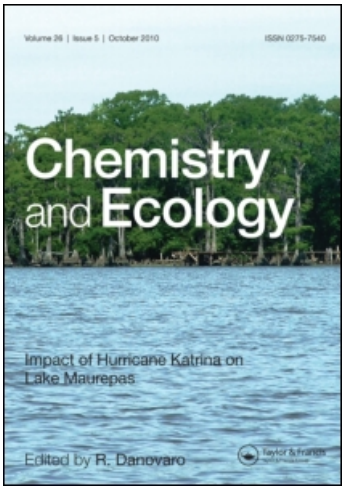

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Ferda Ari[a]; Egemen Dere[a]
[a] Faculty of Science and Art, Department of Biology, Uludag University, Nilüfer, Bursa, Turkey

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Glutathione S-transferase activity in rats exposed to methyl parathion

Ferda Ari* and Egemen Dere

Faculty of Science and Art, Department of Biology, Uludag University, Nilüfer, Bursa, Turkey

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Methyl parathion is an organophosphate insecticide that has been used in agriculture and the domestic sector for several years. This pesticide and others, arriving through different processes, exert significant effects on water quality with serious consequences for environmental and human health. The main objective of this study was to investigate the changes of Glutathione S-transferase enzyme activity in methyl parathion exposed rat tissues. For this purpose, wistar rats (*Rattus norvegicus*) were injected intraperitoneally with 7 mg kg^{-1} dose of methyl parathion, while corn oil was applied to control groups in the same way. The liver, kidneys, brain and small intestine were quickly removed after 0, 2, 4, 8, 16, 32, 64, and 72 hours of injection of methyl parathion and the glutathione S-transferase activity was determined in these tissues. As a result it was seen that glutathione S-transferase activity increases in all tissues in the group of male and female rats to which methyl parathion was given. The increase of glutathione S-transferase activity may be a result of methyl parathion's toxic effect because it is one of the most important enzymes of detoxification metabolism.

Keywords: methyl parathion; glutathione S-transferase; rat

1. Introduction

Organophosphate insecticides (OPIs) are a group of compounds that have been used as pesticides as well as chemical agents. This group of chemicals includes insecticides such as malathion, diazinon, chlorpyrifos, azamethiphos, dichlorvos, parathion and methyl parathion (MP) [1]. The uncontrolled use of these insecticides in agriculture can cause the change of ecological balance and thus many non-target organisms will become victims [2]. Also exposure of humans to MP may result in a lethal cholinergic poisoning [3].

Once OPIs enter the body, they are metabolised and are distributed into various areas of the body where they can cause damage [4]. Pesticide exposure in humans has been associated with an increase in spontaneous abortion, infertility, malformations, infectious and autoimmune diseases, as well as cancer [5].

MP is one of the most widely used OPIs in agriculture and the domestic sector (to kill insects with spray) [6]. MP is classified by the World Health Organisation [7] as a Category

*Corresponding author. Email: ferdaoz@uludag.edu.tr

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Ia (extremely toxic) and by the United States Environmental Protection Agency [6] as a Toxicity Category I (most toxic) insecticide. MP is also one of the most used OPIs in the region of Bursa, Turkey.

MP and its active metabolite, methyl paraoxon, exert their profound toxic effect by inhibiting the activity of acetylcholinesterase in the nervous system [8]. In addition, MP has increased erythrocyte carbonic anhydrase activity in Saanen goats [9].

Glutathione S-transferases (GSTs) are a family of phase II enzymes that have been shown to play an important role in the disposition of a wide range of environmental chemicals, pesticides and other reactive intermediates [10]. GST primarily catalyses the conjugation of electrophilic compounds with the thiol group of reduced glutathione (GSH), generally making the resultant products more water soluble and extractable than the non-GSH conjugated substrates [11]. Fujioka and Casida [12] have shown that GST is an important contributor to pesticide detoxification.

