealed that, oral administration of endosulfan induced microcytic hypochromic anemia, thrombocytopenia, leukocytosis with lymphocytosis, significant hypoproteinemia, hypoalbuminemia with decreased A/G ratio, increased activities of ALT and ALP, total hypercholesterolemia, hypertriglyceridemia and increased BUN and creatinine concentrations. Moreover, endosulfan induced decreased activities of antioxidant enzymes (SOD, GPX and CAT) in hemolysates and tissue homogenates. The present study concluded that oral administration of vitamin E improved the adverse effects of oxidative stress induced by endosulfan.

Key words: Clinical Hematology • Clinical Biochemistry • Oxidative Stress • Antioxidant Enzymes
• Endosulfan • Vitamin E • Rats

INTRODUCTION

Endosulfan is a toxic broad-spectrum insecticide and acaricide against great variety of insects and mites in agriculture and allied sectors. It is used on food and non-food crops [1]. Endosulfan belongs to organochlorine group, under the cyclodiene subgroup. U.S. Environmental Protection Agency (EPA) and European Union (EU) classify endosulfan as highly hazardous [2]. In USA, endosulfan is categorized as a restricted use pesticide (RUP) and is also listed in the acute toxicity I category, due to its high toxicity [3].

Exposure to endosulfan may be through various avenues, especially, ingestion of contaminated food, water and cow's milk [4]. Also, inhalation and skin absorption are occupational risk factors [5]. Endosulfan residues were detected in imported and locally raised chicken and bovine meat in Egypt [6]. Endosulfan has

also been detected in human tissues, cord blood samples obtained at the time of delivery, human sera and adipose tissue. Also it has been traced in human milk samples obtained from healthy lactating women in Egypt, Colombia and Nicaragua and from cotton pickers in Pakistan [7].

Excessive improper application and handling of endosulfan have been linked to congenital physical disorders, mental retardations and deaths in farm workers and villagers in developing countries [8].

Recent studies indicate that, pesticide intoxication (including endosulfan) produce oxidative stress by generation of free radicals and induce tissue lipid peroxidation in mammals and other organisms [9].

Oxidative stress is defined as a disruption of prooxidant-antioxidant balance leading to potential damage [10]. It is a result of one of three factors; an increase in reactive oxygen species (ROS), an impairment

of antioxidant defense systems or an insufficient capacity to repair oxidative damage. Under normal condition, excessive formation of free radicals and concomitant damage at cellular and tissue concentrations is controlled by cellular defense systems. These preventive defense systems can be accomplished by enzymatic and non-enzymatic mechanisms [10].

The role of oxidative stress and lipid peroxidation in endosulfan toxicity has been shown in erythrocytes, blood mononuclear cells, liver kidney [11] and heart [10] which reflected on the hematologic and biochemical parameters.

Vitamin E as lipid soluble, chain-breaking antioxidant plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals in biological membranes and its administration may be useful in controlling the toxic effect of insecticides and chemicals [12].

Superoxide dismutase (SOD), glutathione peroxidase (GPX) and Catalase (CAT) were measured as antioxidant enzymes in RBCs hemolysates and tissue homogenates to evaluate the antioxidant effect of vitamin E in endosulfan toxicity. Liver is one of tissues showing high rate of free radical generation, kidney is the body's most important organ of excretion and homeostasis and it is a frequent target for a variety of toxic agents due to its metabolic capacity for concentrating toxicants and/or metabolites during the excretory process. Heart is the main organ of circulatory system. Spleen is the largest organ in lymphatic system and it stores blood and releases it in circulatory system when body immediately needs [13].

The present study aimed to evaluate the clinicopathological changes associated with the oxidative stress induced by endosulfan in male rats and to evaluate the possible protective effect of vitamin E.

MATERIALS AND METHODS

Chemicals: All chemicals including Vitamin E (alpha-tocopherol) and Endosulfan were purchased from Sigma-Aldrich® Co. USA.

