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# EVALUATION OF *BACOPA MONERI* FOR ANTI -ANALGESIC ACTIVITY

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**Abstract**

Pain is a complex and uniquely personal experience, involving various physiological and perceptual pathways and factors The currently available standard drugs for pain and inflammation remain the mainstay for managing and treating these disorders. However, they are associated with many side effects Recently, the interest in the use of herbal products has grown dramatically in the western world as well as in developed countries *Bacopa monnieri* locally known as brahmi or Jalanimba in India, has been used for centuries in the Ayurveda the treatment of asthma, insanity and epilepsy. The plant has been utilized extensively as a nootropic, digestive aid and to improve learning, memory and respiratory function. The aim of this study is to evaluate anti analgesic activity of *Bacopa moneri*. Thee leaves of plant were collected and subjected to extraction. Further oualitative, quantitative studies along with in vivo studies for detecting anti analgesic activity was performed. The results showed that The HABM (Hydroalcoholic extract of *Bacopa moneri)* at 300mg/kg & at 500mg/kg showed significant increase in latency time ($P < 0.01$) and percentage protection was found to be 44.82.0% and 38.458% respectively. At 500 and 300mg/kg, i.p. the extract showed significant increase in reaction time ($P < 0.01$). The tail withdrawal reflex time in Hydroalcoholic extract of *Bacopa monnier*i was found to be significant in mice at 30 min. HABM at 500 mg/kg significantly inhibited the acetic acid-induced writhing responses. From these results it can be concluded that *Bacopa monnieri* exhibits potent anti -analgesic activity.

## Introduction

Pain is a complex and uniquely personal experience, involving various physiological and perceptual pathways and factors. Pain is distinct from nociception, the latter being defined as the transmission of noxious stimuli to the brain, and the numerous processes that drive this transmission.[1] Pain is then accordingly described as perceiving nociception, whether arising from the nociceptors (nociceptive pain), the nerve itself (neuropathic pain), or a combination of the two (mixed pain). Pain is further defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage," and is therefore an experience by an individual that involves both perceptual and physiological input [1-3].

The currently available standard drugs for pain and inflammation remain the mainstay for managing and treating these disorders. However, they are associated with many side effects and toxicities, such as gastric irritation, gastric ulcer, alterations in renal function, effects on blood pressure, hepatic injury, and platelet inhibition which may result in increased bleeding. Using NSAIDs results in increased risk of cardiovascular adverse events especially, in patients taking COX-2 inhibitors. Opioid analgesics are also associated with many unwanted side effects and toxicities, including drowsiness, nausea and vomiting, pruritus, constipation, disturbing hormonal homeostasis, hearing loss, tolerance, physical dependence, addiction, and respiratory problems. In view of this, there is a need for the intensification of research into medicinal plants which are claimed to be effective in the management of pain and inflammation [4,5].

Recently, the interest in the use of herbal products has grown dramatically in the western world as well as in developed countries. It is now becoming exceedingly apparent that available psychotherapeutics does not properly meet therapeutic demands of a vast majority of patients with mental health problems, and that herbal remedies remain to be the ultimate therapeutic hope for many such patients in the western world and elsewhere [6-8].

*Bacopa monnieri* locally known as brahmi or Jalanimba in India, has been used for centuries in the Ayurveda the treatment of asthma, insanity and epilepsy.The plant has been utilized

extensively as a nootropic, digestive aid and to improve learning, memory and respiratory function [9,10].

## Experimental

### Materials

### Plant Material

The leaves of *Bacopa monnieri* were collected from medicinal garden of our institute in the period of January 2022 considering the seasonal conditions for obtaining maximum phytoconstituents.

### Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=3) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India, prior to experimentation.

### Methods

### Determination of physicochemical parameters

Dried material of the plant were ground in a grinder and used for evaluation of physicochemical parameters. Foreign matter was determined visually using lenses, while loss on drying was determined by drying a specified quantity of plant material in an oven to constant weight at 110°C. Total ash, acid-insoluble ash, and water-soluble ash values were determined by incinerating the plant material at a temperature between 500-600◦C until it was white, indicating the absence of carbon. Extractive values in solvents of the rhizomes were evaluated by soaking in respective solvents for ~18 h. Physicochemical

parameters such as percentage of total ash, acid insoluble ash, water soluble ash and sulphated ash were calculated based upon standard procedures [11].

