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# Neurotoxic effects of subacute exposure of dichlorvos and methyl parathion at sublethal dosages in rats

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ABSTRACT

The present study was designed to understand the effects of sublethal dosages of dichlorvos (DIC) and methyl parathion (MP) on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in various tissues of rats exposed to 5 and 10 ppm of DIC and MP in drinking water *ad libitum* for 28 days continuously. According to the results, AChE activity was significantly decreased in all the tissues of rats treated with both dosages of DIC and MP except for in the lungs treated with both dosages of DIC. With regard to the BChE, MP caused a significant decrease in the liver, heart and lungs with 5 ppm dosage whereas it did not change the BChE activity in the other tissues with two dosages. Also, DIC caused a significant decrease in BChE activity in the heart tissue treated with both dosages and in the brain of rats treated with 5 ppm. The observations presented led us to conclude that the administrations of MP and DIC at sublethal concentrations inhibited AChE and BChE activities in the rats. These results suggest that inhibition of AChE may be a better biomarker for the assessment of neurotoxic effects in the living.



## 1. Introduction

A considerable literature exists describing the effects of pesticides on populations and communities of organisms under field conditions. Major effects of pesticides on animal and insect populations result primarily in significant changes in species abundance and associated shifts in dynamics; thus they have been resulted in an imbalance in the natural system [1]. Dichlorvos (DIC) and methyl parathion (MP) are two organophosphate (OPIs) insecticides which widely used in agriculture. OPIs are some of the most useful and diverse classes of insecticides in use for almost five decades. However, the uncontrolled use of these insecticides in agriculture and public health operation has increased the scope of ecological imbalance and thus many non-target organisms have become victims [2]. AChE and BChE share 65% of amino acid sequence homology and have similar molecular forms and active center structure despite being products of different genes on human chromosomes [3]. The main function of AChE is rapid hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses, and it is one of the fastest enzymes known [4]. But individuals whose BChE is absent does not correlate with any physiological abnormality. Its importance as a detoxification enzyme is growing interest in recent years. BChE is of pharmacological and toxicological importance, because it hydrolyzes ester-containing drugs and scavenges cholinesterase inhibitors including potent organophosphorus nerve agents before they reach their synaptic targets [5].

In the literature, it is reported that OPIs are neurotoxic in nature by acting as inhibitors of neuronal cholinesterase (ChE) activity [6]. However, some studies reported that OPIs caused lipid peroxidation [7–10] in vertebrates. Feeding studies indicate that dosages of DIC very much larger than doses which inhibit cholinesterase are needed to produce illness [11,12]. Campbell and Ofurum [13] investigated effects of DIC on serum and liver enzyme such as glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) changes in rats after short and long term exposure to dichlorvos. The study showed that DIC significantly elevated serum and liver GOT and GPT activities in rats after short and long term exposure to dichlorvos. Hasan and Ali [14] found a dose-dependent increase in the rate of lipid peroxidation in various regions of the brain of the rat after intraperitoneal administration of dichlorvos at concentrations ranging from 0.6 to 3 mg/kg body weight, daily for 10 days. In addition, Celik and Suzek [15] found that the administrations of DIC promote MDA content and fluctuate in the ADS in rats during the exposure.

On the other hand, mutagenic effects of MP have been studied to determine the chemical's ability to cause a change in the DNA sequence of a gene. A study involving three OPIs was completed and it was determined that dimethoate, DIC and MP, when administered to Wistar rats for a 6-week period of five treatment days per week at doses of 1/100, 1/75 and 1/50 of the $LD_{50}$, displayed no significance in mutagenicity [16]. The cytogenetic and cytotoxic

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effects of OPIs and organochlorine compounds following a single dose administration were studied. It was found that the frequency of chromosomal aberrations and micronuclei in bone marrow cells and an assay of the liver expressed the genotoxic capabilities of these chemicals [17]. It was reported that MP caused DNA damage in rats [18]. Further, it was found that MP, as a result a single-exposure, was the most hazardous tested organophosphate showing definite pathology in the livers of treated rats. It was also concluded from genotoxicity studies of organophosphorus pesticides that MP had some genotoxic effects [19]. On the other hand, the administrations of subacute MP elevate tissue damage serum marker enzymes, and increase the number of WBC [20] and promote MDA content and fluctuate in the antioxidant defense system [21] in rats. A significant increase in the percentage of abnormal sperm was observed in mice treated orally with MP [22]. MP caused a significant decrease in sperm count of rats [23].

DIC and MP are two of the most widely used OPIs in agriculture and public health programmes. DIC and MP are also two of the most used OPIs in the region of Van, Turkey. The aim of this study was to investigate the effects of subacute exposure of DIC and MP at sublethal dosages on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in various tissues of rats exposed to 5 and 10 ppm DIC and MP in drinking water *ad libitum* for 28 days continuously. To this end, the treatment of DIC and MP were done orally because of the effect of chemicals, which represent a well characterized *in vivo* toxicity model system. This study was conducted in accordance with national and institutional guidelines for the protection of human subjects and animal welfare. This study was approved by The Ethic Committee of the Faculty of Medicine, Yüzüncü Yil University. Further, these studies were supported by the University Grant Commission of Yüzüncü Yil University.

