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The protective effect of ascorbic acid and β–carotene against toxicity induced by malathion in male albino rats

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ABSTRACT

The objectives of the present study were to evaluate the possible protective effects of ascorbic acid (vitamin C) and β–carotene as antioxidants against the toxicity induced by malathion on male albino rats. Sixty of adult male albino rats were randomly assigned to six equal groups including control group. Animals were orally treated with either ascorbic acid (10 mg / kg b.w), β–carotene (10 m / kg b.w), acute dose of malathion (470 mg / kg b.w which equivalent ½ LD_{50}), ascorbic acid plus malathion or β–carotene plus malathion. Ingestion of acute dose of malathion caused a significant increase in levels of MDA (malondialdhyde) in liver and kidney of male rats, SOD (superoxide dismutase) in erythrocytes and CAT (catalase) in liver of male rats. It significantly decreased GSH (reduced glutathione) activity in liver and kidney of male rats after 4, 8, 16, 32 and 64 hours of treatments. The treatments with ascorbic acid and β–carotene plus malathion have not cause any significant change in all parameters in treated male rats, compare with untreated group. The treatment with ascorbic acid plus malathion resulted pronounced fall in that values of MDA, SOD and CAT activities in treated male rats as compared to those malathion treatment. On the other hand, an elevation in GSH had been recognized over these of malathion administered male rats. Also, the male rats that treated with β–carotene showed a significant improvement in the level and activities of all parameters compared with male rats which treated with malathion.

Keywords: Malathion, toxicity, MDA, CAT, SOD, GSH, ascorbic acid, β-carotene.

INTRODUCTION

The powerful insecticidal properties of the organophosphorous compounds promoted their wide spread use for control of agricultural pests, invertebrate vectors of animals and human diseases. Unfortunately, the problem of residual toxicity has led to significant damage to many ecosystems and concern that their presence in all types of food might present a carcinogenic hazard to man (Concon, 1988).

The organophosphate malathion is a contact insecticide. It is used to control the insects on crops, fruits, vegetables and stored products. Also, it is used in public health programs. Previous studies have revealed that most commonly used organophosphorus pesticides are known to interfere with important biochemical processes in the body (Datta *et al*., 1992; Gupta *et al.,* 1992 and Berber *et al.,* 1999).

Fedorov and Fedorova (1988) proved that the short and/or long application of either carbophos or dimethy dichlorvinyl phosphate on shaven–skin out bred rats caused intensified lipid peroxidation. Datta *et al*. (1992) demonstrated the deleterious effects of the organophosphorus insecticides, phosphamidon at doses of 250 and 500 ppm on the antioxidant defense mechanism of human erythrocyte and plasma. They noticed depression in the activities of glutathione reductase while the levels of reduced glutathione, superoxide desmutase and catalase were stimulated. Also, Gupta *et al.* (1992) reported that under *in vitro* conditions, malathion at a concentration of 250 ppm and more altered the activities of enzymes associated with antioxidant defense system in human fetal brain and liver. Moreover, Julka *et al*. (1992) reported that dichlorovos caused a significant increase in the activities of antioxidant enzymes, superoxide dismutase and catalase, accompanied with a decrease in lipid peroxidation and levels of reduced and oxidized glutathione. Lukaszewicz and Moniuzk (1999) reported that chlorfenvinphos caused an elevation in activity of blood antioxidant enzymes and serum concentration of malondialdhyde (MDA) in rats after 1 and 24 h of treatment. The same results were sighted by Gultekin *et al*. (2000) and Prakasam *et al.* (2001).

Early studies have been shown that an antioxidant has a direct effect on oxygen free radicals which caused lipid peroxidation in the liver and also glutathione precursor (Kojima *et al*., 1992 and Sen, 1999).

