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The Effect of Endosulfan Pesticide in Some Biochemical Parameters of white Mice

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Abstract

The present study aimed to examine the effect of endosulfan insecticide on some molecular and biochemical parameters in white mice. Thirty mice were separated randomly into three groups for treatment with endosulfan. One group (G1) served as the control, while the other two groups received intraperitoneal injections of endosulfan G2 (3 mg/kg) and G3 (17 mg/kg) twice a week for 21 and 45 days, respectively. A biochemical study by measuring liver function parameters, including (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) and kidney function parameters, including (Blood Urea and Creatinine) and malondialdehyde (MDA), catalase activity (CAT). This study also tested DNA damage by comet assay (normal%, low%, medium%, high%). The results of renal function parameters (Blood Urea and Creatinine) were significantly increased in all treated groups after 21 and 45 days exposed to endosulfan compared with control groups. The highest value of blood urea recorded was (49.33 ±0.88 mg/dl) at 17 mg/kg for 45 days compared with the control group, and the highest value of Creatinine recorded was (1.81 ±0.13 mg/dl) at 17 mg/kg for 45 days compared with the control group. Liver function parameters (ALT and AST) significantly increased in all treated groups compared with control groups. The results of MDA, CAT enzyme, were significantly increased in all treated groups after 21 and 45 days compared with control groups. The highest value of MDA recorded was (3.93 ±0.07 µM) at 17 mg/kg for 45 days compared with the control group. Tail DNA (%) showed a significant increase at high concentrations, and the results showed a considerable increase in the severe damage of DNA in the treated group 17 mg\kg b.wt. (25.00 ±1.00)% for 45 days, compared with the control group (3.00 ±1.00) %.

Key words: Liver function, Comet assay, Creatinine, Sub-lethal doses

1.Introduction

Endosulfan is a broad-spectrum organochlorine pesticide used to suppress insect pests in homes and farms worldwide [1]. Endosulfan is sold as a 7:3 blend of alpha and beta isomers, with the alpha isomer having a longer half-life (approximately 157 days) and a more significant environmental impact. It has been outlawed in many nations, including the United States [2], but emerging nations like Ghana continue to utilize it [3]. It has been extensively used along with modern agriculture methods during the last few decades, contaminating various environmental spaces, such as the soil, the water, and the air. It can pass through the body through the oral cavity when consumed orally, through the skin's pores, and through the nose when inhaled as vapor, dust, or spray particles [6-8]. The contaminated environmental framework has various adverse effects on non-targeted organisms, including people [4]. Pesticides harm non-target organisms in two ways: First, pesticides harm non-target species when they come into direct contact with them; second, pesticide residuals may cause non-target organisms to suffer harm down the road [5]. Endosulfan has been linked to cardiovascular toxicity [9,10], neurotoxicity [11], hepatotoxicity [12], immunotoxicity [13], reproductive toxicity [14,15], and DNA damage [16]. Other studies revealed a considerable loss of weight, a change in hematological parameters, and histological modification confirming endosulfan's hematological toxicity [17].

2. Materials and Methods

2.1. Animals Housing

White mice aged 6–8 weeks on average, weighing approximately (25±5 g) were used in the present study. The animals were held in polypropylene cages in a controlled situation with 12±2 hrs of light and dark cycles. The temperature was 25±5 °C with a relative humidity of 50–60%. The food sources were available 24 hrs a day. Overall, mice were left for one week before beginning the investigation for adaptation to the research laboratory environment.

2.2. Experimental Animal's Treatment

Thirty male mice were randomly separated into three groups and treated with endosulfan (EN) to investigate the toxicological effects (G1, G2, and G3). Two intraperitoneal injections of endosulfan G2 (3 mg/kg) and G3 (17 mg/kg) were given twice a week, while one group served as the control.

2.3. Biochemical parameters

2.3.1. Renal function measurement:

Blood urea levels were measured using the colorimetric method and a Human kit (Human Gesellschaft fur Biochemica and Diagnostica mbH, Germany). While serum creatinine was estimated using a Jaffe reaction that was depending the colorimetric method (Bonsnes and Taussky, 1945) by using a Human kit (Human Gesellschaft fur Biochemica and Diagnostica mbH, Germany).

