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Preliminary evaluation of different components of *Bacopa monnieri* for laxative effect

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Abstract

Bacopa monnieri (*B. monnieri*) has been used for various therapeutic purposes in traditional medicine including treatment of constipation and stomach disorders. The aim of the present study was to investigate and identify the fractions of *B. monnieri* extract with maximal laxative effect. Three different types of Bacopa fractions and one extract were prepared: Fraction 1 (non-polar fraction), Fraction 2 (approx. 60-70% bacosides containing fraction), Fraction 3 (polar fraction) and Extract 4 (40% bacosides containing extract). In Study 1, rats and mice were given p.o. different doses of the four different test substances (40, 120 and 500 mg/kg b. wt.). Vehicle, loperamide (5 mg/kg b. wt.) and castor oil (2 ml/rat and 0.4 ml/mouse) were used for comparison. The stools were observed and graded. Animals given vehicle were observed to have normal stools, while all animals showed diarrhea with castor oil and loperamide caused constipation. Significant diarrhea ($p < 0.05$) was observed in rats administered with Fraction 1 and 2 (100%, and 71% respectively) at a dose of 500 mg/kg. Diarrhea was observed in one mouse administered Fraction 1 at 500 mg/kg. In Study 2, when the four different test substances were given in combination with loperamide, Fraction 1 (500 mg/kg) resulted in non significant diarrhea in 29% of rats. Studies on the rate of small intestine propulsion in mice also indicate that Fraction 1 showed the maximal distance traversed by carbon black ($p < 0.05$). It could be noted here that Fraction 1 (non-polar fraction) exhibited the maximum diarrhea while Fraction 3 (polar fraction) exhibited no diarrhea.

Key words: Bacopa monnieri, laxative effect, polar fraction, non polar fraction, bacosides, rats

1. Introduction

The drug Brahmi is derived from the dried whole plant of *Bacopa monnieri* L. (Scrophulariaceae). However Centella asiatica L. (Apiaceae) and few other species are also used and sold as Brahmi in North India [1]. *B. monnieri* has been extensively used for various therapeutic purposes in Ayurvedic system of medicine in India. It has found use in treatment of epilepsy, insanity and neurasthenia [2], abdominal pain [3] liver troubles [4] and rheumatism [5]. Studies show that it is useful in treating memory loss [6]. It is also found to have laxative effect and the whole plant is used in treatment of constipation [7] and stomach disorders [8]. The

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herb contains alkaloids Brahmine, Herpestine, ($C_{34}H_{46}N_2O_6$, mp 116-117°C), saponins, monnierin ($C_{51}H_{82}O_{21}.3H_2O$ mp 262-263ºC), hersaponin (mp 232-234ºC) Bacoside A ($C_{41}H_{68}O_{13}$ $5H_2O$ mp 250°C) and Bacoside B ($C_{41}H_{68}O_{13}.5H_2O$ mp 203°C) [9, 10]. The aim of the present study was to understand the laxative constituents of *B. monnieri.*

2. Materials and methods

2.1 Experimental animals

Adult, male Wistar albino rats weighing 225 to 350 gm and adult male Swiss albino mice weighing 15 to 40 gm were used for the experiments. They were housed 3 per cage and maintained at a temperature of 28+/-5°C under standard conditions. The animals were maintained at 12 h light/dark cycle. Food (Hindustan Lever chow pellets) and water were provided *ad libitum*. Twelve hours before initiation of experiments, food was withheld but the animals were provided with water. The Institutional Animal Ethics Committee approved the protocol for all experiments, which were conducted in humane conditions.

2.2 Drugs

Whole plants of *Bacopa monnieri* Linn. (Syn: *Herpestis monniera*; Family: Scrophulariaceae) were collected in the fresh condition and taxonomically identified. Voucher specimens were deposited at the Department of Phytochemistry, Natural Remedies Pvt. Ltd. Voucher specimens were also sent to the NISCAIR, New Delhi for authentication. Loperamide was from Torrent, India.

Preparation of Bacopa Fractions and Extract

Three different types of Bacopa fractions and one extract were provided by Natural Remedies Pvt. Ltd., Bangalore India as fine powder, greenish to brownish in colour: Fraction 1 (non-polar fraction), Fraction 2 (approx. 60-70% bacosides containing fractions), Fraction 3 (polar fraction) and Extract 4 (40% bacosides containing extract). All the four were suspended in 1% Carboxy Methyl Cellulose (CMC).

