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Lactate Dehydrogenase Enzyme Activity in Some Tissues of Rats Exposed to Methyl Parathion

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Abstract

Insecticides are of great importance in combating insects that damage agricultural products. However, the risks they pose for humans and the environment cannot be ignored. One of these frequently used insecticides is methyl parathion (MP), an organophosphate compound. In this article, the effect of methyl parathion on lactate dehydrogenase enzyme activity was investigated. Wistar rats were used in experimental studies. Small intestine, kidney, brain and liver tissues were removed 2, 4, 8, 16, 32, 64 and 72 hours after methyl parathion was injected intraperitoneally in male and female rats at a dose of 7mg/Kg. Lactate dehydrogenase enzyme activities were determined from the supernatants obtained. It is noteworthy that lactate dehydrogenase activity was significantly increased in almost all experimental periods in all tissues studied. Small increases in lactate dehydrogenase activity generally do not indicate any harm. However, significant increases can indicate many major conditions such as liver disease, circulatory failure and muscle damage. Our research results and discussion show that methyl parathion can be dangerous to human health.

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Anahtar Kelimeler

Methyl parathion, Organophosphate insecticide, Lactate dehydrogenase, Rat

Öne Çıkanlar

It has been observed that lactate dehydrogenase, which is an important enzyme of anaerobic glycolysis, is affected by methyl parathion. It should be kept in mind that this insecticide can cause damage to the small intestine, kidney, brain and liver. When methyl parathion is to be used in agricultural control, the application doses must be followed.

Metil-Parathion'a Maruz Kalmış Sıçanların Bazı Dokularında Laktat Dehidrojenaz Enzim Aktivitesi

Abstract

Tarımsal ürünlere zarar veren böceklerle mücadelede insektisitlerin büyük önemi vardır. Buna rağmen, insan ve çevre için oluşturdukları riskler göz ardı edilemez. Sıklıkla kullanılan bu insektisitlerden birisi de bir organofosfat bileşiği olan metil parathiondur. Makalede metil parathionun laktat dehidrogenaz enzim aktivitesi üzerine olan etkisi araştırılmıştır. Deneysel çalışmalarda wistar sıçanlar kullanılmıştır. Erkek ve dişi sıçanlara 7mg/Kg dozunda metil parathion intraperitonal olarak enjekte edildikten 2, 4, 8, 16, 32, 64, 72 saat sonra ince bağırsak, böbrek, beyin ve karaciğer dokuları çıkarılmıştır. Elde edilen süpernatantlardan laktat dehidrogenaz enzim aktiviteleri tayin edilmiştir. Çalışılan bütün dokularda hemen hemen tüm deney periyotlarında laktat dehidrogenaz aktivitesinin önemli ölçüde artmış olması dikkat çekicidir. Laktat dehidrogenaz aktivitesindeki küçük artışlar genellikle herhangi bir hasara işaret etmez. Ancak, önemli artışlar, karaciğer hastalıkları, dolaşım yetmezliği ve kas hasarı gibi birçok önemli durumu gösterebilir. Araştırma sonuçlarımız ve tartışma metil parathionun insan sağlığı için tehlikeli olabileceğini göstermektedir.

Keywords

Metil-parathion, Organofosfat insektisit, Laktat dehidrogenaz, Sıçan

Highlights

Anaerobik glikolizin önemli enzimi olan laktat dehidrogenazın metil parathiondan etkilendiği görülmüştür. Bu insektisidin ince bağırsak, böbrek, beyin ve karaciğerde hasar oluşturabileceği unutulmamalıdır. Metil parathion zırai mücadelede kullanılacağı zaman uygulama dozlarına mutlaka uyulmalıdır.