L-Ascorbic acid (Vitamin C) occurs widely in nature. It is used as oxygen scavenger and also as synergists in a wide variety of food products. It had been allocated an ADI "not limited" by JECFA (FAO /WHO, 1981). Geetanjali *et a*l (1993) supported the protective role of ascorbic against toxicity of the organophosphate, dimthoate. John *et al.* (2001) reported that Vitamine E decreased lipid peroxide in erythrocytes, while it elevated the activities of both superoxide dismutase and catalase in rats following single low dose of 0.01 % of dimethoate and/or malathion.

β-carotene (Provitamin A) is mainly used as food colornate. It is effective quencher of singlet oxygen and can acts as antioxidant by preventing formation of hydrogen peroxide. It is not toxic to rats, dogs and humans. An ADI of 0.5 mg / kg of body weight has been allocated by the GECFA (FAO/WHO, 1974). Lawlor and O'Brien (1997) demonstrated the antioxidant efficiency of β–carotene to modulate paraquat–induced oxidative stress in chicken embryo fibr blasts. β–carotene increased superoxide dismutase (SOD) and catalase (CAT) activities. On the other hand, glutathione peroxidase activity recorded a decrease relative to that of control. Kadry *et al.* (2001) found that female rats treated with β–carotene plus fenitrothion hadn't been caused a significant change in level of reduced glutathione (GSH) comparing with untreated animals.

The objectives of the present study were to evaluate toxicity of malathion to male rats and the possible protective effects of the antioxidants, ascorbic acid and β–carotene against the toxicity induced by malathion.

MATERIALS AND METHODS

Materials: Malathion [(dimethoxy thiophosphoryl thio) succinate] (Fig. I) was purchased in the form of emulisifiable concentrate (57EC) from El–Nasr Intermediate Chemicals, Giza, Egypt. The oral LD_{50} of malathion was

determined according to Weil (1952) using the technical form. The selected dose was 475 mg / kg of body weight (equivalent of ½ LD_{50}).

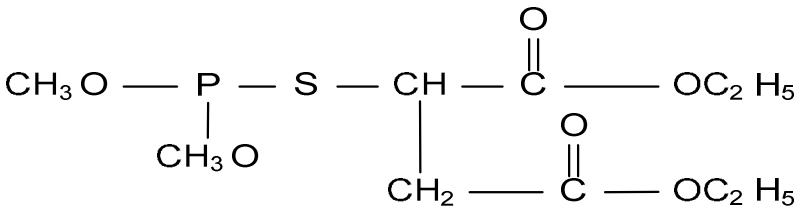

Fig. (1): Malathion

L- Ascorbic acid (Vitamin C), 3- Keto – 1 – glucofuranolactone (Fig. 2) was obtained in the form of tablets from Memphis Co. Pharm. & Chemicals Ind. (Cairo – A.R.E).

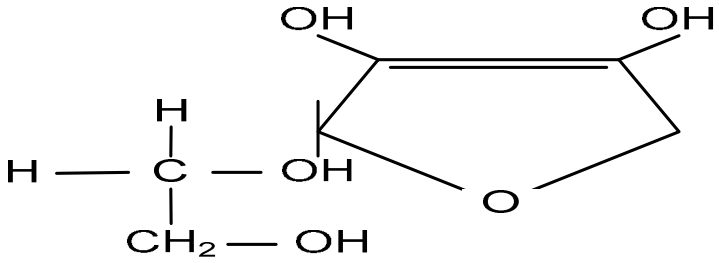

Fig. (2): L- Ascorbic acid (vitamin C)

β–carotene (Provitamin A) (Fig. 3) was purchased from Memphis Co. Pharm. & Chemical Ind. (Cairo – A.R.E).