2.3 Experimental Design

2.3.1 Study 1: Drug administration and Observation of stool consistency [11]

Different groups of rats (n=7 in each group) were administered *p.o.* with one of the following:

1) Vehicle 2) Fraction 1 (40, 120, or 500 mg/kg b.wt.), 3) Fraction 2 (40, 120 or 500 mg/kg b.wt.), 4) Fraction 3 (40, 120 or 500 mg/kg b.wt.), 5) Extract 4 (40, 120 or 500 mg/kg b.wt.), 6) Loperamide (5 mg/kg b.wt.) and 7) Castor oil (2 ml/animal). The rats were put in individual cages for observation of stool consistency at 1 h, 3 h, 6 h and 24 h after test substance administration that was scored as: Grade 0 – no stools, 1- normal, 2- soft and 3- unformed.

The experiment was repeated with different groups of mice (n=7 in each group) but the dose of castor oil was 0.4 ml/animal. The mice were put in individual cages for observation of stool consistency at 15 min, 30 min, 1 h and 2 h after test substance administration and scored as described above.

2.3.2 Study 2: Drug-induced constipation [11]

In rats, after the administration of loperamide (5 mg/kg, p.o) all feces expelled within 24 h was observed for stool consistency and scored as above. Test substances (Fraction 1, Fraction 2, Fraction 3, and Extract 4 at 40 and 500 mg/kg) or vehicle were administered orally 30 min before loperamide.

In mice, the experiment was carried out at only 500 mg/kg of the test drug.

2.3.3 Study 3: Effect on small intestinal propulsion [12, 13].

This experiment was performed in mice only. Seven groups of mice (n=9) were given *p.o.* vehicle, one of the Bacopa fractions and extract (500 mg/kg), loperamide (5 mg/kg) or castor oil (0.4 ml/animal). After 20 min, 10% carbon black suspended in 1% CMC (10 ml/kg) administered *p.o.* to the mice. All mice were sacrificed 20 minutes after administration of carbon black, and the entire small intestine was removed. The rate of small intestine propulsion was calculated by dividing the distance of the carbon black migration by the total length of the small intestine.

2.4 Statistics

In Study 1 and 2, the number of animals showing different scores of stool consistency was calculated for each group. The Chi square test was used to calculate the significance. In Study 3, the mean and standard deviation were calculated for all the groups. One way ANOVA was used to calculate the significant differences between the groups followed by the post-hoc Tukey's test. Significance level was set at 0.05.

Table 1. Rate of small intestine propulsion in mice after administration of various drugs (n=9)

Group	Rate of small intestine propulsion (%)
Vehicle control	48.47 ± 7.83
Castor oil	88.33 ± 10.90*
Loperamide	27.60 ± 10.73*
Fraction 1	81.87 ± 15.07*
Fraction 2	46.50 ± 18.95
Fraction 3	60.53 ± 15.55
Extract 4	42.96 ± 15.49

* Significantly different than control ($p < 0.05$)

3. Results

3.1 Observation on rats

3.1.1 Study 1

Control rats were observed to have normal stool consistency up to 24 h after administration, while all rats showed diarrhea with castor oil (2 ml/animal). Loperamide caused constipation up to 24 h after drug administration (Fig. 1).

Fraction 1 at 500 mg/kg dose caused diarrhea in all rats ($p < 0.05$) but at 120 mg/kg and 40 mg/kg no diarrhea was observed (Fig. 2).

With fraction 2, at 500 mg/kg, 71% rats showed diarrhea at 24 h ($p < 0.05$), while at 40 mg/kg none showed diarrhea (Fig.3).

With fraction 3, no significant diarrhea was observed in any of the doses (Fig. 4).

With extract 4, no diarrhea at 40 mg/kg or 120 mg/kg (Fig. 5). At 500 mg /kg though diarrhea was observed it was not statistically significant.

