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*Original Article*

# *Effect of Intravenous Lipid Emulsion Therapy on Serum Pseudocholinesterase in Experimental Model of Diazinon Intoxication*

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***ABSTRACT***

*The aim of this study was to investigate the effect of intravenous lipid emulsion (20% lipid solution) on serum pseudocholinesterase in an toxication model of diazinon. Organophosphate poisoning has a different importance for individuals those admitted to the emergency service due to poisoning. The most commonly used organic phosphorus compounds are diazinon, malathion, and parathion. Intravenous lipid emulsion (ILE) treatment is used as a new treatment method in systemic toxicity cases caused by local anesthetics. Twenty-one male Wistar albino rats, (weighing 180-200 g) were randomly divided into three equal groups. Group I was control, while Group II was diazinon and Group III was diazinon + lipid emulsion treatment. Only 1 ml corn oil was given by gavage to the rats in Group I. A diazinon dose of 335 mg/kg was given by gavage to the rats in Group II. In addition to diazinon, a 20% lipid solution (3 ml/kg) was administered via tail vein to the rats in Group III. At the end of the experimental period, blood samples were taken from animals, and serum pseudocholinesterase levels were measured. When the pseudocholinesterase levels were analyzed, no significant difference was found between diazinon and diazinon + lipid emulsion treatment groups. However, a significant difference was found between control and the others ($p < 0.05$). In our study, there was no positive effect of lipid treatment detected on serum pseudocholinesterase. Toxication models with lower doses can be designed in future studies.*

***Key words:*** *Diazinon, pseudocholinesterase, lipid*

## *Diazinon İle Meydana Getirilen Deneysel Zehirlenme Modelinde Serum Psödokolinesteraza İntravenöz Lipid Emülsiyon Tedavisinin Etkisi*

***ÖZET***

*Diazinon ile oluşturulan intoksikasyon modelinde İntravenöz lipit emülsiyon tedavisinin (% 20 lipid solüsyonu) serum pseudokolinesteraz düzeyi üzerine etkisinin araştırılması amaçlandı. Organofosfat zehirlenmeleri acil servise başvuran hastalar arasında önemli bir yer tutmaktadır. En sık kullanılan organik fosforlu bileşikler diazinon, malation, paration'dur. İntravenöz lipit emülsiyon (ILE) tedavisi lokal anesteziklerin yol açtığı sistemik toksisite durumlarında yeni bir tedavi yöntemi olarak kullanılmaktadır. 21 adet Wistar-albino grubu sıçan yedişerli üç eşit gruba ayrıldı. Grup I, kontrol; Grup II, diazinon; Grup III, diazinon + İntravenöz lipit emülsiyon tedavisi grubu olarak düzenlendi. Grup I'e sadece 1 ml mısır yağı gavaj yolu ile verildi. Grup II'ye 335 mg/kg diazinon (gavaj yolu ile) verildi. Grup III' e diazinon'a ilave olarak kuyruk veninden 3 ml/kg olacak şekilde %20 lipit solüsyonu verildi. Deney sonunda hayvanlardan alınan kanlarda serum psödokolinesteraz düzeyleri bakıldı. Psödokolinesteraz düzeyleri analiz edildiğinde diazinon ile diazinon+lipit grubu arasında istatistiksel açıdan önemli bir fark bulunamadı. Bununla birlikte kontrol grubu ile diğer gruplar arasında önemli farklılık tespit edildi ($p < 0.05$). Çalışmamızda lipit tedavisinin serum pseudokolinesreraz üzerine olumlu etkisi tespit edilemedi. Daha düşük dozlardaki toksikasyon modelinde yeni çalışmalar denenebilir.*

***Anahtar kelimeler:*** *Diazinon, psödokolinesteraz, lipid.*

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## INTRODUCTION

Organic phosphorus poisoning is one of the major cases in emergency admissions. These are toxic compounds mainly used as pesticides. Malathion, diazinon, parathion and chlorpyriphos (1) are the most commonly used organic phosphorus compounds. Exposure can be occurred through skin and inhalation, however the most common acute poisoning is caused by ingestion (2). Symptoms depend on the balance between nicotinic and muscarinic receptors. Clinical manifestations range from non-specific nausea and vomiting to severe situations such as coma (3). It has three clinical features; i.e. cholinergic crisis depending upon the inhibition of acetyl cholinesterase, intermediate syndrome without a fully known mechanism, and prolonged neuropathic findings explained by inhibition of neurotoxic esterase enzyme (4).