**Extraction by Soxhlet Apparatus**

The Collected plant drug was cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drug was converted into moderately coarse powder in hand grinder. Powdered plant drug was weighed (45.7 gm) and packed in soxhlet apparatus. The plant Material was defatted with petroleum ether (40°-60°C) for about 12 hrs separately & complete defatting was ensured by placing a drop from the thimble on a filter paper which did not exhibited any oily spot. The defatted material was removed from the Soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted plant drug was subjected to extraction by (ethanol: water; 70:30) as solvents. The liquid extract were collected in a tarred conical flask. The solvent removed by distillation. Last traces of solvent being removed under vacuum.

**Preliminary Phytochemical Screening**

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of leaves of *Bacopa monnieri*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids [12].

**Estimation of total phenolic content**

Estimation of total phenolic content Total phenolic content of all the extracts was evaluated with Folin-Ciocalteu method. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 0.5mL aliquots of 10, 20, 30, 40 and 50µg/ml methanolic gallic acid solutions were mixed with 2.5 mL Folin–Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantitative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer 1650 Shimadzu,

Japan. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extracts as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

**Estimation of total flavonoids content**

The aluminum chloride colorimetric method was modified from the procedure reported by Woisky and Salatin. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 5 to 25 µg/ml. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of Hydroalcoholic extracts and Flavonoid standard solutions were reacted with aluminum chloride for determination of Flavonoid content as described [13].

**Acute toxicity studies**

Acute toxicity studies were carried out using acute toxic class method as per OECD guidelines 425 (OECD, 2000). Acute toxicity for Hydroalcoholic extract of *Bacopa monnieri* (Leaves) was carried out using groups of three Swiss albino mice by administering a dose 2000 mg/kg, in 1% CMC p.o., while the control group received only the vehicle. The groups were observed mortality and behavioral changes during 48 h [14].

**Experimental Design**

In the experiment, a total of 30 rats were used. The rats were divided into 5 groups comprising of 6 animals in each group as follows:

**Group I:** control, received vehicle 1ml/100gm

**Group II:** Diclofenec sod. (10mg/kg, p.o.) Only one day, before 1hr of placing in hot plate.

**Group III:** 300 mg/kg *Bacopa monnieri* extract , p.o., once daily for 7 days.

**Group IV:** 500 mg/kg *Bacopa monnieri* extract , p.o., once daily for 7 days.

**Hot plate method**

The method originally developed by Woolfe and MacDonald (1944) . The paws of mice and rats are very sensitive to heat at optimum temperature, which are not damaging the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws. The animals were placed on Eddy's hot plate kept at a temperature of 55 ± 0.5 ◦C. A cut off period of 15 s, was observed to avoid damage to the paw. Reaction time and the type of response were noted using a stopwatch. Control rats were treated with vehicle (12% Tween 80, 1 ml/kg) [15-17].

Aspirin was used as positive control (100 mg/kg) and Hydroalcoholic extract of *Bacopa monnieri* (Leaves) (300 and 500 mg/kg, i.p.).The latency was recorded before and after 15, 30, 60 and 120 min following intraperitoneal administration of 300 and 500 mg/kg of each of the extract to different groups of six animals each. Average reaction times were then calculated and the percentage variation calculated

**Tail immersion method**

The procedure is based on the observation that analgesic drugs are selectively prolonging the reaction time of the typical tail withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 ◦C . Control rats were treated with vehicle (12% Tween 80, 1 ml/kg). Aspirin was used as positive control (100 mg/kg) and Hydroalcoholic extract of *Bacopa monnieri* (Leaves) (300 and 500 mg/kg, i.p.). The tail withdrawal reflex was recorded before and after 15, 30, 60 and 120 min following intraperitoneal administration of the extract to different groups of six animals each [18].

