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Full Length Research Paper

Protective effects of vitamin C against biochemical toxicity induced by malathion pesticides in male albino rat

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Malathion is a chemical pesticide, commonly used by Egyptian farmers; however, its contamination of water sources has become an important issue. It is suggested that the role of vitamin C in alteration of enzymes responsible for energy metabolism was induced by administration of Malathion. The effect of malathion and malathion with vitamin C on some enzymes in the rat were recorded in the present study. Significant increases in the level of hepatic enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma glutamyl transpeptidase) were recorded. Furthermore, renal markers such as urea, cholesterol and creatinine were increased in rats treated with malathion. Additionally, serum triglyceride was significantly decreased. No significant changes were observed in total protein, albumin and bilirubin in all treated groups as compared to the control. The present results showed that a significant ($p < 0.001$) decrease in glycolytic enzymes (hexokinase, pyruvatekinase, glucose phosphate isomerase and phosphofructokinase) were observed in treated groups, and lactate dehydrogenase enzyme activity also showed significant ($p < 0.01$) reduction. Co-administration of vitamin C to the groups restored all the parameters cited above to near-normal values. Therefore, the present investigation revealed that vitamin C appeared to be a promising agent for protection against malathion–induced toxicity.

Key words: Malathion pesticide, male albino rat, biochemical toxicity.

INTRODUCTION

Using pesticides is an important procedure for enhancing agriculture yield. However, the great consciousness, brought back upon their deleterious effects on human, animal and environmental health, lead to the shortage of their use by imposing various rules(Dikshit et al., 2003; Petersen et al., 1996). The oral route constitutes the main source of general population exposure to this pesticide which is ingested within food and water (Barlow et al., 2001). Synthetic pyrethroids (also including sumithrin, fenvalerate, d-trans allethrin, permethrin and cypermethrin) have the ability to disrupt biochemistry, haematology and reproduction (Yousef et al., 2003a).

The resulting pesticide pollution of the water becomes a great threat to the aquatic ecosystem (Alam et al., 2005; Gupta et al., 2002). Malathion is an insecticide with a neurotoxin action that causes insect death by inhibiting the acetylcholinesterase enzyme (Kwong, 2002). Besides their neurotoxin effects, the organophosphate insecticides have other properties such as cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity, which can affect human beings (Flessel et al., 1993; Giri et al., 2002).

Some experimental studies have demonstrated that vitamins C and E can be used to counteract pesticide toxicity (Yavuz and Kutsal, 2004; Yousef et al., 2006). Vita-

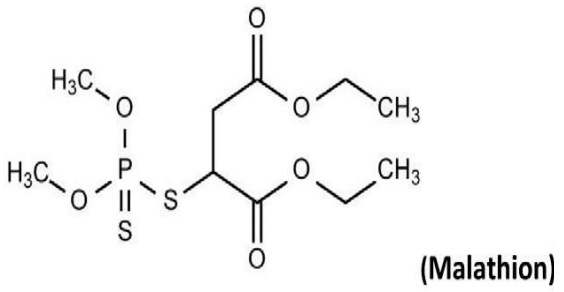

Figure 1. Chemical structure of malathion.

min C is an important water-soluble chain-breaking antioxidant and enzyme cofactor (Wilson, 2002). Supplementation with vitamin C (500 or 1000 mg/day) during 13 weeks in 48 healthy men and women, has shown to increase the lymphocyte ascorbate by 51%, accompanied by an increase of lymphocyte glutathione by 18% (Lenton et al., 2003). Vitamin C tend to reduce the toxicities of these pesticides because of the health problems induced by many environmental pollutants. Much effort has been expended in evaluating the relative antioxidant potency of vitamin C (Lenton et al., 2003).

Therefore, the aim of the present study was to evaluate whether malathion induced biochemical perturbations in rats and to investigate the possible modulatory effects of vitamin C on carbaryl pesticides-induced toxicity.

MATERIALS AND METHODS

Animals

Laboratory-bred strain albino male rats of three months old with an average weight of 27.5±2.5 g obtained from a closed random-bred colony at the National Research Centre, Cairo, Egypt were used in this study. They were maintained in a well ventilated animal house. They were housed in large polypropylene cages with free access to food and water *ad labium* during the course of the experiment.

Malathion

Malathion [diethyl (dimethoxythiophosphorylthio) succinate] (Figure 1) with molecular formula $C_{10}H_{19}O_6PS_2$ and molecular weight of 330.36 g/mol was obtained from ADWIA company, Egypt. The solubility in water was 145 mg/L at 20°C (Tomlin, 1997). The density was 1.23 g/cm^3, and boiling point was 156 to 157°C at 0.7 mm Hg.

