Anti-diarrhoeal activity of friedelin isolated from *Azima tetracantha* Lam. in Wistar rats

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Abstract

The present study was aimed to evaluate the anti-diarrhoeal activity of friedelin isolated from leaves of *Azima tetracantha* Lam. The anti-diarrhoeal effect of friedelin was studied by using castor oil-induced diarrhoea, gastrointestinal motility test, magnesium sulphate-induced diarhoea and castor oil-induced enteropooling in rats. Friedelin (20 mg/kg) showed significant (P < 0.0001) reduction of intestinal transit and gastric emptying which were similar to the anti-motility activity as known compound atropine (0.1 mg/kg). Friedelin (20 mg/kg) also exerted significant anti-enteropooling effects, against castor oil-induced enteropooling in rats. The defeaecation frequencies and the faecal droppings wetness were significantly (P < 0.0001) reduced. Additionally, friedelin (20 mg/kg) revealed significant (P < 0.0001) inhibition (89.64%) of castor oil-induced diarrhoea. The overall results elucidated that the anti-diarrhoeal activity of friedelin may be due to its anti-secretory and anti-motility properties, which consequently provide evidence for the traditional claim.

1. Introduction

Diarrhoea (from the Greek word to “flow through”) is nothing but the speedy passage of gastric stuffing over the bowel. Diarrhoea is restricted not only to the developing world, but is also a significant origin of morbidity was observed on developed world, particularly in the early stage (first year) of life (Whyte and Jenkins 2012). Currently there are varieties of drugs available in order to treat diarrhoea including anti-motility drugs (e.g. loperamide hydrochloride, diphenoxylate), adsorbents (e.g. kaolin, attapulgite) and many more. Nevertheless, these drugs undergoes various side effects including allergic reactions, rash, fever, eosinophilia abdominal discomfort, dry mouth, nausea, epigastric distress, metallic taste, constipation, headache, dizziness, and skin eruptions (Sharma and Sharma 2007). In order to get safer drugs without any unwanted effects, people around the globe seeking to medicinal plants, since they provided number of beneficial phytochemicals for various ailments.

Previous studies reported that *Azima tetracantha* Lam. exhibited that number of pharmacological activities against rheumatism, dyspepsia, dropsy, chronic diarrhoea. *A. tetracantha* also shows significant anti-inflammatory, astringent, antiperiodic and expectorant activities (Nadkarni 1976). Friedelin isolated from *A. tetracantha* shows significant gastroprotective (Antonisamy et al., 2015) anti-inflammatory, analgesic and anti-pyretic effects (Antonisamy et al., 2011). Conventionally the *A. tetracantha* used as anti-diarrhoeal drug. Conse-
sequently, the present study was aimed to assess the anti-diarrhoeal activity of friedelin on animal model.

2. Materials and methods

2.1. Chemicals used

Chlorpromazine HCl, loperamide and Castor oil were acquired from Sigma-Aldrich (St, Louis, MO, USA). Magnesium sulphate, atropine and carboxymethyl cellulose (CMC), were acquired from Himedia (Mumbai, Maharashtra, India).

2.2. Identification and characterization of friedelin

Previously we have reported about the isolation and identification of friedelin (Antonisamy et al., 2011). Chemical structure of friedelin is shown in Fig. 1.

![Fig 1. Chemical structure of friedelin.](image)

**Table 1.** Effect of friedelin (05–20 mg/kg, p.o.) on castor oil-induced diarrhoea in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o)</th>
<th>Total number of faeces in 4 h</th>
<th>Total number of wet faeces in 4 h</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (0.5% CMC)</td>
<td>-</td>
<td>6.82±0.47</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>8.40±0.61</td>
<td>7.68±0.55</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>03</td>
<td>1.82±0.12*</td>
<td>0.60±0.05*</td>
<td>84.28</td>
</tr>
<tr>
<td></td>
<td>05</td>
<td>6.88±0.49*</td>
<td>4.60±0.34*</td>
<td>45.23</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3.56±0.26*</td>
<td>2.14±0.13*</td>
<td>57.61</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.47±0.11* NS</td>
<td>0.87±0.07*</td>
<td>89.64</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. (n = 6).

*P< 0.0001 vs. control.

**Table 2.** Inhibitory activity of friedelin (05–20 mg/kg, i.p.) on intestinal motility in rats (charcoal meal study).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, i.p.)</th>
<th>Maximal distance traveled by charcoal plug (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.5% CMC)</td>
<td>-</td>
<td>91.62±6.92</td>
</tr>
<tr>
<td>Atropine Friedelin</td>
<td>0.1</td>
<td>31.25±2.34*</td>
</tr>
<tr>
<td></td>
<td>05</td>
<td>76.83±5.81*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>49.19±3.76*</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>28.62±2.18*</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. (n = 6).

*P<0.0001 vs. control.

