Original Paper

Comparison of anti-diarrheal activity of aqueous extracts of root, stem and leaves of *Murraya Koenigii* in castor oil induced diarrheal rats

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**Running Title:** Evaluation of anti-diarrheal activity of *Murraya Koenigii*

**Received:** 27 January, 2015; **Accepted:** 16 February, 2015

**Abstract**

The present study deals to evaluate comparatively the anti-diarrheal activity of an aqueous extract of root, stem and leaves of *Murraya Koenigii* in castor oil induced diarrhea in the Wistar albino rats. Wistar albino rats (About 180-200 gm) (Either sex) were taken. The animals were divided in to five groups with six rats in each group. Significant anti-diarrheal activity was observed in root, stem as well as leaves of *Murraya Koenigii*. In this experiment, the extracts treated rats showed significant reduction in the diarrheal fecal materials formation while the normal vehicle control or untreated group showed more frequently passage of watery diarrhea fecal materials with mucous secretions. The loperamide group showed significant (p<0.001) reduction in both number 0.66 (Mean), 0.82 (SD),*** p<0.001 and weight (1.14(Mean), 0.49 (SD),** p<0.01) of the faecal materials after 4 hr with respect to control group and its % of protection was 90.47% . All the three (root, stem and leaf of *Murrya koenigii*) extract successfully and significantly (*p<0.05. **p<0.01 and *p<0.05 respectively) reduced the number of diarrhea faeces formation but the aqueous stem extract of *Murrya koenigii* shown better % of protection(66.66%) as compared to other extracts (root and leaf).The stem shows maximum reduction in both number (1.33(Mean), 1.21 (SD),** p<0.01) and weight (1.42(Mean), 0.15 (SD),* p<0.05) of the faecal materials in comparison to the root and leaves of Murraya Koenigii respectively. The percentage protection of the stem was found to be 66.66 %.On the basis of these findings; it can be assumed that *Murraya koenigii* could be a potential source for novel lead discovery of newer anti-diarrheal herbal drugs.

**Keywords:** Anti-diarrheal activity, *Murraya Koenigii*, Wistar albino rats, Faecal materials.

**Introduction**

Diarrhoea is a condition in which loose, watery stools are passed more often than normal. It is a common symptom of a gastrointestinal disturbance. It is characterized by frequent loose stools and may be accompanied by abdominal pain, cramping, nausea, and fatigue. Sometimes there will be blood and/or mucous in the stool [1].

Rapid movement of fecal matter through intestine occurs and results in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools in diarrhea. According to world health organization, it is one of the most common causes of morbidity and mortality in many developing countries effecting mainly the infants and children [2]. It is often caused by enterotoxins which are produced by certain bacteria such as Escherichia coli, Salmonella typhi, Salmonella typhimurium, Clostridium difficile, Clostridium freundii, Aeromonas hydrophila, Campylobacter jejuni and Vibrio cholerae etc [3].
These bacteria cause the influx of water and ions to the intestinal lumen and thus increase the intestinal motility, thereby causing watery stools. Such secretory diarrhea is treated by the administration of oral rehydration salts in children or adults to reduce the loss of essential electrolytes and maintain the body fluids osmolality. Alternatively, many opioid drugs like Diphenoxylate, Loperamide, Diloxanidefuroate for protozoal infections induced diarrhea and dysentry, racecadotril, muscarinic receptor blockers like atropine sulfate etc; are available in the market for treating diarrhea. But all of the existing drugs suffer from adverse effects like the induction of bronchospasm, vomiting by racecadotril; intestinal obstruction and constipation by loperamide [4].

