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Preventive Effects of Black Seed (*Nigella Sativa*) Extract on Sprague Dawley Rats Exposed to Diazinon

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Abstract: Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of a plant's seeds, leaves, roots, bark, flowers or fruits for medicinal purposes. Long practiced outside of conventional medicine, herbalism is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show their value in the treatment and prevention of disease. Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary health care. The use of herbal supplements for medicinal purposes has increased dramatically over the past thirty years. Therapeutical properties of medical herbs are very useful in healing various diseases and the advantage of these medicinal herbs is being 100% natural. Nowadays people are being bombarded with thousands of toxicants, pollutants and unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal herbs can represent the best solution. The capability of an ethanol extract of *Nigella sativa* seeds to attenuate the severe influences induced by diazinon intoxication in rats has been evaluated. Sixty male rats of Sprague Dawley strain were used in the present study and they were divided into four groups. Rats of first group were untreated and served as control. Animals of second group were orally given daily a dose of diazinon at the level of 12.8 mg/kg body weight for six weeks. Rats of third group were orally supplemented with *Nigella sativa* seeds extract (300mg/kg/day) and after two hours received diazinon at the same dose given to second group. Animals of fourth group were supplemented with *Nigella sativa* seeds extract at the same dose given to third group. Hematological and serum biochemical parameters were estimated after three and six weeks. Red blood corpuscles (RBC) count, hemoglobin (Hb) concentrations, hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) values and percentage of neutrophil showed significant reduction, while the values of mean corpuscular volume (MCV), white blood corpuscles (WBC) count and percentages of lymphocyte and monocyte were statistically increased in rates exposed to diazinon. Administration of diazinon for three and six weeks resulted in marked increases in the values of serum creatinine, urea, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK). Also, the results of this study showed that *Nigella sativa* seeds extract given orally for three and six weeks attenuated the extensive changes of hematological and biochemical parameters in diazinon-treated rats. Based upon these results, we suggest that *Nigella sativa* seeds can be considered as a promising therapeutic agent against hematotoxicity, immunotoxicity, hepatoxicity, nephrotxicity and cardiotoxicity induced by diazinon and may be against other chemical pollutants, environmental contaminants and pathogenic factors.

Key words: Diazinon, *Nigella sativa*, blood, rats

INTRODUCTION

Environmental pollution caused by pesticide residues is a major concern due to their extensive use in agriculture and in public health programs (Waliszevski *et al*., 1996). The environmental impact of pesticide

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use is related to several fundamental properties essential to their effectiveness as pesticides. Firstly, pesticides are toxicants, capable of affecting all taxonomic groups of biota, including non-target organisms, to varying degrees depending on physiological and ecological factors. Secondly, many pesticides need to be resistant to environmental degradation so that they persist in treated areas, thus enhancing their effectiveness. This property also results in long-term effects in the natural ecosystem (Christensen and Tucker, 1976). Since pesticides are recommended for plant protection, there has been an improvement in the control of pest populations and the spread of infection-born disease vectors. Public health programs in many developing countries also utilize pesticides to control disease-transmitting organisms (Arslan *et al*., 1997). Of the some 1500 chemicals used in agriculture, pesticides are the most important. They are known to be potentially dangerous to the environment, nature, and for the human beings (Grant, 1982). Pesticides are chemical substances used to control any type of unwanted organisms. Consequently the term include a wide variety of chemicals, applied in many places, however they are an integral and necessary component of the technological world in which we live. Organophosphorous insecticides represent a major class of pesticides in use today. They exhibit less persistence than the organochlorine pesticides and exhibit greater toxicity to mammals because they are cholinesterase inhibitors (Menn *et al*. 1976; LÓbpez *et al*., 1986; Reigart and Roberts, 1999; Ware, 2000). Diazinon ($C_{12}H_{21}N_2O_3PS$), O,O-diethylO-(2-isopropyl-6-methyl4-pyrimidinyl) phosphorothioate (Fig. 1) is a nonsystemic organophosphate insecticide which has been used since 1956 for the control of soil insects and pests, on ornamental plants, and on fruit and vegetable field crops. Now it is used to control flies around animal facilities, greenhouses, fairgrounds and other businesses and public places where food or animal wastes might accumulate (Dikshith and Diwan, 2003). Diazinon affects mainly the nervous system regardless of the route of exposure. Some mild signs and symptoms of poisoning include headache, dizziness, weakness, feelings of anxiety, constriction of the pupils, and blurred vision. More severe symptoms include nausea and vomiting, abdominal cramps, slow pulse, diarrhea, pinpoint pupils, difficulty breathing, coma, and possibly death. These effects also occur in animals exposed to high doses of diazinon. There is no evidence that long-term exposure to low levels of diazinon causes harmful effects in people. Diazinon has not been shown to affect fertility in humans (ATSDR, 2008).

