Original Article
Protective effects of the antihistamine promethazine against acute paraxon-methyl and dicrotophos toxicity in adult rats

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Abstract: Organophosphorus compound poisoning (OPC) is a global issue. The problem is aggravated with the threats of terrorist use, unintentional use and irresponsible practice as happened recently in turmoil countries. The purpose of the current study was to investigate the old-generation antihistamine promethazine (PROM), a drug with multi pharmacological actions, as an antidote to extremely and highly toxic (WHO’s class IA and IB) OPC poisoning in experimental animal models conducted on adult male wistar rats. Experimental groups were treated intraperitoneal (i.p.) with LD70 of methyl paraoxon (MPOX), class IA and dicrotophos (DCP), class IB alone and a combination of simultaneously i.p. injection of PROM. Mortality was recorded at 30 minutes, 1, 2, 3, 4, 24, 48 hours post injections. RBC-AChE was measured in survivals. MPOX was chosen for further studies with atropine (ATR) and pralidoxime (PAM). In addition to Kaplan-Meir survival analysis, serum lactate dehydrogenase (LDH) and creatinine kinase (CK) from serum were measured in all experimental groups with MPOX. The results revealed significant protection by PROM in both MPOX and DCP intoxicated rats, though the inhibition of RBC-AChE was high. The observed results show that groups treated with a combination of MPOX and PROM or MPOX, PROM, and PAM were protected higher than those treated with MPOX and ATR or MPOX, ATR, and PAM though statistically not significantly different (P ≤ 0.05). No effect was observed on the activity of LDH and CK. The study concludes that PROM may be effectively used in OPC poisoning. However, risk/benefits trials and further studies with different doses and other OPC groups are warranted.

Keywords: Promethazine, antihistamine, paraoxon, dicrotophos, atropine, pralidoxime, organophosphorus poisoning

Introduction

Organophosphorus compound (OPC) poisoning is a global issue. The compounds have a wide variety of applications. Thousands of casualties and death are reported each year due to unintentional and intentional use, apart from undesirable exposure in the environment, particularly in agriculture sector. Situation further intensifies by the terrorist use of these compounds which happened several times in the past [1-3]. The use of sarin was suspected in the Syria turmoil recently (August 2013) where thousands of casualties were reported (http://www.theguardian.com/world/2014/mar/06/sarin-gas-attack-civilians-syria-government-un. Last accessed 14.7.15). OPC nerve agents pose a permanent threat for the civilian population and military forces. In the event of mass casualty, shortage of standard antidotes may be anticipated, especially in the developing countries. In addition, the agriculture region with inadequate healthcare facilities is affected in many of the developing countries. Most of the unintentional or intentional OPC poisoning occurs commonly in these countries. The standard therapy which is not changed for many decades includes mainstay treatment of antimuscarinic agent atropine, an oxime and a benzodiazepine along with supportive measures. In addition, many different approaches and alternatives have been proposed in the literature [4] but none could practically replace the existing standard treatment. On the other hand, the
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The increasing threat of nerve agents use against civilian population and forecasted increase in the use of OPCs in coming years calls for effective preparedness, and search for the effective, easily available, and cost effective/inexpensive antidotes.