Experimental Design: A total of 40 male Albino rats (weighing 150-200 g) were obtained from Faculty of Medicine, Qassim University, KSA. Rats were acclimated for a period of 7 days in laboratory condition prior to the experiment. Rats were housed in plastic cages under standard condition with free access to drinking water and basal diet. Rats were randomly divided into 4 groups, 10 rats each as follows:

- Control group (Group A): each rat was given 0.2 ml corn oil once a day.
- Endosulfan treated group (Group B): each rat was given 2 mg/kgb.wt. endosulfan in corn oil once a day.
- Vitamin E treated group (Group C): each rat was given 200 mg/kgb. wt. vitamin E in corn oil twice a week.
- Vitamin E and endosulfan treated group (Group D): each rat was given 200 mg/kgb.wt. vitamin E (twice a week) and 2 mg/kgb.wt. endosulfan (in corn oil) once a day.

The experiment continued for 4 weeks and all treatments were administered to rats through stomach tube.

Blood Samples: Blood samples from each group were collected at weekly intervals. The obtained blood sample from each rat (retro-orbital venous plexuses) was divided into two parts. The first part was anticoagulated by di-potassium salt of ethylene diamine tetra-acetic acid (EDTA) and used for hematological procedures and hemolysate preparation. The second part was collected in a clean tube and allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation. The clear non hemolysed supernatant serum was harvested for biochemical studies.

RBCs Hemolysates: Hemolysates were prepared, at the end of experiment, according to the method of Bogdanska *et al.* [14] after determination of Hb concentration. The blood was centrifuged at 2600 rpm for 15 minutes and plasma was removed. Erythrocytes were carefully sampled from the bottom of tubes to minimize contamination with leukocytes, washed three times with isotonic saline solution and lysed by addition (1/4, by vol.) of double distilled water containing 5 ml/L Triton x-100, followed by vigorous vortex-mixing and storage on ice for 10 minutes. Membrane-free hemolysate was obtained by centrifugation at 10000x g for 5 minutes.

Tissue Homogenates: At the end of the experiments rats were sacrificed by cervical dislocation and tissue specimens (liver, heart, kidney and spleen) were collected for preparation of tissue homogenates by the method described by Jalili *et al.* [10]. These tissue specimens were immediately rinsed in ice-cold saline, homogenized in 10% w/v ice-cold 0.05 M potassium phosphate buffer (pH 7.4), centrifuged and the

supernatants were used for the determination of protein concentration [15] and antioxidant enzymes activity.

Hematological and Serum Biochemical Studies

Hematological Studies: Erythrocytes, total leukocytes (TLC) and platelets counts were done using improved Neubauerhemocytometer. Differential leukocytic count was performed on Giemsa stained blood smears [16]. Packed cell volume (PCV) was estimated by microhematocrit technique. Hemoglobin (Hb) concentration was colorimetrically determined using cyanmethemoglobin method. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated as described by Weiss and Wardrop [16].

Serum Biochemical Studies: Serum samples were prepared to assay the following biochemical studies; serum total proteins was determined according to Weichselbaun [17], serum albumin was determined according to Dumas and Biggs [18] and serum globulins were determined by subtracting value of serum albumin from the value of serum total proteins. A/G ratio was obtained by dividing values of serum albumin by those of serum globulins. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were performed according to Reitman and Frankel [19] and Tietz [20], respectively. Blood urea nitrogen (BUN) and serum creatinine were determined according to Tabacco *et al.* [21] and Fabiny and Ertingshausen [22], respectively. The above mentioned serum biochemical parameters were assayed using reagent kits supplied by StanBio Laboratories incorporation, USA.

Antioxidant Enzyme Assays: Antioxidant enzymes assays [superoxide dismutase (SOD), glutathione peroxidase (GPX) and Catalase (CAT)] were performed in the same day of preparation of hemolysates and tissue homogenates. SOD and GPX activities were measured with Randox kits according to Bogdanska *et al.* [14] and Paglia and Valentine [23], respectively. CAT activity was measured by method of Aebi [24].

