

Antidiarrhoeal activity of methanolic extract of *Justica procumbens* by magnesium sulphate induced method in rats

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ABSTRACT

Methanolic extract was prepared by using Leaves of *Justica procumbens* were tested for anti-diarrhoeal activity in magnesium sulphate induced model in rats. The toxicity values were established and 1/10th dose of the Methanolic extracts i.e. 300mg/kg and 500mg/kg were used for the entire study of anti diarrhoeal activities in rats. Antidiarrhoeal potential of extracts was evidenced by a significant reduction in faecal output in castor oil induced diarrhea and also a significant decrease in propulsive movement of gastrointestinal contents.

Key words: *Justica procumbens*, anti-diarrhoeal activity

INTRODUCTION:

Herbal drugs constitute a major share of all the officially recognized systems of health in India. Today estimate that 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a very repository of medicinal plants that are used in traditional medicine treatments. Traditional system of medicine continues to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments¹.

A large proportion of world population, especially in the developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred plant genera are used medicinally mainly in the form of herbal preparation in indigenous system of medicine in different countries. Plant products are also a source of very potent and powerful

drugs that have stood the test of times and modern chemistry has not been able to replace most of them².

Diarrhoea is a symptom marked by rapid and frequent passage of semisolid or liquid fecal material through the gastrointestinal tract and involve both an increase in the motility of the gastrointestinal tract along with increased secretions and a decrease in the absorption of fluid and thus a loss of electrolytes particularly sodium and water. Diarrhoea is also called loose motions. Diarrhoea is not itself a disease but can be a symptom of several diseases. It is one of the most common clinical signs of gastrointestinal disease but also can reflect primary disorders outside the digestive system³.

Diarrhoea is considered to be present if one of the following applies

- I) Stool weight of greater than 200g per day.
- II) More than 2 stools per day for more than 30 days.
- III) More than 3 stools per day for more than 7 days.
- IV) More than 3 stools per day, looser than usual, for more than 3 days or
- V) More than 3 stools per day with a change in frequency or consistency

In developing countries a majority of people living in rural areas almost exclusively use traditional medicine for treating diseases of different types including diarrhoea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the pediatric ward die of diarrhoea. Diarrhoea is responsible for deaths in adults and especially children all over the world, particularly in developing countries. Worldwide distribution of diarrhoea accounts for more than 5-8 million death each year infants and children below 5 years old especially in developing countries in general chronic diarrhoea can develop from congenital problems, neoplastic syndrome, human immunodeficiency virus, adverse effects of medications and most commonly is caused by gastrointestinal infections including viral, bacterial gastroenteritis. According to W.H.O estimates for 1998, about 7.1 million deaths were caused by diarrhoea⁴.

The main causes of diarrhoea are over eating or eating of wrong foods, putrefaction of food in the intestinal tract, fermentation caused by incomplete carbohydrate digestion, nervous irritability and uses of antibiotic drugs and excessive intake of laxatives. Intestinal infection is the most common cause of diarrhoea worldwide. Causes of diarrhoea include infectious agents such as virus, parasites, bacteria certain medications such as antibiotics, anticancer drugs and antacids containing magnesium plant and animal toxics, GIT disorders and substances that increase gastrointestinal tract secretions. It can also be caused by the ingestion of poorly absorbable materials or inflammatory and dysmotility⁵ problems of the

gastrointestinal tract. Mucositis or inflammation of the mucous membranes lining the alimentary tract is potential adverse effect of chemotherapeutic agents and diarrhea is the symptomatic result of mucositis of the large bowel. For some chemotherapy regimens like 5-fluorocil and IFL, diarrhoea has been linked to excess hospitalization and death.

MATERIALS AND METHODS

Plant description:

Justicia is a genus of flowering plants of the family Acanthaceae. It roughly 420 species and is native to tropical to warm temperate regions of the Americas, with two species occurring north into cooler temperate regions. Common names include **water-willow** and **shrimp plant**, the latter from the inflorescences, which resemble a shrimp in some species.