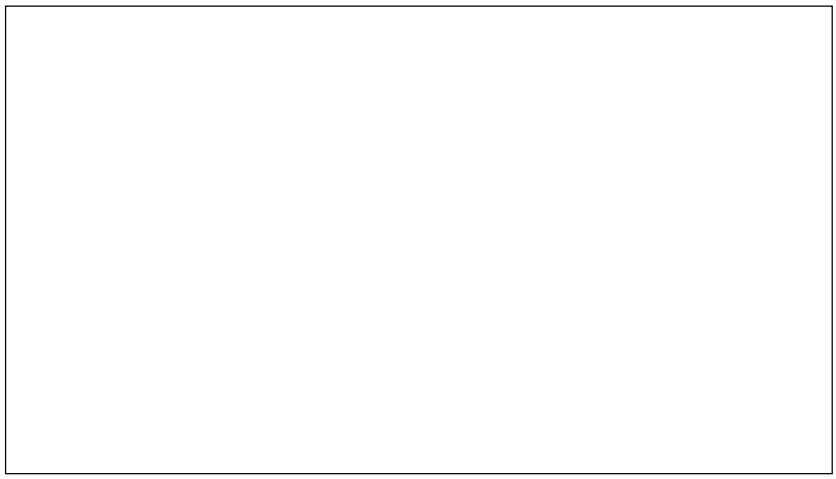
3.1.2 Study 2

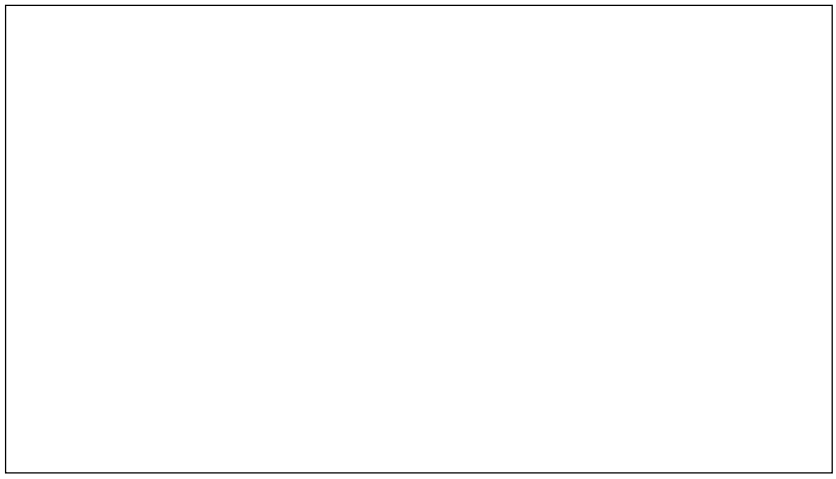
When the three different fractions and one extract were administered in combination with loperamide, stools were observed in all the groups of rats. When administered at 40 mg/kg, 14% of rats showed Grade 2 stools with fraction 2 and fraction 3 (Fig 6). At 500 mg/kg 29% of rats showed diarrhea with fraction 1 and 14% Showed Grade 2 stools with fraction 2 (Fig. 7).

