ANTI GIT MOTILITY, TOXICOLOGICAL AND PHYTOCHEMICAL STUDIES ON *BACOPA MONNIERI*

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Summary

A hydroethanolic extract (HE-ext) of *Bacopa monnieri* (BM) was tested for anti GIT motility effect and toxicological studies in rats and mice respectively. Gross phytochemical investigation was also carried out on the extract. The extract exhibited highly significant anti GIT motility effect ($P<0.001$), antagonized by Alpha2 receptor antagonist, yohimbine and a non selective GABA receptor antagonist, picrotoxine but unaffected by opioid receptor antagonist, naloxone. The Gross Phytochemical studies showed that *Bacopa monnieri* possesses saponins, tannins, flavonoids, and triterpenoids. The LD_{50}, ED_{50} and therapeutic index of hydroethanolic extract of *Bacopa monnieri* was calculated to be 67.778, 67.22 and 232, 232 and 3.42, 3.45 in writhing hot plate tests respectively.
These results indicate that *Bacopa monnieri* possesses anti GIT motility effect due to the presence of saponins, tannins, flavonoids and triterpenoids and that may involve the interaction of plant constituents with Alpha 2 and GABA receptors.

Key words: *Bacopa monnieri,* Anti GIT motility effect, Toxicological studies; Gross phytochemical investigation

Introduction

Bacopa monnieri (family: Scrophulariaceae[1]) also known as *Bacopa monniera*, water hyssop, *Herpestis monnieri* is a perenial creeping, succulent herb found in marshy areas of Indo-Pak subcontinent[2]. In India, It is commonly known as "Brahmi" is an ancient and renowned medicinal plant with legendary reputation as a memory vitalizer[3].

Bacopa monnieri is held in high repute to be the brain booster and is highly valued in conditions affecting CNS. In ancient traditional system of medicine, it is often prescribed for epilesy, insomnia, and psychiatric disorders such as mental breakdown in alzheimer's disease[4], neuralgia, and memory[5]. It is known to possess cardiotonic, sedative, analgesic, anticonvulsant, antiinflammotry[6], antioxidant[7], anticancer, antipyretic, laxative, diuretic, antistress[8], and anxiolytic[9] properties. In this study we have examined *Bacopa monnieri for* anti GIT motility effect and toxicological studies in animal models and carried out its gross phytochemical studies.

Materials and Methods

Bacopa monnieri

Bacopa monnieri was collected from Ramli stream near Quaid-e-Azam University Islamabad, Pakistan and authenticated by Dr. Muhammad Ibrar, Professor of Botany University of Peshawar. A reference specimen was submitted to the herbarium of the Botany Department, University of Peshawar and a voucher specimen (029006/Bot. University of Peshawar) was obtained.

Preparation of *Bacopa monnieri extract*

Aerial parts were separated from roots, dried under shade, coarsely grinded. The coarsely ground material was extracted with 70% ethanol and was concentrated on rotary evaporator at 60 ^{0}C, and then to semisolid form (% yield: 37.25).

Chemicals and Drugs

Ethanol was obtained from Khazana Sugar Mills Mardan through proper channel. Morphine was secured through proper channel (PDH Lahore through proper channel, Pakistan). . Opioid antagonist, naloxone and picrotoxine were purchased from Sigma, USA. For experiments, all drugs and extracts were dissolved in water for injection.

Animals

Balb-C mice and Sprague dawley rats bred in the animal house of the Department of Pharmacy, University of Peshawar, were used in this study. Animals were housed in groups of eight in cages with sawdust bedding. Experiments were carried out during the light phase between 9.00 am and 3.00 pm strictly in accordance with procedures laid down under the Animal Scientific Procedure Act (1986). GIT motility was tested in rats of either sex weighing 130-150 grams while antinociceptive and toxicity studies were carried out on mice of either sex weighing 18-22 g and 20-40 g respectively. Control animals received equal volume of normal saline (0.9% NaCl). Animals were marked for their proper identification.

