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RESEARCH ARTICLE

Immunotoxicity of sub-chronic doses of diazinon in male albino Wister rats

Hany Mohammed Ibrahim

Department of Zoology, Faculty of Science, Minufiya University, Shebin El-kom, Minufiya, Egypt.

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Corresponding Author

Hany Mohammed Ibrahim
hanyibrahimeg@gmail.com

Abstract

Diazinon is one of the most organophosphate pesticides that widely used in agriculture. In the current study, diazinon was administrated at doses of 10, 1, 0.1mg/kg and different humoral and cell mediated immunity parameters were examined in male Wister rats. Results showed that high dose of diazinon (10mg/kg) could inhibit the hemagglutination titer, the total serum immunoglobulin, quantitative hemolysis of SRBC (QHS), phagocytic function of neutrophils, delayed-type hypersensitivity response, proliferative response of the blood mononuclear cell and decrease the percentage of $CD4^+$ and $CD8^+$ blood T cells without affecting CD4/CD8 ratio significantly. Diazinon at moderate dose (1mg/kg) show only reduction in QHS, phagocytic function of neutrophils and delayed-type hypersensitivity response. Diazinon at low dose (0.1mg/kg) did not produce any significant changes on the humoral and cellular immune tested parameters. In conclusion high dose of diazinon affect both humoral and cell-mediated immunity in rats. On contrary, low dose of diazinon has no adverse effect on the immune response.

1. Introduction

Diazinon is a broad spectrum organophosphorous (OP) pesticide, effective against many pests of fruits, vegetables, pasture, field crops, tobacco, forage and grasslands. It presents toxic effects on the immune system in different organisms (Vial et al., 1996, Banerjee, 1999). Moreover, many reports on fish models or mice showed that diazinon caused changes in immunological parameters (Barnett et al., 1980; Cho et al., 1989; Abd-Elnassaer, 1995; Dutta et al., 1997, Khalaf-Allah et al., 1999; Galloway and Handy, 2003; Neishabouri et al., 2004; Khoshbavar-Rostami et al., 2006). The diazinon immunotoxicity revealed severe effect on C57bl/6 mice that received 25 mg/kg. It elicited severe histological changes in spleen and thymus as well as suppression of humoral and cell mediated immunity (Neishabouri et al., 2004). Moreover, diazinon treatment elicited severs reduction of T-dependent and T-independent antibody responses in mice. Diazinon not only decreases the B-lymphocytes, but also decreases the non-mature thymic T-lymphocytes (Kump et al., 1996). According to the author knowledge, there is a little information regarding its immunotoxicity potentials in rats as an animal model. The historical importance of rats to scientific research is reflected by the amount of literature on it, which is roughly 50% more than that on mice (Krinke, 2000). This study was intended to investigate the status of the humoral and cell-mediated immune response to sub-chronic diazinon intoxication in male albino Wister rats.

2. Material and methods

2.1. Chemicals

Diazinon technical grade was obtained commercially at a concentration of 95% from El-Help Pesticides and Chemical Company, Free zone, New Damietta, Egypt. The desired concentrations were prepared freshly when needed by diluting the pesticide with corn oil. Concanavalin A (Con A) from *Canavalia ensiformis* [5 mg/ml stock solution in RPMI 1640 medium] was obtained from Sigma (Sigma, St Louis, MO, USA).

2.2. Animals

Male albino Wister rats (weighting approximately 200-230 g) were obtained from the Egyptian Organization for Serology and Vaccination, Ministry of Health, Cairo, Egypt. Female guinea pigs (250 g) were used for the preparation of complement for quantitative hemolysis of SRBC (QHS) assay. All animals were kept under controlled laboratory conditions in the animal room, Zoology Department, Faculty of Science, Minufiya University. Animals were housed in standard plastic rodent cages with enough space for their activity. Standard rodent food and clean water were supplied *ad libitum*. The animals were acclimatized to laboratory condition for at least one week before the initiation of the experiments. The animals were used after approval of Institutional Animal Ethical Committee.

2.3. Experimental protocol

The experimental animals were divided into four groups; ten rats per group were treated by appropriate volumes of diazinon solutions orally to receive 10, 1 and 0.1 mg/kg of diazinon, respectively. The negative control group received only the vehicle, corn oil, for 30 days.

2.4. Blood sampling:

At the end of the period, animals were anesthetized, dissected immediately, and blood was collected from the hepatic portal vein. Each blood sample was divided into two tubes, one of them was mixed with convenient anticoagulant and the other was permitted to clot. Then serum samples were separated by centrifugation, 3000 rpm for 15 min. The separated serum was sampled into clean tubes and kept in a deep-freezer at -20 ^{o}C.

2.5. Evaluation of haematological parameters

Haematological parameters [red blood cell (RBC) counts, hemoglobin, hematocrit, white blood cell (WBC) total and relative differential counts, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)]; was done manually according to method described by Dacie and Lewis, (1991).

2.6. Assessment of humoral immunity:

2.6.1. Antigens

Sheep red blood cells (SRBC) were collected in Alsever's solution, and then it washed three times in large volumes of pyrogen-free, sterile saline.