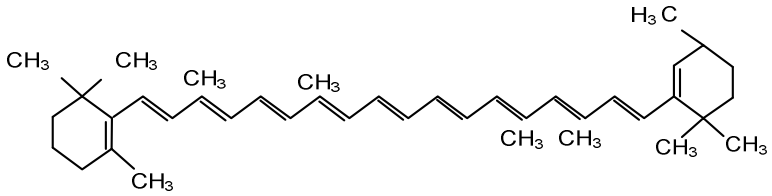

Fig. (3) β–carotene

Experimental animals: Three months old sexually mature male albino rats (derived from Spragu Darwley) weighing 150 ± 10 g were purchased from animal house colony, Giza, Egypt. The animals were maintained on standard laboratory diet (protein: 16.04 %, fat: 3.36 %, fiber: 4.01 % and metabolic energy: 2887 Kcal/kg) and water *ad libtum* for one week, to acclimatize under the laboratory conditions (27 °C ± 3 and 60 ± 5 RH). After acclimatization period, the animals were divided into six groups (10 rats / group) and housed in glass cages (65 × 70 × 20 cm).

Acute toxicity studies: The experimental animals were given oral doses as follows: group 1, untreated control; group 2, were given ½ of LD_{50} of malathion (475 mg / kg b.w), which where determined according to method of Weil, (1925); group 3, were given β–carotene (10 mg / kg b.w); group 4, were given ascorbic acid (10 mg / kg b.w); group 5, were given malathion plus β–carotene (475 and 10 mg / kg b.w, respectively), group 6, were given malathion plus ascorbic acid (475 and 10 mg / kg b.w, respectively).

After 2, 4, 8, 16, 32 and 64 hrs of oral administration of the tested group of chemicals, the living animals were killed and dissected to obtain the desired liver and kidney. The blood was collected in clean and sterile tubes, containing heparin. Centrifugation at 10000 rpm for 10 min. takes place. The erythrocyte was taken to determine activity of the antioxidant enzyme, superoxide dismutase (SOD).

Biochemical analysis: Reduced glutathione (GSH) in liver and kidney tissues was quantified according to the method of Beutler *et al*. (1963). The principle depended on the reaction of GSH with 5,5 –dithio bis –(2–nitrobenzoic acid) (DTNB) giving a stable yellow color which denoting to the amount of GSH (mg/g). Malondialdhyde (MDA), a parameter of lipid peroxidation, was determined according to method of Uchiyama and Mihara (1978). In such method, 0.5 g of liver, homogenized with 5 ml of ice- cold 1.15 % KCl solution and centrifuged at 10000 rpm for 10 min. The mixtures were boiled at 100°C for 45 min. Then, were cooled under tap water, the reaction solution was extracted with 4 ml of *n*–butanol. Absorbance at 535 and 520 nm against reagent blank which contain 0.5 ml of distilled water by using spectrophotometer (Shimadzu

UV- 120- 01). The difference in absorbance's between the two measurements was calculated. The specific level of MDA was expressed as nmole / ml.

The activity of superoxide dismutase (SOD) erythrocyte was determined according to method of Maral *et al*. (1977). Two ml of heparinized blood was centrifuged for 10 min. at 3000 rpm, and the plasma was separated. The resulting red cells were washed several times with cold 0.9 % saline. One ml of distilled water was then added and the erythrocytes and re-suspended by the agitation and lysed for 2 hrs at 4 $^{\circ}$C. Then 0.8 ml of chloroform/ethanol and 0.3 ml of distilled water were added to 1.88 ml of hemolysate to precipitate hemoglobin which was centrifuged at 300 rpm for 10 min. Five µl of chloroform/ethanol extract were added to 2 ml of the reaction medium. The mixture was then irradiated by white light for 10 min. in the light box. A control experiment took place following exactly the same procedure, except with the addition of the erythrocyte extract. The optical density were the measured at 560 nm against air. The specific level of SOD was expressed as units / ml.

The activity of catalase (CAT) in liver was assayed according to method Cohen *et al.* (1970), in which one g of liver tissue were homogenized in phosphate buffer pH 7.4 and centrifuged at 10000 rpm. for 10 min. One ml of the supernatant was added to 0.01 ml of ethanol and incubated in ice water for 30 min. Then, 0.01 ml of 10 % Triton X-100 followed by 99 ml of phosphate buffer pH 7.4 was added to the supernatant to produce the original homogenate. 0.5 ml of cold 6 mM $H_2 O_2$ were mixed with 0.5 ml of original homogenate, after exactly 3 min., 1 ml of 6 N H_2SO_4 was added. A blank was prepared by adding 0.5 ml distilled water instead of sample. Finally, 7 ml of $KMnO_4$ (0.01N) reagent were added and mixed to each of the sample and blank. The absorbance was measured at 480 mm against air. A standard was prepared by adding 7 ml of 0.01 N $KMnO_4$ to mixture of 55 ml buffer and 1 ml 6 N H_2SO_4. The specific level of CAT was expressed as unit/gm.