Activity of these enzymes was expressed as U/ g hemoglobin and U/ mg protein in hemolysates and tissue homogenates, respectively.

Statistical Analysis: Values were expressed as mean \pm SD. Statistical comparisons among the means of tissue homogenates (antioxidant enzymes) for different

experimental groups were made with one way ANOVA. For all other parameters, statistical comparisons among the means of different experimental groups were made with completely randomized two ways ANOVA "Student-Newman-Keuls test" by COSTAT program version one. A probability "P" value of <0.05 was assumed for statistical significance.

RESULTS AND DISCUSSION

Clinicopathological Findings

Erythrogram and Platelets: Mean values of erythrogram (RBCs count, PCV, Hb concentration, MCV and MCHC) and platelets count of different experimental groups are illustrated in Tables 1, 2.

Erythrogram and platelets counts of different experimental groups, in comparison to those of control group, revealed presence of microcytic hypochromic anemia and significant thrombocytopenia in groups (B) and (D) started from the 1st week post treatment till the end of the experiment. Anemia was determined by significant decreases in values of RBCs counts, PCV, Hb concentration, MCV and MCHC. This anemia may be attributed to dysfunction of erythropoiesis, hemosynthesis and osmoregulation or due to increased rate of erythrocyte destruction in hematopoietic organs associated with endosulfan treatment [25-28]. Also, endosulfan can induce oxidative stress in blood cells (or in cell membranes) and disturb their structure playing a key role in lysis of erythrocytes and platelets which decreased their numbers in circulation [29, 30].

Leukogram: Mean values of leukogram (TLC, neutrophil, lymphocyte, eosinophil and monocyte counts) of different experimental groups are illustrated in Tables 3, 4.

Compared to control group, results of groups (B) and (D) showed significant leukocytosis due to significant lymphocytosis started from the 1st week post treatment till the end of the experiment. Insignificant changes in neutrophil, eosinophil and monocyte counts were observed in all experimental groups.

Leukocytosis observed in endosulfan treated groups (B) and (D) can be attributed to the oxidative effect of endosulfan on stem cells and multi-tasks cells that is the cause of their hyperactivities [30] or it has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue [31, 32].

Serum Biochemistry: Mean values of serum biochemical parameters of different experimental groups are illustrated in Tables 5-8.

Table 1: RBCs count, packed cell volume (PCV) and hemoglobin (Hb) concentration of different experimental groups (means \pm SD).

Week	RBCs count ($\times 10^9/\mu\text{l}$)				PCV (%)				Hb concentration (g/dl)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0	6.43 \pm 0.19	6.52 \pm 0.69	6.46 \pm 0.21	6.76 \pm 0.69	40.69 \pm 1.53	40.81 \pm 0.62	40.49 \pm 0.89	40.78 \pm 0.43	14.83 \pm 1.63	14.69 \pm 0.03	13.51 \pm 1.09	14.44 \pm 0.86
1	6.61 \pm 0.71	5.46 \pm 0.73	6.37 \pm 0.62	5.91 \pm 0.98	41.15 \pm 0.83	30.12 \pm 2.56	40.50 \pm 0.42	33.93 \pm 1.33	14.98 \pm 0.38	9.23 \pm 1.56	14.77 \pm 0.86	11.11 \pm 1.11
2	6.54 \pm 0.09	5.29 \pm 0.75	6.66 \pm 0.96	5.83 \pm 0.18	40.74 \pm 0.33	28.89 \pm 1.10	41.71 \pm 0.88	32.47 \pm 0.91	15.33 \pm 0.56	9.01 \pm 0.97	14.91 \pm 0.16	10.91 \pm 0.25
3	6.81 \pm 0.11	5.17 \pm 0.38	6.56 \pm 0.12	5.84 \pm 0.65	42.20 \pm 0.86	27.67 \pm 3.18	39.94 \pm 0.40	32.76 \pm 1.52	15.41 \pm 0.57	8.22 \pm 1.78	14.88 \pm 0.4	10.86 \pm 0.47
4	6.53 \pm 0.21	4.95 \pm 0.6	6.23 \pm 0.42	5.69 \pm 0.6	40.78 \pm 0.66	25.75 \pm 2.12	38.55 \pm 0.92	30.56 \pm 0.51	14.63 \pm 1.23	7.49 \pm 0.79	14.59 \pm 0.97	9.58 \pm 1.28
LSD	0.62				4.19				2.94			

