Egypt. J. Exp. Biol. (Zool.), 6(2): 219 – 230 (2010)

RESEARCH ARTICLE

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DIMETHOATE-INDUCED HEPATOTOXICITY IN RATS AND THE PROTECTIVE ROLES OF VITAMIN E AND N-ACETYLCYSTEINE

ABSTRACT:

Dimethoate, an organophosphates pesticide, is used in controlling the pests of a variety of crops. Thus it is important to investigate the possible ways to ameliorate its toxicity. The aim of this study was to investigate the ameliorative effects of vitamin E alone and in combination with an antioxidant N-acetylcysteine (NAC) on some hepatic tissue enzyme activities and selected indices of oxidative stress in dimethoate-exposed rats. Eight groups of male Dawley rats were used in this study: control; dimethoate (D) group (orally administrated in a concentration of 21 mg/kg body weight); vitamin E group (200 mg/kg BW orally); NAC group (100 mg/kg BW i.p) and vitamin E+NAC group. The other three groups were orally administrated dimethoate in a concentration of 21 mg/kg BW followed by either vitamin E, NAC or vitamin E plus NAC in the same previous doses for 7 weeks. Oral administration of dimethoate induced a significant decrease in body weight and an increase in liver weight. Also, a highly significant increase in level of hepatic lipid peroxidation; significant decrease in the activities of antioxidant enzymes [superoxide dismutase, catalase and glutathione-S-transferase] were noticed at the end of the 7th week. Serum total protein, albumin, triglyceride, very low density lipoprotein cholesterol (VLDL-cholesterol) levels were decreased, and serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl-transferase (GGT), lactate dehydrogenase (LDH), and total cholesterol levels were increased significantly in the dimethoate-intoxicated rats. There was a statistically significant difference for all biochemical parameters except LDH when the vit.E + NAC + dimethoate-treated group was compared with the dimethoate-treated group. The present study also examined the genotoxic effect of vit.E and NAC in liver tissue of dimethoate-intoxicated rats. Analysis of DNA fragmentation (DNA damage) by gel electrophoresis was used. Agarose gel electrophoresis of DNA of hepatocytes resulted in a characteristic ladder pattern in dimethoate-intoxicated rats. Also it has been observed that vit.E and NAC selectively altered the extent of DNA damage. In combination, vit.E and NAC completely ameliorated the dimethoate-induced oxidative damage. Either compound alone was partially protective against dimethoate damage. In conclusion, the present results provide an evidence of beneficial effect of the antioxidant NAC in conjunction with vit.E in re-balancing the impaired prooxidant/ antioxidant ratio in sub-chronic dimethoate intoxication in rats.

KEY WORDS:
Dimethoate, vitamin E, N-acetylcysteine, hepatotoxicity.

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ARTICLE CODE: 26.01.10

INTRODUCTION:

Organophosphorus insecticides (OPIs) are some of the most useful and diverse classes of insecticides in use for almost five decades. However, the uncontrolled use of these insecticides in agriculture and public health operation has increased the scope of ecological imbalance and thus many non-target organisms have become victims (Das and Mukherjee, 2000). It has been reported that OPIs are neurotoxic in nature by acting as inhibitors of neuronal cholinesterase (ChE) activity (Ecobichon, 1996) and serum cholinesterase (SChE) (Singh *et al*., 2002). Some studies reported that OPIs caused lipid peroxidation (Dasgupta *et al*., 1992; Bagchi *et al*., 1995; Gultekin and Akdogan, 2000; Kalender *et al*., 2007) in vertebrates.

Dimethoate (*O*, *O* – dimethyl – *S* – methyl – carbamoyl - methyl phosphorodithioate) is an organophosphorus insecticide with a contact and systemic action. The extensive use of dimethoate poses a health hazard to animals and humans because of its persistence in soil and crops (WHO/IPCS, 1996). Majority of population is exposed to lower doses of dimethoate via food, contaminated drinking water, or by application of household insecticides containing dimethoate (Sharma *et al*., 2005). Exposure to low level organophosphate pesticides is known to produce a variety of biochemical changes, some of which may be responsible for the adverse biological effects reported in humans and experimental animals (Ogutcu *et al*., 2008).

The body has developed several defence mechanisms against oxidative damage. These defence mechanisms are composed of enzymatic and non-enzymatic systems. Enzymatic defence systems involve certain enzymes whereas non-enzymatic defence systems involve certain endogenous compounds found in the body and certain exogenous compounds taken into the body (Halliwell, 1999). Defence systems not only reduce the level of lipid peroxidation by means of the elimination of active endogenous and exogenous compounds that may cause peroxidation, but also prevent lipid peroxidation by means of the conversion of these radicals into less harmful and harmless compounds (Gutteridge, 1995). When free radical damage overwhelms compensation of cells, peroxidation occurs, and a series of adverse reactions, including alteration of cell permeability, impairment of intracellular and extracellular transport systems, and intracellular energy metabolisms are observed. Constituting a problem for cells itself, peroxidation is also capable of causing adverse effects on living organisms through intermediate and final products of peroxidation (Comporti, 1993). The level of MDA, a final product of peroxidation, and the activities of antioxidant enzymes that play active role in the alleviation of peroxidation take place among the parameters which are referred to the assessment of the occurrence and intensity of lipid peroxidation (Gutteridge, 1995). Studies that have been conducted on many OPIs have similarly demonstrated the occurrence of oxidative damage (Kalender *et al*., 2007; Ogutcu *et al.*, 2008). OPIs have also been described as potent alkylating agents and cause genotoxic effects (Eto and Ohkawa, 1970). Epidemiological studies have reported that occupational exposure to pesticide mixtures, including OPIs, significantly increased chromosome damage (Dulout *et al.*, 1985; Pakldy *et al.*, 1987; Vijayaraghavan and Nagarajan, 1994).

