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Histopathological and Biochemical Studies on the Effect of Diazinon with Ameliorating Effect of Garlic Extract in Rats

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Abstract: This study was carried out to determine the antioxidant effect of garlic against diazinon toxicity. Thirty albino rats were equally divided into 3 groups (n= 10). Group 1 was received soybean oil (1.4 mg/kg) and used as control. Group 2, 3 was injected intraperitoneally (ip) with (0.1mg/kg b.wt) of diazinon (DZN). Group 3 was injected ip with diazinon plus 0.5 mg/kg b.wt garlic extract. All rats in all groups were treated daily and sacrificed at 35 days from the beginning of experiment. Blood and serum were collected for hematological and biochemical examination. Samples from liver and kidneys were collected for histopathological detection. DZN-treated animals (group.2) exhibited significant increase in red blood cells, white blood cells and thrombocytes count. While, there was significant decrease in hemoglobin concentration. Furthermore, neutrophilia, lymphopenia, monocytopenia and eosinophilia were noticed in differential leukocytic count when compared with group1. However, group 3 revealed an improvement in the hematological parameters. Biochemical finding showed significant increases in liver and kidneys parameters in group 2,3 (ALP), AST, ALT.) and (urea, uric acid and creatinine), respectively in compared to group 1. Histopathologically, DZN toxicity (group 2) induced necrosis in the renal tubular epithelium and hepatocytes associated with inflammatory cells infiltration. In addition, congestion was seen in blood vessels with thrombus. On the other hand, garlic extracts treatment (group 3) moderate regeneration in liver and kidneys tissues. These findings demonstrated that garlic extract treatment can progress to recovering the toxic effects of DZN in albino rats.

Key words: Albino Rats · Diazinon · Garlic Extract · Hematology · Biochemical · Histopathological Examination

INTRODUCTION

Diazinon is the most common name for a synthetic organophosphate pesticide [1, 2]. Diazinon is widely used in agriculture to control insects and pests on a variety of fruit, vegetable, nut and field crops. It also used non-lactating cattle in an insecticidal ear tag. It also applied to lawns for insect control simultaneously with nitrogenous fertilizers such as urea [3]. Diazinon when reach human and animals through inhalation, ingestion and/or dermal exposure [4] it could induce signs of toxicity like lacrimation, salivation, anorexia, coughing [5] cardiac signs as tachycardia, convulsions and mydriasis with massive oral exposures [6]. Nervous system signs and symptoms may also be arisen such as restlessness and/or hyperactivity, depressed [7] anxiety depression, seizures [8] and coma [9]. Diazinon toxicity caused hematological deteriorations [10] and also induced histopathological changes in different organs and other related biochemical changes completely [11]. There are some natural antioxidant agents as garlic has the ability to neutralize toxic effect generated from diazinon through scavenge reactive oxygen and nitrogen species, increase enzymatic and non-enzymatic antioxidants level, also it activate Nrf2 factor, moreover, inhibit some prooxidant enzymes (Xanthine oxidase, cyclooxygenase and NADPH oxidase) [12]. The aim of this study is to investigate the antioxidant effect of garlic against diazinon toxicity.

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MATERIALS AND METHODS

Thirty (30) adult female albino rats were used in this experiment, weighting about 200gm, were obtained from the laboratory animal house of the National Research Center, Dokki, Cairo, Egypt. The animals were housed in stainless steel cages on 12 h. light/dark cycle at a room temperature of 20±2°C and 50-70 % relative humidity. The animals were given water and standard pellet diet for 2 weeks for adaptation.

Chemicals:

- Diazinon was obtained from produced by Almasryia Co., Egypt.
- Soyabean oil was obtained from Company for organic for natural oils.
- Garlic was purchased from local market belonging to Qena governorate, Egypt.
- Biochemical determination including liver function tests as AST(Aspartate Aminotransferase) [13] ALT (Alanine Aminotransferase) [14] and ALT(Alanine Aminotransferase) [15] Purchased from Randox Laboratories Ltd, 55 Diamond Road, Crumlin, Country Antrim, BT29 4QY, United Kingdom; kidneys function tests as urea [16] creatinine [17] and uric acid [18] were purchased from bio-diagnostic Co. (Dokki, Giza, Egypt).

Experimental Design: Thirty albino rats were divided into 3 groups (n= 10). All rats in all groups treated daily for 35 days.

Group (1): The rats were received intraperitoneally (ip) with soybean oil (1.4 mg/kg) and used as control.

