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A pharmacological evaluation of antidiarrhoeal activity of leaves extract of *Murraya koenigii* in experimentally induced diarrhoea in rats

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ABSTRACT

Objective: To evaluate anti-diarrhoeal activity of aqueous and alcoholic extract of the leaves of *Murraya koenigii* (*M. koenigii*)by using models of castor oil induced diarrhoea, charcoal meal test and PGE_2 induced diarrhoea. **Methods:** Alcoholic extract (400 mg/kg) and aqueous extract (200 mg/kg) of leaves of *Murraya koenigii* were used with loperamide as standard. Albino Wistar rats of both sexes weighing between 150-250 g were used for the anti-diarrhoeal activity. **Results:** The result suggested that it could act centrally and inhibit the PGE_2 to give anti-diarrhoeal effects. Result of charcoal meal test also suggested its anti-muscarnic activity. **Conclusions:** These findings indicate that aqueous extract of the leaves of *M. koenigii* displays good anti-diarrhoeal activity, corroborating the folk use of *M. koenigii* preparations and contributing for its pharmacological validation.

1. Introduction

Diarrhoea is one of the major health threats to populations in tropical and subtropical countries, responsible for about 5 million deaths annually, of which 2.5 million are children less than 5 years. A study by Martinez, who looked at what form of treatment is administered by primary care-takers of young children, demonstrated that herbal treatments are still important in the home treatment of diarrhoea[1].On the contrary, most herbal drugs reduce the offensive factors and proved to be safe, clinically effective, better patient tolerant, relatively less expensive, and globally competitive. Plant extracts, however, are some of the most attractive sources of new drugs and have shown promising results in the treatment of diarrhoea. Aqueous extract of the leaves of *Murraya koenigii* (*M. koenigii*) possesses alexeteric, antihelmintic, analgesic, dysentry, purgative and blood disorders. Also they are reported to be useful in inflammation, healing of wounds, injuries, antioxidative activity[2,3]. In folklore practice, the decoction of *M. koenigii* leaves has been reported to be useful in diarrhoea. There is no scientific report on the effect of *M. koenigii* on the diarrhoea. The present investigation was undertaken to evaluate the effect of *M. koenigii* on experimentally induced diarrhoea in rats.

2. Materials and methods

Fresh leaves of *M. koenigii* (5 kg) were collected locally from the Indore district of Madhya pradesh and got identified by Department of Botany, Saifia college of science and education, Bhopal. Specimen voucher no. is 168/Bio/saifia/10. The leaves were shade dried and were crushed to moderately coarse powder. Aqueous solution of *M. koenigii* was prepared in distilled water and was administered orally. Loperamide was procured from Micro Lab, Bangalore India. Rats were divided in four group containing six rats. Group I was control and given

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distilled water as vehicle. Group II and III were given *M. koenigii* (200 and 400 mg/kg, *p.o.*). Group IV received loperamide as standard (2 mg/kg, *p.o.*).

2.1. Preparation of extract

The powder was extracted with distilled water using soxhlet at boiling temperature (100 ℃) up to 10 h. A dark brown colour extract was obtained. This dark brown extract was cooled and filtered to remove the residue. The extract was concentrated on rotavapour under reduced pressure and then lyophilized to get a powder weighing about 7.5 g[4]. The preliminary phytochemical screening was carried out on the aqueous extract of the leaves of *M. koenigii* for qualitative identification[5,6].

2.2. Experimental animals

Albino Wistar rats of both sex weighing between 150-250 g were used. The experimental protocol was approved from Institutional Animal Ethics Committee. Animals were housed under standard conditions of temperature [(24±2) ℃] and relative humidity (30%-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water *ad libitum*.

2.3. Acute toxicity study

The acute oral toxicity study was carried out for aqueous extract of *M. koenigii* leaves using fixed dose method according to OECD (1993) guideline no.420. Healthy adult female Swiss albino mice weighing between 25 to 35 g were used for the study. Animals were divided into four groups of three animals each and fasted overnight. 5, 50, 300 and 2 000 mg/kg b.w. doses were administered to the Group I, II, III, IV respectively. After administration of extracts various parameters like body temperature, CNS activity, micturation, defecation *etc*. were observed for 24 h. Four groups of rats of both sex (six animals per group) were administered orally a single dose of either 5, 10, or 15 times of effective dose of aqueous extract of *M. koenigii* leaves. The rats were observed for gross behavioral, neurologic, autonomic, and toxic effect continuously. Food consumption, faeces and urine were also examined at 2 h and then at 6 h intervals for 24 h[7,8].