2.6.2. Total Immunoglobulin (Ig) estimation (zinc sulfate turbidity test)

Total serum immunoglobulins were estimated by using the zinc sulfate turbidity test (Gawade et al., 2013; Sedlinska et al., 2005; McEwan et al., 1970). Briefly, 25 µl of the tested serum was mixed with 1.7ml of 0.7mM zinc sulphate, pH 5.8 (Z tube). The mixture was shaken and left to stand at room temperature for 1 h. Blood serum mixed with PBS at the same ratio was used as the blank or control (C tube). The turbidity at wavelength 545 nm was expressed as 20 zinc sulfate turbidity (ZST) units. The obtained ZST value was converted to g/L immunoglobulin using the following formula:

Zinc sulfate turbidity (ZST units) = O D. of Z tube - O D. of C tube × 10

Total immunoglobulin (g/L) = 0.04 + 0.98 × ZST units.

2.6.3. Hemagglutination (HA) titer

Eight days before the end of treatment period, animals were immunized by intraperitoneal injection of 1×10^{8} SRBC in saline. At the end of experiment after preparing sera from blood samples, aliquots (25µl) of two-fold diluted sera in saline were challenged with 25µl of 1% (V/V) SRBC suspension in microtiter plates. The plates were incubated at 37 ^{o}C for 2 h and then observed for hemagglutination (Bin-Hafeez et al., 2003). The highest dilution giving hemagglutination was considered as antibody titer.

2.6.4. Quantitative hemolysis of SRBC (QHS) assay

QHS assay was performed using the methods of Simpson and Gozzo (1978) with some modification. Spleens were removed and a cell suspension of 10×10^{6} ml was prepared in PBS. One milliliter of 0.2% SRBC and 1 ml of 10% guinea pig serum were mixed with cell suspension and incubated for 1 h at 37°C. After centrifugation

at 3000 rpm for 3 min, optical density of the supernatant was measured at 413 nm using spectrophotometer (Helios alpha, Unicam Ltd., England).

2.7. Assessment of cell-mediated immunity:

2.7.1. Delayed hypersensitivity reaction (DTH)

Eight days before the end of treatment period, rats were primed with 0.1 ml of SRBC suspension containing 1×10^8 cells intraperitoneally. Animals were challenged on day 29 with 1×10^8 SRBC in right-hind foot pad. The contra-lateral paw received saline alone. The thickness of foot pad was measured at 24 h after challenge using micrometer (Kunkel, Germany). The difference in the thickness of right hind paw and left hind paw was used as a measure of DTH reaction (Bin-Hafeez et al., 2003).

2.7.2. Blood mononuclear cells proliferation assay

Blood mononuclear cells proliferative responses to mitogen Con A were determined by a micro-tissue culture system as described by Ibrahim et al. (2010). The mononuclear cells (majority of cells are lymphocytes) were isolated from control and treated animals by HISTOPAQUE®-1077 (Sigma) according to the manufacturer's instructions. Mononuclear cells were suspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 U/ml streptomycin. Mononuclear cells (5×10^5) from treated or control animals were cultured for 24 h with Con A. Then 10 µl of Cell Counting Kit-8 (Sigma, St Louis, MO, USA) reagent was added. After 3 h incubation at 37°C in 5% CO_2, the optical density was determined at 450 nm (Seac, Radim Company, Italy).

2.8. Assessment of non-specific immunity phagocytic activity of the neutrophils:

Briefly, neutrophils were isolated from the heparinized blood sample according to the method of Markert et al. (1984) 0.1 ml of aliquot of cell having density of 10×10^6 cells/ml was mixed with 0.1 ml RPMI-1640 containing 20% fetal calf serum (FCS) and 100×10^6 cells/ml heat treated yeast cells. The mixture was incubated for 60 min at 37^{o}C with occasional shaking. The tubes were then immersed in ice cold water to stop the reaction. The phagocytic index was estimated by checking the Gimsa stained phagocytic cells under ordinary light microscope according to the method of (Fujiki and Yano, 1997).

2.9. T cell sub-typing

T cell sub-typing was done as previously described (El-Chennawi et al., 2008). Briefly, Blood mononuclear cells in RPMI-1640 (10^6 cell/ml) were prepared and after counting viable cells by trypan-blue dye exclusion method, Blood mononuclear cells cellularity was determined. Then, $CD4^+$ and $CD8^+$ T cell subtypes were measured using FACS flow cytometer (Becton Dickinson, Sunnyvale, CA, USA) using mice anti-CD4 and anti-CD8 monoclonal antibodies conjugated with fluorescein-iso-thiocyanate (FITC; BD Biosciences, San Jose, CA).

2.10. Statistical analysis:

For statistical analysis the SPSS computer program was used. The statistical analysis was carried out by one-way ANOVA setting the probability level to $P<0.05$, *post hoc* analysis of group differences was performed by LSD test. The treated groups were compared both with each other and with control group.

