Antidiarrhoel activity of methanolic extract of *Achillea millefolium* L. leaves in albino rats

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ABSTRACT

Based on the traditional folk use, the anti-diarrheal activity of the methanolic extract of *Achillea millefolium* L. (AM) leaves was evaluated on castor oil-induced diarrhoea and assessment of gastrointestinal propulsion of charcoal meal in rats. *Achillea millefolium* L. commonly known as yarrow belonging to the family Asteraeae, is an ancient traditional herb native to Europe and is used to treat wounds, hepatic disorders, gastrointestinal disorders, spasmodic diseases, headaches, pain, inflammation and diarrhoea. The three doeses of *Achillea millefolium* L. methanolic extract has been selected (150 mg/kg, 300 mg/kg and 450 mg/kg). Among three dosages of AM leaves, the two dosages (300 mg/kg-IIIb and 450 mg/kg IIIc) showed a significant reduction in various parameters like distance travelled (IIIb-25± 1.679 cm; IIIc-17± 2.534 cm) and % average travelled (IIIb-47.16; IIIc-32.69) travelled in charcoal meal model when compared to control group. Phytochemical screening of the plant extract revealed the presence of flavonoids, tannins, steroids and terpenes. Results showed that the methanolic extract of *Achillea millefolium* Leaves possess anti-diarrhoal activity possibly mediated by inhibiting the intestinal motility, hydroelectrolyte secretion and by making intestinal mucosa more resistant to chemical alteration and hence reduce secretions.

Keywords: *Achillea millefolium* L.; Anti-diarrhoeal; Castor oil; Loperamide; Charcoal meal test.

INTRODUCTION

Diarrhoea is a leading cause of high morbidity and mortality globally responsible for two million deaths under 5 years of age [1]. Overall diarrhea related deaths were 15% of 10.5 million, under 5 years in developing countries. In India it was second killer disease responsible for 20% deaths whereas deaths due to respiratory problem were 30% [2]. Many infectious diseases such as diarrhoea, urinary tract infection, bronchitis, parasitic diseases and cutaneous abscesses are being treated by the use of many plants as folk medicine from the ancient period of time [3]. According to the survey by world health organization 80% of the world’s population mainly depends on the traditional medicine and the use of the traditional plants as well as their extracts to treat various diseases [4]. Medicinal plants have been used for their accessibility, economical viability and ancestral experience, due to this reason they constitute major component of the traditional medicine [5]. Hence, medicines obtained from the plant origin, and to know their safety and effective profile for the use of humanity being an important consideration and to be an important area of active research. Depending on traditional medical practices, World Health Organization motivates studies for the prevention and treatment of diarrhoeal diseases [6].

*Achillea millefolium* L., common known as yarrow belonging to the family Asteraceae, is an ancient traditional herb native to Europe and is used to treat wounds, hepatobiliary and gastrointestinal disorders, spasmodic diseases, headaches, pain and inflammation [7, 8]. Extensive literature survey revealed the presence of active secondary metabolites of the chemical constituents, including terpenoids, steroids, sesquiterpenes derivatives, resins, saponins, coumarins, fatty acids, alkaloids, flavonoids, essential oil and glycosides present in the leaves and flowers of *Achillea millefolium* L. [9]. Different extracts of *Achillea millefolium* L. (AM) in experimental studies confirmed their traditional uses including antiulcer [10], hepatoprotective and anti-spasmodic [11], hypotensive [12], diuretic [13], anxiolytic [14], anti-inflammatory [15], hypoglycaemic and hypolipidemic [16], anti-oxidant and anti-microbial [17], and anti-mutagenic activity [18].

Due to its folk use in the treatment of diarrhoea and also it has proven its anti-microbial activity [17], and microbes are one of the major causes of diarrhoea. The present study aims to evaluate the anti-diarrhoeal effect of the methanolic extract of *Achillea millefolium* L. leaves (MEAM) in castor oil induced diarrhoea and assessment of gastrointestinal propulsion of charcoal meal in experimental animal models.
MATERIALS AND METHODS

2.1. Plant Material and drugs
The methanolic extract of Achillea millefolium L. (AM) (Yarrow) leaves was procured from Amsar Pvt. Ltd., Indore (M. P.), loperamide (Torrent, Ahmedabad, India), Castor oil and activated charcoal powder were purchased from S.D. Fine Chem. Ltd.

