Some in vitro and in vivo Pharmacological Observations on Paraphenylenediamine (Hair Dye)

Haseeba A. Saad, Badreldin H. Ali, Hassan M. Moussa and Mohammed B. Ali

Abstract: Objectives: Paraphenylenediamine (PPD) is commonly used in our region as hair dye, and to intensify Henna color. Several cases of poisoning with this compound have been reported. Therefore, we have examined the actions of PPD on a variety of pharmacological preparations in an attempt to determine the basis of its toxicity. Methods: Several isolated rat, rabbit, frog and Guinea pig preparations were used, together with anesthetized cat for blood pressure measurement. Results: After incubation of PPD with chopped G. Pig lung tissue, the supernatant was found to contract G. Pig ileum. This action was abolished by chlorpheniramine, suggesting that PPD released histamine. PPD, or the chopped lung tissue preparation, given alone, did not contract G. ileum. Various doses of PPD did not affect striated muscle preparations, nor did it affect isolated tissues preparation, given alone, did not contract G. ileum. Various doses of PPD was also without effect on prostaglandin synthase or receptors. No significant change in blood pressure was observed following PPD injection at low doses in anesthetized cats, whereas high doses were lethal. Conclusion: PPD was ineffective in significantly altering the reactivity of several pharmacological preparations. It suggested the PPD releases histamine.

Key words: Paraphenylenediamine, hair dye, pharmacodynamics

Introduction
Paraphenylenediamine (PPD) is a synthetic compound that is widely used as a hair dye, in photochemical measurements, and also in manufacturing of tire cords and photographic developer (Macnab and Podger, 1975; Burnett and Corbett, 1977). Several cases of accidental, homicidal, and suicidal poisoning with PPD have been reported in the Sudan, where it is mainly syed to intensify the black color produced by Henna (Lawonia inermis), and to reduce the time required for dying and decorating hands and feet with Henna (El-Ansary et al., 1983; Suliman et al., 1983; Yagi et al., 1991; Abdel Karim et al., 1982). PPD has also been identified as one of the constituents of ‘home doctors’ (Averbukh et al., 1989).

As far as we are aware, there is no published work on the basic pharmacology of PPD. Therefore, we assessed its effect on some pharmacological preparations in vitro and in vivo. Our results may elucidate, at least partially, some of the toxicological actions of the dye.

Materials and Methods
Animals: Rabbits (White New Zealand strain, about 1.5 Kg), cats (local breed, 1.5-2.0 Kg), rats (Wistar strain, 180-200 g), and frogs (40 g) of both sex were used. They were supplied and maintained in the facilities of the Animals House of the Medicinal and Aromatic Plant Research Institute, Khartoum, Sudan). Nutritionally adequate feed and water were supplied to the animals ad libitum, except where mentioned.

In vitro experiments (isolated preparations): All the pharmacological preparations tested in this work were conducted essentially as described by Kitchen (1984). These include: Frog rectus abdominus muscle, Isolated rat uterus, Rat fundus strip, Isolated perfused rabbit heart, Rabbit aortic strip, Rat ascending colon, Guinea (G) pig ileum, Chopped G. Pig lung and Rat phrenic nervehemidiaphragm.

In vivo experiments: Anesthetized at blood pressure (Kitchen, 1984).

Drugs and Chemicals: All chemicals were analytical reagent grade. PPD and serotonin were bought from Sigma (St. Louis, MO, USA).

Statistical analysis: Values reported are means ± SEM (number of observations). Differences between means were estimated by the Student’s test, and probability (P) given. P less than 0.05 was considered significant.

Results
In vitro experiments
Frog rectus abdominus: PPD (1-500 µg/ml) had no effect when added alone to this preparation. However pre-addition of PPD (>10 µg/ml) reduced the sensitivity of the tissue to carbachol. At a concentration of 300-500 µg/ml, the tissue lost its sensitivity to carbachol (0.5-2.0).

Isolated rat uterus: PPD (100-200 µg/ml) did not produce an effect on this preparation when added alone. Pre-addition of PPD (>500 µg/ml) reduced the sensitivity of the tissue to carbachol (1 µg/ml). At a concentration of 600-2000 µg/ml of PPD, the tissue lost its sensitivity to carbachol (2 µg/ml).