1. Introduction

Pesticides are chemicals used against agricultural pests. These are formulated to control or remove pests that destroy agricultural products and spread disease. The term agricultural pest can be defined as any organism that harms people’s agricultural activities and farmland. The term pesticide defines the chemicals used against these pests. However, viruses or bacteria are sometimes used against agricultural pests. This situation is defined as biological control (Tadeo 2008). These substances can leak into streams, rivers and the sea, and directly or indirectly harm people (Konstantinou et al. 2006). The main goal in the use of pesticides is to destroy harmful organisms. Thus, pesticides can increase product stability and quality, while facilitating the use of the product (WHO 2006). Care should be taken when using pesticides that are harmful to human health. A dose that is toxic to humans should not be administered in the use of each pesticide. Attention should be paid to the doses used against pests (WHO 2006).

Pesticides can be classified in various ways, taking into account their different properties. For example, according to the pest groups they affect, insecticides kill insects, rodenticides kill rodents, fungicide kills fungi, etc. Pesticides are also classified according to the active ingredient group in their compounds. There are several chemical families of insecticides such as organophosphates, carbamates, synthetic pyrethroids and organochlorines. Insecticides containing phosphorus atoms in the hydrocarbon molecular structure are called Organophosphorus (OP) insecticides. OP pesticides are the most preferred chemical group among pesticides in the world. They are widely used all over the world in agriculture, veterinary medicine, gardens and homes. OP compounds are detoxified relatively fast in biological systems (Tadeo 2008). However, OP insecticides can remain in the form of basic molecular structure or hydrolysis products in the air, soil and surface water for a long time after application to the field. This situation creates acute health problems for living things (Kavikarunya and Reetha 2012; Pailan and Saha 2015), because OP compounds can easily reach people through the respiratory tract, skin and gastrointestinal tract (Eskenazi et al. 2007). OP poisoning causes significant morbidity and mortality in many countries (Peter and Cherian 2000).

In a study conducted on *Allium cepa*, it was suggested that catalase (CAT), glutathione S-transferase (GST) and superoxide dismutase (SOD) activities were increased, while ascorbate peroxidase (APX) and glutathione reductase (GR) activities were decreased with the effect of malathion. In the same study, malathion was shown to cause DNA damage in *Allium cepa* stem cells (Srivastava and Singh 2020).

OP compounds are neurotoxic in target organisms. These insecticides also show similar effects when they reach humans (Klaassen and Doull 1996). These are all irreversible acetylcholinesterase inhibitors (synaptic and globular). OPs also affect many esterases and some enzymes, such as butyrylcholinesterase (AFSSAPS 2010).

Although OP pesticides affect many enzymes, it especially inhibits acetylcholinesterase enzyme (Howard and Pope 2002). Inhibition of this enzyme causes an increase in the amount of acetylcholine, and increased acetylcholine causes too much stimulation of cholinergic receptors (Klaassen and Doull 1996; Samuel et al. 2007). This situation is also seen as a serious cause of poisoning (Brown 2013).

Methyl parathion (MP) [O, O-dimethyl O-4-nitrophenylphosphorothioate (IUPAC)], $C_8H_{10}NO_5PS$ is an odorless substance in white crystals. It undergoes detoxification in the liver and turns into methyl paraoxon (Figure 1) (Celik and Süzek 2008).

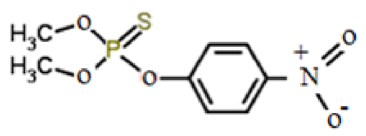

Figure 1. Molecular structure of methyl-parathion

LDH is an important enzyme responsible for pyruvate formation in anaerobic glycolysis and NADH to NAD^+ during glycolysis under hypoxia conditions. Although this enzyme is found in all cells, it is found more in muscle, liver and kidney. Changes in LDH levels occur in association with a wide range of diseases. That is why an increase in LDH levels is analyzed in many clinical laboratories as an early indicator of tissue damage.

In this study, we used MP, an insecticide from the organophosphate family, to detect the effect on LDH enzyme activity in some organs (brain, liver, kidney and small intestine) of rats. This pesticide has a negative effect on human health. As Celik and Süzek (2008) noted, subacute MP administration increases the level of tissue damage serum markers and the number of white blood cells in rats. These findings led us to consider the effects of MP on enzyme activities (Celik and Süzek 2008).