The antihistamines as a tentative antidote for OPC poisoning have been scarcely studied. Some of the studies [5-13] and recent review by Ojha et al. [14] are noteworthy in this connection. In these studies, focus was mainly given on experimental studies conducted with old-generation antihistamine, e.g. diphenhydramine. Albeit, the conventional thinking of OPC toxicity is the inhibition of AChE, it is well established that many other non-cholinergic factors play a crucial role in mortality and morbidity. One of these factors is the stimulation of mast cell degranulation, possibly causing the release of histamine or histamine-like compounds that can precipitate the inflammatory processes [15, 16]. Accordingly, histamine released from mast cells binds to histamine H1 receptors which increases capillary permeability, initiate vasodilation and cause inflammatory responses [17]. For instance, Sarin, an OPC nerve agent has recently been reported to elevate histamine levels in the broncho-alveolar lavage of guinea pigs [18]. In addition, malathion metabolites were reported to induce histamine release from basophils and peritoneal mast cells [16]. Antihistamines have been described to act as anti-inflammatory agents by preventing histamine release from mast cells and/or stabilizing histamine receptors in an inactive conformation. Interestingly, neuro-inflammations with severe neuronal disorders by acute or chronic exposure of OPC have been widely documented in the literature [19, 20]. Hence, centrally acting old-generation antihistamines, e.g. PROM, may be useful in preventing the delayed deleterious effect of OPC poisoning, through its multi-pharmacological actions. The antihistamine PROM has been used for several decades, and its pharmacokinetics and toxicity are well documented and established. Also, it is supposed to be available in almost all the clinical settings. A potent anti-cholinergic (both muscarinic and nicotinic), antihistaminic, a centrally acting drug, an inhibitor of human α 7 nicotinic acetylcholine receptor, and an adrenergic blocker, this drug was considered as a proficient antidote for OPC poisoning. Moreover, the compound is easily and widely available, inexpensive and obtainable in large quantities in most of the hospitals and pharmacies in the event of mass casualty. In the present study, PROM is tested against paraoxon-methyl, (extremely toxic OPC; WHO class IA) and dicrotophos (highly toxic OPC; WHO class IB; Figure 1) [21]. MPOX is the most potent among AChE inhibiting OPCs, and about 70% as potent as the nerve agent sarin. The proposed study will provide an alternative to existing atropine therapy, especially in...

Figure 1. Schematic presentation of the work plan. Additional studies with MPOX includes survival studies, RBC-AChE, creatinine kinase and lactate dehydrogenase measurement.
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<table>
<thead>
<tr>
<th>Compound</th>
<th>Structural formula</th>
<th>Empirical formula (Hill Notation)</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraoxon-methyl (O,O-Dimethyl O-(4-nitrophenyl) phosphate)</td>
<td><img src="image" alt="Paraoxon-methyl" /></td>
<td>C₈H₁₂NO₆P</td>
<td>247.14</td>
</tr>
<tr>
<td>Dicrotophos 3-(dimethoxy phosphinyloxy)-N,N-dimethylcis-crotonamide</td>
<td><img src="image" alt="Dicrotophos" /></td>
<td>C₈H₁₆NO₅P</td>
<td>237.19</td>
</tr>
<tr>
<td>Promethazine 10-[2-(Dimethylamino)propyl]phenothiazine hydrochloride</td>
<td><img src="image" alt="Promethazine" /></td>
<td>C₈H₁₆N₂SxHCl</td>
<td>320.88</td>
</tr>
<tr>
<td>Atropine endo-(±)-α-(Hydroxymethyl)benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester</td>
<td><img src="image" alt="Atropine" /></td>
<td>C₂₇H₂₃NO₃</td>
<td>289.37</td>
</tr>
<tr>
<td>Pralidoxime Pyridine-2-aldoxime methochloride</td>
<td><img src="image" alt="Pralidoxime" /></td>
<td>C₇H₉N₂OₓCl</td>
<td>172.61</td>
</tr>
</tbody>
</table>

the event of shortages of supplies. In addition, the study will be a newer approach of using an antihistamine with multi pharmacological actions as antidote of OPC poisoning instead of prevailing anti-muscarinic agent.

**Material and methods**

**Experimental animals**

During the entire experiment, the “Guiding principles in the Care of and Use of Laboratory Animals” have been observed. Animals were handled, ethically treated, and humanly killed as per the rules and instructions of the Ethical Committee. All studies were performed with the approval of the Institutional Ethical Committee (A15/14). The original stock of Wistar rats was purchased from Harlan Laboratories (Harlan Laboratories, Oxon, England). The animals used in the present studies were bred at our own Animal Facility from the original stock. Adult male rats were housed in polypropylene cages (43 × 22.5 × 20.5 cm³; six rats/cage) in climate- and access-controlled rooms (23±1°C; 50±4% humidity). The day/night cycle was 12 h/12 h. Food and water were available ad libitum. The food was purchased from Emirates Feed Factory (Abu Dhabi, UAE) which is a standard maintenance diet for rats.

**Chemicals**

Methyl Paraoxon (MPOX) and dicrotophos (DCP) stock solution (100 mM) was prepared in dry acetone. Working solution for i.p. application
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was prepared ex tempore by diluting stock solution with saline. The other solutions were prepared before experiment. All the chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Chemical structures of the compounds used in the present study are provided in Table 1.

