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# Hematotoxicity of diazinon pesticide at different time intervals in male albino rats

Faten R. Abdel Ghaffar; Hany M. Ibrahim; Imam A. Hassouna; Ibrahim A. Elelaimy and Heba M. Abd El latif.

Zoology Department, Faculty of Science, Menoufia University, Shebin El Kom, Egypt.

(Corresponding author e.mail:abdelghafar_fr@yahoo.com)

## Abstract

Diazinon, an organophosphate insecticide has been used in agriculture for several years. The aim of the present study was to analyze the hematotoxic effects of diazinon (DZN) at different time intervals (10, 20 and 30 days) on adult male albino rats. The changes in some hematological parameters; RBCs count, Hb content, Ht%, RBCs indices, total and differential count of WBCs, Platelets count, PT, APTT and bleeding time; were investigated. The antioxidant system in RBCs membrane; GSH, GST, GR, GPx, CAT and SOD; as well as LPO, as oxidative stress marker, were estimated. The current study showed that; DZN (14.88 mg/kg b.w.) at different time intervals resulted in decline in RBCs count, Hb content, Ht value, total WBCs count, platelets count, and relative lymphocytes and monocytes counts when compared with their control groups. On the other side, DZN caused increase in MCV, MCH, relative granulocytes count, bleeding time, PT and APTT. Furthermore, DZN decreased the activity of erythrocyte antioxidant enzymes (GST, GR, GPx, CAT and SOD) and GSH level. On the other hand, DZN caused increase in LPO. Generally, these all changes were directly proportional to the development of intoxication time. So, the current research concludes that DZN has hematotoxic effects which appeared in time-dependent manner.

***Keywords:*** Diazinon, Hematotoxicity, Antioxidant, Oxidative stress.

## 1 Introduction

Pesticides are usually used indiscriminately in large amounts causing environmental pollution, and therefore are a cause of concern (Kalender et al., 2005). Fleischli et al. (2004) reported the effect of acute pesticide poisoning at wildlife and domestic animals. The adverse health effects of pesticides includes acute and persistent injury which produces blood disorders (Köprücü et al., 2006), dysfunction of the immune and endocrine systems (Liu et al., 2006), lung damage (Hoppin et al., 2007), injury to the reproductive organs (Andersen et al., 2008), development of cancer (Hohenadel et al., 2011) and disorders of the nervous system (Wang et al., 2013).

Organophosphorus pesticides (OPs) were first synthesized in Germany before the Second World War. Common uses of OPs are in public health and agriculture (Garfitt et al., 2002). There have been an estimated 300,000 severe pesticide poisoning events reported worldwide, mostly due to them (Shadnia et al., 2005; Rahimi et al., 2006). Mechanism of action of OPs based on their neurotoxic action, whereas they are adapted to be effector inactivators of acetylecholinesterase through the covalent interaction of the nucleophilic active site serine of the enzyme with the OP to form a phosphorylated enzyme derivative. Reactivation rates of these inhibited enzymes differ according to the chemical structure of the OP, the cellular location and the form of the enzyme (Galloway and Handy, 2003).

Diazinon (DZN) is a synthetic chemical substance with a broad spectrum of insecticidal activity. It is one of the most frequently used OPs in agriculture and horticulture for controlling insects in crops, lawns, fruits, ornamentals and vegetables and as a pesticide in domestic and public buildings (Garfitt et al., 2002).

Reactive oxygen species (ROS) are unstable and highly reactive particles that are products of normal metabolic and signal transduction events in cells but may also play an important role in a pathological process (Matés et al., 1999). ROS may be produced as a result of the metabolism of organophosphates by cytochrome P450s (Chambers et al., 2001).

Blood cells are affected by the stress caused by environmental pollution (Larsson et al., 1984). The hematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Larsson et al., 1985). It is well documented that pesticides are of the

environmental pollutants that cause damage in the circulatory system and all blood components (Ahamed et al., 2006; Gabbianelli et al., 2009; Malla et al., 2009; Chatterjee et al., 2013).The present study is interested in evaluating the effects of DZN on hematological parameters and antioxidant activities of erythrocytes at different time intervals (10, 20 and 30 days).

## 2 Materials and Methods

### *2. 1 Chemicals*

Diazinon(O,O-diethyl-O-[2-isopropyl-4-methyl-6-pyrimidinyl]phosphorothioate), 95% purity, was obtained from EL-Help Company, for pesticides production, Free Zone, New Damietta, Egypt. The desired concentration was freshly prepared by diluting the pesticide with corn oil. The oral lethal dose of diazinon (1340 mg/kg b.w.) was obtained according to (Akturk et al., 2006). All other chemicals and reagents were of the highest purity available.