Group (2): The rats were injected ip with (0.1mg/kg) of diazinon.

Group (3): it received diazinon ip (0.1mg/kg) plus garlic extract ip (0.5 mg/kg).

The experiment was established for a period 35 days. The clinical signs and mortality rate of the animals were daily recorded. All rats were anaesthetized by halothane, blood and serum samples were taken from orbital vinous plexus using glass capillaries for hematology and biochemical examinations.

Histopathology Examination: Tissues specimens from liver and kidneys were collected and fixed in 10% phosphate buffer formalin, dehydrated in alcohols and embedded in paraffin wax. Sections of 5µm thickness were prepared and stained with hematoxylin and eosin stain (H&E) for microscopic examination.

Statistical Analysis: The obtained data were subjected to one-way analysis of variance (ANOVA). It was done to compare between groups, followed by post-hoc analysis (Dunnett's test) using SPSS (Statistical Package for Social Sciences) version 17 [19]. The data was expressed in form of Mean ± Standered Error (SE).

RESULTS

Hematological Findings

Blood Parameter: Table (1) was shown significant increase in RBCs of group 2 when compared with group (1, 3), while the group 3 recorded non-significant increases with group 1, 2. WBCs count showed significant increase in group 2 when compared with control but group 3 displayed significant decrease in comparison with group 2. Hemoglobin was significant decrease in group 3 when compared group 1, 2. PCV % of group 3 recorded non-significant change when compared with group 1, 2. MCV values recorded significant decrease in group 2 when compared with control, while there was significant increase in group 3 when compared with group 2. MCH recorded significant decrease in group 3 when compared with control. MCHC recorded significant decrease in group 3 when compared with control and group 2. Additionally, platelets count were significantly changes when compared with control and diazinon groups.

Table (2) was shown, neutrophils percentage exhibited significant increase in group 2, 3 when compared with control, while it significant increase in group 3 in comparison with group 2. Lymphocytes exhibited significant decrease in groups 2, 3 when compared with control, while significant decrease was detected in group 3 in comparison with group 2. Monocytes percentage displayed significant decrease in group 2 when compared with control as well as, significant increase in group 3 when compared with group 2. There was significant increase was detected in eosinophils percentage groups 2& 3 when compared with control, while significant increase in group 3 in compared with group 2. Basophils percentage was significantly increased in group 3 when compared with control 1, 2. Band cells percentage exhibited significant increase in group 2 in comparison with group (1, 3).

Table 1: The Mean ± Standard Error of Blood parameters of albino rats of Group 1 (Control), Group 2 (Diazinon), Group 3 (D+ garlic)

	RBCs ($x10^6$)	WBCs ($x10^3$)	Hb. (gm/dl)	PCV %	MCV (Fl)	MCH (Pg)	MCHC %	Platelets ($x10^9$)
Group (1)	4.8±0.17	6.8±0.61	9.8±0.74	26.3±2.2	49.3±0.53	18.4±0.48	37.3±0.83	130.7±3.3
Group (2)	6.3±0.26[a]	11.1±0.54[a]	10.8±0.38	25.6±1.5	44.5±0.6[a]	16.7±0.31	37.7±0.72	418.7±6.9[a]
Group (3)	5.5±0.28	7.6±0.73 [b]	8.1±0.43[a]	25.9±1.2	47.5±1.7	15.7±1.1[a]	31.3±3.4[ab]	280.7±20.5[ab]

a → The mean difference is significant in comparison with control (Group 1) at 0.05.

b →The mean difference is significant in comparison with Diazinon group (Group 2) at 0.05.

Table 2: The Mean ± Standard Error of the differential leucocytic counts of the albino rats of Group 1 (Control), Group 2 (Diazinon), Group 3 (D+ garlic).

	Neutrophil %	Lymphocytes %	Monocytes %	Eosinophil %	Basophils %	Band %
Group (1)	15.3±0.33	77.3±1.4	4.6±1.4	2.6±0.33	0 ±0.0	0 ±0.0
Group (2)	36.0±3.0 [a]	57.3±1.4 [a]	1.0±0.57 [a]	4.0±1.0 [a]	0 ±0.0	1.6 ±0.88 [a]
Group (3)	47.6±5.8[ab]	38.6±3.1[ab]	5.3±2.4 [b]	5.6±1.2 [a]	2.0±1.1[ab]	0.66±0.66

a →The mean difference is significant in comparison with control (group 1) at 0.05.

b →The mean difference is significant in comparison with Diazinon group (group 2) at 0.05.