Statistical analysis: Statistical analysis was performed for all the estimated parameters by student t-test using the computer program (Sigma Plot for Windows, version 2.0).

RESULTS

Data in Table (1) showed that the oral administration acute dose of malathion alone (475 mg / kg b.w) gave a significant decrease in level of reduced glutathione (GSH) in liver of male rats after 4, 8, 16, 32 and 64 hrs of treatment, comparing with control group. The highest decreases were recorded after 16 and 32 hrs of treatment, with GSH level of 1.09 and 0.91 while, it was 1.41 and 1.25 mg / g in untreated animals after the same time intervals, respectively.

Table (1): Effect of malathion alone and in combination with β–carotene or ascorbic acid on level of reduced glutathione (GSH) in liver of male rats.

Time after treatment (hrs)	Level of GSH (mg/g)					
	Control	Malathion	β–carotene	Ascorbic acid	Malathion + β–carotene	Malathion + Ascorbic acid
4	1.35 ± 0.03	1.19 ± 0.05*	1.56 ± 0.05	1.54 ± 0.05	1.41 ± 0.07	1.45 ± 0.05
8	1.29 ± 0.02	1.12 ± 0.02*	1.62 ± 0.11**	1.68 ± .02**	1.48 ± 0.05	1.53 ± 0.04
16	1.41± 0.04	1.09 ± 0.03**	1.58 ± 0.15*	1.71 ± 0.11**	1.52 ± 0.10	1.63 ± 0.07
32	1.25 ± 0.03	0.91 ± 0.04**	1.55 ± 0.06*	1.53 ± 0.04*	1.31 ± 0.02	1.42 ± 0.03
64	1.35 ± 0.05	1.11 ± 0.06*	1.41 ± 0.09	1.49 ± 0.13	1.39 ± 0.02	1.47 ± 0.47

* Significant (P≥0.05) ** Significant (P≥0.01)

Treatment with β–carotene or ascorbic acid alone resulted in a significant increase in level of reduced glutathione (GSH) in liver of treated animals, compared to control group. Also, treatment with malathion plus β–carotene or ascorbic acid exhibited significant increase in level of GSH, compared with malathion alone, at all times after treatment. The levels of GSH were 1.52, 1.31 and 1.39 mg / g in liver of malathion treated-animals plus β–carotene and 1.63, 1.42 and 1.47 mg / g in liver of group that treated with malathion plus ascorbic acid after 16, 32 and 64 hrs of treatment, respectively. While, the levels of GSH were 1.09, 0.91 and 1.11 mg / g after treatment with malathion alone at the same times, respectively.

Data in Table (2) recorded that level of GSH in kidney of male rats exhibited a significant gradual decrease after treatment with malathion alone. The level of GSH in kidney revealed a highest significant decrease after 32 and 64 hr of treatment, compared to control group.

Table (2): Effect of malathion alone and in combination with β–carotene or ascorbic acid on the level of reduced glutathione (GSH) in kidney of male rats.

