

## Protection by *Murraya koenigii* leaf extract against ethanol-induced gastric lesions in rats

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**Introduction:** The mature leaves of *Murraya koenigii* (Spreng.) (Family, Rutaceae, Karapincha in Sinhala and Karuvembu in Tamil) are commonly used in Sri Lanka for flavouring soups, vegetables and meat dishes [1]. Many Ayurvedic physicians in Sri Lanka also prescribe these leaves for constipation, abdominal colic, diarrhoea and hic-cough [1]. On the other hand, some practitioners of Sri Lanka folk medicine make staggering claims that *M. koenigii* has the ability to impair the development of gastric ulcers. The validity of these claims, hitherto, has not been scientifically proven.

The aim of the present study was to investigate whether the mature leaves of *M. koenigii* possess gastroprotective activity as is claimed, and if present, to evaluate the possible mechanisms of action. This was done with a water extract of *M. koenigii* leaves given to rats, using alcohol as the gastric ulcerogen.

**Materials and methods:** Mature and fresh *M. koenigii* leaves were plucked from a tree at a home garden in Polgasowita, Sri Lanka. The identity of the leaves was authenticated by Professor R.N.F. de Foenseka, Department of Botany, University of Colombo, Sri Lanka.

2 kg of the leaves were crushed with 750 mL of distilled water using a domestic crusher (John Oster Manufacturing Co., New York, USA). The resulting slurry was then squeezed and filtered through muslin, and the filtrate (extract) stored at 4°C until use. The concentration of the extract was determined by evaporating 250 mL with excess methanol (Petroleum Corporation, Sapugaskanda, Sri Lanka) at 60 ± 5°C to dryness and measuring the residual weight. The methanol makes a low boiling azeotropic mixture, which facilitates evaporation.

Cross-bred female albino rats (175-225 g) from our own colony were used. They were housed individually in raised mesh bottomed cages (to prevent coprophagia) under standardised animal house conditions (temperature: 28-30°C, photoperiod about 12 h light and 12 h dark, relative humidity 50-55%) with free access to pelleted food (Oils and Fats Co., Ltd. Seeduwa, Sri Lanka) and tap water. Food was withheld for 36 h and tap water for 12 h, before the commencement of each experiment. All studies were carried out using 5-12 rats per group.

Gastric lesions were induced as described by Robert *et al.* [2], using oral administration (1 mL/animal) of absolute ethanol (Fluka Chemicals Co., Buchs, Switzerland). 1 mL of the extract (final concentration 200, 400 or 800 mg kg<sup>-1</sup>) or distilled water was administered either orally or intraperitoneally to different groups of rats (see Table 1 for numbers) 30 min before ethanol administration. 1 h following ethanol treatment the animals were killed by an overdose of ether (BDH Chemicals Ltd., Poole, UK).

The stomachs were removed immediately and were instilled with 5 mL of 10% formalin (v/v) and left immersed

for 6-10 min in a beaker containing 10% formalin. The stomachs were then slit opened along the greater curvature and examined macroscopically for linear haemorrhagic lesions in the mucosa of the glandular portion. The length (mm) of the linear lesions was measured using a vernier caliper (Fisons Scientific Equipment, Loughborough, UK) fixed with two pointed fine pins at the arms. These measurements were summed up and divided by the number of animals which received a specific treatment.

The concentrations of the extract (given orally and intraperitoneally) that were required for 50% inhibition of lesions (EC<sub>50</sub>) were calculated with 95% confidence limits using probit analysis. The dose dependency of the gastroprotective activity of the extract was determined using linear regression analysis.

18 rats were pre-treated with a 10 mg kg<sup>-1</sup> dose of indomethacin (Sigma Chemical Co., St Louis, MO, USA) (dissolved in 1% sodium bicarbonate solution) given subcutaneously, before they were orally treated with 400 mg kg<sup>-1</sup> (*n* = 6), 800 mg kg<sup>-1</sup> of extract (*n* = 6) or distilled water (*n* = 6). These rats were given orally 1 mL of absolute ethanol 30 min following the administration of the extract or vehicle. 1 h after this ethanol treatment the rats were killed using an overdose of ether and the lengths of the linear lesions were measured as described previously.

13 rats were orally given a 400 mg kg<sup>-1</sup> dose of extract (in 1 mL) and another 16 rats with 1 mL distilled water. 1 h later, the rats were anaesthetised using ether, their abdomens were opened under aseptic precautions and their pylori were ligated, taking care not to interfere with the blood supply to the stomachs. The abdomens were closed and animals allowed to regain consciousness.

4 h after pylorus ligation the animals were killed by an overdose of ether. The external appearance of the stomach was noted and the gastric contents collected using a plastic syringe and the volume was recorded. After centrifugation for 15 min at 430 g the total acidity of each sample was determined by titration against 0.1 N NaOH to pH 7. The acid content was expressed in mM. The amount of gastric acid secretion was calculated as the product of the amount of gastric solution and the acidity [3].

The thickness of the gastric mucus layers was monitored indirectly using the method of Corne and Wood as described by Onoda *et al.* [4]. Briefly, 6 rats were orally administered 400 mg kg<sup>-1</sup> dose of extract (in 1 mL) and another 6 were given 1 mL distilled water. 1 h later, the animals were killed by an overdose of ether.

The stomachs were excised, slit opened along the greater curvature and rinsed in 0.25 M sucrose solution. Each stomach was then incubated in a 10 mL aliquot of 0.1% Alcian blue solution (w/v) containing 0.15 M sucrose and 0.05 M sodium acetate (pH = 5.8) for 2 h at 30°C. The stomachs were then washed twice in 0.25 M sucrose solution and each was immersed in 10 mL of 0.5 M magnesium chloride for 2 h at 30°C to elute the Alcian blue bound to the mucus. The solution was thoroughly shaken with 10 mL of