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Detoxification of Dietary Diazinon by Clay, Vitamin C and Vitamin E in Rabbits

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Abstract: This work was carried out to evaluate the ability of clays, vitamin C and E on detoxification of Diazinon (DZN) in rabbit diets. Eighty growing New Zealand White (NZW) male rabbits were randomly divided into 8 equal groups (10 in each). The first group served as control (without contamination), the second group was contaminated with 12.5 mg DZN / kg diet (1/8 LD₅₀). The other experimental groups were contaminated by the same level of DZN plus clays (at levels 0.5 or 1 % / kg diet), vitamin C (at levels 500 or 750 mg / kg diet) and vitamin E (at levels 25 or 50 mg/kg diet). The obtained results showed that addition clays (at levels 0.5 or 1% / kg diet), vitamin C (at levels 500 or 750 mg / kg diet) and vitamin E (at levels 25 or 50 mg / kg diet) were significantly (p<0.05 or 0.01) improved daily feed intake, feed conversion, water intake, final body weight, daily body gain, digestibility of all nutrients, dressing percentage, liver weight, kidney weight, rectum temperature, respiration rate, blood biochemical (including serum total protein, albumin, blood glucose, uric acid, createnine, ALT and AST) and blood haematological (including RBC's, Hb and WBC's). The serum globulin insignificantly decreased in rabbits fed polluted with DZN only in comparison with fed DZN plus additives and control. On the other hand, the weights of liver and kidney as % of carcass weight were significantly (p< 0.01) increased with DZN. In conclusion, the addition of clays (at levels 0.5 or 1\% / kg diet), vitamin C (at levels 500 or 750 mg/kg diet) and vitamin E (at levels 25 or 50 mg/kg diet) to growing rabbit diets were safe and practical method to minimize the DZN toxicity.

Key words: Diazinon · Clay · Vitamin C · Vitamin E · Production · Carcass · Blood · Rabbits

INTRODUCTION

Pesticides are commonly used agricultural chemicals. The effects of pollutants on nature became a subject of interest for scientists beginning in the second half of the 20 century and, subsequently, investigations of the effects of these pollutants on human beings, plants and animals were initiated. Pesticides use has risen considerably in the recent past. In addition to its primary target, pesticides can also affect human and animal in the vicinity of insecticide sprayed area. Once the insecticides enter the body, it is transported to different parts of it through the blood [1].

Diazinon (DZN), is a commonly used organophosphorous (OP) pesticide to control a variety of insects in agriculture and in the environment. DZN, is an organophosphorous insecticide with antcholinesterase

mode of action [2,3]. Mild structural and functional change in liver as well as in test of experimental mice was observed after single intraperitoneal administration of DZN [4]. The exposure of zebra fish to DZN for up to 168 hours a significantly reduced the total protein in liver [5]. Jyostana et al. [6], observed a significant biochemical and hematological alterations due to the exposure to the various pesticides, also significant damage in hepatic cells and glucose metabolism in liver was observed as the result of DZN administration [7]. Clay materials is a crystalline aluminosilscates characterized by its ability to exchange cations without major changes in structure; it is used in animal diets to improve digestibility of nutrients [8-10], daily gain and feed intake [11]. It can absorb toxic products of digestion and decreases the accumulation of toxic substances in tissues, thus decreasing the incidence of internal disorders [12,13]. Vitamin C as antioxidant

nutrient protects the tissues from the harmful effect of ROS through its free radical scavenging activity [14-17]. Vitamin C supplementation can help the stressed animals by maintaining the normal metabolic functions of the body [18,19] and/or by improving disease resistance via optimizing the function of the immune system [20]. Vitamin E is a primary antioxidant that plays an important role in protecting cells against toxicity by inactivating free radicals generated following pesticides exposure. At the last 10 years in developing countries, the number of peoples suffering from various liver and kidney diseases were increased. Therefore, the present study was undertaken to investigate the possible protective effect of clay, vitamin C and vitamin E against DZN- induced adverse effects on the performance of growing rabbits, digestibility and some physiological traits, hematological and biochemical indices.