Time after treatment (hrs)	Levels of GSH (mg/g)					
	Control	Malathion	β–carotene	Ascorbic acid	Malathion +β–arotene	Malathion +Ascorbic acid
4	0.78 ± 0.03	0.71 ± 0.04	0.81 ± 0.08	0.95 ± 0.08	0.77 ± 0.01	0.82 ± 0.07
8	0.80 ± 0.05	0.69 ± 0.07	0.85 ± 0.06	0.98 ±0.06	0.81± 0.03	0.86 ± 0.03
16	0.83 ± 0.03	0.62 ± 0.05*	0.95 ± 0.08	1.10 ± 0.09*	0.85 ± 0.05	0.92 ± 0.05
32	0.84 ± 0.19	0.55 ± 0.09**	1.02 ± 0.05*	1.25 ± 0.05**	0.91± 0.05	1.11 ± 0.12*
64	0.86 ± 0.03	0.49 ± 0.11**	1.10 ± 0.10*	1.29 ± 0.03**	0.96 ± 0.07	1.17 ± 0.1*

* Significant (P≥0.05) ** Significant (P≥0.01)

Treatment with β–carotene or ascorbic acid caused significant increases in the level of GSH in kidney of treated organs, especially, after 16, 32 and 64 hrs of treatment. Also, the data in Table (2) revealed that malathion plus β–carotene or ascorbic acid showed significant increases in level of GSH in kidney of male rats, compared with malathion-treated group, at all times after treatment. The levels of GSH were 0.85, 0.91 and 0.96 mg/g in kidney of group animals treated with malathion plus β–carotene and 0.92, 1.11 and 1.17 mg / g in kidney of malathion plus ascorbic acid-treated group, after 16, 32, 64 hrs of treatment, respectively. While, the level was 0.620, 0.55 and 0.49 mg/g in malathion-treated animals, at the same times, respectively.

Levels of malalondialdyde (MDA) in liver of male rats after treated with malathion alone and in combination with β–carotene or ascorbic acid were presented in Table (3). Data recorded that level of MDA in liver of malathion treated group exhibited a significant increase after 16, 32 and 64 hrs of treatment, compared with control group. Treatment with β–carotene or ascorbic acid alone didn't cause any significant changes in the level of MDA in liver of

treated groups, compared with control group. Data also showed that levels of MDA were significantly decreased in liver of malathion plus β–carotene - treated group, compared with malathion-treated group. The same results were observed in liver of malathion plus ascorbic acid-treated group.

Table (3): Effect of malathion alone and in combination with β–carotene or ascorbic acid on the level of malondialdhyde (MDA) in liver of male rats.

Time after treatment (hrs)	Level of MDA (nmole/mg)					
	Control	Malathion	β–carotene	Ascorbic acid	Malathion + β–carotene	Malathion + Ascorbic acid
4	3.85 ± 0.39	3.98 ± 0.35	3.88 ± 0.65	3.79 ± 0.41	3.94 ± 0.54	3.89 ± 0.43
8	3.80 ± 0.53	4.15 ± 0.44*	3.83 ± 0.55	3.81 ± 0.53	3.85 ± 0.47	3.83 ± 0.33
16	3.95 ± 0.55	4.95 ± 0.63*	3.91± 0.57	3.85 ± 0.45	4.25 ± 0.62	3.99 ± 0.57
32	4.11± 0.59	5.88 ± 0.73**	3.98 ± 0.62	3.92 ± 0.62	4.21± 0.57	3.95 ± 0.47
64	4.35 ± 0.62	6.56 ± 0.56 **	4.21 ± 0.38	3.98 ± 0.45	4.62 ± 0.72	4.12 ± 0.32

* Significant (P≥0.05) ** Significant (P≥0.01)

The levels of MDA were 4.25, 4.21 and 4.62 nmole / mg in liver of malathion plus β–carotene-treated group and 3.99, 3.95 and 4.12 nmole / mg in liver of malathion plus ascorbic acid-treated group after 16, 32 and 64 hrs of treatment, respectively. While, it was 4.95, 5.88 and 6.56 nmole / mg in liver of malathion-treated group, at the same times, respectively.

Data in Table (4) showed that the levels of MDA in kidney of malathion-treated group were significantly increased after treatment, compared to the control group. The highest increase of MDA was noticed after 16 and 32 hrs of treatment with 2.09 and 2.51 nmole / mg, respectively. Also, significant decrease in level of MDA was observed in kidney of malathion plus β–carotene-treated groug after all times of treatment, compared with control group. The same trend was recorded in kidney of malathion plus ascorbic acid-treated group.

