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Effects of Acute Malathion Exposure on Liver Mitochondrial Respiratory Chain Enzymes in Rats

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Abstract

Malathion is an organophosphorus insecticide widely used to control pests. Previous toxicological studies attributed to the ability of Malathion to effect on cellular metabolism. The main objective of present study was to find out what actually happens on the activities of key enzymes of the mitochondrial respiratory chain after acute exposure to Malathion. To design an acute toxicity study, all male Wistar rats were given Malathion at a single oral dose of 400 mg/kg. Activities of plasma acetylcholinesterase (AchE) and liver mitochondrial respiratory chain enzymes were measured after 24 h. Inhibition of AchE was accompanied by depressed nicotinamide adenine dinucleotide-ubiquinone oxidoreductase, and cytochrome C oxidase enzymes activity in the mitochondrial respiratory chain. On the other hand, Malathion did not significantly change succinate-ubiquinone oxidoreductase, and adenosine triphosphate synthase enzymes activity. It can be concluded that Malathion by extra energy demand in cholinergic storm phase reduced glycogen content of liver to supply glucose. On the other hand, inhibition of the mitochondrial respiratory chain key enzymes activity shift cellular glucose metabolism from mitochondrial respiration to anaerobic glycolysis pathway to provide demanded energy.

Keywords: Liver, Mitochondrial Respiratory Chain Enzymes, Organophosphorus

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1. Introduction

Malathion as an organophosphorus (OP) pesticide has been used in agriculture and domestic for many years. The main toxic mechanism of these pesticides is the elevation of acetylcholine in synaptic spaces. In addition, more studies have shown the effect of OP on induction of oxidative stress[1] and change in metabolic pathways especially in the liver.[2] These compounds induce ultrastructural,[3] biochemical,[4] and mitochondrial5 changes in the liver as a target tissue for OP pesticides. However, mechanisms contributing to the metabolic disorder of Malathion in liver tissue have not been thoroughly clarified. Mitochondria in addition to involvement in metabolic pathways and adenosine triphosphate (ATP) generation have pivotal roles in the induction of oxidative stress and control of apoptotic pathways.6 Histological findings such as mitochondrial swelling and changes in mitochondrial cristae and sinusoidal spaces support the involvement of mitochondria in Malathion toxicity in liver tissue.[7,8] There are several studies have focused on a possible role of mitochondrial function in OP toxicity and have shown that OP can change mitochondrial respiration and activities of respiratory chain enzymes[9] energy production,[10,11] and cell death pathways.[12] Since, there is only single study has investigated the effects of Malathion on mitochondrial respiratory chain enzymes activities in chronic exposure;[13] we decided to evaluate this hypothesis in liver tissue of adult male Wistar rats in acute poisoning with Malathion.

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2. Materials and Methods

2.1 Materials

Pure Malathion with a technical grade of 95% was purchased from Shimi Keshavarz Pesticide Production Co., (Tehran, Iran). Mitochondria isolation kit was from BioChain Institute Inc., (Newark, USA). All other chemicals were of the highest purity available and were obtained from Sigma-Aldrich (St. Louis, USA).

2.2 Animals

The healthy male albino Wistar rats with weight of 250 ± 5 g were housed in the cages with the temperature range of 20–25°C and fed by commercially available pellet diet. All experiments were performed according to the Animal Welfare Act (P.L. 89–544), and the study protocol was approved by pharmaceutical sciences research center Ethics Committee of Tehran University of Medical Science.

3. Methods

3.1 Experiment I: Selection of the effective dose of Malathion

A pilot study was performed to find out an appropriate dose to design an acute poisoning, model. For this purpose, Malathion was dissolved in corn oil and administered (0.1 ml/100 g) to rats by gavage. Treatment groups ($n = 6$ for each dose) received doses of 50, 200, 400, 600 mg/kg of Malathion and controls ($n = 6$) received only corn oil (0.1 ml/100 g). Malathion treated rats were anesthetized by ketamine/xylazine 24 h after treatment and then 1 ml blood was taken by cardiac puncture. The blood was immediately centrifuged 20 min in 5000 × g at 4°C and plasma was separated from red blood cells. Acetylcholinesterase (AchE) activity in the plasma was measured according to Ellman method as previously described.[14]

3.2 Experiment II: Assessment of mitochondrial respiratory chain enzymatic activities

3.2.1 Treatment of Animal

Male albino Wistar rats were divided into two groups: Treatment group ($n = 6$) and control group ($n = 6$) received, respectively, a suspension of Malathion at the dose of 400 mg/kg in corn oil and only corn oil. Twenty-four hours after administration of Malathion, the ats were anesthetized by ketamine/xylazine and liver tissue was removed for isolation of mitochondria.