MATERIALS AND METHODS

A total of eighty weaning male New Zealand White (NZW) rabbits at 35 days of age and nearly equal average initial live body weight were used in the present study. Rabbits were picked up from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia. They were housed in groups of 5 per plastic cage, maintained under standard laboratory conditions (temperature 22±1°C, 12:12 h light: dark cycle) and offered balanced standard diet (The animals were feed basal diet consisted of 28% alfalfa hay, 18% barley, 18% soybean meal (44%), 25% wheat bran, 6% yellow corn, 3 % molasses, 1.1% limestone, 0.3% sodium chloride, 0.6 % vitamin and mineral premix. The basal diet contained of 18.18 % crude protein, 13.43% crude fiber, 2.29% ether extract, 2656.00 digestible energy (kcal/kg).) with free access of water. The protocol of the present study was approved by the Animal Care and Use Committee of King Abdul Aziz University.

Diazinon 60 EC (Fig. 1) was applied as a commercial emulsifiable concentrate formulation containing 60% active ingredient. It was diluted in deionized water for the final concentration. The acute oral LD50 for male rabbits was determined by Elisa M.A. Salih [21] and it was found to be 100 mg/kg body weight. The animals were randomly assigned to 8 treatment groups (10 in each) to study the ability of clay, vitamin C and E to detoxify of DZN in diet as following:

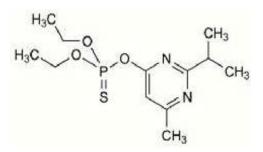


Fig. 1: The chemical structure of Diazinon.

The Experimental Groups Were:

T1 = Control (untreated)

 $T2 = 2.5 \text{ mg DZN } (1/8 \text{ of LD50}) \setminus \text{kg diet}$

 $T3 = D + 0.5 \text{ g clay } \setminus \text{kg diet}$

 $T4 = D + 1.0 \text{ g clay } \setminus \text{kg diet}$

T5 = D + 500 mg vitamin C \kg diet

 $T6 = D + 750 \text{ mg vitamin } C \setminus kg \text{ diet}$

T7 = D + 25 mg vitamin E \kg diet

 $T8 = D + 50 \text{ mg vitamin } E \setminus kg \text{ diet}$

Live body weight was recorded individually for each rabbit at 5, 9 and 13 weeks of the age, then weight gain was calculated. Feed intake was determined precisely and calculated as gram per rabbit per day. Unused feed from each cage was collected daily, weighed and taken into consideration for calculation of feed intake. Feed conversion was also estimated (g feed / g gain). The rectal temperature and respiration rate were measured in rabbits once every two weeks at 9-11 a.m. Respiration rate was recorded by a hand counter, which counts the frequency of the flank movement per minute. Internal body temperature was taken by medicine thermometer inserted into the rectum for 2 minutes at depth of 2 cm. At the end of the experimental period three male rabbits from each group were randomly taken for slaughter, after complete bleeding, pelt, visceras and tail were removed and the carcass and some carcass components were weighted. The blood samples were collected from rabbits during the slaughter. The blood in the heparinized tubes was immediately used for hematological measurements including red blood corpuscles (RBC) count, hemoglobin (Hb) concentration and white blood corpuscles (WBC) count. Hematological parameters were assessed using AD VIA Hematology Automatic System (USA). The blood in non-heparinized tubes was centrifuged at 3000 rpm for 20 minutes. The clear supernatants sera were frozen till the time of various biochemical estimations including the levels of creatinine, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST). Total protein, albumin, creatinine and glucose concentration in plasma were estimated using commercial kits (Bio Merieux, France) according to the procedure outlined by the manufacturer. The globulin values were obtained by subtracting the values of albumin from the corresponding values of total protein.

The obtained data were statistically analyzed by using completely randomize design according to Snedecor and Cochran (1982) by the following model: $Xij = \mu + Ti + eij$ where, $\mu = general mean$, Ti = fixed effect of the treatments (1,....8) and eij = random error. The differences between experimental groups were separated by Duncan s multiple range test [22].

