# Dose-Dependency of Toxic Signs and Outcomes of Paraoxon Poisoning in Rats

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## ABSTRACT

Organophosphorus compounds induce irreversible inhibition of acetylcholinesterase, which then produces clinically manifested muscarinic, nicotinic and central effects. The aim of the study was to analyse the clinical signs of acute paraoxon poisoning in rats and to determine the relationship between the intensity of signs of poisoning and the dose of paraoxon and/or the outcome of poisoning in rats. Animals were treated with either saline or atropine (10 mg/kg intramuscularly). The median subcutaneous lethal dose ($LD_{50}$) of paraoxon was 0.33 mg/kg and protective ratio of atropine was 2.73. The presence and intensity of signs of poisoning in rats (dyspnoea, lacrimation, exophthalmos, fasciculations, tremor, ataxia, seizures, piloerection, stereotypic movements) were observed and recorded for 4 h after the injection of paraoxon. Intensity of these toxic phenomena was evaluated as: 0 – absent, 1 – mild/moderate, 2 – severe. Fasciculations, seizures and tremor were more intense at higher doses of paraoxon and in non-survivors. In unprotected rats piloerection occurred more often and was more intense at higher doses of paraoxon as well as in non-survivors. In atropine-protected rats, piloerection did not correlate with paraoxon dose or outcome of poisoning. The intensity of fasciculations and seizures were very strong prognostic parameters of the poisoning severity.



## KEYWORDS

organophosphate; insecticide; paraoxon; poisoning; acetylcholinesterase inhibitor; atropine



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## INTRODUCTION

Acetylcholinesterase (AChE, EC 3.1.1.7) is a very potent enzyme whose role is to break down acetylcholine (ACh) in the synaptic cleft (1, 2). Inhibition of AChE results in the accumulation of ACh and excessive stimulation of cholinergic receptors. AChE inhibitors (AChEI) can be reversible (carbamate compounds) and irreversible (organophosphorus compounds – OPCs) (3–5). OPCs form a stable covalent bond with AChE, which is not spontaneously hydrolysed (6). They are divided into two major groups, nerve agents (tabun, sarin, soman, VX) (7) and organophosphorus insecticides (OPI). Paraoxon (diethyl (4-nitrophenyl) phosphate) is an active metabolite of the highly toxic OPI parathion (8). Among the OPIs, paraoxon is very similar to nerve agents, in terms of its median lethal dose ($LD_{50}$), profile of inhibition of cholinesterases and general toxicity (9).

Acute OPC poisoning manifests itself with muscarinic effects (bronchoconstriction, bronchorroea, bradycardia, hypotension, nausea, vomiting, increased motility of bowels and bladder, miosis, hypersalivation, lacrimation), nicotinic effects (tachycardia, hypertension, fibrillation, fasciculations, skeletal muscle necrosis, mydriasis) and CNS effects (tremor, convulsions, coma, respiratory depression) (10). Intermediate syndrome can occur after 1–4 days and, 1–2 weeks later, organophosphate-induced delayed neuropathy (OPIDN) can be seen.

Treatment of OPC poisoning is based on a triple regimen: symptomatic anticholinergic therapy (atropine), AChE reactivators (oximes) and anticonvulsants (mainly diazepam) (11). Atropine as an antimuscarinic drug, alleviates the muscarinic effects of OPC poisoning, but has no impact on the nicotinic ones. Oximes bind to OPC already bound to AChE, which leads to the reactivation of AChE, with variable affinity for different OPCs between oximes. Diazepam inhibits the excitability of the neurons in the CNS; by increasing the effect of GABA, it increases cAMP, decreases the level of cGMP, leading to the cessation of convulsions (11).

The aim of the study was to analyse the clinical signs of acute paraoxon poisoning in rats and to determine whether there is a relationship between the intensity of toxicity signs and the dose of paraoxon and/or outcome of poisoning.

## MATERIAL AND METHODS

### ANIMALS

The study was conducted in adult Wistar rats weighing 200–240 g, purchased from the Faculty of Natural Sciences and Mathematics, University of Banja Luka, the Republic of Srpska. The animals were given water and food *ad libitum*, kept at a temperature of 20–22 °C, with a 12 h cycle of light and darkness. The study was approved by the Ethics Committee for the Protection and Welfare of Experimental Animals in Biomedical Research, Faculty of Medicine, University of Banja Luka (Decision No 18/1/20). The animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals (12). The study was conducted at the Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka.

### CHEMICALS

Paraoxon was purchased from Sigma Aldrich, St Louis, MO, USA. Paraoxon was dissolved in isopropyl alcohol up to a concentration of 100 mg/mL and final dilution to the desired concentration was made with saline (0.9% NaCl). Atropine sulphate was dissolved in saline to a concentration of 10 mg/mL. The volumes of administered paraoxon and atropine were 1 mL/kg. Paraoxon and atropine were administered subcutaneously (sc) and intramuscularly (im), respectively. Final dilutions were made immediately before application.

### STUDY DESIGN

The $LD_{50}$ of paraoxon was determined by the "up and down" method according to Litchfield and Wilcoxon (1949) (13). In the first part of the experiment, then following doses of paraoxon were administered: 0.2, 0.3, 0.35, 0.4 mg/kg sc. One minute after paraoxon the saline (1 mL/kg, im) was administered. In the second part of the experiment, the following doses of paraoxon were administered: 0.6, 0.9 and 1.2 mg/kg sc. Atropine 10 mg/kg im was injected 1 minute after paraoxon application.

