A Comparative Histological and Biochemical Study on the Use of Vitamins C, E and Alpha - Lipoic Acid Either Separately or in Combination on Acute Hepatic Toxicity with Malathion

Dalia Abdo El-Gamal, Hala M. Fathy¹, Nagwa M. Ghandour¹ and Mona A. Elbaz²

Departments of Histology, ¹Forensic Medicine and Clinical Toxicology, ²Medical Biochemistry Faculty of Medicine. Assiut University

ABSTRACT

Introduction: Malathion is one of the most popular organophosphorous insecticides. Free radical damage is an important direct or indirect factor involved in malathion poisoning.

Aim of the Work: The objective of the present study was to estimate the role of vitamin C, vitamin E and alpha-lipoic acid either individually or in combination, in amelioration of acute hepatic toxicity induced by malathion.

Materials and Methods: Sixty adult male albino rats were divided into six equal groups. Group 1 served as control. Group 2 received malathion (1000 mg/kg body weight) once orally. Group 3 received malathion + vit.C (200 mg/kg) once i.p. Group 4 received malathion + vit. E (150mg/kg) once i.m. Group 5 received malathion + alpha-lipoic acid (25mg/kg) once i.p. Group 6 received malathion+ vit. C + vit.E + alpha-lipoic acid. Animals of all groups were sacrificed after 24 hours. Histological examination of the liver was performed. Biochemical assay of superoxide dismutase (SOD) activity and total thiols as antioxidant indices, thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation (oxidative stress indices), aspartate aminotransferase (AST), alanine amino transferase (ALT), total protein, albumin and globulin as liver function tests was done.

Results: Light and electron microscopic examination of liver of group 2 exhibited foci of altered cells with dense nuclei and vacuolated cytoplasm, mononuclear cell infiltrations in portal areas, electron lucent areas in the cytoplasm of the hepatocytes, margination of nuclear chromatin. Biochemical analysis showed a significant increase in the serum levels of SOD, total thiols, TBARS, AST, ALT, total protein and globulin as compared to control. Treatment by any of the antioxidants variably reduced the hepatic structural changes induced by malathion, while combined treatment resulted in a significant degree of recovery. There was significant decrease in serum levels of all biochemical parameters when treated with one or combination of antioxidants (vitamin C, E or α lipoic acid).

Conclusion: Combination of the previous antioxidants could be used as helpful therapeutic line in treatment of acute hepatic toxicity with malathion rather than their use separately.

Key Words: Malathion, antioxidants, liver.

INTRODUCTION

Malathion [-S-(1,2-bisethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate] is one of the most widely used organophosphorous pesticides for agriculture and public health programs¹. It has been postulated that OPs produce oxidative stress in different tissues through the formation of reactive oxygen species (ROS)²,³.

Liver is the major metabolizing and the most active organ for mediating bio-activation of thiono-organophosphates⁴. It is considered among the primary targets for malathion toxicity⁵. Reported toxicity of malathion might be attributed to its metabolite malaxon, which unlike its parent compounds could damage DNA⁶.

Living organisms have a complex antioxidant (enzymatic and non-enzymatic) system to protect against the deleterious effects of free radicals. Vitamin C is non enzymatic antioxidant act to overcome the oxidative stress, being part of the antioxidant system⁷. Vitamin E is the most effective chain breaking lipid soluble antioxidant in the biological membranes and protects cellular structures against damage from oxygen free radicals and reactive products of lipid peroxidation⁸.
Many studies have reported that a combination of vitamin C and E could reduce lipid peroxidation caused by toxic substances. Alpha lipoic acid is a "non-vitamin" nutrient involved in oxidative decarboxylation of α-keto acids mainly derived from dietary sources. Most α-lipoic acid in food is derived from lipoamide-containing enzymes and is bound to the amino acid, lysine (lipoyllysine). Plant sources that are rich in lipoyllysine include spinach, broccoli and tomatoes. Animal sources include kidney, heart and liver. Alpha lipoic acid has metal chelating, free radical scavenging, antioxidant-regenerating abilities with no serious side effects.

Thus, the aim of the present investigation was the evaluation of the ability of vitamin C, vitamin E and alpha-lipoic acid individually or in combination to ameliorate acute hepatic toxicity with malathion.