2.3.2. Measurement of malondialdehyde (MDA):

Malondialdehyde was determined in serum using kit from BioVision\USA.

2.3.3. Measurement of catalase activity (CAT):

Bioassay Systems' improved assay directly measures catalase degradation of H_2O_2 using a redox dye.

2.3.4. Measurement of ALT & AST:

Liver enzymes were measured by using kits provided by Biomerieux-Frans for the colorimetric determination of ALT and AST in serum.

2.3.5. Measurement of deoxyribonucleic acid (DNA) damage:

DNA damage related to the cultured lymphocytes from the spleen was evaluated for three groups utilizing the single-cell electrophoresis (Comet assay) approach.

2.4 **Statistical analysis**

The statistical analysis system- SAS (2012) program was used to detect the effect of different factors on study parameters. The least significant difference –the LSD test (Analysis of Variation-ANOVA), was used to significantly compare the means in this study.

3. Results and Discussion

3.1 Effect on renal function parameters

The treated groups with EN showed a difference in renal function parameters, as shown in **Tables (1) and (2).** A significant (P≤0.01) increase in blood urea level was found in the treated group with 17 mg\kg of b. wt. (40.00 ±2.64) mg/dl after 21 days compared with the control and treatment groups with three mg\kg of b. wt. (24.67 ±1.45and 27.33 ±1.76) mg/dl. As the results showed in **Table (1)**, there was a significant (P≤0.01) increase in the level of urea in the two treated groups with 3 and 17 mg\kg of b. wt. (33.67 ±2.60and49.33 ±0.88) mg/dl after 45 days of the treatment, respectively, compared with the control group (24.66 ±1.45)mg\dl. At the same time, the results showed a significant (P≤0.01) increase in the level of blood urea in the treated group with three mg\kg of b. wt. (33.67 ±2.60) mg\dl for 45 days compared with the level of urea for the same group after 21 days (27.33 ±1.76) mg\dl, and the same significant(P≤0.01) increase was present in the group treated with 17 mg\kg of b. wt. (49.33 ±0.88 and 40.00 ±2.64) after 45 and 21 days of treatment, respectively. The results showed a significant(P≤0.01) increase in creatinine concentration in the treated group with 17 mg\kg of b. wt. (1.30 ±0.23) mg/dl after 21 days compared with the control and treatment groups with three mg\kg of b. wt.(0.433 ±0.08 and 0.76 ±0.08) mg/dl.

The results in **Table (2)** obtained a significant (P≤0.01) increase in the Creatinine concentration in the two treated groups with 3 and 17 mg\kg of b. wt. (0.86 ±0.03and 1.81 ±0.13) mg/dl after 45 days, respectively, compared with the control group(0.433 ±0.08)mg\dl.

The results showed a nonsignificant (P≤0.01) increase in the Creatinine concentration in the treated group with three mg\kg of b. wt. (0.76 ±0.08 and0.86 ±0.03) mg\dl for 21 and 45 days, while the results showed a significant (P≤0.01) increase in the group treated with 17 mg\kg of b. wt. (1.81 ±0.13) mg/dl after 45 days compared with the group treated for 21 days(1.30 ±0.23) mg/dl. This study's results agreed with Bouhafs et al. [18] and Sebastian and Raghavan [15], suggesting that the kidney is affected by pesticides when orally exposed to 3 mg/kg for 1 and 21 days.

Table 1. Effect of doses and period in Blood Urea

Groups	Mean ± SE of Blood Urea (mg/dl)		LSD value
	21 Days	45 Days	
Control	24.66 ±1.45 B a	24.66 ±1.45 C a	3.82 NS
3 mg/kg	27.33 ±1.76 B b	33.67 ±2.60 B a	6.02 **
17 mg/kg	40.00 ±2.64 A a	49.33 ±0.88 A b	5.73 **
LSD value	6.98 **	6.21 **	---
Means with different Capital letters in the same column and small letters in the same row are significantly different. . ** (P≤0.01).			