Loperamide is an opioid-receptor agonist and acts on the µ-opioid receptors of the myenteric plexus of the large intestine. It works similarly to morphine, by decreasing the activity of the myenteric plexus, which in turn decreases the tone of the longitudinal and circular smooth muscles of the intestinal wall [5, 6]. This increases the amount of time substances stay in the intestine, allowing for more water to be absorbed out of the fecal matter. Loperamide also decreases colonic mass movements and suppresses the gastrocolic reflex [7]. Loperamide does cross the blood brain barrier [8]. The use of loperamide (Imodium) in children under 2 years is not recommended [9]. There are rare reports of fatal paralytic ileus associated with abdominal. Treatment should be avoided in the presence of high fever or if the stool is bloody (dysentery) [10].

For this reason, present days there has been great interest in herbal remedies for the treatment of such ailments. Although, several medicinal plants have gained importance for the treatment of diarrhea, many remain to be evaluated scientifically.

The plant has been reported to possess wound healing activity [11], antioxidant [12], antibacterial and cytotoxic [13], anti-cancer [14], anti-microbial [15], anti-diabetic [16] and anti-diarrheal properties [17]. However, literature review failed to offer any scientific validation on the antidiarrheal activity (only few) of Murraya Koenigii leaves. In view of this, the present study is undertaken to justify the traditional use of the root, stem and leaves of Murraya Koenigii as an anti-diarrheal agent.

Taking all this into consideration, the topic entitled “Comparative Evaluation of Antidiarrheal Activity of Aqueous Extract of Root, Stem and Leaf of Murraya Koenigii in castor oil Induced Diarrhea in Rats” will be a new herbal approach of treating diarrhea.

**Materials and Methods**

**Plant material**

The root, stem and leaves of Murraya Koenigii were obtained from Visakhapatnam in the month of January. The identification and authentication of the root, stem and leaf was done at the Department of Botany, St. Joseph women’s College, Visakhapatnam.

**Chemicals:**

Pure drug sample of Loperamide was purchased from YARROW chemicals Ltd., Mumbai; India. The Tween 80(1%) was procured from Desai chemicals, Visakhapatnam, AP.

**Preparation of the root, stem and leaf extract**

Root, stem and leaves of Murraya Koenigii (~75 g) were thoroughly powdered and kept airtight in cool, dry and dark conditions. An approximately 50 gm of the powdered root, stem and leaves were subjected separately to maceration with 280 ml of distilled water for 24 hours. The filtrate was evaporated to dryness in a hot water bath then stored at room temperature, and protected from direct sunlight. Aqueous extracts from different parts obtained are shown in Figure 1.

**Experimental animals**

Wistar albino rats of either sex weighing about 180-200 gm were obtained from the Central Animal House. The animals were fed a normal Chow diet, with water ad libitum. Then they were maintained under standard conditions of temperature, humidity and light (12 hours light / 12 hours dark cycle). The experiment complied with...
the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethics Committee (IAEC) with registration number 285 / CPCSEA.

Grouping of animals
The experimental animals were divided into the following groups such as:

Group 1: Control, treated with 1% Tween 80.
Group 2: Standard drug (Loperamide)
Group 3: Aqueous root extract of the Murraya Koenigii (AREMK)
Group 4: Aqueous stem extract of the Murraya Koenigii (ASEMK)
Group 5: Aqueous leaf extract of the Murraya Koenigii (ALEMK)

Selection of doses and route of administration of the drugs

Acute Toxicity Study
Different doses (50–2000mg/kg, p. o) prepared from the aqueous extract of root, stem and leaves of Murraya Koenigii were administered to the different groups of rats and were observed continuously for 1 hour and then at half – hourly intervals up to 4 hours. The gross behavioral changes were observed up to 72 hours, followed by observation of mortality rate up to 14 days as per the OECD Guideline 425. From the toxicity study; it was observed that plant extract is non-toxic and caused no death up to the dose of 2000 mg/kg (1/10th of the maximum dose i.e. 200 mg/kg was selected). It is safe and used for further experiment.

The dose and route of the administration of drugs were taken from the literature and acute toxicity study.

