

DIMETHOATE-INDUCED CHANGES IN BIOCHEMICAL PARAMETERS OF EXPERIMENTAL RAT SERUM AND ITS NEUTRALIZATION BY BLACK SEED (*NIGELLA SATIVA* L.) OIL

AHMED M. ATTIA*, HODA M. NASR

Alexandria University, Alexandria, Egypt

ABSTRACT

There is a considerable interest in detailed study of free radical-mediated damage to biological systems due to pesticide exposure. However, there is a lack of consensus as to which determinations are best used to quantify future risk of xenobiotic exposure and the use of natural plant products as antioxidant interventions. A purpose of this study was to investigate the effect of dimethoate on oxidative stress, biochemical parameters and enzyme activities in rat males as well as possible role of *N. sativa* oil (NSO) in attenuation of dimethoate-induced changes. The animals were divided randomly into 4 groups and kept at 10 rats per group. The first group was served as a control and administered with corn oil per orally; the group 2 was injected with a single dose of dimethoate (75 mg.kg⁻¹ BW, per orally) 24 h prior to decapitation, the group 3 rats were pretreated per orally with NSO (2 ml.kg⁻¹ BW, three times a week for three weeks), the group 4 rats were pretreated per orally with NSO (2 ml.kg⁻¹ BW, three times a week for three weeks) prior to oral administration of a single dose of dimethoate (75 mg.kg⁻¹ BW). Dimethoate induced ($P<0.001$) increase in serum thiobarbituric acid-reactive substance (TBARS) levels, superoxide dismutase (SOD) and catalase (CAT) activities and inhibited AChE activity. Treatment with NSO alone increased the activities of SOD and catalase, while decreased TBARS levels. NSO protected against dimethoate-induced lipid peroxidation and oxidative stress in these rats. Total lipid (TL), cholesterol, triglyceride (TG), and low density lipoprotein (LDL), were significantly ($P<0.01$) increased, while high density lipoprotein (HDL) level was decreased. NSO alone decreased levels of lipids and lipoproteins, and neutralized harmful effects of dimethoate. Dimethoate increased ($P<0.01$) levels of serum aminotransferases, phosphatases, lactate dehydrogenase (LDH) and gamma-glutamyl transferase (γ -GT). Urea, creatinine, uric acid and total bilirubin levels were increased, whilst serum total protein, albumin and globulin were significantly ($P<0.01$) decreased. The results show that NSO may neutralize dimethoate-induced changes in biochemical parameters, lipid peroxidation by activation of the antioxidant defense system in rats.

Keywords: *Nigella sativa* L. oil; dimethoate; oxidative stress; antioxidant; lipid peroxidation

INTRODUCTION

Pesticides are occasionally used indiscriminately in large amounts causing environmental pollution and therefore, are a cause of concern. Residual amounts of organochlorines and organophosphate pesticides (OPI) have been detected in the soil, water reservoirs, vegetables, grains and other food products (John et al., 2001). OPI are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase (AChE) and subsequent activation of cholinergic receptors (Costa, 2006). Involvement

of oxidative stress following acute exposure to OPI has been reported recently (Sharma et al., 2005a,b; Sivapiriya et al., 2006; Kamath et al., 2008) and it has been demonstrated unequivocally that lipid peroxidation is one of the molecular mechanisms involved in OPI-induced toxicity (Akhgari et al., 2003; Abdollahi et al., 2004; Kamath et al., 2008).

Numerous studies indicate that dimethoate intoxication can cause oxidative stress by the generation of free radicals and induce hepatic lipid peroxidation in mice (Sivapiriya et al., 2006) and rats (John et al., 2001; Sharma et al., 2005a, b; Kamath et al., 2008). As some

Correspondence: E-mail: attiaamm@yahoo.com
Department of Environmental Studies, Institute of Graduate Studies & Research, Alexandria University, 163, Horrya Av. El-Shatbey, P.O. Box 832, 21526, Alexandria, Egypt; Tel.: +203 42 95 007; Fax: +203 42 85 792

Received: November 27, 2008
Accepted after corrections: January 23, 2009

OP's may be present in blood of exposed humans and animals, it may cause oxidative stress in erythrocytes. The antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) as well as total-SH content in erythrocytes, however, many neutralize the oxidative stress (John et al., 2001).