**Tail Flick Method**

The tail of the rat was placed on the nichrome wire of an analgesiometer and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. Hydroalcoholic extract of *Bacopa monnieri* (Leaves) (300 and 500 mg/kg, i.p.) and Aspirin (100 mg/kg) were injected intraperitoneally. Distilled water served as control. Analgesic activity was measured at 30, 60, 90, 120 and 150 min after the administration of test and

standard drugs. The analgesic activity was classified as positive if the rat failed to withdraw its tail within 10 sec of exposure

**Acetic Acid-induced Writhing Test**

This was based on the method described by. Distilled water (control), Hydroalcoholic extract of *Bacopa monnieri* (Leaves) (300 and 500 mg/kg, i.p.) and aspirin (100 mg/kg) were administered intraperitoneally 60 min before i.p. injection of 0.6 % v/v acetic acid solution in water at a dose of 10 ml/kg. Immediately after administering acetic acid, the number of writhing or stretches (a syndrome, characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) were counted for 15 min. A reduction in the writhing number as compared to the control group was considered as evidence for the presence of analgesia which was expressed as percent inhibition of writhings [19].

**Statistical analysis**

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet's multiple comparisons using graph pad in stat 3 Demo and all the results obtained in the study were compared with the vehicle control group. P values <0.05 were considered statistically significant.

**Results & Discussion**

The total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying and foreign matter of *Bacopa monnieri* (Leaves) was found 8.55, 0.89 %, 4.12%, 6.47%, 0.49% and 0.71 % respectively. The yields were found to be (13.58 % w/w of crude drug) of petroleum ether extract with semisolid mass of brown colour, (19.44% w/w of crude drug) of hydroalcoholic extract with orange black colour semisolid mass for *Bacopa monnieri* leaves. Phytochemical test reveal the presence of alkaloid, glycoside, tannins, flavonoid, steroids, proteins & phenol. Total phenol & flavonoid content was found to be 0.856mg/100mg & 0.746mg/100mg respectively.

Analgesic activity was evaluated using Hot plate methods, Tail immersion method, Tail flick method and Acetic acid-induced writhing in rats. HABM (300mg/kg), HABM (500mg/kg). At 500 and 300mg/kg, i.p. the extract showed significant increase in latency time ($P < 0.01$)

and percentage protection was found to be 44.82.0% and 38.458% respectively. At 500 and 300mg/kg, i.p. the extract showed significant increase in reaction time ($P < 0.01$). The tail withdrawal reflex time in Hydroalcoholic extract of *Bacopa monnieri* was found to be significant in mice at 30 min. It was found that Hydroalcoholic extract of *Bacopa monnieri* at 500 mg/kg significantly inhibited the acetic acid-induced writhing responses

**Table 1: Physicochemical parameters of *Bacopa monnieri* (Leaves)**

| S. No. | Parameter | Value obtained (w/w) |
|---|---|---|
| 1 | Total ash | 8.55% |
| 2 | Acid insoluble ash | 0.89% |
| 3 | Water-soluble ash | 4.12% |
| 4 | Sulphated ash | 6.47% |
| 5 | Loss on drying | 0.49% |
| 6 | Foreign matter | 0.71% |

**Table 2: Preliminary phytochemical screening of *Bacopa monnieri***

| S. No. | Phytoconstituents | Test Name | Hydroalcoholic Extract |
|---|---|---|---|
| 1 | Alkaloids | Mayer's Test | **+(ve)** |
| | | Dragendorff's Test | **-(ve)** |
| 2 | Glycosides | Raymond's Test | **+(ve)** |
| | | Killer Killani Test | **+(ve)** |
| 3 | Carbohydrates | Molisch's Test | **-(ve)** |
| | | Fehling's Test | **-(ve)** |
| 4 | Tannins | Vanillin- HCl Test | **+(ve)** |
| | | Gelatin Test | **-(ve)** |
| 5 | Flavonoids | Lead acetate | **+(ve)** |
| | | Shinoda Test | **+(ve)** |
| 6 | Resins | Color detection with ferric chloride | **-(ve)** |
| | | Turbidity Test | **-(ve)** |
| 7 | Steroids | Libermann- Bur chard Test | **+(ve)** |
| | | Salkowski Reaction | **+(ve)** |
| 8 | Proteins & Amino acids | Biuret Test | **+(ve)** |
| | | Precipitation test | **-(ve)** |
| | | Ninhydrin Test | **+(ve)** |
| 9. | Phenols | Ellagic Acid Test | **+(ve)** |