Vitamin E (Alpha-tocopherol) primarily induces antioxidant effect and is known to be the compound with highest biological activity (Kalender *et al.*, 2005). Although the biological activity of this compound has not been exactly defined, additional effects other than its antioxidant effect have been reported. Vitamin E has been used in pesticide toxicity studies (Kalender *et al.*, 2007; Uzunhisarcikli *et al.*, 2007). Many pesticides are hydrophobic molecules which bind extensively to biological membranes, especially to phospholipid bilayers (Lee *et al.*, 1991). Vitamin E is the major lipid-soluble antioxidant and protects cellular membranes and lipoproteins against peroxidation (Yavuz *et al.*, 2004). Studies carried out with antioxidants such as α-tocopherol have shown inhibition of free radical formation (Kalender *et al.*, 2005), which may effectively minimize lipid peroxidation in biological systems (Akca *et al.*, 2005). The reaction of α -tocopherol with free radicals generates tocopherol radicals.

Being a glutathione precursor, *N*-acetylcysteine (NAC) is converted into cysteine *in vivo*, and increases glutathione (GSH) production. The antioxidant property of GSH is due to the thiol group of cysteine. Glutathione is a sweeper of reactive oxygen species (ROS). N-acetylcysteine is also a radical binder when administered alone. In addition to its antioxidant effects, it is also used in toxicology for the elimination of certain metals through chelation. Its chelating property is directly related to the –SH groups that exist in its structure. On the other hand, its mucolytic effect is due to free sulfhydryl groups. The sulfhydryl groups react with the disulphide bonds of mucoprotein, and decrease the consistency of mucus. NAC is one of the antidotes preferred in case of acetaminophen intoxications and it is also used in cardiovascular diseases (Azad *et al.*, 2001).

Previous studies have reported a protective effect for vitamins with different class of OPIs such as methyl parathion, methidathion, phosalone, malathion and diazinon (Altuntas and Delibas, 2002; Kalender *et al.*, 2005, 2006). But, no available data about the protective effect of vitamin E, NAC, and vitamin E + NAC in dimethoate toxicity as OPIs. The objective of the present study was to investigate the possible protective role of vitamin E (α-tocopherol), NAC and vitamin E plus NAC in dimethoate-induced OP toxicity. This study was interested to evaluate the hepatotoxicity effects of dimethoate probably through the generation of free radicals and the possible preventive effects of vitamin E and NAC after 7-weeks (subchronic exposure). Indicators of hepatic function (total protein, albumin, ALT, AST, ALP, GGT, and LDH), lipid profile (total cholesterol, triglycerides, VLDL-cholesterol), oxidative stress and hepatic antioxidant enzyme activities (SOD, CAT, and GST) as well as quantitative determination of level of DNA fragmentation in liver tissue were evaluated as indicators of dimethoate hepatotoxicity.

MATERIAL AND METHODS:

Chemicals:

Dimethoate (purity 98%), N-acetylcysteine were purchased from Sigma Chemicals, St. Louis, MO, USA. Vitamin E (α-tocopherol acetate) was supplied by Merck (Germany). Corn oil was used for preparing suspensions of dimethoate. All other chemicals and solvents that required for the biochemical assays were of highest purity and analytical grade and purchased from either Merck

(Darmstadt, Germany), or Sigma-Aldrich Chemie (Deisenhofen, Germany).

Animals:

Fifty-six adult male albino rats (body weight of 130± 20 g) were obtained from the animal house of Medical Research Institute, Alexandria University, Alexandria, Egypt. Animals were housed in stainless steel cages at an environmentally controlled room (temperature ≈ 25-27°C, with 12h light/dark cycle) for one week prior to starting the experiments and they were provided with tap water and standard rat diet (protein 24%, fat 5%, fibre 4%, carbohydrates 55%, calcium 0.6%, moisture 10%, and ash 9%).

Experimental design:

The animals were randomly assigned into eight groups of seven animals each:

• Group I served as control and animals were fed corn oil by oral gavages.

• Group II (dimethoate-treated group, D) in which dimethoate in corn oil was orally administrated in a concentration of 21 mg/kg body weight.

• Group III (Vitamin E) administrated vitamin E (200 mg/kg BW orally) once daily.

• Group IV (N-acetylcysteine, NAC) administrated at dose (100 mg/kg BW i.p) once daily.

• Group V (Vit.E + NAC).

• The other three groups were orally administrated dimethoate in a concentration of 21 mg/kg BW followed by either vitamin E {Group VI (D+Vit.E)}, NAC {Group VII (D+NAC)}, or vitamin E plus NAC {Group VIII (D+Vit.E+NAC)} in the same previous doses for 7 weeks.

The test doses of dimethoate, vitamin E and N-acetylcysteine were determined on the basis of findings from previous studies (Sivapiriya *et al.*, 2006; Kalender *et al.*, 2005; Eraslon *et al.*, 2007, respectively).

Measurement of body and organ weights:

Body and liver weights of the control and treated rats were measured by employing an automatic balance (AND GX-600, Japan). Livers were removed from the adipose tissue before weighing. Rats were anesthetized with diethyl ether after measurement of body weights, and livers were removed and their weights were measured.

Biochemical analysis:

At the end of the experimental period, blood samples of the rats were taken from the heart and collected into sterile tubes. Blood samples were centrifuged at 3500 rpm for 20 min, and serum was separated. Livers were removed immediately and washed with sodium phosphate buffer (pH 7.2).

Total protein, albumin, total cholesterol, triglyceride, and VLDL-cholesterol (very low density lipoprotein-cholesterol) levels were assessed in serum using a commercially available spectrophotometric-enzymatic kit (Thermo Trace-BECGMAN) and analyzed by an autoanalyzer (Bayer ope-RA). ALP (alkaline phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), GGT (γ-glutamyltransferase), and LDH (lactate dehydrogenase) activities were assessed in serum with a commercially available enzymatic-kinetic kit (SpinReact-BECGMAN) and analyzed by autoanalyzer (Bayer ope-RA).