2.2. Animals
Wistar rats (150–200 g) of either sex, procured from the Lokan Thermometers, Ambala Cantt. The animals were maintained under standard environmental conditions and had free access to normal diet (Ashirwad industries, Kharar) and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.3. Acute toxicity study (LD₅₀)
Lethal dose (LD₅₀) was determined according to the guidelines of organization for economic cooperation & development (OECD) following the up and down method (OECD guideline no. 423) and fixed dose method (OECD guideline no. 420). Based on these guidelines a limit test was to categorize the toxicity class (LD₅₀) of the compound. The limit test was performed at 2000 mg/kg, p.o. A dose range of 150 mg/kg, 300 mg/kg and 450 mg/kg was selected for the pharmacological activity. For all the studies overnight fasted rats of either sex were used.

2.4. Antidiarrhoel activity test by castor oil induced diarrhoea
The method described by Teke (2010) was used to access the anti-diarrhoel activity [19]. The animals were starved for 18 hrs prior to the experiments and were randomly distributed into five groups of 5 animals in each. Groups 1 had received normal saline (1ml/100 g of body weight) and group 2 had received loperamide (2.5 mg/kg, p.o.). Rats of the last three groups were administered orally by gavage 150, 300 and 450 mg/kg body weight of the methanol extract, respectively. Sixty minutes after drug treatment, each animal was administered castor oil orally (1 ml/100 g body weight). The latent period (the time between castor oil administration and appearance of first diarrheic drop) was recorded. Observation for defecation continued up to 4 h on filter paper placed beneath the individual perforated rat cages. This paper was replaced every hour after noting its weight (W₁). Finally, the filter paper was exposed in the laboratory for drying over a period of 14 hrs and it was reweighed (W₂). The fecal water content was calculated as (W₂−W₁) g. The percentage of rats that responded to diarrhea, the latent period, the mean stool frequency, frequency of diarrheic drops and water content were recorded [20]. The purging indices [21], the percentage inhibition of defecation and diarrheic drops [22] were evaluated by following formula.

\[
\text{Purging index} = \frac{\% \text{ respondents} \times \text{ average number of stool}}{\text{Average latent period}}
\]

\[
\text{Inhibition of defecation} (\%) = \frac{\text{Mc} - \text{Md} \times 100}{\text{Mc}}
\]

\[
\text{Water Content} = \frac{(W₂ - W₁) g}{\text{Mc}}
\]

Where

Mc= mean no of defecation caused by castor oil
Md= mean no of defecation caused by drug or extract

2.5. Assessment of gastrointestinal propulsion of charcoal meal
The method used to assess the effect of plant extract on the gastrointestinal transit of charcoal meal [23, 24]. Animals were used in groups of five per dose of plant extract or loperamide, a standard drug after fasting for 16 hrs. Control group was pretreated with 0.3 ml of physiological saline given orally, for 20 min and then given 0.4 ml of charcoal meal (an aqueous suspension of 5% charcoal and 5% gumacacia) orally; 20 min after the charcoal meal administration, the animals were killed by ether inhalation and the intestine was removed from the cardia to the rectal end. The distance travelled by the charcoal meal was measured and expressed as a percentage of the total length of the intestine. Experiments were repeated with other groups of animals pretreated with either the methanolic extract (150 mg/kg, 300 mg/kg and 450 mg/kg) of the plant or the standard drug, loperamide (20 mg/kg), both given orally in a volume of 1 ml/100 g of animals, prior to the administration of 0.4 ml of charcoal meal. All experiments were carried out between 08:00 and 17:00 hrs in a quiet laboratory with an ambient temperature of 22±2 °C.

2.6. Statistics
The statistical analysis was performed by using graph pad prism 6, using one-way Analysis of Variance (ANOVA) with level of statistical significance taken as p>0.05*, p<0.05, p<0.01, p<0.001 with Dunnett’s multiple comparison test. Results obtained were expressed as mean± SEM. (n=5).

