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Ameliorative effect of vitamin C and curcumin on malathion induced hepatorenal toxicity in male mice

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ABSTRACT

Malathionis an organophosphorous pesticide that is widely used in agricultural and household applications to control pests. The purpose of this study was to evaluate the ameliorator property of vitamin C or/and curcumin againstmalathion-induced hepatotoxicity in male mice at the biochemical and histological levels. The mice were divided into nine groups. The first three groups were served as a control, while, the second two groups were treated with two different doses of malathion (Low and High doses). The third and fourth groups were treated with high and low dose of malathion with Curcumin.The fifth and sixth groups were treated with malathion (Low and High doses) combined with vitamin C. Meanwhile the eighth and ninth groups were treated with vitamin C and curcumin combined with malathion (Low and High doses).The experiment was continuous for a period of 30 days.Exposure to malathion at the twoexamined doses to mice led to an alteration of liver functions and lipid parameters. The effects of malathion on the biochemical parameters of mice were dose-dependent. Administration of curcumin or/and vitamin C to Mal-treated mice attenuates the toxicity of this compound, objectified by biochemical and histological improvement of liver. But, the alleviation is more pronounced with the both antioxidants. Thus, the synergistic effect of curcumin and vitamin C together is most powerful in reducing the toxicity induced by malathion and improving the hepatic and renal activities of mice.

Keywords: Malathion,Vitamin C, Curcumin, Liver Functions, Histopathology.

INTRODUCTION

There is a jump in the total number of used pesticides in the world **[1]**.Pesticides play an important role in sustaining the agricultural production by protecting all kinds of crops from pest attack and vector-borne diseases **[2]**. Organophosphate compounds are one of the most commonly used insecticides in agriculture and public health, accounting for 50% of the global insecticidal use **[3]**.

malathion [O,O- dimethyl-S - (1,2 - dicarbethoxyethyl) phosphorodithioate] is an OP pesticide that is widely used in agricultural and household applications to control pests. malathion is also extensively used for mosquito eradication and as an animal ectoparasiticide and human miticide **[4].**

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene- 3,5-dione), the active portion of turmeric, has been shown to have significant antioxidant activity, both *in vitro* and *in vivo***[5]**, curcumin is a potent scavenger of reactive oxygen and nitrogen species such as hydroxyl radicals and nitrogen dioxide radicals **[6]**.

In addition curcumin has various other health-benefiting properties, such as anti-diabetic, antioxidants, anti-inflammatory, anticarcinogenic, antiviral, hypolipidemic, and anti-infectious effects **[7-8]**.

vitamin C (Ascorbic acid) is the most important vitamin in fruits and vegetables, and has been regarded as the most potent natural antioxidant and thus, vitamin C has become an essential dietary component for human survival **[9]**. vitamin C has anti-inflammatory effects, prevents endothelial dysfunction and apoptosis, and reduces the risk of arteriosclerosis, cardiovascular disease and some forms of cancer **[10]**.

Vitamin C enters mitochondria by means of facilitative glucose transporter and provides mitochondrial protection against oxidative damage. vitamin C exerts its antioxidant action by inhibiting lipid peroxidation and oxidative cell damage. vitamin C can directly metabolize reactive oxygen species. Pathogenic dysfunction of tissues owing to cell death via apoptosis is one of the important outcomes of oxidative stress that could be diminished by vitamin C **[11]**. There are multitudes of reports available on the protective effects of curcumin, vitamin C individually against various xenobiotics induced oxidative stress in experimental animals. Still to date the reports are scanty regarding the combined alleviated efficacy of curcumin in combination with vitamin C on malathion induced toxicity.

Therefore, the present study was undertaken to evaluate the ameliorator property of vitamin C or/and curcumin on toxic status during Mal-induced injury in male mice at the biochemicaland histological levels.

EXPERIMENTAL SECTION

2.1 Chemicals

Vitamin Cand curcumin were purchased from Roche (Germany), KKWRiedel (Germany) and Applichem GmbH (Germany), respectively. malathion was obtained from the Agricultural Center, Cairo, Egypt.Other chemicals and reagents were of the highestanalytical grade and were bought from standard commercial suppliers.