**Table 3: Total phenolic and flavonoid content of hydroalcoholic extract of *Bacopa monnieri***

| Sample | Total phenolic content mg/100mg | Total Flavonoid content mg/100mg |
|---|---|---|
| Hydroalcoholic extract | 0.856 | 0.746 |

**Table 4: Effects of Hydroalcoholic extract of *Bacopa monnieri* and aspirin administered intraperitoneal on the latency of rat exposed to the hot plate**

| Treatment | Mean latency time (s) ± S.E.M | % Protection |
|---|---|---|
| Vehicle control | 2.16 ± 0.166 | -- |
| Aspirin (100mg/kg) | 6.83 ± 0.401** | 68.3 |
| HABM (300mg/kg) | 4.83 ± 0.477** | 55.2 |
| HABM (500mg/kg) | 6.33 ± 0.614** | 65.8 |

**Table 5: Effect of Hydroalcoholic extract of *Bacopa monnieri* and aspirin administered intraperitoneal, tail withdrawal reflux of mice induced**

| Treatment | After 30 min of drug treatment | % Protection |
|---|---|---|
| | Mean reaction time ± S.E.M. | |
| Vehicle control | 2.66 ± 0.210 | ----- |
| Aspirin (100mg/kg) | 5.66 ± 0.210** | 52.94 |
| HABM (300mg/kg) | 4.33 ± 0.333** | 38.45 |
| HABM (500mg/kg) | 4.833 ± 0.3073** | 44.82 |

**Table 6: Effect of Hydroalcoholic extract of *Bacopa monnieri* and aspirin administered intraperitoneal, tail flick method**

| Treatment | Response time after drug treatment (mean ± S.E.M) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 |
| Vehicle control | 3.62±0.05 | 3.43±0.07 | 3.59±0.05 | 3.57±0.04 | 3.42±0.03 | 3.49±0.05 |
| Aspirin (100mg/kg) | 3.42±0.05 | 9.80±0.03** | 10.00±0.00** | 10.00±0.00** | 10.00±0.0**0 | 10.00±0.0**0 |
| HABM (300mg/kg) | 3.52±0.07 | 3.42±0.06 | 3.56±0.07 | 3.63±0.07* | 3.72±0.063* | 3.46±0.07** |
| HABM (500mg/kg) | 3.59±0.03 | 3.52±0.07 | 3.50±0.06 | 3.61±0.07* | 3.60±0.08* | 3.49±0.08** |

**Table 7: Effect of hydroalcoholic extract of *Bacopa monnieri* and aspirin on acetic acid-induced writhing in mice**

| Treatment | No. of writhings per 15 min (mean ± sem) | Inhibition of writhing (%) |
|---|---|---|
| Vehicle control | 34.20 ± 1.82 | |
| Aspirin (100mg/kg) | 8.30 ± 0.8 1 * | 75.74 |
| HABM (300mg/kg) | 12.80 ± 1.07* | 62.58 |
| HABM (500mg/kg) | 8.90 ± 0.98* | 73.98 |

## Conclusion

Hydroalcoholic extract of *Bacopa monnieri* was found to be potent analgesic. In accordance to the present study, it has been observed that Hydroalcoholic extract of *Bacopa monnieri* has beneficial effects against centrally, peripherally and inflammatory pain models. This protective action may be attributed towards the presence of flavonoids and Phenols. We would like to conclude that it is worthwhile to think, to use *Bacopa monnieri* (leaves) as drugs and further studies should be initiated to establish exact mechanism of action and elaborative phytochemical investigations to find out which active constituents responsible for analgesic activity. These reports may serve as a foot step in the research of potent analgesic drug.

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