3. Results

3.1. Changes in hematological parameters

As shown in Table (1), no statistically significant changes were observed in total leukocyte count, relative lymphocyte and granulocyte counts and MCHC among all diazinon treated groups and control group. Diazinon at dose 10mg/kg significantly reduced ($P<0.05$) the hemoglobin concentration, PCV, RBCs and relative monocyte count compared to control group. On the other hand, Diazinon at dose 10mg/kg resulted in a significant elevation MCV and MCH ($P<0.01$ and $P<0.05$, respectively) compared to control group. In all parameters doses 1mg/kg, 0.1mg/kg per day of diazinon could not produce statistically significant changes compared to vehicle.

3.2. Humoral immunity

Diazinon at dose 10mg/kg significantly decreased ($P<0.01$) the serum anti-SRBC titer and the total serum immunoglobulin compared to control (Fig.1A, B). Doses 1mg/kg, 0.1mg/kg per day of diazinon could not produce statistically significant changes compared to control group. Moreover, effect of diazinon on QHS assay, is showed a

significant reduction at both doses 10mg/kg and 1mg/kg (P<0.01 and P<0.05, respectively), while no significant effect was demonstrated at dose 0.1mg/kg when compared to vehicle (Fig. 1C).

3.3. Phagocytic activity of neutrophils

Figure (2), illustrated the effect of diazinon on the phagocytic function of neutrophils, expressed as phagocytic index. An obvious significant decrease (P<0.001) was observed in rats upon the treatment with diazinon at dose 10mg/kg when compared to control animals. Furthermore, treatment with diazinon at dose 1mg/kg resulted in a significant decrease (P<0.05) when compared to control animals. On contrary, no significant difference was detected in rats treated with diazinon at dose 0.1mg/kg.

3.4. Cell-mediated immunity:

Cell-mediated immunity was conducted by both the delayed-type hypersensitivity response, and the proliferative response of the blood mononuclear cells to Con A (Fig. 3). Diazinon at doses of 10mg/kg and 1mg/kg per day showed a significant decrease (P<0.01 and P<0.05, respectively) in DTH response as compared to the control group. However, diazinon at dose of 0.1mg/kg did not show any significant differences when compared to the control animals. The results also indicated that diazinon at dose 10mg/kg suppressed significantly (P<0.05) the proliferative response of the blood mononuclear cells to Con A (Fig 3B). Diazinon at the other doses (1mg/kg and 0.1mg/kg) did not show any significant changes when compared to the control group.

3.5. Blood T cells sub-typing

Diazinon at dose of 10mg/kg per day was able to significantly (P<0.05) decrease the percentage of $CD4^+$ and $CD8^+$ T cells without affecting CD4/CD8 ratio significantly. However, diazinon did not show any significant differences at doses1mg/kg and 0.1mg/kg per day (Table 2).

Table 1. Effects of diazinon treatment on some hematological parameters in blood of male Wister rats

Parameter	Control (vehicle)	Diazinon 10mg/kg	Diazinon 1mg/kg	Diazinon 0.1mg/kg
Hemoglobin	13.80±0.25	11.07±0.13*	13.52±0.14	13.83±0.24
PCV	41.83±0.70	33.50±0.43*	40.50±0.43	41.67±0.76
RBCs	7.15±0.23	4.80±0.12*	6.46±0.30	6.94±0.30
MCV	58.77±1.92	70.07±2.34*	63.23±2.36	60.43±2.34
MCH	19.42±0.64	23.13±0.71*	21.12±0.81	20.10±0.80
MCHC	32.98±0.11	33.05±0.25	33.38±0.13	33.20±0.08
WBCs	8291.66±719.53	8008.33±712.91	8256.67±789.73	8383.33±725.68
Lymphocyte	64.67±1.43	62.83±1.51	65.17±1.33	65.67±1.67
Granulocyte	26.00±1.71	30.50±1.31	25.33±1.63	25.33±1.84
Monocyte	9.33±0.67	6.67±0.42*	9.50±0.89	9.00±0.68

Number of animals/group = 10, Data are expressed as: mean ± standard error (STE).
* P<0.05 indicate significant difference compared to the control group (vehicle, corn oil)

Table 2. Effects of sub-chronic exposure to diazinon on rat blood T cell subtypes

Treatment group	$CD4^+$ T cell (%)	$CD8^+$ T cell (%)	CD4/CD8 ratio
Control (vehicle)	64.92±2.85	21.48±1.40	3.03±0.09
Diazinon 10mg/kg	45.31±2.15*	15.32±1.54*	2.99±0.17
Diazinon 1mg/kg	64.39±2.27	21.82±1.27	2.95±0.06
Diazinon 0.1mg/kg	67.20±1.16	22.91±1.01	2.94±0.09

Number of animals/group = 10, Data are expressed as: mean ± standard error (STE).
* P<0.05 indicate significant difference compared to the control group (vehicle, corn oil)

Fig 1. Effects of sub-chronic exposure to diazinon on humoral immune response. (**A**) Total immunoglobulin level, (**B**) Hemagglutination titer, and (**C**) Quantitative hemolysis of SRBC (QHS) assay. Data are expressed as: mean ± standard error (STE). Statistical difference was calculated compared to control group with an ANOVA and follow-up test (LSD). * P<0.05, ** P<0.01 indicate significant difference compared to the control group (vehicle, corn oil), n=10