Table 3: Mean ± Standard Error of liver function tests including SPOT/AST, SGPT/ALT and ALP (IU/l) of the albino rats of Group 1 (Control), Group 2 (Diazinon) and Group 3 (D+ garlic)

	Liver Function Tests		
	SPOT/AST (IU/l)	SGPT/ALT (IU/l)	ALP (IU/l)
Group (1)	22.3±1.4	118.7±4.6	84.6±2.4
Group (2)	43.3±0.88 [a]	167.0±2.1 [a]	103.3±4.4 [a]
Group (3)	42.6±1.4	197.7±5.0 [a]	74.6±2.3 [ab]

a →The mean difference is significant in comparison with control (Group 1) at 0.05

b → The mean difference is significant in comparison with Diazinon group (Group 2) at 0.05.

Table 4: Mean ± Standard Error of kidney function tests including Urea, Creatinine and uric acid (gm/dl) of the albino rats of Group 1 (Control), Group 2 (Diazinon), Group 3 (D+ garlic)

	Kidney Function Tests		
	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group (1)	37.3±0.88	0.63±0.03	1.9±0.03
Group (2)	41.6±1.2 [a]	0.86±0.03	2.4±0.1 [a]
Group (3)	38.6±0.66	0.76±0.1	2.3±0.17

a →The mean difference is significant in comparison with control (group 1) at 0.05.

b →The mean difference is significant in comparison with Diazinon group (group 2) at 0.05.

Table (3) showed the effect of diazinon toxicity on the liver enzyme activity. It exhibited significant increase in AST, ALT and ALP enzyme of groups 2 and 3 when compared with control.

Table (4) exhibited significant increase in urea level of group 2 in comparison with control group, while group 3 exhibit significant decrease of urea when compared with diazinon group 2. Creatinine level was detected in significant changes in comparison with both control and group 2. Moreover, uric acid of group 2 displayed significant increase in comparison with control. Group 3 recorded non-significant changes in uric acid level when compared with group 2.

Histopathological Finding: Histopathological finding showed vacuolization which detected in hepatocytes with congestion in central vein in group 2 (Fig. 1a). Moreover, severe congestion in blood sinusoids and portal vein causing atrophy in hepatocytes with inflammation in portal areas was also detected (Fig. 1b). Focal aggregation of inflammatory cells around portal areas with congestion in portal vein was detected in group 3 (Fig. 1c). Some inflammatory cells also found around the necrotic cells. Severe necrosis in the hepatocytes and there are dilatation of portal vein , where it fibrosed with an increase in inflammatory cell around it (Fig. 1d). Kidneys, interstitial nephritis characterized by inflammation in interstitial tissue was detected in cortex in group 2, edema in bowman's capsule led to atrophy in glomerular tuft with focal aggregation of mononuclear cells replaced the necrotic tubules, besides renal blood vessels were congested. Vacuolation in the epithelial lining renal tubules was noticed in some rats. Congestion found in all renal blood vessels.

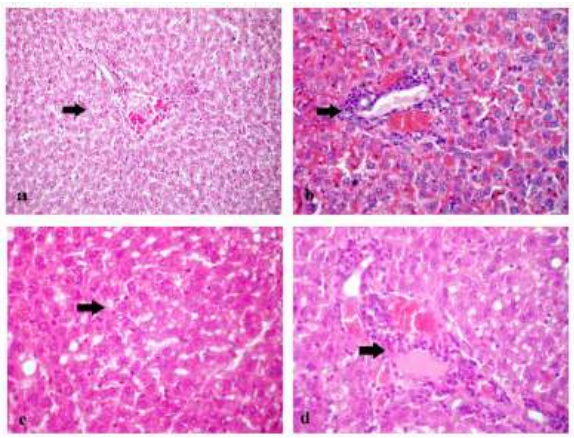

Fig. 1a-d: Liver of rats received diazinon gp (2) showing vacuolization in hepatocytes with congestion in central vein (a). Severe congestion in blood sinusoids and portal vein causing atrophy in hepatocytes with inflammation in portal areas in gp 2 (b). Focal area of necrosis with vacuolar degeneration in hepatocytes in gp 3 (c). Aggregation of inflammatory cells around portal areas with congestion in portal vein in gp 3 (c) (H& E., 80 and 150)