RESULTS

3.1. Antidiarrhoel activity test by castor oil induced diarrhoea
The effect of leaf extract on castor oil induced diarrhoea in rats showed decrease in the water content in stool which will lead to change in consistency of faeces from watery to solid at the different doses of Achillea millefolium L. (AM) (150 mg/kg, 300 mg/kg, and 450 mg/kg p.o.) respectively. There is percent inhibition of wet stool when compared to control group.

Animals treated with castor oil (control group) at the dose of (1ml/100gm of body weight) significantly induced the diarrhea in Rats. The different parameters observed in castor oil induced diarrhea were i.e. latent period (63± 3.136 min), Total stool frequency (8.01±1.00 min), Frequency of wet stool (7.29± 0.56), Water content (11.80±0.4595 min), purging indices (12.69) and % inhibition of defecation (100) (Table. 1).
Animals treated with standard drug (loperamide) at the dose of (2.5 mg/kg, p.o.) showed most significant changes in different parameters i.e. latent period (183±3.536 min; P<0.001), Total stool frequency (2.6±0.40 min, P<0.01), Frequency of wet stool (0.80±0.98 min; p<0.001), Water content (1.28±1.762 min; p<0.001) and there was reduction in purging indices (1.42) and % inhibition of defecation (35.51). There was significant increase in inhibition of wet stool (89.02) when compared with negative control group.

In group III (a), animals treated at the dose 150 mg/kg, p.o., group III (a) significantly delay the various parameters i.e latent period (92.5±25.66 min; p > 0.05”), Total Stool Frequency (3.8±0.7348 min; P < 0.01), Frequency of Wet Stool (1.8±0.7348 min; p < 0.01), Water Content (4.097±0.3522; p < 0.001) and reduction in Purg Indices (4.108) and % inhibition of defecation (20.68). MEAM (150 mg/kg p.o.) also increase the inhibition of Wet Stool (73.66) when compared to negative control group.

In group III (b), animals treated at the dose of 300 mg/kg, p.o., group III (b) significantly delay the various parameters i.e latent period (138±2.630 min; p < 0.01), Total Stool Frequency (3.9±0.4427 min; p < 0.001), Frequency of Wet Stool (1.01±0.74 min; p < 0.001), Water Content (3.046±0.3042; p < 0.001) and reduction in Purg Indices (3.10) and % inhibition of defecation (50.06). MEAM (300 mg/kg p.o.) also increase the inhibition of Wet Stool (86.14) when compared to negative control group.

In group III (c), animals treated with dose 450 mg/kg, p.o., group III (b) significantly delay the various parameters i.e latent period (116±8.260 min; p < 0.05”), Total Stool Frequency (3.4±0.5099 min; p < 0.01), Frequency of Wet Stool (1.92±0.76 min; p < 0.001), Water Content (4.097±0.3522; p < 0.001) and reduction in Purg Indices (4.108) and % inhibition of defecation (20.68). MEAM (450 mg/kg p.o.) also increase the inhibition of Wet Stool (86.69) when compared to control group.

All results showed the reduction in water content in stool will lead to changed in consistency of faeces from watery to solid at the different doses of Achillea millefolium L. (AM) (150 mg/kg, 300 mg/kg, and 450 mg/kg p.o.) respectively.

3.2. Assessment of gastrointestinal propulsion of charcoal meal

The distance travelled by charcoal in small intestine in control group was determined by different parameters like length of small intestine, distance travelled by charcoal and % average travelled.

In control group, the distance travelled by charcoal in small intestine was determined by different parameters like length of small intestine (58±0.97 cm). Distance travelled by charcoal (51±1.54 cm) and % average travelled (87.93) in control group.

Animals treated with standard loperamide at the dose (20 mg/kg p.o.) showed a highest significant reduction in distance travelled by charcoal (13±2.876; p < 0.001), length of small intestine (57±1.3) and highly decrease in % average travelled (22.80) by charcoal when compared to control group.

