Bacopa monnieri inhibits locomotor hyperactivity induced by morphine without altering noradrenaline


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Abstract

Bacopa monnieri, (BM) a perennial herb has centuries old clinical utility as a nootropic herb in ayurvedic system of medicine. We investigated the effect of methanolic extract of Bacopa monnieri (Mt-ext BM) on locomotor activity in saline and morphine (10 mg/kg) treated mice. Mt-ext BM was first analyzed on High performance Liquid Chromatography (HPLC) with UV for quantification of Bacoside A<sub>3</sub>, Bacopaside II and Bacopasaponin C. Locomotor activity was recorded sixty minutes after oral administration of 10, 20 and 30mg/kg dose of Mt-ext BM. To separate group morphine (10 mg/kg) was administered intraperitoneally and after 30 minutes locomotor activity was recorded. Mt-ext BM (10, 20 and 30mg/kg) was administered orally to separate groups and after one hour morphine (10 mg/kg) was administered intraperitoneally and locomotor activity was recorded after 30 minutes. Our results indicate that Mt-ext BM all three doses significantly lowered locomotor activity in both saline and morphine treated mice. Additionally Mt-ext BM significantly lowered morphine induced upsurge of DA, DOPAC, HVA, and 5HIAA, in morphine treated mice. While Mt-ext BM did not alter neurotransmitters in saline treated mice striatum. Mt-ext BM failed to modulate noradrenaline (NA) in both saline and morphine treated animals. The lowering of neurotransmitters in morphine treated groups imply an antidopaminergic/antiserotnergic effect which might have a promising role in morphine dependence management.

Keywords: Bacopa monnieri; Locomotor hyperactivity; Dopamine; Serotonin; Morphine; Bacoside A
Introduction

Opiates dependence is an international multifaceted health issue that afflicts directly or indirectly a large population across the globe including adult age groups of both genders. Prescription drug abuse and subsequent dependence among patients using opiates add a new horrific dimension to the opiates addiction challenge (1-4). All opiates upon first and intermittent exposure cause a locomotor hyperactivity in rodents, which is a display of animals’ euphoric behavior (5-6). This behavioral sensitization may last for a year and animals this sensitization is one parameter for quantifying abuse potential of abuse liable drugs in rodents (7-8). Brain dopaminergic pathways crucial role have been primarily associated with rewarding effects of abuse liable drugs including opiates (5). Dopamine has been considered main neurotransmitter involved in rewarding effects of opiates, and expression of locomotor effects as dopamine receptor antagonists have been reported to antagonize expression of morphine induced hyper locomotor effects (9). Although there are some converse evidences that speaks higher for role for noradrenergic pathways major role in expression of morphine sensitization (10-11). Acute morphine administration increases DA, 5-HT, DOPAC, HVA, and 5HIAA, concentration in mice striatum (12-16). Chronic morphine administration also leads to post synaptic dopamine receptor sensitivity and enhancement in (µ) mu receptor density in specified brain regions. (17-25)

Bacopa monnieri is a small perennial creeping herb from Scrophulariaceae family found in shady marshy places and fresh water streams in both Asia and Europe including Pakistan (26). Bacopa monnieri has a long and historic use a nootropic drug in ayurvedic system of medicine since time immemorial (27). Bacopa monnieri folkloric utility includes as cardio tonic, anxiolytic, antidepressant, antiepileptic antiulcer, anti asthmatic, as energizer and diuretic (27) Many compounds have been reported from Bacopa monnieri, including alkaloids, saponins and sterols (28). Bacopa monnieri main active moiety that is responsible for its major pharmacological profile is Bacoside A, which is actually a mixture of four compounds i.e., Bacoside A₃, Bacopasaponin II, Bacopasaponin C and isomer of Bacopasaponin C (29). Currently Bacopa monnieri is available in various herbal formulations (30) for memory enhancement and other indications(31) and has been found to be safe in both pre clinical and clinical models(31-32). Bacopa monnieri has been reported to have a protectant effect against morphine induced hepatotoxicity (33-35), and has recently been reported to have antinociceptive effect comparable to morphine and has been reported to inhibit acquisition and expression of morphine tolerance (36) Bacopa monnieri has also been reported to enhance morphine analgesia (36) Bacopa monnieri has been found to be having an inhibitory effect on morphine withdrawal in isolated tissues of guinea pig ileum and has also been reported to be effective in lowering morphine induced hyperlocomotion, dopamine receptor sensitivity and apomorphine induced climbing behavior in rodents (37-38). As BM is a renowned nootropic, has a clinical and folkloric standing for memory enhancement, scientists are exploring newer role for nootropic and cognitive enhancers for the management of Opioids dependence (24-25).

