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# EFFECT OF *DESMOSTACHYA BIPINNATA* EXTRACTS ON CASTOR OIL AND MAGNESIUM SULPHATE INDUCED DIARRHEA IN WISTAR RATS

Anupama Singh [*1, 2], Vikas Anand Saharan [2], Verma Ram [2] and Anil Bhandari [1]

Faculty of Pharmaceutical Sciences [1], Jodhpur National University, Boranada, Jodhpur - 342012, Rajasthan, India.
Sardar Bhagwan Singh PG Institute of Biomedical Sciences and Research [2], Balawala, Dehradun - 248008, Uttarakhand, India.

**Keywords:**

*Desmostachya Bipinnata*, Magnesium Sulphate, Acute Diarrhea, Castor Oil, Toxicity

**Correspondence to Author:**
**Anupama Singh**

Faculty of Pharmaceutical Sciences, Jodhpur National University, Boranada, Jodhpur - 342012, Rajasthan, India.

**E-mail:** anupama.cognosy@gmail.com

**ABSTRACT:** *Desmostachya bipinnata* (L.) Stapf [Gramineae (Poaceae)] is used in Indian traditional medicines system for treatment of various diseases such as asthma, kidney stone, diarrhea, wound healing, *etc*. These traditional claims require authentication by performing animal testing. This study aims to confirm the antidiarrheal activity of *D. bipinnata* in Wistar rats. Underground parts of *D. bipinnata*was extracted in alcohol, water, a hydroalcoholic solution, chloroform, and ethyl acetate. Acute toxicity test was performed in albino mice and antidiarrheal activity was estimated in Wistar rats. Castor oil and magnesium sulfate-induced diarrhea in Wistar rats were treated with single oral administration of prepared *D. bipinnata* extracts (500 mg/kg) and antidiarrheal drug loperamide (3 mg/kg). Diarrhoeal droppings were observed for 6 h in all treated animals. One way ANOVA followed by Dunnet's '*t*' test was used to identify significant differences among obtained results. Extracts of *D. bipinnata* showed neither mortality nor any toxic effect in albino mice up to the dose of 5 g/kg in 48 h to 14 days. The alcohol extract of *D. bipinnata* was 61.54% ($P<0.01$) effective in reducing feces in castor oil induced-diarrheal rats and 64.52% ($P<0.01$) feces reduction in magnesium sulphate induced-diarrheal rats. Extracts of *D. bipinnata* were found effective in reducing diarrhea in Wistar rats Therefore, it is anticipated that *D. bipinnata* contains pharmacologically active substances responsible for antidiarrheal activity.

**INTRODUCTION:** *Desmostachya bipinnata* (L.) Stapf (syn: *Eragrostis cynosuroides*) Gramineae (Poaceae)] is commonly known as sacrificial grass, kusha [1], drabh [2], and dab [3]. *D. bipinnata* contains flavonoids, viz., kaempferol, quercetin, quercetin-3-glucoside, trycin, trycin-7-glucoside; coumarins, *viz*., scopoletin and umbelliferone; sugars; amino acids; and carbohydrates [4].

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Camphene, β-eudesmol, eseroline, and calarene are the major components of *D. bipinnata* oil. Diphenyliodinium bromide, limenone, 2-cyclohexene-1-one, and 8-nitro-12-tridecanolide are minor constituents present in *D. bipinnata* oil [5]. Leaf paste of D. bipinnata is used to cure cuts and wounds [6]. Roots of *D. bipinnata* are used in the treatment of asthma, rheumatism [7], carbuncles, piles, cholera, dysuria [2], diuretic, galactagogue, astringent [1], dysentery, leucorrhoea, and wounds [1]. Diarrhea is the frequent passing of loose, watery, and unformed feces. Loss of fluids in diarrhea can cause dehydration and electrolyte imbalance.

Herbal treatments for diarrhea in traditional medicinal practices utilize various plant extracts or

plants such as *Semicarpus anacardium* Linn. Anacardiaceae, *Achyranthus aspera* Linn. Amaranthaceae, *Rhus semialata* Murr. Anacardiaceae [8], *D. bipinnata* [7], *Elytraria acaulis* Lind. Acanthaceae, *etc*. [9, 10]

The antidiarrheal activity of *D. bipinnata* has been investigated with alcoholic and aqueous extracts of *D. bipinnata* using castor oil induced-diarrhea in wistar rats, and charcoal meal stimulated gastrointestinal transit in albino mice [11].