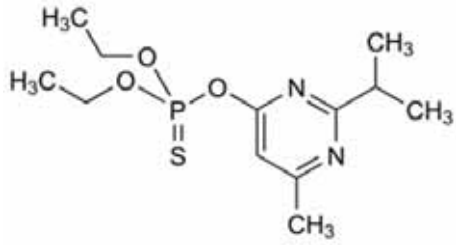

Fig. 1: The chemical structure of Diazinon.

There is an increasing interest towards medicinal plants and their active ingredient since 1980. One of the most important motives was the synthetic drugs which show off their dangerous side effect by time, whereas medicinal plants have generally centuries-long use and little unknown side effects (Hanefi *et al*., 2004). The use of plants as medicines dates from the earliest years of man's evolution (Dattner, 2003). Medicinal plants serve as therapeutic alternatives, safer choice , or in some cases, as the only effective treatment. People in different cultures and places have used particular plants for to treat certain medical problems. A larger number of these plants and their extract have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, and immunomodulatory effects (Thatte *et al*., 1992; Rege *et al*., 1999; Salem and Hossain, 2000; Chattopadhyay *et al*., 2002; Dattner, 2003; Huffman, 2003; Miller *et al*., 2004; Aherne *et al*., 2007; Qin *et al*., 2008; Brown *et al*., 2009). Among the promising medicinal plants, *Nigella sativa* (commonly known as black seed and black cumin), a dicotyledonous of *Ranunculaceae* family, is an annual herb with finly dissected leaves sepals 5, petaloid petals 5, growing in the western part of Saudi Arabia (Migahid,1996), amazing herb with a rich historical and religious background (Goreja, 2003). *Nigella sativa* L. is found in southern Europe, northern Africa, and Asia Minor. It is a bushy, self-branching plant with white or pale to dark blue flowers. *Nigella sativa* reproduces with itself and forms a fruit capsule which consists of many white trigonal seeds. Once the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air, becoming black in color (Schleicher and Saleh, 1998). The seeds of *Nigella sativa* are the source of the active ingredients of this plant. The oil and seed constituents have shown potential medicinal properties in traditional medicine (Salem, 2005). Historically, it has been recorded that *Nigella sativa* seeds

were prescribed by ancient Egyptian and Greek physician to treat headache, nasal congestion, toothache and intestinal worms, diuretic and to increase milk production (El-Dakhakhny, 1965; Goreja, 2003). The seeds of *Nigella sativa* have long been used in the middle and far east as a traditional medicine for a wide range of illnesses including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems (Schleicher and Saleh, 1998; Al-Rowais, 2002). Its use in skin condition as eczema has also been recognized worldwide (Goreja, 2003). Additionally, seeds of *Nigella sativa* have been employed for thousands years as a spice and food preservative (Aboutabl *et al*., 1986; Hanafy and Hatem, 1991). *Nigella sativa* seeds contain 36%-38% fixed oils, proteins, alkaloids, saponin and 0.4%-2.5% essential oil (Lautenbacher, 1997). The fixed oil is composed mainly of unsaturated fatty acids, including the unusual C20:2 arachidic and eicosadienoic acids (Houghton *et al*., 1995). The essential oil was analysed by Burits and Bucar (2000) using GC-MS. Many components were characterized, but the major ones were thymoquinone (27.8%-57.0%), ρ-cymene (7.1%-15.5%), carvacrol (5.8%-11.6%), t-anethole (0.25%-2.3%), 4-terpineol (2.0%-6.6%) and longifoline (1.0%-8.0%). Thymoquinone readily dimerizes to form dithymoquinone (El-Dakhakhny, 1963). Four alkaloids have been reported as constituents of *Nigella sativa* seeds. Two, nigellicine and nigellidine have an indazole nucleus, whereas nigellimine and its N-oxide are isoquinolines (Atta-ur-Rahman *et al*., 1985; 1992;1995).