LPO was ascertained in liver tissue by the assay of thiobarbituric acid reactive substances (TBARS) spectrophotometrically as described by Conrad *et al.* (2000). The level of lipid peroxidation is expressed as nmol/mg protein, using 1,1,3.3-teteraethoxypropan as standard.

Catalase activity (CAT) was assayed following the procedure of Aebi (1984). In a cuvette containing 200 µl phosphate buffer and 50 µl of tissue extract, 250 µl of 0.066 M H_2O_2 was added (in phosphate buffer) and a decrease in optical density was measured at 240 nm for 60s. The molar extinction coefficient of 43.6 M/Cm was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded/ min per mg protein.

Superoxide dismutase (SOD) activity was assayed spectrophotometrically as described by Durak *et al.* (1996). Briefly, 2.8 ml of reactive mixture (xanthine 0.3 mM, EDTA 0.67 mM, 150 M nitrotetrazolium blue chloride (NBT), sodium carbonate 0.4 M, bovine albumin 30 mg/30 ml) is added to 0.1 ml sample and 50 µl xanthine oxidase (10 µl in 2M ammonium sulphate), incubated at 25 °C for 20 min and mixed with 0.1 ml 8 M copper chloride. The colour reaction was measured at 560 nm.

Glutathione -S- transferase (GST) activity was determined using aromatic substrate by monitoring the change in absorbance due to thioether formation at 340 nm with a standard curve of 1-chloro-2,4-dinitrobenzene as described by Habig *et al.*, (1973).

Protein content was measured in the liver homogenates according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Determination of DNA fragmentation level:

Quantitatively the level of DNA fragmentation was measured by the method of Sellins and Cohen (1987). The ratio between the total DNA to fragmented DNA was determined at 280 nm. Also, DNA fragmentation was analysed using agarose gel electrophoresis. Tissue samples (30 mg) were incubated overnight at 37°C in salt EDTA (150 mM), Proteinase K (40 mg/ml) and 20% SDS

solution. The DNA was then extracted by phenol–chloroform method. Samples were loaded into wells of 1% a garose gel in 1× TBE. DNA was visualized by ethidium bromide staining. A control lane of DNA molecular weight standards from 100 to 650 bp was run. Banding patterns were visualized with the Foto/EclipseUV transilluminator and photographed

Statistical Analysis:

The values are expressed as mean ± SE. The results were computed statistically (SPSS software package, version 8) using one- way analysis of variance (ANOVA). Post hoc testing was performed for inter-group comparison on using the LSD. P<0.05 was considered mild significant and < 0.001 was considered highly significant.

RESULTS:

Evaluation of body and organ weights:

Death was not observed in any of the experimental groups during the experimental period. However, food intake of the dimethoate- and vitamin E + NAC + dimethoate-treated rats was reduced. Body weight, absolute liver weight, and relative liver weight did not significantly differ during the experiment between the vitamin E, NAC treated groups and the control group. At the end of the 7th week, there was a statistically significant decrease in body weight and an increase in the absolute and relative liver weights when the dimethoate-intoxicated group and vitamin E + NAC + dimethoate-treated group were compared to the control group ($P < 0.01$). Administration of either vit.E or NAC alone resulted in a highly significant increase ($P < 0.01$) in body weight and decrease in relative liver weight as compared to dimethoate group. When the vitamin E + NAC + dimethoate -treated group was compared to the dimethoate -treated group, no statistically significant differences were observed for body weight or the absolute and relative liver weights (Table 1).

Table 1. Body weight, liver weight, and relative liver weight of control and experimental rats

Groups	Body weight			Absolute liver wt.(g)	Relative liver weight (g/100 g body weight)
	Initial (g)	Final (g)	%Change		
Control	124.42 ±1.26	145.21 ±1.72	16.70 ±1.20	4.81 ±0.04	3.31 ±0.01
Dimethoate	123.31 ±2.21	101.21 ±2.54	-17.92 ±0.65 a#	5.89 ±0.08 a#	5.81 ±0.02 a#
Vit.E	126.42 ±1.11	141.35 ±2.71	11.80 ±1.43	4.62 ±0.03	3.26 ±0.02
NAC	125.21 ±1.27	139.27 ±1.55	11.22 ±0.74	4.91 ±0.13	3.52 ±0.08
Vit.E + NAC	125.91 ±0.91	140.18 ±1.56	11.33 ±0.88	5.01 ±0.02	3.57 ±0.05
Dimethoate +Vit.E	123.56 ±1.27	147.21 ±2.43	19.14 ±1.45 b#	5.71 ±0.06	3.87 ±0.01 b#
Dimethoate +NAC	124.62 ±1.44	148.33 ±3.12	19.02 ±1.85 b#	5.83 ±0.01	3.93 ±0.02 b#
Dimethoate +Vit.E +NAC	125.61 ±2.01	103.34 ±1.42	-17.72 ±1.11 a# c# d#	5.92 ±0.03 a# c*	5.72 ±0.04 a# c# d#

- Values represent the mean ± SE for seven rats.

- a: significantly different from control group; b: significantly different from (dimethoate) group; c: significantly different from (dimethoate+Vit.E) group and d: significantly different from (dimethoate+NAC) group.

- *: P<0.05 and #: P< 0.001.

Evaluation of biochemical results:

The control group was compared with all other groups at the end of the 7th week. In addition to this, the dimethoate treated group was compared to the vit.E and/or NAC individually or in combination with dimethoate treated groups. Biochemical changes are shown in tables 2-4. No statistically significant differences were observed when both the vit. E or NAC alone-treated groups were compared with the control group. The biochemical parameters were investigated in order to assess the changes in hepatic function, the changes in the lipid profile and also to evaluate the oxidative damage and the changes of antioxidant enzyme levels.

Changes in hepatic function:

Total protein and albumin levels and ALP, ALT, AST, GGT, and LDH activities are indicators of hepatic function. Table 2 shows the levels and activities of these parameters in serum.