MATERIALS AND METHODS

Animals and treatments:

The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethics Committee of Assiut University.

Adult 60 male albino rats (age 2–3 months; weight 250–300 g) were divided into 6 groups of 10 rats each, with free access to food and water.

Group (1): Control group, kept without treatment through the period of the experiment (one day).

Group (2): Received malathion in a dose of 1000 mg/kg once orally. LD50 of malathion is 1350 mg/kg body weight. The doses were selected to provide low level of pesticide intoxication.

Group (3): Received vitamin C in a dose of 200 mg/kg once intraperitonially, 30 min after administration of malathion orally.

Group (4): Received vitamin E in a dose of 150 mg/kg once intraperitonially, dissolved in corn oil, 30 min after administration of malathion orally.

Group (5): Received alpha-lipoic acid in a dose of 25 mg/kg once intraperitonially dissolved in a 1:1 ratio with ethyl alcohol, 30 min after administration of malathion orally.

Group (6): Received all the previous treatment, 30 min after administration of malathion orally.

The animals were sacrificed by cervical decapitation 24 hours after the last treatment.

Histological examination:

I- light microscopic study:

After sacrifice, liver specimens were rapidly removed, preserved in 10% buffered formalin for 48 hours. Paraffin sections were prepared and stained with hematoxylin and eosin and Masson’s Trichrome.

II- Electron microscopic study:

The liver specimens were immediately fixed in 2.5% buffered gluteraldehyde at 4°C for 24 h and post fixed in osmium tetroxide for one hour. Semithin sections were cut and stained with Toluidine blue. Ultrathin sections (80-90 nm) were stained with uranyl acetate and lead citrate, examined and photographed with a JEOL transmission electron microscope in Assiut University E.M. unit.

Biochemical assay:

Blood samples were collected via retro-orbital puncture (from optic vein). Serum was removed by centrifugation for 10 min at 3000 rpm.

Serum superoxide dismutase (SOD) activity, Serum total thiols and thiobarbituric acid reactive substance (TBARS) was measured in serum by chemical methods. Aspartate aminotransferase (AST) and alanine amino transferase (ALT) were determined by colorimetry Kit purchased from SPECTRUM, MDSS, Hannover, Germany. Total protein content in serum was determined by colorimetric method. Serum albumin was determined by colorimetry kit purchased from Diamond Diagnostics, Cairo, Egypt. The serum globulin level was calculated by subtracting the albumin from total protein levels.

Statistical analysis:

The data are expressed as mean and standard error (M±SE). Differences among experimental groups were determined by one-way ANOVA and t test. In all experiments, p values less than 0.05 were considered to be statistically significant.

RESULTS

Histological findings:

A) Light microscopic results:

Examination of H and E stained sections and semithin sections of the control group (Group 1) showed normal hepatic architecture. Hepatocytes appeared polyhedral cells with granular cytoplasm and vesicular nuclei running in cords radiating from centrilobular veins (Fig. 1). Blood sinusoids separated liver cords and lined with endothelial cells. Kupffer cells were seen...
projecting into their lumina (Fig. 2). Animals treated with malathion (Group 2) displayed obvious hepatic changes. The alterations were noted in the form of foci through the lobules rather than being dispersed (Fig. 3). These foci of altered cells showed dense nuclei and vacuolated cytoplasm (Fig. 3 inset). Increased staining intensity was evident in other areas where hepatocytes showed deeply stained cytoplasm and their nuclei showed peripheral localization of dense chromatin clumps (Fig 4). There were mononuclear cell infiltrations in portal areas and thickening in the wall of some bile ductules (Fig. 5). Kupffer cells were more frequently observed in semithin sections compared to the control group (Fig. 6). Vascular dilatation and congestion were observed particularly in the central veins (Figs. 3&7).

Less gross degenerative changes were noted in the liver of the animals received combined malathion and vitamin C group 3. Increased frequency of binucleated hepatocytes, sinusoidal dilatation, mild mononuclear infiltration were observed (Fig. 8). After supplementation of malathion intoxicated rats with vitamin E group 4, the normal histological pattern of the liver was restored to a great extent, except for fine foci of mononuclear infiltration located between hepatocytes. Also, frequent binucleated cells were observed (Fig. 9). In the case of combined treatment with malathion and alpha-lipoic acid group 5, most of the hepatocytes showed vacuolated cytoplasm (Fig. 10-A). There was variation in the staining intensity and the size of hepatocyte nuclei (Fig. 10-B).