It has been shown that glutathione forms conjugates with MP. This conjugation is carried out by cytoplasmic GSTs [13]. GST is involved in the dealkylation of MP forming S-methylglutathione [14].

In our study, the effect of MP on the activity of GST enzyme in the liver, kidney, brain and small intestines is investigated. By defining the changes in the GST enzyme activity, the aim is to contribute to studies researching the detoxification metabolic means of MP, one of the most important chemicals in the agricultural struggle with pests.

2. Materials and methods

2.1. *Animals*

Wistar rats (*Rattus norvegicus*) weighting 200–250 g were used. They were purchased from the Experimental Animals Feeding and Research Centre of Uludag University Medical Science Faculty Bursa-Turkey. Animals were acclimatised in a 12-h light/dark cycle at 21–23 °C. Animal care was conducted according to institutional guidelines.

2.2. *Animal treatment*

For each trial period four rats (2 male; 2 female) from the control group and eight (4 male; 4 female) from the experimental group were used (totalling 32 for control and 64 for experimental group). Control groups were treated with corn oil while experimental groups were injected intraperitoneally with 7 mg kg^{-1} (LD_{50}) dose of MP. The rats were left without food and water for 24 h before injection, ensuring the start of metabolism of animals in both groups at the same time. Following injection, food and water were regularly given to the animals until the trial periods were completed. Treated and control rats were kept in plastic metabolic cages. Animals were killed, via cervical dislocation, at each time point of 0, 2, 4, 8, 16, 32, 64, and 72 h after injection. The liver, kidneys, brain and small intestine were quickly removed and were perfused in ice-cold 0.15 M KCl. The homogenates were prepared and homogenised at 2000 rpm in a T-line laboratory stirrer type homogeniser. Each homogenate was centrifuged in a Dupont Instruments Sorvall 'RC-5 super speed refrigerated centrifuge' at 48000 g for 30 min.

2.3. *Protein determination*

Protein concentration was determined with the method of Bradford [15] and bovine serum albumin was used as protein standard.

2.4. *Glutathione S-transferase activity*

The activities of GST were determined according to the method of Habig et al. [11]. One unit (U) of activity was defined as the formation of 1 μ mol/min of conjugated product.

2.5. *Statistical analysis*

Data were analysed using SPSS 13.0 for windows. Independent t-test was applied between data of control and experiment periods. The significance was calculated using one-way analysis of variance (ANOVA) and Student's t-test. A value of $p < 0.05$ was taken as statistically significant.

3. Results

3.1. *Liver GST activity*

When GST activities are examined in the liver of rats for the effect of MP, it is generally observed that there is activation in all experiment periods in male and female. While these activations increase until the 4th hour in both male and female, they decrease at the 8th and 16th hours. Afterwards it is seen that activations are increased again (Figure 1). While activities at 4, 32, 64, 72 h on males and at 32, 64, 72 h on female are significant ($p < 0.05$), in other experiment periods they are not significant ($p > 0.05$).

3.2. *Kidney GST activity*

There is an increase in GST activity in all experimental periods in the kidney in male and female rats compared with the control group. These increases are significant ($p < 0.05$) except for at the beginning and 16th hour. Another situation that attracts attention is that activation of GST occurring in females because of MP is higher than in males (Figure 2).

3.3. *Brain GST activity*

It is observed that GST activity increased in all experimental periods in male and female rats. Significant results in GST activity were recorded in other experiment groups except for during

