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Analgesic and sedative action of monocrotophos following oral administration in rats

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Introduction: Organophosphorous compounds that inhibit cholinesterase are now widely available and commonly used as agricultural insecticides in many countries including Sri Lanka. Although claimed to be selectively toxic to insect pests [1], with sufficient exposure they are potentially harmful and often toxic to humans [1]. Organophosphates are the commonest group of chemicals involved in suicidal poisoning in Sri Lanka [2], perhaps due to their easy availability.

Methamidophos and monocrotophos, commonly used organophosphorous insecticides in Sri Lanka, induce both immediate pseudoneuropathy and delayed neuropathy in humans following deliberate oral intake [3]. Recently, we demonstrated, for the first time, analgesic and sedative effects in rats with sublethal doses of methamidophos [4], which may be indicative of neurological disturbances.

Such activity may also be present with sublethal doses of monocrotophos, but has not been reported so far. The present work was carried out to investigate this possibility.

Materials and methods: Healthy, adult cross bred male albino rats (weight: 200–250 g) from the Department of Zoology colony were used. The rats were housed in plastic cages under standardised animal house conditions (temperature: 28–30°C; photoperiod: approximately 12 h light and 12 h dark daily; relative humidity: 50–55%) and fed with standard pelleted food (Oils and Fats Corporation, Seeduwa, Sri Lanka) and tap water *ad libitum*.

Monocrotophos was obtained from Lankem Ltd, Colombo, Sri Lanka, and was dissolved in distilled water to obtain the required concentration in 1 mL solution (3.5 mg kg^{-1}; 1.75 mg kg^{-1}, 0.85 mg kg^{-1} and 0.438 mg kg^{-1}).

Five groups of rats (n = 6/group) were orally administered (via gastric intubation) 1 mL distilled water (vehicle) and 1 mL of monocrotophos solution (different concentrations) for three alternative days (between 10.00 and 10.30 h) in the following manner. Group 1 received 1 ml distilled water. Group 2 received 3.5 mg kg^{-1} day^{-1}. Group 3 was given 1.75 mg kg^{-1} day^{-1}. Group 4 received 0.85 mg kg^{-1} day^{-1} and Group 5 0.45 mg kg^{-1} day^{-1}. The day of initiation of treatment was designated as day 1.

The treated rats were observed twice daily for mortality and changes in appearance or behaviour. Body weight, food consumption and water intake were determined daily during the period of investigation. The consistency of the faeces was also noted. Each rat from Groups 1, 2, 3 and 4 was then placed in the centre of a rat hole-board apparatus and was given a 7.5 min trial period. During this time, the number of head-dips (poking into holes), rears and locomotor activity were scored as described in detail by File and Wardill [5]. The time spent for head dip was then computed.

1–2 h following this test, the animals were individually placed in the tail flick analgesia meter (Model MK 330A, Muromachi Kikai Co. Ltd, Tokyo, Japan) at a beam level of 43 and the time taken (in s) for the rat to flick the tail away from the light source (reaction time) was determined. The animals were then individually placed on a hot plate analgesia meter (Model MK 350A, Muromachi Kikai Co. Ltd, Tokyo, Japan) maintained at 55°C and the time taken (in s) to lick the forepaws (reaction time) was measured.

On day 8, righting reflexes (in s) were tested in vehicle-treated rats and in rats treated with the highest dose of monocrotophos (3.5 mg kg^{-1} day^{-1}) as described by Martin *et al.* [6].

The results are represented as means ± SEM. Statistical comparisons were made using the Mann Whitney U Test and Trend Test. The significance level was set at $p < 0.05$.

Results: Monocrotophos caused marked piloerection within 5–10 min of administration, and this effect subsided within 30–60 min. In addition, all of the doses of monocrotophos induced moderate salivation (wet zone almost half the sub maxillary area) and mild lacrimation 0.5–1 h following administration. This effect was followed by moderate to mild redening of the mucus membrane along the oral cavity and nose tip. These symptoms were, however, transient (lasting 3–4 h). No deaths were recorded during the study period. The appearance of the faecal pellets of the monocrotophos-treated rats was almost identical to that of the control rats.