Earlier we have reported that BM n butanol extracts inhibits morphine induced locomotor hyperactivity and inhibits morphine induced upsurge of DA and 5-HT. As methanolic extract of BM is used extensively across the globe for various ailments (39). The aim of this study was to quantify Bacoside A major components i.e., Bacoside A₃ (Fig.1), Bacopasaponin C (Fig 2) and Bacopaside II (Fig 3) in methanolic extract of locally available Bacopa monnieri, and to assess the effect of Bacopa monnieri methanolic extract on morphine induced locomotor activity, and its effect on striatal NA, DA, DOPAC, HVA, Serotonin, and 5HIAA in mice.
Material and Methods

Animals

Balb-C mice weighing 23-28 g of either sex were used in all procedures of locomotor activity. Animals were acquired from Animal House and Bioassay Laboratory of the Department of Pharmacy, University of Peshawar, where animals are bred and housed under standard conditions of temperature and light i.e. 22 ± 2°C and 12h light /12 h dark cycle, with free access to food and water. All experimental procedures were performed with the prior approval of the Ethical Committee of the Department of Pharmacy University of Peshawar also conforming to UK Animal Scientific Procedure ACT 1986.

Drugs

Chemicals for High performance Liquid Chromatography (HPLC) procedures including HPLC grade, 1-octane sulphonic acid sodium salt, acetonitrile, , sodium dihydrogen orthophosphate sodium (Fisher scientific U.K) and EDTA (Electrochemical detector grade), were supplied by the Merck local distributor in Peshawar, Pakistan. Morphine sulphate was generously gifted by PDH Laboratories Lahore Pakistan with prior approval ministry of Health and ministry of Narcotics control. Commercial grade methanol n-hexane, acetone and n-Butanol used for plant extraction were purchased from Haq chemicals Peshawar, Pakistan. Bacopaside II, Bacoside A, and Bacopasaponin C were gifts from Prof Dr Ikhlas A. Khan, School of Pharmacy University of Mississippi, U.S.A. All drugs were dissolved in normal saline.

Plant Material

Bacopa monnieri plant was collected from Rumalee stream near Quaide Azam University, Islamabad, Pakistan. Prof. Dr. Muhammad Ibrar, Department of Botany University of Peshawar authenticated the plant, Voucher No 7421. The plant aerial parts were washed and shade dried. The coarsely powdered shade dried aerial parts weighing 500 grams were extracted with n-Hexane, followed by acetone to remove fats and the chlorophyll type pigments. The powder was further extracted with commercial grade methanol using Soxhelet apparatus yielding 19 grams methanolic extract. This methanolic extract of the plant was used in all experiments. The extract was dissolved in normal saline.