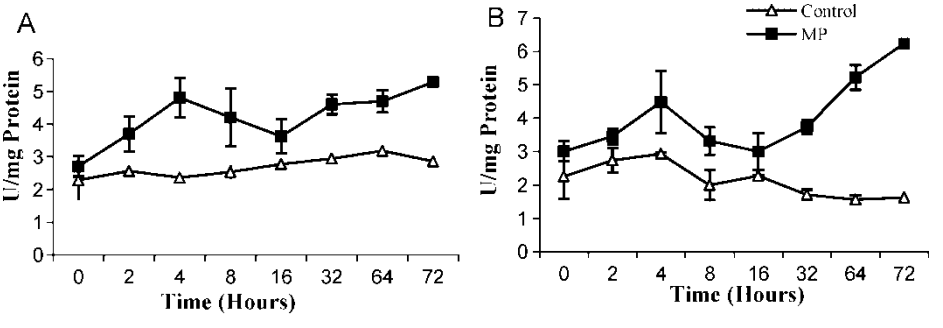

Figure 1. Change in GST activity in liver of MP-treated group. (A) Male, (B) Female.

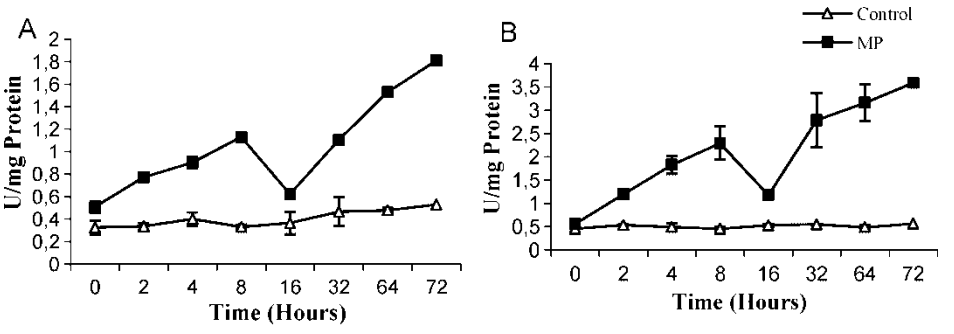

Figure 2. Change in GST activity in kidney of MP-treated group. (A) Male, (B) Female.

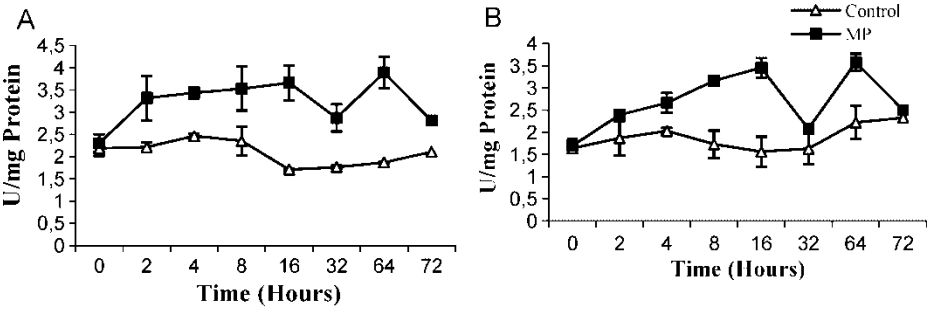

Figure 3. Change in GST activity in brain of MP-treated group. (A) Male, (B) Female.

the first period and 72nd hour in male rats ($p < 0.05$). However in female rats, it is seen that significant results occurred only after 8, 16 and 64 hours ($p < 0.05$) (Figure 3).

3.4. *Small intestine GST activity*

The GST activity of small intestine tissue increased in male and female rats compared with the control group. These results were found to be significant at all experiment periods except for during the first hour in male and female rats ($p < 0.05$) (Figure 4).

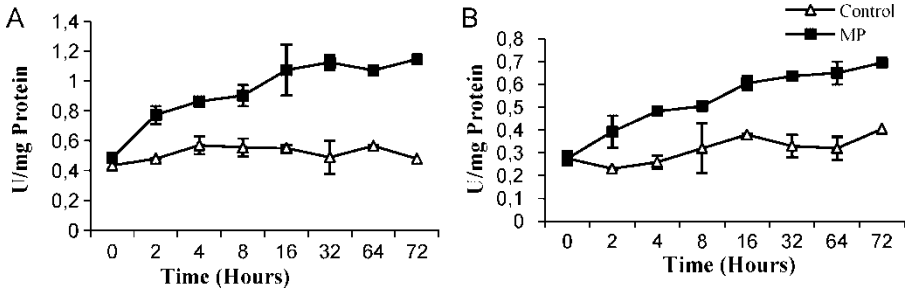

Figure 4. Change in GST activity in small intestine of MP-treated group. (A) Male, (B) Female.