Morphology

Stems 8–24 in. long, weak, hairy. Leaves 1 1/2 by 1/2– 3/4 in., elliptic or ovate, narrowed at either end, sparsely hairy; petiole 0– 1/5 in. long. Spikes 1–1 1/2 by 1/4– 1/3 in., sparsely hairy; bracts 1/5 in. long, narrow-lanceolate, green with scarious margins; bracteoles similar to the bracts but smaller. Sepals 4, 1/6 in. long, narrow-lanceolate. Corolla 1/4 in long, purple or rose. Filaments glabrous; one anther-cell below the other, tailed; pollen ellipsoid, with 2 longitudinal bands, not tubercled. Ovary nearly glabrous, style thinly hairy, branches 2, subequal, minute. Capsule 1/5 in. long, 4-seeded, minutely hairy (at least on the top and sutures), seeds with simple tubercles all over.

Geographical distribution

In open places at low and medium altitudes, from the Batan Islands and northern Luzon to Mindanao, inmost islands and provinces. Also in China and southward to Australia.

Functional uses

- I) Used as ingredient in a Taiwan herbal tea.
- II) In the Philippines, leaves are used externally as astringent for the treatment of certain skin eruptions.
- III) Alterative and expectorant, given as Infusion for asthma, coughs and rheumatism.

- IV) Juice of leaves squeezed in the eyes for treatment of ophthalmia.
Used as laxative and diuretic.
- V) Whole plant decoction used for backache, plethora and flatulence.
- VI) In traditional Chinese medicine, used for fever and pain associated with pharyngolaryngeal swelling. Also used for cancer.

Collection and authentication of plant material:

The plant material was collected in the month of June 2013 from Srichalam hills and a specimen was dropped in the herbarium and the leaves was authenticated by Professor Dr. Madhavachetty S. V. University, Trupathi. The collected powdered material was shade dried and pulverized.

Solvents used for extraction: Petroleum ether and methanol

Preparation of the extract:

The dried powders of leaf of *Justica procumbens* were defatted with petroleum ether (60-80°C) in a Soxhlet Apparatus by continuous hot-percolation. The defatted powder material (marc) thus obtained was further extracted with methanol with same method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

PHYTOCHEMICAL SCREENING :

The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).

Test for reducing sugars (Fehling's test): The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for anthraquinones: The individual plant extract (0.5 g) was boiled with 10 ml of sulphuric acid (H₂SO₄) and filtered while hot. The filtrate was shaken with 5 ml of

chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for terpenoids (Salkowski test): To 0.5 g each of the individual extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

Test for flavonoids: A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins: To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins: About 0.5 g of the individual extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride (FeCl₃) was added and observed for brownish green or a blue-black coloration

Test for alkaloids: 0.5 g of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test): To 0.5 g of individual plant extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric

chloride solution. This was under layer with 1 ml of concentrated H₂SO₄. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

PHARMACOLOGICAL SCREENING

Acute pharmacological studies

The acute pharmacological studies are carried out at Teegala Ram Reddy College of Pharmacy (Reg. No. 1447/PO/a/11/CPCSEA) the form B was approved by IAEC members for the animal species, and further parameters of the study has been evaluated at origin laboratory, Coimbatore.

Selection of dose for the study

The dose for *Justicia procumbens* was not found in the retrospective data. So toxicity study has been planned for the plant extract *Justicia procumbens*.

Screening methods for antidiarrhoeal activity

1. Castor oil induced diarrhoea model
2. Magnesium sulphate induced diarrhoea model
3. Enterpooling test
4. Serotonin induced diarrhoea model
5. Small intestine transit time model
6. Irinotecan induced diarrhoea model

Models chosen for the study: Magnesium sulphate induced diarrhoea model

Magnesium sulphate induced diarrhoeal model

6.2.1 Magnesium sulphate induced diarrhoea model

Magnesium sulphate has been reported to induce diarrhea by increasing the volume of intestinal content through prevention of reabsorption of the water. It has also been demonstrated that it promotes the release of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of the small intestine and thereby prevents the reabsorption of sodium chloride and water.

Rational need for the chosen models

The models were selected for the study by considering the infrastructure and availability of the lab, animal facility, time constraint, accuracy of the results, result obtained in short span of time and cost consideration. Magnesium sulphate is a suitable model as it allows the observation of measurable changes in the number of stools and frequency of defecation. The osmotic properties of magnesium sulphate prevent the reabsorption of water and ions leading to an increase in the volume of the intestinal content. This product also promotes cholecystokinin from duodenal mucosa.

Toxicity study

Pharmacology studies have been performed worldwide for many years as part of the nonclinical evaluation of drugs for human use.