The presence and intensity of signs of paraoxon poisoning in animals were observed and recorded for 4 h. The following signs have been observed: dyspnoea, lacrimation, exophthalmos, fasciculations, tremor, ataxia, seizures, piloerection, stereotypic movements. Their presence and intensity were noted at the minutes: 5, 10, 15, 30, 60, 90, 120, 150, 180, 210 and 240 after paraoxon application. Intensity was evaluated as: 0 – absent, 1 – mild/moderate, 2 – severe. Signs of poisoning were observed in relation to the dose of paraoxon, as well as the outcome of the poisoning (survival or death).

**Tab. 1** Time of death from paraoxon administration depending on paraoxon dose.

| POX dose (mg/kg sc) | | Time of death (minute) | |
|---|---|---|---|
| | | Mean ± SD | 95% CI |
| With saline | 0.2 | – | – |
| | 0.3 | 16.67 ± 7.51 | -1.98–35.31 |
| | 0.35 | 22.00 ± 3.61 | 18.67–25.33 |
| | 0.4 | 18.09 ± 6.99 | 13.39–22.78 |
| | All | 19.19 ± 6.19 | 16.37–22.01 |
| With atropine | 0.6 | – | – |
| | 0.9 | 14.00 ± 3.83 | 7.91–20.09 |
| | 1.2 | 14.00 ± 4.24 | 7.25–20.75 |
| | All | 14.00 ± 3.74 | 10.87–17.13 |
| **Total** | | **17.76 ± 6.04** | **15.46–20.06** |

SD: standard deviation; CI: confidence interval; POX: paraoxon; Administered volumes of paraoxon, atropine and saline were 1 mL/kg; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

## STATISTICS

The $LD_{50}$ and the PR were analysed by the method of Litchfield and Wilcoxon (1949) on Pharm/PCS statistical software. Other analyses were performed on IBM SPPS for Windows, Version 18.0. After the Kolmogorov-Smirnov test showed an unequal distribution of data, appropriate nonparametric tests were applied: Chi-square test (or Fisher exact test) and Kruskal-Wallis test. Statistical significance level was set at $p < 0.05$.

## RESULTS

The $LD_{50}$ of paraoxon was 0.33 mg/kg sc (95% CI: 0.31–0.36). The $LD_{50}$ of paraoxon when atropine was administered was 0.91 mg/kg sc (95% CI: 0.67–1.25). Therefore, the PR of atropine was 2.73. All deaths occurred during the first hour of poisoning (Table 1).

## CLINICAL SIGNS OF POISONING

### *1. Fasciculations*

Frequency of fasciculations was not correlated with the dose of paraoxon (Table 2).

In atropine-protected rats, fasciculations occurred more often in non-survivors ($p = 0.023$) (Table 3).

Fasciculations occurred earlier and were more intense at higher doses of paraoxon (Figure 1). Although the intensity of fasciculations were related to the dose of paraoxon throughout the observed period, the difference was significant in the minutes 10, 15, 30, 210 and 240 (Kruskal-Wallis test, $p = 0.035$, $p = 0.045$, $p = 0.038$, $p = 0.014$ and $p = 0.034$, respectively).

Due to atropine protection, higher doses of paraoxon could be administered. The intensity of fasciculations depending on paraoxon dose when atropine was administered is shown in Figure 2. Atropine did not influence the

**Tab. 2** Frequency of signs of poisoning related to paraoxon dose.

| Sign | Paraoxon + Saline | | | | | | Paraoxon + Atropine | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paraoxon (mg/kg, sc) | | | | Total | p* | Paraoxon (mg/kg, sc) | | | Total | p* |
| | 0.2 | 0.3 | 0.35 | 0.4 | | | 0.6 | 0.9 | 1.2 | | |
| Fasciculations | 100.00 | 66.67 | 100.00 | 83.33 | 85.71 | 0.080 | 100.00 | 83.33 | 50.00 | 77.78 | 0.250 |
| Seizures | 66.67 | 75.00 | 83.33 | 100.00 | 83.33 | 0.225 | 83.33 | 100.00 | 100.00 | 94.44 | 1.000 |
| Tremor | 83.33 | 100.00 | 100.00 | 100.00 | 97.61 | 0.143 | 100.00 | 100.00 | 100.00 | 100.00 | 1.000 |
| Piloerection | 0.00 | 33.33 | 66.67 | 75.00 | 50.00 | **0.009** | 50.00 | 50.00 | 0.00 | 33.33 | 0.149 |
| Exophthalmos | 83.33 | 91.67 | 100.00 | 100.00 | 95.23 | 0.498 | 83.33 | 100.00 | 100.00 | 94.44 | 1.000 |
| Lacrimation | 83.33 | 50.00 | 50.00 | 50.00 | 54.76 | 0.501 | 16.67 | 33.33 | 16.67 | 22.22 | 1.000 |
| Ataxia | 83.33 | 58.33 | 75.00 | 58.33 | 66.67 | 0.691 | 66.67 | 33.33 | 33.33 | 44.44 | 0.589 |
| Stereotypy | 100.00 | 66.67 | 83.33 | 41.67 | 69.05 | **0.044** | 66.67 | 0.00 | 50.00 | 38.89 | 0.095 |
| Dyspnoea | 33.33 | 33.33 | 25.00 | 16.67 | 26.19 | 0.862 | 50.00 | 50.00 | 33.33 | 44.44 | 1.000 |