The present study deals with the examination and verification of traditional claims for the anti-diarrheal activity of *D. bipinnata*. Extracts of *D. bipinnata* were prepared, and their antidiarrheal activity was investigated by castor oil induced and magnesium sulfate-induced diarrhea models in Wistar rats.

## MATERIALS AND METHODS:

### Plant material:

**Collection and Authentication:** Whole plants of *D. bipinnata* were collected in September 2009 from Chirawa, district Jhunjhunu, Rajasthan, India. Dr. R. P. Pandey authenticated the collected plant from Botanical Survey of India, Jodhpur, India. A voucher specimen, JNU/PH/2010/Db D2 was deposited in the herbarium of Jodhpur National University, Jodhpur, India.

**Extraction:** Different underground plant parts, *viz*., roots, stem, *etc*., were dried in the shade for one month, ground in an electric mixer-grinder, and screened using BSS standard sieve no. 22 (average aperture size 710 μm). The powdered crude drug (10 g) was extracted in Soxhlet extractor with petroleum ether, ethyl acetate, chloroform, ethanol, ethanol (50%), and water, separately, to extract non-polar and polar compounds. The obtained extracts were filtered through Whatman filter paper, concentrated, and dried by evaporating the solvent on a water bath. The residual moisture in these extracts was removed by heating in an oven followed by storage of the powdered extracts in a desiccator.

**Animals:** The antidiarrheal studies were conducted on healthy female Wistar rats, weighing 150-200 g. Albino mice of either sex, weighing 25-30 g, were used in acute toxicity studies. Approval by Institutional Animal Ethical Committee (IAEC), vide registration number 1258/ac/09/CPCSEA, was obtained for the conduct of animal experiments. The experimental animals were kept in colony cages at standard husbandry conditions. All animals were provided free access to feed and water, *ad libitum*.

**Preliminary Acute Toxicity Test:** *D. bipinnata* extracts were administered orally in doses of 250, 500, 1000, 2000 and 5000 mg/kg body weight to albino mice (one dose per group; five animals in a group). Simultaneously, the control animals received normal saline (5 ml/kg). The general signs and symptoms of toxicity, intake of food and water, and mortality were recorded for a period of 48 h and then for 14 days as per OECD guideline 423 [12].

**Experimental Procedure for Diarrhea:** Healthy Wistar rats were marked and randomly distributed to 7 groups, each group consisting of 5 animals. These groups were given drug treatments as follows:

**Group I:** Normal control (1% CMC 10 ml/kg, body weight)
**Group II:** Standard drug (loperamide 3 mg/kg, body weight)
**Group III:** Diarrheal rats, treated with water extract of *D. bipinnate*
**Group IV:** Diarrheal rats, treated with a hydroalcoholic extract of *D. Bipinnata*
**Group V:** Diarrheal rats, treated with an alcoholic extract of *D. Bipinnata*
**Group VI:** Diarrheal rats, treated with chloroform extract of *D. Bipinnata*
**Group VII:** Diarrheal rats, treated with ethyl acetate extract of *D. Bipinnata*

All animals were initially screened for induction of diarrhea by administering 1 ml of castor oil. Animals, in which diarrhea was developed, were selected for antidiarrheal studies.

**Castor Oil-Induced Diarrhea in Rats:** Wistar rats weighing 150-200 g were selected and kept for overnight fasting. Loperamide and *D. bipinnata* extracts were administered orally by gavage at 3 and 500 mg/kg doses, respectively. After an hour, 1 ml of castor oil was administered orally to each animal for induction of diarrhea. Animals were placed in cages, where cage floor was lined with non-wetting paper sheets of uniform weight.

These non-wetting paper sheets were changed per hour for up to 6 h. Characteristic diarrheal droppings per hour, up to 6 h, were recorded after draining the urine by gravity. A numerical score based on stool consistency was also assigned. The normal stool was assigned as 1, semi-solid stool as 2, and watery stool as 3. Mean of diarrheal droppings in treated animal groups were compared to the control group [13-15].

**Magnesium Sulfate-Induced Diarrhea in Rats:** Wistar rats weighing 150-200 g were selected and kept for overnight fasting. Loperamide and *D. bipinnata* extracts were administered orally by gavage at 3 and 500 mg/kg doses, respectively. After 30 min, magnesium sulfate at dose 2 g/kg was administered orally to each animal. These animals were placed in cages, where cage floor was lined with nonwetting paper sheets of uniform weight. Non-wetting paper sheets were changed per hour for up to 6 h. Characteristic diarrheal droppings per hour up to 6 h was recorded after draining the urine by gravity. A numerical score based on stool consistency was assigned. The normal stool was assigned as 1, semi-solid stool as 2 and watery stool as 3. Mean of diarrheal droppings in treated animal groups were compared to the control group [14].