GIT motility

Adult Sprague dawley rats of either sex (n=6) weighing 140-180 g were used in GIT motility study. Animals were starved from food for 18 hours prior to experiment, but were allowed free access to water. 25 minutes after BM HE-ext or morphine (MP) or normal saline (SAL) administration, one mL of a 10% charcoal suspension in 5% powdered gum acacia was administered to each rat orally[10]. For antagonism, naloxone (1 mg/kg) or yohimbine (3 mg/kg) or picrotoxine (0.25 mg/kg) was administered subcutaneously (s.c.) 5 minutes before drug administration. Animals were killed 15 min after being administered with charcoal meal, abdomen was opened, and small intestine was dissected out, and was placed on a clean surface. The distance travelled by the charcoal meal from the pylorus was measured. The entire length of the small intestine was also measured. The percentage distance travelled by the charcoal plug along the small intestine (from the pylorus to the ceacum) was then estimated for morphine, *Baccopa monnieri* extract and normal saline-treatment groups. Percent GIT motility was calculated with the help of following formula:

% GIT motility = (Distance travelled by charcoal through small intestine/total length of small intestine) x 100

Acute toxicity test

The test was carried out to determine the lethal and non lethal doses of the extract. Eight groups of animals, containing eight animals in a group, were used for HE ext. Animals were administered the extract at doses (31.25, 62.5, 125, 250, 500, 1000, 2000 mg/Kg; i.p.). The control animals received an equal volume of saline. The mortality rate was measured 24 hours post drug administration[11].

Acetic acid induced writhing test

Balb-C mice of either sex (n=8) weighing 18-22 g were used. Food and water were withdrawn from animals 2 hours before the start of experiment. Writhing behavior was tested, in which 1% acetic acid (AA) was administered i.p. and the number of abdominal constrictions occurring over the period of 20 minutes were counted just after 1% AA (10 mL/kg) administration[12,13]. The hydroethanolic extract of *Bacopa monnieri* or 0.9% sodium chloride were administered intraperitoneally (i.p.) 30 minutes before 1% AA administration. Percent protection against pain was calculated with the help of following formula:

% Protection = (1 - Mean no. of abdominal constrictions of treated drug/Mean number of abdominal constrictions of control) × 100

Hot plate test

Balb-C mice of either sex (n=8) weighing 18-22 g were acclimatized to laboratory conditions one hour before the start of experiment with food and water available *ad libitum*. Animals were then subjected to pre-testing on hot plate (Havard apparatus) maintained at 54 ± 0.1^0C. Animals having latency time greater than 15 seconds on hot plate during pre-testing were rejected (latency time: Time for which mouse remains on the hot plate (54 ± 0.1^0C) without licking or flicking of hind limb or jumping)[14,15]. After 30 minutes of pre-testing, animals were administered with hydroethanolic extract of *Bacopa monnieri* or saline i.p. Animals then were tested for latency on hot plate maintained at 54 ± 0.1^0C at 30 minutes after drug administration.

Percent analgesia was calculated with the help of following formula:

% Analgesia = (Test latency – control latency)/(Cut – off time – control latency) x 100

Gross phytochemical analysis

Preliminary phytochemical analysis of *Bacopa monnieri* was performed for alkaloids[16-19], Saponins[18-22], flavonoids[18,19], tannins[18,19,23,24], triterpenoids[16] and glycosides[17,22,23].

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) with post hoc tests for multiple comparisons and student t test. Effects were considered significant at $p < 0.05$.

Results

Effect of morphine and hydroethanolic extract of *Bacopa monnieri* on GIT motility in rats