## 2. Materials and methods

### 2.1. Chemicals

Acetylthiocholine iodide (ASChI), 5,5-*O*-dithiobis(2-nitrobenzoic acid) (DTNB), butyrylthiocholine iodide (BSChI) and sodium dihydrogenephosphate ($NaH_2PO_4$) of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA). DIC and MP used in experimentation were obtained from Agricultural Struggle Center, Van, Turkey.

### 2.2. Animals

Rats (Sprague–Dawley albino) weighing 150–200 g were provided by the animal house of the Sciences Faculty of Yüzüncü Yil University, and were housed in three groups, each group containing six rats. All animals were fed a group wheat–soybean-meal-based diet and water *ad libitum* in stainless cages, and received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health. The animals were housed at 20 ± 2 °C in a daily light/dark cycle.

### 2.3. Treatment of chemical

This investigation was performed on male rats. DIC [DDVP Bayer 50%, EC] and MP [Parathion (*O*,*O*-diethyl-*p*-nitrophenyl-phosphorothioate, Bayer, 500 g/L)] were given to rats orally with drinking water at two doses. The stock solution was appropriately diluted with the test water to achieve the desired concentrations of DIC and MP. Rats were exposed DIC and MP *ad libitum* during the tests for 4 weeks. Control rats were given only the test water. Daily water consumption of rats was approximately 28 ± 2 ml during the tests.

At the end of the treatments, the rats were anesthetized by inhalation of diethyl ether, and after tissues samples were obtained, they were sacrificed. The tissues were dissected and put in Petri dishes. After washing the tissues with 0.9% NaCl, samples were taken and kept at −78 °C during the analysis. The tissues were homogenized for 5 min in 50 mM ice-cold $NaH_2PO_4$ solution (1:5 w/v) using a glass–porcelain homogenizer (20 kHz frequency ultrasonic, Jencons Scientific Co.) for 5 min and then centrifuged at 7000*g* for 15 min. All processes were carried out at 4 °C. The supernatant was used as the enzyme source.

### 2.4. AChE and BChE activity determination

Cholinesterase activity was quantified in brain, lungs, kidney, liver, heart and spleen following the method developed by Ellman et al. [24] for which we used DTNB and ASChI which is specific for AChE, BSChI which is specific for BChE as substrates. For the colorimetric assay, according to Ellman's method, reaction mixtures were made up in 50 mM Tris, pH 7.4 containing 1 mM DTNB and ASChI or BSChI at a final concentration of 0.8 mM. The reaction was performed at 25 °C and monitored at 412 nm.

### 2.5. Analysis of data

All data were expressed as mean ± standard deviation (SD). The statistical analyses were made using the minitab 13 for windows packet program. Means and standard deviations were calculated according to the standard methods for all parameters. One-way ANOVA statistical test was used to determine the differences between means of the treatments and the control group accepting the significance level at $P \leqslant 0.05$.

## 3. Results

Following the exposure to 5 and 10 ppm dosages of DIC and MP, the effects of DIC and MP administration on rats' neurotoxic index were evaluated as the activity of AChE and BChE in the tissues samples from control and treated rats. DIC and MP treatment caused a marked effect on ChE (Figs. 1–4). According to the results, AChE significantly decreased in all the tissues of rats treated with both dosages of DIC and MP except for in the lungs tissue exposed with both dosages of DIC. Meanwhile, MP significantly decreased BChE

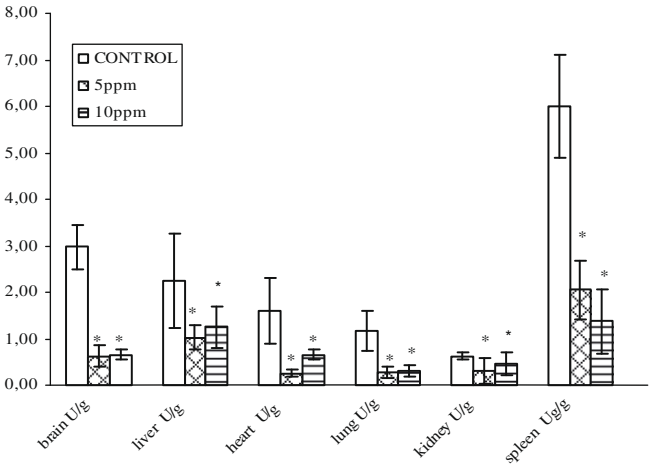


**Fig. 1.** Effects of subacute treatment of MP on AChE enzyme (U/g tissue) of rats. Values are means ± SD. *Significantly different from control rats at $P \leqslant 0.05$ (one-way ANOVA).