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Research Article

Evaluation of Oxidative Stress Indices after Exposure to Malathion and Protective Effects of Vitamin C in Ovarian Tissue of Adult Female Rats.

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ABSTRACT:

Background: Malathion is an organophosphate pesticide which is known to induce oxidative stress in the target tissues such as the reproductive system.The aim of present study was to evaluate the effects of malathion on malondialdehyde(MDA) and glutathione(GSH) levels in female rat reproductive tissue (ovary) and to assess the protective role of vitamin C.

Materials and Methods: In this experimental study, 30 adult female Wistarrats (weight range: 200-250 g) were divided into five groups,consisting of six rats: control group (no interventions), sham group (normal saline), experimental group 1 (malathion+normal saline 50 mg/kg), experimental group 2 (200 mg/kg of vitamin C), and experimental group 3 (similar dosesof vitamin C and malathion). The animals were scarified after two weeks and MDA as a marker of lipid peroxidation and GSH content were measured in ovarian tissue.

Results: Malathion reduced GSH content and increased MDA level in ovary compared with the control group ($P<0.001$). Vitamin C plus malathion increased GSH content but decreased malathion induced MDA elevation in rat ovarian tissue**.**

Conclusion: Oxidative stress contributes to DZN-induced ovarian toxicity.Our results concluded that vitamin E may have a protective role in this toxicity.

Keywords: Malathion, Glutathione, Malondialdehyde, Vitamin C , Ovary,Rat,

INTRODUCTION

Considering the widespread use of organophosphoruscompounds in agriculture, industry, and veterinary medicine, studies on these compounds and theirmechanisms of action are still under way (1, 2). So far, a wide range ofeffects have been attributed to organophosphorus compounds. The major mechanism of action in these compounds involves the inhibition of acetylcholinesterase and development of cholinergic crisis. However, many effects of these compounds are notassociated with the inhibition of acetylcholinesterase (2). In Iran, organophosphorus compounds are considered asthe third cause of toxicity and the main cause of toxicity-inducedmortality (3). One of the mechanisms involved inpesticide effects is the production of free radicals,followed by changes in the antioxidant system and lipid peroxidation of cell membranes. Under normal conditions, there is a balance between the production and elimination of free radicals.Imbalance in this process leads to oxidative stress and multiple pathological changes in cellular macromolecules such as DNA, lipids and enzymes. (4). Oxidative stress leads to the production of ROSand causes changes in enzymatic activities and antioxidant defense mechanisms in the body (5, 6). However, excessive production of ROS can damage the cells through lipid peroxidation

(7-10).Malondialdehyde (MDA) is a product of lipid peroxidation. MDA is in fact one of the important products resulting from the oxidation of polyunsaturated fatty acids. MDA measurement is one of the important indices of lipid peroxidation (11, 12). Thiol groups are reducing agents, existing at a concentration around 5 mM in animalcells(13).Thiol groups are sensitive to oxidation, and lipid peroxidation often results in a decline in their level (14).Glutathione belongs to thiolgroups andplays an essential role in protecting body cells against damages such as oxidative stress (15). Antioxidants are a family of diverse compounds, which inhibit the excessive production of toxic free radicals and theinduced damages (16, 17).Vitamin C as a vital antioxidant can change lipid oxidation parameters through reducing the productionof oxidative stress (18). The purpose of this study was to evaluate the level of glutathione and MDA in ovarian tissues of malathion-induced female rats and to assess the protective effects of vitamin C.

MATERIALS AND METHODS

In this experimental study, 30 adult female Wistarrats (agedtwo months), with a weight range of 200-250 g, were randomly divided into five groups,consisting of six rats: experimental group 1 (daily administration of 50 mg/kg of malathion in normal saline solution for two weeks), experimental group 2 (daily administration of 200mg/kg of vitamin C), experimental group 3 (daily administration of 50mg/kg of malathion and 200mg/kg of vitamin C), control group (no interventions), and sham group (administration of 50mg/kg of normal saline for two weeks).Normal saline solution was used in this study, and malathionand vitamin C were intraperitoneally injected. After two weeks, the rats were sacrificed and MDA level was measured as an index of lipid peroxidation; also, the amount of glutathione in the ovaries of rats was estimated.