Finally, in the group of animals received malathion plus vitamin C, E and alpha-lipoic acid group 6, histopathological alterations were markedly reduced. The degree of degeneration, intracellular vacuolation, vascular congestion and inflammatory infiltration appeared to be reduced (Figs. 11&12), apart from the frequent presence of Kupffer cells (Fig. 12).

Examination of Masson’s trichrome stained sections showed the few collagen fibers around the central veins and portal tracts in the control group (Figs. 13- A,B). In group 2 (malathion treated), there was an increase in collagen deposition around the wall of central veins, and more obviously around the portal tract (Fig. 14). The stained sections of liver of group 3 (malathion + vit C), group 4 (malathion + vit E) and group 5 (malathion+alpha-lipoic acid) and group 6 (malathion + all antioxidants) revealed few collagen fibers around portal tracts compared to group 2 (Figs. 15&16).

B) Electron microscopic results:

Ultrastructural examination of hepatocytes of control group showed large rounded euchromatic nuclei with prominent nucleoli (Fig. 17). The cellular organelles were uniformly distributed throughout the cytoplasm, where numerous mitochondria, cisternae of rough endoplasmic reticulum and glycogen granules were observed (Fig. 17). Kupffer cells were seen in the hepatic sinusoids with irregular outlines, kidney shaped nucleus and electron dense cytoplasm containing lysosomes (Fig. 18). Ito cells were also distinguished, located in the space of Disse being characterized by numerous lipid droplets in their cytoplasm (Fig. 19).

Electron microscopic examination of hepatocytes of malathion treated group (Group 2) revealed the appearance of many electron lucent areas of the cytoplasm and large lipid droplets (Fig. 20). Other electron dense areas contained mitochondria and cisternae of rough endoplasmic reticulum (Fig. 21). The nuclei were variable in size and showed peripheral localization of heterochromatin clumps (Figs. 20&21). Kupffer cells prevailed in the hepatic sinusoids very common with their characteristic cytoplasmic processes (Fig. 22). Ito cells, located in the space of Disse, showed decrease in the amount of lipid droplets and surrounded by collagen fibers (Fig. 23).

Regarding group 6 (animals received malathion and treated by all previous antioxidants), most hepatocytes regained their normal appearance (Fig. 24). However, other hepatocytes displayed electron lucent areas of the cytoplasm, large lipid droplets as in group 2. Kupffer cells were oftenly observed having multiple cytoplasmic processes and their cytoplasm appeared studded with lysosomes (Fig. 25).

Biochemical findings:

The biochemical assay revealed that serum superoxide dismutase (SOD) activity, total thiols (total-SH), thiobarbituric acid reactive substances (TBARS), aspartate amino transferase (AST) and alanine amino transferase (ALT) in malathion treated group (Group 2) were highly significantly increased as compared to control (Table 1) and these levels in all groups (Groups 3,4,5&6) were significantly decreased as compared to malathion treated group (Group 2) (Table 1 and Figs. 1-I-V). Table 1 showed that the levels of serum total protein and globulin in malathion treated Group (Group 2) were significantly high as compared to control. Table 4 and Fig. VI showed that the levels of serum total protein and globulin in all (Groups 3,4,5&6) were significantly decreased compared to the malathion treated group. The serum level of albumin was not changed either in malathion treated group (Table 3) or in all Groups (Groups 3,4,5&6) as compared to the control group.
### Table 1: Effect of acute treatment with malathion on levels of serum activity of SOD, total-SH, TBARS, AST and ALT compared to control.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/ml)</th>
<th>Total-SH (nmol/ml)</th>
<th>TBARS (nmol/ml)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.0±0.289</td>
<td>4.4±0.39</td>
<td>1.7±0.356</td>
<td>73.8±0.601</td>
<td>16.2±0.401</td>
</tr>
<tr>
<td>Malathion (2)</td>
<td>52.4±0.369***</td>
<td>8.67±0.394***</td>
<td>6.54±0.357***</td>
<td>203.17±0.601***</td>
<td>61.3±0.382***</td>
</tr>
</tbody>
</table>

Values are presented by mean±SE
P probability for significance has been performed for malathion treated group compared to control group; p values are shown as:
N.S (non significant).
*P<0.05 (significant).
**P<0.01 (highly significant).
***P<0.001 (very highly significant).

