



Research Article

EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF *CRATAEVA NURVALA* ROOT BARK IN EXPERIMENTAL ANIMALS

INAYATHULLA^{1*}, SHARIFF W. R.¹, KARIGAR ASIF A². SIKARWAR MUKESH S³

¹Sharada Vilas College of Pharmacy, Mysore, Karnataka ²Principal, Genba Sopanrao Moze College of Pharmacy, Pune

³Department of Pharmacognosy, K.L.E. S College of Pharmacy Ankola, Karnataka, India. Email:mukeshsikarwar@gmail.com, inayathuk@yahoo.co.in

ABSTRACT

Crataeva nurvala stem bark has been used traditionally in Ayurveda for the treatment of diarrhoea and dysentery. However, the claims of Ayurveda need to be validated by a suitable experimental model. Therefore, the present study was undertaken to evaluate the effect of ethanolic extracts of *Crataeva nurvala* for its antidiarrhoeal potential against several experimental models of diarrhoea in Albino Wistar rats. The antidiarrhoeal activity of ethanol extracts of *Crataeva nurvala* stem bark was evaluated using castor oil-induced diarrhoea model in rats. The gastrointestinal transit rate was expressed as the percentage of the longest distance traversed by the charcoal divided by the total length of the small intestine. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. Like atropine (3mg/kg, i.p.) there were significant reductions in fecal output and frequency of droppings when the plant extracts 500 mg/kg doses were administered intraperitoneally compared with castor oil treated rats. This dose of the plant extracts significantly retarded the castor-oil induced enteropooling and intestinal transit. It significantly inhibited ($P < 0.001$) weight and volume of intestinal content.

Keywords: Anti-diarrhoeal, *Crataeva nurvala*, Castor oil-induced diarrhoea, Small intestinal transit

INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problem in the developing countries. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and small children less than 5 year. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea. Secretary diarrhoea is the most dangerous symptom of gastrointestinal problems and is associated with excessive defecation and stool outputs, the stools being of abnormally loose consistency.

There are large numbers of epidemiological and experimental evidence pertaining to worldwide acute-diarrhoeal disease, which is one of the principal causes of death in the infants, particularly in malnourished and which is of critical importance in developing countries. It thus becomes important to identify and evaluate commonly available natural drugs as alternative to currently used anti-diarrhoeal drugs, which are not completely free from adverse effects

The family Cappariaceae comprises about 45 genera and 700 species of trees, which are distributed mainly in the warmer (tropical) parts of the world. *Crataeva nurvala* (Family: Cappariaceae) is a small tree with a much branched head. Leaves are deciduous 3 foliolate; petioles 3.8-7.6 cm long; leaflets 5-15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath and reticulately veined. It is usually cultivated

in the vicinity of temples in Central India, Bengal and Assam. Its bark is hot, bitter at first and then sweet sharp taste, easy to digest, stomachic, laxative, antilithic, anthelmintic, expectorant and antipyretic. Researches shown that its bark contains saponins which are especially useful in urinary complaints such as kidney and bladder stones³⁻⁴. The present study was taken up as no such effort have made until time to evaluate, the traditional claim on this drug.

Material and methods

Plant material

Stem bark of *Crataeva nurvala* were collected in and around local forest area of Sirsi in Western Ghats, Karnataka and authenticated by the Botanist Prof. G. S. Naik, Department of Botany, G. C. Science and Art College, Ankola. A voucher herbarium specimen number GCSAC/CN/02 was also preserved in the same college. The collected leaves were dried under shade and powdered to coarse consistency in grinder mill. The powder was passed through 40 # mesh particle size and stored in an airtight container at room temperature.

Physicochemical evaluation⁵

Physicochemical parameters were evaluated for powder material to determine of ash value, extractive value, moisture content, foaming index and ash values. All experiments were repeated five times for precision and values were expressed in mean \pm standard deviation in terms of air dried material.

Extraction and preliminary phytochemical analysis

2.5 kg of the fresh air-dried, powdered crude drug of *Crateva nurvala* was extracted with 95% ethanol by adopting simple maceration procedure at room temperature for seven days in conical flask with occasional shaking and stirring. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites⁶. The yield of the extracts was 10.65 % w/w. Extract was preserved in a refrigerator till further use. Preliminary phytochemical analysis was carried out for extracts by different methods of phytochemical analysis⁷.