2.2 Experimental animals design

ICR male mice, weighing approximately 35-40 g were maintained in solid bottom shoe box, type polycarbonate cages with stainless steel wire-bar lids, using a wooden dust-free litter as a bedding material. Animals were located in air-conditioned room and were allowed free access to a pellet diet and tap water for a week before starting the experiment. The European Community Directive (86/609/EEC) and National rules on animal care have been followed. After 2 weeks of acclimation, animals were randomly divided into nine groups with 10 animals in each one as following:

Groups 1 wasserved as untreated control (1ml/Kg of vehicle (Demso) orally daily for 30 successive days),Groups 2 and 3 were received malathion (200 and 400 mg/Kg); respectively (Possamai*et al.*,2007**[12]**and Lasram *et al.*, 2009**[13])**. Groups 4 and 5 were treated with different doses of malathion (200 and 400 mg/kg/day) and followed by curcumin (60 mg/Kg), respectively**(**Abdul-Hamid and Moustafa (2013)**[14]**. Groups 6 and 7 were administered different doses of malathion (200 and 400 mg/Kg) and concotimined by vitamin C (100 mg/Kg) (Rana and Ahmad, (2012)**[15]**.Groups 8 and 9 were treated with combinations of curcumin and vitamin C with different doses of malathion (200 and 400 mg/Kg)for 30 successive days.

2.3 Collection of blood samples

At the end of the experimental period**,** blood samples of the fasted mice were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, Mucaps) under ether anesthesia **[16]**. Then, the blood was centrifuged at 3000 rpm for 15 min and serum was collected for different biochemical analyses.

2.4 Hepato-renal-biomarkers determination

Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities were determined with kits from Human diagnostic worldwide, Germany. The serum total cholesterol (TC) and triglycerides (TG) were determined by the method of Carr *et al*. **[17]**. High density lipoprotein–cholesterol (HDL–c) was determined according to the methods of Warnick *et al*. **[18]**. Serum low density lipoprotein–cholesterol (LDL–c) level was calculated according to Friedewald **[19]**formula:

LDL-c = total cholesterol—(HDL-c + triglycerides)/5.

Very low density lipoprotein cholesterol (VLDL-c) levels were calculated by using the following formula of Prakasam *et al*. **[20]**: VLDL-c = triglyceride/5.

The protein content was determined by the method described by the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules CA. USA) using bovine serum albumin as the standard.

The levels of uric acid and creatinine in serum were estimated spectrophotometricaly using commercial diagnostic kits according to the manufacturer's instructions. The data were expressed as mg/dl.

2.7Histological evaluations

Histological examination of the tissues was conducted after removal of liver tissues from mice. The tissues were gently rinsed with a physiological saline solution (0.9% NaCl) to remove blood and adhering debris. They were then fixed in 5% formalin for 24 hr, and the fixative was removed by washing overnight with running tap water. After dehydration through a graded series of alcohols, the tissues were cleared in methyl benzoate and embedded in paraffin. Sections were cut by a microtome at 6-µm thickness and stained with haematoxylin staining as described by Gabe (1968) **[21]**and counter-stained with eosin dissolved in 95% ethanol (H&E). After dehydration and clearing, sections were mounted with DPX (digital picture exchange) and observed under a microscope.

2.8Statistical analysis

Statistical analysis was performed using SPSS for Windows version 17.0. Data was given in the form of arithmetical mean values and ± standard error (S.E). Differences between groups were evaluated by one-way ANOVA according to $P<0.05$ and post–hoc Duncan test.

RESULTS

3.1 Biomarkers of liver and kidneyassessment

All the parameters of lipid profile (TG, TC, LDL-c, VLDL-c and risk ratio) were increased except the HDL-c was decreased when the mice exposed to the different doses of malathion (Table 1). Administration of vitamin C or/and curcumin to the Mal-treated group with low dose (200 mg/kg) restored all the parameters cited above.

Table (1):Effect of Malathion, Curcumin, vitamin C and their combinations on Lipid profile in male mice (mean± SE)