Choice of dosage and treatment

The acute dose (≈ LD₇₅) of MPOX and DCP and half of LD₀₁ of PAM was selected to administer to the animals. PAM, PROM and ATR dosage was selected according to previous studies [10, 22, 23]. Kan et al. [10] used 40 mg/kg body weight against soman (a nerve agent) poisoning. Since our study deals with a lesser toxic OPC than nerve agent, hence we selected a slightly reduced dose that is 30 mg/kg body weight. Sanderson [23] investigated the effect of i.p. administered atropine (17.4 mg/kg) given alone, or combined with oximes against different OPCs in rats and found promising survival results. We opted for 18 mg/kg body weight based on Sanderson work. A schematic plan of conducted experiments is shown in Figure 1.

Reference groups

Only MPOX exposure: 1.98 mg/kg average body weight diluted in 500 µl saline solution. Only DCP exposure: 14 mg/kg average body weight diluted in 500 µl saline solution. PAM: 35 mg/kg average body weight diluted in 500 µl saline solution. PROM: 30 mg/kg average body weight diluted in 500 µl saline solution. ATR: 18 mg/kg body weight diluted in 500 µl saline solution.

Experimental groups and exposure of compounds

There were eight groups of experimental rats. The experiments were repeated four times (3-4 cycles; 6 rats/group/cycle). The 1st and 3rd group was given MPOX and DCP i.p. alone respectively. Group 2 & 4 received MPOX and DCP injection and, thereafter (within one minute) i.p.

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Table 2. Percentage of mortality after treatment with MPOX (G1) and DCP (G3) alone and simultaneous application of PROM (G2 & G4). First row in each box shows percent mortality with standard error of mean. Second row is the 95% confidence interval of the mean. Data is derived from n=18 to 24 treated rats in 3-4 cycles of experiments

<table>
<thead>
<tr>
<th>Groups</th>
<th>30 min.</th>
<th>60 min.</th>
<th>120 min.</th>
<th>180 min.</th>
<th>240 min.</th>
<th>1440 min.</th>
<th>2880 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: MPOX only</td>
<td>73±10 (46-100)</td>
<td>73±10 (46-100)</td>
<td>73±10 (46-100)</td>
<td>73±10 (46-100)</td>
<td>73±10 (46-100)</td>
<td>73±10 (46-100)</td>
<td>73±10 (46-100)</td>
</tr>
<tr>
<td>G2: MPOX+PROM</td>
<td>17±7 (0.38)</td>
<td>17±7 (0.38)</td>
<td>17±7 (0.38)</td>
<td>17±7 (0.38)</td>
<td>17±7 (0.38)</td>
<td>17±7 (0.38)</td>
<td>17±7 (0.38)</td>
</tr>
<tr>
<td>G3: DCP only</td>
<td>56±119 (0-143)</td>
<td>78±11 (32-166)</td>
<td>78±11 (32-166)</td>
<td>78±11 (32-166)</td>
<td>78±11 (32-166)</td>
<td>78±11 (32-166)</td>
<td>78±11 (32-166)</td>
</tr>
<tr>
<td>G4: DCP+PROM</td>
<td>11±6 (0.35)</td>
<td>17±10 (0.58)</td>
<td>17±10 (0.58)</td>
<td>17±10 (0.58)</td>
<td>22±6 (0.46)</td>
<td>22±6 (0.46)</td>
<td>22±6 (0.46)</td>
</tr>
</tbody>
</table>

Table 3. Percentage of mortality after treatment with different treatment regimens of MPOX and PROM, ATR, PAM. First row in each box shows percent mortality with standard error of mean. Second row is the 95% confidence interval of the mean. Data is derived from n=18 to 24 treated rats in 3-4 cycles of experiments