3.2.2 Preparation of Liver Mitochondria

One hundred mg of fresh liver tissues were processed according to mitochondria isolation kit protocol for preparation of mitochondria by differential centrifugation. Finally, mitochondrial samples were stored in –80°C for downstream processing.

3.2.3 Determination of Nicotinamide Adenine Dinucleotide-Ubiquinone Oxidoreductase Activity

The principal of this method is based on measuring the oxidation rate of nicotinamide adenine dinucleotide (NADH) via reduction of ubiquinone by spectrophotometer (BioTek® Instruments, Inc.). This reaction was measured by addition of 0.1–0.2 mg/ml of mitochondria protein to reaction mixture with and without rotenone (0.36 mM) at 340 nm (extinction coefficient of 6.22/mM/cm) for 3 min. Reaction mixture was contained potassium phosphate buffer (25 mM; pH 7.4), 25% bovine serum albumin, magnesium chloride ($MgCl_2$) (5 mM), decylubiquinone (2.8 mM), NADH (5.7 mM), antimycin A (0.0037 mM), and potassium cyanide (KCN) (0.002 mM). Finally, rotenone-sensitive activity was calculated as the total activity minus the rotenone-insensitive activity and was reported as mM of NADH/min/mg of mitochondrial protein.[15]

3.2.4 Succinate-Ubiquinone Oxidoreductase Activity Assay

The activity of this enzyme was measured by measurement of the reduction rate of 2,6-dichlorophenolindophenol (DCPIP) at 600 nm. Briefly, after incubation of mitochondria (0.01–0.05 mg of protein) for 10 min in potassium phosphate buffer (25 mM; pH 7.2), $mgCl_2$ (5 mM), and succinate (20 mM) AQ2 at 30°C, the baseline rate was recorded for 3 min by addition of antimycin A (0.002 mg/ml), rotenone (0.002 mg/ml), KCN (2 mM), and DCPIP (0.05 mM). The reaction was started with ubiquinone (0.065 mM) for 3–5 min. The activity was calculated using a DCPIP standard curve as mM of DCIP/min/mg of mitochondrial protein.[16]

3.2.5 Determination of cytochrome C oxidase activity

This assay was performed by adding 0.005–0.015 mg of mitochondrial protein to a solution containing

reduced cytochrome C and lubrol PX (0.45 mM) in potassium phosphate buffer (25 mM). The activity of this enzyme (marker of complex IV) was measured by the decrease of optical density at 550 nm for 3–6 min and reported as the first order rate constant (k)/min/mg of mitochondrial protein.[17]

3.2.6 Determination of adenosine triphosphate synthase activity

ATP synthase activity was measured by adding mitochondrial protein (8 mg/ml) in a solution containing tris-acetate (0.025 mM; pH 7.5), potassium acetate (0.025 mM), sucrose (0.3 mM), potassium chloride (100 mM), $MgCl_2$ (0.002 mM), ATP (0.002 mM), pyruvate kinase (3 U/ml), lactate dehydrogenase (LDH) (2.8 U/ml), phosphoenol pyruvate (0.001 mM), and NADH (400 nM) at 340 nm for 8 min. For elimination of oligomycin-insensitive enzyme activity, which is not localized in mitochondrial inner membranes the activity of this enzyme was measured in presence and absent of oligomycin (0.006 mg/ml) and reported as mM of oxidized NADH/min/mg of mitochondrial protein.[18]

3.2.7 Determination of Glycogen Content

According to the phenol-sulfuric acid method, 100 mg of liver tissue was homogenated with 1 ml of 30% potassium hydroxide solution. After centrifugation, 0.2 ml of a 5% phenol solution was added to 0.1 ml of supernatants of samples and glycogen standards. One ml of concentrated sulfuric acid was added rapidly to each tube and finally the absorbance was read at 490 nm after 20 min.[19]

3.2.8 Determination of lactate content

One hundred milligram liver sample in 0.5 ml of 8% perchloric acid was homogenated and for assessment of lactate content, 0.1 ml of was added to 2.9 ml of reaction mixture containing hydrazine buffer (0.5 mM; pH 9.2), NAD^+ (2 mg/ml), LDH (12.5 U/ml). Finally, the absorbance changes was read at 340 nm in 50 min with 1-min intervals.[20]

3.2.9 Determination of protein content

Protein concentration of tissue and mitochondrial samples were measured by bicinchoninic acid protein assay.[21]

4. Statistical Analysis

All values were expressed as mean ± standard error of mean. The significance of differences among experimental groups was determined using the Student's *t*-test. A level of $P < 0.05$ was considered as a significant difference.