Groups	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)(mg/dl)	HDL(g/dl)	LDL(g/dl)	VLDL(g/dl)
1-Control group	133.58±4.25^{h}	73.52±3.25^{i}	38.65±1.25^{a}	28.98±1.74^{h}	27.11±1.22^{f}
2-Malathion (Low dose)	165.36±3.65^{b}	140.48±4.25^{e}	27.54±1.11^{g}	35.64±1.85^{b}	33.07±1.44^{b}
3-Malathion (High dose)	194.39±4.85^{a}	222.74±3.87^{a}	25.36±1.06^{h}	38.65±1.97^{a}	38.87±2.01^{a}
4- malathion (Low dose) + Curcumin	139.68±1.63^{f}	122.32±2.22^{fg}	29.52±1.25^{ef}	32.25±1.54^{d}	27.93±1.68
5-Malathion (High dose) + Curcumin	149.36±2.65^{d}	184.74±1. 75^{b}	28.36±2.36^{f}	33.65±1.68^{cd}	29.87±2.04^{de}
6-Malathion (Low dose) + vitamin C	134.25±2.22^{gh}	120.36±2.65^{g}	30.15±1.87^{d}	31.25±1.55^{ef}	26.85±1.77^{g}
7-Malathion (High dose) + vitamin C	154.48±2.68^{c}	174.65±2.41^{c}	29.35±2.39^{ef}	32.14±1.65^{d}	30.89±2.32^{c}
8- malathion (Low dose) + curcumin + vitamin C	132.36±2.79^{h}	119.25±2.69^{h}	31.25±1.47^{c}	30.89±2.15^{f}	26.47±1.98^{g}
9- malathion (High dose) + curcumin + vitamin C	140.94±2.55^{e}	152.36±1.68^{d}	32.68±2.36^{b}	29.87±2.06^{gh}	28.18±1.09^{ef}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

The total protein content decreased in Mal-treated groups (Low and High doses) depend on the dose, but increased significantlyin the groups treated with the low dose and vitamin C and Cur, (Fig.1). Serum ALT activities of malathion groups (Low and High doses)wereincreased significantly when compared with control group (Fig. 2). Treatments of the mice with low and high doses of malathion in combination with vitamin C and/or curcumin were found to decrease the activity of ALT more than malathion compound treatment alone. The same observation has been noticed in the AST and LDH activities (Fig.3) that increased by increasing the dose of malathion and decreased by the treatment with each antioxidant separately.But the best combined group amelioration of enzyme activities was malathion (Low dose) with vitamin C and curcumin which decrease the elevated enzyme markers noticed in malathion treated group only either at high or low dose.

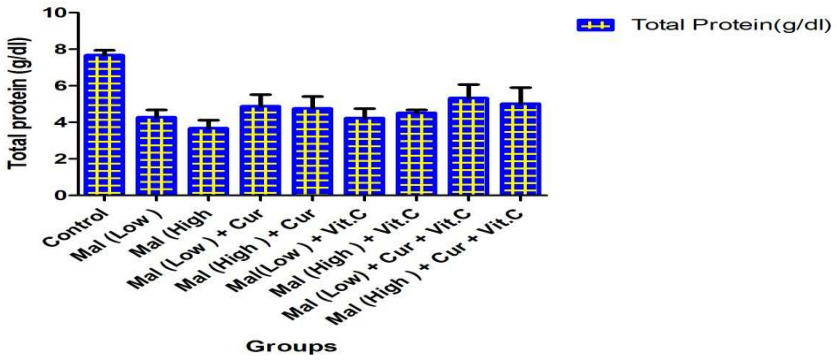


Fig. (1): Effect of Malathion, curcumin (50 mg/ and their combinations on Total protein (g/dl) in male mice

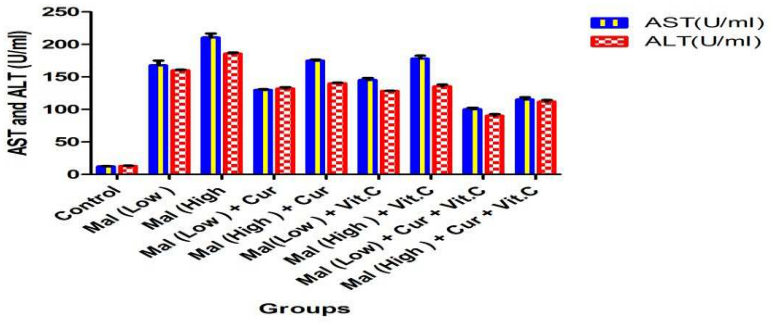


Fig. (2): Effect of Malathion, Curcumin, vitamin C and their combinations on AST & ALT (U/ml) in male mice

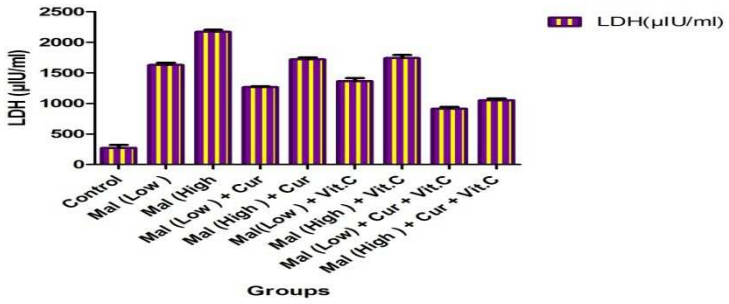


Fig. (3): Effect of Malathion, Curcumin, vitamin C and their combinations on LDH enzyme (µIU/ml) in male mice