**Statistical Analysis:** The data obtained in antidiarrheal studies were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's '*t*' test using Graph Pad Prism Version 5.01 (Graph Pad Software Inc., U.S.A) to compare results of treated animal groups to control. P-value <0.01 was considered significant, and results were expressed as mean ± SD.

**RESULTS:** Acute toxicity studies for *D. bipinnata* extracts were performed with extracts prepared from underground plant parts. Extracts of *D. bipinnata* showed neither mortality nor any toxic effects up to the dose of 5 g/kg in 48 h to 14 days. Behavior, breathing, cutaneous effects were found normal. The obtained results indicated that in a single dose, there is no acute toxicity of *D. bipinnata* extracts. Therefore, these extracts are considered safe in acute toxicity studies, since general toxicity dose for rodents is limited upto 2 g/ kg/day for rodents and 1g/kg/day for non-rodents [16].

In castor oil-induced diarrhea model, the extracts of *D. bipinnata* showed an antidiarrheal effect in Wistar rats. Loperamide, being a standard antidiarrheal drug, was most effective in reducing the number of feces by 70.94%, while among studied extracts alcoholic extract was found most effective, which reduced the number of feces by 61.54%. The least potent antidiarrheal effect was observed in chloroform extract, which was able to reduce diarrhea by 26.50%. All the tested extracts significantly (P<0.01) reduced the total number of feces when compared to a control group using one way ANOVA followed by Dunnett's '*t*' test **Table 1.**

**TABLE 1: ANTIDIARRHEAL EFFECT OF *DESMOSTACHYA BIPINNATA* UNDERGROUND PARTS EXTRACTS ON CASTOR OIL-INDUCED DIARRHEA IN WISTAR RATS**

| Treatment | Doses (mg/kg, p.o.) | Mean of total number feces in 6 h | Feaces Reduction (%) |
|---|---|---|---|
| Water | 500 | 16.4 ± 1.14* | 29.91 |
| Hydroalcholic | 500 | 10.4 ± 1.14* | 55.56 |
| Alcohol | 500 | 9.0 ± 1.00* | 61.54 |
| Chloroform | 500 | 17.2 ± 1.48† | 26.5 |
| Ethyl Acetate | 500 | 14.2 ± 1.30* | 39.32 |
| Loperamide | 3 | 6.8 ± 0.84* | 70.94 |
| Control | 10‡ | 23.4 ± 2.07 | |

* Significant difference at P<0.01 *vs*. control and P<0.001 *vs*. control; one- way ANOVA followed by Dunnett's '*t*' test. The †Significant difference at P<0.01 *vs*. control; No significant difference at P<0.001 *vs*. control; one-way ANOVA followed by Dunnett's '*t*' test, ‡ In ml/kg

In magnesium sulfate-induced diarrhea model, the extracts of *D. bipinnata* showed antidiarrheal effect in Wistar rats **Table 2**. Alcoholic extract reduced 64.52% feces, which outperform slightly to loperamide effect with 71.77% feces reduction. The least antidiarrheal effect of 25.81% feces reduction was observed with chloroform extract. All extracts significantly (P<0.01) produced an antidiarrheal effect and reduced number of feces when compared to the control group, using one way ANOVA followed by Dunnett '*t*' test.

**TABLE 2: ANTIDIARRHEAL EFFECT OF *DESMOSTACHYA BIPINNATA* UNDERGROUND PARTS EXTRACTS ON MAGNESIUM SULPHATE-INDUCED DIARRHEA IN WISTAR RATS**

| Treatment | Dose (mg/kg, p.o.) | Mean of the total number of mean feces in 6 h | Feaces Reduction (%) |
|---|---|---|---|
| Water | 500 | 15.0±1.00* | 39.52 |
| Hydroalcholic | 500 | 9.0±1.00* | 63.71 |
| Alcohol | 500 | 8.8±1.10* | 64.52 |
| Chloroform | 500 | 18.4±1.14† | 25.81 |
| Ethyl Acetate | 500 | 14.0±1.58* | 43.55 |
| Loperamide | 3 | 7.0±0.71* | 71.77 |
| Control | 10‡ | 24.8±1.92 | |