<table>
<thead>
<tr>
<th>Groups</th>
<th>30 min.</th>
<th>60 min.</th>
<th>120 min.</th>
<th>180 min.</th>
<th>240 min.</th>
<th>1440 min.</th>
<th>2880 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G5: MPOX+PAM</td>
<td>29±24 (0-100)</td>
<td>42±22 (0-100)</td>
<td>42±22 (0-100)</td>
<td>42±22 (0-100)</td>
<td>42±22 (0-100)</td>
<td>42±22 (0-100)</td>
<td></td>
</tr>
<tr>
<td>G6: MPOX+ATR</td>
<td>13±8 (0.38)</td>
<td>13±8 (0.38)</td>
<td>13±8 (0.38)</td>
<td>13±8 (0.38)</td>
<td>13±8 (0.38)</td>
<td>13±8 (0.38)</td>
<td></td>
</tr>
<tr>
<td>G7: MPOX+PROM+PAM</td>
<td>9±5 (0.24)</td>
<td>9±5 (0.24)</td>
<td>9±5 (0.24)</td>
<td>9±5 (0.24)</td>
<td>9±5 (0.24)</td>
<td>9±5 (0.24)</td>
<td></td>
</tr>
<tr>
<td>G8: MPOX+ATR+PAM</td>
<td>39±15 (0-100)</td>
<td>50±10 (8-92)</td>
<td>50±10 (8-92)</td>
<td>50±10 (8-92)</td>
<td>50±10 (8-92)</td>
<td>50±10 (8-92)</td>
<td>50±10 (8-92)</td>
</tr>
</tbody>
</table>
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injection of PROM. Groups 5-8 received i.p. injections of PAM, PROM or ATR according to the groups mentioned below.

The animals were monitored for 48 hours and mortality was recorded at 30 min, 1, 2, 3, 4, 24 and 48 hours. There were 3 control groups with 6 rats each. Control groups received only the PROM, PAM and ATR respectively but no MPOX injections.

**Test groups**

- Group 1 (G1): MPOX only.
- Group 2 (G2): MPOX+PROM.
- Group 3 (G3): DCP only.
- Group 4 (G4): DCP+PROM.
- Group 5 (G5): MPOX+PAM.
- Group 6 (G6): MPOX+ATR.
- Group 7 (G7): MPOX+PROM+PAM.
- Group 8 (G8): MPOX+PAM+ATR.

**Control groups**

- Group 9 (G9): PROM ONLY.
- Group 10 (G10): PAM only.
- Group 11 (G11): ATR only.

**RBC-AChE activity**

The blood samples for RBC-AChE measurement was collected from the tail vein. The RBC-AChE activity was measured in diluted whole blood samples in the presence of the selective butyryl-cholinesterase inhibitor, ethopropazine as described previously [24]. The values were normalized to the hemoglobin (Hb) content (determined as cyanmethemoglobin) and expressed as mU/µmol Hb.

**Blood collection for biochemical tests**

After forty eight hours of treatment, blood samples from rats were collected by decapsulation, centrifuged at 3000 rpm for 10 minutes. Serum obtained was then stored frozen at -80°C. CK and LDH were performed by auto-analyzer, COBAS INTEGRA 400 PLUS from Roche Diagnostics, Germany.

**Statistical analysis**

Statistical analysis was performed on the mortality data of four cycles. Kaplan-Meier survival analysis was performed using SPSS 21.0 statistical software, SPSS Inc., Chicago, IL, USA. Kaplan-Meier survival analysis allows estimation of survival over time. The time starting from a defined point to the occurrence of a given event, for instance death is called as survival time. For each interval, survival probability is calculated as survivors divided by experimental individuals at risk. Experimental individual who have died, are not counted as “at risk”. Non-parametric Manwhitney-U test was used for statistical significance and *P* ≤ 0.05 was considered significant. SPSS 21.0 software package was used for all statistical evaluations.

**Results**

**Mortality/survival analysis**

Tables 2 and 3 show the percentage of animals died at different time points over the period of 48 hours in MPOX and DCP treatment and after application of PROM. The results observed clearly show that PROM significantly provided a protective effect in both extremely and highly toxic OPC intoxicated groups (Tables 2 and 3). The mean survival times estimated by Kaplan Meir survival analysis showed 2405.00±216.81 minutes in MPOX+PROM treated group as compared to 623.75±236.26 in MPOX only group. Similarly, mean survival time in DCP+PROM treated group was 2265.00±271.34 minutes as compared to 670.00±278.45 min-