RESULTS AND DISCUSSION

The present study was undertaken to investigate the possible protective effect of clay, vitamin C and vitamin E against DZN- induced adverse effects on the performance of growing rabbits, digestibility and some physiological traits, hematological and biochemical indices. Following 9 weeks of DZN administration, changes of several parameters indicating the occurrence of injury were observed

Evaluation of Performance Traits: The average daily feed intake, feed conversion, water intake, final body weight and daily body gain (Tables 1 and 2) were decreased (p<0.01, 0.05) significantly by addition DZN (12.5mg/kg diet) when compared with the control group. In Feed utilization the same trend was observed by Berry and Gore [23], who found that the feed efficiency ratio decreased treated rats by pesticide. The decrease of feed intake agreed with those obtained by Berry and Gore [23]. Rajini et al. [24], Omayakhi and Ohrheruata, 2009) who reported that DZN toxicity caused loss of appetite. Also, the decrease in body weight and body weight gain agree with the Rajini et al. [24]; Rajini and Krishnakumari [25], Omayakhi and Ohrheruata, [26] who reported that pesticide additions decreased the body weight of rats. Decreasing the feed intake, live body weight and body daily gain may be due to bad effect of DZN on rabbits health (headache, nausea, skin irritation, runny nose, abdominal pain, vomiting, disease of nervous, hematopoietic, cardiovasculs system, liver and kidneys function), loss of appetite (Dahlgren, et.al. 2004).

Clay, vitamin C and vitamin E (Tables 1 and 2) addition to rabbit contaminated diets improved (p<0.01) significantly daily feed intake, feed conversion, water intake, live body weight and gain compared with those fed DZN diet alone. This results were agreed with those obtained by [27] who found that addition clay to rabbit contamination diets by insecticide improved the feed intake, feed conversion, body weight and body gain. Also, Simn and Eriksson (1997), found the same trend when treated rats by vitamin C and, Sibel oimoek, et.al. (2006), in vitamin E. Clay materials is a crystalline aluminosilscates characterized by its ability to exchange cations without major changes in structure; it is used in animal diets to improve digestibility of nutrients [8-10], daily gain and feed intake [11]. It can absorb toxic products of digestion and decreases the accumulation of toxic substances in tissues, thus decreasing the incidence of internal disorders [12,13]. Vitamin C as antioxidant nutrient protects the tissues from the harmful effect of ROS through its free radical scavenging activity [14-17]. Vitamin C supplementation can help the stressed animals by maintaining the normal metabolic functions of the body [18,19] and/or by improving the function of the immune system [20]. Vitamin E is plays an important role in protecting cells against toxicity by inactivating free radicals generated following pesticides exposure.

By comparing to control group, the lowest statistically significant changes were detected in the intake for both feed and water for groups treated with clay 1%/kg diet plus DZN (Table 1). While there was a significantly improve in the feed conversion. Nevertheless, this effect could have be improved by treatment with DZN and vitamin E (25 mg \kg diet) where feed conversion was improved to 6.10, intake for feed and water to 90.4 and 90.7 respectively.

Evaluation Digestibility Traits Changes: In Table (3) the result showed a significant decrease in the ability of the animal to digest the food with Diazinon treatment. This results were agreed with obtained by Bamikole *et al.* [28], Maigandi and Hadejia [29], Omayakhi and Ohrheruata, [26] and Shehata, [30], who reported that feeding growing rabbits by toxic materials decreased significantly (p<0.01) all digestibility traits. Bersenyi *et al.* [31]. Who, reported that crude protein (CP) digestibility decreased when rabbits fed diets contain high level of toxic. Also, Fekete *et al.* [32] reported decrease in digestibility of organic matter (OM) and nitrogen free extract (NFE) in rabbits fed diet containing high level of lead. Decreasing the

Table 1: Means and standard errors ($\bar{x}\pm$ S.E.) for feed intake, feed conversion and water intake of NZW male rabbits as affected by diazinon contamination and its decontamination.

Items	Feed intake	Feed conversion	Water intake
Control	118.1ª ±3.0	6.07 b±0.20	128.1 a ±6.46
Diazinon 1/8 DL50 (D)	$61.3^{e} \pm 2.1$	7.96 a ±0.46	$76.5^{e} \pm 6.99$
D+ caly (0.5% \kg diet)	$98.4^{\text{cd}} \pm 4.5$	$6.12^{\mathrm{b}} \pm 0.20$	100.7 b ± 3.66
D+ caly (1.0% \kg diet)	113.0 ab±4.0	$6.37^{\text{ b}} \pm 0.24$	105.4 bc±3.88
D+ vitamin C (500 mg \kg diet)	$101.4^{cd} \pm 3.5$	$6.36^{\text{ b}} \pm 0.17$	99.2 bc ±3.91
D+ vitamin C (750 mg \kg diet)	$105.0^{\text{bc}} \pm 4.0$	6.43 b ±0.31	95.4 bc ±3.88
D+ vitamin E (25 mg \kg diet)	$90.4^{d} \pm 4.5$	$6.10^{\text{ b}} \pm 0.32$	$90.7^{bc}\pm3.66$
D+ vitamin E (50 mg \kg diet)	93.6 d ±3.5	$6.21^{\text{ b}} \pm 0.38$	89.2 ce ±3.91
Sig.	**	*	**

NS = Not significant, * = P < 0.05 and ** = P < 0.01

Means bearing different letters in the same column within each factor differ significantly ($P \le 0.05$).