2.4. Castor oil induced diarrhea[9]

Rats of either sex (150-250 g) were fasted for 18 h. They were divided into four groups (n=6). The first group, which served as control was administered with aqueous 1% tragacanth suspension. The second group received standard drug, loperamide (2 mg/kg) orally as suspension. The extract was administered orally at 200 mg/kg dose to third group and 400 mg/kg dose to fourth group as suspension. After 60 min of drug treatment, the animals of each group received 1 mL of castor oil orally and the watery faecal material and number of defecation was noted up to 4 h in the transparent metabolic cages with filter paper at the base. Weight of paper before and after defecation was noted.

2.5. Charcoal meal test[10]

Rats of either sex (150-235 g) were fasted for 18 h. They were divided into four groups (n=6). The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group receives standard drug atropine (0.1 mg/kg) subcutaneously. The extract was administered orally at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. The animals were given 1 mL of 10% activated charcoal suspended in 10% aqueous tragacanth powder *p.o.*, 30 min after treatment. Animals were euthanized 30 min after charcoal meal administration by ether anesthesia. The abdomen was cut off and the small intestine carefully removed. The distance travelled by charcoal plug from pylorus to caecum was measured, and expressed as percentage of the distance traveled by charcoal plug for each of animal.

2.6. PGE_2 induced enteropooling[11]

Rats of either sex (150-235 g) were fasted for 18 h. They were then divided into four groups (n=6). A solution of PGE_2 was made in the 5%v/v ethanol in the normal saline. The first group, which served as control, was administered with PGE_2 (100 μg/kg *p.o.*) only. The second group, which served as vehicle control was administered with aqueous 1% tragacanth suspension by oral route. The extract was administered orally at 200 mg/kg to third group and 400 mg/kg to fourth group as suspension. Immediately after extract administration PGE_2 was administered. After 30 min following administration of PGE_2 each rat was sacrificed and whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

2.7. Statistical analysis

The data are represented as mean ± SEM, and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Dunnett t-test where P<0.05 was considered statistically significant using Graph pad 5 software.

3. Results

Preliminary phytochemical investigation of aqueous extracts of leaves of *M. koenigii* revealed the presence of phenols, carbazole, alkaloids, flavonoids and tannins.

Both doses of extract showed protection against castor oil and PGE_2 induced diarrhea. Aqueous extract at 200 and 400 mg/kg significantly decreased the total number of faeces, total number of diarrheal faeces and delay in defecation time, which was comparable with the effect of loperamide ($P<0.05$) (Table 1). Aqueous extracts of leaves of *M. koenigii* (200 and 400 mg/kg) and the anti-muscarinic drug, atropine (0.1 mg/kg) significantly decreased the propulsive movement in the charcoal meal study, atropine being less potent than the aerial part extract of 200 mg/kg ($P<0.05$) (Table 2). The extracts significantly decreased volume of intestinal fluid ($P<0.05$) (Table 3).

Table 1

Evaluation of anti-diarrhoeal activity of aqueous extract of *M. koenigii* by castor oil induced diarrhea.

Treatment	Total number of faeces	Total number of diarrheal faeces	Delay in defecation time (min)
Control	10.33±0.31	7.68±0.42	6.41±0.08
Loperamide (2 mg/kg)	5.18±0.18*	2.48±0.17**	2.76±0.17**
Aqueous extract (200 mg/kg)	6.53±0.18*	4.35±0.73**	3.24±0.62**
Alcoholic extract (400 mg/kg)	5.80±1.09**	4.20±0.20**	3.35±0.14**

* $P<0.05$, ** $P<0.01$ *vs.* control group.

Table 2

Evaluation of anti-diarrhoeal activity of aqueous extract of *M. koenigii* by charcoal meal test.