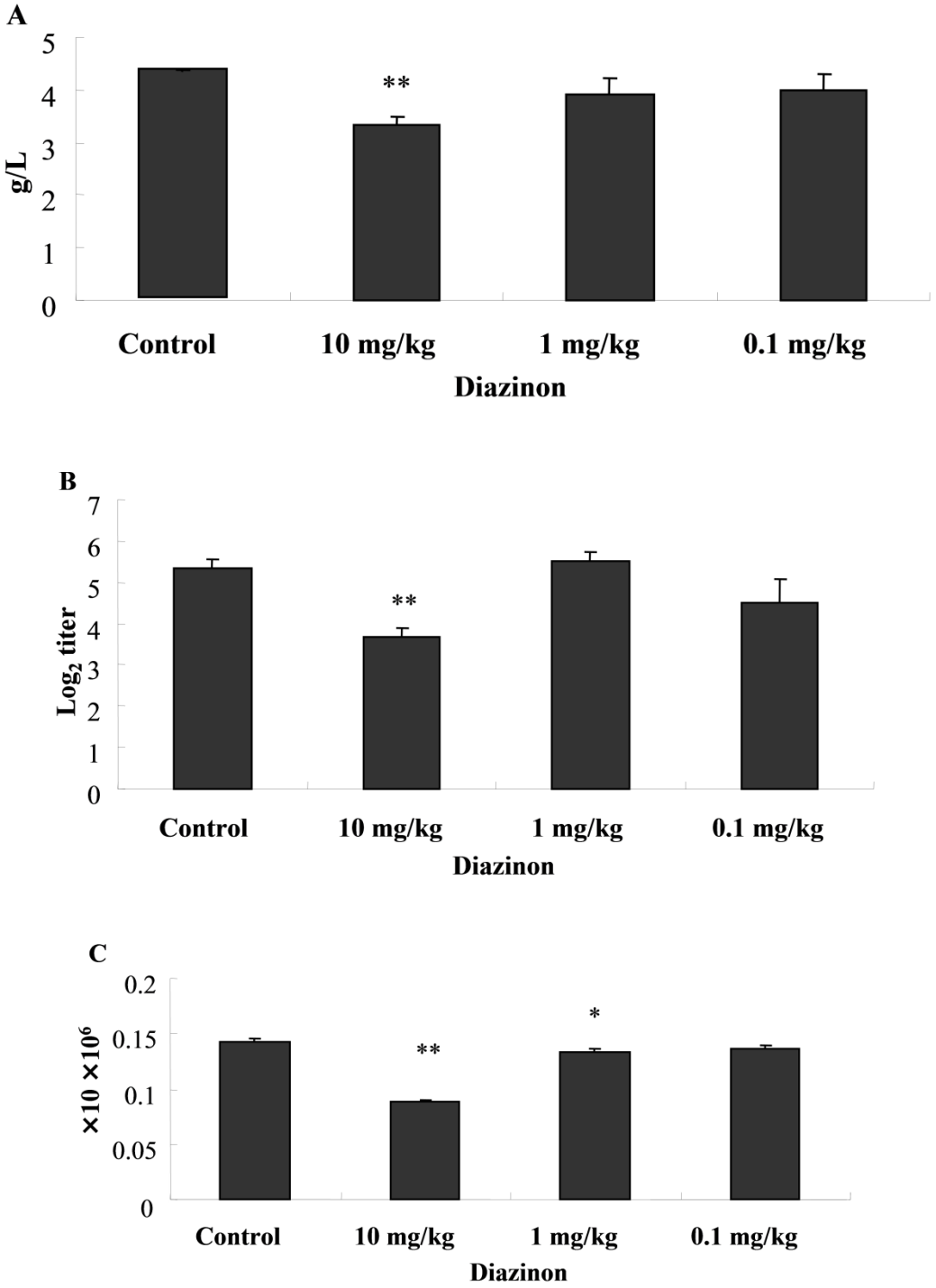


Fig. 2. Effect of sub-chronic exposure to diazinon on phagocytic activity of neutrophils. After counting at least 300 cells, the percentage of cells that engulf the yeast cell was determined. Data are expressed as: mean ± standard error (STE). Statistical difference was calculated compared to control group with an ANOVA and follow-up test (LSD). * P<0.05, *** P<0.001 indicate significant difference compared to the control group (vehicle, corn oil), n=10

