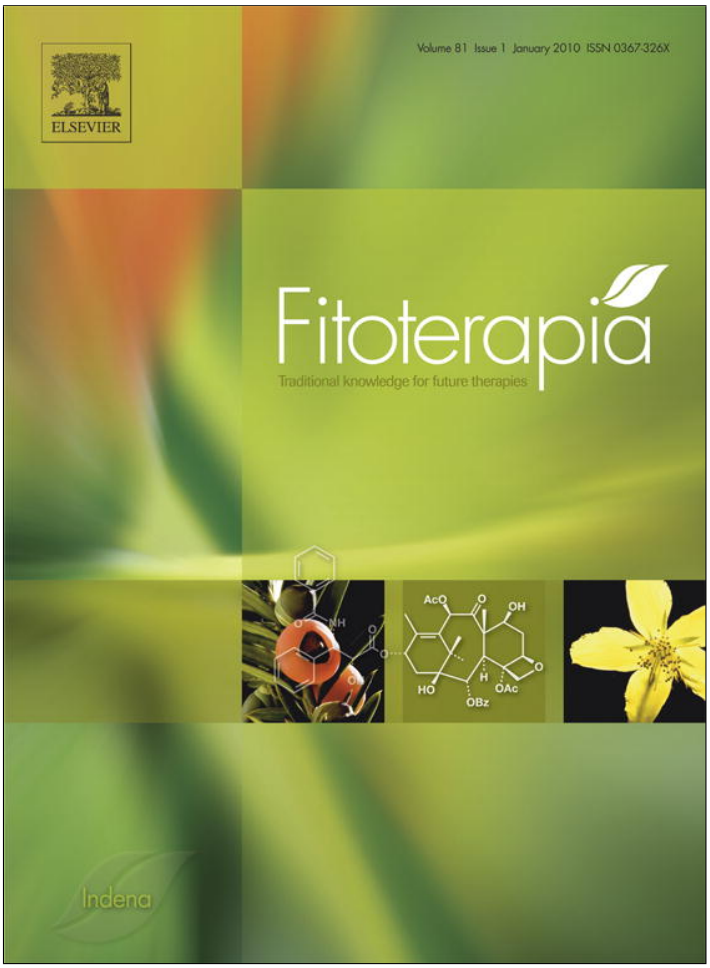

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Antidiarrhoeal activity of carbazole alkaloids from *Murraya koenigii* Spreng (Rutaceae) seeds

Suvra Mandal [b], Anupam Nayak [a], Manoj Kar [d], Samir K. Banerjee [b], Ashes Das [b], S.N. Upadhyay [c], R.K. Singh [c], Avijit Banerji [a], Julie Banerji [a,*]

[a] Centre of Advanced Studies on Natural Products including Organic Synthesis, Department of Chemistry, Calcutta University, 92, A.P.C. Road, Kolkata – 700009, India
[b] Department of Chemistry, National Research Institute of Ayurveda for Drug Development, Bidhannagar, Kolkata – 700091, India
[c] Department of Pharmacology, National Research Institute of Ayurveda for Drug Development, Bidhannagar, Kolkata – 700091, India
[d] Biochemistry Unit, Neonatology, Institute of Post Graduate Medical Education and Research, Kolkata – 700020, India

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ABSTRACT

The bioassay guided fractionation of the *n*-hexane extract of the seeds of *Murraya koenigii* Spreng (Rutaceae) resulted in the isolation of three bioactive carbazole alkaloids, kurryam (**I**), koenimbine (**II**) and koenine (**III**). The structures of the compounds were confirmed from their ^{1}H-, ^{13}C-, and 2D-NMR spectral data. Of the three compounds (**I**) and (**II**) exhibited significant inhibitory activity against castor oil-induced diarrhoea and PGE_2-induced enteropooling in rats. The compounds also produced a significant reduction in gastrointestinal motility in the charcoal meal test in Wister rats.

1. Introduction

The plant *Murraya koenigii* (L) Spreng (Sanskrit name: Surabhinimba) belonging to the family Rutaceae is native to India but is now distributed in most of southern Asia. The leaves of this plant are well-known as curry leaves and have been used as one of the important herbs in South Indian food [1]. Various parts of the plant have been used in traditional or folk medicine as an antidysentric as well as an astringent [2].

Since the first report of the carbazole alkaloid, murrayanine, from the stem bark of *M. koenigii* [3], a number of carbazole alkaloids have been isolated from this species, possessing C_{13}, C_{18} and C_{23} skeletons [4–7]. Our bioassay directed investigations of the seeds of *M. koenigii* has led to the isolation of three carbazole alkaloids, Kurryam (**I**) [8] Koenimbine (**II**) and Koenine (**III**) [9] (Fig. 1). The activity of compounds (**I**) and (**II**) were studied on castor oil-induced diarrhoea in rat model and then compared with the standard antidiarrhoeal medicine, diphenoxylate. This is the first report of the biological activity of these carbazole alkaloids.

2. Experimental

2.1. Plant material

Seeds of *Murraya koenigii* Spreng (Rutaceae) were collected from the Ministry of Food and Supplies, Government of India, and compared with a voucher specimen maintained in the herbarium of Calcutta University. The seeds were crushed in a mechanical grinder.