For rodents, the LD_{50} dose of methyl parathion ranges from 6-24mg/kg. The use of this pesticide is banned in many countries because it is dangerous. Despite this, there are reports of acute methyl parathion poisoning in the world and in our country (Isbister et al. 2007). Oral dose of LD_{50} in rats is 6.9 mg kg^{-1} (Liu et al. 2007; Sun et al 2000). The United States environmental protection agency has announced the LD_{50} doses, chronic and acute interactions, and changes in tissues of methyl parathion in various experimental animals and humans through inhalation, inhalation, skin, etc. (Morrow 1998). In our study, 7 mg kg^{-1} was used as a dose.

In our previous study, we revealed the effect of MP on the activity of glutathione S-transferase. We found that glutathione S-transferase activity was increased in all studied tissue types of male and female rat groups to which MP was administered (Arı and Dere 2008). Our study will contribute to knowledge of the negative effects of MP on health, especially in the field of anaerobic glycolysis studies.

2. Material and method

2.1. Material

2.1.1. Animals

We used Wistar rats (*Rattus norvegicus*) with a weight ranging between 200 and 250g that were purchased from the Center for Experimental Animal Nutrition and Research at the Faculty of Medical Sciences at Bursa Uludağ University, Bursa, Turkey. Care of animals before the experiment was carried out in accordance with the rules. The animals were acclimatized at a temperature of 21-23 °C. The rats used in the study were tested by an expert authorized with the certificate numbered 2007/129.

2.2. Method

2.2.1. Animal treatment

Two groups of rats were used: A control group composed of 4 rats (2 males; 2 females) and an experimental group composed of 8 rats (4 males; 4 females). Rats were allocated to experimental periods for 2, 4, 8, 16, 32, 64 and 72 hours. A total of 28 control rats and 56 experimental rats were used. The control groups were injected with corn oil while the experimental groups received a dose of 7mg kg^{-1} (LD_{50}) of MP via intraperitoneal injection. Methyl parathion in analytical standard was purchased from Sigma-Aldrich, St. Louis, MO. The rats were left without food and water for 24 h before injection, ensuring that the animal metabolism in both groups was synchronous. After the injection, the animals were regularly fed with food and water until the end of the test periods. The animals were sacrificed by cervical dislocation at 2, 4, 8, 16, 32, 64 and 72 h after the injection.

After the tissues (small intestine, brain, kidney, liver) to be tested were removed, they were infused into 0.15M KCl. Care was taken to remove the tissues in a cold environment. Tissues were homogenized at 2000 rpm in a T-line laboratory stirrer type homogenizer. Each homogenate was centrifuged in an “RC-5 High-Speed Refrigerated Centrifuge” (Dupont Instruments Sorvall) at 48000g for 30min.

2.2.2. Protein determination

Protein amounts of homogenates were determined by the Bradford method using bovine serum albumin as standard (Bradford 1976).

2.2.3. Lactate dehydrogenase activity

LDH activity was determined according to the method of Bohringer and Mannheim, (1973). One unit of activity (U) was defined as the formation of 1μmol/min of conjugated product. All comparisons were made with the control group in order to reveal the methyl parathion effect.

2.2.4. Statistical analysis

The data obtained from the experimental results were analyzed using the GraphPad Prism viewer mode 8 program for Windows, and the experimental and control groups were evaluated with independent t-test. The significance was calculated using one-way analysis of variance (ANOVA) and Student’s t-test. Values of $p<0.05$ were considered statistically significant.

3. Results and Discussion

3.1. Liver LDH activity

The graph given in Figure 2 shows that LDH activity in the liver in rats injected with MP increased at 8, 16, 32, 64 and 72 hours. The activities at 8, 16, 32, 64 and 72 h in male and in female rats were significant (p<0.01; p<0.001). The change in LDH activity was not significant at 2 and 4 h after MP administration (p>0.05). It is noteworthy that the highest activity for LDH in both males and females was at the 8th hour.