5. Results

5.1 Experiment I

Malathion reduced the activity of AchE at the dose of 50, 200, 400, and 600 mg/kg by 87, 61, 34, and 14%, respectively [Figure 1]. The dose of Malathion reducing 70% of AchE activity can be used for the design of an acute model of poisoning in rats.[22] Malathion at a dose of 400 mg/kg inhibited the activity of this enzyme to 30% in comparison to control group, and this dose of Malathion was used for the design of an acute model of poisoning.

5.2 Experiment II

After 24-h, Malathion at the dose of 400 mg/kg caused a significant (*P*: 0.048) decline in the NADH-ubiquinone oxidoreductase activity as a marker for complex I of mitochondrial respiratory chain [Figure 2] and cytochrome C oxidase activity [Figure 3]. On the other hand, the activity of succinate-ubiquinone oxidoreductase as the key enzyme of complex II of mitochondrial respiratory chain [Figure 4] and ATP synthase did not have a significant change [Figure 5] in treated animals in comparison to control group.

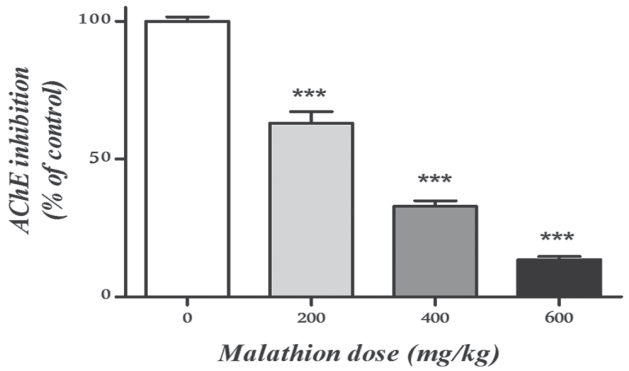


Figure 1. Inhibition of acetylcholinesterase 24 h postoral administration of different doses of Malathion. Values are mean ± standard error of mean (*n*=6).

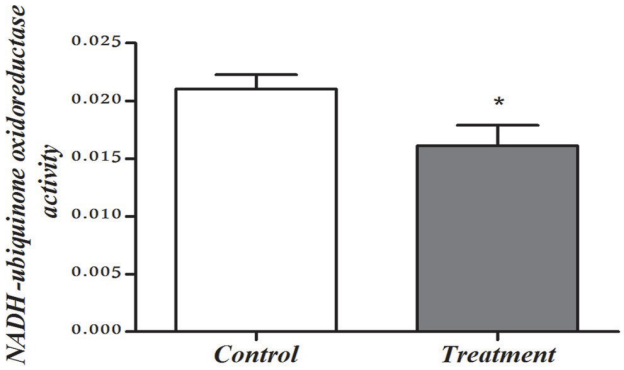


Figure 2. Effect of acute Malathion exposure on nicotinamide adenine dinucleotide-ubiquinone oxidoreductase in the rat liver tissue. Values are mean ± standard error of mean (*n*=6).

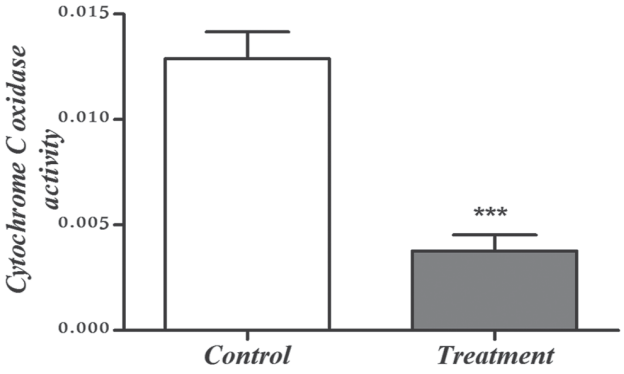


Figure 3. Effect of acute Malathion exposure on cytochrome C oxidase in the rat liver tissue. Values are mean ± standard error of mean (*n*=6).

In addition, Malathion (400 mg/kg) decreased the glycogen content of liver (12%) and raised the lactic acid content of liver (9%), after 24 h [Table 1].