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

LSD: least significant difference between different groups at probability $P < 0.05$.Table 2: Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets count of different experimental groups (means \pm SD).

Week	MCV (fl)				MCHC (%)				Platelets count ($\times 10^9/\mu\text{l}$)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0	63.28 \pm 2.25	62.59 \pm 3.42	62.68 \pm 0.51	60.33 \pm 0.92	36.45 \pm 1.87	36.01 \pm 1.80	33.37 \pm 2.06	35.41 \pm 1.53	456.23 \pm 50.95	472.48 \pm 21.68	428.64 \pm 45.11	424.22 \pm 43.79
1	62.25 \pm 1.91	55.16 \pm 1.73	63.58 \pm 2.36	57.41 \pm 3.98	36.40 \pm 0.43	30.64 \pm 0.39	36.47 \pm 1.25	32.74 \pm 0.77	473.74 \pm 47.66	290.11 \pm 14.46	465.29 \pm 35.11	330.56 \pm 25.95
2	62.29 \pm 3.55	54.61 \pm 1.58	62.63 \pm 1.51	55.69 \pm 4.35	37.63 \pm 1.13	31.19 \pm 2.16	35.75 \pm 1.57	33.60 \pm 2.07	495.38 \pm 41.90	256.28 \pm 31.9	444.87 \pm 41.84	293.82 \pm 31.58
3	61.97 \pm 4.65	53.52 \pm 0.17	60.88 \pm 0.47	56.1 \pm 2.05	36.52 \pm 1.24	29.71 \pm 3.28	37.26 \pm 0.93	33.15 \pm 3.78	474.71 \pm 33.27	208.68 \pm 18.12	476.59 \pm 27.52	269.14 \pm 24.21
4	62.45 \pm 1.54	52.02 \pm 2.27	61.88 \pm 0.73	53.71 \pm 1.61	35.88 \pm 0.86	29.09 \pm 0.07	37.85 \pm 3.35	31.35 \pm 1.00	469.81 \pm 37.2	199.33 \pm 11.9	461.23 \pm 35.26	226.03 \pm 28.03
LSD	4.03				3.15				68.37			

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

LSD: least significant difference between different groups at probability $P < 0.05$.Table 3: Total leukocyte (TLC), neutrophil and lymphocyte counts of different experimental groups (means \pm SD)