- Loperamide- 3mg/kg orally [18]
- Aqueous root extract of the Murraya Koenigii (AREMK) - 200mg/kg orally
- Aqueous stem extract of the Murraya Koenigii (ASEMK) - 200mg/kg orally
- Aqueous leaf extract of the Murraya Koenigii (ALEMK) - 200mg/kg orally

Castor Oil Induced Diarrhea
The animals were screened initially by giving 0.5ml of castor oil and only those showing diarrhea were selected for final experiment. The animals were divided in to five groups like control, standard and three test groups, with six rats in each group. The animals of five groups were fasted for 18 hr. After 18 hr the control group received vehicle (1% tween 80 in water) at a dose of 1 ml/kg orally. The standard group received Loperamide at a dose of 3 mg/kg orally. The first test group received aqueous root extract of the Murraya Koenigii at a dose of 200 mg/kg orally. Second test group received aqueous stem extract of the Murraya Koenigii at a dose of 200mg/kg. Third test group received aqueous leaf extract of Murraya Koenigii at a dose of 200mg/kg. Diarrhea was induced by oral administration of 1 ml of castor oil; 30 min after the above drug pre-treatments. The animals were kept in separate metabolic cages with a plain sheet of paper placed on the floor to collect their droppings. They were observed every hour for 4 h after castor oil administration. The floor lining was changed every 2 hour. During an observation period of 4h total number of faeces, the weight of diarrheic fecal materials excreted by animals were recorded and the percentage inhibition of diarrhea were calculated. The numerical score based on stool consistency was assigned as follows; normal stool=1, semisolid stool=2, and watery stool=3.

Table 1: Preliminary phytochemical tests for identification of the phytoconstituents from aqueous extract of the root, stem and leaves of M. Koenigii.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Name of the test</th>
<th>Root</th>
<th>Stem</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer's test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner's test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hager's test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff's</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Salkowaski test</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch's test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling’s solution test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>_</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol and Tannins</td>
<td>Ferric-chloride test</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Gelatin test</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Proteins</td>
<td>Millon’s test</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Biuret test</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>
Results

Extraction and the Phytochemical Investigations

Percentage yield of the extracts

After drying, the dried mass of the aqueous extract of root, stem and leaves of Murraya Koenigii were weighed in a digital balance. The yields were found to be 2.45 gm (root), 8.09 gm (stem), 14 gm (leaf) (from 50gm Murraya Koenigii). The percentage yields were found to be 4.9% (root), 16.18% (stem), 28% (leaf).

Preliminary phytochemical screening of the extracts

The results of different phytochemical tests of the crude aqueous extract showed that components like alkaloids, phenol, carbohydrates, tannins, saponin, phytosterol (steroid), flavonoids and proteins are represented in Table 1.

Castor oil induced diarrhea

In this test, aqueous root, stem and leaf extract of Murraya Koenigii (200 mg / kg) showed significant reduction in number and weight of faecal materials when compared to the control conforming its anti diarrheal activity. The standard drug (Loperamide) having significant effect with respect to the control (**P < 0.01 and 90.47% inhibition). The result obtained from both the standard and extract treated groups were compared with the control group. The aqueous stem extract of Murraya Koenigii shown better antidiarrheal activity in comparison to that of root and leaves respectively; as the results of significance for root and leaf (*P < 0.05, 62.5% protection and *P < 0.05, 50% protection respectively) are less than that of stem (***P < 0.01, 66.66%). The result of above activity is shown in Table 2, Figure 2, 3 respectively.

Statistical Analysis:

The result was expressed as Mean ± S.E.M. Statistical analysis was carried out by using the Analysis of Variance (ANOVA) followed by Dennett’s multiple comparison tests using prism software version 6 (2011). P-values < 0.05 were considered as significant.