### *2. 2 Animals*

Male albino rats were purchased from the animal house of National Research Center, Dokki, Egypt. Animals were kept under standard housing conditions with a 12 hr. dark/light cycle. The animals were acclimatized to standard laboratory conditions for two weeks before the commencement of the experiment. They had free access to a rodent lab diet and water *ad libitum*. The animals were used after approval of Institutional Animal Ethical Committee of Faculty of Science, Menoufia University.

### *2. 3 Experimental design*

In order to perform the experiment, 30 adult male albino rats ranging from 150-200 gram were used. Animals were divided into 2 groups as follows:

Group (1): 15 rats received corn oil (3ml/kg b.w./day, orally) as control.

Group (2): 15 rats received diazinon dissolved in corn oil. The insecticide was administered with dose of (1/90 LD50 = 14.88 mg/kg b.w./day, orally).

Five rats of each of the previous groups were sacrificed, 24 hr after the last day of treatment, at intervals of 10, 20 and 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 three tubes, one of them was mixed with EDTA, another one was mixed with sodium citrate and the last one was mixed with heparin. Then plasma of citrated blood samples was separated by centrifugation, 2000 g for 5 min. and drained into clean test tube.

### *2. 5 Evaluation of hematological parameters:*

Hematological parameters; red blood cells (RBCs) count, hemoglobin, hematocrit, white blood cells (WBCs) total and relative differential counts, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets count; were done manually using blood sample mixed with EDTA according to method described by Dacie and Lewis (1984).

### *2. 6 Evaluation of coagulation process:*

Bleeding time was determined using a cutting-tail rat model according to the method described by Song et al. (2012). Citrated plasma was used to determine activated partial thromboplastin time (APTT) and prothrombin time (PT), using kit of BioMed-Liquicellin-E for APTT determination (BioMed Diagnostics, Germany) and Phosphoplastin RL prothrombin time reagent for PT determination (R2 Diagnostics, UK).

### *2. 7 Preparation of hemolysate:*

Erythrocytes of heparinized blood samples were sedimented, by centrifugation at 1500 g for 10 min, washed three times with cold isotonic saline and the Buffy coat was discarded. Erythrocytes suspension was destroyed by using the same volume of deionized water and centrifugation at 10,000 g for 10 min at 4 °C. The supernatant (hemolysate) was obtained and stored at -20 °C until measurements within 3 days (Sinha et al., 2007).

### *2. 8 Biochemical analysis:*

The biochemical parameters were determined using erythrocyte hemolysate as follows:

Reduced glutathione (GSH) (Buetler and Kelley, 1963), glutathione-s-transferase (GST) (Habig et al., 1974), glutathione reductase (GR) (Carlberg and Mannervik, 1975), glutathione peroxidase (GPx) (Hafeman et al., 1974), catalase (CAT) (Goth, 1991), superoxide dismutase (SOD) (Beauchamp and Fridovich, 1971) as well as lipid peroxidation products, malonaldehyde (MDA), content (Ruiz-Larrea et al., 1994).

### *2. 9 Statistical analysis:*

The results were expressed as mean ± standard error of means (SEM). The data were statistically analyzed using one way analysis of variance test (ANOVA) with the program Statistical Package for Social Sciences (SPSS) version 11. The values of $P<0.05$ were considered statistically significant. Post Hoc analysis of group differences was performed by LSD test.

## 3 Results

### 3. 1- Hematological parameters:

#### 3.1.1- Red blood cells count, Hemoglobin content and Hematocrit value:

Figure (1,2 and 3) demonstrated that the daily oral administration of DZN into rats produced significant reduction in red blood cells (RBCs) count, hemoglobin (Hb) content and hematocrit (Ht) value gradually after 10, 20 and 30 days of toxicity.

#### 3.1.2- Red blood cell indices:

Data presented in figures (4, 5 and 6) clearly illustrated that, after 10 days of toxicity with DZN there was no significant effect on RBCs indices when compared with control group. On the other hand, after 20 days of toxicity, DZN resulted in a significant ($P<0.05$) increase in MCV value when compared with control group. Moreover, DZN caused significant ($P<0.05$) increase in MCV and MCH values after 30 days of toxicity each when compared with its corresponding control group. There was no significant effect on MCHC value at the three time intervals.

**3.1.3- Total white blood cells count:**

Time dependent effect of DZN on total white blood cells (WBCs) count was illustrated in figure (7). Ten days of intoxication with DZN had no significant effect on total WBCs count of rats when compared with control group. While, 20 and 30 days of toxicity resulted in highly significant (P<0.001) decrease in total WBCs count when compared with the corresponding control group.

**3.1.4- Differential white blood cells count:**

Changes in differential WBCs count of the adult male albino rats due to intoxication with DZN at three time intervals (10, 20 and 30 days) were demonstrated in figures (8, 9 and 10).