In spite of large number of pharmacological studies carried out world wide on *Nigella sativa* seed, recent references like Ahmed *et al*. (2004) explained that *Nigella sativa* is truly an amazing combination, but there are still many components that haven't been yet identified, but research is going on around world. Therefore, the objective of the present study was to test the possible protective effects of *Nigella sativa* seeds extract against diazinon-induced hematological and biochemical disturbances in normal rats.

MATERIALS AND METHODS

Animals:

A total of sixty male rats of Sprague Dawley strain weighing 190-210 g were used in the present study. Rats 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 maintenance diet 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.

Preparation of Seeds Extract:

The extraction of *Nigella sativa* seeds was prepared according to the method of Hadjzadeh *et al*. (2007) with special modification. *Nigella sativa* seeds were purchased from a local market with a highly degree of quality assurance. The seeds were botanically authenticated and its identification was confirmed by a specialist of plant taxonomy. The seeds were cleaned, dried and powdered in an electrical grinder and stored at 5 ºC until further use. Seed powder was extracted with a sufficient volume of 96% ethanol using Soxhlet extraction apparatus. Ethanol was evaporated at 40°-50°C under reduced pressure and the yield of extract was dissolved in water before use.

Treatments:

Diazinon 60 EC 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 LD_{50} for male albino rats (Sprague Dawley) was determined by Ibrahim and El-Gamal (2003) and it was found to be 128 mg/kg body weight. Rats were divided into four groups, each group consisting of 15 animals. Rats of group 1 were untreated and served as control. Animals of group 2 were orally given daily a dose of diazinon at the level of 12.8 mg/kg body weight (1/10 of LD_{50}) for six weeks. Rats of group 3 were orally supplemented with *Nigella sativa* seeds extract (300mg/kg/day) in aqueous solution and after two hours received diazinon at the same dose given to group 2. Animals of group 4 were supplemented with *Nigella sativa* seeds extract at the same dose given to group 3.

Blood Sampling:

After three and six weeks, rats were anaesthetized with ether. Blood samples were collected from orbital venous plexus in heparinized and non-heparinized tubes. The blood in the heparinized tubes was immediately used for hematological measurements including red blood corpuscles (RBC) count, hemoglobin (Hb)

concentration, hematocrit (Hct) value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood corpuscles (WBC) count, differential count of leukocytes such as lymphocyte (%), neutrophil (%) and monocyte (%). Hematological parameters were assessed using ADVIA Hematology Automatic System (USA). The blood in non-heparinized tubes was centrifuged at 2000 rpm for 20 minutes. The clear supernatants sera were frozen till the time of various biochemical estimations including the levels of creatinine, urea, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK). All of these biochemical parameters were measured using an automatic analyzer (Reflotron® Plus System, Roche, Germany).

Statistical Analysis:

Data are expressed as means± standard deviation (SD). Statistical comparison between different groups were done using one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test to judge the difference between various groups. Significance was accepted at $P< 0.05$.