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. The cellular antioxidant action is reinforced by the presence of dietary antioxidants (Kamath et al., 2008). Accordingly, interest has recently grown in the role and usage of natural antioxidants as strategy to prevent oxidative damage in various health disorders with oxidative stress as factor in their pathophysiology (Kamath et al., 2008). Natural antioxidants from fruits and vegetables are reported to provide substantial protection that slows down the process of oxidative damage caused by reactive oxygen species (ROS) (Jacob and Burri, 1996). Among the promising medicinal plants, *N. sativa*, a dicotyledon of the Ranunculaceae family, is an amazing herb with rich historical and religious background. The seeds of *N. sativa* are the source of the active ingredients of this plant (Salem, 2005). The seeds of *N. sativa* known as black seed, black cumin or ‘Habatul-Barakah’, have long been used in folk medicine for a wide range of illnesses, including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems (Al-Rowais, 2002). Of the studies that have been performed to evaluate different effects of *N. sativa*, majority (more than 35) of the studies have confined to address its antitoxic properties both *in vitro* and *in vivo* (Salem, 2005). For instance, essential oil obtained from six different extracts of *N. sativa* seeds and from a commercial fixed oil showed antioxidant effects with almost identical quantitative effects (Ramadan et al., 2003). The crude *N. sativa* oil (NSO) and its fractions (neutral lipids, glycolipids, and phospholipids) showed potent *in vitro* radical scavenging activity that is correlated well with their total count of polyunsaturated fatty acids, unsaponifiables, and phospholipids, as well as the initial peroxide values of crude oils (Ramadan et al., 2003). Both hepatotoxicity and nephrotoxicity are associated with alteration in the levels and activities of certain mediators such as L-alanine aminotransferase (ALT), alkaline phosphatase (AIP), lipid peroxide (LPO), and the oxidant scavenger enzyme system including glutathione (GSH) and SOD. The antioxidant effects of *N. sativa* have been examined using different hepatic and kidney toxicity *in vivo* murine models induced by tert-butyl hydroperoxides, carbon tetrachloride (CCl₄), doxorubicin, gentamicin, methionine, potassium bromate (Salem, 2005).

In view of this, it is much interest and practical importance to study the influence of plant products on oxidative stress induced by pesticide exposure. Hence, it

prompted us to investigate the acute dimethoate exposure on oxidative stress by assessing lipid peroxidation, antioxidant enzymes as well as other biochemical parameters. Simultaneously, protective action of *N. sativa* oil on dimethoate-intoxicated rats was investigated.

MATERIALS AND METHODS

Chemicals

Dimethoate (98%) was purchased from Chemical Service (West Chester, PA, USA). The LD₅₀ of dimethoate when given orally to rats was reported to be 300 mg/kg BW (Gallo and Lawryk, 1991). The tested dose of dimethoate was 75 mg.kg⁻¹ (1/4 LD₅₀) (Sharma et al., 2005a). Use of dimethoate was approved by the Animal Care Committee and met all guidelines for its use. Nigella sativa fixed oil was purchased from Asala Chemical Company, Alexandria, Egypt. Effective dose of Black seed oil (Nigella sativa fixed oil, NSO) was 2 ml.kg⁻¹.day⁻¹ (Ali, 2004), as it was tested against oxidative stress and toxicity. Thiobarbituric acid and all other chemicals were purchased from Sigma Chemical Company (St. Louis, Mo, USA).

Animals

Albino rat males weighting 160 ± 20 g were randomly drawn from the stock colony of National Research Institute, Cairo, Egypt. The animals were housed in universal galvanized wire cages at room temperature (22 ± 2 °C) and in a photoperiod of 12:12 light/dark cycle, 50% ± 5% humidity. The rats were acclimatized for 2 weeks prior to the start of the experiment. Rats were maintained on commercial pellet diet (protein 18%, fat 6%, fiber 6%, carbohydrates 56%, calcium 0.6%, moisture 10% and ash 11%) and water ad libitum. All procedures with animals were conducted strictly in accordance with guidelines approved by the Institute Animal Ethical Committee. Permission to handling with laboratory animals was obtained from the Institute's animal committee. During the experiments, maximum care was taken to minimize animal suffering and, in addition, the number of rats used was kept at minimum.

Animal treatment and experimental protocol

Adult rat males were grouped randomly into four groups (n=10). The group I - (control): rats received corn oil 24h prior to decapitation; the group II - (NSO): rats received *N. sativa* seed oil (NSO) per orally (2 ml.kg⁻¹.day⁻¹ for 21 days; the group III - dimethoate: rats received a single dose (75 mg.kg⁻¹) of dimethoate per orally 24h prior to decapitation; the group IV - dimethoate + NSO: rats received NSO at 2 ml.kg⁻¹.day⁻¹ for 21 days and a single dose (75 mg.kg⁻¹) of dimethoate per orally 24h prior to decapitation.

Sample collection

The animals were starved overnight for 12h before blood was collected. Rats were anaesthetized with light ether and venous blood samples were collected by direct heart puncture into sterilized vials. Blood samples allowed setting to clot at 4 °C and centrifuged at 1000 g for 30 min. Then 1000 µl aliquots of serum were placed in microfuge tubes and frozen on dry ice. Labeled bags were placed into a -20 °C freezer until the time of the assay.

Assay systems

Serum thiobarbituric acid reactive substances (TBARS) were measured at 532 nm by using 2-thiobarbituric acid (2,6-duhydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 cm⁻¹ mole⁻¹ was used for calculation (Tapel and Zalkin, 1959). Superoxide dismutase (SOD) (EC. 1.15.1.1) activity was measured in serum according to Flohe and Otting (1984). Catalase (EC. 1.11.1.6) activity in serum was assayed by monitoring the decomposition of H₂O₂ at 240 nm as described by Aebi (1984). The activity of acetylcholinesterase (AChE) (EC 3.1.1.7) was measured according to the method of Blawen et al. (1983) using commercial kit obtained from Quimica Clinica Aplicada S.A. Spain. Serum alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were determined using commercial kits obtained from BioM'erieux, France. The principle reaction of the colorimetric determination of AST or ALT activity is based on the reaction of aspartate or alanine with α-ketoglutarate to form oxaloacetate or pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine (Reitman and Frankel, 1957). Serum alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured at 405 nm by the formation of para-nitrophenol from para-nitrophenylphosphate as a substrate (Rosalki and Foo, 1993). Acid phosphatase activity (ACP; EC 3.1.3.2) was determined according to Tietz (1986) using commercial kits obtained from Bio ADWIC, Egypt. Serum lactate dehydrogenase (LDH; EC 1.1.1.27) was determined according to the method of Friedman and Young (1997).