### Table 2: Serum levels of SOD activity, total-SH, TBARS, AST and ALT in groups 3,4,5,6 compared to group 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/ml)</th>
<th>Total-SH (nmol/ml)</th>
<th>TBARS (nmol/ml)</th>
<th>AST (U/L)</th>
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<td>61.3±0.382</td>
</tr>
<tr>
<td>Malathion+VitC (3)</td>
<td>24.5±0.428***</td>
<td>5.3±0.376*</td>
<td>3.3±0.363***</td>
<td>104.5±0.764***</td>
<td>22.5±0.35***</td>
</tr>
<tr>
<td>Malathion+VitE (4)</td>
<td>19.3±0.382***</td>
<td>3.4±0.031***</td>
<td>1.91±0.362***</td>
<td>88.5±0.764***</td>
<td>16.8±0.382***</td>
</tr>
<tr>
<td>Malathion + α lipoic acid (5)</td>
<td>32.3±0.357***</td>
<td>6.9±0.34**</td>
<td>3.1±0.363***</td>
<td>144.8±0.601***</td>
<td>26.1±0.342***</td>
</tr>
<tr>
<td>Malathion+Vit C.+ Vit E.+ alpha -lipoic acid(6)</td>
<td>23.2±0.477***</td>
<td>5.8±0.367**</td>
<td>3.9±0.367***</td>
<td>100±0.577***</td>
<td>20.2±0.477***</td>
</tr>
</tbody>
</table>

Values are presented by mean±SE
P probability for significance has been performed for each group compared to malathion group; p values are shown as:
N.S (non significant).
*P<0.05 (significant).
**P<0.01 (highly significant).
***P<0.001 (very highly significant).

### Table 3: Effect of acute treatment with malathion on levels of serum total protein, albumin and globulin and compared to control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Globulin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.367±0.088</td>
<td>2.587±0.003</td>
<td>1.812±0.002</td>
</tr>
<tr>
<td>Malathion (2)</td>
<td>8.633±0.088**</td>
<td>2.681±0.021†</td>
<td>5.951±0.103†</td>
</tr>
</tbody>
</table>

Values are presented by mean±SE
P probability for significance has been performed for malathion treated group compared to control group; p values are shown as:
N.S (non significant).
*P<0.05 (significant).
**P<0.01 (highly significant).
***P<0.001 (very highly significant).

### Table 4: Serum levels of total protein, albumin and globulin in groups 3,4,5,6 compared to group 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein (g/dL)</th>
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</tr>
</thead>
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<tr>
<td>Malathion (2)</td>
<td>8.633±0.088</td>
<td>2.681±0.021†</td>
<td>5.951±0.103†</td>
</tr>
<tr>
<td>Malathion+Vit C (3)</td>
<td>5.067±0.088***</td>
<td>2.618±0.032 NS</td>
<td>2.449±0.057***</td>
</tr>
<tr>
<td>Malathion+Vit E (4)</td>
<td>3.6±0.115***</td>
<td>2.405±0.003**</td>
<td>1.195±0.114***</td>
</tr>
<tr>
<td>Malathion+alpha- lipoic acid (5)</td>
<td>5.6±0.058****</td>
<td>2.494±0.094 **</td>
<td>3.106±0.056**</td>
</tr>
<tr>
<td>Malathion+Vit C.+ Vit E.+ alpha- lipoic acid (6)</td>
<td>4.8±0.058****</td>
<td>2.589±0.012*</td>
<td>2.211±0.047***</td>
</tr>
</tbody>
</table>

Values are presented by mean±SE
(*) P probability for significance has been performed for each group compared to malathion group; p values are shown as:
N.S (non significant).
*P<0.05 (significant).
**P<0.01 (highly significant).
***P<0.001 (very highly significant).
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Fig. I: The mean ± SE of the serum SOD activity levels in the different studied groups (1= Control 2= malathion 3= malathion+vit. C 4= malathion+vit. E 5= malathion + alpha - lipoic acid 6= malathion + vit. C + vit. E + alpha - lipoic acid).

