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STUDY OF PROTECTIVE EFFECT OF VITAMIN C IN REDUCTION TOXICITY OF MALATHION IN WHITE ALBINO MICE

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Abstract

Of the present study aime to study malathion effects in some biochemical and molecular marker of mice and protective activity of vitamin C in reduction toxicity , white albino mice administrated malathion and vitamin C+ malathion with dose (50-40) mg/kg b. wt. /day some biochemical markers used in present study. the result shown the biochemical markers Urea and Creatinine concentration in blood with treated malathion were significantly increased with elevated the dose of malathion as compared with control group and vitamin C+ malathion. While the total protein concentration in blood was significantly decreased in malathion treatment as compared with control and vitamin C+ malathion .Activities of alanine transferase ALT, aspartate transferase AST, Alkaline phosphatase ALT in liver were significantly increased in malathion treatment While these enzyme activities in vitamin C + malathion treatment were found to be close to level of control , and the activity of lactate dehydrogenase LDH was significantly decreased in malathion and vitamin C+ malathion treatments, when compared with control.

Key words : Toxicity of malathion, Biochemical and Molecular Markers, Vitamin C.

Introduction

Malathion Malathion is an organophosphate insecticide, which acts as an acetylcholinesterase inhibitor. It have been used in agriculture to improve food production via eliminating unwanted pests and controlling disease vectors, among common pesticides (Srivastava *et al*., 2012). Malathion (O,O-dimethyl-S-1,2-bisethoxycarbonylethylphosphorodithioate) is a non-systemic, which used excessively to protect crops. It metabolized by cytochrome P450 enzymes which play essential role in oxidized Malathion to malaoxon and converted it to be more toxic than original structure (Bonilla *et al*., 2008). The primary action site of malathion is a central and peripheral nervous systems because they inhibit acetylcholinesterase (AChE), which hydrolyses of the neurotransmitter acetylcholine (ACh). The Bioaccumulation of toxic substances stimulate redox reactions by generating free radicals, especially reactive oxygen species (ROS) that induce biochemical changes in biomolecules (Howitz *et al*., 2003). Oxidative stress occur when an imbalance between production and removal of these ROS. It can be detoxified by defense system enzymes, like superoxide dismutase (SOD), catalase (CAT), while organic peroxides can be detoxified by the activity of glutathione-S-transferase (GST) (Friedman and Lawrence, 2002).

Vitamin C is a major circulating water-soluble antioxidant. It is well absorbed by the gastrointestinal tract and required for multiple biological functions and biochemical reactions in humans and animals and it is an important element for the body by neutralize ROS and reduce oxidative DNA damage which causes genetic mutations (Li and Schellhorn, 2007). Harapanhalli *et al*. (1996) shown hydrophilic and a very important free-radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protecting bio membranes from per oxidative damage. The anticarcinogenic and antimutagenic roles of vitamin C have been tested in a variety of *in vivo* and *in vitro* systems exposed to radiation and pesticides (Durak *et al*., 2009). It prevents the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues (Sies *et al*., 1992).

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Vitamin C accelerates the degradation of intra and extracellular proteins targeted to the lysosomal lumen by autophagic and heterophagic pathways, relevant for the removal of abnormal proteins that accumulate with aging (Martin *et al*., 2002).

Materials and Methods

Animals : Thirty adult mice (weight 25-30g) were housed at the animal house in the faculty of Science of Babylon University fed standard mice chow, and given free access to water, the mice acclimation to the laboratory conditions for one weeks.

Experimental design : They were divided into three groups of 10 mice in each group. Control group were given tap water only. Second group were given malathion at a dose 50 mg/kg b. wt. /day. Third group (Vitamin C+ Malathion) were given Vitamin C 40 mg/kg b.wt before three hours of malathion administration and the same dose of malathion in second group via oral gastric tube .This dose equal to 1/10 of the LD 50 for an oral dose according to Gallo and Lawryk (1991). At the end of the experimental period (three weeks) and after overnight fasting blood samples were obtained from hearts of each mice The blood samples were centrifuged at 3000 rpm for 10 min. to separate plasma and to detect some biochemical markers (Savithri *et al*., 2010). The liver tissues of mice were homogenized by using pestle motor mixer provided by Agros Technologies (U.S) Cat No A0001 in 50mM Potassium Phosphate buffer (pH7.0) then, centrifuged at 14000 r.p.m, 4^0C for 5 min for antioxidant and liver function measurements (Aljuboori, 2017).

Biochemical assay

The urea, creatinine and total protein concentrations were measured by using a specific kit .