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Table 4. Mean survival time in minutes over the period in different groups. All the treatment groups are statistically significantly improved in survival than no treatment group that is MPOX and DCP only.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean ± SEM</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPOX only</td>
<td>623.75±236.26</td>
<td>160.68-1086.82</td>
<td></td>
</tr>
<tr>
<td>MPOX+PROM</td>
<td>2405.00±216.81</td>
<td>1980.06-2829.94</td>
<td></td>
</tr>
<tr>
<td>DCP only</td>
<td>670.00±278.45</td>
<td>124.24-1215.76</td>
<td></td>
</tr>
<tr>
<td>DCP+PROM</td>
<td>2265.00±271.34</td>
<td>1733.18-2796.82</td>
<td></td>
</tr>
<tr>
<td>MPOX+PAM</td>
<td>1347.50±287.81</td>
<td>783.39-1911.61</td>
<td></td>
</tr>
<tr>
<td>MPOX+ATR</td>
<td>2523.75±192.40</td>
<td>2164.65-2900.85</td>
<td></td>
</tr>
<tr>
<td>MPOX+PROM+PAM</td>
<td>2642.50±160.78</td>
<td>2327.36-2957.65</td>
<td></td>
</tr>
<tr>
<td>MPOX+PAM+ATR</td>
<td>1458.33±335.10</td>
<td>801.54-2115.12</td>
<td></td>
</tr>
</tbody>
</table>

*All the treatment groups are statistically significantly improved in survival than no treatment group i.e. MPOX only. *statistically significant than G1 (MPOX).

Table 5. Pairwise comparison using Log-Rank (Mantel-Cox) test in Kaplan-Meir survival analysis.

<table>
<thead>
<tr>
<th></th>
<th>MPOX</th>
<th>MPOX+PROM</th>
<th>DCP</th>
<th>DCP+PROM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPOX</td>
<td>0</td>
<td>0.000</td>
<td>0.065</td>
<td>0.000</td>
</tr>
<tr>
<td>MPOX+PROM</td>
<td>0.000</td>
<td>0</td>
<td>0.000</td>
<td>0.032</td>
</tr>
<tr>
<td>DCP</td>
<td>0.065</td>
<td>0</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>DCP+PROM</td>
<td>0.000</td>
<td>0.699</td>
<td>0.000</td>
<td>0</td>
</tr>
</tbody>
</table>

**Statistical analysis**

Statistical analysis was performed on the mortality data of four cycles. Kaplan-Meier survival analysis was performed using SPSS 21.0 statistical software, SPSS Inc., Chicago, IL, USA. Kaplan-Meier survival analysis allows estimation of survival over time. The time starting from a defined point to the occurrence of a given event, for instance death is called as survival time. For each interval, survival probability is calculated as survivors divided by experimental individuals at risk. Experimental individual who have died, are not counted as “at risk”. Non-parametric Manwhitney-U test was used for statistical significance and *P* ≤ 0.05 was considered significant. SPSS 21.0 software package was used for all statistical evaluations.

Mortality/survival analysis

Tables 2 and 3 show the percentage of animals died at different time points over the period of 48 hours in MPOX and DCP treatment and after application of PROM. The results observed clearly show that PROM significantly provided a protective effect in both extremely and highly toxic OPC intoxicated groups (Tables 2 and 3). The mean survival times estimated by Kaplan Meir survival analysis showed 2405.00±216.81 minutes in MPOX+PROM treated group as compared to 623.75±236.26 in MPOX only group. Similarly, mean survival time in DCP+PROM treated group was 2265.00±271.34 minutes as compared to 670.00±278.45 min-

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Table 4 shows the survival analysis with MPOX, PAM and ATR (G5 TO G8). The mortality of control rats that had only received PAM, PROM and ATR, was 0%. Kaplan Meier survival curves are shown in Figure 2. The maximum survival was achieved in Group 7 (MPOX+PROM+PAM) followed by Group 6 (MPOX+PAM) and then Group 2 (MPOX+PROM). The standard combination, namely, MPOX+ATR+PAM (Group 8) ranked fourth. Based on the standard treatment protocol, it is evident that Group 8 is less effective than PROM administered regimes (Table 4 and Figure 2). The notion that “administration of PAM along with ATR is not worthy” is also noted in the results of survival times in minutes after treatment. Interestingly, the P-values calculated for the pair-wise comparison (Tables 5 and 6) revealed that administration of PROM instead of ATR produced higher survival times which are also statistically significant from control group.