Fig. II: The mean ±SE of the serum total thiols levels in the different studied groups (1= Control 2= malathion 3= malathion + vit. C 4= malathion + vit. E 5= malathion + alpha - lipoic acid 6= malathion + vit. C + vit. E + alpha - lipoic acid).

Fig. III: The mean ±SE of the serum TBARS levels in the different studied groups (1= Control 2= malathion 3= malathion + vit. C 4= malathion + vit. E 5= malathion + alpha - lipoic acid 6= malathion + vit. C + vit. E + alpha - lipoic acid).

Fig. IV: The mean ± SE of the serum AST activity levels in the different studied groups (1= Control 2= malathion 3= malathion + vit. C 4= malathion + vit. E 5= malathion + alpha - lipoic acid 6= malathion + vit. C + vit. E + alpha - lipoic acid).

Fig. V: The mean ± SE of the serum ALT activity levels in the different studied groups (1= Control 2= malathion 3= malathion + vit. C 4= malathion + vit. E 5= malathion + alpha - lipoic acid 6= malathion + vit. C + vit. E + alpha - lipoic acid).

Fig. VI: The mean ±SE of the serum Globulin levels in the different studied groups (1= Control 2= malathion 3= malathion + vit. C 4= malathion + vit. E 5= malathion + alpha - lipoic acid 6= malathion + vit. C + vit. E + alpha - lipoic acid).
Fig. 1: A photomicrograph of a section in the liver of control group showing; the typical architecture of tightly packed pink-stained cords of hepatocytes around centrilobular venules. H&E X 100. Inset shows: The polyhedral hepatocytes with acidophilic cytoplasm and vesicular nuclei separated by hepatic sinusoids H&E X 1000.

Fig. 2: A photomicrograph of a semithin section of liver of control group showing; polyhedral hepatocytes (↑) with rounded nuclei and prominent nucleoli. Notice: The blood sinusoids (s) and Kupffer cells are projecting into their lumina (K). Toluidine blue X 1000.

Fig. 3: A Photomicrograph of a section in the liver of group 2 (malathion treated) showing; focal degenerative changes in the liver parenchyma (*) and portal venule congestion (↑). H& E X 100. Inset: shows a higher magnification of the previous section with marked intracellular vacuolation of the affected cells (↑). H&E X 1000.

Fig. 4: A Photomicrograph of a semithin section in the liver of group 2 (malathion treated) showing; some hepatocytes with dark cytoplasmic granules and peripheral localization of dense chromatin clumps (↑) in their nuclei, others with vacuolated cytoplasm (v) and numerous lipid droplets (L). Toluidine blue X 1000.

Fig. 5: A photomicrograph of a section in the liver of group 2 (malathion treated) showing; periportal mononuclear cell infiltration (▲) and thickening in the wall of the bile ductule (↑). H&E X 100.

Fig. 6: A Photomicrograph of a semithin section in the liver of group 2 (malathion treated) showing; Kupffer cells in the lumina of blood sinusoids (K). Toluidine blue X 1000.
Fig. 7: A photomicrograph of a section in the liver of group 2 (malathion treated) showing; marked dilatation in the centrolobular venules. 
H&E X 100.

Fig. 8: A photomicrograph of a section in the liver of group 3 (malathion + vitamin C) showing; increase in the binucleated cells (↑) and dilated hepatic sinusoids (*). 
H&E X 400.

Fig. 9: A photomicrograph of a section in the liver of group 4 (malathion + vitamin E) showing; apparent healthy hepatocytes, some of which are binucleated (↑) and localized mononuclear cell infiltration (▲). 
H&E X 400.

Fig. 10-A: A photomicrograph of a section in the liver of group 5 (malathion + alpha-lipoic acid) showing; most of the hepatocytes with vacuolation of their cytoplasm. 
H&E X 400.

Fig. 10-B: A photomicrograph of a section in the liver of group 5 (malathion + alpha-lipoic acid) showing; variation in the size of hepatocyte nuclei, some hepatocytes have deeply stained nuclei and cytoplasm (↑) and mononuclear cell infiltration (▲). 
H&E X 400.