Serum samples were analyzed for total protein (TP) by the Biuret method according to Armstrong and Carr (1964). Albumin concentration was determined by the method of Doumas et al. (1977). Globulin concentration was determined as the difference between total protein and albumin. Serum total bilirubin was measured using the method of Walters and Gerade (1970). Also, low density lipoprotein (LDL) cholesterol was measured according to the method of Assmann et al. (1984), high density lipoprotein (HDL) cholesterol was measured according to the method of Burstein et al. (1984). Cholesterol and triglycerides (TG) was measured according to the method

Carr et al. (1993), and serum gamma glutamyl transferase (γ-GT) was measured according to the method of Tietz (1986), using commercial kits obtained from BioSystems Co., Spain. Total lipids were measured according to the method of Knight et al. (1972) using commercial kits obtained from Bio ADWIC, Egypt. Serum creatinine was measured according to the method of Henry (1974), uric acid was estimated according to the method of Guder et al. (1996), using commercial kits obtained from Biocon® Diagnostik, Marienhagen, Germany. Urea was measured according to the method of Patton and Crouch (1977) using commercial kit obtained from Diamond Co., Egypt.

Statistical procedures

Data are expressed as the means ± S.E.M. The data were analyzed using a one-way analysis of variance and the statistical significance between specific means was determined using the Newman-Keuls multiple range test.

RESULTS

Rats administered with *N. sativa* oil (NSO) showed no signs of toxicity and gained body weight compared to control rats. Dimethoate did not induce any distinctive clinical signs of toxicity or mortality. NSO *per se* had no effect on serum levels of TBARS when it compared with controls (Table 1). In dimethoate treated rats, a significant ($P<0.001$) increase of lipid peroxidation in serum was observed, as evidenced by the enhanced TBARS levels in serum (+79.5%) compared with those of controls (Table 1). With NSO pretreatment in dimethoate treated rats, a dramatic reduction ($P<0.001$) in TBARS levels was recorded compared to dimethoate treated group.

SOD activity in NSO treated rats (group 3) was significantly ($P<0.01$) increased as compared to control (Table 1). Treatment with dimethoate (group 2) showed significant ($P<0.001$) increase in serum SOD activity as compared to control. NOS pretreated rats administered with dimethoate (group 4) showed significant ($P<0.01$) decrease in serum SOD activity compared to dimethoate-treated group (group 2).

NSO-treated rats (group 3) showed serum CAT activity comparable to control (Table 1). Treatment with dimethoate (group 2) showed significant ($P<0.001$) increase in serum CAT activity as compared to control. NSO-pretreated rats administered with dimethoate (group 4) showed decrease in serum CAT compared to dimethoate-treated group (group 2).

AChE activity in serum was significantly ($P<0.01$) inhibited in dimethoate-treated rats (group 2) as compared to control (Table 1). NSO-pretreated rats administered with dimethoate showed high recovery rate compared to NSO-pretreated rats administered with the pesticide (group 4).

Data represented in Table 2 show that oral treatment with dimethoate caused significant ($P<0.05$) increase in serum total lipid, cholesterol, triglycerides and LDL concentrations, whilst HDL levels were decreased compared to control animals (Table 2). NSO

alone caused significant ($P<0.05$) decrease in lipid profile compared to control. NSO-pretreated rats administered with dimethoate showed reduction in the serum lipid level caused by dimethoate.

Table 1: Effect of *N. sativa* seed oil (NSO) on thiobarbituric acid reactive substances (TBARS) levels, and superoxide dismutase (SOD), catalase (CAT) and acetylcholinesterase (AChE) activities in serum from dimethoate-treated rats

Parameters	Control	Dimethoate	NSO	Dimethoate + NSO
TBARS (nmol/ml)	2.212 ± 0.233	3.970 ± 0.313 ^z	2.070 ± 0.224 ^{x, c}	2.962 ± 0.095 ^{y, c}
SOD (U/ml)	1.166 ± 0.258	3.80 ± 0.656 ^z	1.974 ± 4.52 ^{y, b}	2.620 ± 0.227 ^{z, b}
Catalase*	505.8 ± 38.664	737.0 ± 13.50 ^z	664.6 ± 23.57 ^{y, b}	634 ± 70.59 ^{y, a}
AChE µmol/min/ml	3.449 ± 0.264	1.876 ± 0.687 ^y	3.647 ± 0.571 ^b	3.328 ± 0.181 ^b

Values are expressed as Mean ± SEM; n= 10 for each treatment group. ^x p<0.05, ^y p<0.01, ^z p<0.001 compared with control, respectively; ^a p<0.05, ^b p<0.01, ^c p<0.001 in comparison between dimethoate and dimethoate + NSO respectively. * µmoles of H₂O₂ decomposed/min/mg protein

Table 2: Effect of *N. sativa* seed oil (NSO) on lipid and lipoprotein profiles of serum from dimethoate-treated rats