Table 6. Pairwise comparison using Log-Rank (Mantel-Cox) test in Kaplan-Meir survival analysis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MPOX</th>
<th>MPOX+PROM</th>
<th>MPOX+PAM</th>
<th>MPOX+ATR</th>
<th>MPOX+PROM+PAM</th>
<th>MPOX+PAM+ATR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPOX</td>
<td>--</td>
<td>0.000</td>
<td>0.046</td>
<td>0.000</td>
<td>0.000</td>
<td>0.388</td>
</tr>
<tr>
<td>MPOX+PROM</td>
<td>0.000</td>
<td>--</td>
<td>0.008</td>
<td>0.686</td>
<td>0.388</td>
<td>0.23</td>
</tr>
<tr>
<td>MPOX+PAM</td>
<td>0.046</td>
<td>0.008</td>
<td>--</td>
<td>0.003</td>
<td>0.001</td>
<td>.859</td>
</tr>
<tr>
<td>MPOX+ATR</td>
<td>0.000</td>
<td>0.686</td>
<td>0.003</td>
<td>--</td>
<td>0.640</td>
<td>0.009</td>
</tr>
<tr>
<td>MPOX+PROM+PAM</td>
<td>0.000</td>
<td>0.388</td>
<td>0.001</td>
<td>0.640</td>
<td>--</td>
<td>0.003</td>
</tr>
<tr>
<td>MPOX+PAM+ATR</td>
<td>0.038</td>
<td>0.023</td>
<td>0.859</td>
<td>0.009</td>
<td>0.003</td>
<td>--</td>
</tr>
</tbody>
</table>

Figure 2. Kaplan-Meier survival plot: Survival is statistically significantly increased by all treatment groups. Legends are shown according to efficacy in plot. Higher protection was achieved when PROM and PAM is given together (G7; topmost line) followed by G6, G2, G4, G8, and G5. The standard application (PAM+ATR; G8) yielded less protection than other treatment groups.

RBC-AChE activities

The enzyme activities (% of baseline) in different groups are shown in Figures 3 and 4. The enzyme activities were measured in the survived animals (n=3-10) at 30 minutes, 24 and 48 hours after treatment. The mortality was high in MPOX and DCTP treatment groups, assuming less than 10% RBC-AChE activity which is not incorporated in the data, and hence, could not be measured practically. The RBC-AChE at 30 minutes was almost the same in all groups except the group which received MPOX+PROM, and was found to be with values that were less than the values observed for animals in Group 1 (P-value=0.020). Conversely, it was one of the most promising protection groups in terms of survival. Similarly, MPOX+PAM+ATR group showed increased reactivation of RBC-AChE at all-time points but number of survivor was less than the PROM administered groups. Overall, reactivation of RBC-AChE was progressively higher at 24 hours and 48 hours, even in the groups where PAM was not injected. A ranking of the reactivators according to their ability to increase enzyme activity is difficult since the data available are, for obvious reasons, from surviving animals only and may thus be biased.

Serum creatinine and LDH

Creatinine and LDH levels in serum are shown in Table 7. Though variations may be noted in
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The values but statistically no significance was found when compared with MPOX treatment group. The results suggest that administration of PROM failed to produce profound effect leading to cardiac toxicity. On the other hand, when PROM was administered alone (control group), it produced markedly but statistically not significant increase in CK level. The only statistical significance was noted in LDH level of MPOX+PROM+PAM group where it was found to be decreased when compared with the MPOX treated group.

Discussion

The treatment of poisonings produced by OPC-AChE inhibitors has remained unchanged for many decades with the muscarinic antagonist atropine (ATR) being used as a primary antidote. Meanwhile, application of OPC pesticides has been increased and anticipated to increase multifold in future. Likewise, in the event of mass casualty, shortage of medicine may be anticipated. Keeping all such circumstances, and based on the reality that the standard ATR is being mainly anti-muscarinic, the present study was designed to investigate the protective potency of PROM, an old-generation antihistamine with well-known centrally acting pharmacological profile which includes anti-muscarinic and anti-nicotinic effects. Two structurally (Table 1) and functionally (potentially) different OPCs were used to assess the protective effect of PROM.