* Chi-squared test (Fisher exact), **bold**: statistical significance. Values in the table are in percentages; sc: subcutaneously; im: intramuscularly; Administered volumes of paraoxon, atropine and saline were 1 mL/kg; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

**Tab. 3** Frequency of signs of poisoning related to poisoning outcome.

| Sign | Paraoxon + Saline | | | | Paraoxon + Atropine | | | |
|---|---|---|---|---|---|---|---|---|
| | Rat survived | | Total | p* | Rat survived | | Total | p* |
| | Yes | No | | | Yes | No | | |
| Fasciculations | 85.71 | 85.71 | 85.71 | 1.000 | 50.00 | 100.00 | 77.78 | **0.023** |
| Seizures | 66.67 | 100.00 | 83.33 | **0.009** | 90.00 | 100.00 | 94.44 | 1.000 |
| Tremor | 95.23 | 100.00 | 97.61 | 1.000 | 100.00 | 100.00 | 100.00 | 1.000 |
| Piloerection | 23.81 | 76.19 | 50.00 | **0.002** | 40.00 | 25.00 | 33.33 | 0.638 |
| Exophthalmos | 95.23 | 95.23 | 95.23 | 1.000 | 90.00 | 100.00 | 94.44 | 1.000 |
| Lacrimation | 71.43 | 38.10 | 54.76 | 0.062 | 20.00 | 25.00 | 22.22 | 1.000 |
| Ataxia | 71.43 | 61.90 | 66.67 | 0.744 | 70.00 | 12.50 | 44.44 | **0.025** |
| Stereotypy | 85.71 | 52.23 | 69.05 | **0.043** | 60.00 | 12.50 | 38.89 | 0.066 |
| Dyspnoea | 28.57 | 23.81 | 26.19 | 1.000 | 60.00 | 25.00 | 44.44 | 0.239 |

* Chi-squared test (Fisher exact), **bold**: statistical significance; Values in the table are in percentages; im: intramuscularly; Administered volumes of paraoxon, atropine and saline were 1 mL/kg; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

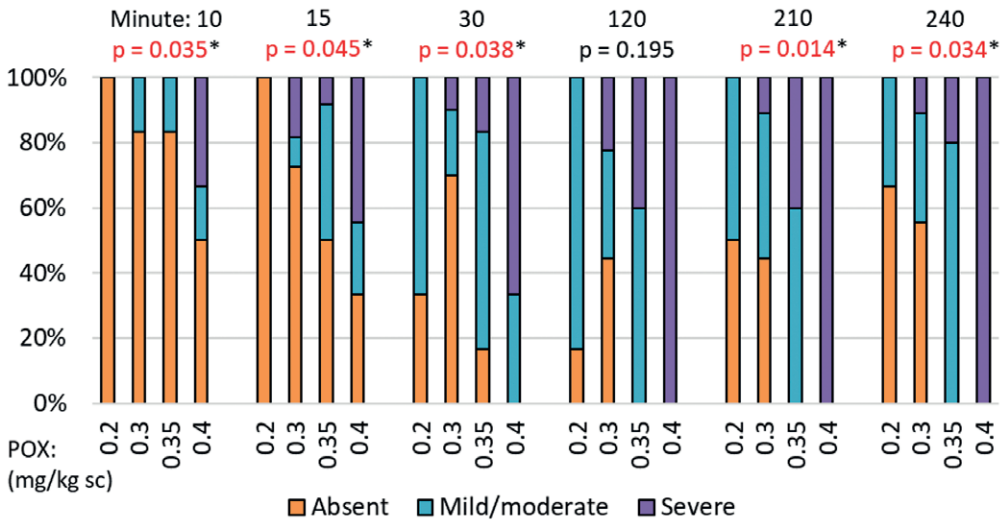


**Fig. 1** Intensity of fasciculations in rats depending on paraoxon dose.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 10, 15, 30, 120, 210 and 240 after paraoxon application.

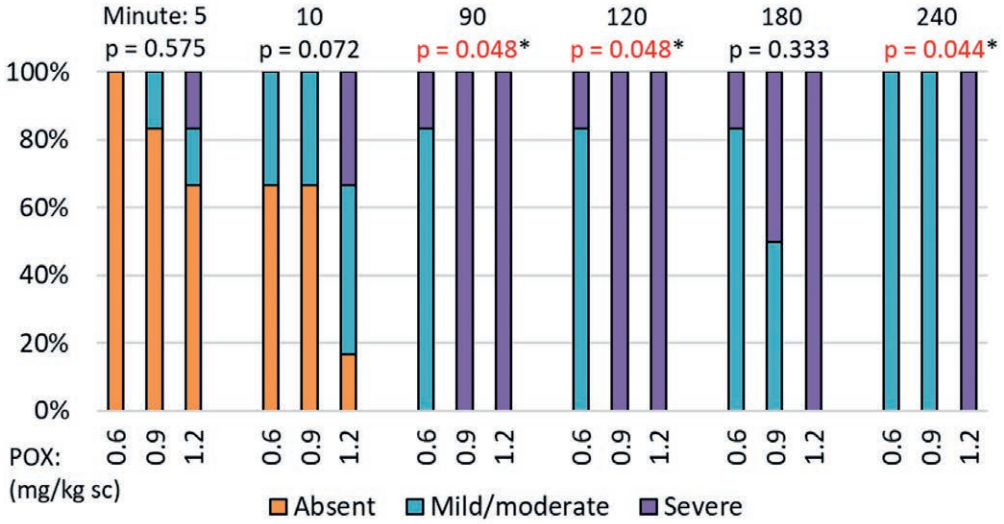


**Fig. 2** Intensity of fasciculations depending on paraoxon dose in rats protected with atropine.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 5, 10, 90, 120, 180 and 240 after paraoxon application; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