Experimental animals

Adult Albino rats of wistar strain (150-200gm) of either sex were procured from Government Veterinary College, Bangalore and were housed in the animal house of K L E S College of Pharmacy, Ankola under standard laboratory condition ($25 \pm 2^\circ\text{C}$ temperature, $55 \pm 5\%$ relative humidity, and 12 hrs light and dark cycles. Standard pellets obtained from Goldmohar rat feed, Mumbai India, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water ad libitum. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by institutional ethical committee in resolution no. 1/18/2007 held on 23rd November 2007 at J N Medical college, Belgaum (Ethical committee IAEC reg. no.: 627/02/a/CPCSEA).

Acute-toxicity studies⁸

The acute oral toxicity studies of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17th December 2001 received from CPCSEA, Ministry of social justice and empowerment, Govt. of India. Administration of the stepwise doses ethanolic extracts of *Crateva nurvala* from 50 mg/kg b.w. up to the dose 5000 mg/kg b.w. caused no considerable signs of toxicity in the tested animals. One tenth of upper limit dose were selected as the levels for examination of antidiarrheal activity.

Castor oil-induced diarrhoea

Rats were divided into three groups of six animals each, diarrhoea was induced by administering 1 ml of castor oil orally to rats. Group 1 served as control (2 ml/kg, i.p. saline), group 2 received atropine (3mg/kg, i.p.) served as standard and group 3 received ethanolic extract (500 mg/kg, i.p.) 1 h before castor oil administration. The number of both wet and dry diarrhoeal droppings were counted every hour for a period of 4 h. Mean of the stools passed by the treated groups were compared with that of the positive

control group consisted of animals given an intraperitoneal injection of saline (2ml/kg, ip)⁹.

Castor oil-induced enteropooling

Intra-luminal fluid accumulation was determined by the method of Robert et al.1976. Overnight fasted rats were divided three groups of six animals each. Group 1 received normal saline intraperitoneally (2 ml/kg, i.p.) served as a control, group 2 received atropine (3mg/kg, i.p.) and groups 3 received the ethanolic extract of 500 mg/kg intraperitoneally respectively 1h before the oral administration of castor oil. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines¹⁰.

Small intestinal transit¹¹

Rats were fasted for 18 h divided into four groups of six animals each, Group1 received 2 ml normal saline orally, group 2 received 2 ml of castor oil orally with saline 2 ml/kg intraperitoneally, group 3 received atropine (3 mg/kg, i.p.), group 4 received 500 mg/kg intraperitoneally of the plant ethanolic extract respectively, 1 h before administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The rats were sacrificed after 1h and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum.

Statistical analysis

The data were analyzed statistically using one-way analysis of variance followed by Dunnett's 't' test. Results obtained

RESULTS AND DISCUSSION

Standardization parameters for *Crateva nurvala* stem bark were determined and all the parameters were found to be within pharmacopoeial standards limit. Crude powder taken for extraction was of yellowish brown colour with bitter taste. Loss on drying, total ash, acid insoluble ash, water soluble ash were found to be 4.37, 9.76, 0.647 and 1.56 % w/w respectively. Thin layer chromatography of *Crateva nurvala* stem bark revealed bright brownish red spot ($R_f = 0.30$), $R_f = 0.71$ (Magenta color), $R_f = 0.15$, 0.90 (light violet spots) which turns magenta on keeping.

Phytochemical screening of extract of *Crateva nurvala* showed the presence of various chemical constituents mainly triterpenoids and flavonoids which may be responsible for its antilithic and anti-diarrhoeal activity respectively. The results obtained were comparable and satisfied the standard literature. In

acute toxicity study, all the extracts of *Crataeva nurvala* stem bark did not show significant toxicity signs when observed for the parameters during the first four hours and followed by daily observations for 14 days and no mortality was also observed, the drug was found to be safe at the tested dose level of 5000 mg/kg b.wt.

Castor oil-induced diarrhoea

30 min after administration of castor oil the diarrhoea was clinically apparent in all the animals of control group, for the next 4 h. This was markedly reduced by the intraperitoneal injection of atropine, 3 mg/kg (Table 1). A similar marked reduction in the number of defecations over four hours was achieved with dose of 500 mg/kg i.p. *Crataeva nurvala* stem bark. This dose of ethanolic extract delayed the onset of diarrhoea and only 20% of animals showed diarrhoea at first hour ($P < 0.01$).