Antioxidants defense measurements

Superoxide dismutase activity SOD was determined by autooxidation of Pyrogallol according to Marklund and Marklund (1974). While Catalase activities was measured according to procedure of Clariborn (1985) and Aebi (1974), Glutathione activity was determined according to the method of Moron *et al*. (1979), the acid soluble sulfhydryl groups form a yellow colored complex with dithionitrobenzene (DTNB).

Lipid peroxidation and Acetylcholinesterase assay

Lipid peroxidation was estimated by the Thiobarbituric acid assay for Malondialdehyde (MDA) concentration according to Aust (1985) and Burtis (1999) and A colorimetric determination of acetylcholinesterase activity in tissues of liver according to the method described by Ellman *et al*. (1961).

Liver function and Lactate dehydrogenase assay

The activity of alkaline phosphatase ALP, Alanine transaminase ALT and Aspartate transaminase AST were measured for supernatant of liver tissue of mice by reflotron plus roche and lactate dehydrogenase by a specific Kit BioScience for each biomarker.

DNA damage

DNA damage was detected using comet assay accoriding to Conners (2004) by taken a small portion of liver tissue (50 mg) was serially washed and this piece of liver was placed in a 2 ml microcentrifuge tube containing 1.5 ml of phosphate buffer solution then homogenization by homogenized (15-20S) by pestle motor mixer, then added 40µl of proteinase K for liver tissue and Centrifuged at 13000 r.p.m for 15min., 4°C, about 2-5 µl of suspension cell was added to a clean 1.5 ml tube and mixed on Comet slide with 40µl of low melting agarose , then lysis solution was prepared (consist of 2.5M NaCl, 100 mM EDTA, 10 mM Tris-base and 8g NaOH), all compenants were dissolved in dH_2O, then it completed to 700 ml after this about 110 ml of (55 ml 1% triton X and 55 ml 10% dimethyl Sulphoxide) was added and volume completed to 100 ml with deionized water, mixture was chilled at 4^0C or by ice for at least 20 min before used, then it combined 7.5µl with 75µl of low melting agarose and immediately spread the mixture onto the clear part of a comet slide, comet slide was wormed by heating plate at 42-50ºC slides could store in lysis solution at 4Cº for 60 minutes, then, lysis solution was removed and replaced by alkaline solution (6 g NaOH and 500 µl 0.5% Na_2EDTA) for 5-60 minutes at room temperature in dark, slide removed from alkaline solution gently, the excess buffer was removed from slide and washed by immersing in 1X TBE buffer for 5 minutes, the slides were transfered to an horizontal electrophoresis apparatus, it covered by 1X TBE buffer. vol 70 V, for 60 min, excess TBE was gently removed with added drops of 70% ethanol the slides were stained by immersed in ethidium bromide solution for 24 hour slides were viewed by fluorescence microscope.

Statistical analysis

Data of present study was analyzed according to the system of statistical package for social science (SPSS) to found means, Standard deviation, Least Significant differences by ANOVA at p value<0.05

Results

Biochemical markers

The statistical analysis shown significant differences in all biochemical markers among control and treatments at ($P<0.05$) (table 1). The urea concentration in blood control was 16.7 mg/dl, while its concentration in treated blood by malathion was significantly increased 27.8 mg/dl and in Vitamin C+malathion the urea concentration was 24.6 mg/dl. The creatinine concentration showed significant differences between control and treatment in blood concentration was 0.35 mg/dl in control, whereas Creatinine concentration in treated blood with malathion was elevated to 2.5 mg/dl after 21 days and its concentration in treated group with vitamin C + malathion reached to 0.53 mg/dl. The largest inhibition of total protein in treated blood with malathion was 1.2 mg/dl when compared with control and vitamin C+ malathion (8.9-4.1) mg/dl, respectively (fig. 1).

Result of statistical analysis was showed significant differences in activities of alanine transferase ALT, aspartate transferase AST, Alkaline phosphatase ALT in liver, and alanine transferase activity in liver control was (20.2) U/l while in malathion treatment elevated to (88.9) U/l and in vitamin C + malathion treatment reduced to (50.6) U/l when compare with malathion treatment. Aspartate transferase activity was 30.7 U/l in control. Whereas it's activity in malathion treatments was (97.2) U/l and in vitamin C + malathion treatment was (66.4)U/l. Alkaline phosphatase activity in malathion and vitamin C+malathion treatments were 61.8 U/l and 57.8 U/l respectively when compare with control 35.6 U/l. the activity of lactate dehydrogenase LDH in the control was 25.7 U/l and its activity in malathion and vitamin C+malathion treatments were 12.5-18.4 U/l, respectively (fig. 2)