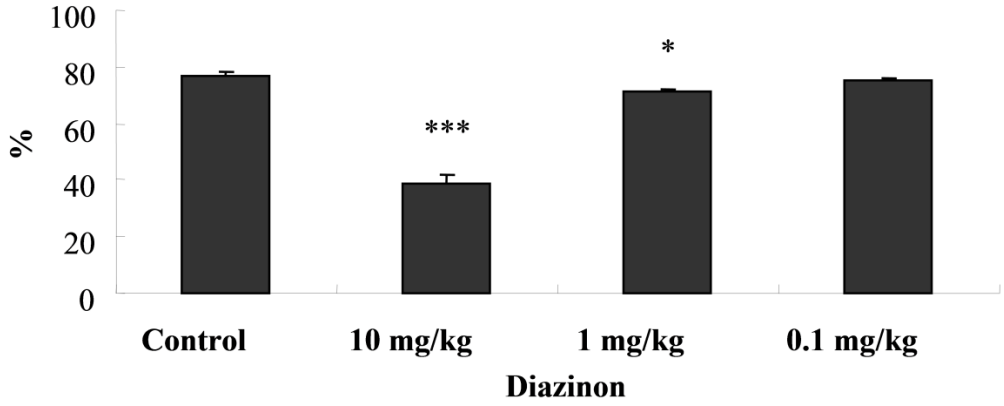
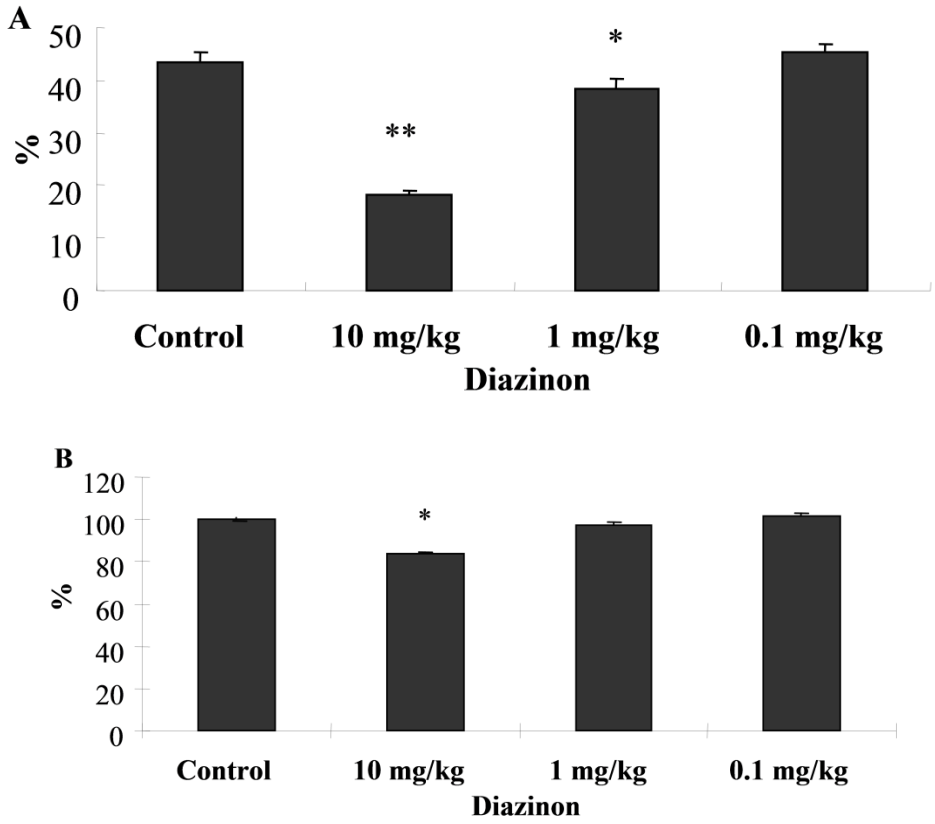


Fig. 3. Effects of sub-chronic exposure to diazinon on cell-mediated immunity. (**A**) Delayed-type hypersensitivity response (percentage of increase in the paw thickness, edema), (**B**) Blood mononuclear cell proliferative response, upon treatment with diazinon or vehicle, cells (5×10^5) were cultured for 24 h with 5µg/ml Con A. Then 10µl of Cell Counting Kit 8 reagent was added. After 3 h of incubation at 37°C in 5% CO_2, the optical density was determined at 450 nm. The percentage of growth was calculated by dividing each value (control or tested) by the average mean of the control samples (vehicle) multiplied by 100. Data are expressed as: mean ± standard error (STE). Statistical difference was calculated compared to control group with an ANOVA and follow-up test (LSD). * P<0.05, ** P<0.01 indicate significant difference compared to the control group (vehicle, corn oil), n=10



4. Discussion

Organophosphates poisoning occurs as a result of agricultural use or accidental exposure, the mode of exposure to diazinon includes oral, inhalation or dermal way (Garfitt et al., 2002; Yurumez et al., 2007). It is readily absorbed from the gastrointestinal tract and is rapidly metabolized within a few hours (FAO/WHO, 1970). The present study investigated the immunotoxic effect of diazinon that commonly used in agricultural purposes in male Wister rats.

In the current study, decrease of RBCs, PCV, hemoglobin concentration and relative monocyte counts were observed in rats treated with 10mg/kg of diazinon. According to Kalender et al. (2006) the decrease in hemoglobin along with the decrease in the RBC might be due to the effect of pesticide on the erythropoietic tissue in rats. The poisoning of pesticide residues resulted in development of anemia due to interference of hemoglobin biosynthesis and shortening of life span of circulating erythrocytes (Patil et al., 2003). In the present study, increase in MCV in rats treated with 10mg/kg of diazinon was demonstrated. The resultant effect of this would be osmotic stress, erythrocyte swelling and increased MCV, thus the observed increase in the mean of MCV in rats exposed to diazinon was manifestation of swollen erythrocytes and macrocytic anemia. In this study the results show a little pit increase in the level of MCH without any significant change in the level of MCHC, total WBC count, and relative lymphocyte counts and relative granulocyte counts upon treatment with diazinon at dose 10mg/kg compared to the control group. No functional changes in rat's hematological parameters were observed with diazinon sub-chronic administration at middle (1mg/kg) and low doses (0.1mg/kg).