Table 2. Effect of vitamin E and N-acetylcysteine (NAC) on liver damage markers in dimethoate- exposed rats.

Groups	ALP	ALT	AST	GGT	LDH	Total Protein	Albumin
			(U/L)			(g/dl)	
Control	140.05±4.76	55.54±2.10	60.41±3.41	3.02±0.07	1400.71±30.15	10.43±1.08	4.81±1.02
Dimethoate	205.87±13.45 a#	73.86±2.81 a#	84.57±4.71 a#	3.83±0.09 a#	1904.96±35.71 a#	7.30±1.02 a#	3.55±0.91 a#
Vit.E	144.24±1.62	55.91±1.24	61.05±1.81	3.03±0.02	1420.14±15.11	10.21±0.98	4.87±1.02
NAC	142.11±2.15	56.11±2.17	60.92±2.19	3.06±0.01	1429.29±22.17	10.35±1.01	4.90±1.03
Vit.E + NAC	142.91±1.92	56.01±1.48	61.28±1.44	3.04±0.02	1436.15±19.82	10.28±1.21	4.88±1.87
Dimethoate +Vit.E	170.05±2.62 b#	60.51±2.82 b#	77.35±3.18 b*	3.20±0.09 b#	1866.75±26.13	8.41±0.74 b*	4.10±1.12 b*
Dimethoate +NAC	175.16±3.58 b#	69.67±1.71 b*	79.17±2.22 b*	3.11±0.41 b#	1950.61±41.32	7.95±1.02 b*	4.02±0.95 b*
Dimethoate +Vit.E+NAC	150.23±1.87 b# c* d*	58.41±2.34 b# c* d#	63.52±1.48 b# c* d*	3.03±0.21 b# c#d*	1930.11±27.19	10.11±1.65 b# c*d#	4.63±1.22 b# c* d#

- Values represent the mean ± SE for seven rats.
- a: significantly different from control group; b: significantly different from (dimethoate) group; c: significantly different from (dimethoate+Vit.E) group and d: significantly different from (dimethoate+NAC) group.
- *: P<0.05 and #: P< 0.001.

Oral administration of dimethoate caused a highly significant (P<0.001) increase in the activities of ALP (+47%), ALT (+33%), AST (+40%), GGT (+27%), and LDH (+36%) but statistically significant reductions were found in total protein (-30%) and albumin levels (-26%) compared to the control group. Treatment with vit.E alone caused a highly significant decrease (P<0.001) in ALP, ALT, and GGT activities, mild decrease (P<0.05) in AST activity while a mild significant increase (P<0.05) was obtained in the total protein and albumin levels as compared to dimethoate exposed group. However, the Treatment with NAC alone also significantly decreased the enzyme activities (ALP & GGT: P<0.001; AST& ALT: P<0.05) and significantly (P<0.05) ameliorated the inhibition in levels of total protein and albumin fraction which induced by dimethoate administration but the treatment with both compounds caused a highly significant decrease (P<0.001) in the activities of enzymes (ALP: 27%, ALT: 21%, AST 25%, and GGT 21%) and highly significant increase (P<0.001) in the level of total protein and albumin as compared to dimethoate group and restored them near to the control. Increased LDH activity remained unchanged on any treatment with vit.E or NAC at the end of 7th week.

Changes in lipid profile:

Table 3 shows levels of serum total cholesterol, triglyceride and VLDL-cholesterol. When the dimethoate- treated group was compared with the control group, there was a highly significant (P<0.001) increase in the total cholesterol level (+52%) and highly significant decreases (P<0.001) in the triglyceride (-60%) and VLDL-cholesterol (-64%) levels. Administration of both vit.E and NAC alone resulted in a mild significant decrease in total cholesterol level and a mild significant increase in triglyceride and VLDL-cholesterol levels as compared to dimethoate exposed group. Treatment with both compounds caused a highly significant decreased in the total cholesterol level and a highly significant increase in triglyceride and VLDL-cholesterol levels.

Table 3. Changes in levels of lipid profile in rats exposed to subchronic dimethoate and protective effect of vitamin E and N-acetylcysteine (NAC).

Group	Total Cholesterol	Triglycerides	VLDL-Cholesterol
		(mg/dl)	
Control	70.34 ± 3.53	120.68 ± 6.25	20.22 ± 1.32
Dimethoate	106.91 ± 6.62 a#	48.27 ± 1.23 a#	7.27 ± 1.67 a#
Vit.E	73.82 ± 2.23	122.01 ± 3.92	18.39 ±1.82
NAC	71.11 ± 4.61	118.92 ± 2.96	18.92 ± 1.30
Vit.E + NAC	72.56 ± 2.67	121.62 ± 8.33	21.63 ± 2.01
Dimethoate + Vit.E	90.21 ± 2.31 b*	66.42 ± 3.72 b*	9.32 ± 2.24 b*
Dimethoate + NAC	93.68 ± 4.79 b*	71.31 ± 2.62 b*	10.68 ± 1.83 b*
Dimethoate + Vit.E + NAC	78.45 ± 3.32 b#c*d*	110.46 ± 9.21 b#c*d*	17.36 ± 3.31 b#c*d*

- Values represent the mean ± SE for seven rats.
- a: significantly different from control group; b: significantly different from (dimethoate) group; c: significantly different from (dimethoate+Vit.E) group and d: significantly different from (dimethoate+NAC) group.
- *: P<0.05 and #: P< 0.001.