Fig. 11: A photomicrograph of a section in the liver of group 6 (malathion + all antioxidants) showing; normal portal tract. Still some hepatocytes have densely stained nuclei (↑) 
H&E X 200.
Fig. 12: A photomicrograph of a semithin section of the liver of group 6 (malathion + all antioxidants) showing; apparently normal hepatocytes with large vesicular nuclei (N) and lipid droplets in the cytoplasm (L). Notice: Kupffer cell in blood sinusoids (K). Toluidine blue X 1000.

Fig. 13-A: A Photomicrograph of a section in the liver of control group showing; few collagen fibers around the central vein (↑). Masson's trichrome, X 100.

Fig. 13-B: A Photomicrograph of a section in the liver of control group showing; few collagen fibers around the portal tract (↑). Masson's trichrome, X 100.

Fig. 14: A photomicrograph of a section in the liver of group 2 (malathion treated) showing; increase in the collagen fibers around portal tract compared to the control (↑) while around the central vein, they are relatively normal. Masson's trichrome X 100.

Fig. 15: A photomicrograph of a section in the liver of group 5 (malathion + alpha- lipoic acid) showing; few collagen fibers around portal tract compared to group 2 (↑). Masson's trichrome X 100.

Fig. 16: A photomicrograph of a section in the liver of group 6 (malathion + all antioxidants) showing; few collagen fibers around portal tract and central vein compared to group 2 (↑). Masson's Trichrome X 100.
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Fig. 17: An electron micrograph of a hepatocyte of control group showing; an euchromatic nucleus (N), variable sized mitochondria (m), rough endoplasmic reticulum cisternae (rer) and glycogen granules (g). X 8000.

Fig. 18: An electron micrograph of a Kupffer cell of control group showing; irregular outlines, kidney shaped nucleus (N) and lysosomes (ly). X 6700.

Fig. 19: An electron micrograph of an Ito cell (IC) of control group showing; numerous lipid droplets (L). X 5000.

Fig. 20: An electron micrograph of hepatocytes of group 2 (malathion treated) group showing; variable sized nuclei (N), electron lucent areas of cytoplasm (*) and large lipid droplets (L). X 2700.

Fig. 21: An electron micrograph of a hepatocyte of group 2 (malathion treated) showing; electron lucent areas of cytoplasm (*) between other dense areas containing rough endoplasmic reticulum (rer) and mitochondria (m). The nucleus shows aggregation of heterochromatin clumps (↑). X 8000.

Fig. 22: An electron micrograph of a Kupffer cell of group 2 (malathion treated) showing; well defined cytoplasmic processes (K). X 6700.
DISCUSSION

Environmental liver injury has been studied extensively at both the biochemical and morphological levels. Malathion, one of the most popular OPs insecticides showed a relationship between its distribution in the internal organs and its route of administration. It was previously confirmed that oral administration of malathion showed its highest activity in the cytosol of liver cells\(^{20}\). Some authors stated that the greatest accumulation of the pesticide in the liver and kidneys was noted after I.V. administration followed by an oral administration\(^{21}\).

In the present work, oral administration of malathion to rats brought about significant liver damage in the form of parenchymatous degeneration in the hepatocytes, vascular dilatation, congestion in addition to mononuclear cell infiltration and increased Kupffer cell appearance intrasinusoidally. These Changes indicated that disturbed integrity of protein – lipid membranes had occurred. That was manifested by multiple vacuoles observed in the cytoplasm of the hepatocytes. These vacuoles were due to swelling of the cell which eventually produced hydropic degeneration. With degeneration, the protein content was watered down and the cell was less well stained\(^{22}\).

Masson’s trichrome – stained sections showed marked increase in the collagen deposition around the portal tract and to a lesser extent around the wall of central veins. In agreement with the present findings, previous researchers observed that in inflammatory liver diseases, extra cellular matrix components in fibrotic liver are similar regardless of the underlying cause\(^{23}\). One cause is the Kupffer cells which are the potent mediators of inflammatory response by the secretion of a variety of bioactive factors\(^{24}\). Kupffer cells can stimulate matrix synthesis through the actions of cytokines (especially transforming growth factor - B) and reactive oxygen intermediates / lipid peroxides\(^{25}\). The release of these cytokines modulates gene expression in non parenchymal and parenchymal cells to increase production of procollagen and inhibitors of enzymes that breakdown collagen. So, the synthesis of collagen exceed its break down resulting in liver microvascular fibrosis\(^{26}\). The appearance of Kupffer cells in semithin section in the present study coincides with the observations of the mentioned authors.