In the current study, the high dose of 10mg/kg per day of diazinon showed inhibition in the humoral response in all tested parameters (total serum immunoglobulin, hemagglutination titer and QHS assay). While the moderate dose of 1mg/kg significantly decreased only the QHS assay. No significant effect was demonstrated at low dose 0.1mg/kg when compared to vehicle. Similar obtained results were reported by Barnett et al. (1980) the level of immunoglobulin-G2b (IgG2b) was significantly depressed in female mice offspring that were exposed to 9 mg/kg diazinon at 101 days of age. The diazinon elicited severs suppression of humoral immunity in C57bl/6 mice that received 25 mg/kg (Neishabouri et al., 2004). Diazinon treatment elicited severs reduction of T-dependent and T-independent antibody responses and caused slight decrease in the B-lymphocytes (Kump et al., 1996).

Neutrophil phagocytic function showed a significant reduction in diazinon-intoxicated rats (high and moderate doses) compared to the control group. In this respect few reports have been done to evaluate the effects of diazinon on phagocytosis therefore, the current data come in agreement with other studies especially those recorded by Queiroz et al. (1999) who reported that there was a considerable reduction in the ability of neutrophils from exposed workers to OP pesticides to kill *Candida albicans*. In the pervious report the authors concluded that exposure to OP insecticides may lead to changes in neutrophils function even in workers presenting no impairment in the cholinesterase (ChE) activity (Queiroz et al. 1999). Also, Wysocki et al. (1987) showed a significant decrease in the neutrophils activity in workers exposed to OP pesticides as demonstrated using nitroblue-tetrazolium test and this reduction was in linear correlation with a reduced ChE activity. The phagocytic index was significantly reduced in *tilapia* exposed to 7.830 ppm and 3.915 ppm of diazinon (Giron-Pe´rez et al., 2007). In the present study, the low dose of diazinon did not reduce the neutrophil phagocytic activity.

Previous data showed that diazinon at high dose (20 mg/kg per day) caused gross changes in mice thymus and spleen (Handy et al., 2002). In the present study, the high dose of 10mg/kg per day of diazinon showed suppression in the cell-mediated immunity (DTH response and the proliferative response of the blood mononuclear cells). Moreover, it significantly decreases the percentage of $CD4^+$ and $CD8^+$ T cells. The moderate dose of 1mg/kg significantly decreased only the DTH response. No significant effect was demonstrated at low dose 0.1mg/kg when compared to control animals. These results were consistent with previous reports. Significant reduction in the lymphocytes blastogenesis to Con A and lipopolysaccharide and the proliferation of lymphocytes in mixed lymphocyte reaction were evident in Balb/c mice, which were treated with diazinon (Abd-Elnassaer, 1995). Diazinon elicited severs histological changes in thymus and spleen as well as suppression of cell mediated immunity (Neishabouri et al., 2004). Interestingly, diazinon intoxication altered the *tilapia* immune system, as it reduced the relative spleen weight and showed a significant splenocytes proliferation reduction (Giron-Pe´rez et al., 2007).

The immunotoxic effect of diazinon could be attributed to different mechanisms. The first mechanism related to the ability of diazinon to modulate the cytokine production. It was previously reported that mice intoxicated with 50 mg/kg for 30 days indicated gradual decrease in the level of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-12 (IL-12) and interferon-γ (IFN-γ) in the splenocytes cultures that were pulsed with phytohaemagglutinin (PHA). Sever suppression of these cytokines was confirmed by the RT-PCR (Alluwaimi and Hussein 2007). These cytokine control both humoral and cellular immune response. This mean

diazinon could reduce both humoral and cell-mediated immunity through its inhibitory effect on these cytokines. The second mechanism contributed to the diazinon oxidative stress. It is possible that free radicals generated by diazinon could inhibit the immune response. Oxidative stress induced by organophosphates, especially diazinon, has been reported as the main mechanism of its toxicity (Ranjbar et al., 2005, Oruc and Usta, 2007). Moreover, it has been reported that intoxication with diazinon increases glucose release from liver into blood and leads to enhancement of lipid peroxidation as well as changes in the activity of glutathione peroxidase in the liver (Teimouri et al., 2006). Reactive oxygen interacts with receptors, second messengers and transcription factors, altering gene expression and influencing cell growth and survival (Palmer and Paulson, 1997). Thus, oxidative stress caused by diazinon is possibly an important mechanism of diazinon-induced immunotoxicity. The third mechanism deled with the ability of diazinon to alter the immunity through its cholinergic responses. Diazinon is well known to exert its toxic effects by inhibiting cholinesterase in plasma, erythrocytes and brain (Tomokuni and Hasegawa, 1985). Cha et al. (2000) showed that treatment of mice with diazinon completely blocked the serum cholinesterase activity in mice. In addition to the inhibition of acetylcholinesterase, any of the serine hydrolase class of enzyme could be target for inhibition (Galloway and Handy, 2003). Serine hydrolase plays vital role in the immune system. Esterases associated with the cell membrane of lymphocytes and monocytes (Becker and Henson, 1973, Stepanovic et al., 1998) may likewise be inhibited and may lead to structural or functional changes in the immunocyte populations (Galloway and Handy, 2003). It's possible that one mechanism or more than one mechanism is contributed to the immunotoxicity of diazinon.