**a. Granulocytes (%):**

The statistical analysis of data in figure (8) revealed that, after 20 and 30 days of toxicity with DZN there was a highly significant (P<0.001) increase in the relative granulocytes count when compared with the corresponding control group. On the other hand, 10 days of DZN toxicity had no significant effect on the relative granulocytes count when compared with control group. The increase in the relative granulocytes count was in time dependent manner.

**b. Lymphocytes and Monocytes (%):**

As shown in figure (9 and 10) there was time dependent significant (P<0.05) decrease in the relative lymphocytes and monocytes count, when compared with the corresponding control group, after 20 and 30 days of toxicity with DZN. On the other hand, 10 days of DZN toxicity had no significant effect on the relative lymphocytes and monocytes count when compared with control group.

**3.1.5- Total platelets count:**

Data presented in figure (11) clearly illustrated that, DZN caused gradual decrease in the total platelets count at three time intervals. But, this decrease was not significant after ten days of rat's intoxication when compared with control group. On the other hand, after 20 or 30 days of toxicity, there was a highly significant (P<0.001) decrease in the total platelets count when compared with the corresponding control groups.

**3.1.6- Bleeding time:**

DZN caused time dependent increase in bleeding time as shown in figure (12). The effect of DZN on bleeding time was not significant after ten days of rats' intoxication when compared with control group. On the other hand, after 20 or 30 days of intoxication there was a highly significant (P<0.001) increase in the bleeding time when compared with the corresponding control groups.

**3.1.7-Prothrombin time and activated partial thromboplastin time:**

Gradual effect of DZN on prothrombin time (PT) and activated partial thromboplastin time (APTT) was illustrated in figures (13 and 14). After 20 or 30 days of intoxication with DZN, there was a highly significant (P<0.001) increase in PT and APTT when compared with the corresponding control groups. On the other hand, DZN had no significant effect on PT and APTT after 10 days of intoxication when compared with corresponding control groups.

**2- Antioxidant defense system:**

**2.1- Reduced glutathione:**

From the data shown in figure (15), it is clear that RBCs' reduced glutathione (GSH) level exhibited marked significant (P<0.001) gradual decrease upon treatment with DZN after 20 or 30 days of intoxication when compared with corresponding control groups. On the other hand, ten days-toxicity had no significant effect on GSH level in comparison with its control group.

**2.2- Glutathione-S-transferase, Glutathione reductase and Glutathione peroxidase:**

RBCs' glutathione-S-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPx) enzyme activities were highly affected in rats intoxicated with DZN as shown in figures (16, 17 and 18), respectively. DZN showed a highly significant (P<0.001) decrease in GST, GR and GPx enzyme activities after 10, 20 and 30 days of toxicity when compared with corresponding control groups. Furthermore, these declines in enzyme activities were in time dependent manner.

**2.3- Catalase and Superoxide dismutase:**

Figure (19 and 20), illustrated the changes in RBCs' catalase (CAT) and superoxide dismutase (SOD) enzyme activities of DZN-intoxicated rats. Generally, DZN intoxication induced a significant (P<0.001) decrease in CAT and SOD activities when compared with corresponding control group. Furthermore, this decrease was in time dependent manner.

**2.4- Lipid peroxidation:**

Data presented in figure (21) showed that DZN caused a significant (P<0.05) elevation of lipid peroxidation level (LPO) when compared with control group at 10 days time interval. Moreover, after 20 and 30 days of toxicity DZN resulted in marked significant (P<0.001) increase in LPO level when compared with control groups. The elevation in LPO level was in time dependent manner.

## 4 Discussion

In the present study changes were observed in hematological parameters and antioxidant defense system in erythrocytes. Marked significant decrease of RBCs count, hemoglobin content and hematocrit value were observed in a time dependent manner due to intoxication with DZN. On the other side, the results showed time dependent increase in MCV and MCH values however, there was no abnormal effect on MCHC.

Several previous reports are consistent with the current results. Kalender et al. (2006) reported that DZN caused time dependent decline in Hb content and Ht value and also time dependent increase in MCV value, although no changes were observed in MCHC in all treated groups.

Köprücü et al. (2006) studied the effect of DZN on some hematological parameters of Fingerling European catfish (*Silurus glanis* L.). The authors found that DZN caused time dependent decrease in RBCs count, Hb content and Ht value. In the same manner, Hariri et al. (2011) have reported that 20 mg/kg of DZN for 4 weeks resulted in in significant decrease in RBCs count, Hb content and Ht value when compared to control animals.