While no chemical can be Certified “Safe” (free of risks), since every chemical is toxic at some level of dosage, it is usually possible to estimate the risk associated with exposure to the chemical under specified conditions, if appropriate tests are performed³².

It is important to adopt a rational approach when selecting and conducting safety pharmacology studies. Pharmacology studies can be divided into three categories: primary pharmacodynamic, secondary pharmacodynamic and safety pharmacology studies. The objectives of safety pharmacology studies are.

- I) To identify undesirable pharmacodynamic properties of a substance that may have relevance to its human safety.
- II) To evaluate adverse pharmacodynamic and/or pathophysiological effects of substance observed in toxicology and/or clinical studies
- III) To investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected.

The investigational plan to meet these objectives should be clearly identified and delineated. Toxicology is the science that deals with the adverse effect of chemicals on living organisms. Traditional uses of herbal drugs may be broadly divided into three categories which are well known and have been widely used for many years, which are not well known in the country but for which international experience is available and those represent a new compound hereto not evaluated as to its safety and efficacy.

The first category consists chiefly of food stuffs which have been in use for a long time as traditional herbal remedies and the requirements are limited. In general it seems unnecessary to require the proof of safety of these products.

For the second category, views concerning the type of documents required to be presented may differ from country to country. So, it is necessary that varieties of requirements will be elaborated for these products covering anything from reference in scientific literature confirming the product is safe. To satisfy the demands for limited or shortened toxicological testing of these products, an investigation must be carried out on toxicity profile.

The third group, where the authority is faced with a product not previously screened for its toxicological properties, toxicity studies of these products must have to be undertaken.

Although adverse effects of a substance may be detectable at exposures that fall within the therapeutic range in appropriately designed safety pharmacology studies, they may not be evident from observations and measurements used to detect toxicity in conventional animal toxicity studies.

Acute oral toxicity studies was performed as per OECD-423 guidelines (acute toxic class method), with methanolic extract of *Justicia procumbens* using albino mice (n=6) of either sex, selected by random sampling for acute toxicity study. Animals are fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night with the rat, food but not water should be withheld for 3-4 hr). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hr in rats or 1-2 hr in mice. Six animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight) then, exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 3500 mg/kg and 5000 mg/kg body weight may be considered. Animals are observed individually after dosing at least once during the first 30 min, periodically during the first 24 hr, with special attention given during the first 4 hr, except where they need to be removed from the study and humanely killed for animal welfare reasons are found dead. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in animal, then the same dose was repeated again to confirm the toxic dose, if mortality was not observed, the procedure was repeated for higher doses⁶.

Experimental procedure:

Selection of animals :

Inbred Swiss albino rats of either sex of 2 months age, weighing 20 ± 5 g, were used for the present study. The rats were obtained from the stock in breed colony. They were housed at room temperature of $23\pm 1^{\circ}\text{C}$, relative humidity $55\pm 55\%$ less than 12 hr light/12 hr dark cycles in the animal house. Mice were fed with commercial pellet diet and water *ad libitum* freely throughout the study. The animals were transferred to the laboratory at least 1 hr before the start of the experiment. All animal procedures were performed after approval from the IAEC (institution of animal ethical committee) and in accordance with the recommendations for the proper care and use of laboratory animals.

Antidiarrhoeal activity ⁷

Treatment protocol

Group 1: Receives Tween 80 (10 mL/kg).

Group 2: Receives reference standard drug, loperamide (3 mg/kg) orally as suspension.

Group 3: Receives the extract of *Justica procumbens* orally at 300 mg/kg.

Group 4: Receives the extract of *Justica procumbens* orally at 500mg/kg dose.

Magnesium sulphate induced diarrhoeal model

Rats of either sex (150-250gm) were fasted for 18 hr. They were divided into four groups (n=6). The first group of animals, which served as control was administered with Tween-80 (10 mL/kg). The second group received standard drug, loperamide (3 mg/kg) orally as suspension. The *Justica procumbens* was administered orally for group 3 and with a dose level of 300mg/kg and 500mg/kg. After 60 min of drug treatment, the animals in each group received magnesium sulphate (2 gm/kg) orally. Again, the fecal material and the frequency of defecation were noted up to 4 hr in the transparent metabolic cages with pre weighed plastic dishes placed at the base. Weight of plastic dish before and after defecation was noted and compared to control.