Oxidative damage to lipids and the activities of antioxidant enzymes in hepatic tissue:

Table 4 shows the TBARS concentration an-end product indicative of the extent of lipid peroxidation- and the activities of antioxidant enzymes: CAT, SOD and activity of glutathione metabolizing enzyme GST in the hepatic tissue. Dimethoate administration caused a highly significant (P<0.001) increase in TBARS level (+56%), while a highly significant decrease (P<0.001) was obtained in SOD, CAT and GST activities by (-60%), (-44%), and (-37%) respectively as compared to the control. Treatment with vit.E alone significantly (P<0.001) reduced the increased TBARS level, whereas it significantly increased (P<0.001) the CAT, SOD and GST activities in comparison with dimethoate group, while treatment with NAC caused a mild (P<0.05) changes in these parameters. Combined treatment with vit.E and NAC restored their levels back to normal values.

Table 4. Effect of vitamin E and N-acetylcysteine (NAC) on lipid peroxidation and antioxidant status in hepatic tissue of dimethoate- exposed rats.

GROUP	TBARS	SOD	CAT	GST
	nmol/mg protein	µmol/min/mg protein		nmol/min/mg protein
Control	160.14 ± 9.56	8.79 ± 0.34	101.57 ± 3.72	1.13 ± 0.02
Dimethoate	249.81± 10.21 a#	3.51± 0.02 a#	56.87 ± 1.23 a#	0.71± 0.01 a#
Vit.E	162.72 ± 6.22	8.51± 1.02	104.11± 5.21	1.12 ± 0.01
NAC	160.81± 8.11	8.91± 1.23	100.91± 2.98	1.11± 0.02
Vit.E + NAC	158.81± 10.10	8.66 ± 1.01	102.19 ± 7.11	1.16 ± 0.01
Dimethoate + Vit.E	205.71± 13.11 b#	6.41± 0.08 b#	77.34 ± 2.81 b#	1.01± 0.01 b#
Dimethoate + NAC	221.82 ± 11.87 b*	5.12 ± 0.04 b*	68.25 ± 1.72 b*	0.93 ± 0.02 b*
Dimethoate + Vit.E + NAC	164.85 ± 9.27 b# c* d#	8.12 ± 1.01 b# c* d*	98.22 ± 5.12 b# c* d#	1.08 ± 0.04 b# c* d#

- Values represent the mean ± SE for seven rats.

- a: significantly different from control group; b: significantly different from (dimethoate) group; c: significantly different from (dimethoate+Vit.E) group and d: significantly different from (dimethoate+NAC) group.

- *: P<0.05 and #: P< 0.001.

Evaluation of level of DNA fragmentation:

Figure 1 represents the level of DNA fragmentation in liver of control and experimental rats at the end of experiment. Dimethoate Challenged rats showed a highly significant increase (P<0.001) in the level of DNA fragmentation as expressed in % DNA fragmentation (26.67 ± 4.04). Such an increase was almost decreased significantly (P<0.001) upon treatment with vitamin E and NAC. The decrease being 14.17 ± 0.76 and 16.50 ± 0.50 in liver was observed respectively. Combined treatment with vit.E and NAC significantly decrease DNA cleavage (16.17± 1.04) in comparison with dimethoate group. The DNA fragmentation was further confirmed by agarose gel electrophoresis of DNA isolated from liver samples of control and experimented rats, which represented in figure 2. Dimethoate exposed rat liver DNA was found to be fragmented as evidence by the DNA ladder formation upon agarose gel electrophoresis. Dimethoate caused DNA cleavage into fragments and manifested as DNA laddering a hallmark of apoptosis. The results indicate induced hepatocellular apoptosis.

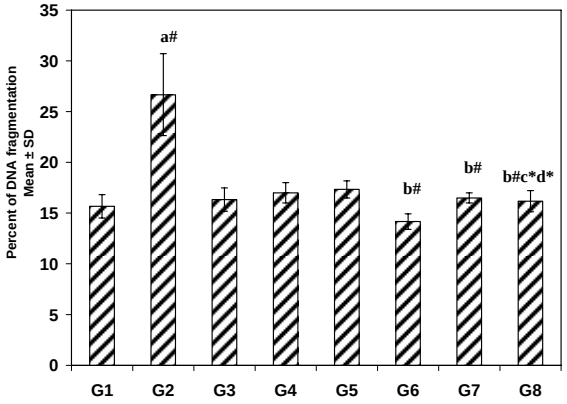

Fig. 1. Illustrating the percentage of DNA fragmentation in the liver cells of control and experimental groups. G 1: Control, G 2: Dimethoate, G 3: Vitamin E, G 4: NAC, G 5: Vitamin E + NAC, G6: Dimethoate + Vitamin E G7: Dimethoate,+ NAC G8: Dimethoate + Vitamin E + NAC.

a: significantly different from control group.
b: significantly different from (dimethoate) group.
c: significantly different from (dimethoate+Vit.E) group.
d: significantly different from (dimethoate+NAC) group.
*: P<0.05 and #: P< 0.001.

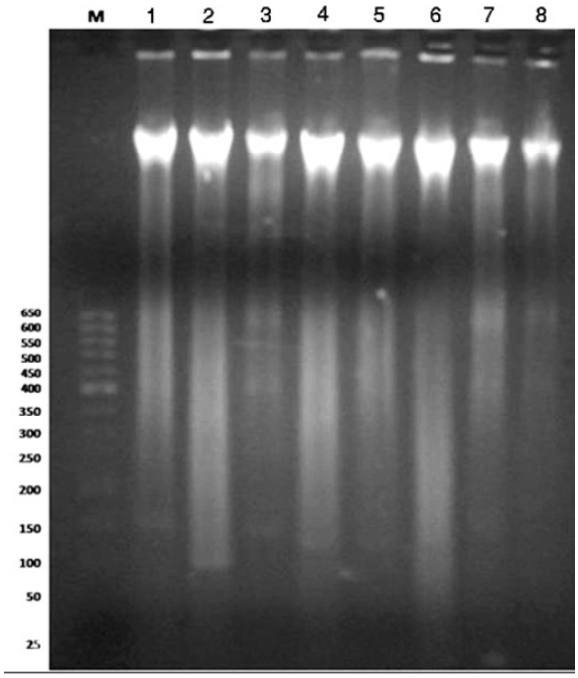

Fig. 2. Agarose gel electophoretic pattern of DNA fragmentation in control and experimental rats at the end of experiment.M is a DNA size marker, Lane1: control, Lane2: Dimethoate, Lane3: Vitamin E, Lane4: NAC, Lane5: Vitamin E + NAC, Lane6: Dimethoate + Vitamin E , Lane 7:Dimethoate+NAC and Lane8: Dimethoate + vitaminE + NAC.