Chromatographic analysis of Bacopa monnieri methanolic extract for Bacopaside

The methanolic extract was screened for Bacoside A major three components, i.e., Bacopaside II, Bacoside A, and Bacopasaponin C using High performance Liquid Chromatography with UV detection using Phrompitayarat method (40) with slight modifications. HPLC system consisted of LC-20AT double pump (Shimadzu, Japan) and SPD-20A UV Visible detector , and C18 column (250 mm x 4.6 mm, 5 µm particle size) a Rheodyne injector with 20 µL loop. Briefly Mt-ext BM 50 mg was dissolved in 10 ml HPLC Grade methanol and was then centrifuged at 3000 rpm for fifteen minutes. After centrifugation this solution was filtered through 0.45 µ filter before injecting into HPLC system. The mobile phase was prepared of phosphoric acid 0.2% and acetonitrile (60:40, v/v), and pH adjusted to 3.0 with 3 M NaOH. The HPLC system was run at wavelength of 205 nm having 0.6 mL/min flow rate. All the peaks were acquired in 22 minutes run time. The peaks were first confirmed by addition of standards Bacosides to the samples.

Measurement of locomotor activity

Locomotor activity was measured in Bioassay laboratory quite room using a box measuring 50cm × 40cm × 44cm (length × width × height) with floor divided by dark lines into four equal rectangular zones as described by Subhan (41). One hour before the start of the experiment animals Mice (n=6) were acclimatized under red light (40 watt) to the laboratory conditions. Mice (n=6) were administ
red morphine (10 mg/kg) or saline intraperitoneally, or Mt-ext BM (10, 20 and 30 mg/kg) orally. Locomotor activity evaluated as line-crossings was performed 30 min after intraperitoneal drug administration and 60 min after oral drug administration. Selected groups received doses of saline or Mt-ext BM (10, 20 and 30 mg/kg orally) 60 min before morphine (10mg/kg) dosing. Thirty min after intraperitoneal administration of morphine, or saline, the mice were placed in the recording box and group mean line-crossing counts were subsequently recorded between 1 and 30 min interval.

Chromatographic analysis of mice striatum for NA, DA, 5-HT and their metabolites, DOPAC, HVA and 5-HIAA.

DA, DOPAC, HVA, 5HT, 5HIAA, and NA were quantified by HPLC coupled with Electrochemical Detection. Briefly, the system consisted of an HPLC system (Shimadzu, Japan), Communication Bus Module (model 20 A), two independently working pumps (model LC-20AT), an analytical column MD _150; (3mm x 150 mm, 3µm), a Rheodyne injector with 20 µL loop attached to an electrochemical detector (ESA Choulchem III model 5300) equipped with an analytical cell (model 5011 A). Electrodes 1 and 2 of the analytical cell were set at +200 and -200 mV respectively, with a sensitivity of 2 uA, while the guard cell (model 5020) potential was set at 500 mV. The mobile phase consisted of 94 mM sodium dihydrogen orthophosphate, 40 mM Citric acid, 2.3 mM sodium 1-octane sulphonic acid, 50 uM EDTA, and 10 % acetonitrile (pH 3).

Sample Preparation

Immediately after measuring locomotor activity, animals were killed by decapiticaion and whole brain excised onto an ice chilled plate and striata were separated and stored at -80°. For analysis, individual striata were weighed and homogenized in ice cold 0.2 % perchloric acid at 5000 rpm with a Teflon-glass homogenizer (Wise stir HS 30E). The samples were then centrifuged at 12000 g/minute (4°C) (Centurion UK) for twenty minutes and filtered through a 0.45 micron filter. The samples obtained were injected directly into the HPLC system.

Results

Chromatographic analysis of Mt-ext BM for Bacopasides

The HPLC analysis revealed that Mt-ext BM contained Bacoside A, major components i.e Bacoside A3, Bacopasaponin C and Bacopaside II. Our results further indicated that the quantity of these Bacopasides were 1.6 µg (Bacopasaponin C), 5 µg (Bacoxide A3), and 1.8 µg (Bacopaside II), in each milligram of Mt-ext BM.