Statistical analysis

The results obtained in biochemical assays were given in terms of mean \pm SEM. The statistical significance of the data was assessed by one way analysis of variance (ANOVA) followed by Dunnett's 't' test between different groups. Toxic, Standard and Test groups were compared with normal group. Standard and all Test groups were compared with the toxic group. $p < 0.05$ was considered as statistically significant.

RESULT AND DISCUSSION

Phytochemical investigation

Result

In the preliminary phytochemical investigation on the *Justica procumbens* it reveals the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides and sugars. The result of chemical test of *Justica procumbens* was shown in the Table

Table : Preliminary phytochemical screening of *Justicia procumbens* leaves.

S.No	Constituents	Test	Methanolic extract
1.	Alkaloids	a) Mayer's reagent b) Wagner's reagent c) Dragendroff's reagent d) Hagner's reagent	Present
2	Carbohydrates	a) Molisch's reagent b) Benedicts reagent c) Fehling' s solution	Present
3	Glycosides	a) Modified bortrager's test b) Legal test	Present
4	Phytosterol	a) Salkowski's test b) Libermann Burchard's test	Absent

5	saponins	a) Froth test b) Foam test	Absent
6	Tannins	Gelatin test	Absent
7	Proteins	a) Xanthoprotein test b) Ninhydrin test	Absent
8	Flavonoids	a) Alkaline reagent test b) Lead acetate test c) Shinoda test	Present
9	Diterpenes	Copper acetate test	Present

Discussion

Preliminary phytochemical investigation of the Methanolic extracts of *Justica procumbens*. Show positive results for alkaloids, flavonoids, terpenes, glycosides and tannins.

Acute toxicity studies

Acute toxicity result of *Justica procumbens*

According to the above toxic studies the Methanolic extract of with a *Justica procumbens* 3000 and 5000mg/kg body weight /oral route are regarded as safe or low toxicity and found to possess no mortality . The 1/10th of this dose was consider as effective i.e 300mg/kg and 500mg/kg. The results are shown in Table

Table : Acute toxic studies of *Justica procumbens*.

S.no	Treatment	Signs of toxicity	Onset of toxicity	Weight variation	Duration of observation
1	2500mg/kg	Nil	Nil	Negligible	14 days
2	3500mg/kg	Nil	Nil	Negligible	14 days

3	4500mg/kg	Nil	Nil	Negligible	14 days
4	5000mg/kg	Nil	Nil	Negligible	14 days

Discussion

The acute oral toxic study was done according to the OECD guidelines 423 and the results are shown in Table there was no death of rat administered at 5000mg/kg dose of methanolic extract of *Justica procumbens* and with in short and long term outcome of the limit dose test up and down procedure. However the observed behavior signs of toxicity include tremors, convulsions, salivation, lethargy, sleep, coma, behavior pattern, irritation, restlessness, tachyphonia, anorexia, bilateral narrowing of eyelids and abnormal posture was also observed during the course of the study.

Antidiarrhoeal activity

Magnesium sulphate induced diarrhoeal model

Results:

The extract of *Justice procumbens* showed significant reduction in the total number of feces as well as in the weight of feces at 300mg/kg and 500mg/kg dose mg/kg dose when compared to the control group. The standard drug loperamide 3mg/kg protect diarrhoea against magnesium sulphate induced model. The extract showed antidiarrhoeal activity but found to be high significant with maximum dose i.e 500mg/kg. The date are represented in the Table.

Table : Effect of methanol extracts of *justice procumbens* leaves on magnesium sulphate induced diarrhoea in rats.

S.No	Groups	Mean frequency of diarrhea \pm SEM	Mean wt of fecal drops \pm SEM	Mean wt of faces \pm SEM after 4 hrs	% protection
1	Control	4.066 \pm 0.225	11.6 \pm 0.669	1.45 \pm 0.076	-
2	Standard	0.63 \pm 0.136***	1.56 \pm 0.105***	0.125 \pm 0.007***	91.37%
3	MEJP(300mg/kg)	1.53 \pm 0.18***	3.2 \pm 0.17***	0.23 \pm 0.16***	84.13%
4	MEJP(500mg/kg)	1.1 \pm 0.15***	2.16 \pm 0.47***	0.14 \pm 0.016***	90.34%