DISCUSSION:

The extensive use of different pesticides in agriculture and for public health purposes, has led to drastic effects in many non-target species including man (WHO/PCS, 1996; Karanth *et al.*, 2004). The current study was performed to investigate the hepatic toxicity of a commonly used organophosphorus pesticide, dimethoate, in rats following seven weeks subchronic exposure. In addition, the study was designed to investigate the effect of NAC, vitamin E and NAC plus vitamin E on the dimethoate hepatotoxicity in rats.

Organophosphorus (OP) pesticides cause a reduction of body weight in experimental animals (Kalender *et al.*, 2007; Uzunhisarcikli *et al.*, 2007). In the present study, at the end of 7thweek, there was a decrease in the body weight of the dimethoate-intoxicated rats compared to the controls. This decrease may occur as a consequence of reduced food intake. In addition, there was an increase in the absolute and relative liver weights of the dimethoate-treated rats. OP pesticides cause an increase in the absolute liver weight (Sharma *et al.*, 2005; Jeong *et al.*, 2006) and relative liver weights (Undeger *et al.*, 2000; Johnson *et al.*, 2002; Kang *et al.*, 2004) in experimental animals. In toxicological studies, organ and relative organ weights are important criteria for evaluation of organ toxicity (Ogutcu *et al.*, 2008). Amacher *et al.* (1998) reported that the increased liver weight was related to enzyme induction of cytochrome-p450 and might be due to edema in the tissue. Johnson *et al.* (2002) stated that the increase in liver weight stemmed from a metabolic reaction induced by the OP.

In the current study, the biochemical studies show that seven weeks after dimethoate administration, serum ALP, ALT, AST, GGT, and LDH activities increased compared to those of the control group. Several OP pesticides may cause increases in these serum enzymes (Morowati, 1997; Srivastava and Raizada, 1999; Banerjee *et al.*, 1999; Gomes *et al.*, 1999; Altuntas and Delibas, 2002; Khan *et al.*, 2005). The changes in the levels of these enzymes may differ depending on the exposure time and dose of OP. These enzymes are primarily used to evaluate hepatic damage. These enzymes are secreted into the blood after hepatocellular injury, resulting in an increase their levels (Kalender *et al.*, 2005).

OP Pesticides generally cause an increase in total cholesterol level (Hassan *et al.*, 1988; Kalender *et al.*, 2005). In this study, dimethoate caused an increase in the serum total cholesterol level. Increased serum cholesterol can be attributed to the effects of the pesticide on the permeability of liver cell membranes (Adham *et al.*, 1997). Also, the increase in the level of serum total cholesterol may be attributed to the blockage of the liver bile ducts, causing a reduction or cessation of cholesterol secretion into the duodenum (Zaahkouk *et al.*, 2000). An increase in the serum cholesterol level may be a sign of liver damage (Lucic *et al.*, 2002). In the present study, dimethoate caused decreases in the triglyceride and VLDL-cholesterol levels. Clinically, in parenchymal liver diseases, levels of these parameters decrease. Some different pesticides cause a decrease in the VLDL-cholesterol and triglyceride levels (Joshi *et al.*, 2003; S. Kalender *et al.*, 2005).

A decrease in biosynthetic function of the liver of dimethoate-intoxicated rats, as shown by decreased levels of total protein and albumin, is the end result of this phenomenon. In this study, a decrease was observed in the serum total protein and albumin levels. The results of the present study suggest that the exposure to dimethoate may influence total protein and albumin metabolism. Albumin is the most abundant blood serum protein and is produced in the liver and often transports or binds the drugs or chemicals. In the present study, level of albumin was decreased after dimethoate administration, this is in agreements with the report of Peeples *et al.* (2005) on different OP. Normally, the reduction of albumin level indicates a liver disease. This reduction could be attributed to changes in the protein and

free amino acids metabolism and their synthesis in the liver (Ncibi *et al.*, 2008). In the same field, Li *et al.* (2007) suggested that albumin could be used as a biomarker of OP toxicity.