Table 2. Effect of doses and period in Creatinine

Groups	Mean ± SE of Creatinine (mg/dl)		LSD value
	21 Days	45 Days	
Control	0.433 ±0.08 B a	0.433 ±0.08 C a	0.147 NS
3 mg/kg	0.76 ±0.08 B a	0.86 ±0.03 B a	0.207 NS
17 mg/kg	1.30 ±0.23 A b	1.81 ±0.13 A a	0.378 **
LSD value	0.524 **	0.320 **	---
Means with different Capital letters in the same column and small letters in the same row are significantly different. . ** (P≤0.01).			

3.2. Effect on malondialdehyde (MDA)

The results of the determination of malondialdehyde showed a significant (P≤0.01) increase in the treated group with 17 mg\kg b.wt. (3.23 ±0.22 µM) for 21 days compared with the control group and treated group with three mg\kg b.wt. (1.73 ±0.16 µM) for 21 days. The results also

showed a significant (P≤0.01) increase in the concentration of (MDA) in the treated group with 3 and 17 mg\kg b.wt. (2.72 ±0.44 and 3.93 ±0.07)µM for 45 days compared with the control group (1.32 ±0.24 µM). The results did not show a significant difference in the concentration of malondialdehyde within the same groups and for the different treatment periods in table (3). Malondialdehyde (MDA) is one of several by-products of the lipid peroxidation process and is a biomarker that indicates lipid peroxidation level [19]. The level of the MDA biomarker reflects the degree of oxidative stress [20,21]. In this study, the reason for the high MDA is due to the toxic effect of endosulfan. The same results were observed by Guo et al. [22] after intraperitoneal injection of EN (1, 5, and 10 mg kg−1) in wistar rats and by Oyovwi et al. [23], and by Hussein et al. [24] when Nile tilapia (Oreochromis niloticus) was exposed to 1/20 (12.795 µg/L) 96 h LC50 of the same insecticide.

Table 3. Effect of doses and period in MDA

Groups	Mean ± SE of MDA(µM)		LSD value
	21 Days	45 Days	
Control	1.32 ±0.24 B a	1.32 ±0.24 C a	0.403 NS
3 mg/kg	1.73 ±0.16 B a	2.72 ±0.44 B a	0.822 **
17 mg/kg	3.23 ±0.22 A a	3.93 ±0.07 A a	0.794 NS
LSD value	0.748 **	1.027 **	---
Means with different Capital letters in the same column and small letters in the same row are significantly different. . ** (P≤0.01).			

3.3. Effect on catalase enzyme (CAT)

The results in **Table (4)** showed a significant (P≤0.01) increase in the level of catalase enzyme in the two groups treated with concentrations 3 and 17 mg\kg b.wt. (1.90 ±0.12 and 3.27 ±0.29)IU/L, respectively, after 21 days compared with the control group (1.12 ±0.22 IU/L). It also showed a significant increase in the concentration in the treated group with 17 mg\kg b.wt. (3.27 ±0.29 IU/L) compared with 3 mg\kg b.wt. (1.90 ±0.12 IU/L). On the other hand, the results showed a significant increase in the enzyme concentration in the two treated groups, 3 and 17 mg\kg b.wt. (2.15 ±0.26 and 2.83 ±0.12)IU/L for 45 days compared to the control group (1.12 ±0.22 IU/L). The results did not show a significant difference in the level of Catalase enzyme within the same groups and for the different treatment periods. Many pesticides were associated with the induction of oxidative stress via the formation of ROS and alterations in antioxidant or free oxygen radical scavenging enzyme systems [25-27]. This may be a rise in CAT in treatment groups due to the overproduction

of free radicals and H2O2 that lead to Oxidative stress. These results did not agree with Hussein et al. [24] after Nile tilapia (Oreochromis niloticus) was exposed to 1/20 (12.795 µg/L) 96 h LC50 of EN and study of pregnant rats exposed to EN caused a significantly decreasing CAT activity both in liver and kidney [18]. And my results not agreed with Yan et al. [28] and [23].

Table 4. Effect of doses and period in CAT

Groups	Mean ± SE of CAT (IU/L)		LSD value
	21 Days	45 Days	
Control	1.12 ±0.22 C a	1.12 ±0.22 B a	0.226 NS
3 mg/kg	1.90 ±0.12 B a	2.15 ±0.26 A a	0.473 NS
17 mg/kg	3.27 ±0.29 A a	2.83 ±0.12 A a	0.598 NS
LSD value	0.775 **	0.741 **	---
Means with different Capital letters in the same column and small letters in the same row are significantly different. . ** (P≤0.01).			