The values are mean \pm SEM (n=6). Statistical significant test for comparison was done by one way ANOVA followed by dunnetts's test. Symbols statistical significant *P<0.05, **P<0.01,***P<0.001

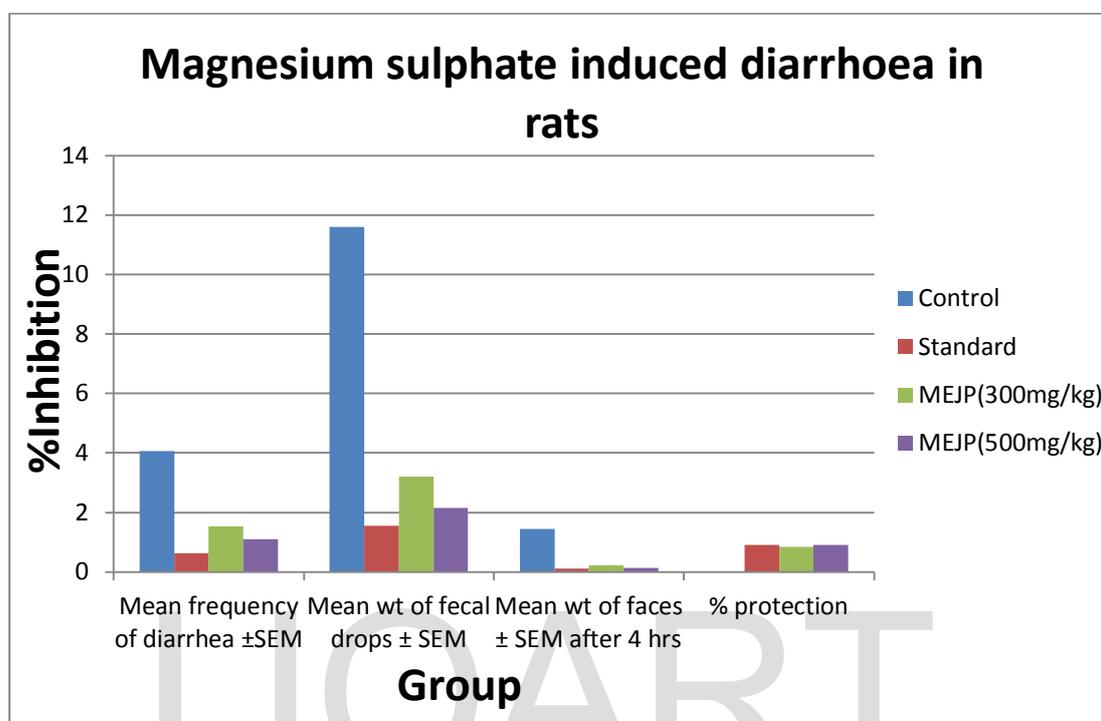


Fig: Effect of methanol extracts of *justice procumbens* leaves on magnesium sulphate induced diarrhoea in rats.

Discussion

The mechanism of action of the plant extract is through antipropulsive and antisecretory effects. The plant extracts showed a significant effect against magnesium sulphate induced diarrhoea. The chemical cause to act by its osmotic pressure and by cholecystokinin production from the duodenal mucosa, which increases the secretion and motility of small intestine and there by prevents the reabsorption of sodium chloride and water. Therefore mechanism other than inhibition of prostaglandin synthesis must also be considered as a number of factors that contribute to the functions of the gut⁶.

Conclusion:

The present study was under taken to evaluate the antidiarrhoeal activity of methanolic extracts of *Justica procumbens* leaves in rats using castor oil, castor oil

enteropaling and magnesium sulphate induced diarrhoea in rats, the extracts showing significant antidiarrhoeal activity.

The bioactivity-guided phytochemical screening of methanolic extract of *Justica procumbens* revealed the presence of flavonoids, saponins, steroids, terpenoids, tannins, and glycosides which may be responsible for the antidiarrhoeal effect and can be further fractionated and investigated for their role and utility in any of the antidiarrhoeal mechanisms.

In conclusion, with this preliminary investigation it can be concluded that the methanolic extract of *Justica procumbens* having good antidiarrhoeal activity. However the isolation of fraction from the extract and evaluation of the activity for that fraction is required for the characterization of active constituents responsible for the activity.

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