Among the objectives of the present study was to assess the oxidative damage sustained by the liver following subchronic exposure to dimethoate. Liver plays a central role in detoxification and is chronically exposed to xenobiotics and their toxic derivatives. It has been previously reported that the exposure to OP pesticides may induce oxidative stress in rats (Koner *et al.*, 1997). The present study strengthens the hypothesis and suggests that the induction of oxidative stress is perhaps the central mechanism by which OP pesticides exert their cellular action. Oxidative damage primarily occurs through production of reactive oxygen species, including hydroxyl radicals and hydrogen peroxide that subsequently react with biological molecules as well as causing damage to membranes and other tissues (Banerjee *et al.*, 1999). LPO and antioxidant defence systems were used in this study as important biomarkers for detection of toxic nature of dimethoate. Three antioxidant markers (CAT, SOD, and GST) and LPO were evaluated for oxidative stress. A significant increase in hepatic LPO level, as evidenced by increased level of TBARS following administration of the dimethoate dose in the present study, suggests participation of free radical as a result of stress condition induced oxidative cell injury in mediating the toxicity of dimethoate. These intentionally introduced environmental xenobiotics (OP) are known to have a strong affinity for interaction with membrane phospholipid (Joshi *et al.*, 2003) and phospholipid component of biomembrane is believed to be the site of action of OP insecticides (Dasgupta *et al.*, 1992). It is known that OH can initiate lipid peroxidation in tissues (Srivastava and Narain, 1985). Many different OP caused an increase in lipid peroxidation level in many organs (Bagchi *et al.*, 1995; Gultekin and Akdogan, 2000; Kalender *et al.*, 2007). It has been reported previously that during liver damage there is an observed decrease in antioxidant defenses in the liver (Seven *et al.*, 2004). The by-products of oxygen metabolism initiate different subcellular outcomes. The superoxide radical has been shown to directly inhibit the activities of catalase enzyme (Kono and Fridovich, 1982); likewise, singlet oxygen and peroxyl radicals have been shown to inhibit SOD and CAT activities (Escobar *et al.*, 1996). These observations explain the significant inhibition of SOD and CAT activity in dimethoate-intoxicated rats observed in the present study. These results are in agreement with previous studies (Tappel., 1973; Doyotte *et al.*, 1997) where alterations in lipid peroxidation and changes in the activity of SOD and CAT have been noted following exposure to pesticides. Confirming the data in the current study, Dimitrova *et al.* (1994) suggested that the superoxide radicals by themselves or after their transformation to H_2O_2 cause an oxidation of the cysteine in the enzyme and decrease SOD activity. Consequently, the decreased SOD activities might have reflected a cellular oxidative stress due to dimethoate exposure. There have been reported that the long term intoxication with OP leads to a gradual exhaustion of SOD, CAT and GST (Dasgupta *et al.*, 1992; Bagchi *et al.*, 1995; Kalender *et al.*, 2007). Also, Ogutcu *et al.* (2008) found that the molecule of GST binds pesticides in a sequestering mechanism adding to the defense of the organism either as a passive way of detoxification or as a facilitating one. Therefore, it is reasonable to suggest that the GST functions as a binding protein contributing to the action of other enzymes involved in degradation of pesticides.

Under normal circumstances apoptosis is a naturally occurring process by which organisms intentionally eliminate damaged cells (D'Agostini. *et al*., 2001). The DNA ladder formation can be considered as the biochemical indicator of apoptotic cell death. The observations in the present study demonstrate that DNA fragmentation analysis illustrates increased percentage of DNA fragmentation and DNA ladder formation upon agarose gel electrophoretic separation of DNA, indicating apoptotic cell death. The increase in the DNA fragmentation profile observed in dimethoate-intoxicated rats during this study might be attributed to the increase in the activity of oxidative stress. From these findings it was clear that the oxidative stress is the pathway that initiates apoptosis in dimethoate exposed rats. This was further confirmed by the DNA fragmentation assay. Consistent with these results, Several OP have been extensively studied as genotoxic agents in mammalian *in vivo* systems (Behera and Bhunya, 1989; Vijayaraghavan and Nagarajan, 1994).

The antioxidant properties of NAC are well documented and its potential as an antioxidant *in vivo* has been reported recently (Kamboj *et al.*, 2006). In the current study, a moderate protection conferred by NAC against dimethoate-induced hepatotoxicity as evidenced by the decreases in the activities of measured serum enzymes and the levels of total cholesterol and LPO. Also hepatic CAT, SOD, and GST activities as well as serum total protein content were increased. Examination of the TBARS levels of the group administered NAC and dimethoate demonstrated a decrease in the level of LPO. However, the levels were above the values recorded in the control group. Therefore it can be suggested that NAC alleviates oxidative damage to a certain level. This protective

effect may be related to the increase in the level of intracellular glutathione (Kelly, 1998). Glutathione acts as a radical sweeper due to the SH groups found within its structure, and plays a role in the elimination of free radicals by binding to them (Meister, 1983). The activity increase observed in CAT, SOD, and GST, in comparison to the group administered dimethoate alone is again related to the decrease in the level of free radicals. Thereby, the decrease in the level of free radicals has alleviated the intensity of enzyme inhibition. Amongst studies carried out on lipid peroxidation of pesticide origin and the use of NAC, a study carried out by Azad *et al.* (2001), in which Wistar rats were administered aluminium phosphide at a dose of 12.5mg/kg, and it has been demonstrated an increase in the level of myocardial MDA as well as decrease in CAT activity. In groups administered NAC, a decrease in MDA levels and an increase in CAT activity were observed in the same periods. In another study carried out by Kamboj *et al.* (2006), Wistar rats were first administered carbofuran at a dose of 1mg/kg, and secondly NAC at a dose of 200 mg/kg, half-a-hour later the administration of carbofuran. The authors reported that carbofuran caused an increase in the level of cerebral LPO levels, and a decrease in the SOD and CAT activities. Decrease in MDA levels and increase in CAT and SOD activities were detected in the group administered carbofuran plus NAC. As indicated above, the findings obtained for various pesticides display similarity to the findings obtained in this study.

From the present results it can be assumed that the hepatotoxic effects of dimethoate are mainly attributed to oxidative stress although these effects were largely prevented by a vit.E supplementation. The antioxidant activity promoted by vit.E is essentially performed in the lipid membrane environment. Vit.E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions (Akca *et al.*, 2005). Furthermore, due to its antioxidant activity it could increase the antioxidant activity in the hydrophilic environment. Previous studies have also shown the protective effect of vit.E against several xenobiotics (Altuntas and Delibas, 2002). Cotreatment of dimethoate with vit.E allowed recovering some of the antioxidant enzymatic activity lost by dimethoate treatment only. In this study, a decrease in hepatic thiobarbituric acid reactive substance (TBARS) levels was determined in the group administered a combination of dimethoate+vitamin E, in comparison to the group administered dimethoate alone. This decrease is related to the sweeping of free radicals generated by dimethoate, by treatment with vit.E. Vit.E treatment could be useful to decrease dimethoate toxicity by quenching oxidative stress imposed by dimethoate. Amongst research carried out on other pesticides, the study conducted by El-Demerdash *et al.* (2003), who demonstrated that vit.E alleviated the intensity of lipid peroxidation caused by fenvalerate. Also, Giray *et al.* (2001) have reported a decrease in cerebral TBARS levels in the group administered vitamin E, amongst rats that were administered cypermethrin as repeated doses of 75 mg/kg for a period of 5 days. Similar findings were obtained in the group administered vitamin E, in this study.