Effect of Mt-ext BM alone and in combination with morphine on locomotor activity

Methanolic extract of Bacopa monnieri significantly (P<0.05) reduced locomotor activity in saline treated animals. The results indicated (Fig.4) that oral administration of all three doses i.e., 10, 20 and 30 mg/kg Mt-ext BM significantly reduced locomotor activity in saline treated animals. Additionally all three doses i.e. 10, 20 and 30 mg/kg Mt-ext BM significantly (P<0.05) inhibited locomotor activity in morphine treated mice. The 30 mg /kg Mt-ext BM inhibition of ambulation is far more significant (P<0.001) than 10 and 20 mg/kg Mt-ext BM in both saline and morphine treated animals (Fig.4).

see Fig. 4

Effect of Mt-ext BM on striatal DA and its metabolites DOPAC and HVA in mice

Methanolic extract of Bacopa monnieri oral administration in all three doses (10, 20, 30 mg/kg) did not change DA, DOPAC and HVA, in mice striatum as compared to saline treated group as shown in table 1. While in morphine (10 mg/kg) treated groups Mt-ext BM all three doses significantly lowered DA, DOPAC and HVA as compared to
morphine treated animals as shown in table 2. The dose dependent impact of all three Mt-ext BM is evident from changes in dopamine as compared to morphine treated groups. The inhibition of morphine induced upsurge of DOPAC and HVA is highly significant also but the effect did not clearly display dose dependency (Table 2).

**Effect of Mt-ext BM on 5-HT and its metabolite 5HIAA in the striatum**

As shown in table 1, Mt-ext BM all three doses did not alter 5HT and 5HIAA concentration in mice striatum as compared to saline treated. In morphine treated group all three doses of Mt-ext BM significantly lowers 5HIAA concentration although the inhibition picture does not clearly portray a dose dependent response as shown in table 2. A downward trend in 5HT contents has been there in morphine treated groups but this inhibition is statistically insignificant (Table 2).

**Effect of Mt-ext BM on NA in the striatum**

As shown in table 1, Mt-ext BM all three does (10, 20, 30 mg/kg) did not modulate noradrenaline contents in mice striatum as compared to saline treated group. Additionally no significant change in NA content was found in both morphine and Mt-ext BM treated groups as shown in table 2.

- see Table 1.
- see Table 2.

**Discussion**

The findings of the study indicate that acute administration of Mt-ext BM in all three doses does not modulate DA or its metabolites, or serotonin or its metabolites and noradrenaline in saline treated animals. Although All three doses significantly lower ambulation in saline treated animals, without altering DA, DOPAC, HVA, 5HT, 5HIAA and noradrenaline. The depression of ambulation effect in saline treated might be due to calcium channel blocking effect (42) or nitric oxide synthase inhibiting (43-44) effect of Mt-ext BM or both simultaneously might be responsible. It has been reported that L-NAME (L-NG-Nitroarginine methyl ester) nitric oxide synthase inhibitor inhibits locomotion in saline treated animals (45-46).

Current literature highlights a close interdependent interplay between opioidergic and Adenosine 1A receptors agonists (47) and adenosine 1A receptors agonists have recently been reported to inhibit morphine induced sensitization (48) BM has also been recently reported to have adenosine 1A agonist’s activity, and reverses diabetic neuropathic pain through this pathway (49). The inhibition of morphine induced hyperlocomotion might be due to the BM adenosine 1A agonist activity, although it needs further validation in specified experimental paradigms.

Our findings indicate that Mt-ext BM inhibits morphine induced hyperlocomotion highly significantly. Our findings testify the findings of Sumathi (50) that reported that Mt-ext BM inhibits morphine induced hyperlocomotion and reverses apomorphine induced reverse tolerance to locomotion. Our neurotransmitters findings further validate the behavioral findings of Sumathi (50) that concluded on behavioral grounds the antidopaminergic effect of BM in morphine treated animals.