RESULTS AND DISCUSSION

Results:

Data of hematological parameters after three and six weeks are shown in Table 1. In rats treated with diazinon (12.8 mg/kg), group 2, for three and six weeks, RBC count, Hb concentrations, MCHC values and neutrophil percentage showed significant reduction as compared with the untreated control group or groups 3 and 4. The values of Hct were statistically decreased after three weeks in group of rats exposed to diazinon in comparing with groups 3 and 4, while this parameter were significantly declined after six weeks in comparing with groups 1, 3 and 4. In comparison with groups 3 and 4, significant increase of MCV value was recorded after three weeks in diazinon-treated rats, while this parameter was markedly elevated after six weeks as compared with groups 1, 3 and 4. Notable decline in MCH value was observed after three weeks in diazinon-exposed rats in relation to groups 1, 3 and 4, and after six weeks as compared with groups 3 and 4. White blood corpuscles (WBC) count was statistically increased after three and six weeks as compared with the untreated control group or groups 3 and 4. Lymphocyte percentage was significantly elevated after three weeks in group 2 as compared with groups 1, 3 and 4, while this parameter was statistically increased after six weeks as compared with group 1. Mononcyte percentage was significantly declined after 3 weeks as compared with group 1 and after six weeks as compared with groups 1, 3 and 4. In the case of rats treated with diazinon plus *Nigella sativa* seeds extract (group 3), statistically significant decreases in the number of RBC and the concentration of Hb were noted after three and six weeks compared with control group. Hct value was decreased after six weeks as compared with control group. Also, in comparison with control group, neutrophil percentage was markedly decreased after three weeks. MCV, MCH, MCHC, WBC, lymphocyte and monocyte values were statistically unchanged in group 3 compared with group 1 and 4. Hematological parameters were insignificantly changed in rats supplemented with only *Nigella sativa* seeds extract, group 4.

Fig. 2, 3, 4, 5, 6 and 7 show the values of serum biochemical analysis. Administration of diazinon (12.8 mg/kg) for three and six weeks resulted in marked increases in the values of serum creatinine (63.01% and 101.25%), urea (20.42% and 65.33%), uric acid (27.02% and 71.61%), ALT (69.00% and 120.23%), AST (53.51% and 84.42%) and CK (23.98% and 77.77%) which were significantly different from those of groups 1, 3 and 4. The values of creatinine and uric acid were statistically unchanged after three and six weeks in rats treated with diazinon plus *Nigella sativa* seeds extract (group3) as compared with groups 1 and 4. In comparison with control, the value of urea (14.35%) was significantly increased after three weeks in rats treated with diazinon plus *Nigella sativa* seeds extract. Additionally, the activity of ALT (45.71%) was statistically increased after six weeks in group 3. Moreover, the activity of AST (9.51% and 12.60%) was notably increased after three and six weeks, while the activity of CK (10.46%) was significantly increased after three weeks in group 3 as compared with control group. On the other hand, no statistically significant differences were observed in the levels of serum biochemical parameters in rats treated with only *Nigella sativa* seeds extract. From the present results, It was pronounced that the treatment by *Nigella sativa* seeds extract significantly reduced the highly alterations of hematological and biochemical parameters caused by diazinon intoxication.

Table 1. Hematological values of control, diazinon, diazinon plus Nigella sativa seeds extract and Nigella sativa seeds extract treated rats at three and six weeks. Tabulated values are means of seven determinations± standard deviation (SD).