2.2. Extraction and isolation

2.2.1. Identification of components from n-hexane fraction

The air dried seeds of *M. koenigii* were extracted with *n*-hexane in a Soxhlet apparatus for 72 h at room temperature. The total extract was concentrated and kept at room temperature. A yellowish solid (yield: 4.2%) separated out. This was dissolved in chloroform and chromatographed using a silica gel column and eluted successively with *n*-hexane and *n*-hexane-ethyl acetate mixture. The fractions obtained with

* Corresponding author. Department of Chemistry, University of Calcutta, University College of Science, 92, A.P.C. Road, Kolkata – 700009, India. Tel.: +91 33 2350 8386; fax: +91 33 2351 9755.
E-mail address: juliebanerji@yahoo.co.in (J. Banerji).

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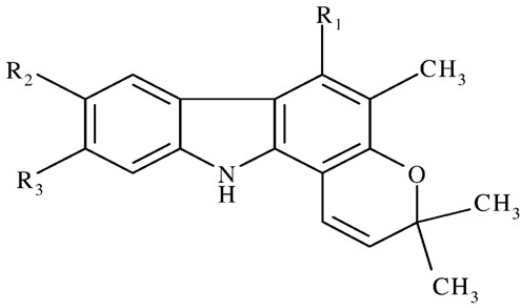

(**I**) $R_1 = OH, R_2 = R_3 = OMe$

(**II**) $R_1 = R_3 = H, R_2 = OMe$

(**III**) $R_1 = R_3 = H, R_2 = OH$

Fig. 1. Structures of compound (I), (II) and (III).

20% ethyl acetate in *n*-hexane afforded a white solid, which on repeated crystallization from ether and *n*-hexane mixture as solvent afforded compound **I** (Kurryam) (0.015%) as a white, crystalline, homogeneous solid. The fraction obtained with 4% ethyl acetate in *n*-hexane afforded a white solid which on repeated crystallization from chloroform-petroleum ether (60–80°) afforded the pure compound **II** (Koenimbine) (0.4%) as white shining crystals. The fraction obtained with 2% ethyl acetate in *n*-hexane afforded a white solid, which on repeated crystallization from methanol-*n*-hexane yielded the pure compound **III** (Koenine) (0.002%) as white needles.

2.2.2. General experimental procedure

Melting points were determined in open capillary tubes in a Köfler block apparatus and remain uncorrected. Silica gel (60–120 mesh, Spectrochem) was used for column chromatography and silica gel G (Spectrochem) for TLC. The UV spectrum was recorded using a Hitachi U-3501 spectrophotometer in spectral grade alcohol (Merck); the IR spectrum in KBr in a Perkin Elmer RX-1 FT-IR spectrophotometer. The 300 MHz ^{1}H-NMR, 75.5 MHz ^{13}C-NMR spectra were recorded in $CDCl_3$ in a Bruker AVANCE 300 DIGITAL MHz NMR spectrometer. (Chemical shifts are in δ ppm and *J* in Hz). The mass spectrum was recorded on GCMS-SHIMADZU-QP5050A.

3. Results and discussion

3.1. Antidiarrhoel activity (ADA)

3.1.1. Test animals

Wister rats weighing between 150 and 200 g of either sex were used. The rats were housed in standard environmental conditions and provided with food and water ad libitum.

3.1.2. Castor oil-induced diarrhoea in rats

Rats of either sex were fasted for 18 h and randomly assigned to five groups with six animals in each group. The doses of koenimbine (**II**), selected on trial basis, were administered orally (10, 30 and 50 mg/kg suspended in 2% v/v aq. tween 80) to three groups of animals. The fourth group received 5 mg/kg of diphenoxylate orally as a standard drug. The fifth group which served as control received 2% v/v aq. tween 80 suspension only. One hour after treatment, each animal received 1 ml of castor oil orally by gavages and then defecation was observed up to 4 h. The presence of characteristic diarrhoeal droppings was noted (Table 1). The same experiment was carried out with kurryam (**I**) (Table 2).

Table 1
Effect of koenimbine (II) on castor oil-induced diarrhoea in rats.

Oral pre-treatment at 1 h	Mean defecation/rat (M ± S.E.M.)
Tween 80 suspension (5 ml/kg)	5.50 ± 0.65
Diphenoxylate (5 mg/kg)	1.35 ± 0.61**
Koenimbine (10 mg/kg)	2.51 ± 0.58*
Koenimbine (30 mg/kg)	1.94 ± 0.81*
Koenimbine (50 mg/kg)	1.29 ± 0.21**

$N = 6$; *$P < 0.01$, **$P < 0.001$; Student's *t*-test.

Table 2
Effect of kurryam (I) on castor oil-induced diarrhoea in rats.

Oral pre-treatment at 1 h	Mean defecation/rat (M ± S.E.M.)
Tween 80 suspension (5 ml/kg)	5.00 ± 0.62
Diphenoxylate (5 mg/kg)	1.38 ± 0.51**
Kurryam (10 mg/kg)	2.35 ± 0.35*
Kurryam (30 mg/kg)	1.88 ± 0.28*
Kurryam (50 mg/kg)	1.21 ± 0.25**

$N = 6$; *$P < 0.01$, **$P < 0.001$; Student's *t*-test.