Parameters	Control	Dimethoate	NSO	Dimethoate + NSO
TL (mg/dl)	279.994 ± 7.00	371.426 ± 53.45 ^y	230.376 ± 10.31 ^{z, b}	308.144 ± 26.14
Cho (mg/dl)	80.6 ± 7.48	107.2 ± 8.976 ^y	63.0 ± 3.521 ^{y, c}	79.8 ± 12.528 ^a
TG (mg/dl)	98.6 ± 2.600	124.3 ± 3.687 ^z	77.4 ± 3.389 ^{z, c}	100.2 ± 7.756 ^b
HDL (mg/dl)	50.474 ± 1.195	31.725 ± 4.044 ^z	65.537 ± 2.692 ^{z, c}	45.822 ± 5.160 ^b
LDL (mg/dl)	47.928 ± 1.13	62.523 ± 2.403 ^z	37.068 ± 2.30 ^{y, c}	54.492 ± 2.38 ^{z, b}

Values are expressed as Mean ± SEM; n= 10 for each treatment group. TL, total lipids; Cho, cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol. ^x p<0.05, ^y p<0.01, ^z p<0.001 compared with control respectively; ^a p<0.05, ^b p<0.01, ^c p<0.001 in comparison between dimethoate and dimethoate + NSO respectively

Table 3: Effect of *N. sativa* seed oil (NSO) on serum enzymes of dimethoate-treated rats

Parameters	Control	Dimethoate	NSO	Dimethoate + NSO
TP (g/dl)	8.270 ± 0.262	5.576 ± 0.610 ^z	7.426 ± 0.509 ^{x, a}	5.524 ± 0.552 ^z
Albumin (g/dl)	5.465 ± 0.229	3.822 ± 0.365 ^y	4.872 ± 0.308 ^{x, b}	4.268 ± 0.201 ^{y, a}
Globulin (g/dl)	2.844 ± 0.108	2.184 ± 0.426 ^y	2.560 ± 0.195	2.244 ± 0.207 ^y
Bilirubin (mg/dl)	5.10 ± 0.21	6.12 ± 0.31 ^y	5.08 ± 0.32 ^b	5.26 ± 0.29 ^a
AST (U/L)	130.6 ± 2.154	203.8 ± 14.918 ^z	107.8 ± 4.749 ^{y, c}	152.6 ± 7.337 ^{y, b}
ALT (U/L)	42.4 ± 1.496	56.2 ± 2.638 ^y	39.4 ± 2.577 ^c	47.6 ± 1.356 ^{y, c}
ALP (U/L)	74.62 ± 11.666	139.6 ± 28.097 ^y	50.60 ± 2.577 ^{y, c}	86.2 ± 4.069 ^b
ACP (U/L)	37.2 ± 0.632	43.4 ± 2.245 ^y	26.4 ± 0.894 ^{z, c}	33.0 ± 3.286 ^{x, b}
LDH (U/L)	213.20 ± 35.6	323.1 ± 8.73 ^y	226.5 ± 30.07 ^b	261.4 ± 35.3 ^a
γ-GT (U/L)	2.664 ± 0.543	4.216 ± 0.274 ^y	2.218 ± 0.609 ^b	2.618 ± 0.407 ^b

Values are expressed as Mean ± SEM; n= 10 for each treatment group. TP, total protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ACP, acid phosphatase; LDH, lactate dehydrogenase; γ GT, gamma glutamyl transferase. ^x p<0.05, ^y p<0.01, ^z p<0.001 compared with control respectively; ^a p<0.05, ^b p<0.01, ^c p<0.001 in comparison between dimethoate and dimethoate + NSO respectively

Table 4: Effect of *N. sativa* oil (NSO) on urea, creatinine and uric acid values in serum from dimethoate-treated rats

Parameters	Control	Dimethoate	NSO	Dimethoate + NSO
Urea (mg/dl)	24.86 ± 2.054	29.37 ± 1.895 ^y	21.47 ± 2.558 ^b	20.48 ± 2.001 ^{x, c}
Creatinine (g/dl)	0.44 ± 0.141	2.71 ± 0.183 ^z	0.31 ± 0.110 ^c	1.86 ± 0.359 ^{y, b}
uric acid (mg/dl)	3.76 ± 0.229	4.27 ± 0.113 ^y	3.73 ± 0.163 ^c	3.71 ± 0.134 ^b

Values are expressed as Mean ± SEM; n= 10 for each treatment group. ^x p<0.05, ^y p<0.01, ^z p<0.001 compared with control respectively; ^a p<0.05, ^b p<0.01, ^c p<0.001 in comparison between dimethoate and dimethoate + NSO respectively.

Treatment with dimethoate significantly ($P<0.05$) increased the activities of AST, ALT, AIP, ACP, LDH and γ -GT in serum compared to control animals (Table 3). The present study demonstrates that treatment with NSO alone did not cause any significant change in enzyme activities in serum and alleviated the toxicity of dimethoate. NSO in combination with dimethoate alleviated its negative effect on activities of the above measured enzymes.

Data represented in Table 3 showed that treatment with dimethoate caused significant ($P<0.01$) decrease in serum TP, albumin and globulin, and increase ($P<0.05$) in total bilirubin compared to control. Treatment with NSO alone significantly increased serum TP, albumin and globulin and decreased bilirubin content. The presence of NSO with dimethoate counteracted its harmful effects.