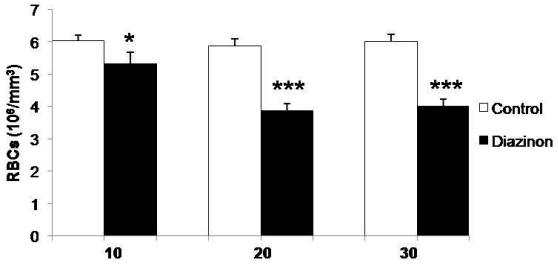


**Figure (1):** Changes in red blood cells (RBCs) count 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). * (P<0.05) against control group, *** (P<0.001) against control group.

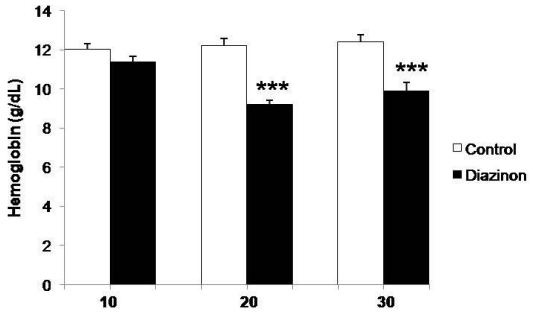


**Figure (2):** Changes in hemoglobin content 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

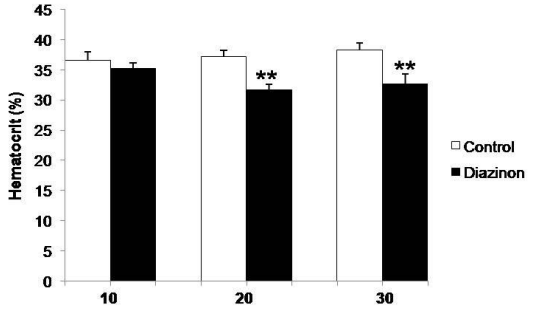


**Figure (3):** Changes in hematocrit value 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). ** (P<0.01) against control group.

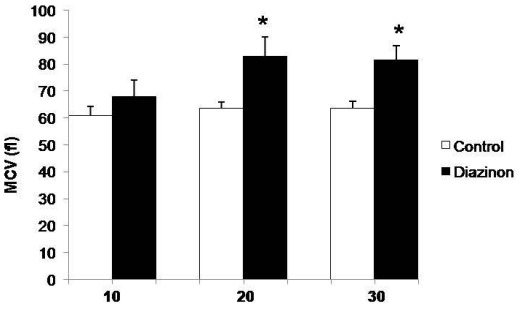


**Figure (4):** Changes in mean corpuscular volume (MCV) 10, 20 and 30 days after diazinon intoxication to rats. Numberof animals/group = 5, Data are expressed as = mean ± standard error (SE). * (P<0.05) against control group.

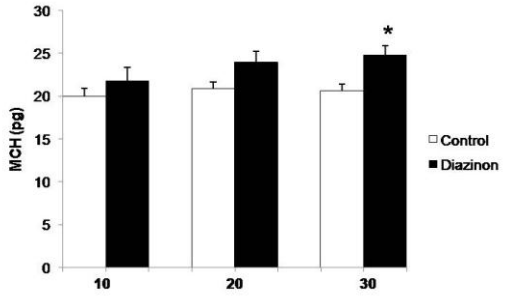


**Figure (5):** Changes in mean corpuscular hemoglobin (MCH) 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). * (P<0.05) against control group.

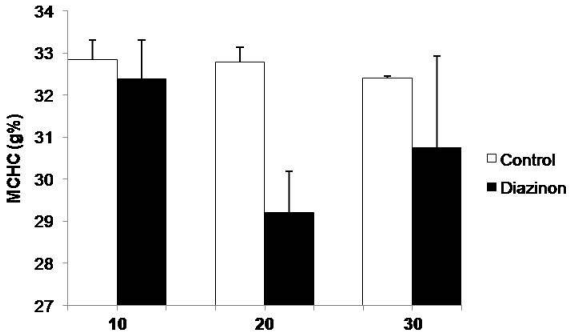


**Figure (6):** Changes in mean corpuscular hemoglobin concentration (MCHC) 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE).