The animals received the plant extract MEAM at the dose of (150 mg/kg, p.o.) (Group III (a)), showed a significant reduction in distance travelled by charcoal (37±3.3; p < 0.01), length of small intestine (56±3.65) and decrease in % average travelled (66.07) when compared to control group.

Table 1: Effect of methanolic extract of Achillea millefolium L. leaves (MEAM) against castor oil induced diarrhoea

<table>
<thead>
<tr>
<th>Groups and Doses</th>
<th>% Respondent</th>
<th>Latent Period (min.)</th>
<th>Total Stool Frequency</th>
<th>Purg Indices</th>
<th>% inhibition of defecation</th>
<th>Frequency of Wet Stool</th>
<th>Water Content (g)</th>
<th>Inhibition of Wet Stool (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control (Normal Saline)</td>
<td>100</td>
<td>63±3.136</td>
<td>8±1.000</td>
<td>12.69</td>
<td>100</td>
<td>7.29±0.56</td>
<td>11.80±0.459</td>
<td>0</td>
</tr>
<tr>
<td>Group II Standard, Loperamide (2.5 mg/kg, p.o.)</td>
<td>60</td>
<td>18±3±3.536 ***</td>
<td>2.6±0.40 ***</td>
<td>1.42</td>
<td>35.51</td>
<td>0.80±0.98</td>
<td>1.28±0.1762</td>
<td>89.02</td>
</tr>
<tr>
<td>Group III a MEAM (150 mg/kg, p.o.)</td>
<td>100</td>
<td>92.5±25.66 **</td>
<td>3.8±0.7348 **</td>
<td>4.108</td>
<td>20.68</td>
<td>1.78±0.1764</td>
<td>4.097±0.3522</td>
<td>73.66</td>
</tr>
<tr>
<td>Group III b MEAM (300 mg/kg, p.o.)</td>
<td>100</td>
<td>116±8.260 °</td>
<td>3.6±0.5099 °</td>
<td>3.10</td>
<td>50.06</td>
<td>1.92±0.74 **</td>
<td>3.046±0.3042</td>
<td>86.14</td>
</tr>
<tr>
<td>Group III c MEAM (450 mg/kg, p.o.)</td>
<td>80</td>
<td>138±2.630 °°</td>
<td>2.9±0.4427 °°</td>
<td>2.10</td>
<td>71.72</td>
<td>0.96±0.54</td>
<td>1.783±0.2277</td>
<td>86.69</td>
</tr>
</tbody>
</table>

*All values are expressed as Mean±SEM (n=5)*** p < 0.001, ** p < 0.01, ° p < 0.05, p > 0.05, ns - non significant as compared to control group, data was analyzed by one way ANOVA followed by Dunnett’s test*
Fig. 1: Effect of methanolic extract of *Achillea millefolium* L. leaves (MEAM) on latent period in castor oil induced diarrhoea model. Values are expressed as Mean±SEM. (n=5). Data was analyzed by one way ANOVA followed by Dunnett’s multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001, ns - non significant as compared to control group.

Fig. 2: Effect of methanolic extract of *Achillea millefolium* L. leaves (MEAM) on total stool frequency in castor oil induced diarrhoea model. Values are expressed as Mean±SEM. (n=5). Data was analyzed by one way ANOVA followed by Dunnett’s multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001 as compared to control group.

Fig. 3: Effect of methanolic extract of *Achillea millefolium* L. leaves (MEAM) on frequency of wet stool in castor oil induced diarrhoea model. Values are expressed as Mean±SEM. (n=5). Data was analyzed by one way ANOVA followed by Dunnett’s multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001 as compared to control group.
Values are expressed as Mean±SEM. (n=5). Data was analyzed by one way ANOVA followed by Dunnett's multiple comparison tests.