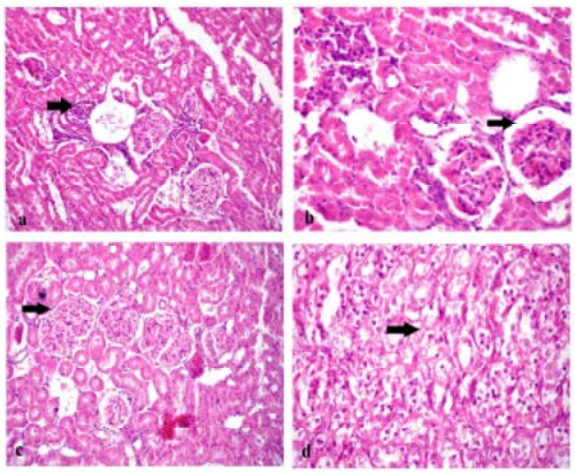

Fig. 2a-d: Kidneys of rats received diazinon gp (2) showing interstitial nephritis characterized by inflammation in renal tubules, besides atrophy in some glomerular cells (a). Edema in bowman's capsule led to atrophy in glomerular tuft with focal aggregation of mononuclear cells replaced the necrotic tubules in gp 2 (b). Congestion with hypercelluarity in glomeruli with necrosis in the adjacent tubules in gp 3 (c). Necrosis in most of renal tubules in gp 3 (d) (H& E., 80 and 150)

Degeneration and necrosis of the renal proximal epithelium were observed with congestion in blood vessels and inflammatory infiltration in the cortical area (Figure 2).

DISCUSSION

In the present study, diazinon toxicity induced some hematological and biochemical changes characterized by significantly increase in RBCs and WBCs count. MCV values exhibited significant decrease, while, MCH and MCHC displayed non-significant changes in our study. EL-Shenawy *et al*. [20] reported that no significant changes of MCH, MCV and MCHC counts were observed after diazinon treatment after 14 days. Additionally, platelets count were significantly increase which related to thrombopoiesis induced by diazinon. Differential leucocytic count showed significant changes. It could be attributed to interference of the free radicals from pesticide (OP) that cause damage in the circulatory system and all blood components [21]. The adverse health effects of OP include acute and persistent injury which produces blood disorders [22] an activation of the animal's defense mechanism and immune system [23].

Liver function showed significant increase in AST, ALT and ALP enzymes. Also, there was increase in renal tests including urea and uric acid. Our results indicated that the toxic effect of diazinon, induced renal and hepatic tissues function impairment [24] in response to an increased over production of endogenous oxygen species [25].

Histopathologically, in diazinon group (2) noticed that diazinon induced severe congestion in blood sinusoids and portal vein causing atrophy in hepatocytes with inflammation in portal areas, vacuolization in hepatocytes with congestion in central vein, dilated sinusoids, cytoplasm vacuolization, Extensive hydropic degeneration, sever mononuclear infiltration in all portal areas. Some inflammatory cells also found around the necrotic cells. There are a high number of necrotic cells in hepatocytes and there are dilatation of portal vein fibrosis around the portal triad with an increase in inflammatory cell infiltration (Figure 1 a-d) [20 , 26]. Also, diazinon toxicity caused many histological damage to renal cortex, interstitial nephritis , edema in bowman's capsule led to atrophy in glomerular tuft with focal aggregation of mononuclear cells replaced the necrotic tubules, beside renal blood vessels were congested. Most of renal tubules were damaged and lost their characteristic appearance and their lining epithelium were destructed

[20, 27, 28]. Co-administration of garlic extract as protective agent against diazinon exhibited minimum ameliorating effect on most hematological, biochemical parameters, in trial to reverse the normal level. Also, it alleviates and corrected the hepatic and renal tissue damages caused by diazinon. These results suggested that garlic extract act as the natural antioxidant that can alleviate DZN induced haematotoxicity, nephrotoxicity and hepatoxicity by the ability to the scavenge reactive oxygen (ROS) and nitrogen (RNS) species, increase enzymatic and nonenzymatic antioxidants level, activate Nrf2 factor; or/and inhibit some prooxidant enzymes (Xanthine oxidase, cyclooxygenase and NADPH oxidase) [12, 29, 30].

CONCLUSION

I could be concluded that diazinon has bad effect on exposed animals and causing impairment in liver and kidneys function. Meanwhile garlic appeared improvement moderate in liver and kidneys cells damage.

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