Table 2: Means and standard errors (5± S.E.) for some NZW male rabbits performance as affected by diazinon contamination and its decontamination.

	Initial body weight	Final body weight (g)	
Items	(g) at 5 weeks of age	at 13 weeks of age	Daily body gain (g)
Control	651.0 ±11.8	1744.0° ±24.5	19.5°±0.32
Diazinon 1/8 DL50 (D)	670.0±26.1	$1113.2^{D} \pm 33.1$	$7.9^{E} \pm 6.99$
D+ caly (0.5% \kg diet)	642.5±19.9	$1544.0^{BC} \pm 27.9$	$16.1^{CD} \pm 0.52$
D+ caly (1.0% \kg diet)	632.2±26.6	$1627.0^{\mathrm{B}} \pm 22.9$	$17.8^{\mathrm{B}} \pm 0.27$
D+ vitamin C (500 mg \kg diet)	662.4±17.6	$1556.1^{BC} \pm 28.1$	$15.95^{CD} \pm 0.37$
D+ vitamin C (750 mg \kg diet)	641.0±26.3	$1562.6^{BC} \pm 27.8$	$16.4^{BC} \pm 0.44$
D+ vitamin E (25 mg \kg diet)	665.4±11.7	$1501.0^{\circ} \pm 26.6$	$14.9^{D} \pm 0.49$
D+ vitamin E (50 mg \kg diet)	665.4±25.6	$1505.0^{\circ} \pm 28.1$	15.4 ^{CD} ±0.75
Sig.	NS	**	**

NS = Not significant, * = P < 0.05 and ** = P < 0.01

Means bearing different letters in the same column within each factor differ significantly ($P \le 0.05$).

Table 3: Means and standard errors (Ext. S.E.) for digestibility traits of NZW male rabbits as affected by diazinon contamination and its decontamination.

Items	DM	OM	СР	CF
Control	65.7° ±1.02	67.3 a ±0.83	73.0° ±0.80	34.40 a ±0.73
Diazinon 1/8 DL50 (D)	$55.0^{d}\pm1.01$	$58.8^{\text{cde}} \pm 1.39$	$65.0^{\circ} \pm 0.86$	$19.90^{\text{ de}} \pm 0.84$
D+ caly (0.5% \kg diet)	$58.7^{bc}\pm0.97$	$60.8^{bc}\pm0.63$	$61.8^{d} \pm 0.74$	$24.50^{\circ} \pm 1.00$
D+ caly (1.0% \kg diet)	$60.7^{b}\pm1.25$	$61.9^{b}\pm1.14$	$67.3^{b}\pm0.83$	$28.90^{ b} \pm 0.67$
D+ vitamin C (500 mg \kg diet)	$58.4^{bc}\pm0.67$	$59.2^{cd} \pm 0.39$	$58.3^{\circ} \pm 0.54$	$21.00^{d} \pm 0.70$
D+ vitamin C (750 mg \kg diet)	$58.2^{bc}\pm1.25$	$58.8^{\text{ cde}} \pm 1.14$	$62.7^{d} \pm 0.98$	26.10°±0.73
D+ vitamin E (25 mg \kg diet)	$56.2^{cd} \pm 0.96$	$57.8^{de} \pm 0.63$	$57.6^{\mathrm{e}} \pm 0.76$	$22.10^{d} \pm 1.01$
D+ vitamin E (50 mg \kg diet)	$55.9^{\text{ cd}} \pm 0.67$	$56.1^{e} \pm 0.35$	$54.1^{\circ} \pm 0.50$	$18.40^{e} \pm 0.68$
Sig.	**	**	**	**

 \overline{NS} = Not significant, * = P<0.05 and ** = P<0.01

Means bearing different letters in the same column within each factor differ significantly ($P \le 0.05$).