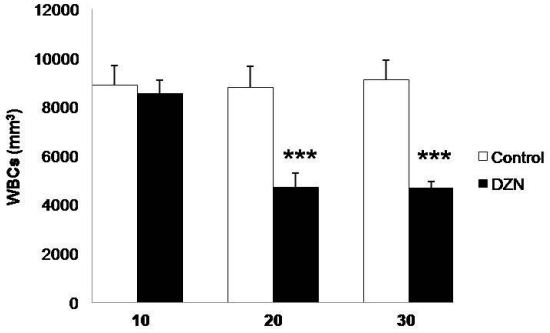


**Figure (7):** Changes in total white blood cells (WBCs) count 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

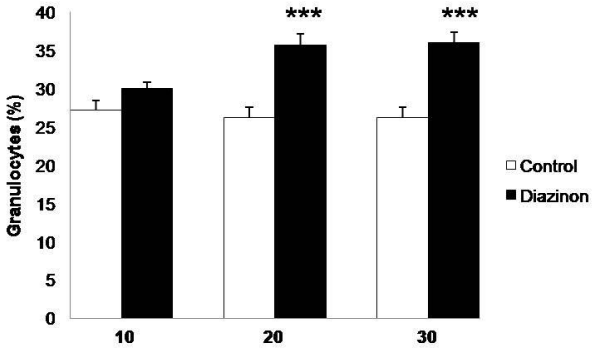


**Figure (8):** Changes in relative granulocytes count 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

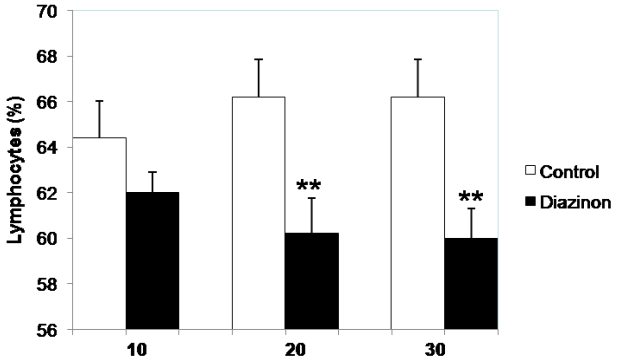


**Figure (9):** Changes in relative lymphocytes count 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). ** (P<0.01) against control group.

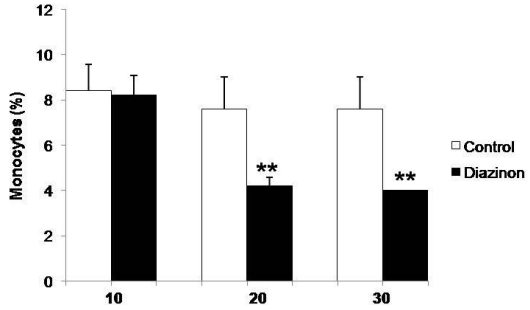


**Figure (10):** Changes in relative monocytes count 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). ** (P<0.01) against control group.

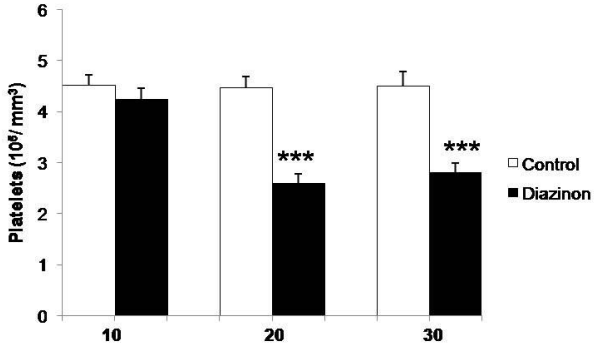


Figure (11): Changes in total platelets count 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

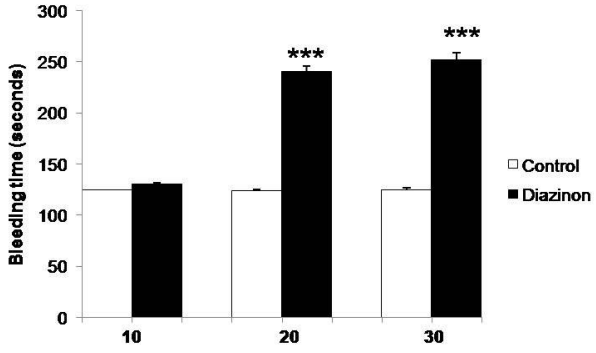


**Figure (12):** Changes in bleeding time 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

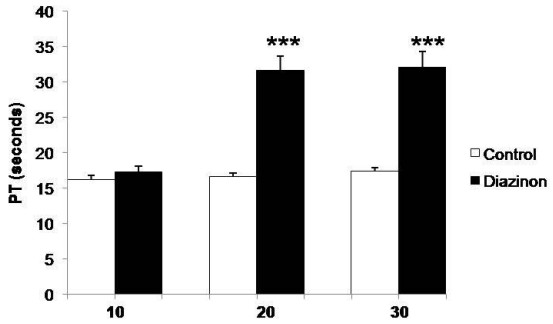


**Figure (13):** Changes in prothrombin time (PT) 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

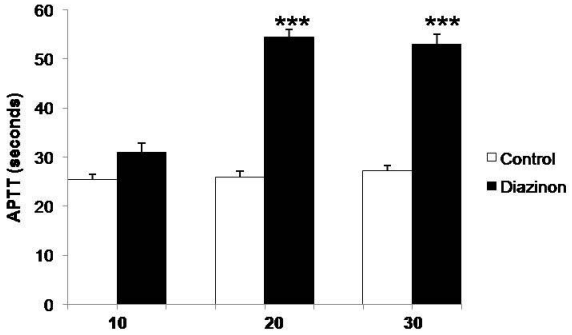


**Figure (14):** Changes in activated partial thromboplastin time (APTT) 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