		Treatments			
Parameters	Periods (weeks)	Control	Diazinon	Diazinon + seeds extract	Seeds extract
RBC	3	7.47±0.36	6.81±0.18*a	7.04±0.36*	7.54±0.28
(10^6/mm^3)	6	7.34±0.28	5.23±0.32*a	6.90±0.19*	7.26±0.12
Hb	3	14.27±1.16	12.24±0.43*a	13.39±0.48*	14.83±0.62
(g/dL)	6	14.77±0.56	10.09±0.52*a	13.27±0.43*	14.80±0.34
Hct	3	40.14±2.27	37.86±1.35a	39.87±1.33	40.43±1.51
(%)	6	42.17±1.51	34.73±1.78*a	37.73±0.99*	41.17±2.19
MCV	3	53.78±3.08	55.57±1.88a	56.63±2.57	53.56±1.20
(µm^3)	6	57.27±2.26	66.71±6.43*a	54.58±2.42	56.52±2.80
MCH	3	19.76±1.62	17.98±0.93*a	19.23±0.65	19.66±0.24
(pg)	6	20.10±0.86	19.31±0.85a	19.32±0.86	20.43±0.38
MCHC	3	35.31±4.00	32.02±2.75*a	33.58±1.96	36.68±1.00
(g/dL)	6	35.12±1.51	28.90±2.61*a	35.47±1.85	35.81±1.55
WBC	3	10.04±0.33	11.47±0.98*a	10.17±0.77	10.01±0.42
(10^3/mm^3)	6	10.36±0.37	16.12±1.92*a	11.36±0.37	10.690.32
Lymphocyte	3	76.43±1.72	80.44±2.5*a	77.57±1.99	75.14±2.41
(%)	6	73.43±2.07	77.57±2.76*	75.00±3.22	75.43±3.21
Neutrophil	3	20.29±1.50	16.29±1.89*a	17.57±2.30*	20.71±2.29
(%)	6	18.03±1.17	14.84±2.66*a	17.29±1.38	18.69±2.06
Monocyte	3	1.71±0.95	2.00±0.82*	2.29±0.94	1.86±0.69
(%)	6	2.86±1.22	5.29±1.11*a	3.43±1.62	3.42±0.98

*: Indicates a significant difference between control and treated groups.
a: Indicates a significant difference between the groups treated with diazinon, diazinon plus Nigella sativa seeds extract and *Nigella sativa* seeds extract.

Discussion:

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 20th 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 (Deichmann *et al*., 1968). The administration of insecticides, therefore, affects the biochemical environment of blood and liver. The present investigation demonstrated that the exposure of rats to diazinon caused extensive changes in hematological and biochemical parameters. Hematological characteristics have been widely used in the diagnosis of variety of diseases and pathologies induced by industrial compounds, drugs, dyes, heavy metals, pesticides and several others (Morgan *et al*., 1980; Ali *et al*., 1988; Ali and Shakoori, 1988; Mansour and Mossa, 2005; Kalender *et al*., 2006; Eraslan *et al*., 2009). Reduction in RBC count is considered due to the direct injurious action of the toxin on the animals. Erythropenia along with decreased hemoglobin concentration is an indication of decrease in oxygen carrying capacity in the animals, resulting in insufficient supply of oxygen to the tissues causing adverse effects on animal health. The present results showed that the administration of diazinon caused highly decrease in RBC count and hemoglobin concentration. Reduction in hemoglobin concentration may be due to increased rate of breakdown of red cells and/or reduction in the rate of RBC formation. Shakoori *et al*. (1990) suggested that the decrease in RBC count is either indicative of excessive damage to erythrocytes or inhibition of erythrocyte formation. Moreover, the hepatic heme biosynthesis has already been reported to be affected by insecticidal exposure, which also contributes to decreased RBC count and hemoglobin concentration (Taljaard *et al*., 1972). Additionally, many investigations showed that RBC count, hemoglobin concentration and the values of Hct, MCH and MCHC were significantly declined, while the levels of MCV were markedly elevated in diazinon and other pesticides-treated experimental animals (Ali and Shakoori, 1990; Yousef *et al*., 2003; Adeniran *et al*., 2006; Kalender *et al*., 2006; Yehia *et al*., 2007; Alahyary *et al*., 2008; Afshar *et al*., 2009). In the present study, the administration of diazinon induced increases in the number of WBC, lymphocyte and monocyte percentages, while the percentage of neutrophil was significantly decreased. Further induction of leukocytosis is considered of immunological significance to meet the adverse situation developed by the introduction of diazinon in the blood. It may also be for the removal of the debris of tissue damaged by the diazinon. Several studies showed that the treatment with different pesticides markedly increased the animal's defense mechanism and immune system (Shakoori *et al*., 1990; Fujitani *et al*., 1997; Yousef *et al*., 2003; Celik and Suzek, 2008; Celik *et al*., 2009).