Dimethoate-intoxicated rats showed a constellation of disorders in renal function witnessed by increased urine output and changes in creatinine, urea and uric acid levels (Table 4). Compared to controls, creatinine levels in treated rats were six-fold higher in serum of dimethoate-intoxicated rats. Dimethoate treatment led to a reduction in creatinine clearance, an indicator of glomerular dysfunction in adult rats. Pretreatment of rats with NSO significantly ($P<0.001$) reduced creatinine concentration compared to dimethoate-treated animals. Urea levels in treated rats were higher (+18.14 %) in the serum and lower (-14%) in antioxidant-challenged groups compared to controls. Uric acid levels were higher in the serum of dimethoate-treated rats (+13.56%) and kept at normal levels in NSO-pretreated intoxicated rats.

DISCUSSION

At present there is a considerable interest in the investigation of free radical-mediated damages to biological systems due to pesticide exposure. However, there is a lack of consensus as to which determinations are best used to quantify future risk of xenobiotic exposure, and the use of natural plant products as antioxidant interventions. A study of some commonly used plant products as antioxidants against xenobiotic-induced oxidative stress therefore appeared to be of interest (Ahmed et al., 2000).

Lipid peroxidation has been suggested as one of the molecular mechanisms involved in organochlorine, carbamate or organophosphorous pesticide. Large numbers of xenobiotics have been identified to have potential to generate free radicals in biological system (Ahmed et al., 2000, Kehrer, 1993). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Some of these free radicals interact with various tissue components, resulting in dysfunction and the question of whether oxidative stress is a major cause of injury remains equivocal. In this study we have investigated the effects of administration of *N. sativa* oil on LPO induced by dimethoate. To our knowledge there is no information concerning protective action of *N. Sativa* oil on oxidative injury induced by dimethoate.

Lipid peroxidation has been extensively used as a marker of oxidative stress. The increase in the level of thiobarbituric acid-reactive substances (the marker of extent of lipid peroxidation) in serum due to dimethoate administration to rats is in agreement with the finding of John et al. (2001). NSO-pretreated rats administered with dimethoate however, showed decreased LPO in serum as compared to organophosphate toxicity. The protective effect of NSO may be in part due to thymoquinone (TQ), the main constituent of NSO. TQ showed to have a cytoprotective action against oxidative damage in many tissues (Badary, 1999).

Under normal physiological conditions, a delicate balance exists between the rate of formation of H_2O_2 via dismutation of O_2^- by SOD activity and the rate of removal of H_2O_2 by CAT and glutathione peroxidase. Therefore any impairment in this pathway will affect activities of other enzymes in the cascade (Kono and Fridovich, 1992). The present study indicates that dimethoate treatment resulted in increased SOD and CAT activities what may be assumed to block the excessive free radical load. Results from this study suggest that pretreatment with NSO exerts an antioxidative effect by decreasing lipid peroxidation, and maintaining normal levels of SOD and CAT activities. Higher levels of antioxidative enzymes have been well correlated with increased lipid peroxidation after dimethoate administration. In support of our finding Salem (2005) reported that when rats were

subjected to ischaemia/reperfusion, the injection of NSO or TQ tended to normalize the SOD level.

AChE activity in plasma of dimethoate-treated rats was significantly reduced compared to control. Moreover, the activity of γ -GT was significantly enhanced. The inhibition of AChE resulted in the accumulation of acetylcholine (ACh) which may cause stimulation of lymphocytes and elevated concentration of cellular cGMP, increased lymphocyte motility and cytotoxicity (Malik and Summer, 1982). Since AChE and γ -GT are both membrane-bound enzymes, γ -GT could interact with amino acid neurotransmitter Ach, which may be removed from the binding with AChE and may result in decreased activity of AChE (Szelenyi et al., 1987). Biological significance of γ -GT-dependent lipid peroxidation *in vivo* might be multifold. Varying levels of γ -GT activity can be detected in erythrocytes and lymphocytes. It is conceivable that the pro-oxidant effects of γ -GT activity are normally balanced by its established role in favoring the cellular uptake of precursors for GSH re-synthesis, thus allowing the reconstitution of cellular antioxidant defense (Banerjee et al., 1999). *N. sativa* oil reversed the changes of AChE and γ -GT

In the present study, oral administration of dimethoate to rats caused a significant hepatic damage, as observed from the elevation of hepatospecific enzyme activities, as well as severe alterations in different liver parameters. Activities of AST, ALT, ALP and ACP in serum were significantly increased in dimethoate-intoxicated rats. Pre-treatment with NSO affords significant protection against dimethoate. A significant rise in ALT and AST might be taken as an indicator of liver damage. The increased transaminase activity was reversed to normal level following NSO-supplementation with healing of hepatic parenchyma and regeneration of hepatocytes. Supporting our finding, *Nigella sativa* seeds protect isolated hepatocytes against tert-butyl hydroperoxides (TBHP)-induced toxicity evidenced by decreased leakage of ALT and AST (Daba and Abdel-Rahman, 1998). The decrease in serum ALP observed in NSO-pretreated rats has also been reported in rats treated with NSO together with vitamin E, *Crocus sativus* and cysteine (El-Daly, 1998; Zaoui et al., 2002).