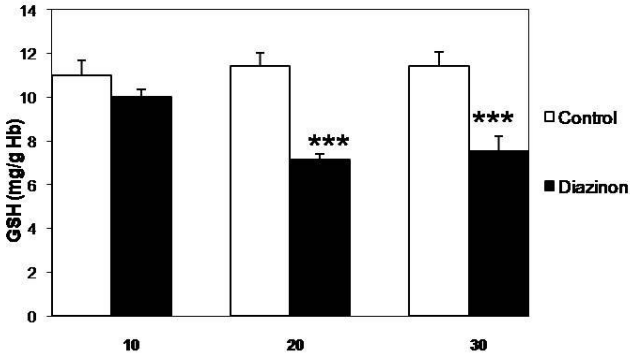


**Figure (15):** Changes in reduced glutathione (GSH) level 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

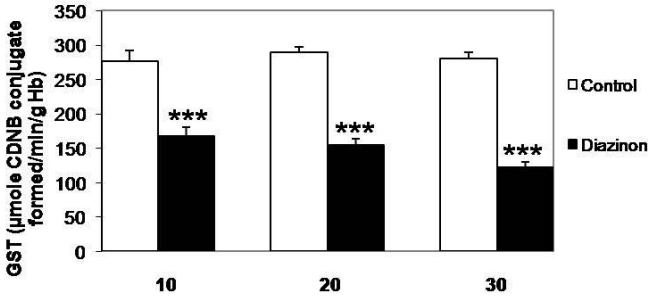


**Figure (16):** Changes in glutathione-S-transferase (GST) activity 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

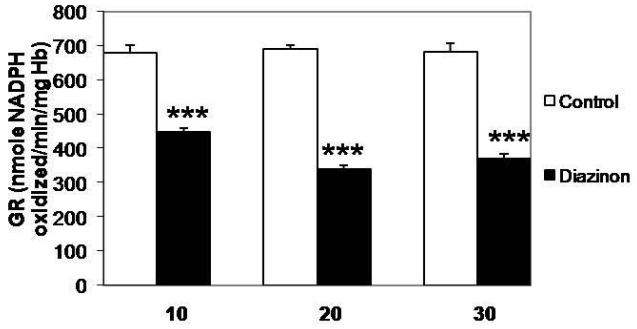


**Figure (17):** Changes in glutathione reductase (GR) activity 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

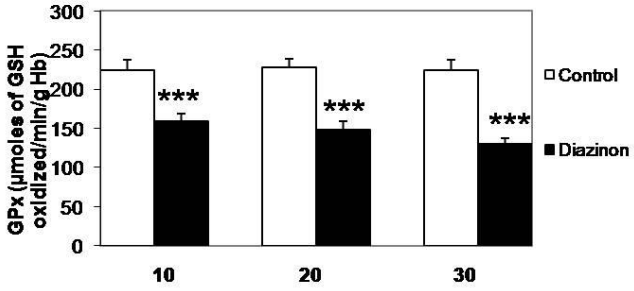


**Figure (18):** Changes in glutathione peroxidase (GPx) activity 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control.

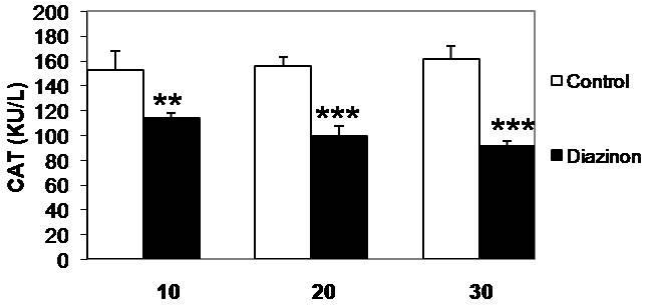


**Figure (19):** Changes in catalase (CAT) activity 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). ** (P<0.01) against control group, *** (P<0.001) against control group.

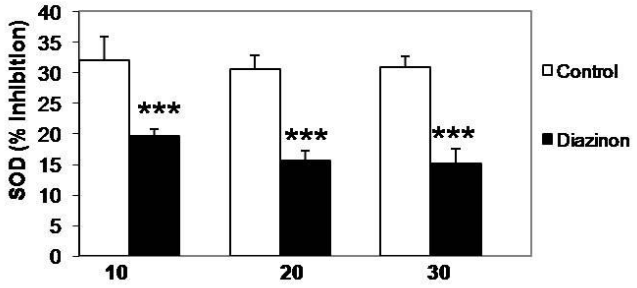


**Figure (20):** Changes in superoxide dismutase (SOD) activity 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). *** (P<0.001) against control group.