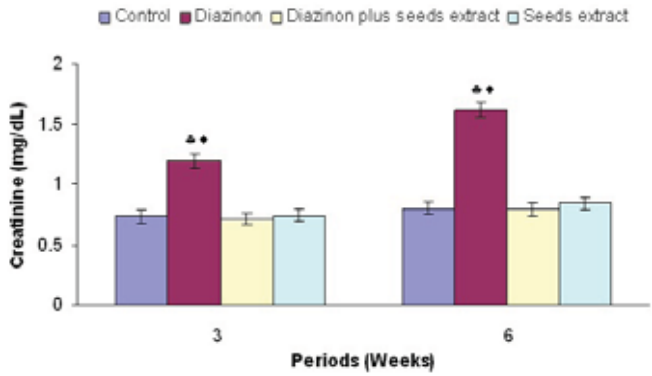

Fig. 2: Serum creatinine levels of all experimental groups at 3 and 6 weeks. Values are means of seven determinations± standard deviation (SD). ♣ indicates a significant difference between control and treated groups. ◆ indicates a significant difference between rats treated with diazinon, diazinon plus *Nigella sativa* seeds extract and *Nigella sativa* seeds extract.

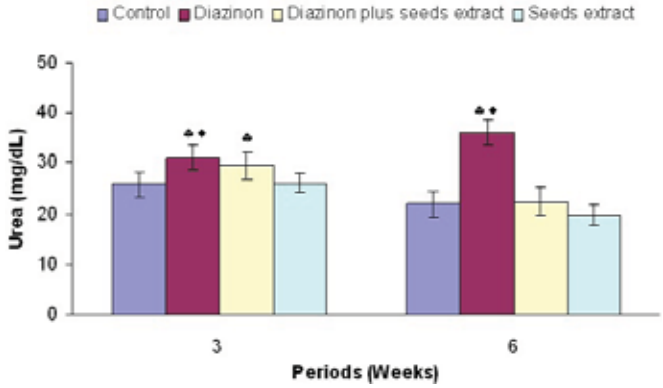

Fig. 3: Serum urea levels of all experimental groups at 3 and 6 weeks. Values are means of seven determinations± standard deviation (SD). ♣ indicates a significant difference between control and treated groups. ◆ indicates a significant difference between rats treated with diazinon, diazinon plus *Nigella sativa* seeds extract and *Nigella sativa* seeds extract.

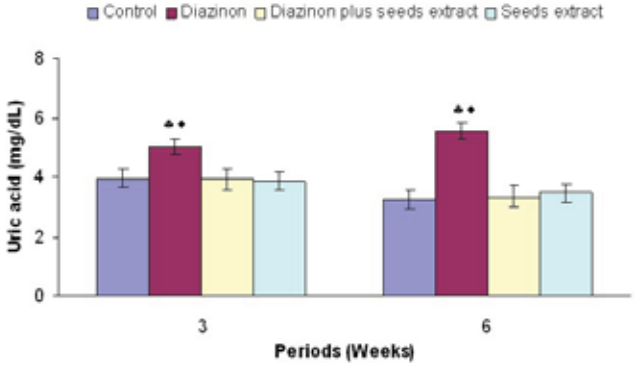

Fig. 4: Serum uric acid levels of all experimental groups at 3 and 6 weeks. Values are means of seven determinations± standard deviation (SD). ♣ indicates a significant difference between control and treated groups. ◆ indicates a significant difference between rats treated with diazinon, diazinon plus *Nigella sativa* seeds extract and *Nigella sativa* seeds extract.