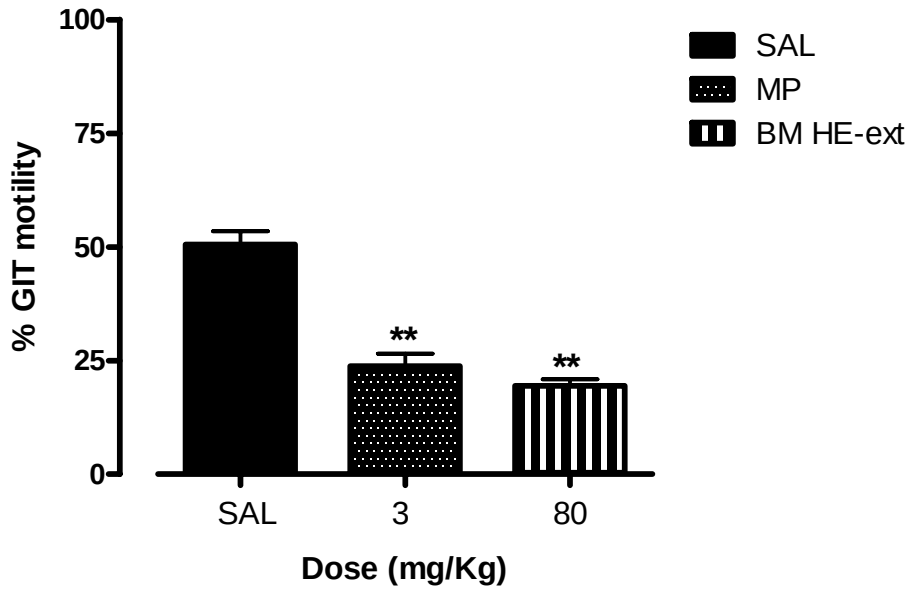

Figure 1. Effect of morphine and hydroethanolic extract of *Bacopa monnieri (*BM HE-ext) on GIT motility in rats. Each column represents percent GIT motility mean ± sem (n=6). ******P<0.001 values significantly different as compared to control (ANOVA with post hoc analysis).

Effect of naloxone (NLX), Yohimbine (YOH) and Picrotoxine (PIC) pre treatment on anti GIT motility effect of morphine, hydroethanolic extract of *Bacopa monnieri* in rats

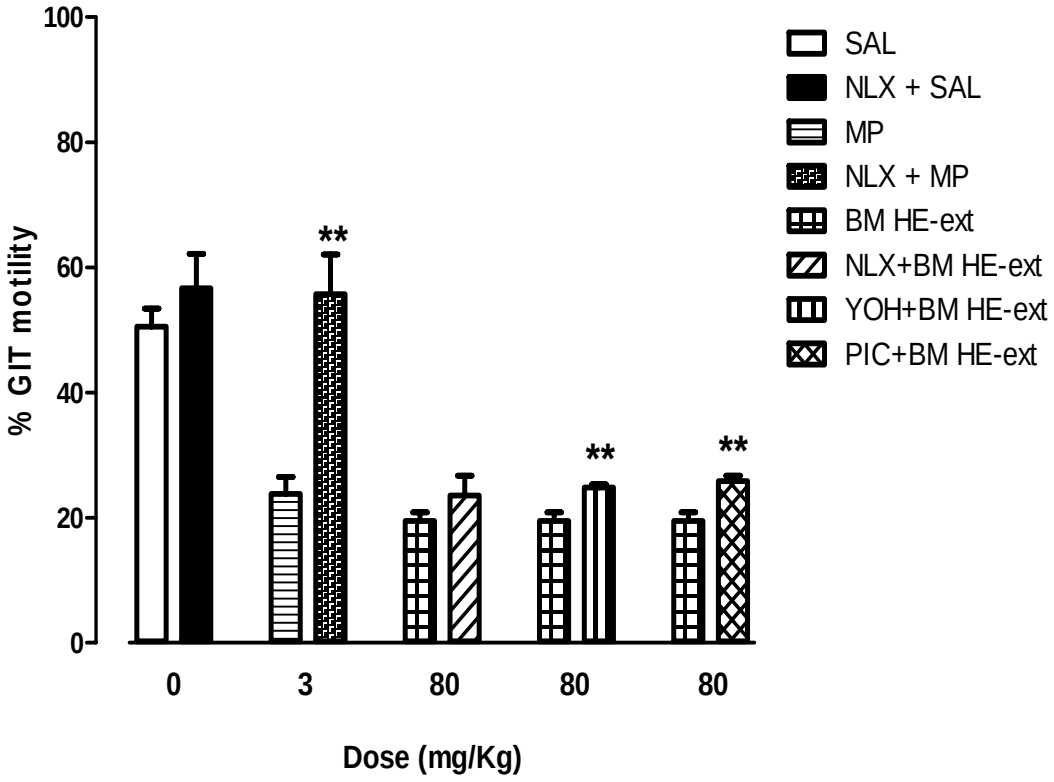

Figure 2. Effect of naloxone, yohimbine and picrotoxine on anti GIT motility effect of hydroethanolic extract of *Bacopa monnieri* in rats. Each column represents mean ± sem (n=6).). **P, value significantly different as compared to control (Student t test).

