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Analgesic activity of *Murraya koenigii* leaf extract in rats

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Introduction: Some native doctors practising the traditional system of medicine in Sri Lanka recommend leaf extracts of *Murraya koenigii* Spreng. Syst. Veg. (Family Rutaceae; known as karapincha in Sinhala and karuvembu in Tamil) for the relief of pain associated with abdominal colic. Thus it is possible that these leaves possess potent analgesic activity, but this has not been evaluated scientifically. Furthermore, if the presence of potent antinociceptive activity in the leaves of *M. koenigii* is proven scientifically, then it could be used as a potential natural source for the development of novel analgesic drugs. Virtually every pharmacological class of drug includes a natural product prototype, most frequently plant-derived [1].

The aim of this study was to evaluate the analgesic activity of a water extract of mature leaves of *M. koenigii* in both males and females using tail flick [2] and hot plate [2] paradigms.

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 Colombo, Sri Lanka. 120 g of the leaves were crushed in a domestic mincer (National, Model MXT110PN, Matsuhita 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 mL.

Healthy adult cross bred albino rats (males weighing 200–250 g and females 200–225 g) from our own stock were used. The animals 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 were allowed free access to pelleted food (Oils and Fat Corporation, Seeduwa, Sri Lanka) and tap water. The rats were deprived of food 1 h before the commencement of the experiment.

The male rats were randomly divided into 5 groups, each consisting of 6 individuals. Rats in group 1 was orally given (between 10.00–10.30 h) 1 mL distilled water, and groups 2, 3, 4 and 5 respectively 0.5, 1, 2 and 4 mL of the leaf extract.

Female rats were subjected to vaginal smearing to select animals in pro-oestrous (n = 6), oestrous (n = 6) and dioestrous (n = 6) conditions of their vaginal cycle and were similarly treated with 1 mL of the extract. Another set of pro-oestrous rats (n = 39) were selected and were individually paired with a sexually experienced male rat (between 17.00 and 18.00 h). Successful mating was confirmed by the presence of sperm in the vaginal smear the following morning (8.00–8.30 h) and was designated as day 1 of the presumed pregnancy.

Six of these pregnant rats were treated with 1 mL of the extract on day 3 (early pregnancy) another six on day 10 (mid pregnancy) and yet another six on day 18 (late pregnancy) of pregnancy. Simultaneously, control rats in the same stage of pregnancy (n = 4 per stage) as the treated group were treated with 1 mL of distilled water.

1 h following the administration of the extract or distilled water the rats were individually placed in the tail flick analgesia meter (Model MK 330A, Muromachi Kikai Co. Ltd., 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 on a hot plate analgesia meter (Model MK 350 A, Muromachi Kikai Co. Ltd., Japan) maintained at 55°C and the time taken (in s) to lick the forepaws (reaction time) was measured. Both these reaction times were then determined at 2, 3, 4 and 6 h post-treatment.

The results are represented as means ± SEM. Statistical comparisons were made between pre-treatment values and treatment values using Mann-Whitney U test. Significance levels was set at $p < 0.05$.

Results: The data obtained with the two algesiometric tests are summarised in Tables 1 (tail flick) and 2 (hot plate). There were no significant differences in baseline latencies (in either test) among the groups, except between the males and females at late pregnancy ($p < 0.01$), where the difference in nociception was significant.

0.5 and 1 of extract (up to 4 h), and 2 mL of the extract (up to 3 h) induced a marked (262%) and significant prolongation of the reaction time in male rats in the tail-flick test. In contrast, the highest dose (4 mL) increased (by 228%) the reaction time significantly ($p < 0.001$) only at the third hour following treatment. The EC50 values for this analgesic effect in the males at 1, 2 and 3 h post-treatment were respectively 18.4, 18.2 and 17.4 mg (in terms of solid content).

In the non-pregnant rats, the extract (1 mL) failed (when assessed by tailflick test) to induce any significant ($p > 0.05$) analgesic activity in the oestrous group. However, the same dose of the extract had significant ($p < 0.001$) hypoalgesic effects in pro-oestrous (by 230% up to 3 h) and dioestrous (by 131% up to 4 h) rats. Similarly, this dose of extract produced profound and significant ($p < 0.002$) analgesia in rats in early- and mid-pregnancy up to 4 h.

None of the extract treated rats displayed overt signs of toxicity or abnormal patterns characteristic of clincal toxicity. Furthermore, the deflection of the tail following thermal stimulation in the tail flick apparatus was not associated with the typical Struab reaction (erection of the tail across the back of the animal in a S-shaped curve due to the contraction of the sacrococcygeus dorsalis muscle) [3].

When the analgesic activity was evaluated in the males, using the hot plate technique, 0.5 mL (by 129%) and 1 mL (by 89%) of the extract induced marked and significant ($p < 0.001$) prolongation in the reaction time (up to 4 h). On the other hand, 2 mL of the extract induced a significant ($p < 0.05$) analgesia only (by 63%) at 2 h and and 3 h post-treatment. In complete contrast, the highest dose failed to induce significant analgesia at any time point.