3.1.3. Gastrointestinal motility test

Rats were fasted for 18 h and divided into five groups containing six animals each. Each animal was administered with 1 ml of charcoal meal orally (3% deactivated charcoal in 10% aq. tween 80). Thereafter the first three groups were treated orally with a suspension of koenimbine at doses of 10 mg, 30 mg and 50 mg per kg respectively. The fourth group

Table 3
Effect of koenimbine (II) on gastrointestinal motility in rats.

Treatment	Movement of charcoal meal (%)
Aq. tween 80 suspension (5 ml/kg)	79.10 ± 2.51
Atropine (0.1 mg/kg)	44.25 ± 2.15**
Koenimbine (10 mg/kg)	69.26 ± 2.11*
Koenimbine (30 mg/kg)	52.25 ± 1.90**
Koenimbine (50 mg/kg)	41.85 ± 2.05**

$N = 6$; *$P < 0.01$, **$P < 0.001$.

Table 4
Effect of kurryam (I) on gastrointestinal motility in rats.

Treatment	Movement of charcoal meal (%)
Aq. tween 80 suspension (5 ml/kg)	78.89 ± 2.34
Atropine (0.1 mg/kg)	43.92 ± 2.26**
Kurryam (10 mg/kg)	67.62 ± 2.13*
Kurryam (30 mg/kg)	48.31 ± 1.98**
Kurryam (50 mg/kg)	38.88 ± 2.44**

$N = 6$; *$P < 0.01$, **$P < 0.001$.

Table 5
Effect of koenimbine (II) on PGE_2-induced enteropooling in rats.

Treatment	Volume of intestinal fluid (ml)
Ethanol in saline (5% v/v)	0.61 ± 0.21
PGE_2 in ethanol (2% v/v)	2.81 ± 0.15*
Koenimbine (10 mg/kg)	2.25 ± 0.17*
Koenimbine (30 mg/kg)	1.55 ± 0.10**
Koenimbine (50 mg/kg)	1.18 ± 0.09**

$N = 6$; *$P < 0.05$, **$P < 0.001$.

Table 6
Effect of kurryam (I) on PGE_2-induced enteropooling in rats.

Treatment	Volume of intestinal fluid (ml)
Ethanol in saline (5% v/v)	0.69 ± 0.25
PGE_2 in ethanol (2% v/v)	2.78 ± 0.16*
Kurryam (10 mg/kg)	2.10 ± 0.21*
Kurryam (30 mg/kg)	1.41 ± 0.17**
Kurryam (50 mg/kg)	0.92 ± 0.19**

$N = 6$; *$P < 0.01$; **$P < 0.001$.

received atropine (0.1 mg/kg i. p.). The fifth group was treated with aq. tween 80 suspension as control. After half an hour each animal was sacrificed and the intestinal distance moved by the charcoal meal from the pylorus was cut and measured and expressed as a percentage of the distance from the pylorus to the caecum (Table 3).

The same experiment was conducted with kurryam (**I**) (Table 4).

3.1.4. PGE_2-induced enterpooling

Three groups of six animals each were treated orally with 10, 30 and 50 mg/kg of koenimbine. The fourth and fifth groups were treated with 1 ml of 5% v/v ethanol in normal saline (i. p.). The fifth group was then treated with 2% v/v tween 80 solution serving as the control. Immediately, PGE_2 (Astra-IDL Limited, India) was administered orally to each rat (100 mg/kg) in 5% v/v ethanol in normal saline. After 30 min, all the rats were sacrificed and the whole length of the intestine from the pylorus to the caecum was dissected in each case. The contents were collected in the test tubes and the volume was measured (Table 5).

The same experiment was conducted with kurryam (**I**) (Table 6).

3.1.5. Statistical analysis

The experimental results are expressed as the mean ± S.E.M., with six animals in each group. Statistical significance tests were performed by Student's *t*-test [10].

Diarrhoea is commonly caused by gastrointestinal infections which results in the death of around 1.8 million people globally each year, mostly children in developing countries. The main cause of death from diarrhoea is dehydration which results from loss of electrolytes in diarrhoeal stools. The carbazole alkaloids, koenimbine and kurryam, isolated from the seeds of *M. koenigii*, when administered orally to rats, exhibited significant and dose-dependent antidiarrhoeal activity. The dose of 30 mg/kg showed an equivalent effect to that of 5 mg/kg of the standard drug, diphenoxylate, and the dose of 50 mg/kg, exhibited better effect than that of 5 mg/kg of diphenoxylate. Moreover, it decreased significantly the propulsion of charcoal meal through the gastrointestinal tract and the PGE_2-induced enteropooling in rats. So the present study shows that *M. koenigii* contains many carbazole alkaloids which can be used as antidiarrhoeal agents. The leaves of this plant also contain appreciable amount of koenimbine [9]. Our observations justify the traditional use of this plant in the management of diarrhoea.

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