Dimethoate administration resulted in a significant decrease in serum TP, albumin and globulin. The reduction in serum protein, particularly albumin, could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Also, the protein level suppression may be due to loss of protein either by reduce in protein synthesis or increased proteolytic activity or degradation (Yeragi et al., 2003). In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of dimethoate on liver cells, as confirmed by the increase in activities of serum AST and ALT. In support of our finding, Al-Gaby

(1998) found that *Nigella sativa* seeds increase serum total protein.

Nigella sativa oil in the present study had a marked protective action against dimethoate-induced hepatic injury, an effect that was associated with suppression in levels of lipid peroxidation and LDH. The anti-ulcerogenic effect of NSO could be attributed to the improvement of the antioxidant status of the animals due to the presence of free radical scavenging substances, such as thymoquinone (Saleh et al., 2000). Thymoquinone is the main active component in the volatile oil of *Nigella sativa* seeds (Houghton et al., 1995), and is able to inhibit lipid peroxidation (Mansour, 2000). Moreover, its ability to preserve the cell membrane integrity could be proven by the restoration of LDH (Salem, 2005).

The biochemical marker employed in this study to evaluate kidney function was uric acid level in serum. Many drugs could affect plasma and/or serum uric acid levels by influencing the net reabsorption of uric acid in the proximal tubule of the nephron (Reyes, 2003). Under our experimental conditions, dimethoate had a poison effect on adult rats increasing serum uric acid levels. Impairment in kidney function could probably occur as a result of kidney oxidative damage. In fact, uric acid in blood is the most important antioxidant (Ames et al., 1981). This compound is the end product of purine catabolism and can reduce oxidative stress by scavenging various ROS (Mahjoubi-Samet et al., 2008).

Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore marked increase in serum urea and creatinine, as noticed in this study, confirms an indication of functional damage to the kidney (Garba et al., 2007). Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, whilst creatinine is, therefore, more specific to the kidney, since kidney damage is the only significant factor that increases serum creatinine level (Nwanjo et al., 2005). Therefore, significant increases in urea and creatinine levels noticed in this study are a classical sign that the kidney was adversely affected by dimethoate administration. Kidney dysfunction and nephrotoxicity induced by dimethoate in present investigation are consequences of oxidative stress. In fact, it has been reported that several OPI compounds caused oxidative stress in different tissues through the formation of ROS (Mahjoubi-Samet, 2008). Pretreatment of dimethoate-intoxicated rats with NSO normalized the levels of urea and creatinine. TQ, the main constituent of NSO, ameliorated the severity of ifosfamide-induced renal damage (Badary, 1999).

Results also showed significant increase in serum HDL of animals pretreated with NSO prior to dimethoate administration compared to dimethoate-treated group. HDL may hasten the removal of cholesterol from peripheral tissue to the liver for catabolism and excretion.

Also, high level of HDL may compete with LDL receptor sites on arterial smooth muscle cells and thus partially inhibit uptake and degradation of LDL. Also, HDL could protect LDL against oxidation in vivo, because the lipids in HDL are preferentially oxidized before those in LDL (Bowry et al., 1992). Supporting our observation, *Nigella sativa* fixed oil, like the fibrates, decreases serum cholesterol and triglycerides, and ameliorates serum HDL. The fibrates act through PPAR α (Peroxisome Proliferator-Activated Receptor α) activation (Zoau et al., 2002).

We have now demonstrated that administration of *N. sativa* oil to rats modulates the antioxidant enzymes in a manner that favors the lowering of lipid peroxidation and suggests a possible adaptive mechanism to counteract oxidative stress. Hence, results of such studies on oxidative/antioxidant status during a free radical challenge can be used as an indicator of protection against the lipid peroxidation in experimental animals for assessing dietary and therapeutic measures. In our study *N. sativa* oil, given to rats, prevented the formation of unwanted free radicals and protected them against acute dimethoate exposure.

In conclusion, the low toxicity of *Nigella sativa* fixed oil, evidenced by high LD₅₀ values, key hepatic enzyme stability, and organ integrity, suggests a wide range of safety for therapeutic use.