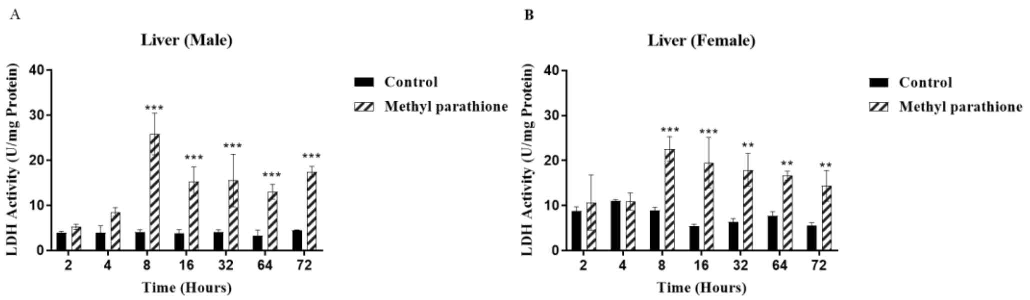

Figure 2. Change in LDH activity in liver of MP-treated group. (A) Male, (B) Female
* Denotes statistical differences compared to control:
** (p<0.01);
*** (p<0.001)
Data are presented as mean±SD

SD: Standard Deviation

3.2. Kidney LDH activity

LDH activity was significantly higher during the experiment hours in male and female rats injected with MP (Figure 3). While change in LDH activities in males was statically significant ($p<0.001$) in all experimental periods, it was significant in females for only 8, 16, 32, 64 and 72 hours ($p<0.001$). Activity increases seen in female rats at 2 and 4 hours were not found to be statistically significant.

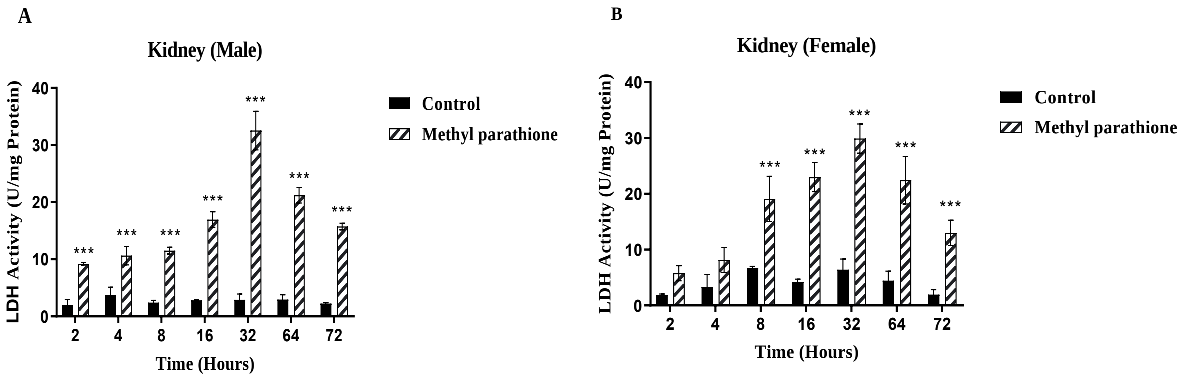

Figure 3. Change in LDH activity in kidney of MP-treated group. (A) Male, (B) Female
* Denotes statistical differences compared to control:
*** ($p<0.001$)
Data are presented as mean±SD
SD: Standard Deviation

3.3. Brain LDH activity

Changes in brain LDH activity are given in Figure 4. Significant increases in activity in both male and female rat brains were observed at 8, 16, 32, 64 and 72 hours ($p<0.01$; $p<0.001$). Change in LDH activity was not significant in the other experimental periods (2 and 4 hours).