Week	TLC ($\times 10^9/\mu\text{l}$)				Neutrophil count ($\times 10^9/\mu\text{l}$)				Lymphocyte count ($\times 10^9/\mu\text{l}$)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0	7.74 \pm 0.34	7.03 \pm 0.40	7.71 \pm 0.71	7.39 \pm 0.37	2.71 \pm 0.05	2.46 \pm 0.46	2.36 \pm 0.68	2.22 \pm 0.63	4.64 \pm 0.24	4.22 \pm 0.95	4.87 \pm 0.96	4.80 \pm 0.93
1	7.37 \pm 0.14	8.76 \pm 0.08	7.64 \pm 0.77	8.36 \pm 0.17	2.58 \pm 0.51	2.67 \pm 0.62	2.56 \pm 0.32	2.70 \pm 0.1	4.42 \pm 0.76	5.71 \pm 0.85	4.59 \pm 0.88	5.39 \pm 0.91
2	7.74 \pm 0.14	8.77 \pm 0.33	7.52 \pm 0.80	8.70 \pm 0.56	2.71 \pm 0.04	2.64 \pm 0.61	2.53 \pm 0.72	2.77 \pm 0.37	4.64 \pm 0.41	5.75 \pm 0.15	4.49 \pm 0.55	5.59 \pm 0.33
3	7.84 \pm 0.93	9.31 \pm 0.77	7.59 \pm 0.01	8.78 \pm 0.08	2.86 \pm 0.52	2.87 \pm 0.12	2.76 \pm 0.76	2.58 \pm 0.76	4.57 \pm 0.76	6.03 \pm 0.88	4.34 \pm 0.43	5.83 \pm 0.46
4	7.88 \pm 0.14	9.51 \pm 0.23	7.85 \pm 0.70	8.92 \pm 0.56	2.76 \pm 0.83	2.80 \pm 0.55	2.60 \pm 0.08	2.42 \pm 0.04	4.73 \pm 0.23	6.31 \pm 0.36	4.74 \pm 0.51	6.15 \pm 0.43
LSD	0.91				0.60				0.89			

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

LSD: least significant difference between different groups at probability $P < 0.05$.Table 4: Eosinophil and Monocyte counts of different experimental groups (means \pm SD)

Week	Eosinophil count ($\times 10^9/\mu\text{l}$)				Monocyte count ($\times 10^9/\mu\text{l}$)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0	0.155 \pm 0.014	0.146 \pm 0.028	0.148 \pm 0.031	0.147 \pm 0.041	0.232 \pm 0.032	0.211 \pm 0.040	0.245 \pm 0.039	0.243 \pm 0.013
1	0.147 \pm 0.025	0.152 \pm 0.013	0.156 \pm 0.034	0.138 \pm 0.019	0.221 \pm 0.019	0.229 \pm 0.028	0.234 \pm 0.021	0.206 \pm 0.016
2	0.155 \pm 0.037	0.151 \pm 0.018	0.153 \pm 0.025	0.144 \pm 0.038	0.232 \pm 0.024	0.226 \pm 0.032	0.242 \pm 0.035	0.233 \pm 0.027
3	0.163 \pm 0.008	0.164 \pm 0.022	0.171 \pm 0.009	0.158 \pm 0.008	0.245 \pm 0.009	0.246 \pm 0.024	0.214 \pm 0.031	0.221 \pm 0.037
4	0.158 \pm 0.028	0.160 \pm 0.011	0.163 \pm 0.033	0.149 \pm 0.015	0.236 \pm 0.034	0.241 \pm 0.017	0.249 \pm 0.051	0.207 \pm 0.035
LSD	0.012				0.30			

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

LSD: least significant difference between different groups at probability $P < 0.05$.

Table 5: Protein profile of different experimental groups (means \pm SD)