**Table 3.** Effect of friedelin (05–20 mg/kg, p.o.) on magnesium sulphate-induced diarrhoea in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Total number of faeces in 4 h</th>
<th>Total number of wet faeces in 4 h</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (0.5% CMC)</td>
<td>-</td>
<td>6.57±0.38</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>9.28±0.62</td>
<td>8.91±0.69</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide Friedelin</td>
<td>03</td>
<td>2.71±0.19*</td>
<td>1.30±0.11*</td>
<td>85.40</td>
</tr>
<tr>
<td></td>
<td>05</td>
<td>7.22±0.53*</td>
<td>3.97±0.29*</td>
<td>55.44</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4.26±0.32*</td>
<td>2.11±0.18*</td>
<td>76.31</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.15±0.21*</td>
<td>1.43±0.10*</td>
<td>83.95</td>
</tr>
</tbody>
</table>
2.3. Animals used
Male Wistar rats (210–220 g) were used for the current experiments. All the animals were maintained at suitable laboratory conditions with proper maintenance and provided standard food and water ad libitum. All the animals utilized for the experiments have been acclimatized for a period of 2 weeks prior to experiments. All the animal mediated experiments were accompanied in accordance with the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines.

2.4. Castor oil-induced diarrhoea in rats
For this experiment, the animals were grouped into six groups of 6 animals each. Castor oil-induced diarrhoea was conducted according the previous methods (Zavala et al., 1998).

2.5. Gastrointestinal motility in rats (charcoal meal)
Male Wistar rats were divided into five groups of six animals each. Charcoal meal mediated Gastrointestinal motility test has been carried out based on the previous method (Gamaniel and Akah 1996).

2.6. Magnesium sulphate-induced diarrhoea in rats
Magnesium sulphate-induced diarrhoea was carried out depending upon previous method (Zavala et al., 1998); this method was similar to that of castor oil-induced diarrhoea.

2.7. Castor oil-induced enteropooling in rats
Castor oil-induced enteropooling analysis has carried out in male Wistar rats. Previous method has been followed for this experiment (Aniagu et al., 2005).

2.8. Statistical analysis
Data were statistically analyzed using analysis of variance (ANOVA), followed by Student's t-test. A probability level lower than 0.05 was considered statistically significant.

3. Results
3.1. Toxicity and effective dose evaluation
In the acute toxicological assessment rats did not shows any behavioural abnormalities against friedelin up to 320 mg/kg of oral treatment.

3.2. Castor oil-induced diarrhoea
Friedelin (20 mg/kg) significantly (P < 0.0001) inhibited (89.64%) castor oil-induced diarrhoea in rats; this effect was higher than the standard drug loperamide (03 mg/kg) (Table 1).

3.3. Gastrointestinal motility and magnesium sulphate-induced diarrhoea
Friedelin (20 mg/kg) exhibited significant (P < 0.0001) anti-motility effects (28.62%); this level of inhibition was near to that of atropine (31.25%) (Table 2). Friedelin (20 mg/kg) also exhibited significant (P < 0.0001) inhibition (83.95%) of magnesium sulphate-induced diarrhoea (Table 3).

3.4. Castor oil-induced enteropooling in rats
In castor oil-induced enteropooling test friedelin (20 mg/kg) shows significant (P < 0.0001) inhibition of intestinal fluid volume compare to control group; this activity was higher than the chlorpromazine HCl (Table 4).

4. Discussion
In the present study, we have assessed the anti-diarrhoeal activity of friedelin on animal model. Ricinoleic acid released from castor oil makes fluctuations in electrolyte transport and mucosal fluid consequently leads to the diarrhoea with hypersecretory response (Ammon et al., 1974; Gaginella et al., 1975). After ricinoleic acid release, prostaglandin synthesis was stimulated in gut lumen at enormous way, which in turn leads to the higher secretion of the water and electrolytes production on small intestine. Furthermore, previous experiments by Luderer et al. (1980) stated that the castor oil administration through orally leads to significant elevation of prostaglandin content and subsequently directs to diarrhoea. Previous report elucidated that the prostaglandin biosynthesis inhibitors considerably inhibits diarrhoea condition (Awouters et al., 1978). In this study, friedelin

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</tr>
<tr>
<td>Friedelin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>76.83±5.81*</td>
</tr>
<tr>
<td></td>
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Values are mean ± S.D. (n = 6).
*P< 0.0001 vs. control.
(20 mg/kg) significantly inhibited diarrhoeal condition, which was identified through the considerable reductions of defecation frequency and faecal droppings wetness when compared to disease control. These outcomes clearly mentioned about the anti-diarrhoeal efficacy of friedelin may be due to its capability on the prostaglandin biosynthesis inhibition.

Friedelin (20 mg/kg) also exhibited significant inhibition of gastrointestinal motility which has been assessed via charcoal meal. This effect was similar to that of standard drug atropine. From these observations we can suggests that the anti-diarrhoeal effects of friedelin at least partially due to its anti-motility capability. Magnesium sulphate-induced diarrhoea is another method applied for the screening of anti-diarrhoeal drugs. Magnesium sulphate can able to induce the diarrhoeal condition through the inhibition of water reabsorption, which in turn leads to elevation of intestinal contents. Magnesium sulphate also stimulates the cholecystokinin release from the mucosa of duodenum and consequently increases the intestinal motility and its secretions; as a result NaCl and H₂O reabsorption has been denied (Galvez et al., 1993). There was another possibility that friedelin provided anti-motility activity may be through its proficiency on the increases of NaCl and water reabsorption.

5. Conclusions
Traditionally A. tetracantha has been used as anti-diarrhoeal drug. Depending upon the outcomes of the existing study, one can suggests that the friedelin may be the major compound responsible for the anti-diarrhoeal activity of A. tetracantha. However, molecular mechanisms responsible for this activity need to be scrutinized in detail.

Conflict of interest statement
We declare that we have no conflict of interest.

References