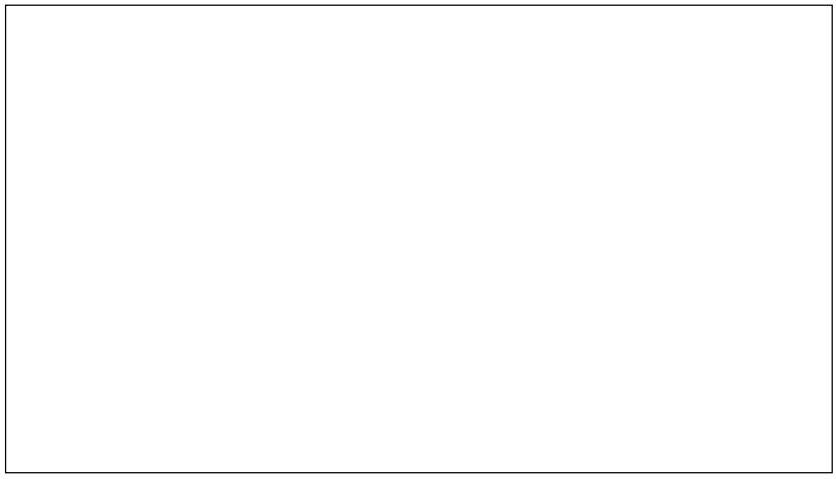
3.2 Observation in mice

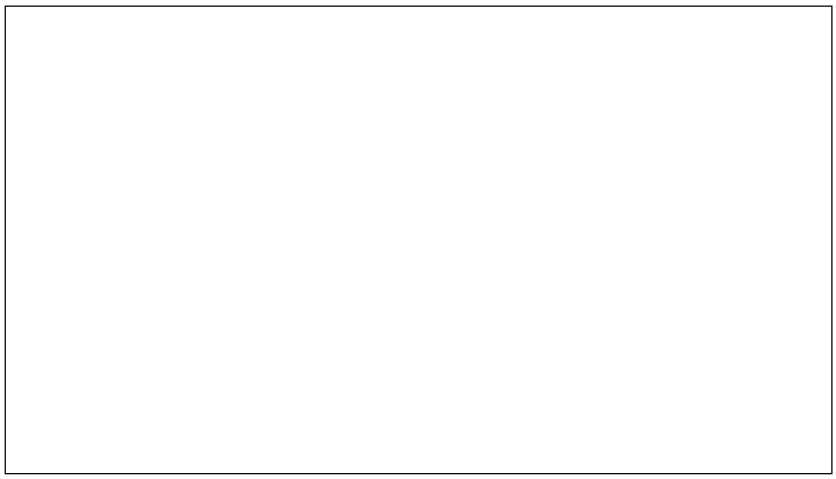
3.2.1. Study 1

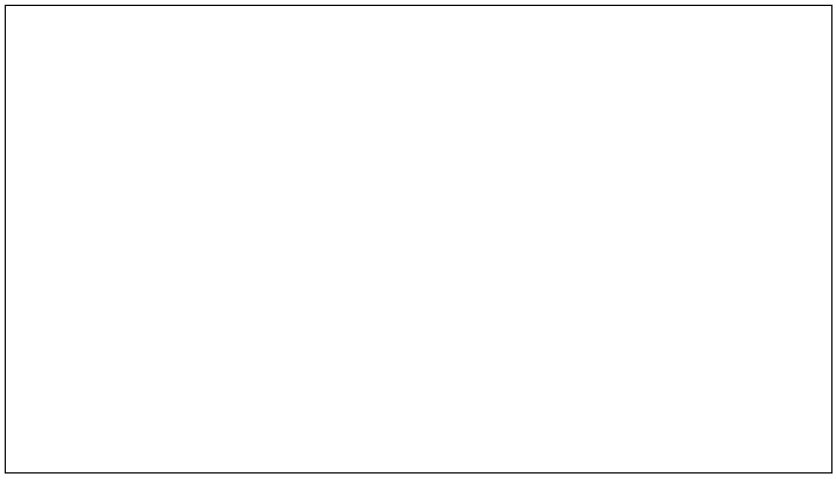
In mice no diarrhea was observed with administration of vehicle and loperamide. 86% had diarrhea after administration of castor oil ($p < 0.05$) (Fig. 8). There was no diarrhea observed with any of the fractions or extract in any dose, except one mouse had diarrhea with fraction 1 at 120 mg/kg and one mouse with extract 4 at 500 mg/kg (Fig. 9, 10, 11 & 12).