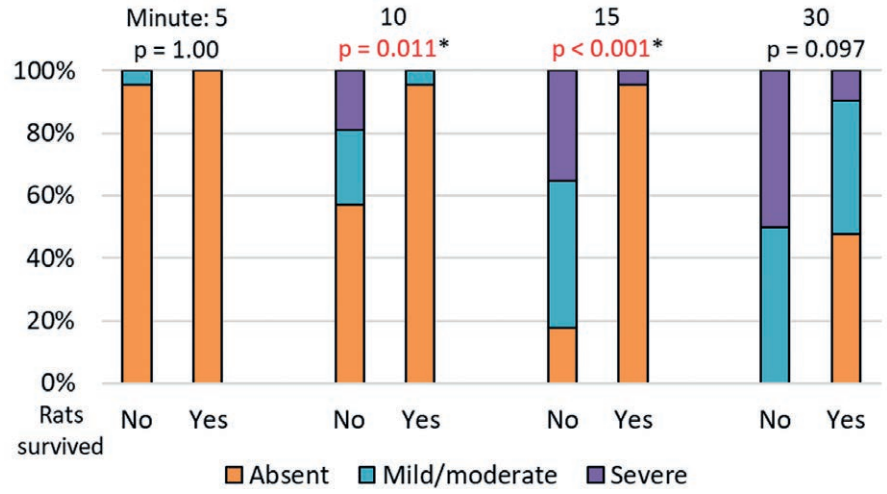


**Fig. 3** Intensity of fasciculations in relation to whether rat treated with paraoxon survived or not.
* Fisher exact test, red colour: statistical significance; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

intensity of fasciculations. Fasciculations occurred earlier and were more intense at higher doses of paraoxon. Although the intensity of fasciculations was related to the dose of paraoxon throughout the observed period, the difference was significant in the minute 60 (data not shown), 90, 120 and 240 (Kruskal-Wallis test, p = 0.033, p = 0.048, p = 0.048 and p = 0.044, respectively).

In unprotected rats intensity of fasciculations was in correlation with the outcome of poisoning (higher intensity was in non-survivors) (Figure 3). In atropine-protected rats, fasciculation intensity did not correlate with the outcome of poisoning.

### *2. Seizures*

Frequency of seizures was not correlated with the dose of paraoxon (Table 2), but seizures were more often in non-survivors compared to survivors (Table 3). Seizures occurred earlier and were more intense at higher doses of paraoxon (Figure 4). Although the intensities of seizures were related to the dose of paraoxon throughout the observed period, the difference was significant only at minutes 15, 180 and 210 (Kruskal-Wallis test, p = 0.002, p = 0.024 and p = 0.015, respectively).

A clear relation between paraoxon dose and seizure intensity can be seen in atropine-protected rats (Figure 5). Although the intensity of seizures was related to the dose of paraoxon throughout the observed period, the difference was significant only at minutes 10, 15, 210 and 240 (Kruskal-Wallis test, p = 0.031, p = 0.014, p = 0.044 and p = 0.011, respectively).

In unprotected rats the intensity of seizures was in correlation with the outcome of poisoning (higher intensity was in non-survivors) (Figure 6). In atropine-protected rats, the intensity of seizures did not correlate with the outcome of poisoning.

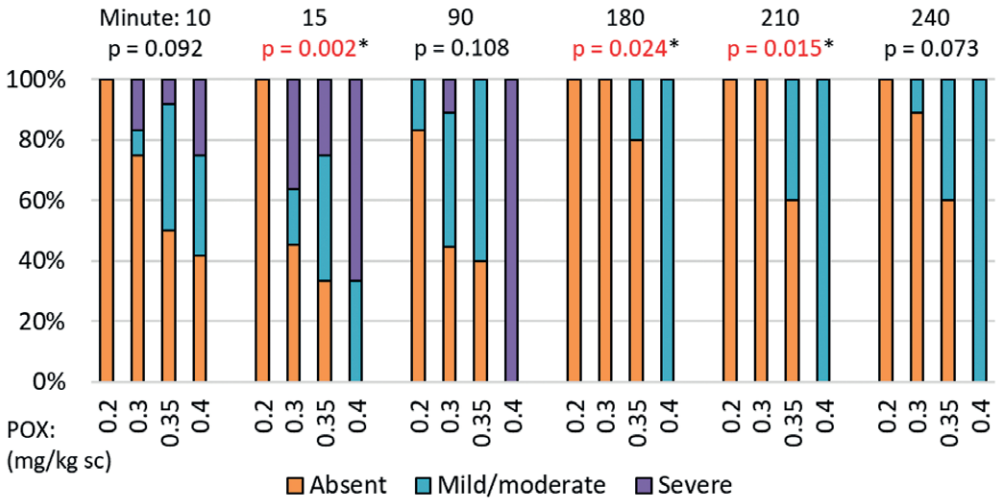


**Fig. 4** Intensity of seizures in rats depending on paraoxon dose.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 10, 15, 90, 180, 210 and 240 after paraoxon application.