Electron microscopic examination of hepatocytes of malathion treated rats revealed the appearance of electron lucent areas of cytoplasm and lipid droplets. These changes confirm the focal degeneration of the cytoplasm which was previously observed\(^{21}\). In vitro studies\(^{27}\) showed that organophosphorous compounds including malathion, changed the physical and chemical properties of the membranes. The physical changes in the structure of plasma membrane disturb the Na+ /
vitamin E and C could have interactive effects. In the present study, measurement of the biochemical markers of oxidative stress assured the histological findings.

Electron microscopic observation of hepatic stellate cells or Ito cells in the perisinusoidal space of livers treated with malathion revealed an obvious change in the morphology of these cells. Their shape changed from the star shape to an oval or rounded ones. They showed few lipid droplets. These findings were consistent with previous observations of some authors who postulated that acute damage to hepatocytes activated transformation of quiescent stellate cells into myofibroblast like cells that play a key role in the development of inflammatory fibrotic response. Our results were in accordance with these postulations as increased collagen deposition and thickening of the basement membranes in the perportal area were observed.

One area of increasing interest is the study of the capability of some vitamins to modulate the effects of environmental toxicants. Therefore, the use of free radical scavengers and antioxidants were used for the treatment of oxidative stress cases. In the present study, vitamin C, E, alpha- lipoic acid were used individually or in combination. Treatment with vitamin C led to a decrease in the histological and biochemical changes induced by malathion toxicity. Previous results clearly indicated that the activities of hepatic microsomal drug metabolizing enzymes are dependent on the vitamin C status of the animal and when rats were supplemented with high level of vitamin C, some of the toxicity signs could be reversed. So, in the group treated with this considerable high dose of vitamin C, features of repair actually were observed. Multinucleation is a distinctive one, meaning cell survival and progressive convalescence.

Compared to combined therapy, the use of vitamin E separately revealed mild improvement in malathion induced histological changes inspite of better biochemical results. This might be explained by poor vitamin E absorption due to liver affection. Vitamin E absorption is delayed in cases of cholestasis which was expected to happen in malathion toxicity. Therefore, the improvement in the histological results retarded beyond the biochemical measurements in our study.

Also some authors reported that a combination of vit E and C restored the histological changes induced by malathion due to their ability to inhibit lipid peroxidation. Vitamin C has the capability to regenerate tocopherol from tocopheroxyl radicals in the biomembranes. Thus, vitamin E and C could have interactive effects. The use of alpha- lipoic acid had been proved by many authors. Its beneficial effect as an antioxidant reducing the damage caused by acetaminophen induced liver injury had been reported. Unlike other antioxidants, lipoic acid is both fat and water-soluble and is easily absorbed and transported across cell membranes. This unique quality offers protection against free radicals both inside and outside the cell. However, in the present study histopathological evaluation failed to show such an effect when used alone. This might be due to the use of a low dose of lipoic acid (25 mg/kg). A higher dose, that should not exceed 1/3 of the animal weight could have a beneficial effect. On the other hand there were features of repair in some hepatocytes. Variation in the nuclear size was observed in rats treated with malathion and alpha - lipoic acid. Increased ratio of nuclear area to cytoplasmic area is found during states of increased activity so we suggest that these cells were making their attempts for repair.

In the present study vitamin C, E and alpha- lipoic acid mixture reduced the histological changes induced by malathion and this was observed by light and electron microscopic examination. Previous authors proved that α-lipoic acid has the ability to regenerate other antioxidants like vitamin E, vitamin C and glutathione(GSH) for further use after they have eradicated free radicals, assisting in flushing toxins from the body. The stained liver sections after the use of combination of antioxidants showed an architecture similar to that of control. Regained cellular organelles and euchromatic nuclei were observed to a great extent. Active Kupffer cells were still observed. Kupffer cells were mentioned to be responsible for removing cellular debris involved in defense. Ito cells were observed regaining their high content of lipid droplets most probably returning to their quiescent state. Previous trials proved that antioxidants can reduce stellate cells (Ito cells) activation in culture. This attractive strategy provides a rationale for antioxidant trials in human.