The results the current study showed promising protective effect (Tables 3 and 4) of PROM against MPOX which is an extremely toxic and dicrotophos, a highly toxic OPC according to WHO, classification of pesticides. There was no statistical significance between MPOX+ATR and MPOX+PROM treatment groups. Noteworthy, the protection observed by the standard clinical application (MPOX+PAM+ATR) was less than by the group treated with MPOX+PROM. The latter finding reflects the concern raised by many researchers and clinicians regarding the use of PAM with ATR [25]. There is only one conference report by Kan et al. [10] who showed promising protection by PROM against OPC Sarin in rats. However, the first study on the use of antihistamine for OPC poisoning was reported in 1963 by Welch and Coon [5]. They concluded that pretreatment with chlorcyclizine significantly reduced the mortality induced by parathion, a parent compound of paraoxon, in mice. Previous antidotal efficacy studies of old-generation antihistamines were mainly focused on diphenhydramine [7-9, 12, 13, 26, 27]. Notably, Gupta et al. [28] reported a clinical case study, where cyproheptadine was tested against an OPC poisoned patient and found effective. Our studies with old-generation antihistamine, PROM, also showed good antidotal efficacy against MPOX and DCP which is in concurrence to earlier studies. Among the various probabilities of effectiveness, PROM is a centrally acting potent anticholinergic compound with both muscarinic and nicotinic components. It is well-known that OPC poisoning produces both muscarinic and nicotinic symptoms whereas the conventional antidotes usually include only antimuscarinic compounds. There
are accumulating arguments that support the usefulness to include anti-nicotinic compounds for a better protection [9]. Besides, it has been observed that early death due to OPC poisoning is considered to be centrally mediated [9]. Also, respiratory failure is a manifestation of acute OPC poisoning [29]. Thus, it may be implicated that the improved survival in the current study by using PROM may be due to its multi-pharmacological and centrally mediated action. The study does not provide clear evidence of role of PROM in the reactivation process of RBC-AChE, though the inhibition of RBC-AChE was almost similar to non-PROM treated group or even less. Since animals survived even at more than 90% of inhibition, a stage where animals do not survive, it may be presumed that there be little enough un-noticeable spontaneous reactivation of AChE that resulted in survival of animals. But the multiple pharmacological action of PROM is indeed a factor for the survival whether by prompting spontaneous reactivation, centrally mediated anti-nicotinic or anti-muscarinic action or by altering the detoxifying enzyme as found by Welch and Coon [5]. Petroianu et al. [22, 30, 31] investigated tiapride for acute paraoxon poisoning against rats and found very improved survival, though the RBC-AChE level was less than paraoxon alone treatment, and concluded that peripheral enzyme activity and mortality are not strongly correlated. Based on the evidences, it may be speculated that merely RBC-AChE is not essential for survival, rather some other mechanisms also play role in the survival after OPC poisoning.

It is noteworthy to mention that many other symptoms and factors are associated with OPC poisoning which may become a co-lethal and mortality factor but by large they are ignored. For instance, OPC intoxication also increases the release of biogenic amines including histamine in addition to many pro-inflammatory cytokines which causes neuro-inflammation [17, 19]. Cowan et al. [32] concluded that non-cholinergic mechanism of OP poisoning often includes anaphylactic shock which is prompted by autacoids such as histamine. Importantly, activation and degranulation of mast cells and basophils lead to the release of histamine, cytokines and other mediators into the extracellular environment and to the development of anaphylaxis. For instance, soman (a nerve agent OPC) has been reported to induce dose-dependently mast cell degranulation in rats and calcium dependent release of histamine from rat peritoneal mast cells. Sarin, (another nerve agent) vapor elevates histamine levels in Broncho alveolar lavage of guinea pigs [33]. Similarly, malathion metabolites have been reported to induce histamine release from basophils and peritoneal mast cells [34]. Furthermore, antihistamines are anti-inflammatory agents that act by preventing histamine release from mast cells and/or stabilizing histamine receptors in an inactive conformation. Therefore, it is suggested that sufferers of OPC poisoning may benefit from the potential anti-inflammatory properties of the antihistamines [35]. An additional promising feature of PROM which is recently reported is its potent antagonist property at various ion-gated channels.