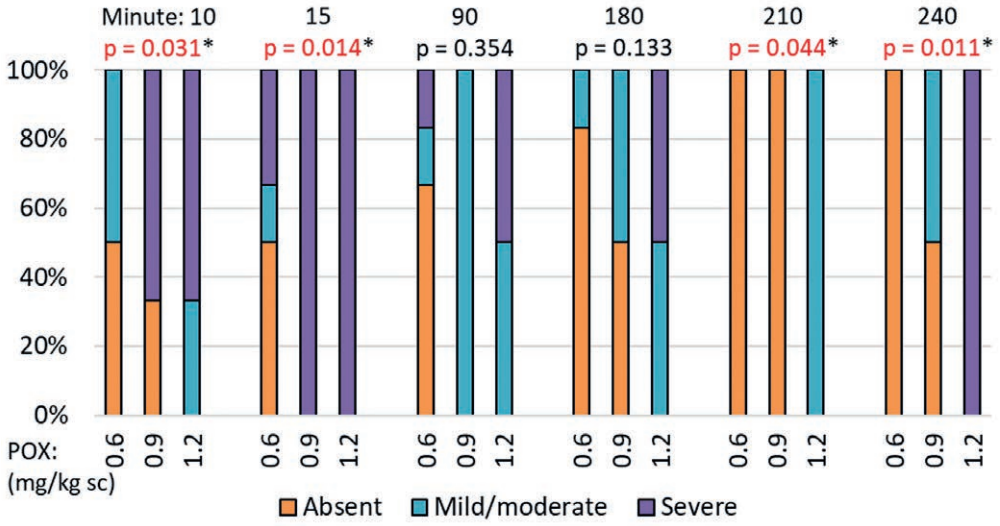


**Fig. 5** Intensity of seizures depending on paraoxon dose in rats protected by atropine.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 10, 15, 90, 180, 210 and 240 after paraoxon application; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

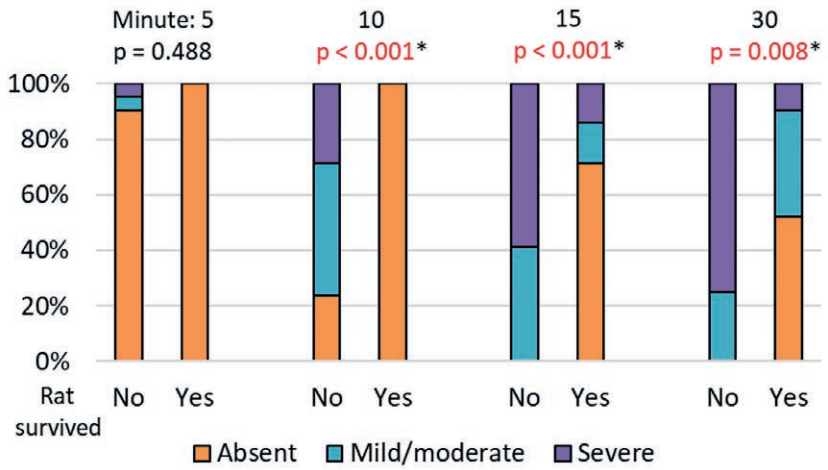


**Fig. 6** Intensity of seizures in relation to whether rat treated with paraoxon survived or not.
* Fisher exact test, red colour: statistical significance; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

### *3. Tremor*

Frequency of tremor was not correlated with the paraoxon dose (Table 2) or the outcome of poisoning (Table 3). Tremor occurred earlier and was more intense at higher doses of paraoxon (Figure 7). Although the intensity of tremor was related to the dose of paraoxon throughout the observed period, the difference was significant at minutes 10, 15 and 240 (Kruskal-Wallis test, $p = 0.001$, $p = 0.002$ and $p = 0.044$, respectively).

In the atropine-protected rats, although a higher intensity of tremor was observed at higher doses of paraoxon, the difference was not significant, except at minute 10 ($\chi^2 = 9.88$, $p = 0.007$) and 30 ($\chi^2 = 6.00$, $p = 0.050$).

In unprotected rats intensity of tremor was in correlation with the outcome of poisoning (higher intensity was in non-survivors) (Figure 8). In atropine-protected rats, the intensity of tremor did not correlate with the outcome of poisoning.

### *4. Piloerection*

Piloerection as a clinical sign of poisoning occurred early (within the first half hour of poisoning) and lasted for a short time (Figure 9). Piloerection occurred more often (Table 2) and was more intense at higher doses of paraoxon administered to unprotected rats. The difference was significant at minutes 10 and 15 (Kruskal-Wallis test, $p = 0.052$ and $p = 0.012$, respectively).

In atropine-protected rats, piloerection did not correlate with paraoxon dose.

In unprotected rats intensity of piloerection was in correlation with the outcome of poisoning. Piloerection was