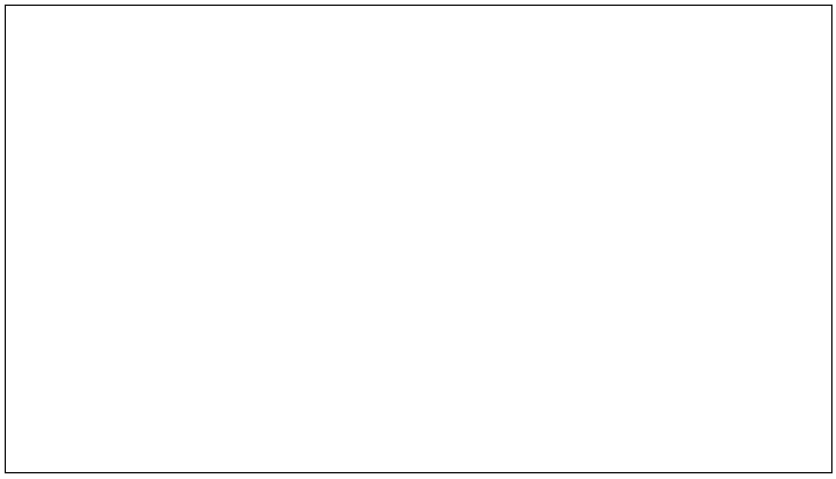


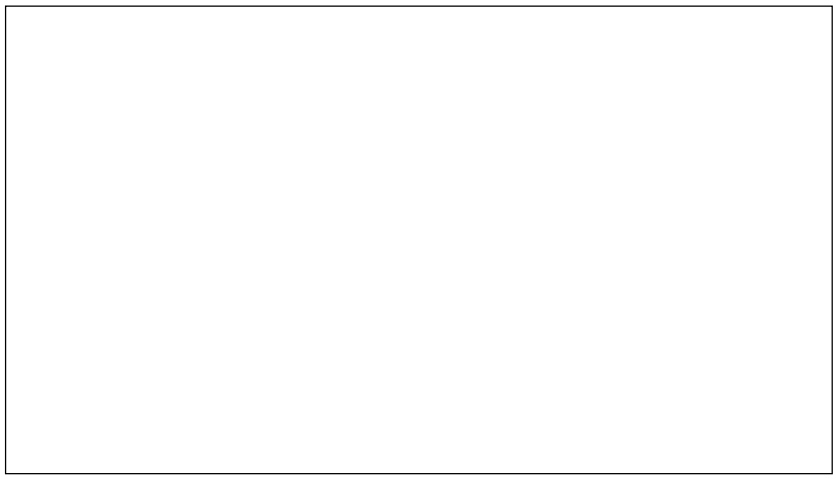


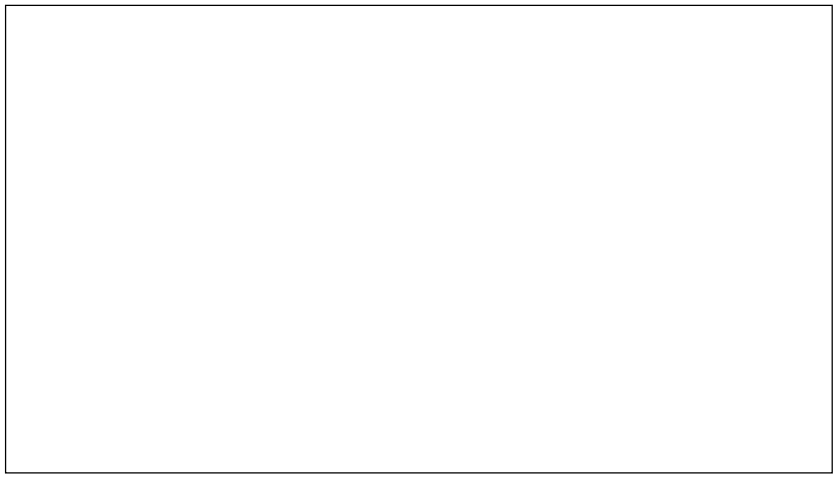


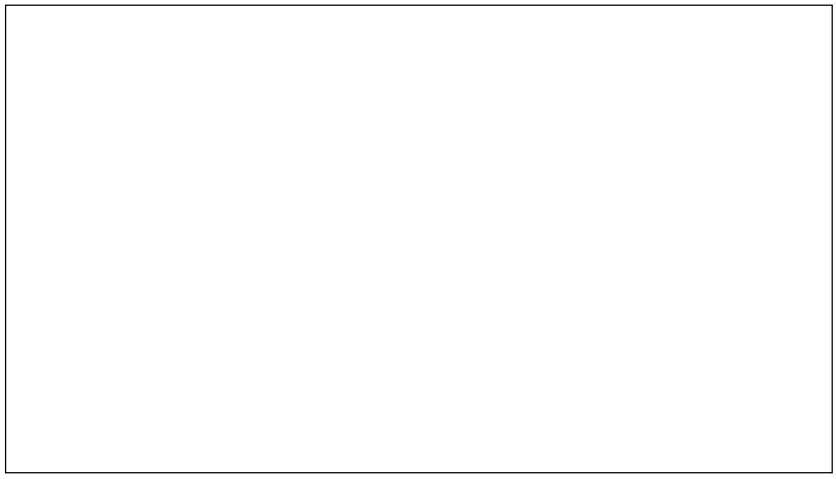


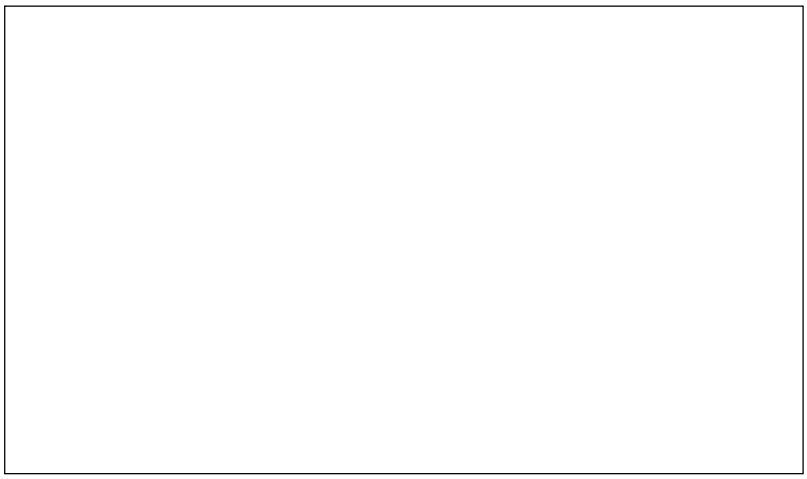


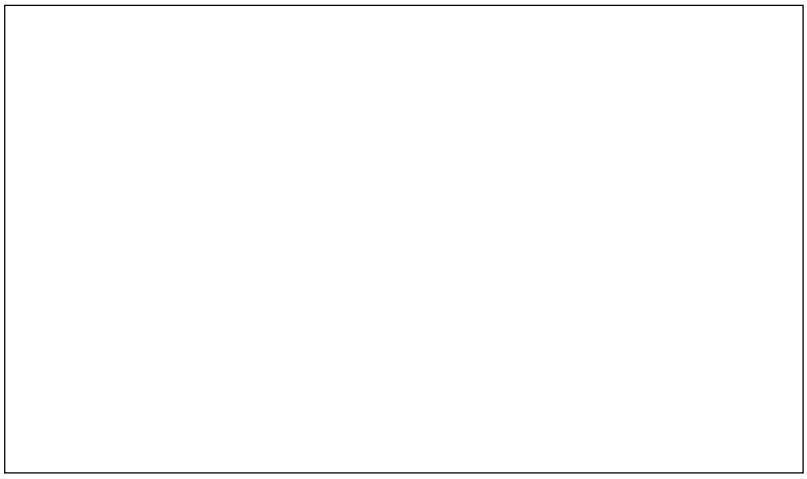