Table (4): Effect of malathion alone and in combination with β–carotene or ascorbic acid on the level of malondialdhyde (MDA) in kidney of male rats.

Time after treatment (hrs)	Level of MDA (nmole/mg)					
	Control	Malathion	β–carotene	Ascorbic acid	Malathion +β–arotene	Malathion + Ascorbic acid
4	1.53 ± 0.07	1.67 ± 0.13	1.49 ± 0.13	1.41 ± 0.08	1.57 ± 0.13	1.54 ± 0.09
8	1.55 ± 0.13	1.89 ± 0.09	1.43 ± 0.11	1.39 ± 0.07	1.61 ± 0.14	1.49 ± 0.07
16	1.61 ± 0.12	2.09 ± 0.04*	1.53 ± 0.12	1.35 ± 0.11	1.67 ± 0.09	1.53 ± 0.11
32	1.59 ± 0.09	2.51 ± 0.16**	1.51 ± 0.08	1.37 ± 0.06	1.62 ± 0.11	1.56 ± 0.13
64	1.65 ± 0.15	1.98 ± 0.14*	1.57 ± 0.10	1.41 ± 0.08	1.63 ± 0.12	1.48 ± 0.09

* Significant (P≥0.05) ** Significant (P≥0.01)

The activities of superoxide dismutase (SOD) in erythrocytes of male rats after treatment with malathion alone and in combination with β–carotene or ascorbic acid were illustrated in Table (5). The results showed that treatment with malathion caused a significant increase in the activity of SOD after 8, 16 and 32 hrs of treatment. On the other hand, treatment with β–carotene or ascorbic acid didn't cause any significant changes in the activity of SOD after 4,

Table (5): Effect of malathion alone and in combination with β–carotene orascorbic acid on activity of superoxide dismutase (SOD) in erythrocytes of male rats.

Time after treatment (hrs)	SOD activity (units/ml)					
	Control	Malathion	β–carotene	Ascorbic acid	Malathion + β–arotene	Malathion +Ascorbic acid
4	235.13 ± 10.13	285.19 ± 8.15	232.12 ± 8.71	228.10 ± 7.19	239.95 ± 7.81	239.95 ± 8.11
8	231.53 ± 9.78	294.98 ± 10.11*	235.10 ± 7.77	221.95 ± 6.12	243.91± 6.91	236.58 ± 6.97
16	225.98 ± 7.56	301.50 ± 12.13**	224.09 ± 6.12	219.19 ± 5.29	263.15 ± 5.71	251.19 ± 9.11
32	222.55 ± 7.78	283.51 ± 11.21*	221.19 ± 5.15	217.17 ± 6.11	241.19 ± 5.95	233.15 ± 5.77
64	229.49 ± 6.59	231.63 ± 6.78	224.59 ± 5.19	220.98 ± 4.45	230.10 ± 4.65	232.92 ± 6.11

* Significant (P≥0.05) Significant (P≥0.01)

8, 16, 32 and 64 hrs of treatment. Also, the results recorded a significant decrease in SOD activity in erythrocytes of malathion plus β–carotene-treated group, compared with malathion-treated group. The activity of SOD was 263.15, 241.19 and 230.1 units/ml in malathion plus β–carotene-treated group, while, it was 301.5, 283.51 and 231.63 units / ml in malathion-treated group, after 16, 32 and 64 hrs of treatment, respectively. The same results were recorded in erythrocytes of malathion plus ascorbic acid-treated group after the same times of treatment.

Activity of catalase (CAT) in liver of treated male rats with malathion alone and in combination with β–carotene or ascorbic acid was recorded in Table (6). Treatment with malathion alone caused a slight increase in activity of CAT in liver of treated groups. This elevation of CAT activity was reduced in malathion plus β–carotene-treated group and in malathion plus ascorbic acid-treated group, compared to malathion-treated group, after all times of treatment. The activity of CAT was 54.89, 53.64 and 52.87 units / gm, in malathion plus β–carotene-treated group, and 52.13, 51.09 and 50.13 units / gm, in malathion plus ascorbic acid-treated group, while it was 60.12, 59.61 and 57.75 units / gm in malathion-treated group, after 16, 32 and 64 hrs of treatment, respectively. Its activity in the control group was 49.91, 50.89 and 49.79 units / gm, after the same times of treatment, respectively.