As evident from Fig. 4 Mt-ext BM significantly reversed morphine induced hyperlocomotion and associated DA, DOPAC, HVA upsurge in morphine treated mice (Table 2). Mt-ext BM also lowered morphine induced upsurge of 5HIAA highly significantly. There are ample evidences that advocate role of serotonin and its turnover in morphine induced hyperlocomotion and subsequent dependence (10, 51-53). Furthermore Adenosine 1A agonists’ have been reported to have a close interplay with serotonin for mediation of its effects via serotonergic pathways mainly involving 5Ht1A receptors (54).

The role of this mechanism in 5HT and 5HIAA inhibition by BM in lowering morphine induced hyperlocomotion cannot be ruled out. BM has been
reported to have calcium channel blocking effect (42) and calcium channel blockers are reported to inhibit morphine sensitization (55), tolerance, dependence, augment opioids analgesia without augmenting respiratory depression (56). Additionally BM has reported to inhibit morphine tolerance and augment morphine analgesia, the calcium channel blocking effect might have the plausible role in antidopaminergic effect of BM (36). Our findings validate the findings of Sumathi that BM has antidopaminergic effect and reverses apomorphine induced climbing behavior in morphine treated mice. The findings are interesting and can further be verified in microdialysis models and through DA receptor studies using western blotting techniques. This antidopaminergic effect of BM may further strengthen the BM candidacy as herbal therapy for opioids dependence. BM has been reported to have protectant effect against morphine induced toxicity, and is an established nootropic herbal drug also (35, 57-58). It’s high time to assess the role of BM a renowned nootropic (27, 59) in drug dependence, as nootropics newer role in drug dependence, augment opioids analgesia without inhibit morphine sensitization (55), tolerance, (42) and calcium channel blockers are reported to inhibit morphine analgesia without augmenting respiratory depression (56). 

Apart from its many centuries’ old clinical utility in ayurvedic system of medicine, recent clinical trials have found BM a safe and well tolerated herbal therapy (60-62).

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Conflict of interest

No conflict of interest to disclose.

References


Fig 2. Bacosaponin C

Fig 3. Bacopside II
Figure 4. Effect of Mt-ext BM (10, 20 and 30 mg/kg orally) on locomotor activity in saline and morphine (10 mg/kg intraperitoneally) in mice (n=6). Values are expressed as mean±SEM, applying ANOVA followed by Tukey’s Post hoc analysis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Striatal tissue concentrations†</th>
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<tbody>
<tr>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Saline</td>
<td>105±23</td>
</tr>
<tr>
<td>Mt-ext BM 10 mg/kg</td>
<td>89±14</td>
</tr>
<tr>
<td>Mt-ext BM 20 mg/kg</td>
<td>110±26</td>
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<td>Mt-ext BM 30 mg/kg</td>
<td>84±12</td>
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</tbody>
</table>

Table 1. Effect of normal saline and Mt-ext BM (10, 20 or 30 mg/kg) on striatal tissue levels of NA, DA, DOPAC, HVA, 5HT and 5HIAA in mice

† Concentration levels are expressed as Mean ± S.E.M ng/gram of wet tissue

<table>
<thead>
<tr>
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<th>Striatal tissue concentrations†</th>
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<tbody>
<tr>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Saline</td>
<td>105±23</td>
</tr>
<tr>
<td>MP (10 mg/kg)</td>
<td>114±22</td>
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<tr>
<td>MP (10 mg/kg) + Mt-ext BM 10 mg/kg</td>
<td>84±28</td>
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<tr>
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<tr>
<td>MP (10 mg/kg) + Mt-ext BM 30 mg/kg</td>
<td>81±11</td>
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Table 2. Effect of normal saline, morphine (MP, 10 mg/kg) and morphine (MP, 10 mg/kg) + Mt-ext BM (10, 20 and 30 mg/kg) on striatal tissue levels of NA, DA, DOPAC, HVA, 5HT and 5HIAA

† Concentration levels are expressed as Mean ± S.E.M ng/gram of wet tissue, ***P<0.001, **P<0.01, *P<0.05. Values are significant as compared to morphine treated group.