Table 6a: Means and standard errors (5± S.E.) for blood components of NZW male as affected by diazinon contamination and its decontamination.

Items	Total protein g/dl	Albumin g/dl	Globulin g/dl	AST	ALT
Control	$7.12^a \pm 0.13$	$4.08^a{\pm}0.08$	3.04 ± 0.12	$35.0^{f} \pm 4.57$	$46.3^{g}\pm1.38$
Diazinon 1/8 DL50 (D)	5.12 ° ±0.10	$2.18^{d} \pm 0.06$	2.94 ± 0.15	78.5 a ±7.89	$174.9^a \pm 1.9$
D+ caly (0.5% \kg diet)	6.57 b ±0.97	$3.50^{bc} \pm 0.09$	3.07 ± 0.17	48.6 ° ±4.43	$88.9^{e} \pm 2.02$
D+ caly (1.0% \kg diet)	$6.67^{ab} \pm 0.17$	$3.40^{\circ} \pm 0.09$	3.27 ± 0.17	$40.8^{\text{ f}} \pm 2.04$	$70.5^{\mathrm{f}} \pm 3.69$
D+ vitamin C (500 mg \kg diet)	$6.86^{ab}\pm0.17$	3.82 ab ±0.11	3.04 ± 0.12	$55.2^{d} \pm 4.83$	$98.2^{d} \pm 2.02$
D+ vitamin C (750 mg \kg diet)	$7.03^{ab} \pm 0.18$	$3.71^{bc} \pm 0.13$	3.32 ± 0.09	$59.3^{cd} \pm 7.82$	109.6° ±2.24
D+ vitamin E (25 mg \kg diet)	$6.98^{ab} \pm 0.19$	$3.69^{bc} \pm 0.14$	3.29 ± 0.09	$68.1^{b}\pm8.52$	$116.6^{b}\pm2.20$
D+ vitamin E (50 mg \kg diet)	$6.97^{ab}\pm0.17$	$3.74^{b}\pm0.10$	3.23 ± 0.19	$62.9^{bc}\pm8.02$	111.4 b±2.47
Sig.	**	**	NS	**	**

 $\overline{\text{NS}} = \text{Not significant}, * = P < 0.05 \text{ and } ** = P < 0.01$

Means bearing different letters in the same column within each factor differ significantly ($P \le 0.05$).

Table 6b: Means and standard errors (x± S.E.) for blood components of NZW male rabbits as affected by diazinon contamination and its decontamination.

Items	Blood glucose g/dl	Uric acid g/dl	Createnine g/dl
Control	104.9 ^d ±2.74	3.98 ^d ±0.06	0.93 f ±0.08
Diazinon 1/8 DL50 (D)	258.7 a ±3.49	8.01 a±0.10	3.95 a±0.07
D+ caly (0.5% \kg diet)	186.3 b ±3.34	$5.43^{\circ} \pm 0.08$	$1.33^{e} \pm 0.08$
D+ caly (1.0% \kg diet)	157.8 ° ±4.25	$5.96^{ b} \pm 0.05$	$1.62^{d} \pm 0.08$
D+ vitamin C (500 mg \kg diet)	185.6 b ±2.84	$6.11^{b} \pm 0.06$	$1.90^{\circ} \pm 0.05$
D+ vitamin C (750 mg \kg diet)	190.7 b ±2.91	$6.03^{b} \pm 0.08$	$2.14^{b} \pm 0.05$
D+ vi	190.2 b ±3.99	$6.10^{b} \pm 0.08$	$2.32^{b}\pm0.06$
tamin E (25 mg \kg diet)	188.6 b ± 3.59	$6.14^{b}\pm0.07$	$2.28^{\mathrm{b}} \pm 0.09$
D+ vitamin E (50 mg \kg diet)	**	**	**
Sig.			

NS = Not significant, * = P < 0.05 and ** = P < 0.01

Means bearing different letters in the same column within each factor differ significantly (P≤0.05).

Table 6c: Means and standard errors (x ± S.E.) for blood hematology of NZW male rabbits as affected by diazinon contamination and its decontamination.