REFERENCES

- ABDOLLAHI, M. – RANJBAR, A. – SHADNIA, S. – NIKFAR, S. – REZAIEE, A. 2004. Pesticides and oxidative stress: a review. *Med. Sci. Monit.*, vol. 10, 2004, p. RA141-RA147.
- AEBI, H. 1984. Catalase In vitro. *Methods Enzymol.*, vol. 105, 1984, p. 121-126.
- AHMED, R. S. – SETH, V. – PASHA, S. T. – BANERJEE, B. D. 2000. Influence of dietary ginger (*Zingiber officinalis* Rose) on oxidative stress induced by malathion in rats. *Food Chem. Toxicol.*, vol. 38, 200, p. 443-450.
- ALI, B. H., 2004. The effect of *Nigella sativa* oil on gentamicin nephrotoxicity in rats. *Am. J. Clin. Med.*, vol. 32, 2004, p. 49-55.
- AKHGARI, M. – ABDOLLAHI, M. – KEBRYAEZADEH, A. – HOSSEINI, R. – SABZEVARI, O. 2003. Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for sub chronic toxicity of malathion in blood and liver of rats. *Hum. Exp. Toxicol.*, vol. 22, 2003, p. 205-211.
- AL-GABY, A. M. 1998. Amino acid composition biological effects of supplementing broad bean and corn proteins with *Nigella sativa* (Black cumin) cake protein. *Nahrung*, vol. 42, 1998, p. 290-294.
- AL-ROWAIS, N. A. 2002. Herbal medicine in the treatment of diabetes mellitus. *Saudi Med. J.*, vol. 23, 2002, p. 1327-1331.
- AMES, B.N. – CATHCART, R., –SCHWIERS, E. 1981. Uric acid provides an antioxidant defense in human against oxidant and radical caused aging and cancer: a hypothesis. *Proc. Natl. Acad. Sci. USA*, vol. 78, 1981, p. 6858-6862.
- ARMSTRONG, W D. – CARR, C. W. 1964. Estimation of serum total protein. In: Physiological Chemistry Laboratory Direction, third ed. Minneapolis, Minnesota, USA : Burges Publishing Co. 1964.
- ASSMANN, G. – JABS, H. U. – KOHNERT, U. – Nolte W. –SCHRIEWER, H. 1984. LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate. *Clin Chim Acta*, vol. 140, 1984, p.77-83.
- BADARY, O. A. 1999. Thymoquinone attenuates ifosfamide-induced fanconi syndrome in rats and enhances its antitumor activity in mice. *J. Ethnopharmacol.*, vol. 67, 1999, p. 135-142.
- BANERJEE, B. D. – SETH, V. – BHATTACHARYA, A. – PASHA, S. T. – CHAKRABORTY, A. K. 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicol. Lett.*, 107, 1999, p. 33-47.
- BLAWEN, D. H. – POPPE, W. A. – TRISCHLER, W. 1983. The effect of subacute doses of organophosphorus pesticide, nuvacron, on the biochemical and cytogenetic parameters of mice and their embryos. *J. Clin. Chem. Biochem.*, vol. 21, 1983, p. 381-386.
- BOWRY, V. W. – STANLEY, K. K. – STOCKER, R. 1992. High density lipoprotein is the major of lipid hydroperoxides in human blood plasma from fasting donors. *Proc. Natl. Acad. Sci.*, vol. 89, 1992, p. 1316-1320.
- BURSTEIN, M. – SCHOLNICK, H. R. – MORFIN, R. 1984. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions, *Scand. J. Clin. Lab. Invest.*, vol. 40, 1984, p. 583-95.
- CARR, T. – ANDRESSEN, C. J. – RUDEL, L. L. 1993. Enzymatic determination of triglyceride, free cholesterol and total cholesterol in tissue lipid extracts. *Clin. Chem.*, vol. 26, p. 39-42.
- COSTA, L. G. 2006. Current issues in organophosphate toxicology, In: *Clin. Chem. Acta.*, vol. 366, 2006, p. 1-13.
- DABA, M. H. – ABDEL-RAHMAN, M. S. 1998. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. *Toxicol. Lett.*, vol. 16, 2006, p. 23-29.
- DOUMAS, B. T. – WATSON, W. A. – BIGGS, H. G. 1977. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chem. Acta.*, vol. 31, 1977, p. 87-96.
- EL-DALY, E. S. 1998. Protective effect of cysteine and vitamin E *Crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicity in rats. *J. Pharm. Belo.*, vol. 53, 1998, p. 87-95.
- FLOHE, L. – OTTING, F., 1984. Superoxide dismutase assays, In: *Meth. Enzymol.*, vol. 105, 1984, p. 101-104.
- FRIEDMAN, K. – YOUNG, D. S. 1997. Effects of disease on clinical laboratory tests, 3rd ed. AACC press
- GARBA, S. H. – ADELAIYE, A. B. – MSHELIA, L. Y. 2007. Histopathological and biochemical changes in the rats kidney following exposure to a pyrethroid based mosquito coil. *J. Appl. Sci. Res.*, vol. 3, 2007, p. 1788-1793.
- GALLO, M. A. – LAWRYK, N. J. 1991. Organic phosphorus pesticides. In: Hayes WJ and Laws ER, editors, Handbook of Pesticide Toxicology, New York, Academic Press, 1991. p. 3-5.