In conclusion, the present study showed that diazinon at high dose has an inhibitory effect on the humoral and cell mediated immunity in the rat model. The current data also indicated that diazinon at low dose (0.1mg/kg) has no immunotoxic potential in rats. Therefore, this low dose of diazinon seems to be appropriate dosage for assessment of NOAEL which is recommended by WHO experts committee for immuno-toxicological consideration.

Conflict of interest

The Author declares no conflict of interest related to this work.

References

Abd-Elnassaer, M. (1995): Immunotoxic response of female Balb/c mice to diazinon, carbofuran and permethrin insecticides. Assiut. Vet. Med. J., 32: 207–215.

Alluwaimi, A.M. and Hussein, Y. (2007): Diazinon immunotoxicity in mice: modulation of cytokines level and their gene expression. Toxicology, 236(1-2): 123-31.

Banerjee, B.D. (1999): The influence of various factors on immune toxicity assessment of pesticide chemical. Toxicol. Lett., 107: 21 e31.

Barnett, J.B., Spyker-cranmer, J.M., Avery, D.L. and Hoberman, A.M. (1980): Immunocompetence over the life span of mice exposed in utero to carbofuran or diazenon. I. Changes in serum immunoglobulin concentrations. J. Envrion. Pathol. Toxicol., 4: 53–63.

Becker, E.L. and Henson, P.M. (1973): *In vitro* studies of immunologically induced secretion of mediators from cells and related phenomena. Adv. Immunol., 17: 93-193.

Bin-Hafeez, B., Haque, R., Parvez, S., Pandey, S., Sayeed, I. and Raisuddin, S. (2003): Immunomodulatory effects of fenugreek (*Trigonella foenum graecum L.*) extract in mice. Int. Immunopharmacol., 3(2): 257-265.

Cha, S.W., Gu, H.K., Lee, K.P., Lee, M.H., Han, S.S. and Jeong, T.C. (2000): Immunotoxicity of ethyl carbamate in female BALB/c mice: role of esterase and cytochrome P450. Toxicol. lett., 115(3): 173-181.

Cho, J.H., Park, J.M. and Jean, Y.H. (1989): Studies on the diagnosis of organophos -phorus insecticide poisoning. Clin. Haemtol. Diagn., 31: 8–13.

Dacie, J.V. and Lewis S.M. (1991): Practical Haematology. Churchill Livingstone UK, 7th Ed.

Dutta, H.M., Quadri, N., Ojha, J., Singh, N.K., Adhakari, S., Munshi, J.S., et al. (1997): Effect of diazinon on macrophages of bluegill sunfish, Lepomis macrochirus, a cytochemical evaluation. Bull. Environ. Contam. Toxicol., 58:135 e41.

El-Chennawi, F.A., Al-Tonbary, Y.A., Mossad, Y.M. and Ahmed, M.A. (2008): Immune reconstitution during maintenance therapy in children with acute lymphoblastic leukemia, relation to co-existing infection. Hematology, 13(4): 203-9.

FAO/WHO (1970): Evaluation of some pesticide residues in food. WHO food additive series No. 42. World Health Organization. Geneva.

Fujiki, K. and Yano, T. (1997): Effects of sodium alginate on the nonspecific defense system of the common carp, *Cyprinus carpio L.* Fish and Shellfish Immunology, 7: 417-27.

Galloway, T. and Handy, R. (2003): Immunotoxicity of organophosphorous pesticides. Ecotoxicology, 12: 345–363.

Garfitt, S.J., Jones, K., Mason, H.J. and Cocker, J. (2002): Exposure to the organophosphate diazinon: data from a human volunteer study with oral and dermal doses. Toxicol. Lett., 134(1-3): 105-13.

Gawade, L., Dadarkar, S.S., Husain, R. and Gatne, M. (2013): A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats. Food Chem. Toxicol., 51: 61-70.

Girón-Pérez, M.I., Santerre, A., Gonzalez-Jaime, F., Casas-Solis, J., Hernández-Coronado, M., Peregrina-Sandoval, J., Takemura, A. and Zaitseva, G. (2007): Immunotoxicity and hepatic function evaluation in Nile tilapia (*Oreochromis niloticus*) exposed to diazinon. Fish Shellfish Immunol., 23(4): 760-9.

Handy, R.D., Abd-El Samei, H.A., Bayomy, M.F., Mahran, A.M., Abdeen, A.M. and El-Elaimy, E.A. (2002): Chronic diazinon exposure: pathologies of spleen, thymus, blood cells, and lymph nodes are modulated by dietary protein or lipid in the mouse. Toxicology, 172: 13–34.