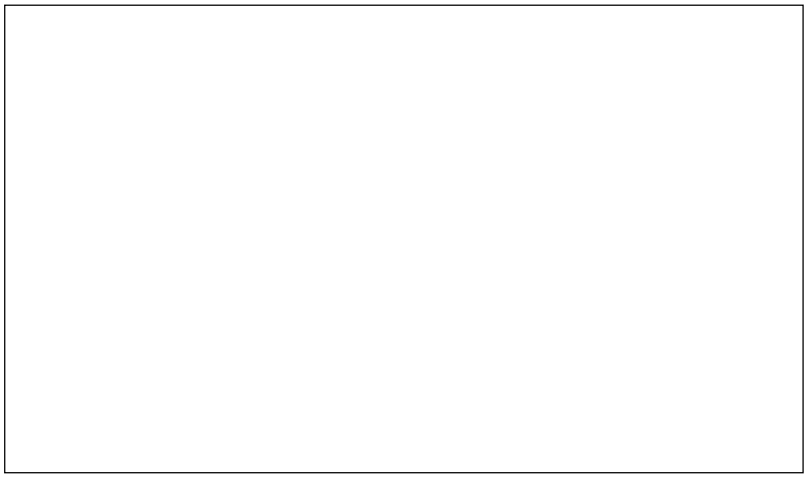


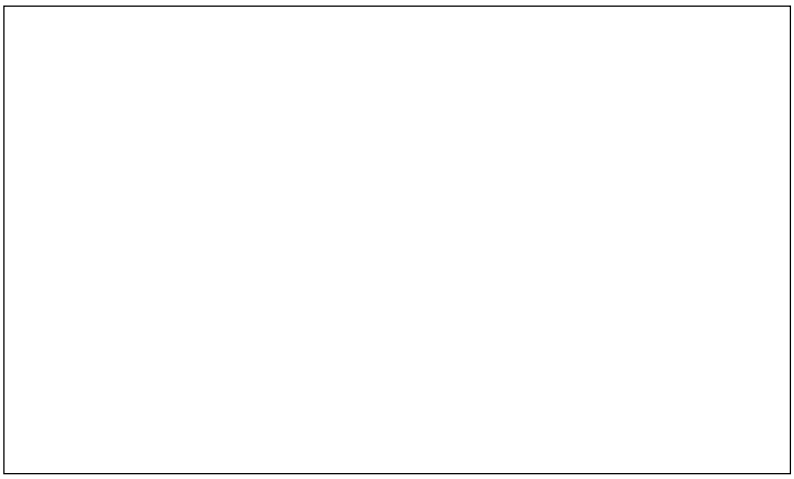












3.2.2. Study 2

When the three different fractions and one extract were administered in combination with loperamide, no Grade 2 stools or diarrhea were observed in any of the groups (Fig. 13).