4. Discussion

Environmental pollution by pesticide residues is a major environmental concern due to their extensive use in agriculture [16]. The continuous and widespread use of insecticides for crop protection is expected to expose non-target organisms through contaminated feed [17] and these will thus be in danger of chronic toxicity [18]. MP is an OPIs that is toxic to several target organs, predominately the central nervous system, especially when metabolised to the active metabolite methyl paraoxon [4].

GSTs are a phase II detoxification enzyme family that can mitigate the cellular toxicity of a number of endogenous and environmental chemicals. GSTs are also a common biomarker implicated in the detoxification of pesticides and in the mechanism of resistance to pesticides by over expression of these enzymes [19–22].

In this study, we employed detoxification of MP by measuring the GST activity in some tissues. It is observed that GST activity is increased in liver, kidneys, brain and small intestine by MP. We think that this enzyme which plays an important role in detoxification increases in order to get rid of the toxic effects of MP. Bammler et al. [13] showed the ability of cytosolic GST to detoxify the MP and that it showed the highest activity in rats. It is suggested that GSTs catalyse the conjugation of a wide variety of electrophilic substrates to reduced glutathione and thus protect the cell against chemically-induced damages in hepatic and extrahepatic tissues [18,23].

In our study, GST activities in liver and kidney are higher than in other tissues. In one study, it is shown that specific activities of liver enzymes such as acid phosphatase, alkaline phosphatase, glutamate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase are increased by MP [24]. In another study, it is seen that MP causes significant increases in activity of acid phosphatase, alkaline phosphatase and amino transferases in plasma in female rats. However the level of esterase activity in plasma decreased significantly [25].

MP causes cellular damage; therefore enzyme activities may change. In one research study, the effect of 2 to 4 mg/kg/day, for 3 months MP dose on brain tissues was studied, and as a result, it was seen that there was cellular damage in rat brain tissues. In addition, it was found that, MP inhibits butrylcholinesterase which may play a role in neuronal development in the nervous system [26]. Our study suggests that the GST activity increased in brain tissues of male and female rats. These increments may result in disruption of MP in the nervous system. The increase in GST activity was decreased in both male and female rats, at 16 h in liver and kidney but only at 32 h in brain. The reason for this inhibition is not only MP itself, but also its metabolites.

Generally, in our study, it can be seen that GST activity is different in male and female rats. This differential action may result from the hormonal status of the rats. A recent work has shown that exposure to cypermethrin and methyl parathion mixture at $1/30$ LD_{50} dose had effects on endocrine hormone levels and immune functions in rats [27]. However, MP has deleterious effects on the reproductive system of male rats [28].

Higher GST activities were found in other cancers, including cancer of the colon, rectum, stomach, lung and breast, but not of the kidney and liver [29–31]. Increased GSH concentration and the GST activity found in primary malignant and metastatic ovarian tumor samples were independent of histopathological and clinical prognostic factors, suggesting that they could be early markers for ovarian carcinomas [32].

In conclusion, based on experimental evidence obtained here we can suggest that GST can be useful biomarkers in field monitoring of the effects of pesticide exposure on wildlife. Further studies are necessary, however, to investigate the kinetics which need to be conducted to explain the effects of MP on GST activity. MP, employed excessively and unconsciously in agriculture, pollutes nature and indirectly results in negative changes to living things. Hence, it is of critical importance to public health that we be careful and selective in using such chemicals, and inform consumers and producers of possible and real damage.

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