Determination of median lethal dose (LD50) of hydroethanolic extract of *Bacopa monnieri* in mice

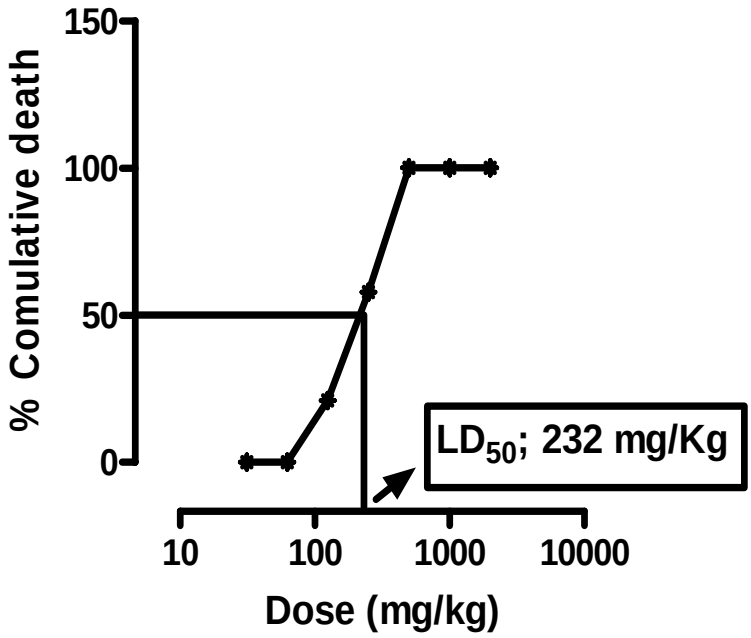

Figure 3. Median lethal dose of hydroethanolic extract of *Bacopa monnieri* in mice.

Determination of median effective dose (ED50) of hydroethanolic extract of *Bacopa monnieri* for antinociceptive effect in acetic acid induced writhing test in mice

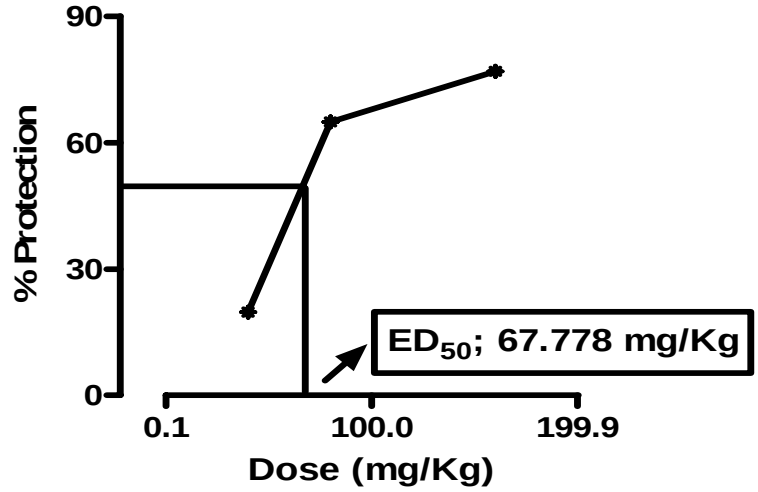

Figure 4. Determination of ED_{50} of hydroethanolic extract of *Bacopa monnieri* for antinociceptive effect in acetic acid induced writhing test in mice.

Determination of median effective dose (ED50) of hydroethanolic extract of *Bacopa monnieri* for antinociceptive effect in hot plate test in mice