The malondialdehyde concentration was 0.97 µmol/ml in liver control. While its concentration in treated malathion was significantly increased 7.8 µmol/ml and MDA concentration in Vit C+ malathion was 3.9 µmol/dl. Whereas, acetylcholinesterase ACHE activity reached in control to 26.6 U/l in liver tissue. but it's activity in treatment of malathion and Vit C+ malathion was significantly decreased to 12.4 - 17.1 U/l

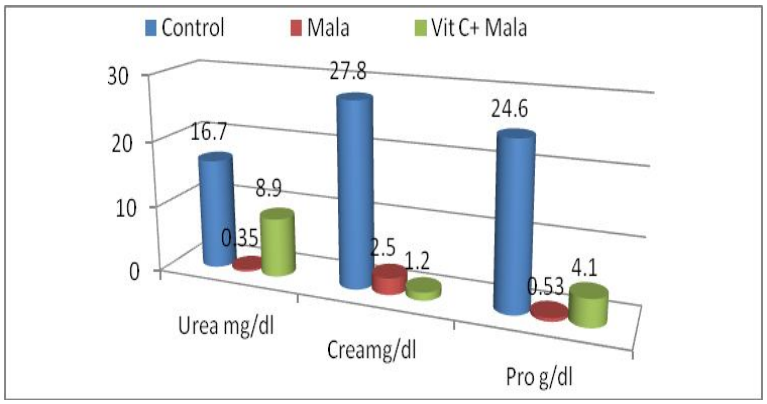

Fig. 1 : Concentrations of urea, Creatinine and total protein in blood of mice treated with malathion and Vitamin C + malathion (mala = malathion , Vit C = vitamin C).

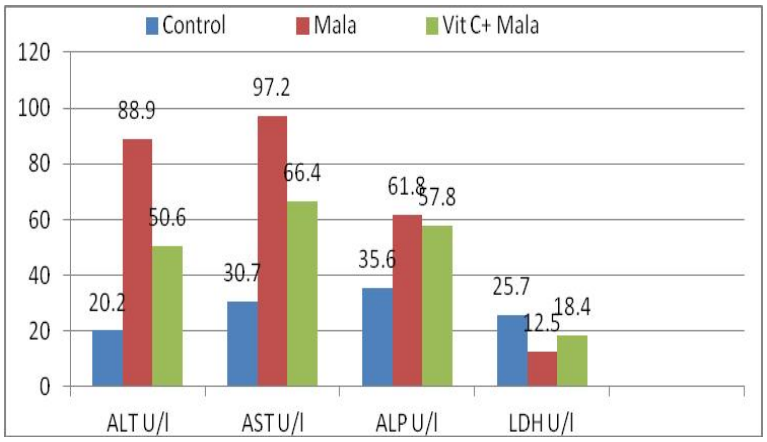

Fig. 2 : Activities of Alanine transferase,aspartate transferase, alkaline phosphatase and lactate dehydrogenase in liver of mice teated with malathion and Vitamin C + malathion (mala = malathion , Vit C = vitamin C).

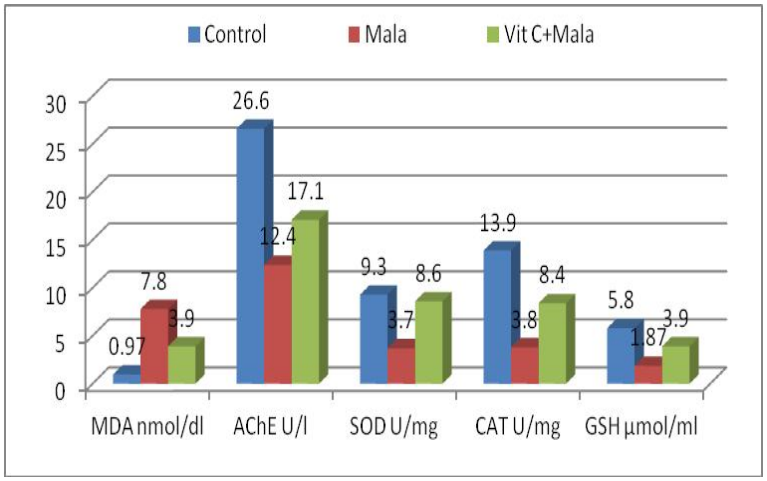

Fig. 3 : Concentration of malondialdehyde, acetylcho-linesterase, superoxide dismutase, catalase and glutathione activities in liver of mice teated with malathion and Vitamin C + malathion (mala = malathion, Vit C= vitamin C).