Week	T. proteins (g/dl)				Albumin (g/dl)				Globulins (g/dl)				A/G ratio			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0	6.23 \pm 0.54	6.2 \pm 0.88	6.19 \pm 0.68	6.21 \pm 0.72	3.82 \pm 0.40	3.8 \pm 0.66	3.77 \pm 0.50	3.8 \pm 0.54	2.41 \pm 0.26	2.4 \pm 0.43	2.42 \pm 0.33	2.41 \pm 0.35	1.63 \pm 0.08	1.62 \pm 0.13	1.6 \pm 0.10	1.62 \pm 0.12
1	6.26 \pm 0.75	5.53 \pm 1.04	6.19 \pm 0.68	5.68 \pm 0.46	3.77 \pm 0.56	3.15 \pm 0.77	3.83 \pm 0.51	3.27 \pm 0.34	2.49 \pm 0.36	2.38 \pm 0.51	2.36 \pm 0.33	2.41 \pm 0.23	1.56 \pm 0.11	1.36 \pm 0.15	1.67 \pm 0.11	1.4 \pm 0.07
2	6.24 \pm 0.90	5.36 \pm 1.10	6.22 \pm 0.55	5.62 \pm 0.44	3.82 \pm 0.67	3 \pm 0.82	3.75 \pm 0.41	3.18 \pm 0.33	2.42 \pm 0.44	2.36 \pm 0.53	2.47 \pm 0.27	2.44 \pm 0.21	1.63 \pm 0.13	1.31 \pm 0.16	1.56 \pm 0.08	1.34 \pm 0.06
3	6.26 \pm 0.67	5.2 \pm 0.95	6.27 \pm 0.61	5.55 \pm 0.41	3.8 \pm 0.49	2.82 \pm 0.70	3.82 \pm 0.45	3.07 \pm 0.31	2.46 \pm 0.32	2.38 \pm 0.46	2.45 \pm 0.29	2.48 \pm 0.20	1.59 \pm 0.10	1.22 \pm 0.14	1.61 \pm 0.09	1.27 \pm 0.06
4	6.22 \pm 0.61	5.24 \pm 0.88	6.25 \pm 0.4	5.31 \pm 0.57	3.86 \pm 0.45	2.79 \pm 0.65	3.84 \pm 0.30	2.96 \pm 0.42	2.36 \pm 0.29	2.45 \pm 0.43	2.41 \pm 0.19	2.36 \pm 0.28	1.69 \pm 0.09	1.17 \pm 0.13	1.64 \pm 0.06	1.29 \pm 0.08
LSD	0.56				0.43				0.29				0.15			

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

LSD: least significant difference between different groups at probability $P < 0.05$.Table 6: Activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes of different experimental groups (means \pm SD)

Week	ALT (U/L)				ALP (U/L)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0	42.55 \pm 8.12	44.75 \pm 12.03	47.15 \pm 9.21	49.12 \pm 9.82	143.43 \pm 6.80	137.60 \pm 11.11	134.40 \pm 8.53	148.20 \pm 9.10
1	47.15 \pm 10.17	80.73 \pm 14.12	41.52 \pm 9.23	74.75 \pm 6.29	131.25 \pm 9.42	343.35 \pm 13.08	143.85 \pm 8.55	317.10 \pm 5.83
2	48.42 \pm 12.17	86.25 \pm 14.91	48.24 \pm 7.52	79.35 \pm 6.22	149.10 \pm 11.27	368.55 \pm 13.81	141.75 \pm 6.97	333.90 \pm 5.56
3	43.01 \pm 9.03	95.45 \pm 12.86	47.38 \pm 8.23	83.15 \pm 5.54	144.90 \pm 8.37	366.45 \pm 11.92	152.25 \pm 7.62	337.05 \pm 5.13
4	44.85 \pm 8.23	97.75 \pm 11.96	48.76 \pm 5.42	86.25 \pm 7.71	128.10 \pm 7.62	375.90 \pm 11.08	138.60 \pm 5.02	349.65 \pm 7.14
LSD	20.19				38.89			

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

LSD: least significant difference between different groups at probability $P < 0.05$.Table 7: Total cholesterol and triglycerides concentrations of different experimental groups (means \pm SD)

Week	Total cholesterol (mg/dl)				Triglycerides (mg/dl)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0	88.75 \pm 13.78	86.94 \pm 14.69	86.1 \pm 14.16	87.9 \pm 7.56	68.98 \pm 12.52	71.57 \pm 13.7	69.79 \pm 6.74	71.66 \pm 8.79
1	90.66 \pm 11.34	119.04 \pm 8.88	89.02 \pm 15.21	105.85 \pm 4.36	62.24 \pm 8.37	177.24 \pm 7.78	63.03 \pm 11.49	148.24 \pm 13.74
2	90.16 \pm 14.33	127.4 \pm 12.13	87.88 \pm 6.37	116.39 \pm 4.89	66.27 \pm 10.04	184.49 \pm 3.31	61.21 \pm 11.66	130.89 \pm 7.89
3	92.19 \pm 11.05	132.28 \pm 7.76	91.36 \pm 8.91	114.45 \pm 3.87	65.11 \pm 14.23	187.44 \pm 3.92	64.13 \pm 8.57	124.73 \pm 12.31
4	91.77 \pm 7.34	128.79 \pm 6.45	90.45 \pm 7.21	113.41 \pm 6.82	63.52 \pm 9.28	185.82 \pm 6.01	61.97 \pm 9.78	114.04 \pm 8.33
LSD	13.13				42.58			