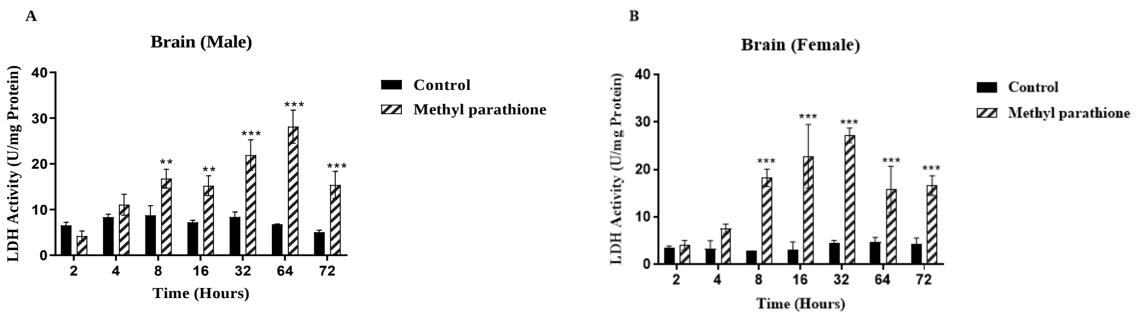

Figure 4. Change in LDH activity in brain of MP-treated group. (A) Male, (B) Female
* Denotes statistical differences compared to control:
** ($p<0.01$)
*** ($p<0.001$)
Data are presented as mean±SD

SD: Standard Deviation

3.4. Small intestine LDH activity

Changes in small intestinal tissue LDH activities are given in Figure 5. The activities at 4, 8,16, 32 and 64 h in males was significant ($p<0.01$; $p<0.001$). In the other experimental periods (2 and 72 h), they were not significant. While statistically significant high LDH activity was observed in female rats at 8, 16 and 32 hours ($p<0.01$; $p<0.001$), increases in other experimental periods (2, 4, 64 and 72 hours) were not significant.

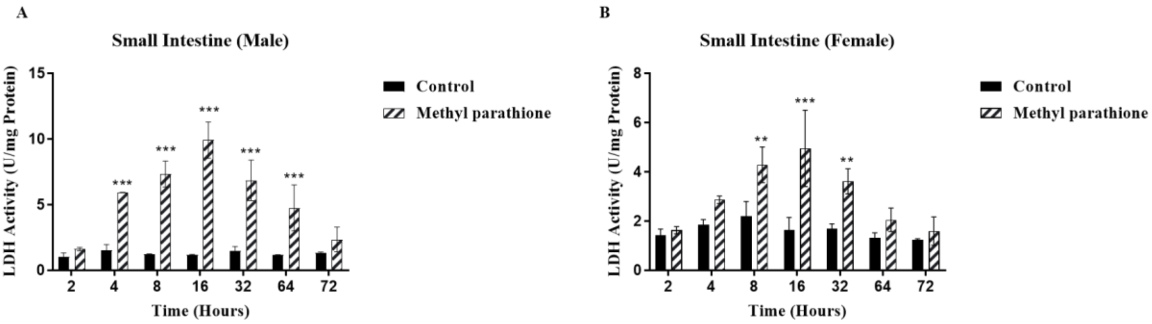

Figure 5. Change in LDH activity in small intestine of MP-treated group. (A) Male, (B) Female

* Denotes statistical differences compared to control:
** ($p<0.01$)
*** ($p<0.001$)
Data are presented as mean±SD
SD: Standard Deviation

Farmers have always sought to protect their agricultural crops from insects, bacterial diseases, parasitic plants and others. Although different methods have been developed to combat pests in agriculture, the direct toxic effects of these pesticides on the consumer and indirect ones on the general environment remain a prominent challenge. In recent years, the indiscriminate and excessive use of pesticides has caused serious harm to humans, animals and the environment in general. Even if OP pesticides are used locally, their widespread use in agriculture, veterinary medicine and at home is a serious problem all over the world (Pimentel 1995; Tripathi and Shasmal 2011; Abhijith et al. 2016).