None of the doses of monocrotophos caused a significant suppression ($p > 0.05$) in the intake of either food or water (data not shown). However, with the highest dose there was significant trend towards suppression of food and water intake (Trend-test, $p < 0.001$). None of the treatments significantly ($p > 0.05$) altered the weight of the animals.

The righting reflexes of control rats and those treated with 3.5 mg kg^{-1} day^{-1} of monocrotophos were respectively 0.4 ± 0.05 and 0.5 ± 0.06 s.

Table 1 summarises the results of the tail flick and hot plate analgesic tests. The two higher doses of monocrotophos induced marked and significant antinociceptive effects (in

Table 1: Effect of monocrotophos on reaction time of rats when evaluated using tail flick and hot plate tests (means ± SEM; ranges in brackets).

Treatment (mg kg^{-1} day^{-1})	Reaction time (s)	
	Tail flick technique	Hot plate technique
Vehicle control (distilled water 1 mL day^{-1})	6.7 ± 1.1 (3.5–10.6)	6.5 ± 1.3 (2.1–10.0)
Monocrotophos		
3.50	18.9 ± 2.6** (12.6–30.6)	17.7 ± 1.2* (12.1–20.4)
1.35	13.7 ± 0.6* (11.7–16.1)	12.7 ± 1.4* (17.8–18.1)
0.88	8.2 ± 0.8 (6.2–11.6)	8.5 ± 1.2 (5.3–13.7)
0.44	6.5 ± 1.6 (2.3–13.3)	10.0 ± 0.7 (6.5–11.8)

*As compared with control, *p < 0.01; **p < 0.001.*

Table 2: Effect of monocrotophos in the rats (n = 6) in the 7.5 min trial in the hole-board (means ± SEM, ranges in brackets).

Treatment (mg kg^{-1} day^{-1})	Number of head dips	Time/dip (s)	Number of rears	Locomotor activity
Vehicle control (distilled water)	11.33 ± 1.20 (8.00–14.00)	6.4 ± 0.61 (4.9–8.20)	45.00 ± 2.80 (36.00–48.00)	24.80 ± 3.80 (11.00–32.00)
Treatment (monocrotophos)				
3.75	5.50 ± 1.40** (1.00–9.00)	3.7 ± 0.92** (1.3–6.00)	13.00 ± 3.10** (1.00–22.00)	8.50 ± 1.90** (2.00–12.00)
1.35	5.80 ± 1.10** (3.00–10.00)	3.8 ± 0.38** (2.4–4.70)	16.20 ± 2.40** (11.00–26.00)	12.10 ± 1.50* (7.00–18.00)
0.87	10.50 ± 1.90 (2.00–16.00)	7.4 ± 0.38 (3.5–12.30)	36.50 ± 3.30 (21.00–45.00)	18.70 ± 3.00 (4.00–29.00)
0.44	9.80 ± 1.40 (6.00–14.00)	6.9 ± 1.00 (4.6–12.00)	34.00 ± 5.80 (12.00–52.00)	22.00 ± 3.50 (9.00–30.00)

*As compared with control treatment, *p < 0.05; **p < 0.01.*

terms of prolongation of reaction time) when tested in both types of analgesic meters (tail flick: 3.5 mg kg^{-1} day^{-1} by 182.08%, $p < 0.001$; 1.7 mg kg^{-1} day^{-1} by 104.48%, $p < 0.002$ and in the hot plate: 3.5 mg kg^{-1} day^{-1} by 172.31%, $p < 0.002$; 1.7 mg kg^{-1} day^{-1} by 95.38%, $p < 0.05$). The analgesic effect was dose-related (tail flick technique, $r = 0.98$ and hot plate test, $r = 0.99$).

The EC_{50} values for the tail flick technique was 2.7 mg kg^{-1} day^{-1} and for the hot plate test was 3.5 mg kg^{-1} day^{-1}. Further, when the analgesic activity was assessed using the tail flick technique, the withdrawal of the tail was not associated with the characteristic Straub reaction (*i.e.* reaction of the tail across the back of the animal in an S-shaped curve due to contraction of the Sacro-coccygeus dorsalis muscle) [7].