Chemicals

For this study, technical malathion was used. Malathion was dissolved in NACL 0.9%. Malondialdehydetetrabutylammonium, reduced GSH, DTNB [5,5 dithiobis-(2-nitrobenzoic acid)] and vitamin C were purchased from Sigma company.

Lipid peroxidation test

The amount of lipid peroxidation was assessed through the measurement of MDA levels in ovary tissue of rats. MDA reacts with ThioBarbituric Acid (TBA) and produces a pink colored complex which has the maximum absorbance at 532 nm. Initially, 1 ml TBA (0.6%) and 3 ml phosphoric acid (1%) were added to homogenate 10% tissue in KCl, then the compound was heated for 45 min in a boiling water bath. After cooling the compound, 4 ml of n-butanol was added to it and thenvortex-mixed was used for 1 min followed by centrifugation at 3000 g for 10 min. After this, the organic layers were removed and transferred to other tubes and absorbance level was read at 532 nm (40). A calibration curve was designed using MDAtetrabutylammonium. MDA levels were expressed by nmol/g tissue.

Reduced GSH test

GSH levelmeasured in ovary by the method of Moron et al (41). The base of the work was the formation of yellow color after adding DTNB [5,5 dithiobis-(2-nitrobenzoic acid)] to compounds containing sulfhydryl groups. Therefore, 300 µl of homogenates tissue were blended with 300 µl of 10% Tricolor Acetic Acid (TCA) and vortexed. After centrifugation at 2500 g for 10 min, the upper layers were removed and mixed with reaction mixtures containing 2 ml phosphate buffer (pH:8) and 500 µl DTNB. After 10 min, the absorbance was read at 412 nm using a spectrophotometer (Jen-way 6105 uv/vis, UK). At the end, the amount of GSH was determined based on a standard curve drawn with commercially available GSH (Sigma). The GSH Levels were expressed by nmol/g tissue

Statistical analysis

Results are expressed as mean±SD. Statistical analysis was performed with ANOVA followed by Tukey-Kramer test to compare the differences between means. Differences were considered statistically significant at $P<0.05$.

RESULTS

The effectsof vitamin C on lipid peroxidation of malathion-treated rats

The results of the present study showed a significant increase in the level of MDA inexperimental group 1 (malathion-induced), compared to the control group. In addition, the level of MDA significantly decreased in experimental group 3 (malathion+vitaminC), compared to experimental group 1. However, the MDA level was not significantly different between the control, sham, and vitamin C groups (Diagram 1).