In this study, changes in LDH enzyme activity, which is one of the important enzymes of anaerobic glycolysis, were investigated in order to contribute to the knowledge on the effects of MP, which is widely used in the agricultural struggle, on living things.

LDH activity, which stands out as an important enzyme in energy production, should be at certain values. This enzyme, which is found in different tissues, is especially important in kidneys, pancreas, liver, heart, brain, small intestine, blood cells and skeletal muscles, because it meets the energy needed by the body with production in these tissues. Changes in LDH activity are considered one of the important markers of tissue damage (Abhijith et al. 2016; Diamantino et al. 2001). LDH activity was observed in the kidney, liver, brain and small intestine, in both experimental rats treated with MP and control group rats at regular intervals (2, 4, 8, 16, 32, 64 and 72 hours). Variations in LDH activities in rat tissues treated with MP were compared with control groups. LDH activity was found to be higher in rats treated with MP in the kidney, liver, brain and small intestine compared to the LDH activity of the control group (Figs. 2, 3, 4 and 5).

Celik and Süzek, (2008) investigated the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), LDH and alanine aminotransferase (ALT) enzymes, which are important markers for tissue damage, after oral administration of 10 ppm MP dose to rats. They showed that the applied dose of methyl parathion increased the activity of serum AST, ALT, ALP and LDH enzymes. They suggested that these increases associated with liver tissue damage were caused by MP administration (Celik and Süzek 2008). MP does not only affect LDH levels. Arı and Dere (2008) showed in their previous studies that glutathione S-transferase activity, which is one of the most important enzymes of detoxification metabolism, was increased in all examined tissues in male and female rats that were given MP. High LDH activity is an indicator for presence of anaerobic glycolysis instead of aerobic glycolysis. Increased anaerobic metabolism can be considered as a defense mechanism for the cell in order to destroy this toxic substance and to deal with the stress that occurs. High levels of LDH indicate tissue damage (Khare et al. 2019; Das et al. 2004, Khan et al. 2008). Increases in LDH enzyme activity in tissues can be considered as one of the signs of toxic damage caused by MP in tissues. Bagchi et. al. (1995) examined the effects of polyhalogenated cyclic hydrocarbons and chlorinated acetamide herbicides in rats *in vivo*, and also looked at the effects of these substances in PC-12 cells, a neuroendocrine cell line developed from rat pheochromocytoma tumors. They found that LDH activity increased in both studies. The increase in LDH activity is considered as an expression of tissue damage. It was reported by Alttas and Haffor (2010) that LDH activity can be a vital biomarker for oxidative stress. Generally, organisms exposed to stress use antioxidant enzymes to overcome stress. In this sense, activities and enzyme amounts vary depending on the dose and type of exposure to stress (Abhijith et al. 2016; Gravato et al. 2006). One of the reasons for the increase in LDH activity may be the effect of MP on the endocrine system. In one study, it was suggested that MP affects the testis functions by affecting the endocrine system (Sharma et al. 2014). Increases and decreases in LDH activities are similar between female and male rats in all studied tissues and experimental groups. There were no statistically significant differences in LDH activities between male and female rats.

Conclusion

Human awareness of the severity of pesticides remains limited. Humans still do not know the correct way to deal with pesticides. In light of the scientific research conducted in this field, the results of the current research indicate that exposure to an MP pesticide leads to a rise in the LDH enzyme in the body. Thus, we can consider changes of LDH activity as a vital indicator for assessing pesticide toxicity to human health and the environment. More studies should be done on the effect of pesticides on all body tissues to provide a clearer picture of how to address health risks.

In conclusion, MP is a pesticide with negative side effects. When it is used for pest control, the recommended usage instructions should be strictly followed and the people who use it should wear protective clothing.

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Conflict of interest statement

The authors declare no competing interests

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