Treatment of organophosphate toxicity showed complexity that consisting of specific and non-specific therapies. The main strategy in treatment is the inhibition of acetyl cholinesterase (5). Intravenous lipid emulsion (ILE) treatment is currently used as a new method in treatment for systemic toxicity cases caused by local anesthetics (6). In experimental studies, intravenous lipid emulsion treatment has shown to be effective in the treatment of cardiac arrest and cardiovascular collapse associated with bupivakain toxicity (7, 8). The aim of this study was to investigate the effect of intravenous lipid emulsion treatment (20% lipid solution) on serum pseudocholinesterase levels and on survival.

## MATERIALS AND METHODS

### *Animals*

This study was performed upon the approval of Local Ethic Committee of Gaziosmanpaşa University, Faculty of Medicine. Twenty-one albino Wistar rats were used in the experiment, and the average weight of the rats were within the range of 180-200 gr. The investigation was conducted based on the guidelines for the use and maintenance of laboratory animals.

### *Experimental procedure*

Rats were divided into three groups randomly, each containing seven rats. Group I was control, while animals in Group II were given diazinon, and the ones in Group III were given diazinon + Intravenous lipid emulsion treatment. Animals in Group 1 were received 1 ml corn oil through gavage. Animals in Group 2 were administered a diazinon dose of 335 mg/kg (a quarter of the lethal dose) via gavage. The animals in Group 3 had 1.5 ml/kg equivalent of 20% lipid solution in addition to diazinon applied 10 minutes later through an intravenous Cannula into the lateral tail vein. The treatment was repeated 30 min later. Each animal had a total of 3 ml/kg lipid treatment.

### *Biochemical Analyses*

The serum pseudocholinesterase levels in blood samples of rats had been planned to be performed 24 hours after the experiment, but the experiments were ceased earlier because the situation of rats were deteriorated 6 hours later in diazinon group, and 12 hours later in diazinon + lipid treatment group. Blood samples kept at −80 until the analyses were centrifuged at 5,000 rpm for 5 min. Pseudocholinesterase commercial kit of Roche Diagnostics (Mannheim, Germany) was used, and pseudocholinesterase measurement was performed in an auto analyzer (Roche Hitachi, Model C-501).

### *Statistical Analyses*

Normality and variance were tested using the One-Sample Kolmogorov-Smirnov, skewness and kurtosis, and histograms for each variable. Quantitative data were presented as means and standard deviation. Comparison between groups were performed by Kruskal-Wallis test, and significance between each group were completed using Mann-Whitney U test. Analyses were completed by using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 20.0 program. The statistical significance for all analyses was set at $p < 0.05$.

## RESULTS

The levels of pseudocholinesterase of the control, diazinon and diazinon+lipid groups are shown in Table 1 and Figure 1. The highest level of serum pseudocholinesterase level was obtained from the control group, followed by diazinon + lipid group. It was shown that there was significant difference found in serum cholinesterase levels between the control group and the diazinon group. However, no difference was detected between diazinon and diazinon + lipid group (Table 1).