- GUDER, W. G. — NARAYANAN, S. — WISSER, H. — ZAWTA, B. 1996. List of Analytes; pre analytical Variables. Brochure in Samples: From the patient to the laboratory, Darmstadt : GIT Verlag, 1996.
- HENRY, R. J. 1974. Clinical chemistry. Principals and Techniques, 2nd ed. Harper and Row. pp 525-531.
- HOUGHTON, P. J. — ZARKA, R. — HERAS, B. — HOULT, J. R. 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.*, vol. 61, 1995, p. 33-36.
- JACOB, R. A. — BURRI, B. J. 1996. Oxidative damage and defense. *Am. J. Clin. Nutr.*, vol. 63, 1996, p. 985S-990S.
- JOHN, S. — KALE, M. — RATHONE, N. — BHATNAGAR, D. 2001. Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J. Nutr. Biochem.*, vol. 12, 2001, p. 500-504.
- KAMATH, V. — RAJINI, P. S. 2007. Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication. *Toxicology*, vol. 231, 2007, p. 137-146.
- KAMATH, V. — JOSHI, A. K. R. — RAJINI, P. S. 2008. Dimethoate induced biochemical perturbation in rat pancreas and its attenuation by cashew nut skin extract. *J. Nutr. Biochem.* vol. 90, 2008, p. 58-65.
- KEHRER, J. P. 1993. Free radicals as mediator of tissue injury and disease. *Crit. Rev. Toxicol.*, vol. 23, 1993, p. 21-48.
- KNIGHT, J. A. — ANDERSON, S. — RAWIE, J. M. 1972. Biochemical effect of chocolate colouring and flavouring like substances on thyroid function and protein biosynthesis. *Clin. Chem.*, vol. 18, 1972, p. 199-203.
- KONO, Y. — FRIDOVICH, I. 1992. Superoxide inhibits catalase. *J. Biol. Chem.*, vol. 257, 1992, p. 5751-54.
- MAHJoubi SAMET, A. — FETOUI, H. — ZEGHAL, N. 2008. Nephrotoxicity induced by dimethoate in adult rats and their suckling pups. *Pest. Biochem. Physiol.*, vol. 91, 2008, p. 96-103.
- MALIK, J. K. — SUMMER, K. M. 1982. Toxicity and metabolism of malathion and its impurities in isolated rat hepatocytes: role of glutathione. *Toxicol. Appl. Pharmacol.*, vol. 66, 1982, p. 69-76.
- MANSOUR, M. A. 2000. Protective-effects of thymoquinone and desferrioxamine against hepatotoxicity of carbon tetrachloride in mice. *Life Sci.*, vol. 66, 2000, p. 2583-2591.
- NWANJO, H. U. — OKAFOR, M. C. — OZE, G. O. 2005. Changes in biochemical parameters of kidney function in rats co-administered with chloroquine and aspirin. *J. Clin. Sci.*, vol. 23, 2005, p. 10-12.
- PATTON, C. J. — CROUCH, S. R. 1977. A simple and rapid micro-Kjeldahl method for total nitrogen analysis. *Anal. Chem.*, vol. 49, 1977, p. 464-9.
- RAMADAN, M. F. — KROH, L. W. — MORSEL, J. T. 2003. Radical scavenging activity of black cumin (*Nigella sativa* L.), Coriander (*Coriandrum sativa* L.), and Niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. *J. Agric. Food Chem.*, vol. 51, 2003, p. 6961-6969.
- REITMAN, S. — FRANKEL, S. 1957. A colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, vol. 28, 1957, p. 56-63.
- REYES, A. J. 2003. Cardiovascular drugs and serum uric acid. *Cardiovasc. Drugs Ther.*, vol. 17, 2003, p. 397-414.
- ROSALKI, S. B. — FOO, A. Y. 1993. Burlina A. Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. *Clin Chem.*, vol. 39, 1993, p. 648-52.
- SALEH, S. — EL-DENSHARY, E. M. S. — MAHRAN, L. G. — SALAH, N. 2000. Anti inflammatory and antioxidant effects of *Nigella sativa* oil in sensitized animals. The 25th International Conference on Science and Technology, New Delhi : India, 2000.
- SALEM, M. L. 2005. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *Int. Immunopharmacol.*, vol. 5, 2005, p. 1749-1770.
- SHARMA, Y. — BASHIR, S. — IRSHAD, M. — DATTA GUPTA, S. — DOGRA, T. D. 2005a. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. *Toxicology*, vol. 206, 2005a, p. 49-57.
- SHARMA, Y. — BASHIR, S. — IRSHAD, M. — NAG, T. C. — DOGRA, T. D. 2005b. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *Toxicology*, vol. 215, 2005b, p. 173-181.
- SIVAPIRIYA, V. — JAYANTHISAKTHISEKARAN, VENKATRAMAN, S. 2006. Effects of dimethoate (*O,O*-dimethyl *S*-methyl carbamoyl methyl phosphorodithioate) and Ethanol in antioxidant status of liver and kidney of experimental mice. *Pesticide Biochem. Physiol.*, vol. 85, 2006, p. 115-121.
- SZELENYI, J. P. — HARIS, P. — HOLLAN, S. 1987. Changes in cholinergic system of lymphocytes due to mitogenic stimulation. *Immunol. Lett.*, vol. 16, 1987, p. 49-54.
- TAPEL, A. L. — ZALKIN, H. 1959. Inhibition of lipid peroxidation in mitochondria by vitamin E. *Arch. Biochem. Biophys.*, vol. 80, 1959, p. 333-336.
- TIETZ, N. W. 1986. Textbook of clinical chemistry, Co. London : W. B. Saunders.
- WALTERS, M. — GERADE, H. 1970. Ultramicromethod for determination of conjugated and total bilirubin in serum or plasma. *Microchem. J.*, vol. 15, 1970, p. 231.
- YERAGI, S. G. — RANA, A. M. — KOLI, V. A. 2003. Effect of pesticides on protein metabolism of Mudskipper *Boleophthalmus Dussumieri*. *J. Ecotoxicol. Environ. Monit.*, vol. 13, 2003, p. 211-4.
- ZAOUI, A. — CHERRAH, Y. — MAHASSINI, N. — ALAOUI, K. — AMAROUCHE, H. — HASSAR, M. 2002. Acute and chronic toxicity of *Nigella sativa* fixed oil. *Phytomedicine*, vol. 9, 2002, p. 69-74.