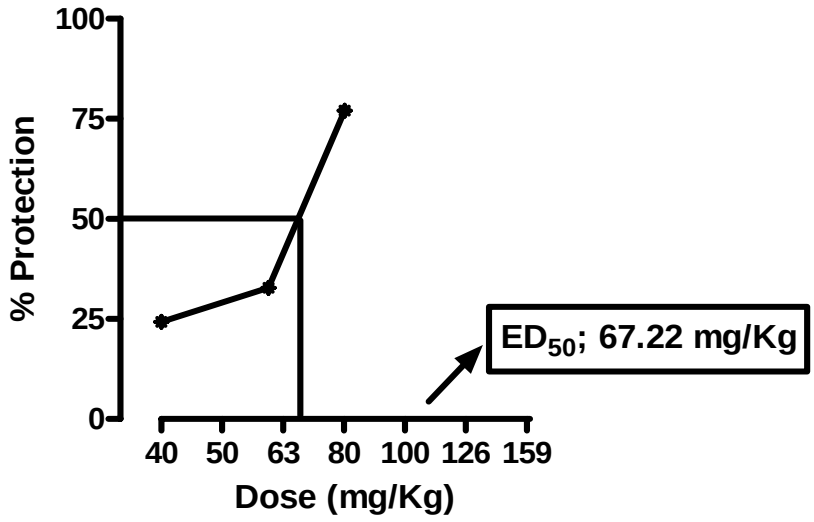

Figure 5. Determination of ED_{50} of hydroethanolic extract of *Bacopa monnieri* for antinociceptive effect in hot plate test in mice.

Calculation of therapeutic index of hydroethanolic extract of *Bacopa monnieri*
Table.1

Treatment group	Antinociceptive test	Median effective dose	Median lethal dose	Therapeutic index (LD_{50}/ED_{50})
HE-ext	Writhing test	67.778	232	3.42
HE-ext	Hot plate test	67.22	232	3.45

Phytochemical screening of hydroethanolic extract of *Bacopa monnieri* for alkaloids
Tab.2

S. No	Test	Observation	Result
1	0.5 g Semisolid HE-ext + 5 mL 1% HCl → Filtration → 1 mL filtrate + dragendorff's reagent	No ppt or turbidity	-
2	0.3 g semisolid HE-ext + 2M HCl + Amyl alcohol → examine the alcoholic layer	No pink color	-
3	0.1 g Semisolid HE-ext + 10% HCl → filtration → 1 mL filtrate + dragendorff's reagent	No ppt or turbidity	-

Phytochemical screening of hydroethanolic extract of *Bacopa monnieri* for saponins
Tab. 3

S. No	Test	Observation	Result
1	300 mg HE-ext + 5 mL Distilled water → boil for 2 minutes and cool→ vigorous shaking	2.5cm (froth length)	+
2	100 mg HE-ext + 2 mL Distilled water → boil for 2 minutes and cool → vigorous shaking	3 cm (froth length)	+
3	0.2 mg HE-ext + 5 mL normal saline → filtration → 1 mL filtrate + 4 mL of blood → centrifuge for 5 minutes	Severe hemolysis	+

Phytochemical screening of hydroethanolic extract of *Bacopa monnieri* for flavonoids

Tab. 4

S. No	Test	Observation	Result
1	100mg HE-ext + 5 mL ethanol → Filter → 1 mL fitrate + 0.5N KOH	Yellowish color	+
2	100 mg HE-ext + 5 ml ethyl aetate → filter → 1 ml filtrate + dilute ammonia solution	Upper alkaline layer turned light green	+

Phytochemical screening of hydroethanolic extract of *Bacopa monnieri* for tannins

Tab.5

S. No	Test	Observation	Result
1	Aqueous aliquot of HE-ext + Ferric chloride reagent	Green black color	+
2	0.1 g of HE-ext. + 10 mL distilled water→Filtered→1 mL filtrate + ferric chloride reagent	Green black color	+
3	0.36 g HE-ext + 10 mL hot 0.9% NaCl solution→Filtered→3 mL filtrate + ferric chloride reagent	Green black color	+

Phytochemical screening of hydroethanolic extract of *Bacopa monnieri* for triterpenoids

Tab.6

S. No	Test	Observation	Result
1	300 mg HE-ext + 5 mL chloroform → warmed for 30 minutes → sulphuric acid was added	Dark red color in lower layer	+

Phytochemical screening of hydroethanolic extract of *Bacopa monnieri* for glycosides

Tab. 7

S. No	Test	Observation	Result
1	500 mg of HE-ext + 2 mL acetic acid + 1 drop $FeCl_3$ +1 mL H_2SO_4	Absence of brown ring color at interphase	-
2	500 mg HE-ext + 5 mL chloroform → mixed for 5 minutes → 10% ammonia solution was added	Absence of light green color in upper ammonia layer	-

Discussion

Both endogenous and exogenous opioids affect the gastrointestinal motility[25,26] and it has been reported that morphine and related drugs inhibit gastrointestinal motility by acting at supraspinal, spinal and peripheral receptors[27-30] and this effect is produced by the release of endogenous serotonin that acts directly on serotonin receptors present in intestinal smooth muscles[31,32].