3.4 Effect on liver function parameters

The results in **Table (5)** showed a significant increase in the ALT enzyme level in the treated groups with 3 mg\kg b.wt.(27.33 ±1.20) (42.67±1.76) IU/L and 17 mg/kg (40.00 ±2.88)(59.33 ±4.97) IU/L after 21 and 45 days respectively of treatment compared to the control group (19.00 ±2.31) IU/L. The results in the table also showed a significant increase in the ALT enzyme in the treated group with 3 mg/kg (42.67 ±1.76) IU/L after the passage of 45 days compared to the enzyme level for the same group after 21 days (27.33 ±1.20) IU/L. Also, the group treated with 17 mg/kg showed a significant increase in the enzyme level after 45 days(59.33 ±4.97) IU/L, compared with the enzyme level for the same group after 21 days of treatment(40.00 ±2.88) IU/L. On the other hand, the results in **Table (6)** showed that there was a significant (P≤0.01) increase in the AST enzyme level in the treated groups with 3 mg\kg b.wt.(30.67 ±1.76) (39.66 ±1.76) IU/L and 17 mg\kg b.wt (37.66 ±2.96)(52.33 ±3.17) IU/L after 21and 45 days, respectively, of treatment compared to the control group (23.66 ±2.02) IU/L as the results of the study showed a significant (P≤0.01) increase in the AST enzyme in the treated group with 3 mg\kg b.wt (39.66 ±1.76) IU/L after 45 days compared to the enzyme level for the same group after 21 days (30.67 ±1.76) IU/L. Moreover, the group treated with 17 mg\kg b.wt showed a significant increase in the enzyme level after 45 days (52.33 ±3.17) IU/L, compared with the enzyme level for the same group after 21 days of treatment (37.66 ±2.96) IU/L. Enzymes of liver activities were employed as vital biomarkers for the discovery of the hepatotoxic nature of this pesticide. In this study, two hepatic serum marker enzymes, ALT and AST, were increased for hepatotoxicity. Results revealed that EN treatment caused an increase in the activities of liver enzymes in the serum of male mice. These results agreed with Hussein et al. [24] and Bouhafs et al. [18].

Table 5. Effect of doses and period in ALT

Groups	Mean ± SE of ALT (IU/L)		LSD value
	21 Days	45 Days	
Control	19.00 ±2.31 C a	19.00 ±2.31 C a	3.75 NS
3 mg/kg	27.33 ±1.20 B b	42.67 ±1.76 B a	6.84 **
17 mg/kg	40.00 ±2.88 A b	59.33 ±4.97 A a	7.03 **
LSD value	7.76 **	11.51 **	---

Means with different Capital letters in the same column and small letters in the same row are significantly different. . ** (P≤0.01).

Table 6. Effect of doses and period in AST

Groups	Mean ± SE of AST (IU/L)		LSD value
	21 Days	45 Days	
Control	23.66 ±2.02 B a	23.66 ±2.02 C a	2.95 NS
3 mg/kg	30.67 ±1.76 AB b	39.66 ±1.76 B a	5.79 **
17 mg/kg	37.66 ±2.96 A b	52.33 ±3.17 A a	7.42 **
LSD value	7.99 **	8.31 **	---

Means with different Capital letters in the same column and small letters in the same row are significantly different. . ** (P≤0.01).

3.5: Effect endosulfan on deoxyribonucleic acid (DNA):

In **Table (7)**, the results showed no significant differences between the groups regarding the low damage of DNA. At the same time, the results showed a significant increase in low damage of DNA in the treated group 3 mg\kg b. wt. for 45 days (14.00 ±1.00)% compared with the same treated group for 21 days (4.50 ±0.50)%.

In **Table (8)**, the results showed no significant differences between the groups regarding the medium damage of DNA. Regarding the considerable damage of DNA, **Table (9)** results showed a significant increase in great damage in the treated group 17 mg\kg b.wt. (22.50 ±2.50)% for 21 days compared to the control group (3.00 ±1.00)% and the treated group 3 mg\kg b. wt. (9.00 ±1.00) for the same period.