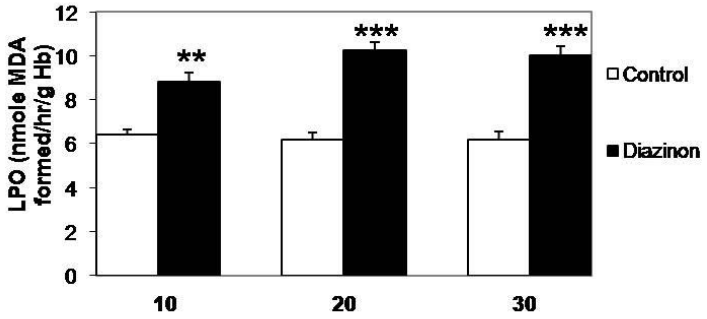


**Figure (21):** Changes in lipid peroxidation (LPO) level 10, 20 and 30 days after diazinon intoxication to rats. Number of animals/group = 5, Data are expressed as = mean ± standard error (SE). ** (P<0.01) against control group, *** (P<0.001) against control group.

Recently, Messarah et al. (2012) found that DZN, at dose 10 mg/kg after 21 days, resulted in significant decrease in RBCs count and Hb content in rats.

Early, Kehrer et al. (1993) discussed that, the decrease of previously mentioned parameters leads to anemia which may be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocyte destruction in hemopoietic organs. Another hypothesis was mentioned by Messarah et al. (2012) who reported that reduction in RBCs count and hemoglobin concentration could be attributed to interference of the free radicals from OP with hemoglobin biosynthesis and shortening of the life span of circulating erythrocytes.

MCV has been reported to provide information on the size and status of erythrocytes (Nussey et al., 1995). Generally, the increased MCV value may be due to DZN-induced osmotic stress that produced erythrocyte swelling leading to increased MCV. Kalender et al. (2006) reported that, the observed increase in MCV may be as a manifestation of swollen erythrocytes and macrocytic anemia.

MCH shows hemoglobin concentration in erythrocytes. It means hemoglobin rate to RBCs. The presented results showed that MCH was increased, with DZN-intoxication, in a time dependent manner. This increased MCH may be explained as the decrease in RBCs count was more than the decrease in Hb concentration so the ratio between them, MCH, was elevated. However, in the current experiment there was no abnormal effect on MCHC that comes in consistent with the results of Messarah et al. (2012). The more recent study of Ibrahim (2014) emphasized that DZN, at a dose of 10mg/kg for 30 days, caused a significant decrease in RBCs count, Hb content, Ht value and significant increase in MCV and MCH, while no significant effect was observed on MCHC.

In the present study, DZN caused significant time dependent decrease in total WBCs count, relative lymphocytes and monocytes counts and significant time dependent increase in relative granulocytes count.

Köprücü et al. (2006) reported that DZN caused decrease in total WBCs count of Fingerling European catfish (*Silurus glanis* L.). Banaee et al. (2008) determined the same effect of DZN on some hematological parameters of common carp, *Cyprinus carpio*. The experimental groups showed, significantly, lower values of leukocytes, lymphocytes and monocytes. While, significant higher values of neutrophils and eosinophils were found compared to the control groups. Moreover, El-Elaimy et al. (2012) indicated that chlorpyrifos, which is an OP pesticide, produced a significant decrease in total WBCs count and relative lymphocytes while it caused an increase in the relative granulocytes.

The decrease in leukocytes count in the current study could be attributed either to the slower rate of production of leukocytes, bone marrow suppression, or due to their inhibited release into the circulation (Goel et al., 2006). Granulocytes, mostly neutrophils, are the first line of defense against infectious agents, tissue injury, parasites and inflammatory or foreign materials and exert their activity by eliminating foreign material by phagocytosis (Kobayashi et al., 2003). So, the increase in relative granulocytes may be due to body need to repair damage that resulted from DZN-intoxication (Luty et al., 1998).

As shown in the results, a significant time dependent reduction in the platelets count was observed. Similar results related to platelets count reduction in rats after subacute exposure to DZN was reported by Kalender et al. (2006) and Hariri et al. (2011). Recent study of Elsharkawy et al. (2013) showed that chlorpyrifos, OP pesticide, resulted in significant decrease in platelets count when compared to control animals. The authors of the previously mentioned report discussed that this decrease in thrombocytes could be a result of free radicals effects on the bone marrow which leads to reduced platelets production or depressed thrombopoiesis.

Generally, in the present study DZN resulted in time dependent increase in bleeding time, PT value and APTT when compared with control groups. Early, Lox and Davis (1983) reported that malathion, OP pesticide, intoxication

caused prolongation in both APTT and PT. Takahashi (2000) attributed this prolongation in bleeding time to the intrinsic coagulation defect or to the decrease in platelets function.