***Table 1**. Comparison of serum pseudocholinesterase level between groups*

| Group | n | df | Mean Rank | Mean±SD | X2 | p |
|---|---|---|---|---|---|---|
| Diazinon + Lipid | 7 | | 7.14 | 21.28±9.89 | | |
| Diazinon | 7 | 2 | 7.86 | 26.57±16.95 | 13.436 | 0.001*† |
| Control | 7 | | 18 | 797.57±187.54 | | |

** p<0,05*
*†Kruskal-Wallis Test*
*Intergroup comparisons:*
*Diazinon+Lipid-Diazinon (p = 0.805§), Diazinon+Lipid-Control (p = 0.001§*), Diazinon-Control (p = 0.001§*)*
*§Mann-Whitney U test.*

## DISCUSSION

The main effect of organophosphates is through the increases in Ach levels that affect cholinergic receptors by the inhibition of acetyl cholinesterase (AChE) that break up acetyl choline. Organophosphate insecticides inactivate the cholinesterase enzyme through chemical changes in the enzyme by phosphorylation. In the absence of pharmacological intervention, they cause irreversible destruction of the enzyme. This is called ageing. The faster the ageing, the less effective the oxime treatments. In order for the oximes to be effective, they should be applied before this process (3). Zhou et al. (9) proposed two-step treatment protocols in organophosphate poisonings. In the first step, a rapid start of lipid treatment takes place, thus toxic material is isolated and its detrimental effect on the patients is lowered. In the second stage, organophosphate is eliminated from the body through hemoperfusion using coal particles (9). The theory used to explain the effect of lipid treatment is called "lipid sink". Based on this theory, lipid molecules make complexes with lipophylic drugs and trap toxic agents, leading to sinking of them within the emulsion (10). There are studies reporting that intravenous lipid emulsion treatment increases lethal dose threshold and reduces mortality in clinical cases of toxicity caused by lipophylic anesthetics (11). In bupivacaine-induced cardiac toxicity in rats, bupivacaine content in cardiac tissue was shown to be lowered in lipid treatment group (12). In a study by Finn et al. lipid treatment was studied in 61 patients who attempted to commit suicide by orally taking sertraline and quetiapine. They found that rapidly administered intravenous lipid emulsion after the beginning of resuscitation resulted in a significant improvement in consciousness (13). In an experimental study on rabbits where bicarbonate, serum physiological and lipid emulsion treatments were investigated in the treatment of clomipramine (a lipid soluble antidepressant) toxicity. Lipid emulsion restored clomipramine-induced hypotension better than sodium bicarbonate (14). Tebutt et al. investigated lipid emulsion for the treatment of verapamile-induced toxicity in rats, and found that it resulted in higher survival rates compared to serum physiological administration (15). In another study, Harvey and Cave reported that lipid emulsion treatment improves hypotension in rats caused by infusion of propranolol which has a high lipid solubility (16).

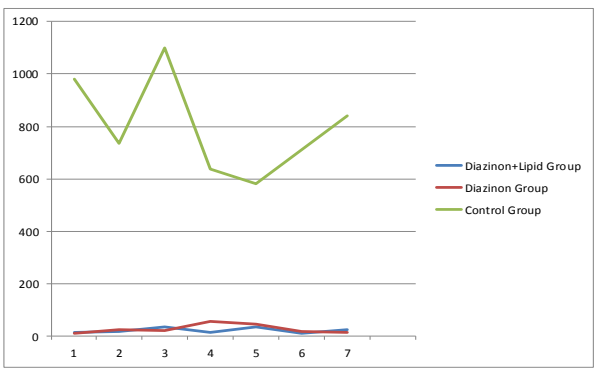


***Figure 1**. Serum pseudocholinesterase levels in the diazinon +lipid, diazinone, and control group.*

In the present study, increased pseudocholinesterase levels observed in rats in lipid treatment group showed no significance compared to diazinon group. This result suggested that failure in lipid treatment could be associated with higher doses of diazinon used in the study.

Several clinical and experimental studies have been shown in the literature dealing with the treatment of various toxicity cases by the administration of lipid emulsions. In the present experimental study on rats with organophosphate poisoning, we couldn't find any

beneficial effect of lipid treatment on serum cholinesterase levels. In contrast to this result, it can be speculated that lipid treatment may be effective in doses lower than the ones used in the present study, in which require further studies to elucidate.

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