The present study also showed that morphine when administered at 3 mg/kg significantly inhibited GIT motility (Fig. 1) reversed with naloxone treatment (Fig 2). It can therefore be suggested that morphine induced anti GIT motility effect through opioids mechanism.

Similarly, BM HE-ext also inhibited GIT motility highly significantly (Fig. 1). However, in contrast to morphine this anti GIT motility effect was not antagonized by the non selective opioid antagonist naloxone (Fig. 2) suggesting non opioid mechanism in the anti GIT motility effect.

Alpha-2 adrenoceptors have an important role in the control of GIT motility[33,34]. Several studies have reported that clonidine (alpha-2 receptor agonist) has anti GIT motility effect in rats[27,35,36] and mice[36,37]. Studies have indicated that yohimbine – alpha-2 receptor antagonist, causes the reversal of inhibition of gastrointestinal motility induced by clonidine. To examine the role of alpha-2 adrenoceptors in the anti GIT motility effect of BM HE-ext, yohimbine, an alpha-2 adrenoceptor antagonist was administered 5 minutes before the BM HE-ext. that resulted in the blockade of anti GIT motility effect of BM HE ext (Fig. 2) thus signifying the role of alpha-2 adrenoceptor in the mechanism of anti GIT motility action of BM HE-ext.

Since GABA receptors also mediate anti GIT motility effect, we tested the GIT motility in the presence of picrotoxine, a non selective GABA receptor antagonist. Interestingly, the anti GIT motility effect of BM HE-ext was antagonized (Fig. 2) suggesting the GABA receptor role as well.

Most of the modern researchers on herbal medicine are involved in traditional system of medicine due to the fact that none of modern medicines is non toxic and quite safe for human consumption. There are number of plants that have been used for the treatment of various disease and ailments. The screening of these plants is therefore very important and necessary in order to know the value of medicinal plants. The identification of specific compound for particular disease is a challenging and lengthy process. The importance of plants is due to their biologically active ingredient. Two types of metabolites are produced by plants, primary metabolites that include sugers, proteins, amino acids and chlorophylls etc

The other type is secondary metabolites that include saponins, tannins, triterpenoids, glycosides and alkaloids etc. These metabolites exert number of significant pharmacological effects on human beings. Phytochemical investigation showed that *Bacopa monnieri* possesses saponins (Tab.3), flavonoids (Tab.4), tannins (Tab.5) and triterpenoids (Tab.6).

Graphs show the quantal dose response curve where % commulative death has been plotted against dose on semilog graph paper by using the package of prism 4. The quantal dose effect curve can be used to calculate LD_{50}, ED_{50}, and therapeutic index. These values provide information about the safety and toxicity of drugs. The curve has also been used to have the information regarding the margin of safety in order to determine range where drug is expected to produce therapeutic effect without having any significant toxic effect. As shown in fig.3 the LD_{50} of hydroethanolic extract of *Bacopa monnieri* is (232 mg/Kg Body weight). One such beneficial measurement is therapeutic index that is the ratio of LD_{50} to ED_{50}. Experiments involving animal models provide convenient way to determine therapeutic index for a particular drug. If the therapeutic index for a drug is very small then drug is more toxic as compared to that is having greater therapeutic index. The therapeutic index calculated for hydroethanolic extract (Tab. 1) is 3.42 and 3.45 for acetic acid induced writhing test and hot plate test respectively. Studies have shown that saponins cause the hemolysis of RBCs[38]. The toxicity of *Bacopa monnieri* may be due to hemolytic effect on RBCs as *Bacopa monnieri* is rich in saponins.

In conclusion, this study has demonstrated that BM HE-ext anti GIT motility reversible with yohimbine and picrotoxine indicate the involvement of alpha-2 and GABA receptors that is due to the presence of saponins, tannins, flavonoids, triterpenoids.

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