Rat fundus strip: PPD (100-2000 µg/ml) did not affect this preparation when added alone. Pre-addition of PPD (>200 µg/ml) reduced the sensitivity of the tissue to serotonin (5-HT). At 600-2000 concentration of PPD, the tissue lost its sensitivity to 5-HT (0.5-2000 µg).

Isolated perfused rabbit heart: PPD, at concentrations of 500-1000 µg/ml, produced a slight increment in the contractility of the heart. At concentrations of 5-40 mg/ml, it significantly and dose-dependently increased contractility (p<0.01). At a concentration of 40 mg/ml the increased contractility (p<0.01). At a concentration of 40 mg/ml the increase in cardiac contractility was followed by an irreversible decrease. The cardiac output tended to decrease with increasing concentrations of PPD. The
Fig. 1: The effect of PPD on perfused rabbit heart. PPD (• Ph 5 mg) increased contractility. Pre-addition of Propranolol (■ Pr 10 µg) did not influence PPD effect. However, pre-addition of chloropheniramine (● Ch 10 µg) blocked the effect.

Fig. 2: The effect of PPD lung incubate on guinea-pig ileum. Administration of authentic PPD (▲ Ph 1 ml) and commercial PPD lung perfusate (∆ Ph 1 ml) on guinea-pig ileum produced marked contraction. These effects were blocked by pre-addition of chloropheniramine (● Ch. µG).

Table 1: The effect of paraphenylene diamine (PPD) on perfused rabbit heart.

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Contractility (g of tension)</th>
<th>Heart rate (beat/min)</th>
<th>Cardiac output (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.57±0.61</td>
<td>121.0±7</td>
<td>6.43±0.2</td>
</tr>
<tr>
<td>0.5</td>
<td>5.77±0.61</td>
<td>118.0±7</td>
<td>6.29±0.3</td>
</tr>
<tr>
<td>01</td>
<td>6.14±0.67</td>
<td>118.0±10</td>
<td>6.21±0.2</td>
</tr>
<tr>
<td>05</td>
<td>7.33±0.70</td>
<td>128.0±12</td>
<td>6.21±0.2</td>
</tr>
<tr>
<td>10</td>
<td>7.51±0.71*</td>
<td>137.0±18</td>
<td>6.07±0.2</td>
</tr>
<tr>
<td>20</td>
<td>6.99±0.88*</td>
<td>108.0±13**</td>
<td>5.21±0.4**</td>
</tr>
<tr>
<td>40</td>
<td>7.24±0.60</td>
<td>81.0±14**</td>
<td>4.67±0.5**</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=6)

*P<0.05, **P<0.01 (compared to control)

Heart rate tended to increase with increasing doses of PPD (5-10 mg/ml). At concentrations of 20-40 mg/ml, however, the heart rate significantly decreased (P<0.01).

Pre-addition of propranolol (10 µg) did not alter the increases in contractility and cardiac output that were produced by PPD (5 mg/ml). Pre-addition of chlorpheniramine (10 µg) abolished the effects produced by PPD, while the pre-addition of propranolol was ineffective (Fig. 1).

Rabbit aortic strip: The addition of PPD (4-1000 µg/ml) did not affect this preparation, nor did it alter the sensitivity of the tissue to noradrenaline (2 µg mL⁻¹).
Rat ascending colon: PPD (100-3000 mg/ml) did not produce significant effects when added alone to the preparation. Pre-addition of PPD (3-200 µg/ml) reduced the sensitivity of the tissue to carbachol (0.5 µg/ml). At a concentration of 3 mg/ml PPD, the tissue became insensitive to carbachol (0.5 µg/ml).

Chopped G. Pig lung and G. Pig ileum: Chopped lung tissues co-incubated with PPD (2 µg/ml) contracted the ileum. Pre-addition of chlorpheniramine (2µg/ml) to the gut bath abolished the contraction (Fig. 2).

Rat phrenic nerve-hemidiaphragm: PPD (10-400 µg/ml) did not affect this tissue. At higher concentrations (>500 µg/ml), PPD irreversibly inhibited the electrically-stimulated muscle twitches.

In vivo experiments
Cat blood pressure: Intravenous (IV) injection of PPD (2-71.5 µg/Kg) did not exert any significant action on blood pressure of anaesthetized cats. However, higher doses (>71.5 µg/Kg) were lethal to the animals.