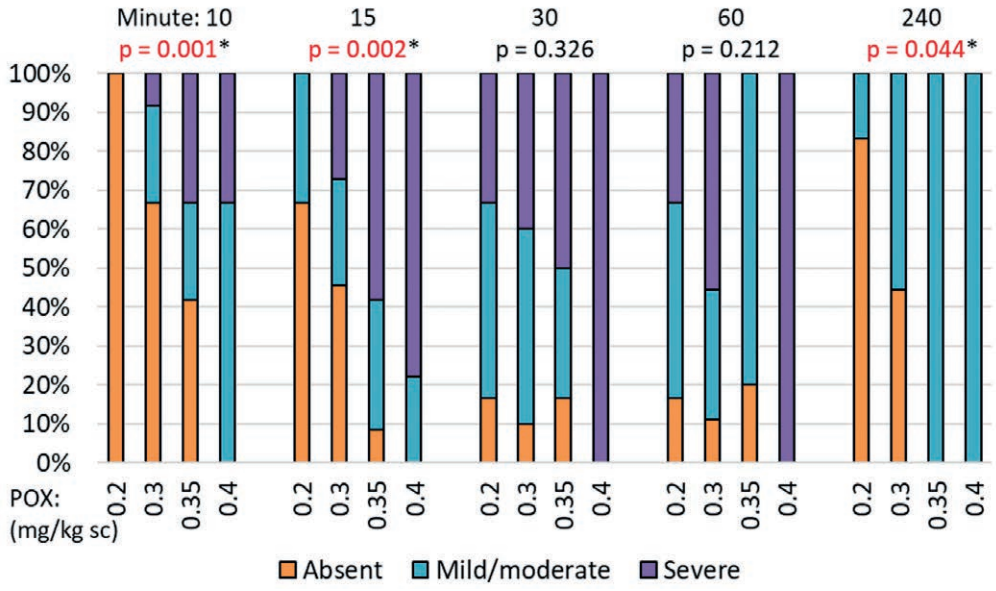


**Fig. 7** Intensity of tremor in rats depending on paraoxon dose.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 10, 15, 30, 60 and 240 after paraoxon application.

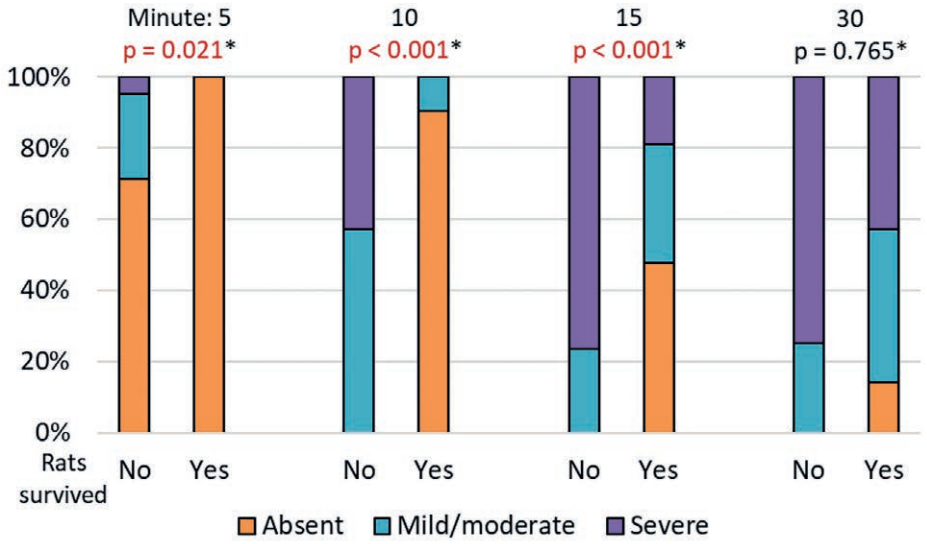


**Fig. 8** Intensity of tremor in relation to whether rat treated with paraoxon survived or not.
* Fisher exact test, red colour: statistical significance; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

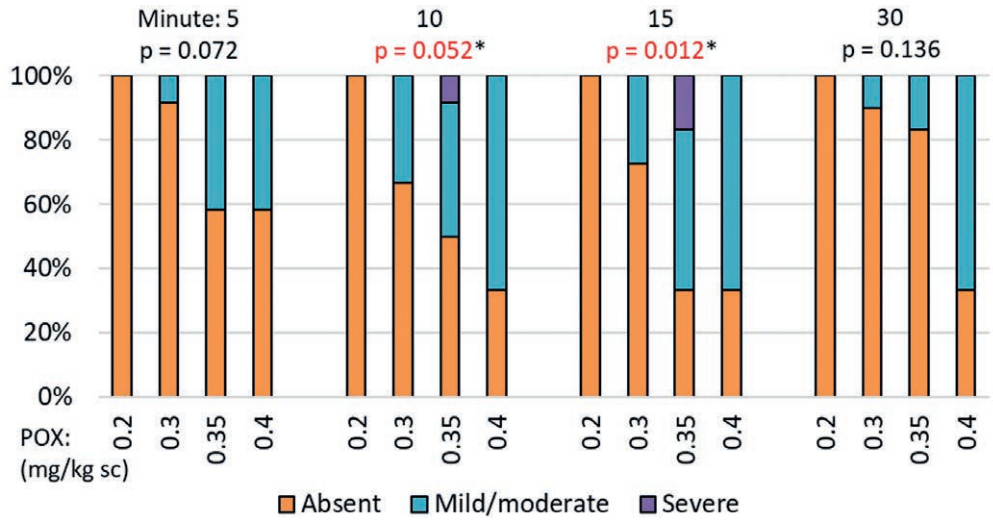


**Fig. 9** Intensity of piloerection in rats depending on paraoxon dose.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