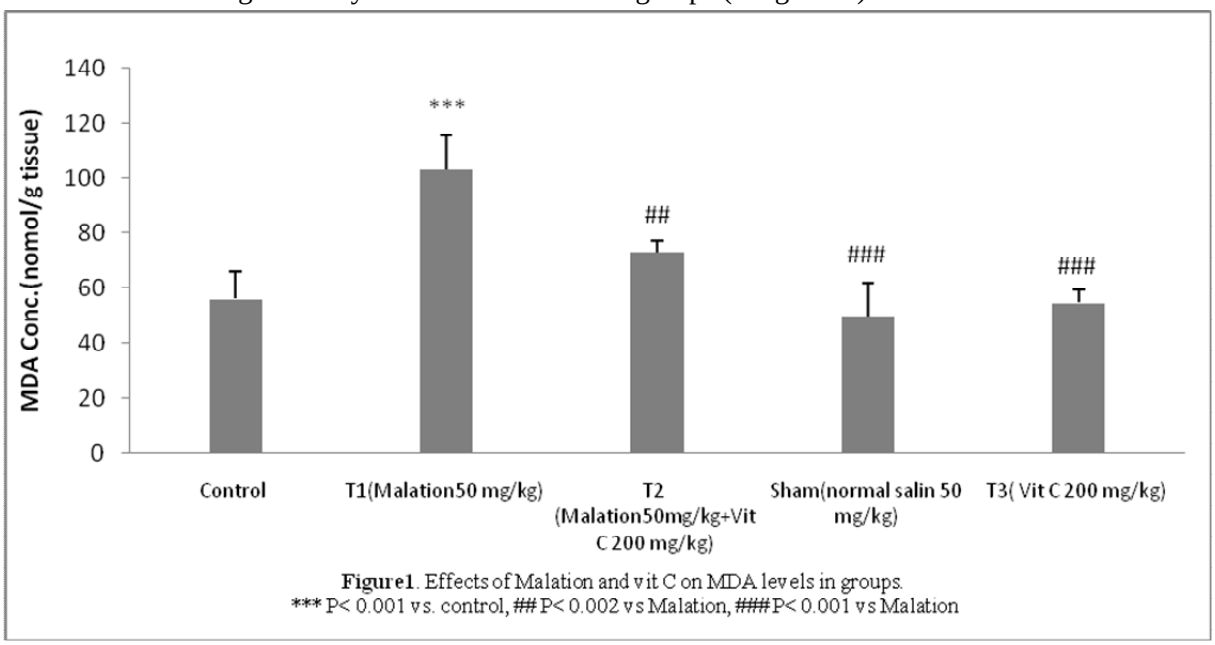

Figure1. Effects of Malation and vit C on MDA levels in groups.
*** P< 0.001 vs. control, ## P< 0.002 vs Malation, ### P< 0.001 vs Malation

The effect of vitamin C on the level of glutathione in ovarian tissues of rats following the injection of malathion

In the present study,malathion-treated rats experienced a significant decline in the level of glutathione in experimental group 1, compared to the control group.Also, the level of glutathione significantly increased in experimental group 3 (vitamin C+malathion), compared to experimental group 1. Also, the control, sham, and vitamin C groups were not significantly different in terms ofglutathione level (Diagram 2).

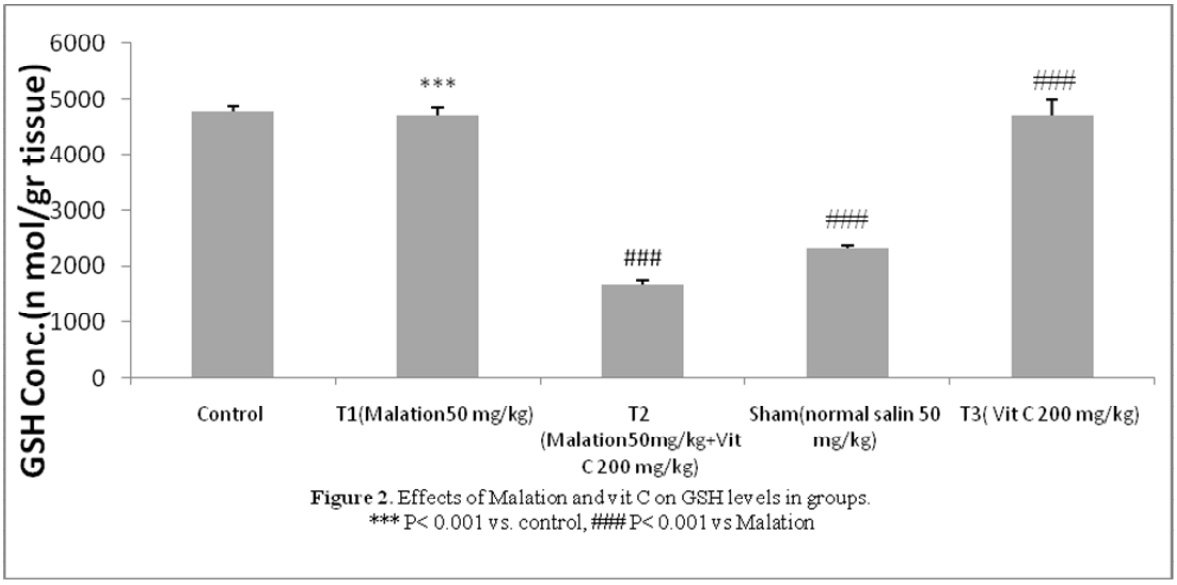

Figure 2. Effects of Malation and vit C on GSH levels in groups.
*** P< 0.001 vs. control, ### P< 0.001 vs Malation