Table 1: Effect of *Crataeva nurvala* ethanolic extract on castor oil-induced diarrhoea

Treatment	Mean defecation in 4 h
Castor oil (1ml p.o.)+ Saline (2 ml/kg.i.p.)	18.04±1.754
Castor oil + atropine (3mg/kg i.p.)	9.42±0.516**
Castor oil + Extract (500mg/kg i.p.)	11.67±1.273**

Values are expressed as mean ± SEM from the experiments. * $P < 0.01$, ** $P < 0.001$ when compared with CO + saline-treated group.

Castor oil-induced enter pooling

Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil-induced enteropooling is not influenced by atropine in rats (3 mg/kg, i.p.). Dose of the ethanolic extract produced a dose-dependent reduction in intestinal weight and volume. 500 mg/kg, i.p. dose of extract produced 61.25% inhibition of volume of intestinal content ($P < 0.001$). The weight of intestinal content was also reduced significantly with this dose (Table 2).

Table 2: Effect of *Crataeva nurvala* extract on castor oil induced enter pooling in rats.

Treatment	Weight Intestinal content	% inhibition wt. intestinal content
Castor oil 2 ml p.o.) + Saline (2 ml/kg.i.p.)	2.71±0.215	----
Castor oil + Atropine (3mg/kg i.p.)	2.395±0.115**	11.81
Castor oil + Extract (500mg/kg i.p.)	1.056±0.134**	61.25

Values are expressed as mean ± SEM from the experiments. * $P < 0.01$, ** $P < 0.001$ when compared with CO+ saline-treated group.

Small intestinal transit

The percent intestinal transit was increased with castor oil (85.35±1.475), but was reduced in with treatment of ethanolic extract, and much more markedly by atropine (41.47±2.764). 500 mg/kg, i.p. dose of ethanolic extract of *Crataeva nurvala* produced 64.56±2.479 of castor oil induced charcoal meal transit (Table 3).

DISCUSSION AND CONCLUSION

In developing countries, a quarter of infant and childhood mortality is related to the diarrhoea. The highest mortality rates have been reported to be in children less than five years of age. During the past decade oral dehydration therapy has reduced mortality from acute diarrhoeal disease, whereas chronic diarrhoea remains a life-threatening problem in those regions, in which malnutrition is a common co-existing and complication factor. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhoea and dysentery. Indigenous plants used for this purpose are: *Andrographis paniculata*, *Asparagus racemosus*, *Butea monosperma*, *Cassia auriculata*, and others others.

The results of the present study show that the ethanolic extract of *Crataeva nurvala* stem bark produced a statistically significant reduction in the severity and frequency of diarrhoea produced by castor oil. It is also noted that the extract significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content was more than atropine. The extract significantly reduced the castor oil induced intestinal transit. In this study, atropine produced a significant reduction in the number of stools and increased intestinal transit time possibly due to its anti-cholinergic effect. However, it did not inhibit castor oil induced enteropooling. Gain in weight of intestinal content suggesting thereby that mediators other than acetylcholine are involved in castor oil induced enteropooling. An increase in intestinal transit time with atropine could also result due to reduction in gastric emptying.

It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea. The experimental studies in rats demonstrated a significant increase in the portal venous PGE₂ concentration following oral administration of castor oil. Ricinoleic acid markedly increased the PGE₂ content in the gut lumen and also caused an increase of the net secretion of the water and electrolytes into the small intestine. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion. Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhoea. The extract appears to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; the extracts also significantly inhibited the PGE₂ induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandins cause diarrhoea in experimental animals as well as human beings. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport.

Table 3: Effect of *Crataeva nurvala* ethanolic extract on castor oil-induced small intestinal transit in rats.

Treatment	Total length of intestine	Distance traveled by marker	% intestinal transit
Saline (2 ml/kg. p.o.)	78.60±2.623	75.54±2.984	95.75±1.732
Castor oil 2 ml p.o.) + Saline (2 ml/kg.i.p.)	72.41±1.985	68.21±2.175	85.35±1.475
Castor oil + Atropine (3mg/kg i.p.)	88.74±2.468**	32.59±2.345	41.47±2.764
Castor oil + Extract (500mg/kg i.p.)	82.52±2.043**	42.76±2.751	64.56±2.479

Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with CO + saline-treated group.

Previous reports have demonstrated the antidiarrhoeal activity of tannin, flavonoids, alkaloids, saponins, reducing sugars and sterols and/or terpenes containing plant extracts. The phytochemical analysis of the extracts showed the presence of flavonoids and terpenes and sugars. These constituents may responsible for the anti-diarrhoeal activity of *Crataeva nurvala* ethanolic extracts.

The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion, which are known to be altered in this intestinal condition.

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