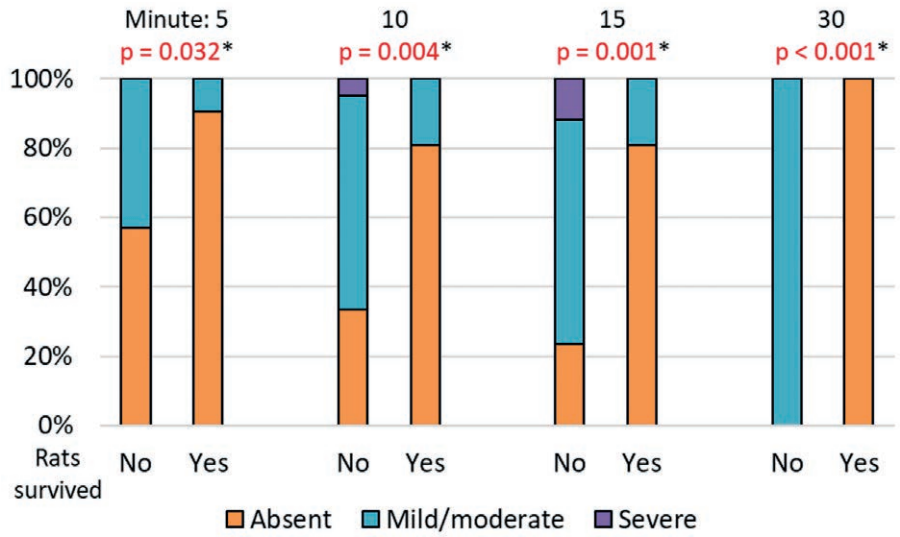


**Fig. 10** Intensity of piloerection in relation to whether rat treated with paraoxon survived or not.
* Fisher exact test, red colour: statistical significance; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

more often (Table 3) and of higher intensity in non-survivors (Figure 10). In atropine-protected rats, piloerection did not correlate with the outcome of poisoning.

The intensity of stereotypical movements, exophthalmos, lacrimation, ataxia and dyspnoea was not related to the dose of paraoxon or to the outcome of poisoning. The above results are not shown. Of the listed signs, stereotypical movements were less often with higher doses of paraoxon ($p = 0.044$) (Table 2). Related to poisoning outcome, ataxia ($p = 0.025$) was observed more often in survivors from the group of atropine-protected rats, while stereotypical movements ($p = 0.043$) were observed more often in survivors from the group of unprotected rats (Table 3).

## DISCUSSION

Due to its extreme toxicity, the World Health Organization (WHO) has classified parathion, parent compound of paraoxon, as a class Ia (extremely hazardous) pesticide (14). Due to its toxicity, it is banned in most developed countries. Tabun, sarin, soman and VX represent OP compounds similar to parathion and paraoxon, with the same mechanism of action (inhibition of acetylcholinesterase) and their extreme toxicity classifies them as nerve agents. Their production, stockpiling, weaponosing and use is strictly prohibited by the 1993 Chemical Weapons Convention (CWC) (7). In undeveloped and developing countries, OPI poisonings, both accidental and intentional, are common (15, 16). In Sri Lanka and China, pesticide poisoning is the most common method of fatal self-harm (17).

The $LD_{50}$ of paraoxon obtained in this study was 0.33 mg/kg sc, which corresponds with the results of other researchers (18). The PR of atropine was 2.73, which is in accordance with the known publications (19). Atropine is effective in blocking the effects of muscarinic but is ineffective against the nicotinic signs of OPC poisoning (20). This antimuscarinic drug is liposoluble and passes the blood-brain barrier (21). Therefore, it to some extent, antagonises the toxic effects of excessive cholinergic stimulation in the brain (22). It seems that a more lipophilic antimuscarinic drug would be more effective than atropine (23). Krutak-Krol and Domino (24) found that the atropine dose of 10 mg/kg im is optimal in experimental studies. The minimum absolute lethal dose of OPCs is 1.3 $LD_{50}$ (25). The administration of atropine made it possible for rats to survive the absolute lethal dose of paraoxon. That enabled monitoring of signs of poisoning at high doses of paraoxon. As expected, atropine blocked to some extent the muscarinic effects, but not the nicotinic ones. Since different doses of paraoxon were administered in rats treated with saline or atropine, the results are not comparable. However, this makes it possible to compare the signs of poisoning in future studies with other antidotes.

In clinical settings, mainly muscarinic signs of OPC poisoning are expected. Bronchoconstriction and bronchorrhea are life-threatening muscarinic effects. Most studies have cited respiratory failure as the leading cause of death (26–28). Dyspnoea was observed as a sign of poisoning in the present experiment. No clear relationship was found between the intensity of dyspnoea and the dose of paraoxon. However, in the recent study, a clear relationship was found between the onset rate of dyspnoea, as well as the overall intensity of dyspnoea and lethal outcome of poisoning (29). Respiratory failure is a consequence of both peripheral and central cholinergic effects (30). Therefore, it is very important to administer an antidote that can cross the blood-brain barrier and prevent central respiratory depression (31).

Another muscarinic sign of poisoning that was observed is lacrimation. It is a sign that is easily noticeable. It is not a sign that directly implies whether the animal is endangered or not, but it is a good indicator of excessive muscarinic stimulation. In the treatment of OPC poisoning, the lack of lacrimation is one of the signs of achieving the so-called patient atropinisation (5). The results of this study also support this assumption. Although significantly higher doses of paraoxon were administered, the lacrimation occurred significantly less frequently in rats treated with atropine (22% vs 55%).

ACh is also found in the preganglionic nerve endings of the sympathetic nervous system (32). Stimulation of alpha-1-adrenergic receptors also leads to piloerection (33). Therefore, piloerection can serve as an indirect indicator of sympathetic stimulation. The results of this study showed a clear relationship between both the frequency and intensity of piloerection and the dose of paraoxon. Besides, piloerection occurred more often and was of stronger intensity in non-survivors.