Ibrahim, H.M., Xuan, X. and Nishikawa, Y. (2010): *Toxoplasma gondii* cyclophilin 18 regulates the proliferation and migration of murine macrophages and spleen cells. Clin. Vaccine Immunol., 17(9):1322-9.

Kalender, Y., Uzunhisarcikli, M., Ogutcu, A., Acikgoz, F. and Kalender, S. (2006): Effects of diazinon on pseudocholinesterase activity and haematological indices in rats: The protective role of vitamin E. Environ. Toxicol. Pharmacol., 22 (1):46-51.

Khalaf-Allah, S.S. (1999): Effect of water pollution on some hematological, biochemical and immunological parameters in *Tilapia nilotica* fish. Deutsche Tierarztliche Wochenschrift., 106:67 e71.

Khoshbavar-Rostami, H.A., Soltani, M. and Hasan, H.M.D. (2006): Immune response of great sturgeon (*Huso huso*) subjected to long-term exposure to sub-lethal concentration of the organophosphate, diazinon. Aquaculture, 256:88 e94.

Krinke, George J. (2000): "History, Strains and Models". The Laboratory Rat (Handbook of Experimental Animals). Gillian R. Bullock (series ed.), Tracie bunton (series ed.). Academic Press. pp. 3–16.

Kump, D.F., Matulka, R.A., Burton, G.F., Jordan, S.D. and Holsapple, M.P. (1996): Alternations in splenocyte and thymocyte subpopulations in B6C3F1 mice exposed to cocaine plus diazinon. J. Pharmacol. Exp. Ther., 277: 1477–1485.

Markert, M., Andrews, P.C. and Babior, B.M. (1984): Measurement of O2 production by human neutrophils. The preparation and assay of NADPH oxidase-containing particles from human neutrophils. Meth. Enzymol., 105:358-65.

McEwan, A.D., Fisher, E.W., Selman, I.E. and Penhale, W.J. (1970): A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clin. Chim. Acta., 27(1):155-63.

Neishabouri, E.Z., Hassan, Z.M., Azizi, E. and Ostad, S.N. (2004): Evaluation of immunotoxicity induced by diazinon in C57bl/6 mice. Toxicology, 196:173–179.

Oruç, E.Ö. and Usta, D. (2007): Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. Environ. Toxicol. Pharmacol., 23(1):48-55.

Palmer, H.J. and Paulson, K.E. (1997): Reactive oxygen species and antioxidants in signal transduction and gene expression. Nutr. Rev., 55 (10): 353-61.

Patil, J.A., Patil, A.J. and Govindwar, S.P. (2003): Biochemical effects of various pesticides on sprayers of grape gardens. Indian J. Clin. Biochem., 18(2):16-22.

Queiroz, M.L., Fernandes, M.D. and Valadares, M.C. (1999): Neutrophil function in workers exposed to organophosphate and carbamate insecticides. Int. J. Immunopharmacol., 21(4):263-270.

Ranjbar, A., Solhi, H., Mashayekhi, F.J., Susanabdi, A., Rezaie, A. and Abdollahi, M. (2005): Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. Environ. Toxicol. Pharmacol., 20(1):88-91.

Sedlinska, M., Krejci, J. and Vyskocil, M. (2005): Evaluation of field methods for determining immunoglobulins in sucking foals. Acta. Vet. Brno., 74:51-58.

Simpson, M.A. and Gozzo, J.J. (1978): Spectrophotometric determination of lymphocyte mediated sheep red blood cell hemolysis *in vitro*. J. Immunol. Methods., 21(1-2):159-65.

Stepanovic, R.M., Jokanovic, M. and Maksimovic, M. (1998): Toxicological importance of lymphocyte neuropathy target esterase. Arch. Pharmacol., 358 (2):5413.

Teimouri, F., Amirkabirian, N., Esmaily, H., Mohammadirad, A., Aliahmadi, A. and Abdollahi, M. (2006): Alteration of hepatic cells glucose metabolism as a non-cholinergic detoxication mechanism in counteracting diazinon-induced oxidative stress. Hum. Exp. Toxicol., 25(12):697-703.

Tomokuni, K. and Hasegawa, T. (1985): Diazinon concentrations and blood cholinesterase activities in rats exposed to diazinon. Toxicol. Lett., 25:7-10.

Vial, T., Nicolas, B. and Descotes, J. (1996): Clinical immunotoxicology of pesticides. J. Toxicol. Environ. Health., 48:215e29.

Wysocki, J., Kalina, Z. and Owczarzy, I. (1987): Effect of organophosphoric pesticides on the behaviour of NBT-dye reduction and E rosette formation tests in human blood. Int. Arch. Occup. Environ. Health., 59(1):63-71.

Yurumez, Y., Cemek, M., Yavuz, Y., Birdane, Y.O. and Buyukokuroglu, M.E. (2007): Beneficial effect of N-acetylcysteine against organophosphate toxicity in mice. Biol. Pharm. Bull., 30(3):490-4.