Table (6): Effect of malathion alone and in combination with β–carotene or ascorbic acid on the catalase activity (CAT) in liver of male rats.

Time after treatment (hrs)	CAT activity (unit/gm)					
	Control	Malathion	β–carotene	Ascorbic acid	Malathion + β–carotene	Malathion + Ascorbic acid
4	50.13 ± 2.15	54.91 ± 2.11	53.63 ± 1.45	50.23 ± 1.09	51.56 ± 1.13	51.13 ± 1.09
8	51.36 ± 1.90	58.73 ± 1.94	54.72 ±2.11	49.11 ± 1.21	53.63 ± 1.56	51.11 ± 1.31
16	49.91 ± 1.13	60.12 ± 5.21*	52.81 ± 11.31	50.09 ±1.53	54.89 ± 2.11	52.13 ± 2.11
32	50.89 ± 1.53	59.61 ± 3.91	53.72 ± 1.41	51.75 ± 1.19	53.64 ± 2.09	51.09 ± 1.41
64	49.79 ± 1.41	57.75 ± 2.31	52.53 ±1.50	48.73 ±1.61	52.87 ± 1.81	50.13 ± 2.45

* Significant (P≥0.05) ** Significant (P≥0.01)

DISCUSSION

Several investigators reported that malathion as other organophosphate pesticides has been shown to produce oxidative stress through the generation of free radicals (Datta *et al*., 1992; John *et al*., 2001; Kalender *et al*., 2004 and Brocardo *et al*., 2005). The most of specific biological targets of the toxicity of free radicals are glutathione, lipid peroxidation and the antioxidant enzyme system which include superoxide dismutase (SOD) and catalase (CAT) (Palaniappan, 2002; Mohammed *et al.,* 2004 and Barlow *et al.,* 2005).

In the present study, the results indicated that, malathion caused a significant decrease in levels of reduced glutathione (GSH) while, it gave a significant increase in level of malondialdhyde (MAD), as indicator of lipid peroxidation, in liver and kidney of treated group, compared with control group. Also, malathion caused a significant increases in superoxide dismutase (SOD) activity in erythrocytes and a slight ncreases was found in catalase (CAT) activity in liver of male rats after all times of treatment.

The study suggests that the acute intoxication with malathion administrated at a dose of ½ LD_{50} leads to livers and kidney's function disturbance, which is a likely result of increased generation of reactive oxygen species as reported by Kalender *et al*., (2004); Barlow *et al*., (2005) and Brocardo *et al*., (2005).

It is of interest to mention that, treatment of male rats with malathion plus β–carotene or ascorbic acid didn't cause a significant change in level of GSH, MAD, CAT, in liver and kidney and SOD activity in erythrocytes, compared with control group. GSH level in liver and kidney increased after treatment with malathion combined with β–carotene or ascorbic acid. However, ascorbic acid was more efficient than β–carotene in formation of GSH in both tested organs. Moreover, β–carotene and ascorbic acid prevented the increases in MDA, as index of lipide peroxidation, SOD and CAT activity caused by malathion intoxication. The results suggest that β–carotene and ascorbic acid, as antioxidant agents can increase of GSH level in liver and kidney which acts as a free radicals scavenge. El-Demerdash *et a.l* (2004); Kalender *et al.* (2004); Sohn *et al.* (2004) and Brocardo *et al.* (2005) demonstrated the action of

antioxidant agents (vitamin E, β–carotene and ascorbic acid) alone or in combination in reducing the harmful effects of pesticides were recorded.

The obtained results demonstrated the beneficial influence of β–carotene and ascorbic acid alone or in combination in reducing the harmful effects of malathion.

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