Experimental design

The rats were randomly sub divided into four groups of eight animals each. Group 1 served as a control and received distilled water *ad libitum*. They were sham-injected daily with saline. Group 2 received through drinking water 1.28 mg/kg BW of Malathion (Yousef et al., 2006) during 4 weeks of treatment. Groups 3 and 4 received malathion plus vitamin C (200 mg/kg BW) (Aksoy et al., 2005) and vitamin C by intraperitoneal injection, respectively. The rats were treated with repeated doses of vitamin C and malathion for four weeks. At the ends of the experimental periods (4 weeks), the rats were sacrificed under diethyl ether anesthesia at fasting state. Each liver was fragmented into 0.25 g portions used for homogenization and biochemical assays. Serum samples were obtained by the centrifugation of blood at 4000 rpm for 15 min at 41°C, and were then divided into Eppendorf tubes. Isolated sera were stored at –20.1°C until analysis.

Biochemical parameters were determined, and analyzed spectrophometrically, using kits purchased from Bio- Merieux Company, France. Hexokinase (HK) was assayed according to the method of Uyeda and Racker (Uyeda and Racker, 1965). Pyruvatekinase (PK) was assayed according to the method of McManus and James (McManus and James, 1975). Glucose phosphate isomerase (GPI) was assayed according to the method of King (King, 1974), phosphofructokinase (PFK) according to the method of Zammit et al. (1978), and lactate dehydrogenase (LDH) activity according to the method of Cabaud and Wroblewski (1958). Aspartate and alanine aminotransferases according to the method of Reitman and Frankel (1957). Alkaline phosphatase activities were assayed according to Bessey et al. (1946) and Fishman and Ferner (1953) respectively. Total protein was assayed according to the method for experimentation of Lowry et al. (1951).

Statistical analysis

The results obtained in the present work are represented as means ± standard deviation (SD), and were analyzed using analysis of variance (ANOVA). The significance of difference between means was calculated using the Duncan Multiple Range Test (Steel and Torrie, 1980).

RESULTS

The effects of Malathion, vitamin C and their combination on some biochemical parameters in the rats are shown in Table 1. Results indicate that Malathion caused a significant increase (-32.12, 304.60 and -23.22% respectively) in the cholesterol, urea and creatinin serum concentrations, while triglyceride decreased (33.29%) significantly as compared to the control group. No significant changes were observed in total protein, albumin and bilirubin in all treated groups as compared to the control. After treatment of rats by Malathion plus vitamin C, cholesterol, urea, creatinin and triglyceride were statistically similar to their control values.

The effects of Malathion, vitamin C and their combination on some biochemical parameters in the rats are shown in Table 2 .Results indicate that Malathion caused a significant increase (-53.48, -86.49, -54.30 and -90.1%, respectively) in AST, ALT, ALP, and gGT activities as compared to the control group. After treatment of rats by Malathion plus vitamin C, AST, ALT, ALP and gGT activities were statistically similar to their control values.

The present results in the Table 3 show very highly significant ($p \leq 0.01$) reduction in Lactate (LDH) enzyme activity in rat treated with Malathion (39.65%) as compared to the normal control, while significant ($p \leq 0.001$) decrease was noticed in other glycolytic enzymes hexokinase (HK), pyruvatekinase (PK), phosphofructokinase (PFK) and glucose phosphate isomerase (GPI) as compared to the normal healthy control. The percentage of enzyme activities in treated rats were 67, 36.2, 39.65

Table 1. Serum biochemistry changes of the control and rats treated with malathion, vitamin C or their combination (malathion and vitamin C).

Parameter	Experimental group						
	Control	Malathion		Malathion and vitamin C		Vitamin C	
	Mean±SD	Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change
Cholesterol (mg/dl)	77.2 ±7.2	102±6.4*	-32.12	82.4±3.2	-6.74	75.8±1.4	1.81
Triglyceride (mg/dl)	111.23 ± 1.1	74.2±3.5*	33.29	98.8±5.12	11.18	112.4±7.2	1.05
Total protein (g/dl)	8.4± 0.21	8.6±0.22	-2.38	8.5±0.41		8.4±0.31	-1.19
Urea (mmol/l)	5.22±0.16	21.12±4.11****	-304.60	13.41±1.12**	-156.9	5.86±0.43	-12.16
Creatinin (mmol/l)	85.32±3.31	105.13±2.32*	-23.22	92.2±2.54	-8.06	84.85±2.11	-8.06
Albumin (g/dl)	2.22±3.14	2.05±0.12	7.66	1.95±0.12	12.60	2.01±0.16	9.46
Bilirubin (mg/l)	3.21±0.4	3.16±2.4	1.55	3.04±1.4	5.29	2.95±0.6	-8.1

Table 2. Enzyme activities in the serum of control and rats treated with malathion, vitamin C or their combination (Malathion and vitamin C).