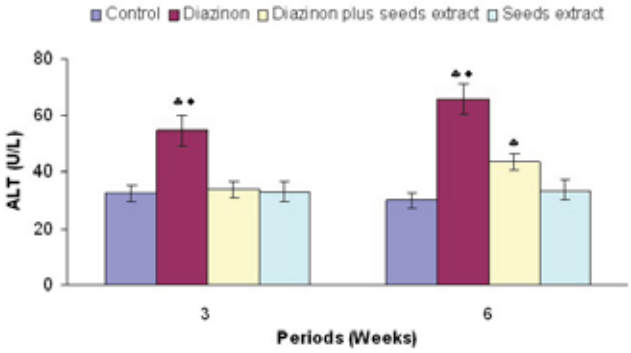

Fig. 5: Serum ALT activities of all experimental groups at 3 and 6 weeks. Values are means of seven determinations± standard deviation (SD). ♣ indicates a significant difference between control and treated groups. ◆ indicates a significant difference between rats treated with diazinon, diazinon plus *Nigella sativa* seeds extract and *Nigella sativa* seeds extract.

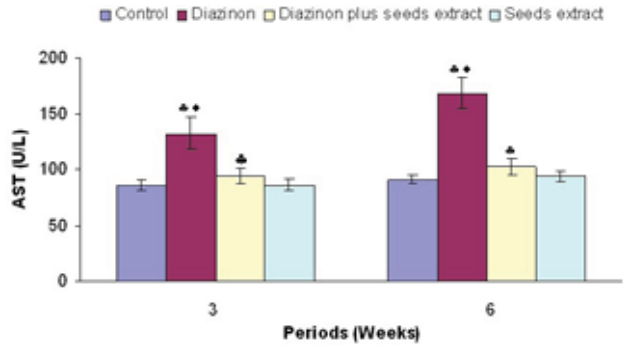

Fig. 6: Serum AST activities of all experimental groups at 3 and 6 weeks. Values are means of seven determinations± standard deviation (SD). ♣ indicates a significant difference between control and treated groups. ◆ indicates a significant difference between rats treated with diazinon, diazinon plus *Nigella sativa* seeds extract and *Nigella sativa* seeds extract.

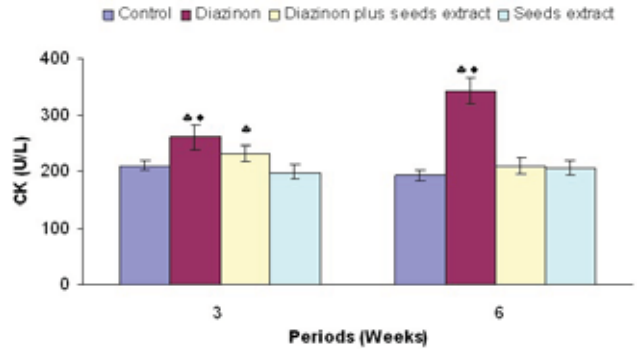

Fig. 7: Serum CK activities of all experimental groups at 3 and 6 weeks. Values are means of seven determinations± standard deviation (SD). ♣ indicates a significant difference between control and treated groups. ◆ indicates a significant difference between rats treated with diazinon, diazinon plus *Nigella sativa* seeds extract and *Nigella sativa* seeds extract.

Serum levels of creatinine, urea and uric acid, enzymes activity of ALT, AST and CK are useful tools in diagnosis as the pick any disturbances to the system early enough to allow for projection and possible remedies. This study evaluated kidney function by measuring serum creatinine, urea and uric acid values, that of liver by activities of ALT and AST, and that of heart by activity of CK. Diazinon treatment is found to elevate cratinine, urea and uric acid levels in serum. These observations are generally in agreement with other investigators on pesticides induce relative effects (Yousef *et al*., 2003; Eraslan *et al*., 2007; Omurtag *et al*., 2008). Creatinine, urea and uric acid are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase of these parameters, as observed in this study, confirms an indication of functional damage to the kidney (Panda, 1999). Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases the serum creatinine level (Cheesbrough , 1988). We postulate that the damaging effect of diazinon on the liver is manifested by increases in serum ALT and AST activities. These results were in accordance with the findings of many studies showing elevations of these enzymes in experimental animals exposed to diazinon and other pesticides (Videla *et al*., 1995; el-Demerdash *et al*., 2003; Kalender *et al*., 2005; Etim *et al*., 2006; Gokcimen *et al*., 2007; Omurtag *et al*., 2008; Eraslan *et al*., 2009). CK is the first heart enzyme to appear in the blood after a heart attack and it also disappears quickly from the blood. In the present study, the activity of serum CK was increased in diazinon-treated rats. This is may be due to leakage from the heart as a result of diazinon-induced necrosis. Several studies showed that diazinon and other pesticides induced cardiotoxicity in experimental animals (Courtney and Ebron, 1981; Sauviat and Pages, 2002; Kalender *et al*., 2004; Ogutcu *et al*., 2006; Jalili *et al*., 2007).

