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Anxiolytic effect of *Murraya koenigii* leaf extract in rats

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Introduction: Use of anxiolytic drugs is increasing. Several classes of such agents are available, benzodiazepines forming the most frequently prescribed group [1], However, most, if not all of these drugs are likely to induce physical or psychological dependency with regular use, while withdrawal symptoms may develop if they are stopped suddenly [2]. Thus there is a need for the development of novel anxiolytic drugs without such undesirable side effects.

Plants can be a potential source of anxiolytic drugs. However, the potential of plants as anxiolytic agents has not been fully investigated.

We have now evaluated the anxiolytic activity of a water extract of mature leaves of *Murraya koenigii* (Family Rutaceae; known as karapincha in Sinhala and Karuvembu in Tamil), using rats and the shock-induced suppression of drinking test [3]. We selected this plant because some Ayurvedic physicians in Sri Lanka claim that a water extract of *M. koenigii* leaves possesses anxiety relieving action. It is, therefore, used in the treatment of hiccough and other conditions. However, this has not been scientifically validated.

Materials and methods: Mature fresh leaves of *M. koenigii* were purchased from the main vegetable market in Colombo, Sri Lanka. The identity of the leaves was authenticated by Professor R.N. de Fonseka, Department of Botany, University of Columbo, Sri Lanka. 120 g of the leaves were crushed in a domestic mincer (National, Model MX – T110PN, Matsushita Electric Co. Ltd., Taiwan) with 625 mL distilled water. The slurry was squeezed and filtered through muslin and the extract stored at 4°C until use. The extract had a brownish colouration and contained 20 mg of solid matter per millilitre.

Healthy adult male cross bred albino rats (mean weight 250 ± 20 g; means ± SEM) from the Department colony were used. They were housed under standardised animal house conditions with free access to tap water and pelleted food (Oils and Fats Co. Ltd., Seeduwa, Sri Lanka). The rats were deprived of water for 22–24 h before the commencement of the experiment.

The rats were randomly divided into eight groups. Four of these (groups 1, 2, 3, 4) consisted of seven animals each, while the other four (groups 5, 6, 7, 8) consisted of six animals each. Rats in groups 1 and 5 were administered orally with 1 mL of the extract and rats in groups 3 and 7 with 2 mL of the extract using a gastric tube, between 9.00 and 9.30 h. Rats in groups 2 and 6 and 4 and 8 were given 1 and 2 mL of distilled water respectively by the same route.

3 h following the administration, the rats in groups 1 and 3 (extract-treated) and in groups 2 and 4 (vehicle-treated) were individually placed in the plexiglass box (20 × 20 × 30 cm) of a Vogel conflict test system apparatus (Model VC – 001, Muromacchikikai, Co. Ltd, Tokyo, Japan) and the anxiolytic effect was evaluated in a 15 min testing session.

The intensity and the duration of the electric shock used were 0.5 mA for 0.5 s. The time taken for a rat to accept 25 shocks was noted and the average number of shocks received per min was computed. The delivery of shocks began after the rat was allowed to drink water for 1 min. This test system is based on shock-induced suppression of drinking and the number of shocks the rat accepts per unit time is taken to indicate the level of anxiety (*i.e.* higher the number of shocks accepted by rat per minute, the less anxious it is).

3 h following the administration the rats in groups 5 and 7 (extract-treated), and in 6 and 8 (vehicle-treated) were placed individually at the middle of a rat hole-board and observed for 7.5 min. During this period the number of head dips, number of rears and locomotory activity was recorded. This test was done to evaluate the sedative potential of the extract [4].

The results are expressed as means ± SEM. Statistical comparisons were made using Mann-Whitney U test and $p < 0.05$ was considered as significant.

Results: As shown in Table 1, compared to control treatments, the lower dose of the extract had no significant effect ($p > 0.05$) on the rate of acceptance of shocks by the rat. By contrast, the higher dose significantly ($p = 0.017$) increased (by 76%) the number of shocks accepted per minute.

In the rat hole-board technique, none of the parameters investigated was significantly changed by either the lower or higher dose of the extract ($p > 0.05$) (see Table 2).

Table 1: Effect of Murraya koenigii *leaf extract in rats in a 15 min trial session in the Vogel conflict test (means ± SEM; range in brackets).*

Treatment regimen	*n*	Average number of shocks per minute
Control (distilled water) 1 mL	7	5.00 ± 0.43 (2.50–6.25)
2 mL	7	4.02 ± 0.84 (1.32–8.30)
Leaf extract 1 mL	7	4.56 ± 0.45 (3.13–6.25)
2 mL	7	7.46 ± 1.01* (3.57–12.50)

* *As compared with control,* p = *0.017.*

Table 2: Effects of Murraya koenigii *leaf extract in rats in a 7.5 min trial in the hole-board (means ± SEM; range in brackets).*

Treatment regimen	*n*	Number of head dips	Number of rears	Locomotory activity
Control (distilled water) 1 mL	6	7.00 ± 1.61 (0.00–12.00)	8.83 ± 1.25 (6.00–14.00)	11.50 ± 2.98 (2.00–21.00)
2 mL	6	4.83 ± 0.95 (2.00–8.00)	12.50 ± 3.41 (0.00–26.00)	8.16 ± 3.00 (0.00–23.00)
Leaf extract 1 mL	6	7.83 ± 1.91 (0.00–15.00)	9.83 ± 1.38 (4.00–14.00)	12.16 ± 2.69 (6.00–24.00)
2 mL	6	10.50 ± 2.75 (3.00–20.00)	15.33 ± 4.75 (0.00–37.00)	12.00 ± 4.22 (1.00–30.00)