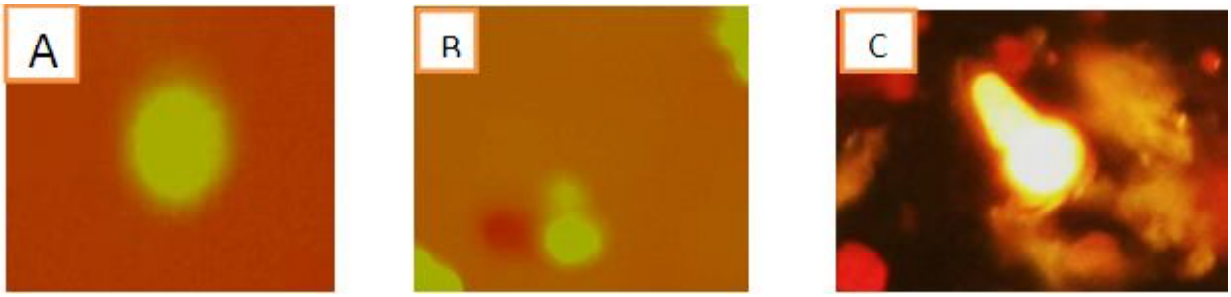

Fig. 4 : Classes of DNA damage in liver of mice according to comet assays. A: Control, B : medium damage and C: high damage (40X).

Table 1 : The concentration of biochemical markers in mice after exposure to malathion and vitamin C + malathion (Mean ±SD).

Biochemical Makers	Control	Malathion treated	Vitamin C + Malathion
Urea mg/dl	16.7a	27.8b	24.6a
Creatinine mg/dl	0.35a	2.5b	0.53a
Total protein g/dl	8.9a	1.2b	4.1c
Alanine transferase U/l	20.2a	88.9b	50.6c
Aspartate transferase U/l	30.7a	97.2b	66.4c
Alkaline phosphatase U/l	35.6a	61.8b	57.8c
Lactate dehydrogenase U/l	25.7a	12.5b	18.4b
Malondialdehyde nmole/dl	0.97a	7.8b	3.9c
Acetylcholinesterase U/l	26.6a	12.4b	17.1c
Superoxide dismutase U/mg	9.3a	3.7b	8.6a
Catalase U/mg	13.9a	3.8b	8.4c
Glutathione µmol/ml	5.8a	1.87b	3.9b

respectively .The superoxide dismutase SOD activity in liver tissue control was 9.3 U/mg. While activity of SOD in treated liver tissue with malathion was significantly decreased to (3.7) U/mg and in Vit C+ malathion reach to 8.6 U/mg. the catalase activity was (13.9)U/mg , Whereas in treated liver, the activity of CAT were reached to (3.8)U/mg in malathion treatment and in Vit C+ malathion treatment 8.4 U/mg. glutathion activity GSH in control of liver tissue was (5.8) µmol/ml, While GSH activity in liver tissue was significantly decreased (1.87) µmol/ml in malathion treatments as compared with Vit C+ malathion treatment reach to 3.9 µmol/ml (fig. 3).

DNA damage

The DNA damage markers were showed significant differences in liver according to statistical analysis at

Table 2 : The DNA damage markers in liver of mice treated with malathion and vitamin C + malathion (Mean ± SD).

DNA damage Markers	Control	Malathion treated	Vitamin C + Malathion
Comet length µm	172.61±5.45a	13651±11.7b	541±10.9c
Tail length µm	13.1±1.21a	4671±7.8b	134±16.7c
% DNA in tail	4.13±0.7a	384±20.7b	56±5.1c
Tail moment µm	0.55±0.01a	174.5±7.4b	12.3±2.8c

Different letters show significant differences between groups. ANOVA one way, significant at p value<0.05

($p<0.05$) (table 2 and fig. 4). The Comet length significantly increased in malathion treatment to 13651 µm and in vitamin C + malathion treatment, comet length was 541 µm as compared with control 172.61 µm. The tail length was significantly increased in malathion and vitamin C + malathion treatment of liver (4671 - 134) µm respectively when compared with control 13.1 µm. the percentage of DNA in tail was significantly increased to (384-56)% in malathion and vitamin C + malathion treatment respectively as compare with control 4.13%, While the highest levels that was observed in tail moments in malathion treatment 174.5 µm and 12.3 µm in vitamin C + malathion treatment when compare with control 0.55 µm.