To investigate the renal functions, we measured blood biochemical parameters (Urea, uric acid and creatinine). Table (2) represents significant increased levels of urea, uric acid and creatinine in malathion administration groups at the two tested-doses. The increase in creatinine level was noticed by increasing the doses to be 0.97 - and 1.05-fold as compared to control for low and high doses of Mal, respectively. Mice treated with high dose of malathion as well as that treated with the two examined doses in combination with vitamin C and curcumin both of them noticed decreasesin the creatinine level to be 0.8, 0.5- and 0.64 fold, respectively. Uric acid level decreased in mice treated with low and high doses of malathion combined with vitamin C and curcumin as compared to the mice exposed to 200 and 400 mg/kg malathion alone by almost the same value (56.25 and 71.54,respectively).

Table (2): Effect of Malathion, Curcumin, vitamin C and their combinations on kidney functions in male mice (mean ± SE)

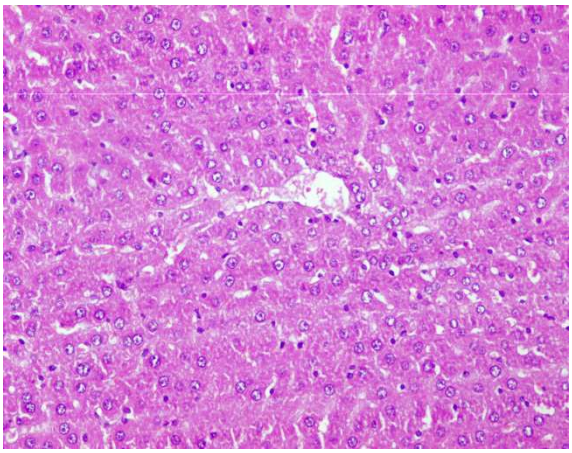
Groups	Urea(mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
1-Control group	21.53±1.60^{f}	0.38±0.01^{h}	26.57±1.02^{h}
2-Malathion (Low dose)	33.40±3.08^{c}	0.97±0.02^{b}	56.25±1.52^{de}
3-Malathion (High dose)	40.20±1.88^{a}	1.05±0.02^{a}	71.54±1.63^{a}
4-Malathion (Low dose) + Curcumin	28.10±1.51^{e}	0.77±0.02^{e}	51.25±2.01^{e}
5-Malathion (High dose) + Curcumin	35.54±0.70^{b}	0.81±0.01^{cd}	66.65±1.98^{b}
6-Malathion (Low dose) + vitamin C	29.10±2.77^{d}	0.65±0.01^{f}	45.79±1.77^{f}
7-Malathion (High dose) + vitamin C	35.08±2.10^{b}	0.80±0.01^{d}	62.41±1.68^{c}
8- malathion (Low dose) + curcumin + vitamin C	28.54±2.01^{e}	0.51±0.01^{g}	38.74±1.96^{g}
9- malathion (High dose) + curcumin + vitamin C	29.15±1.68^{d}	0.64±0.04^{f}	44.68±1.87^{f}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