Discussion

Diarrhea is a very common ailment and national problem in many tropical countries and the cause of 4-5million deaths throughout the world annually. Apart from modern medical therapy, the use of herbal drugs in the treatment of diarrheal diseases is a common practice in many countries of Asia including India. Castor oil induced diarrhea is secretory diarrhea since ricinolic acid, the active ingredient of castor oil, induces diarrhea by a hypersecretory response. Since the aqueous root, stem and leaf extract of Murraya Koenigii successfully inhibited castor oil induced diarrhea. It can be assumed that the anti-diarrheal action was mediated by an ant secretory mechanism. In this castor oil-induced diarrhea experiment, the extract treated rats showed significant reduction in the diarrheal fecal materials formation while the normal vehicle control or untreated group showed more frequently passage of watery diarrhea fecal materials with mucous secretions. The loperamide group showed significant (p<0.01) reduction in both number and weight of the faecal materials after 4 hr with respect to control group and its % of protection was 90.47%. All the three (root, stem and leaf of Murraya Koenigii) extract successfully and significantly (p<0.05, p<0.01 and p<0.05 respectively) reduced the number of diarrhea faeces formation but the aqueous stem extract of Murraya Koenigii shown better (66.66%) % of protection as compared to other extracts (root and leaf). From the
Evaluation of anti-diarrheal activity of Murraya Koenigii

It was found that the bioactive alkaloids, kurryam and koenimbine exhibit inhibitory activity against castor oil-induced diarrhoea. The stem part of Murraya Koenigii contains four carbazole alkaloids, identified as mahanimbine, girinimbine, murrayaninemurrayafoline-a, (whereas the root and leaf part contains only one triterpene alkaloids) which are probably responsible for the better anti-diarrheal actions of stem extract.

Conclusion

The results of the study provide support for the traditional use of different parts (root, stem and leaf) of Murraya Koenigii as anti-diarrheal agents. This test showed that all the three extracts significantly reduced the frequency and weight of watery fecal material excretion and showed good % protection of diarrhea.

But by comparing the results of different parts, it can be concluded that the aqueous stem extract of Murraya Koenigii possesses marked anti-diarrheal activity than that of other two extracts (root and leaf). On the basis of these findings, it can be assumed that Murraya Koenigii could be a potential source for novel lead discovery of newer anti-diarrheal herbal drugs. Future study is necessary to evaluate and isolate the active principle of root, stem and leaves of Murraya Koenigii, responsible for the above activity.

Acknowledgements

The authors would like to acknowledge the contributions of MNR College of Pharmacy, Fasalwadi, Sangareddy, Medak, Telangana, India for providing necessary facilities to carry out the research work.

Conflict of Interest

The authors declare that there is no conflict of interest to reveal.

Table 2: Antidiarrheal Activity of aqueous root, stem and leaf extract of Murraya Koenigii in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose Mg/kg</th>
<th>Total no. of faeces in hr. ± SEM</th>
<th>Weight of faeces in gm± SEM</th>
<th>Mean wt of faeces ± SEM after 4hr</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>1 mL</td>
<td>9.5±1.25</td>
<td>2.34±0.12</td>
<td>1.68±0.27</td>
<td>0.00</td>
</tr>
<tr>
<td>II</td>
<td>Loperamide</td>
<td>3</td>
<td>2.00±0.44 **</td>
<td>0.66±0.33***</td>
<td>0.16±0.05**</td>
<td>90.47</td>
</tr>
<tr>
<td>III</td>
<td>AREMK</td>
<td>200</td>
<td>4.16±1.07*</td>
<td>1.54±0.06**</td>
<td>0.63±0.28*</td>
<td>62.5</td>
</tr>
<tr>
<td>IV</td>
<td>ASEMK</td>
<td>200</td>
<td>2.33±0.71**</td>
<td>1.31±0.11**</td>
<td>0.56±0.22**</td>
<td>66.66</td>
</tr>
<tr>
<td>V</td>
<td>ALEMK</td>
<td>200</td>
<td>2.5±0.56*</td>
<td>1.48±0.07**</td>
<td>0.84±0.17*</td>
<td>50</td>
</tr>
</tbody>
</table>

**P < 0.01, ***P < 0.001, *P < 0.05"
References