Enzyme (U/L)	Experimental group					
	Control	Malathion		Malathion and vitamin C		vitamin C	
		Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change
AST	112.2±6	172.2±5.2**	-53.48	132.2±2.2	-17.83	115.4±1.6	-2.85
ALT	33.3±1.6	62.1±8.1***	-86.49	42.4±3.2**	-27.33	35.1±1.4	-5.41
ALP	44.2±1.65	68.2±1.6**	-54.30	52.3±4.2	-18.33	45.2±1.6	-2.26
gGT	3.23±0.4	6.14±0.6***	-90.1	4.64± 0.3	-43.65	3.4±0.6	-5.26

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; gGT, gamma glutamyl transferase.* P<0.05 ** P < 0.01., *** P < 0.001.

and 64.5, respectively. Moreover, treatment of rat with vitamin C and Malathion plus vitamin C recorded no significant difference in all glycolytic enzymes as compared to control group.

DISCUSSION

In this study, a significant increase was observed in AST, ALT, ALP and gGT activities in Malathion -treated rats. The increase in the activities of these enzymes may be due to the increase in the secretory activities of the hepatocyte cell which were in accordance with the findings reported in sheep [Abdulaziz and Hristev, 1996; Yousef et al., 1999). Also, Malathion may cause increase in serum AST, ALP, and ALT activities (Sharma et al., 2005). The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane (Fan et al., 2009). The increased levels of serum enzymes indicate an enhancement of permeability, damage or necrosis of hepatocytes. However, vitamin C prevents the increase in the activities of these enzymes which is the primary evidence of their hepatoprotective activity (Ergul et al., 2010).

Serum levels of creatinine and urea were used as indicators of renal function. Elevated blood urea is known to be linked with an increased protein catabolism to urea as a result of increased synthesis of arginase enzyme involved in the urea production (Yanardag and Sacan, 2007). In the present study, increased serum creatinine and urea levels reflect the diagnosis of renal failure. The observed cholesterol level increase which is associated to downstream of triglycerides, in the rats exposed to Malathion may be a potential indicator for fatty acid metabolism, and implicitly of a possible membrane lipid peroxidation. To disclose the matter, MDA tissular quantification was tested in both hepatic and renal tissues. These results show an important levelling up of MDA, indicating lipid peroxidation resulting from exposure to Malathion. Thus, it becomes conceivable that the observed alteration in circulating cholesterol and triglycerides levels may be a consequence of membrane lipid peroxidation and free radical release. Because of the approved protective effects of vitamin C dietary supplementation against various pathologies, a part of this work was focused on its effects in counteracting the Malathion induced alteration.

Interestingly, the observed results, herein, show that vitamin C re-establish the disordered conditions. Such a preventive ability had been thoroughly evidenced (Ergul et al., 2010).

In the present study, significant decrease in glycolytic enzymes HK, PK, GPI and PFK were observed in the group treated with Malathion, while LDH enzyme activity

Table 3. Glycolytic enzymes in the serum of control and rats treated with Malathion, vitamin C or their combination (Malathion and vitamin C).

Treatment	Enzyme activity (µ mol/min/mg protein)									
	Hexokinase (HK)		Pyruvate kinase (PK)		PFK		Lactate dehydrogenase (LDH)		Glucose phosphate isomerase (GPI)	
	Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change
Control	6.43± 0.46		2.1±0.5		7.1±5.2		5.8±1.2		3.1 ± 0.91	
Malathion	2.12±0.33***	67	1.34±0.44***	36.2	3.5±1.6***	50.7	3.5±0.1***	39.65	1.14±0.62***	64.5
Malathion and vitamin C	5.3±1.1	17.8	1.7± 0.82	19	6.6±2.1*	7	5.1±1.3**	12	2.6±0.92*	16
Vitamin C	58.2±0.4	9.4	1.9±0.55	9.5	6.8±0.66	4.2	5. 6±1.22	3.4	2.95±0.84	4.8

* P<0.05 ** P < 0.01., *** P < 0.001.

showed significant reduction. The enhancement in the activities of glycolytic enzymes in treated group could be attributed to increase metabolic activities of treated liver tissues to compensate the inhibition of host Krebs cycle caused by the treatment with tamoxifen (Ahmed and Gad 1995; Tielens, 1997].

LDH inhibition revealed the aerobic anaerobic switch induced by treatment with tamoxifen (Tielens, 1997). Kuser et al. (2000) indicated that lactate is accumulated and glycogen depleted confirming inhibition of aerobic respiration and stimulation of anaerobic glycolysis through hexokinase, a rate limiting enzymes of glycolysis. Some authors have reported that tissue damage followed the release of cellular enzymes such as LDH (Paul et al., 1990; Parasad et al., 1991). Besides the decrease in LDH activity, there was a insignificant change in D-lactate and pyruvate level as compared to untreated snails, as reported by Reddy et al., (1995). Concerning gluconeogenic enzymes activities (fructose- 1,6-diphosphatase and phosphoenolpyruvate carboxykinase), it is concluded that chronic exposure to Malathion causes haemolysis, and hepatic and renal toxicities. The mechanism of such pathological facts may be prompted by the free radical release and the lipid peroxidation that it induces. The use of vitamin C was ascertained to reduce the harmful effects of Malathion in the mentioned parameters. The vitamin C supply is a putative protector against such effects, and should prevent farmers, agriculture workers and consumers intoxication by this product.

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