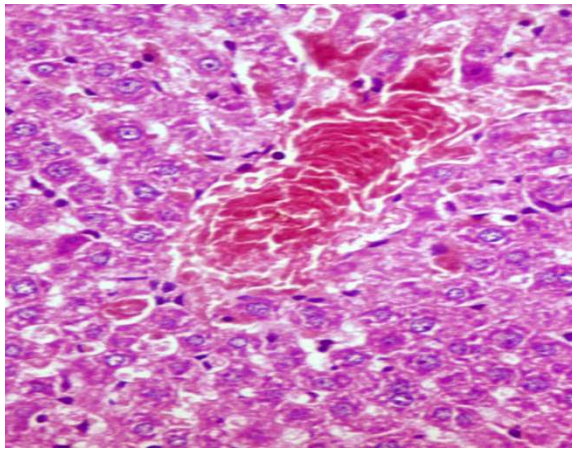
3.3 **Histological assessment**

Light microscopic examination indicated a normal structure of the liver in the control animals (Fig.4A). Liver of control mice is organized into lobules which are roughly hexagonal in shape, with portal triads at the vertices and a central vein in the middle. Within each lobule, hepatocytes are arranged into hepatic cords running radiantly from the central vein and are separated by adjacent sinusoids. Treatment the mice with malathion (Low dose) induced markedly dilated congested central vein (Orange arrow) filled by large number of red blood cells and surrounded by hepatic cords with congested dilated central parts of sinusoids and markedly pyknotic nuclei (Black arrow) (H and E x400) (Fig.4B). Exposure to malathion (High dose) showed markedly dilated congested portal veins, (Black arrow) with congested dilated central parts of sinusoids (Orange arrow) with focal necrosis. (H and E x200)(Fig.4C).Group treated with malathion (Low dose) and curcumin showed dilated central vein (Green arrow) surrounded by some dilated sinusoids (H and E x200) (Fig.4D). Exposureto high dose of malathion (High dose) followed by treatment with curcumin inducedcongested central veins with mild portal inflammation (H and E x400) (Fig.4E).A dilation with mildly congested central veins with ground glass hepatocytes (Blue arrow) (H and E x400) was noticed in group treated with malathion (Low dose) and vitamin C (Fig. 4F). Exposure to malathion (High dose) and vitamin C

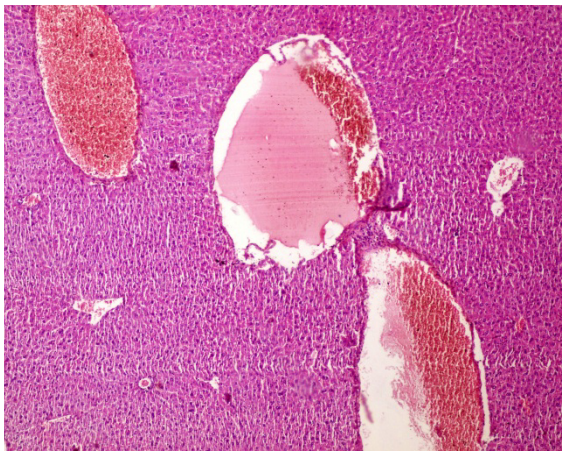
inducedmild lobular inflammation with few scattered intra lobular aggregates of chronic non-specific inflammatory cells (Black arrow) (H&E x400).(Fig. 4G) and the severity of damage increased with increasing the dose. A dilation of central veins with hydrobic degeneration (Blue arrow) (H and E x400) was noticed in group treated with malathion (low dose) with combination of curcumin and vitamin C (Fig.4H). A mildly congested central vein with mild congested sinusoids with fatty change (H and E x400)were also evident in all animals of the malathion (High dose) in combination with curcumin and vitamin C group (Fig.4I).



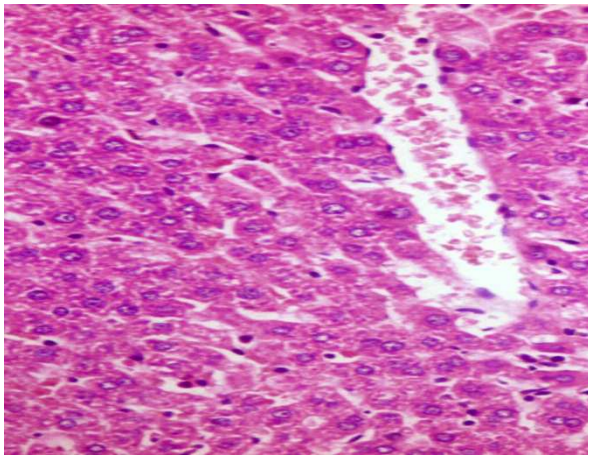
(A) Control group



(B) Malathion (Low dose)

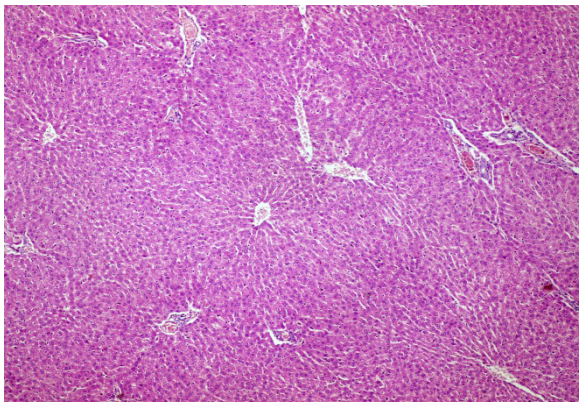


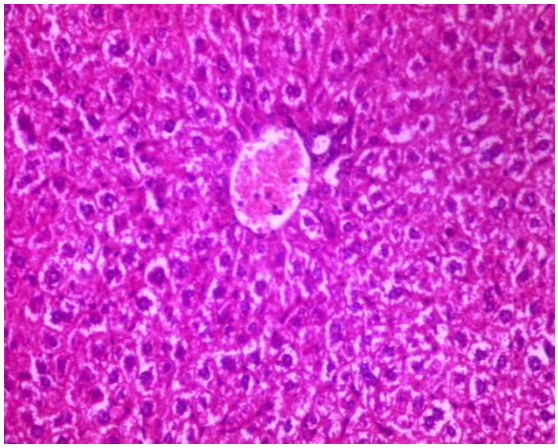
(C) Malathion (High dose)