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

LSD: least significant difference between different groups at probability $P < 0.05$.Table 8: Blood urea nitrogen (BUN) and creatinine concentrations of different experimental groups (means \pm SD)

Week	BUN (mg/dl)				Creatinine (mg/dl)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
0	43.05 \pm 7.96	41.35 \pm 13.01	42.11 \pm 9.98	40.25 \pm 9.65	0.47 \pm 0.023	0.43 \pm 0.038	0.45 \pm 0.029	0.42 \pm 0.031
1	40.01 \pm 11.03	51.45 \pm 15.31	40.95 \pm 10.01	48.41 \pm 6.82	0.42 \pm 0.032	0.70 \pm 0.045	0.40 \pm 0.029	0.68 \pm 0.021
2	40.95 \pm 13.19	53.55 \pm 16.16	43.05 \pm 8.16	50.51 \pm 6.51	0.45 \pm 0.038	0.81 \pm 0.047	0.48 \pm 0.024	0.69 \pm 0.019
3	41.31 \pm 9.80	55.65 \pm 13.95	40.95 \pm 8.92	49.81 \pm 6.03	0.48 \pm 0.029	0.84 \pm 0.041	0.43 \pm 0.026	0.74 \pm 0.017
4	42.42 \pm 8.92	61.95 \pm 12.97	44.31 \pm 5.88	52.71 \pm 8.35	0.43 \pm 0.026	0.87 \pm 0.038	0.46 \pm 0.017	0.79 \pm 0.024
LSD	7.16				0.22			

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

LSD: least significant difference between different groups at probability $P < 0.05$.

Table 9: Activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) of different experimental groups (means \pm SD)

Sample	CAT				SOD				GPX			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
Hemolysate												
(U/g hemoglobin)	3699 ^a \pm 124.1	2148 ^b \pm 89.64	3723 ^a \pm 156.53	3097 ^a \pm 101.17	860 ^a \pm 63.47	386 ^b \pm 28.26	873 ^a \pm 47.5	561 ^a \pm 26.36	3216 ^a \pm 103.58	1735 ^b \pm 92.29	3357 ^a \pm 132.6	2689 ^b \pm 110.43
Heart												
(U/mg protein)	33.4 ^a \pm 1.43	18.4 ^b \pm 2.11	35.6 ^a \pm 1.35	27.4 ^a \pm 2.24	26.15 ^a \pm 2.01	9.69 ^b \pm 3.18	28.41 ^a \pm 4.82	18.28 ^b \pm 2.75	53.07 ^a \pm 2.91	33.22 ^b \pm 3.71	58.46 ^a \pm 2.31	43.28 ^b \pm 3.72
Liver												
(U/mg protein)	186.09 ^a \pm 4.93	95.52 ^b \pm 3.08	204.39 ^a \pm 5.38	152.75 ^a \pm 4.37	29.01 ^a \pm 2.64	12.06 ^b \pm 4.88	31.24 ^a \pm 3.19	20.57 ^b \pm 3.67	60.11 ^a \pm 2.53	23.74 ^b \pm 4.03	63.96 ^a \pm 3.62	49.86 ^b \pm 3.89
Kidney												
(U/mg protein)	140.47 ^a \pm 2.17	64.39 ^b \pm 2.38	152.68 ^a \pm 3.73	117.42 ^a \pm 3.45	46.72 ^a \pm 3.47	26.11 ^b \pm 6.18	49.94 ^a \pm 3.59	35.94 ^b \pm 3.98	58.33 ^a \pm 2.87	29.01 ^b \pm 2.93	60.38 ^a \pm 2.75	43.33 ^b \pm 3.14
Spleen												
(U/mg protein)	24.67 ^a \pm 1.62	10.89 ^b \pm 2.03	26.23 ^a \pm 1.55	19.23 ^a \pm 2.14	18.14 ^a \pm 1.38	3.42 ^b \pm 2.12	20.05 ^a \pm 1.68	12.38 ^b \pm 1.43	71.27 ^a \pm 4.65	45.33 ^b \pm 4.24	73.12 ^a \pm 1.19	60.72 ^b \pm 3.07