Discussion
The present results indicate that PPD doses not affect striated muscles, since treatment with low concentrations of this substance did not produce agonistic or antagonistic effects on frog rectus abdominus muscle or diaphragm. However, higher concentrations caused irreversible reduction or loss of tissue sensitivity to carbachol or to electrical impulses. This may be related to the nectotic action of PPD on muscles, nerves, or both (Yabe, 1992). PPD, likewise, did not affect other preparations that are rich in typtaminergic, adrenergic or muscarinic receptors, indicating the lack of activity at these receptors. Previously, PPD was tested on skinned muscles, and it was postulated that PPD might cause leakage of Ca^{2+} from the sarcoplasmatic reticulum, which consequently causes changes such as continuous contractions, that may lead final to irreversible damage (Yabe, 1992).

The rat ascending colon did not respond to PPD. The activity of this preparation is known to be mediated via prostaglandins (PG) (Vane, 1971). This indicates that PPD has neither PG-like action, nor the ability to alter the synthesis of endogenous PG. Similarly, the lack of effect of the dye on the colon may indicate that it does not affect the autonomic innervation of the preparation (Table 1). PPD produced a significant positive inotropic and chronotropic effect on the isolated perfused rabbit heart. This effect cannot be attributed to beta receptors stimulation, since it was not blocked by the pre-addition of propranolol. The effect of PPD on the heart was blocked by chlorpheniramine. This effect may be due to the stimulation of H₁ and H₂ receptors of the heart, which have inotropic and chronotropic effects (Douglas, 1985). Cardiac muscles continuously produce histamine, and the isolated heart is considered a suitable preparation for the study of histamine release (Giotti et al., 1966). It is possible that PPD releases histamine from mast cells. G. Pig ileum was clearly contracted by a supernatant of G. Pig lung tissue that had been incubated with PPD. PPD alone (without lung tissue) caused no contraction. This effect was probably mediated by H₁ receptors, because it was blocked by chlorpheniramine. This experiment confirmed the histamine-releasing property of PPD. G. Pig lung was used here because of its high mast cells content, and G. Pig ileum because of the presence of H₁ receptors (Kitchen, 1984). The mechanism by which PPD releases histamine is not certain. It may involve impairment of the cellular events linked to exocytosis (Hazama et al., 1992). Disturbances in cell membranes leading to cell lysis (Lau and Pearce, 1990), or to opening of certain Ca ion channels leading to elevated intracellular Ca^{2+} concentration, which, in turn, may activate mast cell secretion (Eleno et al., 1990). The histamine-releasing property of PPD may provide an explanation for the decrease in the rate and force of rabbit isolated heart, since histamine is known to have direct actions on the heart, by promoting Ca^{2+} influx and hastening diastolic depolarization in the sinoatrial node. It also slows atrioventricular (AV) conduction to increase automaticity and elicit diverse arrhythmia (Douglas, 1985). These actions are ascribed to H₁ receptors, except for the AV conduction, which is attributed to H₂ receptors (Douglas, 1985). As the contractility of the isolated heart was blocked by an H₁ receptor antagonist, it may be that there is a loss of selectivity at higher doses, or that there is a species difference.

PPD, as a histamine releaser, may be without effect on the autonomic nervous system or the neuromuscular junctio. Our contention that PPD is a histamine-releaser, is supported by the observation that PPD exposure has been associated with increased histamine tissue levels, hypersensitivity and allergic reactions in chicks and rats (Zhao and Fan, 1991). It has also been shown that PPD induces contact dermatitis by causing oxidative stress in the keratinocytes (Picardo et al., 1991), whereas Rajaka and Blohm (1970) suggested that benzoquinone formation plays an important role in the allergic reaction to PPD. Ng-Sk, in Singapore, considered PPD as one of the chemicals that can produced a different type of contact dermatitis (Ng, 1990). It was also found that challenging humans with PPD produces a positive reaction (Zhao and Fan, 1991).

This study has shown that PPD was ineffective in altering the reactivity of several pharmacological preparations. However, it apparently is a histamine releaser, and this may explain its toxicity in chicks and rats.

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References
Saad et al.: Paraphenylenediamine pharmacology