Table 2 summarises the results obtained in the rat hole-board technique. The two higher doses of the monocrotophos caused significant impairment in all the four parameters tested: number of head dips (3.5 mg kg^{-1} day^{-1} by 51.45%, $p < 0.01$ and 1.7 mg kg^{-1} day^{-1} by 48.8%, $p < 0.01$), time per head dip (3.5 mg kg^{-1} day^{-1} by 42.19%, $p < 0.01$ and 1.7 mg kg^{-1} day^{-1} by 40.63%, $p < 0.01$), number of rears (3.5 mg kg^{-1} day^{-1} by 71.11%, $p < 0.01$ and 1.7 mg kg^{-1} day^{-1} by 64%, $p < 0.01$) and locomotor activity (3.5 mg kg^{-1} day^{-1} by 65.77%, $p < 0.01$ and 1.7 mg kg^{-1} day^{-1} by 51.27%, $p < 0.05$). These effects were dose-related (number of head dips; $r = -0.844$, $p < 0.01$, time per dip; $r = -0.836$, $p < 0.01$, number of rears; $r = -0.887$, $p < 0.01$, and locomotor activity; $r = -0.943$, $p < 0.001$). EC_{50} values for number of dips, time per dip, number of rears and locomotory activity were respectively 4.3, 4.2, 5.6, and 3.7 mg kg^{-1} day^{-1}.

Discussion: These results show that monocrotophos, like methamidophos [4], has both analgesic and sedative activity in rats when given in sublethal doses (0.438–3.5 mg kg^{-1} day^{-1}). Monocrotophos-induced antinociception was evident at low doses and was dose-dependent. This is usually indicative of a receptor mediation. However, this antinociception activity is unlikely to be operative via an opioid mechanism since there was no Straub reaction: in rats, the Straub reaction is characteristic of morphine-like analgesic action [7]. In view of its reported pharmacology [8], the analgesic activity of monocrotophos (in the tail flick rest) cannot be attributed to changes in tail skin temperature as seen with drugs like desipramaine and zinelidine [9].

However, it is very likely that a cholinergic mechanism has played a major role in monocrotophos-induced analgesia: antichoiline-esterase agents and cholinomimetic drugs [10] possess analgesic activity. The fact that monocrotophos is a acetylcholine-esterase inhibitor [2], and the appearance of cholinergic symptoms following monocrotophos administration, support this motion.

Impairment of locomotor activity was evident following monocrotophos administration. Thus it is possible that a motor impairment and/or deficit in muscle growth may contribute to the observed prolongation of the hot plate and tail flick responses. However, possession of completely normal righting reflexes in the monocrotophos-treated rats argues against the former mode of action. The unimpaired food and water intake, and body weight gain, argue against the latter mode of action. Unlike monocrotophos, metamidophos suppresses food and water intake in rats [4].

In the hole-board test, the two higher does (3.75 mg kg^{-1} day^{-1} and 1.35 mg kg^{-1} day^{-1}) caused significant impairment in all the four parameters investigated. This is indicative of profound sedative activity as this technique is claimed to be remarkably sensitive and selective in evaluating the sedative potential of drugs [5].

On the other hand, the two lower doses (0.85 mg kg^{-1} day^{-1} and 0.38 mg kg^{-1} day^{-1}) failed to inhibit any of the four parameters of sedation. Importantly, on the basis of EC_{50} values, both the analgesic and sedative potential of monocrotophos are lower than that of methamidophos. However, with respect to sedation, if the number of parameters inhibited is considered as a measure of potency, monocrotophos is more potent than methamidophos. Monocrotophos inhibited all four parameters while methamidophos, irrespective of the dose tested, impaired only three parameters [4].

In conclusion, our data show that even relatively low doses of monocrotophos, like methamidophos [4], can induce analgesic and sedative effects in rats. This is a matter for clinical concern in Sri Lanka as both these organophosphorous insecticides are widely used in agriculture, often without proper precautions and adherence to recommended dosages.

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