Table1.Mean of GSH and MDA among various groups

Treatments	Glutathione	Malondialdehyde
Control	4783.861±93.23	56.04±10.07
Sham(normal salin 50 mg/kg)	4711.593±150.45	49.91±12.09
T1(Malation50 mg/kg)	1665.815±89.43	103.13±12.41
T2 (Malation50mg/kg+Vit C 200 mg/kg)	2337.043±44.83	73.02±4.16
T3(Vit C 200 mg/kg)	4720.748±267.42	54.63±4.80

DISCUSSION

In the present study, we evaluated MDA and glutathionelevels in ovarian tissues of female rats byinducing malathionpoisoning and assessed the protective effects of vitamin C. Based on the findings, experimental group 1,which only received malathion, experienced a significant increase in the level of MDA, compared to the control group (P<0.001).These alterations can be explained by malathion-induced changes inthe antioxidant system of cellsandcell membrane lipid peroxidation, caused by the production of free radicals (4). Based on previous studies, organophosphates can cause changes in the biochemistry and histopathology of various organs (19, 20). Ovary is one of the target organs, which plays an important role in reproductive function through producing oocytes and hormones (21). The present findingswere consistent with a similar study, which assessed the effects of malathionand endosulfanon lipid peroxidation of rat ovaries. It can be suggested that these toxins cause a significant increase in the level of MDA, confirming the production of free radicals and lipid peroxidation (22, 23). According to several previous studies, the increased level of MDA, induced by organophosphate intoxication, occurs in various tissues (e.g.,heart, lung, liver, and brain tissues), and is accompanied by an increase in the activities of antioxidant systems in these tissues (24, 25). In addition, in the present study, the level of MDA increased in ovariantissues of rats, treated with malathion, due to the production of free radicals and lipid metabolism (26).In the current study, comparison betweenexperimental group 1 and the control group showed that the mean glutathione level was lower in experimental group 1 (P<0.001).This difference seemed to be statistically significant. Previous studies have also confirmed the reduced amount ofglutathione and increased MDA level in erythrocytesof mice and rats in the presence offenthion, an organophosphate insecticide(25, 27). In our previous study on diazinon is an organophosphate poison was found that glutathione level significantly decreased and MDA level significantly increased in ovarian tissue of femail rats (28). Also, it has been shown that glutathione level is significantly decreased in the blood of malathion-treated rats (29). Moreover, a significant decline has been reported in glutathione level in blood and liver tissues due to malathiontoxicity (30).Due to exposure tomalathion, which is an organophosphate compound, the antioxidant system of the body becomes activated against ROS (31). Vitamin C is a water-soluble vitamin, which can reduce the amount of free radicals via its antioxidant features (32).In the present study, as previously stated, vitamin was intraperitoneally injected for 14 days in rats. In experimental group 3 (malathion and vitamin C), a significant increase in the level of glutathione and a significant decline in MDAlevel were reported, compared to the malathiongroup (P<0.002). Therefore, we can conclude that vitamin C was able to improve oxidative stress.Based on the literature, use of vitamin E and C in rats,exposed todiazinon (which is anorganophosphate toxin),can significantly improve lipid peroxidation in erythrocytes (33). Also, oxidative stress, induced by diazinonin brain tissues,is significantly decreasedby vitamin C and E (34).In addition, it has been shown that vitamin C can prevent hepatotoxicity induced by methyl parathion in rats (35).Based on the findings, we can conclude that oxidative stress in malathion-treated rats can cause cellular toxicity.The need fortreatmentand compliance with the instructions is strongly feltfor preventing the entryof toxins to the body, deterring toxicity-induced tissue damages,and preventing other disorders such as gonadal dysfunction and risk of sterility.

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