### Table 7. Creatinine kinase and Lactate dehydrogenase level in serum of control and treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine kinase (U/L) Mean ± SD (P value)</th>
<th>Lactate dehydrogenase (IU/L) Mean ± SD (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPOX only</td>
<td>8007.0±1831.0 (0.071)</td>
<td>1031.3±394.0</td>
</tr>
<tr>
<td>MPOX+PROM</td>
<td>5736.0±1439.7 (0.071)</td>
<td>674.7±199.5</td>
</tr>
<tr>
<td>MPOX+PAM</td>
<td>8524.6±2319.0 (0.456)</td>
<td>975.4±325.5</td>
</tr>
<tr>
<td>MPOX+ATR</td>
<td>7842.7±1973.1 (0.796)</td>
<td>811.0±282.2</td>
</tr>
<tr>
<td>MPOX+PROM+PAM</td>
<td>7844.0±1183.0 (1.000)</td>
<td>651.3±107.8</td>
</tr>
<tr>
<td>MPOX+ATR+PAM</td>
<td>6834.7±471.5 (0.289)</td>
<td>906.7±819.6</td>
</tr>
<tr>
<td>PROM only</td>
<td>10054.8±1262.4 (0.121)</td>
<td>996.3±240.1</td>
</tr>
<tr>
<td>PAM only</td>
<td>7920.2±1154.9 (0.881)</td>
<td>786.4±369.5</td>
</tr>
<tr>
<td>ATR only</td>
<td>6917.3±1079.9 (0.606)</td>
<td>563.2±193.6</td>
</tr>
<tr>
<td>Saline control</td>
<td>6319.2±937.4 (0.327)</td>
<td>994.8±363.0</td>
</tr>
</tbody>
</table>
Promethazine as antidote for OPC poisoning

including human α-7 nicotinic acetylcholine receptors (α-7 nAChRs) [36]. Similarly, HI-6, an oxime with higher efficacy than other existing oxime-derived compounds used in OPC poisoning, was reported to have anti-nicotinic properties [37, 38]. Moreover, a potent protection was reported against sarin and tabun OPC nerve agents by the use of nicotinic antagonists [39]. Interestingly, α-7 nicotinic receptors have been linked to a wide variety of brain functions including pathological conditions such as Parkinson’s and Alzheimer’s disease which are reported to be delayed effects of OPC poisoning. In conclusion, the multiple pharmacological action of PROM including its anti-muscarinic, anti-nicotinic, antihistaminic, α-7 nAChR antagonistic effects along with its centrally mediated actions have produced the better protection in rat model which warrants further investigation as well. Additional investigations using different doses of PROM with various OPCs are also required to further evaluate the substantial risk/benefits of the clinical use of PROM.

Limitations

PROM has been reported to produce QT-prolongation effect. On the other hand, OPCs also cause bradycardia or tachycardia depending upon the type of the respective OPC, necessitating further investigations for the evaluation of its risks/benefits. However, it may be noted that high atropinaztion in OPC poisoning also causes serious adverse effect [40] and still ATR is the mainstream anti-muscarinic agent for OPC poisoning. In spite of the aforementioned side effect of PROM, the compound is widely available even as an OTC compound in different developing countries. In short, death/survival is still at present the gold standard one has to look at. Also, efficacy can only be defined by an increase in survival/reduction in mortality when looking at antidotal therapy for an agent with high acute toxicity. Any effect whether demonstrated in vitro or in vivo is meaningless unless it is translated to an increase/decrease in survival.

Conclusion

Promethazine, an old-generation antihistamine, has been found to be an effective antidotal agent for methyl-paraoxon and dicrotophos acute toxicity in Wistar rats. The observed efficacy was higher than that of ATR. The protective effect obtained in the present study was different from improving RBC-AChE, a marker enzyme. So, multi-pharmacological actions of PROM may be contributing to its enhanced protection. Additionally, PROM was not able to affect CK and LDH, indicating that there are no apparent signs of cardiac toxicity. Since toxicological profile of different OPC greatly varies, further investigations should be undertaken with other groups of OPCs.

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Disclosure of conflict of interest

None.

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