Discussion

Malathion shown to induce oxidative stress that leads to the changes in the antioxidant defense systems for scavenging free radical in cells, different classes of pesticides induce the production of reactive oxygen species (ROS) and oxidative tissue damage (Bagchi *et al*., 1995). The present study show that the treatment of mice with malathion caused significantly increase in concentration of urea, Creatinine and total protein because malathion cause oxidative stress via generation reactive oxygen species that lead to nephrotoxicity lead to reduction in glomerular filteration in kidney and reflect

dysfunction of kidney tubule and oxidative of protein (Durakd *et al*., 2009). While its concentration in treatments group with Vitamin C+malathion was found to be effective in lowering of elevated concentration to be close to normal level in control group because the vitamin C can be scavenging oxidative free radical lead to reduce oxidative damage (Durakd *et al*., 2009).

Liver plays a significant role in metabolism, detoxification of exogenous toxins and therapeutic agents, and bio regulation of fats, carbohydrates, amino acids and proteins (Chen *et al*., 2003). The insecticides and their metabolites can effect in various enzymes of organism via leak out from the necrotic hepatocytes into the blood stream in abnormal amounts and these enzymes have been considered as markers of liver dysfunction and damage such as Alanine transferase, aspartate transferase, alkaline phosphatase and lactate dehydrogenase (LDH) are the most sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity (Uboh *et al*., 2012; Al-Shaibani *et al*., 2013). The results of present study were show that alanine transferase, Aspartate transferase, Alkaline phosphatase activity in liver were significantly elevated in malathion treatments and in vitamin C + malathion treatment when compared with control, this may be due to hepatotoxicity causing permeability alteration and leakage of lysosomal enzymes enhancing the released of enzymes and in vitamin C + malathion treatment, the liver function activities was significantly reduced when compared with malathion treatment. This may be due to the vitamin C roles in protection of the cell membrane against oxidant agents via reducing the oxidative damage that are endogenously generated by malathion (Jacob, 2002). Lactate dehydrogenase activity reflects the state of energy production, which indicator of mitochondria essential role in provide cell with enough energy to cope with the high rate of oxidative metabolic activities (Schneyer *et al*., 1972). The activity of lactate dehydrogenase LDH in malathion treatment group was significantly decreased may be due to oxidative stress that cause excessive free radical accumulation (Sultana and Najam, 2012) but administration group with vitamin C+malathion treatments was found to be close to activity in the control because vitamin C protect the liver from oxidative damage and inhibits the excessive free radical accumulation (Sultana and Najam, 2012).

Malondialdehyde (MDA) is a low molecular weight end product of lipid peroxidation and is widely used as a biomarker of oxidative stress, increased level of oxidative damage in terms of lipid peroxidation (Ma *et al*., 2014). The malondialdehyde concentration was in treated malathion was significantly increased to reach 7.8 µmol/ml and MDA concentration in Vit C+ malathion was 3.9 µmol/dl as compare with control 0.97 µmol/ml because malathion causes oxidative stress and increased lipid peroxidation in primary of hepatocyte (Ding *et al*., 2003).

Acetylcholinesterase enzyme is a serine protease enzyme that are playing important role in hydrolyzing neurotransmitter acetylcholine (Zbarsky *et al*., 2004). Acetylcholinesterase ACHE activity reached in control to 26.6 U/l in liver tissue ,but it's activity in treatment of malathion and Vit C+ malathion was significantly decreased to 12.4 - 17.1 U/l may be due to the oxidative stress that induce by malathion that cause inhibition acetylcholinesterase activity (Abdollahi *et al*., 2004).

The superoxide dismutase SOD, catalase and glutathione activity in malathion treatment were significantly decreased due to malathion induce oxidative stress that occur through an imbalances between the rate of production of reactive oxygen species and the rate of removal of these reactive oxygen species by antioxidants defense systems (Tripathy, 2016). While in Vit C+ malathion treatment was found to be reached to normal level in control because the vitamin C inhibits oxidative stress and prevents accumulation of free radical in cell (Aljuboori, 2017).

The result of the presents study had been showed molecular markers of comet assay was significantly differences between treatment and control group the highest values of comet length µm, tail length µm, DNA in tail and tail moment µm in malathion treatment may be due to insufficient produce of antioxidant defense systems to scavenge the reactive oxygen species that are generated by malathion which lead to find their way across nuclear membrane indicating DNA strand breakage (Srivastava *et al*., 2012; Khadairi *et al*., 2017). While in vitamin C + malathion treatments was found to be lowering oxidative DNA damage because protective effect of vitamin C and prevent genotoxicity by malathion (Hatjian *et al*., 2000).

Conclusion

The malondialdehyde concentration in treated malathion was significantly elevated. Whereas, acetylcholinesterase ACHE activity, superoxide dismutase SOD, Catalase CAT, glutathione GSH and DNA damage in malathion treatment were significantly decreased as compared with Vit C+ malathion treatment were found to be close to normal level in control.

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