Means values with different superscripts in the same row are significantly different ($p < 0.05$); while those with same superscripts in the same row are not significantly different ($p < 0.05$).

Group A: control group.

Group B: endosulfan-treated group.

Group C: vitamin E-treated group.

Group D: vitamin E and endosulfan-treated group.

Compared to control group (A), protein profile of groups (B) and (D) showed significant hypoproteinemia due to hypoalbuminemia with significant decrease in A/G ratio started from the 1st week post treatment till the end of the experiment. No significant changes were recorded in globulins concentration in all experimental groups. The decreased concentrations of total proteins and albumin may be attributed to their catabolism to liberate energy during the stress induced by endosulfan toxicity [33], or due to increased rate of proteolytic activity or reduced rate of transcription (or translation) associated with endosulfan-induced toxicity [31].

Activities of liver enzymes (ALT and ALP) and concentrations of total cholesterol and triglycerides of groups (B) and (D) showed significant increases in their mean values started from the 1st week post treatment till the end of the experiment. These increases resulted from hepatotoxic effect of endosulfan [34, 35] and the disturbance of liver metabolic and excretory mechanisms [36].

Hypercholesterolemia present may be attributed to increased synthesis and accumulation of cholesterol in liver and/or impaired biliary function [37]. As triglycerides is the main components of cell membrane, Hypertriglyceridemia has been reported to occur as a result of leakage associated with cells apoptosis from endosulfan toxicity [38].

Concentrations of BUN and serum creatinine were significantly increased in groups (B) and (D) as a result of oxidative stress from endosulfan toxicity which play a role in changing cell membrane configuration and seem to account for kidney alteration [39, 40].

Antioxidant Enzyme Assays: Mean values of antioxidant enzymes activities (SOD, GPX and CAT) in hemolysates and tissue homogenates are illustrated in Table 9.

Activities of antioxidant enzymes (SOD, GPX and CAT) in hemolysates and tissue homogenates were significantly lower in groups (B) and (D) than those of group (A). These antioxidant enzymes counteract the insecticide-induced oxidative stress, resulting in their consumption which reflected on their decreased activities [41].

Administration of vitamin E alone (group C) showed no significant effect on all previously mentioned hematological and biochemical parameters, but when administered with endosulfan (group D), it improved the observed changes but still significantly differed from the control group. Vitamin E relatively protected the different organs from the oxidative effect of endosulfan because it acts as free radical scavenger within the membranes. Its location in the membrane provides little protection against cytosolic oxidative injury. It donates reducing equivalents to lipid peroxy radicals, thereby limiting chain reaction oxidations of polyunsaturated lipids and thus protecting the membranes of body organs [42] leading to decreased endosulfan cardiotoxicity [10, 43] and hepatotoxicity [44].

CONCLUSION

It can be concluded that endosulfan induced oxidative stress in RBCs and different organs of rats. This oxidative stress is manifested by the recorded changes in different clinicopathological parameters as well as decreased activities of antioxidant enzymes. However, oral administration of vitamin E reduced the adverse effects of endosulfan-induced oxidative stress. This antioxidant effect of vitamin E may be beneficial in endosulfan toxicity but further investigations are required to determine the suitable dose of vitamin E for more protection.

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