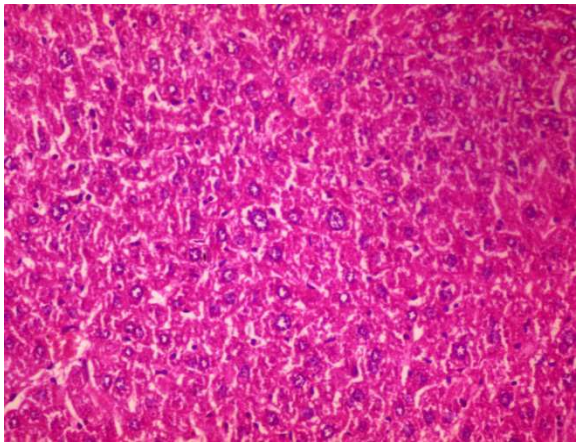
(D) Malathion (Low dose) +Curcumin

(E) Malathion (High dose)+Curcumin

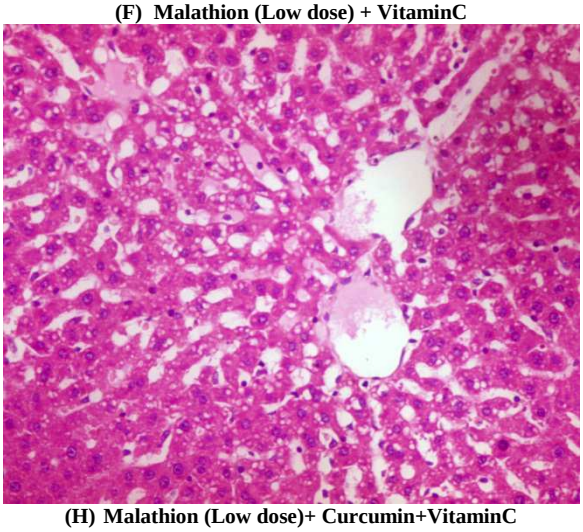




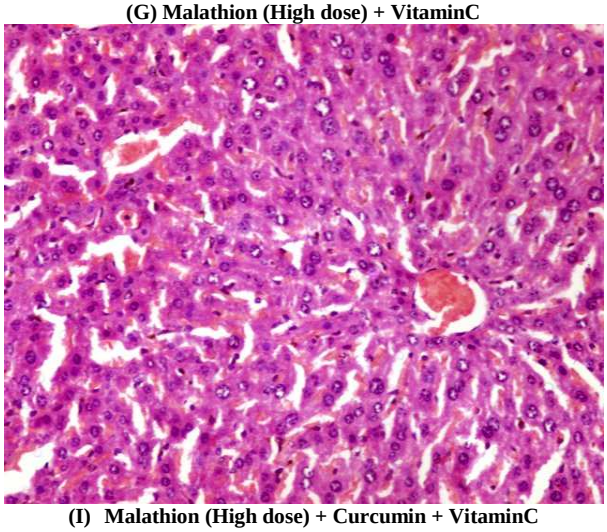
(F) Malathion (Low dose) + VitaminC



(G) Malathion (High dose) + VitaminC



(H) Malathion (Low dose)+ Curcumin+VitaminC



(I) Malathion (High dose) + Curcumin + VitaminC

Fig (4): Histopathological changes in liver sections of different groups treated with two doses of malathion and curcumin and/or vitamin C and their combinations

DISCUSSION

The major goal of this work is to evaluate the potential benefit of vitamin C and curcumin administration on malathion tissue injury.To our knowledge, no study has been conducted on the co-effect of curcumin and vitamin C on malathion toxicity at thebiochemical and histopathological levels for the liver and kidney functions parameters.
Curcumin represents a class of immuno-modulatory, wound-healing, anti-proliferative and antimicrobial activities, anti-inflammatory and antioxidants **[22]**. Curcumin may exert its protective actions against malathion-induced hepatorenal and reproductive toxicity in mice possibly through its antioxidant mechanisms.