Items	RBC's	WBC's	НВ
Control	4.50° ±0.08	6.57 ^d ±0.18	12.61° ±0.14
Diazinon 1/8 DL50 (D)	$2.35^{g} \pm 0.13$	9.99 °±0.22	$8.02^{d}\pm0.12$
D+ caly (0.5% \kg diet)	3.81 ° ±0.06	$7.44^{\circ} \pm 0.22$	$10.87^{b}\pm0.19$
D+ caly (1.0% \kg diet)	4.17 b ±0.04	$9.15^{ab} \pm 0.22$	9.29°±0.11
D+ vitamin C (500 mg \kg diet)	$3.13^{\text{ f}} \pm 0.05$	$9.14^{b} \pm 0.25$	$9.06^{\circ} \pm 0.15$
D+ vitamin C (750 mg \kg diet)	$3.29^{\text{ ef}} \pm 0.06$	$9.56^{ab} \pm 0.24$	9.10°±0.08
D+ vitamin E (25 mg \kg diet)	$3.39^{\text{de}} \pm 0.05$	$9.66^{ab} \pm 0.15$	8.97°±0.11
D+ vitamin E (50 mg \kg diet)	$3.57^{-d} \pm 0.07$	$9.46^{ab}\pm0.19$	$9.05^{\circ} \pm 0.16$
Sig.	**	**	**

NS = Not significant, * = P < 0.05 and ** = P < 0.01

Means bearing different letters in the same column within each factor differ significantly (P \leq 0.05).

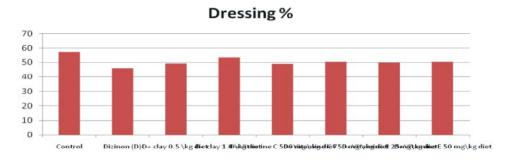


Fig. 1: Dressing % of NZW male rabbits as affected by diazinon and its decontamination.

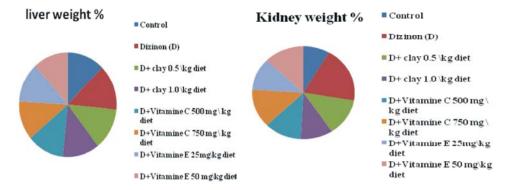


Fig. 2: Liver weight and kidney weight % of NZW male rabbits as affected by diazinon and its decontamination.

Rectum tempratuer Control Dizinon (D) D+ clay 0.5 kg diet D+ clay 1.0 kg diet D+Vitamine C 500 mg kg diet D+Vitamine C 750 mg kg diet D+Vitamine E 25mg kg diet D+Vitamine E 50 mg kg diet

Fig.3: Rectum temperature of NZW male rabbits as affected by diazinon and its decontamination.

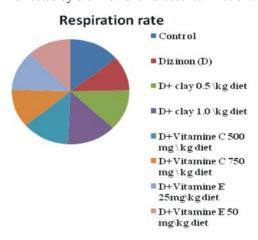


Fig. 4: Respiration rate of NZW male rabbits as affected by diazinon and its decontamination.

digestibility of nutrient by DZN may be due to inhibits the enzymes as acetylcholinesterase and disorders in liver and kidney function.

However, all treated groups with clay and vitamins showed significantly (p<0.01) differences in the raising level of digestibility which pointed by the group of DZN plus clay (1.0% \kg diet). This agreed with the report of Ayyat *et al.* [26] Biobaku and Dosumu [33], who found using clay supplementation was improved the digestibility traits. Increasing the digestibility of nutrient by addition clay and vitamins may be due to the increase of feed intake and reduce the disorders by using pesticide.

Evaluation of Some Carcass Traits Changes: As shown in Fig. (1 and 2) dressing percentage was decreased by DZN treatment. While the percentages for both liver and kidney were increased. This finding agreed with the report of Rivett *et al.* [34] where there were increase in liver

weights and liver body ratios seen in the group of dogs given 10 mg/kg body weight /day organophysphraus pesticide. Wekhe *et al.* [35] reported cardiac hypotrophy in an inverse trend to rising dosage of rabbit with crude oil. The liver and lung also responded in a similar inverse trend with a paler the spleen was statistically different (P < 0.05) among gaits, loss of appetite, dullness, rough fur and loss of the treatment groups. This agreed with the observation hair. Some of these clinical signs were acute, which was not striking but persistent throughout the experimental period.