The results also showed a significant increase in the high damage of DNA in the treated group 17 mg\kg b.wt. (25.00 ±1.00)% for 45 days, compared with the control group (3.00 ±1.00) % and the treated group 3 mg\kg b. wt. (16.00 ±1.00)%. Finally, table (10) results showed that the percentage of normal DNA decreased significantly in the treated group 17 mg\kg b. wt. for 21 days (57.50 ±2.50)%. compared to the control (82.50 ±2.50)% and treated group 3 mg\kg b. wt. for 21days (77.00 ±1.00)%. As well in the two treated groups for 45 days (60.00 ±2.00 and 54.50 ±2.50) % compared with the control group (82.50 ±2.50) %.

Endosulfan is a genotoxic chemical that damages DNA in rats, fish, and clams [29-31]. Damage in DNA observed in the current study could have originated from DNA single-strand breaks, double-strand breaks, DNA-DNA/DNA protein cross-linking, or inhibition of enzymes concerned in DNA repair resulting from the interface of pesticides or their metabolites with DNA [32]. Dose and time-related increase in DNA damage in the form of comet induction was observed during the present investigation. Khisroon et al. [33] surveyed the damage in grass carp after exposure to three different concentrations, 0.75 ppb, 1.00 ppb, and 1.50 ppb, of endosulfan. The DNA damage in 7, 14, 21, and 28 day was more significant in the treated groups than in the control group. As well as Shao et al. [26] noted endosulfan-induced DNA damage in zebrafish. A study by Guo et al. [22] indicated that endosulfan could cause severe DNA damage in spermatogenic cells of rats at concentrations from 12 to 24 µg. ml^{-1}.

Table 7. Effect of doses and period in Comet Assay // Low%

Groups	Mean ± SE of Low %		LSD value
	21 Days	45 Days	
Control	9.50 ±0.50 A a	9.50 ±0.50 A a	1.72 NS
3 mg/kg	4.50 ±0.50 B b	14.00 ±1.00 A a	3.278 **
17 mg/kg	11.50 ±0.50 A a	10.00 ±2.00 A a	1.85 NS
LSD value	2.251 **	5.95 NS	---

Means with different Capital letters in the same column and small letters in the same row are significantly different. . ** (P≤0.01).

Table 8. Effect of doses and period in Comet Assay // Medium %

Groups	Mean ± SE of Medium %		LSD value
	21 Days	45 Days	
Control	5.00 ±1.00 A a	5.00 ±1.00 A a	1.07 NS
3 mg/kg	9.50 ±0.50 A a	10.00 ±2.00 A a	2.69 NS
17 mg/kg	11.00 ±3.00 A a	10.50 ±3.50 A a	2.08 NS
LSD value	8.32 NS	6.79 NS	---
NS: Non-Significant.			

Table 9. Effect of doses and period in Comet Assay // High%

Groups	Mean ± SE of High %		LSD value
	21 Days	45 Days	
Control	3.00 ±1.00 B a	3.00 ±1.00 C a	1.19 NS
3 mg/kg	9.00 ±1.00 B b	16.00 ±1.00 B a	6.21 **
17 mg/kg	22.50 ±2.50 A a	25.00 ±1.00 A a	3.98 NS
LSD value	7.46 **	4.50 **	---
Means with different Capital letters in the same column and small letters in the same row are significantly different**.** . ** ($P \leq 0.01$).			

Table 10. Effect of doses and period in Comet Assay // Normal%

Groups	Mean ± SE of Normal %		LSD value
	21 Days	45 Days	
Control	82.50 ±2.50 A a	82.50 ±2.50 A a	5.83 NS
3 mg/kg	77.00 ±1.00 A a	60.00 ±2.00 B b	7.04 **
17 mg/kg	57.50 ±2.50 B a	54.50 ±2.50 B a	5.69 NS
LSD value	9.54 **	10.56 **	---
Means with different Capital letters in the same column and small letters in the same row are significantly different. . ** (P≤0.01).			

3.Conclusions

The study results show that the endosulfan insecticide poses a toxic role on renal function and liver function parameters in white mice. Furthermore, endosulfan is a genotoxic chemical that induces DNA damage.

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