* Significant difference at P<0.01 *vs*. control and P<0.001 *vs*. control; one- way ANOVA followed by Dunnett's '*t*' test. The †Significant difference at P<0.01 *vs*. control; No significant difference at P<0.001 *vs*. control; one-way ANOVA followed by Dunnett's '*t*' test, ‡ In ml/kg

In both, castor oil-induced diarrhea and magnesium sulfate-induced diarrhea models, the order of antidiarrheal effect was alcohol extract > hydroalcoholic extract > water extract > ethyl acetate extract > chloroform extract. The difference in activity of these extracts in reducing diarrhea is anticipated due to the differences in nature and quantity of phytoconstituents present in these extracts.

**DISCUSSION:** Castor oil hydrolysis produces ricinoleic acid, which induces diarrhea as the hypersecretory response due to changes in the transport of water and electrolytes [17, 18]. Ricinoleic acid causes irritation and inflammation of gastric mucosa resulting in the release of prostaglandins causing stimulation of secretion [19, 20]. Furthermore, ricinoleic acid also sensitizes intramural neurons of the gut. Several other mechanisms, which have been reported to explain the diarrheal effect of castor oil, include adenylate cyclase activation, cAMP-mediated active secretion [21], and inhibition of $Na^+$, $K^+$ ATPase activity [22].

Diarrhea in rats is also induced by administration of oral magnesium sulfate, which increases the accumulation of fluid in the intestinal lumen and enhances flow from the proximal to the distal intestine. This mechanism also involves the release of NO, probably through stimulation of the constitutive form of NO synthase [23]. Magnesium sulfate has also been reported to liberate cholecystokinin from duodenal mucosa increasing small intestine secretions and motility and thus preventing the reabsorption of water and sodium chloride [24, 25]. Alcoholic extract successfully inhibited a total number of feces in castor oil-induced diarrhea model. The antidiarrheal effect is anticipated due to a reduction in secretion by the phytoconstituents, *viz.* alkaloids, flavonoids, glycosides, and steroids, present in this extract [26]. Flavonoids and alkaloids have been reported to inhibit prostaglandins and autacoids release, resulting in a reduction of motility and secretion [27, 28]. Flavonoids also inhibit contraction caused by spasmogens and intestinal secretion, resulting in reduction of intestinal transit. Steroids enhance the intestinal absorption of $Na^+$ and water [28].

Alkaloids, flavonoids, and steroids, in combination, exists in various plant extracts which possess antidiarrheal activity, *viz.*, root extract of *Asparagus racemosus* (Liliaceae), leaf extract of *Clerodendrum phlomidis* (Verbenaceae), stem bark extract of *Cylicodiscus gabunensis* (Mimosaceae), leaf extract of *Emilia coccinea* (Asteraceae), and fruit extract of *Momordica cymbalaria* (Cucurbitaceae) [28]. However, it was also observed that root extracts of *Guiera senegalensis* (Combretaceae) containing an alkaloid, flavonoid and glycosides are effective in controlling diarrhea [28]. Stem bark extracts of *Butea monosperma* (Fabaceae) contains glycosides, flavonoids, and steroids, which are effective in curing diarrhea [28].

It was also observed that plants containing alkaloids, flavonoids, glycosides, and steroids, *viz.*, stem bark extract of *Annona senegalensis* (Annonaceae), *a* root extract of *Combretum sericeum* (Combretacae), and leaf extract of *Dalbergia sisso*o (Fabaceae), are also effective in diarrheal conditions [28].

Therefore, it is proposed that alkaloids, flavonoids, glycosides, and steroids present in extracts of *D. bipinnata* are responsible for the antidiarrheal action. The mechanism seems to be involved may be associated with multiple effects, *viz.*, inhibition of prostaglandins and autacoids release, inhibition of contraction caused by spasmogens, and by

increasing water and electrolyte absorption from the intestine. The extracts of *D. bipinnata* reduced diarrhea by reducing gastrointestinal motility or by increasing reabsorption of electrolytes and water just as loperamide.

**CONCLUSION:** Various extracts of *D. bipinnata* were found effective in reducing diarrhea in Wistar rats. The obtained results indicated that qualitative and quantitative difference of phytoconstituents, among these extracts, may be responsible for the difference in antidiarrheal potency.

Furthermore, studies may be directed to investigate phytoconstituents responsible for the antidiarrheal activity of these extracts. It may be concluded that *D. bipinnata* contain potent pharmacologically active substances that have antidiarrheal potential.

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