Tachycardia and hypertension are rarely expected in patients with OPI intoxications and they often mislead physicians in practice. Saadeh et al found tachycardia in as many as 35–60% of patients poisoned by OPCs (34). It means that tachycardia occurs more often than bradycardia, which indicates that it is a prejudice not to expect nicotinic effects in OPC poisonings. Nicotinic signs of poisoning occur as a consequence of excessive stimulation of ganglionic nicotinic receptors (hypertension, tachycardia, diaphoresis) as well as receptors at the neuromuscular junction (fibrillation and fasciculation) (35). In AChEI poisoning, hypertension and tachycardia can also occur as a consequence of excessive stimulation of the *locus coeruleus*. Stimulation of this cholinergically innervated sympathetic nuclei leads to the centrally-originated hypertension (36, 37).

As already mentioned, ACh is a neurotransmitter of the peripheral nervous system, as well. Excessive stimulation of nicotinic receptors at the neuromuscular junction leads to fasciculations, a toxic phenomenon observed in this study. Fasciculations were the most consistent sign of the severity of rat poisoning. They were more intense at higher doses of paraoxon and in non-survivors throughout the observed period. This is in favour of the fact that nicotinic signs of poisoning appear in severe poisonings (38). When sarin was used in a terrorist attack in the crowded subway in Tokyo, over 5,000 people were injured and 12 people died (7, 39). Published reports cited nicotinic signs of poisoning in severely poisoned patients (40, 41). In rats treated with high doses of paraoxon and atropine, fasciculations were more common in survivors. This can be explained by the rapid lethal outcome of poisoning, which

left non-survivors without this toxic sign. Fasciculations often did not occur in the first 10 minutes of poisoning, but were present even after 4 hours in all survivors. In other words, it means that the non-survivors died too quickly to develop fasciculations. Experimental studies with antinicotinic drugs showed their significant antidotal efficacy against carbamates and OPCs (21). However, nicotinic receptor blockers are rarely used in clinical practice in the treatment of OPC poisonings, due to serious side effects at therapeutic doses of these drugs, primarily the respiratory muscle depression (42).

Tremor is mediated by a variety of neurotransmitters – dopamine, glutamate, serotonin, adenosine and acetylcholine (43). The $M_2$ muscarinic receptors are highly expressed in the nucleus basalis and occipital cortex, then in hippocampus and other cortical regions. Overstimulation of $M_2$ receptors leads to tremor (44). There is a conflicting evidence regarding the role of $M_3$ and $M_4$ receptors in tremor aetiology (45). In this study, a clear relationship was found between the intensity of poisoning, on the one hand and the dose of paraoxon and the outcome of the poisoning, on the other hand. In atropine-protected rats, tremor occurred in all animals. Tremor is often found as a part of the extrapyramidal syndrome that occurs as a consequence of permanent CNS damage in OPC poisoning survivors (21, 46, 47).

Stereotypical movements were registered more often in survivors and at lower dose of paraoxon. The heavily poisoned animals had significantly decreased spontaneous motor activity. Thus, the appearance of stereotypical movements could be a good prognostic sign of a positive outcome of poisoning. At the highest doses of paraoxon (0.9 and 1.2 mg/kg), ataxia was more common in survivors. Atropine prevented the death of rats, but not the skeletal muscle fatigue. As a consequence, only surviving rats could attempt to move in the cage and these movements were ataxic.

Seizures intensity was directly related to the dose of paraoxon and the lethal outcome of the poisoning. A total of 66.67% of survivors vs 100% of non-survivors had seizures. Seizures occur at the beginning of OPC poisoning due to the excessive cholinergic stimulation of the CNS. There are three treatment periods after the onset of OPC-induced convulsions: muscarinic, gamma-aminobutyric acid A ($GABA_A$)/benzodiazepine and glutamatergic ones (48). During the first one, antimuscarinic drugs (atropine and, preferably, more lipophilic drugs, such as scopolamine) can be efficiently used to stop the seizures, provided the right dose is chosen (49). However, beyond this period antimuscarinic drugs become ineffective in counteracting the seizures, irrespective of the dose applied. In the second phase this could be compensated by the administration of the $GABA_A$/benzodiazepine receptor antagonists, such as barbiturates (e.g., pentobarbital, thiopental sodium) and benzodiazepines (e.g., diazepam or midazolam) (50, 51). In the third phase, these seizures can be stopped by the administration of N-methyl-D-aspartate (NMDA) receptor antagonists, such as memantine, dizocilpine (MK-801) or ketamine (52–55). The reason for this is the fact that in the meanwhile the seizures became glutamatergic in its origin (56). Along with fasciculations, seizures were the most constant sign of the severity of the poisoning.

## CONCLUSION

Among all the studied signs of paraoxon toxicity, the intensity of fasciculations and seizures were strong prognostic parameters of the severity of poisoning. They are easily observed and are directly related to both the dose of paraoxon and the lethal outcome of the poisoning. Based on the relationship between the frequency and intensity of muscarinic or nicotinic signs and the doses of paraoxon or outcomes of the poisoning, there are two strong prognostic parameters of the severity of poisoning (fasciculations and seizures) and a good prognostic sign of a positive outcome of poisoning (stereotypical movements). These signs of poisoning may be useful to researchers in monitoring the expected treatment outcome. Also, the appearance of nicotinic and central signs of poisoning in patients indicates the severity of poisoning and provides guidance to clinicians on which potential therapy to use.

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## ABBREVIATIONS

ACh: acetylcholine; AChE: acetylcholinesterase; AChEI: acetylcholinesterase inhibitor; OPC: organophosphorus compounds; OPI: organophosphate insecticide

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