VitaminC is considered as a highly effective antioxidant and an enzyme cofactor for the biosynthesis of many important biochemicals. It activates some enzymes which have an important role in protein, carbohydrates and fat metabolism. It exerts positive effect on lipid and iron metabolism **[23]** and promote immune function.

This study investigated the ability of curcumin and vitamin C to counteract malathion- induced toxicity in mice. Exposure to malathion at the two doses (Low and High doses) to mice led to an alteration of liver and kidney functions. Concomitantadministration of vitamin C or/and curcumin with malathion significantlyprotected most of the altered biochemical variables induced by malathion at its two dosessuggesting their protective efficacy. These two antioxidant compounds also improved the structure of liverthat was evaluated on the basis of histopathological findings.

The biochemical markers used to evaluate liver function wereALT, AST, ALP, total protein and lipid profile. ALT and AST are the most sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity **[24,25]**. The increased in serum enzymes activity in the malathion-treated group may be attributed to a generalized increase in membrane permeability, as reported by Kaczor *et al.***[26]**and Kalender *et al.***[27]**. This elevation could potentially

be attributed to the release of these enzymes from the cytoplasm into the blood circulation **[28]**, indicating a necrosis and inflammatory reactions **[29]**. In the same manner, serum aminotransferases (ALT & AST) are cytosolic enzymes of hepatocytes; an increase in their activities reflecting an increase in the plasma membrane permeability of hepatocyte which in turn associated with cell death **[30]**.

One or more mechanism could explain the malathion-induced hepatic disorders.Malathion may result in mitochondrial membrane rigidification and energetic metabolism impairment through the oxidation of a diverse set of hepatic mitochondrial components, including protein sulfhydryl groups. Additionally, malathion interferes with mitochondrial bioenergetics. This interference is due to the malathion ability to increase mitochondrial inner membrane permeability **[31]**.

With respect to control group, a significant decrease in serum total protein level was indicated as a consequence of malathion intoxication. This indication is consistent with that reported by Shibayama **[32]**.

The reversing of hepatotoxic effect induced by malathion, herewith observed after pretreatment with vitamin C which evaluated by significant decreasing in liver markers ALT, AST and serum proteins comparing with malathion treated group. These results seem to be conceivable with that obtained by Mossa *et al.***[33]**. They reported that vitamin C was able to attenuate hepatic damage induced by some chemical agents, especially in animals.Vitamin C normalized levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase.These may be attributed mainly to the ameliorative effect of vitamin C against the oxidative stress induced by malathion as vitamin C has reported to have antioxidant activity.

On the same basis, El-Gendy*et al.***[34]**revealed that The mechanism by which vitamin C decrease the hepatotoxicity induced by malathion, is embodied in the fact that vitamin C might ameliorate the oxidative damage by decreasing lipid peroxidation and altering antioxidant defense system or by denoting electrons to free radicals and quenching their reactivity.

At the same time, the present results revealed that treatment with curcumin alone or/and in combination with vitamin C after malathion administration was able to normalize the levels of liver enzymes biomarker. These demonstrated by significant decrease in ALS, AST and significant increase in total protein as compared with malathion treated group. These results were supported with that reported by Essam and Ashraf**[35]**. They stated that the protective effects of curcumin against chemically-induced hepatotoxicity are well documented, and have been attributed it to its intrinsic antioxidant properties. It could be suggested that the leakage of enzymes due to liver injury is prevented by the liver cell membrane stabilizing action ofcurcumin(Yousef *et al., 2008*)**[36]**.

Organophosphorous insecticides have been shown to induce hemorrhage, inflammatory cell infiltration **[37]**, tissue damage, and necrosis **[38]**, which could provoke increased white blood cells and thrombocyte counts. This possibility is supported by the histopathological results of the malathion (low and high) treated mice in this study, which revealed hepatic congestion, inflammatory cell infiltration, and liver necrosis.

The elevated serum cholesterol and triglycerides levels herein in malathion treated group may be attributed to one or more of the following explanations. It was stated that, intoxication with malathion could cause centrilobular necrosis, which results in translocation and accumulation of fats from peripheral adipose tissue in the liver, increases hepatic synthesis of fatty acids, impaired the function of smooth endoplasmic reticulum and induce peroxisomes to catalyze β-oxidation of fatty acids converting them into Acetyl-CoA, the precursor of cholesterol biosynthesis, and decreases the release of lipoproteins **[39]**.