Treatment	Total length of intestine	Distance travel by cha rcoal
Control	97.84±0.40	18.48±0.24
Atropine (0.1 mg/kg)	92.84±0.19**	89.04±0.16**
Aqueous extract (200 mg/kg)	97.04±0.31	54. 80±0.82**
Alcoholic extract (400 mg/kg)	84.40±0.20**	71.64±0.16**

* $P<0.05$, ** $P<0.01$ *vs.* control group.

Table 3

Evaluation of anti-diarrhoeal activity of aqueous extract of *M. koenigii* by PGE_2 induced enteropooling.

Treatment	Volume of intestinal fluid (mL)
PGE_2 control	2.24±0.97
Vehicle control	1.98±0.10
Aqueous extract (200 mg/kg)	1. 24±0.19*
Alcoholic extract (400 mg/kg)	1.10±0.11*

* $P<0.05$, ** $P<0.01$ *vs.* control group.

4. Discussion

Both doses of extract showed protection against PGE_2 induced enteropooling, which might be due to the inhibition of synthesis of prostaglandins. Anti-enteropooling effect of the extract iss more relevant because the prevention of enteropooling helps in the inhibition of diarrhea, especially by PGE_2 induced diarrhea as it is involved in the onset of diarrhoea in intestinal mucosal cells. Although intraluminally administered PGE_2 is known to induce duodenal and jejunal secretion of water and of electrolytes such as Cl and Na[12], fluid content is the principal determinant of stool volume and consistency. Net stool fluid content reflects a balance between luminal input (ingestion and secretion of water and electrolytes) and output (absorption) along gastrointestinal tract.

Neurohumoral mechanisms, pathogens and drugs can alter these processes, resulting in changes in either secretion or absorption of fluid by the intestinal epithelium. Altered motility also contributes in a general way to this process, as the extent of absorption parallels the transit time.

Aqueous extracts of leaves of *M. koenigii* (200 and 400 mg/kg) and the anti-muscarinic drug, atropine (0.1 mg/kg) decreased the propulsive movement in the charcoal meal study, atropine being less potent than the aerial part extract of 200 mg/kg. The underlying mechanism appears to be spasmolytic and an anti-enteropooling property by which the extract produced relief in diarrhoea. Tannic acid and tannins are present in many plants and they denature proteins forming protein tannate complex. The complex formed coat over the intestinal mucosa and makes the intestinal mucosa more resistant and reduces secretion[13]. The tannin present in the plant extracts may be responsible for the anti-diarrhoeal activity.

The anti-diarrhoeal effect of the extracts may be related to an inhibition of muscle contractility and motility, as observed by the decrease in intestinal transit by charcoal meal and consequently, in a reduction in intestinal propulsion. Extract also inhibited the onset time and severity of diarrhoea induced by castor oil. Castor oil is reported to cause diarrhoea by increasing the volume of intestinal content by prevention of reabsorption of water. Castor oil contains ricinoleic acid which induces irritation and inflammation of the intestinal mucosa, leading prostaglandin release which, in turn, changes in mucosal fluid and electrolyte transport thereby preventing the reabsorption of NaCl and water results in a hypersecretory response and diarrhea[16-18]. The experimental studies in rats demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil. Ricinoleic acid markedly increased the PGE_2 content in the gut lumen and also caused on increase of the net secretion

of the water and electrolytes into the small intestine. Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhea. The diarrhoeal effect of castor oil may be involved NO that increase the permeability of the epithelial layer to calcium ions, leading to an increase in intracellular Ca^{2+} and enhancement of calmudin stimulation of NO synthatase activity. NO, in turn, could stimulate intestinal secretion. It is well known that nitric oxide and prostaglandins are crucial mediators contributing to generation of inflammatory response to castor oil. Alternatively, the effect of castor oil may be attributed to disordered motility and hence to an increase in intestinal transit of intraluminal material. In this connection, castor oil could alter coordination of intestinal motility and could promote greater loss of fluid from intestine. The reduction of gastrointestinal motility is one of the mechanisms by which many anti-diarrhoeal agents act[19]. Castor oil causes diarrhea due to its active metabolite, ricinolic acid, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa[20]. The diarrhoeal effect of castor oil may be involved NO[21].

Conflict of interest statement

We declare that we have no conflict of interest.

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