Results of the present study showed that SOD activity in serum was significantly increased in malathion treated rats. In addition the treatment with one of the antioxidants (vitamin C, E or alpha- lipoic acid) or a combination of them 30 min after the administration of malathion led to significant decrease in SOD activity than malathion treated rats. These results suggested that malathion exposure provoked oxidative stress and modulate SOD activities. Previous workers reported that SOD activity in erythrocytes was increased in malathion treated rats probably to dismutate superoxide anion (O2−) and to decompose H2O2. The increase in these enzymes was probably a response towards increased reactive oxygen species (ROS) generation in OP toxicity, while in vitamin E pretreated rats toxicated with malathion showed decrease in erythrocyte SOD activity, as vitamin E scavenges ROS and lower oxidative stress. These data agreed with the present results.
In the present study, there was significantly high level of serum total thiols in malathion treated group than control, while treatment with one or combination of antioxidants (vitamin C, E or alpha-lipoic acid) 30 min after the administration of malathion led to significant decrease in total thiols. This was in agreement with the results of previous authors who reported that total thiols were highly statistically increased in malathion treated group and when vitamin E was administered total thiols were decreased. They suggested that the increased erythrocyte total-SH content in malathion treated rats may probably be due to decreased activity of glutathione-S-transferase (GST) in erythrocytes. The decline in erythrocyte GST activity might lower the conjugation of-SH groups thus causing toxic condition in the erythrocytes. Rats pretreated with vitamin E, prior to OP intoxication, showed decrease in total-SH content due to low oxidative stress. Other studies indicated that antioxidant enzyme activity was either increased, reduced or not changed in the liver, brain and erythrocytes of animals treated with OP compound.

It was suggested that lipoperoxidation (LPO) is more affected by malathion exposure than protein oxidation. TBARS content is an excellent biomarker for the estimation of malathion-induced oxidative stress.

The results of the biochemical finding of the present work indicated that malathion in acute exposure at a dose of 1000 mg/kg increased significantly the TBARS level in serum of malathion exposed rats. These results were somewhat similar to those found in the liver of rats exposed acutely or subchronically in liver and blood in which TBARS was highly statistically increased compared to control. Recent studies revealed that the acute administration of malathion provoked an increase in TBARS in high doses (100 and 150 mg/kg) in tissues (kidney, lung, and diaphragm). However, the TBARS contents were decreased in serum at a dose of 100 mg/kg. This result did not agree with the present results in which TBARS levels were increased in serum. While treatment with one or combination of antioxidants (vitamin C, E or alpha-lipoic acid) 30 min after the administration of malathion decreased the level of TBARS to regress to the control level.

AST and ALT are two hepatic enzymes which will be released in blood in the event of cellular destruction proportionally to the intensity of cellular aggression. In the present study the levels of AST and ALT in serum were significantly increased in malathion treated group. However, treatment with one or combination of antioxidants (vitamin C, E or alpha-lipoic acid) 30 min after the administration of malathion caused significant decrease in the activity of AST and ALT than malathion treated rats. The recorded increase of AST and ALT levels may be due to increased LPO. This was in agreement with a recent study which showed a significant increase in serum activities of aspartateaminotransferase (AST) and alanine aminotransferase (ALT) in malathion treated rats.

In the present study the level of total serum protein content was significantly increased in malathion treated group than control. Also, the globulin content was approximately doubled to that of albumin. This reflects the hazardous effect due to such pollutants. It was predicted that the toxic component may be subjected to xenobiotic interaction and the result metabolite became susceptible to be conjugated body protein and such a protein adduct could be acting as a hapten and inducer for immunoglobulin production. This interpretation became verified by observing considerable reduction in the level of serum globulin by treatment with one or combination of antioxidants (vitamin C, E and alpha-lipoic acid).

The differences were found between the present result and others, may be due to the malathion exposure regimen employed, the tissue distribution or the age of the animals.

Our evaluation showed that acute exposure to malathion disrupted the cellular integrity of hepatocytes and hematological system. The present findings reinforced the concept that oxidative stress and particularly LPO were involved in Ops toxicity. Single dose of one or preferably a combination of antioxidants (vitamin C, E or alpha-lipoic acid) 30 min after administration of malathion could reduce LPO caused by malathion. Also, they improved the liver function tests and cellular integrity of hepatocytes.

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