The mechanism of the toxic effect of organophosphate compounds involves the inhibition of acetylcholinesterase and other non-specific esterases through phosphorylation at–OH serine in the esterase centre of the enzyme. This mechanism is the same for all insecticides of the group, irrespective of differences in their chemical structure (Lotti, 2001). The inhibition of the activity of cholinesterase enzymes causes an increase in the level of endogenous acetylcholine in the organism and results in its binding to muscarinic and nicotinic receptors in both the peripheral and central nervous systems(CNS). This increase in the CNS disturbs the balance between neurotransmitters and causes the onset of acute intoxication symptoms (Lotti, 2001). The symptoms of acute intoxication with organophosphates have been well described, while the effects of chronic exposure to these compounds are not completely clear. Many authors postulate that they may have an effect on redox processes in a number of organs, thus leading to disturbances in these processes and causing enhancement of lipid peroxidation, both in acute and chronic intoxication by these compounds (Abdollahi *et al*., 2004; Sharma *et al*., 2005; Costa, 2006; Fortunato *et al*., 2006). As increased generation of reactive oxygen species and lipid peroxidation induced by these species underlies many diseases, it is extremely important to determine the effect of organophosphate insecticides on lipid peroxidation processes (Yagi, 1987; Ueda *et al*., 1997; Matés *et al*., 1999). However, several studies showed that diazinon and other pesticides exposure led to increase lipid peroxidation with tissue specific changes in liver, kidney, heart, testis and brain (Altuntas and Delubas, 2002; Khan, 2005; Akturk *et al*., 2006; Fortunato *et al*., 2006; Mansour *et al*., 2009; Ozden and Alpertunga, 2010). The results of this study indicated that *Nigella sativa* seeds extract given orally for three and six weeks attenuated the extensive changes in hematological and biochemical parameters in diazinon-treated rats. The exact mechanisms by which *Nigella sativa* seeds extract exert their protective effects against diazinon-induced toxicity are not yet known. It has also been shown that compounds isolated from of *Nigella sativa* seeds (including thymoquinone, carvacol, tanethole and 4-terpineol) have appreciable free radical scavenging properties (Burits and Bucar, 2000). Generation of free radicals may be, at least partially, the basis of many human diseases and conditions. Therefore, the antioxidant action of *Nigella sativa*, particularly thymoquinone, may explain its claimed usefulness in folk medicine. This antioxidant property would explain its action against several chemicals-induced liver, kidney, heart, gastric and colon injuries and diseases (Nagi *et al*., 1999; Badary *et al*., 2000; El-Dakhakhny *et al*., 2000; Mansour, 2000; Nagi. and Mansour, 2000; Turkdogan *et al*., 2000; Kanter *et al*., 2006; Al-Johar *et al*., 2008; Uz *et al*., 2008). However, results obtained from the present study showed that the extract of *Nigella sativa* seeds could mostly protect blood, liver, kidney and heart from severe alterations induced by exposure to diazinon. We suggest that these protective effects of *Nigella sativa* seeds could be due to their antiperoxidative and antioxidant influences. Additionally, we suggest that *Nigella sativa* seeds can be considered as a promising therapeutic agent against hematotoxicity, immunotoxicity, hepatoxicity, nephrotxicity and cardiotoxicity induced by diazinon and may be against other chemical pollutants, environmental contaminants and pathogenic factors. Finally, in view of the present findings, the repeated use of *Nigella sativa* seeds as a therapeutic agent in pharmacological and pathological researches should be encouraged.

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