3.3 Study 3

The rate of small intestine propulsion (distance of the carbon black migration divided by the total length of the small intestine x100) in different groups of mice (n=7) 20 min after administration of drug is shown in Table 1.

The rate of small intestine propulsion with fraction 1 and castor oil group was significantly greater than control ($p < 0.05$) and it was significantly lower in the loperamide group ($p < 0.05$).

4. Discussion

B. monnieri has long been used to enhance cognition. The use of *B. monnieri* as a laxative for treatment of constipation was also practiced since long, in ayurvedic system of medicine [7]. In this study, we attempted to understand the laxative components of *B. monnieri*. Significant diarrhea was observed ($p < 0.05$) in rats administered Fraction 1 and 2 (100%, and 71% respectively) at a dose of 500 mg/kg. At lower doses diarrhea was not observed. Diarrhea was observed only in one mouse administered Fraction 1 at 500 mg/kg.

Studies on drug-induced constipation showed that loperamide induced constipation in 100% of rats. When the four different test drugs were administered in combination with loperamide, stools were observed in all the groups of rats and administration of Fraction 1 (500 mg/kg) resulted in 29% of rats exhibiting diarrhea. These results also suggest a laxative effect for *B. monnieri* with maximal activity observed in the fraction 1.

Studies on the rate of small intestine propulsion also indicate the Fraction 1 showed the maximal distance traversed by carbon black ($p < 0.05$)

It is of interest to note that administration of Fraction 1 (non-polar fraction) exhibited the maximum diarrhea while Fraction 3 (polar fraction) exhibited no diarrhea. Fractions 2 (approx. 60-70% Bacoside containing fractions) and Extract 4 (40% bacoside containing extract)

did not show diarrhea up to 120 mg/kg. Evidence shows that the effects of *B. monnieri* on cholinergic system include modulation of acetylcholine release [14] choline acetylase activity and muscarinic cholinergic receptor binding [14]. Although the exact mechanism of action is not known, there is evidence that the laxative effect could be attributed to its cholinergic properties. The findings of the present study reveal that further studies need to be taken up to isolate the laxative components of *B. monnieri*.

References

1. Shah CS, Sukkawala VM, Chavan AR. (1962) *J. Sci. Ind Res*. 21C:22

2. Prem Kishore, Padhi MM. (1983) *Common Healing Herbs*. Central Council for research in Ayurveda and Siddha, Department of ISM&H, Ministry of Family welfare & Health (Govt. of India), New Delhi; 11.

3. Joshi P. (1982) *J. Econ. Tax. Bot.* 3: 257-266.

4. Bhatt RP, Sabnis SD. (1987) *J. Econ. Tax. Bot*. 9:139-145.

5. Bedi SJ. (1978) *Econ. Bot*. 32:221-223.

6. Stough C, Lloyd J, Clarke J, Downey LA, Hutchison CW, Rodgers T, Nathan PJ. (2001) *Psychopharmacol*. 156:481-484.

7. Uniyal MR. (1968) Indian Forester 94:407-420.

8. Shah GL, Menon AR, Gopal GV. (1981) *J. Econ. Tax. Bot*. 2: 173-182.

9. Sastry et al., (1959) *Ind. J. Phramac*., Vol 21, 303.

10. Kulshreshtha & Rastogi. (1973) *Phytochemistry* 12:2074.

11. Saito T, Mizutani F, Iwanaga Y, Morikawa K, Kato H. (2002) *Jpn. J. Pharmacology*, 89:133-141.

12. Satoh K, Kase Y, Yuzurihara M, Mizoguchi K, Kurauchi K, Ishige A. (2003) *J. of Ethnopharmacol*. 86:37-44.

13. Kase Y, Hayakawa T, Takeda S, Ishige A, Aburada M, Okada M. (1996) *Biol.l Pharmaceut. Bull*. 19:1367-1370.

14. Bhattacharya SK, Kumar A, Ghosal S. (2000) In: Siva Sanka DV (Ed) *Molecular aspects of Asian Medicines*. PJD publication: New York.

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