In the present study the co-administration of vitamin C with malathion reduce cholesterol and triglycerides levelsas compared with malathion-treated group. These results strongly supported with that obtained by Uzun *et al.***[40]**. They reported that vitamin C supplementation provided a significant reduction in both LDL cholesterol and triglycerides.Moreover, concomitant vitamin treatment significantly normalized, at least partially, all of the other biochemical parameters that were altered by malathion.This may be explaining thatvitamin C acts as a regulatorof catabolism of cholesterol to bile acid and has been demonstrated to be an important factor in lipid regulation **[41]**.

Furthermore, curcumin could act in several ways to lower plasma LDL-bound cholesterol. First, uptake of cholesterol in the gastrointestinal tract could be inhibited; second, LDL-cholesterol (LDL-c) could be eliminated from the blood via LDL receptor; and finally, the activity of cholesterol-degrading enzymes, namely cholesterol-7-hydroxylase could be increased **[42]**.

Arafa **[43]** suggested that curcumin decreased total cholesterol and LDL-c, while increased HDL-c due to absorption, degradation or elimination of cholesterol. Moreover, Akila*et al.*,**[44]** showed that curcumin reduced cholesterol and increased HDL-c, indicating that curcumin may be mobilizing cholesterol from extrahepatic tissues to the liver where it is catabolized.

One hypothesis is that curcumin prevented increases in serum cholesterol concentrations in the animal studies by inhibiting dietary cholesterol absorption **[43]**. The relatively low absorption efficiency of curcumin is consistent with this hypothesis since the much greater curcumin concentration in the gut than in the blood makes an effect of curcumin on cholesterol absorption somewhat more plausible than an effect on cholesterol synthesis**[43]**.

Uric acid is considered to be a marker of oxidative stress, oxygen leading to the formation of uric acid and more importantly to the generation of free radical as H_2O_2**[45]**. In the present study, there were changes in serum uric acid levels in malathion-treated mice that could be confirmed that the malathion considered as a source of free radical production.Vitamin C was found to improve the effect of high dose of malathion on uric acid level while the curcumin and their combination had not effect
.
Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). Serum creatinine is an important indicator of renal health because it is an easily measured byproduct of muscle metabolism that is excreted by the kidneys. Creatinine is removed from the blood, chiefly by the kidneys, primarily by glomerular filtration. If the filtration in the kidney is deficient, creatinine blood levels rise **[46]**.

The present finding demonstrated that malathion administrated in two doses to mice provoked a marked elevation in serum creatinine levels which used as renal damage biomarker and this value was reduced by using curcumin as previously reported by Al-Harbi *et al.***[47]**.

Concerning the effect of vitamin C, the present result showed that treatment with vitamin C in combination with malathion either at low or high dose administration could reverse malathion nephrototoxic effect, as it reflected by a significant decrease in creatinine, urea and uric acid level in serum. These results supported by Lagowska-Lenard *et al.***[48]**. They demonstrated that vitamin C has a renoprotective effect.

The present results by light microscopic analyses revealed that malathioninduced inflammatory cell infiltration, central and portal vein congestion, hydrobic degeneration, dilation of sinusoids, vascular congestion and necrosis in the mice liver. In this regard, Yehia*et al.* **[49],** stated that theorganophosphorous insecticides are known to induce various histopathological changes in the liver tissues**.**These observed changes are entirely consistent with the changes in various biochemical parameters that were also observed in this study.

The liver damage may arise from the toxic effects of malathion, which disturbs the detoxification mechanisms of the liver. In addition, it is possible that malathion, like several other insecticides, adversely affects the cytochrome P450 system or the mitochondrial membrane transport system of hepatocytes **[50]**.

VitaminC, a low molecular weight antioxidant, defends the cellular compartment against water-soluble oxygen nitrogen radicals. It can also restore the antioxidant abilities and viability of liver tissues **[51]**.

In the present study, all changes in the biochemical parameters that were induced by malathion at either low or high dose exposure were at least partially normalized when vitamin C and curcumin were given together with malathion. Moreover, our light microscopic analyses revealed that vitamin C and curcumin-treated malathion-exposed animals did not exhibit the highly hepatic congestion and necrosis seen in the livers of the malathion-treated group.

In conclusion, this study may constitute the first attempt to evaluate the effects of curcumin combined with vitamin C on malathion -caused hepatotoxicity and nephrotoxicity disturbancesin adult male mice. In fact, mice exposed to malathion in the two studied doses showed an increase in transaminase enzymes activities, uric acid, as well as liverhistological disorders. These disturbances appear to be normalized when vitamin C and curcumin were given together with malathion.

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