**p < 0.01, ***p < 0.001, **p < 0.01, **p < 0.05 as compared to control group

Table 2. Effect of MEAM on assessment of gastrointestinal propulsion of charcoal meal

<table>
<thead>
<tr>
<th>Groups and Doses</th>
<th>Length of Small Intestine (cm)</th>
<th>Distance travelled (cm)</th>
<th>% Average travelled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control Saline (0.5 ml)</td>
<td>58±0.97</td>
<td>53±1.54</td>
<td>87.03</td>
</tr>
<tr>
<td>Group II Loperamide (50mg/kg)</td>
<td>57±1.4</td>
<td>14±2.17</td>
<td>22.80</td>
</tr>
<tr>
<td>Group III (a) MEAM (150mg/kg)</td>
<td>56±3.65</td>
<td>37±3.30</td>
<td>66.07</td>
</tr>
<tr>
<td>Group III (b) MEAM (300mg/kg)</td>
<td>56±3.75</td>
<td>22±1.67</td>
<td>47.16</td>
</tr>
<tr>
<td>Group III (c) MEAM (450mg/kg)</td>
<td>52±3.078</td>
<td>17±2.34</td>
<td>32.69</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM. (n=5) **p < 0.01, ***p < 0.001 as compared to control group, data was analyzed by one way ANOVA followed by Dunnett's test.

Diarrhoea is considered to be changed in frequency of bowel contents at daily rate in comparison with normal person rate [25]. The present study reported the protective effect of methanolic extract of Achillea millefolium L. leaves in various doses against castor oil induced diarrhoea and gastrointestinal transit by charcoal meal transit test.

Castor oil was known to induce diarrhoea by its active compound ricinoleic acid formed in the upper small intestine by reaction with enzyme lipase leads to poor absorption andinduces changes in the mucosal permeability, electrolyte transport, intestinal peristalsis and hypersecretory response [26]. Ricinoleic acid readily forms ricinoleate salts with Na⁺ and K⁺ which causes local irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins which stimulates motility and net secretion of water content and electrolytes in small intestine [27, 28]. There are other several mechanisms have been also reported for castor oil induced diarrhoea which includes reduction in normal fluids absorption by inhibition of intestinal Na⁺, K⁺-ATPase [29-31]. Activation of mucosal CAMP mediated active secretion [29-31], increased prostaglandins synthesis [32-35], platelet activating factor [36-38] and recently NO has been claimed to contribute to the inhibitory non-adrenergic, non-cholinergic neurotransmitter that mediates gastrointestinal motility in physiological and certain non-pathophysiological states, such as in absorptive and secretory processes. NO could lead to gut secretion via elevation of cGMP and cAMP concentration [38-42]. The castor oil significantly induced the diarrhoea at the dose of (1ml/kg, p.o.) [19]. There are several plants have been reported to impant anti-diarrhoeal activity containing bioactive compounds like tannins, flavonoids and terpenes against castor oil induced diarrhoea [43,44]. In present study the preliminary phytochemical test and qualitative estimation showed a presence of flavonoids, tannins, steroids and terpenes. The proposed...
mechanism for plants containing flavonoids and tannins to act as an anti-diarrhoeal by inhibiting the intestinal motility, hydroelectrolyte secretions [45] and by making intestinal mucosa more resistant to chemical alteration and hence reduce secretions [46].

Loperamide, an opioid derivative, has been used in diarrhoea for slowing the intestinal motility by acting on µ receptors and anticholinergic activity on GIT [47-49]. Therefore it also provides a protective mechanism against castor oil induced diarrhoea and intestinal propulsion of charcoal meal in the study.

In another model the extract produced a dose dependent decrease in propulsive movement of the standard charcoal castor oil induced diarrhoea and intestinal propulsion of charcoal meal in the study.

The authors extend their sincere thanks to Director, Rayat institute of Pharmacy for providing the required mechanism for plants containing flavonoids and tannins to act as an anti-diarrhoeal by inhibiting the intestinal motility, hydroelectrolyte secretions [45] and by making intestinal mucosa more resistant to chemical alteration and hence reduce secretions [46].

CONCLUSION

The present study concludes that the methanolic extract of Achillea millefolium L. (AM) leaves possess a significant anti-diarrhoeal activity. The change in motility and its antisecretory property give the experimental basis to understand the use of Achillea millefolium L. (AM) in traditional medicine as anti-diarrhoeal agent. Further studies on the plant extract are required to identify the active constituents responsible for anti-diarrhoeal effects.

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