Table 1. Changes in whole liver glycogen and lactate content in comparison with control group

Groups	Glycogen (ppm/mg of liver tissue)	Lactate (ppm/mg of liver tissue)
Control	319.0±10.5	6.2±0.2
Treatment	282.9±5.2a	6.8±0.1b

6. Discussion

It has previously been reported that Malathion has a potential role in damage of metabolic pathways of carbohydrates, lipids, and proteins.[23] Histological findings of hepatocytes were proved mitochondrial disorders in Malathion toxicity;[7] nevertheless, few studies focused on mitochondrial respiratory chain enzymes and respiratory control ratio.[13,24] The present study revealed the effect of acute Malathion exposure on activity of liver mitochondrial respiratory chain enzymes in rats. The results showed a decrease in activity of NADH-ubiquinone oxidoreductase a key enzyme of respiratory chain complex I with no change in activity of succinate-ubiquinone oxidoreductase a key enzyme of respiratory chain complex II. Massicotte *et al.* also demonstrated an inhibition of the respiratory chain complex I after OP exposure in the human neuronal cell line.[24] Delgado *et al.*[13] also reported same results on respiratory chain complex II. Since both succinate-ubiquinone oxidoreductase and flavin adenine dinucleotide are connection sites between the mitochondrial tricarboxylic acid cycle and the oxidative phosphorylation chain,[25] we can conclude that disorder in NADH supplying pathway due to inhibition of respiratory chain complex I can be a cause for mitochondrial toxicity of Malathion. Besides, decline in the NADH-ubiquinone oxidoreductase activity is in association with induction of oxidative stress [24,26] that several studies proved induction potential of Malathion toward oxidative stress.[27-29]

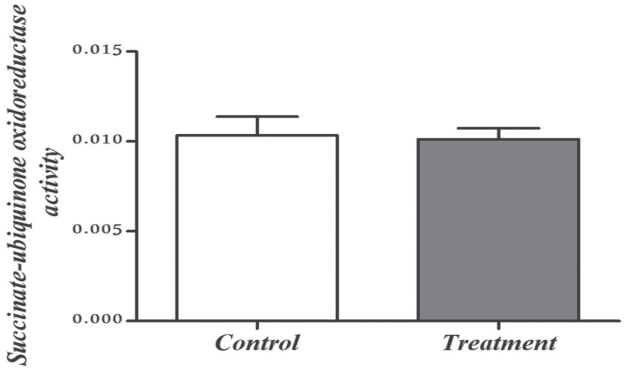


Figure 4. Effect of acute Malathion exposure on succinate-ubiquinone oxidoreductase in the rat liver tissue. Values are mean ± standard error of mean (*n*=6).

The activity of cytochrome C oxidase significantly ($P < 0.001$) decreased in this experiment. Complex I and II of the mitochondrial respiratory chain are responsible for the production of oxidative stress; moreover, reduction in activity of cytochrome C oxidase can also cause depletion in cellular ATP by the collapse of mitochondrial membrane potential.[5] Contrary to what we observed in association with cytochrome C oxidase activity, no significant change was found in the activity of ATP synthase that can be because of a compensatory mechanism to reduce the damage. However, these finding is compatible with the results of a study was done by Delgado *et al.*[13]

Severe muscle fasciculation after the release of acetylcholine make an extra energy demand.[30] It seems that depletion of glycogen stores in liver[31] occurred to supply glucose that can abate harmful effect of mitochondrial disorder in the production of ATP. The first thing that comes to mind about elevation of lactic acid content of liver is shift of glucose metabolism through anaerobic glycolysis pathway as another source of ATP not only in situation of inadequate oxygen supply[9] but also to compensate impairment of mitochondrial oxidative phosphorylation.

7. Conclusion

Totally, it can be concluded that Malathion besides inhibition of activity of AChE has a direct effect on metabolic pathways of glucose, induction of oxidative stress, and depletion of cellular ATP due to change in mitochondrial respiratory chain enzymatic activities. Hence, treatment of mitochondrial injuries by administration of electron donors and exogenous ATP can be therapeutic strategies in future for OP poisoning.

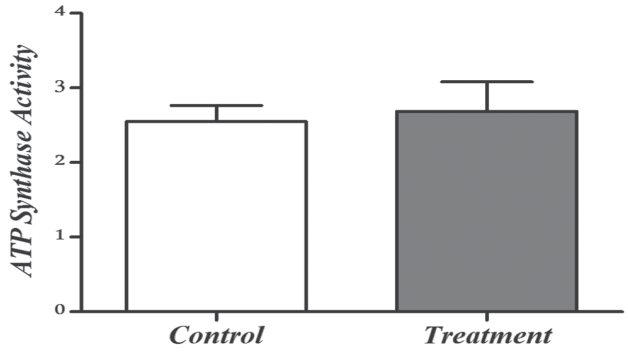


Figure 5. Effect of acute Malathion exposure adenosine triphosphate synthase in the rat liver tissue. Values are mean ± standard error of mean (*n*=6).

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Nil.

Conflicts of interest
There are no conflicts of interest.

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