The protective effect of vitamin E against genotoxic effects of various chemicals has previously been reported (Sugiyama *et al*., 1992 and Lunec *et al*., 2004). The antiapoptotic activity of Vitamin E has been reported by several authors (Barroso *et al.*, 1997; Mobio *et al.*, 2000). The antioxidant vitamins directly inhibit free radical mediated apoptosis by directly eliminating them. Apart from the free radical scavenging property, antioxidants are known to regulate the expression of number of genes and signal regulatory pathways and thereby may prevent the incidence of cell death (Vuchetich *et al.*, 1996).

The current data shows a more significant amelioration in almost biochemical parameters was obtained on treatment with both compounds NAC plus vit.E. NAC administration with vit.E restored most of measurable values suggested a beneficial role of NAC concurrently with vit.E in dimethoate intoxication. In addition, the treatment with vit.E and NAC was observed to reverse the genotoxicity induced by dimethoate. Administration of vit.E and NAC along with dimethoate significantly reduced the extent of apoptosis. Compared to the control group, statistically significant differences were not observed in the groups administered NAC or vit.E alone, with respect to all examined parameters. This situation suggests that none of the two compounds cause lipid peroxidation when administered alone at the indicated doses and for the indicated periods.

According to the results of the present study, it can be concluded that dimethoate toxicity induced LPO and generation of free radicals in liver tissue. Additionally, this study demonstrates that the antioxidant levels were significantly affected by dimethoate toxicity indicating that the oxidative damage can be possible pathway in the mechanism of dimethoate-induced hepatotoxicity. Also, a higher degree of DNA fragmentation of liver cells was evident indicates that a greater number of these cells undergo apoptosis. Moreover, the combined treatment with vit.E and NAC showed a significant protective effect against dimethoate-induced liver injury

at the biochemical level and provide an evidence of beneficial effect of the antioxidant NAC in conjunction with vit.E in re-balancing the impaired prooxidant/ antioxidant ratio in sub-chronic dimethoate intoxication in rats.

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السمية الكبدية المستحثة بواسطة الدايمثوات فى الجرذان و الدور الوقائى لمادة N-أسيتيل سيستين و فيتامين هـ

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يعتبرالدايمثوات واحد من اشهرالمركبات الفسفوعضوية المستخدمة فى مكافحة العديد من الافات الحشرية لانواع كثيرة من المحاصيل الزراعية، لذلك فمن المفيد فحص امكانية علاج سمية هذا المبيد. وتهدف هذه الدراسة إلى فحص الدور العلاجى لكلا من فيتامين هـ و N-أسيتيل سيستين ضد السمية الكبدية المستحثة بواسطة الدايمثوات فى الجرذان. وقد قسمت مجاميع الجرذان الى ثمانية مجاميع: المجموعة الاولى وهى المجموعة الضابطة و المجموعة الثانية (أعطيت مبيد الدايمثوات بتركيز 21 مجم/كيلو جرام من وزن الجسم عن طريق الفم) و المجموعة الثالثة (عولجت بفيتامين هـ بتركيز 200 مجم/كيلو جرام من وزن الجسم عن طريق الفم) والمجموعة الرابعة (حقنت بمادة N-أسيتيل سيستين بتركيز 100 مجم/كيلو جرام من وزن الجسم داخل التجويف البريتونى) و المجموعة الرابعة (عولجت بفيتامين هـ و N - أسيتيل سيستين). كما أعطيت المجاميع الثلاثة الاخرى الدايمثوات بنفس التركيز يتبعها اما بفيتامين هـ فقط أو N - أسيتيل سيستين فقط او كلاهما معا بنفس الجرعات السابقة لمدة سبعة اسابيع. أكدت هذه الدراسه أن التعرض للدايمثوات نتج عنه انخفاض معنوى فى وزن الجسم وزيادة ملحوظه فى وزن الكبد، أيضا ادى الى زيادة ملحوظه فى نسبة الاكسدة الفوقية للدهون فى الكبد و نشاط الانزيمات المضادة للاكسدة (سوبر اوكسيد ديسميوتيز وكتاليز وجلوتاثيون-s- ترانسفيريز) فى نهاية الاسبوع السابع. كما لوحظ أيضا انخفاض معنوى فى نسبة البروتين الكلى والزلال والدهون الثلاثيه ونسبة الكوليستيرول قليل الكثافة فى مصل الدم وكان هذا متلازما مع زياده معنوية فى نشاط انزيم الفوسفاتيز القاعدى و الانين ترانس امينيز، اسبارتيت ترانس امينيز وجاما جلوتاميل ترانسفيريز و انزيم لاكتيت ديهيدروجينيز ونسبة الكوليستيرول الكلى فى الجرذان المعالجه بالدايمثوات. أثبتت هذه الدراسة ايضا اختلاف معنوى فى جميع القياسات الكيميائية الحيوية ما عدا انزيم لاكتيت ديهيدروجينيز عند مقارنة المجموعة الثامنة (دايمثوات + فيتامين هـ + N-أسيتيل سيستين) بالمجموعة الثانية التى أعطيت الدايمثوات فقط. خلصت هذه الدراسة ايضا الى دراسة دور كلا من فيتامين هـ و N-أسيتيل سيستين ضد السمية الوراثية المستحثه بواسطة الدايمثوات و التى كانت متمثلة فى زيادة كمية تقطيع الحامض النووى فى خلايا الكبد مما يدل على تلف الحامض النووى فى المجموعة الثانية. وباختصار قدمت هذه الدراسة حقائق عن الدور العلاجى المفيد لكلا من فيتامين هـ و N-أسيتيل سيستين فى اعادة الاتزان للخلل الذى سببته سمية الدايمثوات فى كبد الجرذان.

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