The dressing percentage, after treatment with DZN plus clay 1.0%/kg diet, was increased by 7.1 when compared with DZN treatment. Also, DZN supplemented with vitamin E (50 mg \kg diet) group was increased by 4.3 when compared with DZN treatment group. While the percentages for both liver and kidney were decreased with DZN plus clays and vitamins . This agreed with the report of Omayakhi and Ohrheruata, [25] and Ayyat et al. [26], who found using clay supplementation was improved the carcass traits. Increasing the carcass traits by addition clay and vitamins may be due to the increase of feed intake and reduce the disorders by using pesticide.

Evaluation of Physiological Changes: DZN-treated groups and control rabbits show a significantly (p<0.01) difference in rectum temperature. In the other side when animals treated with DZN supplemented with (1.0% \kg diet) and vitamin C (500 mg \kg diet) respiration rate was significantly (p<0.05) increased up to 103-and 100.5 respectively when compared with DZN-treated (FIg. 3 and 4). Improving of rectum temperature and respiration rate by addition clays and vitamins may be due to the increase of bio metabolic rate activity, feed intake and reduce the disorders by using pesticide

Evaluation of Biochemical Changes: DZN administration significantly (p<0.01) decreased the level of blood components (protein and albumin), also the blood globulin insignificantly decreased. Meanwhile, a significant (p<0.01) increased in AST and, ALT was detected (Table 6 a). The total protein content significantly increases up to 7.03 in the group supplemented with vitamin C (750 mg \kg diet). As compared to DZN treated level of albumin blood and globulin were improved by treatment with DZN plus vitamin C (500 mg \kg diet). As presented in table 6b, DZN treatment was related to the significant (p<0.01) raise of blood glucose, uric acid and createnine in male rabbits. However, supplemented with

vitamin C and E showed significant (p<0.01) slightly recover on the blood components. Animal blood glucose level was diminished to 157.8 by supply the food with DZN and clay (1.0% \kg diet). The treatment of DZN plus clay (0.5% \kg diet) observed some significant (p<0.01) recovery in both uric acid and createnine levels. This results were agreed with obtained by Dikshith et al. [4], Ansari and Kumar [5], Al-Attar and Wafaa Al-Taisan [36], Salih (2010), who found that exposure of rabbits to diazinon significantly (p<0.01) increased the levels of serum ALT, AST, uric acid, creatinin and blood glucose. Also, Enan et al. [37] showed that the oral administration of diazinon into white rats for four wk exerted a significant inhibition to four serum enzymes. These included glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GTP). The negative changes in blood biochemical of animals administrated by diazinon may be due to reduce the carbohydrate and protein metabolism [38] and affected the amoinotransferase activity [7]. This inhibition was enhanced by the addition of ascorbic acid into the diet. The above mentioned effects of organophosphorus pesticides colud be due to the ability to from free radicals [39,40]. The results of many works on organophosphorus pesticide showed that the using antioxidants vitamins C and E reduce the toxicity of pesticide [41]. This fact may ensure the hypothesis of the ability of organophosphours pesticides to from free radicals, which have been implicated as playing a role in aetiology of many alterations [42].

Evaluation of Hematological Changes: As shown in table 6c, DZN administration significant (p<0.01) decreased Hb concentration and RBCs count. However, food supplemented with clay improve the blood hematology for both DZN plus clay (0.5% \kg diet) and clay (1.0% \kg diet). This results were agreed with obtained by Bhatnagar, [43], Ray, [44], Elias and Saif [41], Al-Attar and Wafaa Al-Taisan [36] and Elias [20], who reported that Red blood corpuscles (RBC) count, white blood corpuscles (WBC) count and hemoglobin (Hb) concentrations were significant (p<0.01) affected in male rabbits exposed to diazinon addition. In rabbits treated by organophosphorus pesticides using antioxidants vitamins C and E significantly (p<0.01) improved the blood hematology [41] and [25]. The poisoning by pesticide residues leads to the development of anaemia due to interference of Hb biosynthesis and shortening of the life span of circulating erythrocytes [45,6].

CONCLUSION

Based upon these results, we suggest that addition clays, vitamins C and E to contaminated rabbit diets can be considered as a promising therapeutic agent against hematotoxicity, immunotoxicity, hepatoxicity and nephrotxicity induced by diazinon and may be against other chemical pollutants, environmental contaminants and disorders factors.

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