It is well documented that DZN have a hepatotoxic effects in rats and mice (El-Shenawy et al., 2010; Messarah et al., 2012) which may affect the synthesis of coagulation factors. So it could be concluded that the increase in APTT and PT could be attributed to a defect in blood coagulation, in both extrinsic and intrinsic coagulation pathways and/or defect in coagulation factors due to liver dysfunction caused by DZN-intoxication.

In general, pesticides have been shown to enhance the production of reactive oxygen species (ROS), which in turn cause oxidative stress, in different tissues (Mehta et al., 2009).

In the present study, treatment of animals with DZN led to the induction of LPO in time dependent way. This result is in agreement with several studies that demonstrated a significant increase in LPO level of rat erythrocytes, pancrease and heart by DZN-intoxication (Gökalp et al., 2005; Ogutcu et al., 2006). Altuntas et al. (2004) examined the effect of DZN at different incubation periods and various concentrations on LPO and the activities of antioxidant enzymes in erythrocytes *in vitro*. The authors concluded that *in vitro* administration of DZN resulted in the induction of erythrocyte LPO and changed the activities of antioxidant enzymes suggesting that ROS may be involved in the toxic effects of DZN. Moreover, time dependent increase in malonaldehyde level in cardiac tissues was observed due to toxicity with 10 mg/kg of DZN at three time intervals 1, 4 and 7 weeks (Ogutcu et al., 2006).

One of the molecular mechanisms underlying the toxicity of some pesticides seems to be lipid peroxidation. As a consequence, these compounds can disturb the biochemical and physiological functions of the RBCs (Mansour and Mossa, 2009). It is well documented that LPO is linked with excessive generation of ROS, which may be contributed by exogenous or endogenous sources. Earlier studies reported that LPO is the most destructive process in the living cells that has been implicated in causing a wide range of biological effects such as osmotic fragility, reduced erythrocyte survival and membrane fluidity (Kaplowitz and Tsukamoto, 1996; Hogg, 1998).

Moreover hematological studies reported that Hb in RBCs is found to be a major source of radical production when it interacts with xenobiotics giving rise to superoxide radicals, hydrogen peroxide and, in certain cases, peroxy radical leading to membrane LPO and hemolysis (Kalender et al., 2002; 2004).

So, the elevated LPO by DZN-intoxication could be attributed to excessive generation of ROS which have been reported to attack various biological molecules including lipids and causing increased LPO (Shah and Iqbal, 2010).

In the present study, DZN-intoxication resulted in time dependent decrease in GSH level. This result was correlated with previous studies of El-Shenawy et al. (2010); Ahmadi et al. (2012) and Messarah et al. (2012) who found that DZN toxicity produced a significant decrease in GSH level in RBCs, liver and spleen. The authors suggested that this depletion of GSH content in RBCs of rats, in the presence of DZN-intoxication, could be explained either by high GSH utilization for conjugation and/or by the participation of GSH as an antioxidant in neutralizing the free radicals. In fact, the decrease in the GSH and the enhanced MDA levels in RBCs reflected the anemia signs observed in the current study, which are probably related to intravascular hemolysis due to lipid peroxidation in the circulating RBCs (Messarah et al., 2012).

In the present study, sever changes in the antioxidant defense system in RBCs of DZN-intoxicated rats were observed. DZN-treatment resulted in time dependent decrease in GST, GR, GPx, CAT and SOD activities.

El-Shenawy et al. (2010) found that mice treated with DZN showed an increase in the liver LPO level and decrease in liver GSH, GR, GPx, CAT and SOD. On the same line, Shah and Iqbal (2010) investigated the effect of DZN on the renal antioxidant defense system in rats. The authors found that after 8 weeks of toxicity there was depletion in GSH and antioxidant enzymes while LPO was increased.

It was well documented that oxidative modification of proteins by ROS may lead to the structural alteration and functional inactivation of many enzyme proteins (Cabiscol et al., 2000). Furthermore, ROS can be detoxified by an elaborated battery of enzymatic defense system, comprising SOD, CAT, GR and GPx or non-enzymatic systems by the scavenging action of GSH, while organic peroxides can be detoxified by the activity of GST (Halliwell, 1994; Halliwell and Gutteridge, 2002).

Consequently, the decline in the levels of GR, GPx, GST, SOD and CAT after DZN-intoxication might come from the involvement of these enzymes in the detoxification process in RBCs, leading to their consumption, and the insufficient level of these antioxidants may damage the cells via the oxidative stress (Abdollahi et al., 2004).

In conclusion, the present study demonstrated that DZN resulted in severe damage in hematological parameters and antioxidant defense system in erythrocytes of treated rats. This effect was in time dependent manner.

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