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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_3, 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 A_3, Bacopaside 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 A_3 (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.

see Fig. 1

see Fig. 2

see Fig. 3

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_3, 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 ll, Bacoside A_3, and Bacopasaponin C using High performance Liquid Chromatography with UV detection using Phrompitayyarat method (40) with slight modifications. HPLC system consisted of LC-20AT double pump (Shimadzu, Japan) and SPD-20A UV Visible detector , and C^{18} 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 *u* 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 administe-

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 decapicitaion 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 (Bacoside A_3), and 1.8 µg (Bacopaside ll), 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 down ward 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 stratum 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 is under investigation and some encouraging results have been obtained (24-25).

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

1. Green TC, Grimes Serrano JM, Licari A, Budman SH, Butler SF. Women who abuse prescription opioids: findings from the Addiction Severity Index-Multimedia Version Connect prescription opioid database. Drug Alcohol Depend. 2009 Jul 1;103(1-2):65-73.
2. Knisely JS, Wunsch MJ, Cropsey KL, Campbell ED. Prescription Opioid Misuse Index: A brief questionnaire to assess misuse. Journal of Substance Abuse Treatment. [doi: DOI: 10.1016/j.jsat.2008.02.001]. 2008;35(4):380-6.
3. Sproule B, Brands B, Li S, Catz-Biro L. Changing patterns in opioid addiction: characterizing users of oxycodone and other opioids. Can Fam Physician. 2009 Jan;55(1):68-9, 9 e1-5.
4. Veilleux JC, Colvin PJ, Anderson J, York C, Heinz AJ. A review of opioid dependence treatment: pharmacological and psychosocial interventions to treat opioid addiction. Clin Psychol Rev. 2010 Mar;30(2):155-66.
5. Koob GF, Sanna PP, Bloom FE. Neuroscience of addiction. Review. Neuron. 1998;21:461-76.
6. Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nature Reviews Neuroscience. 2001;2(2):119-28.
7. Sanchis-Segura C, Spanagel R. Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. Addiction Biology. 2006;11(1):2-38.
8. Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Research Reviews. 1986;11(2):157-98.
9. Pollock J, Kornetsky C. Evidence for the role of dopamine D1 receptors in morphine induced stereotypic behavior. Neurosci Lett. [doi: DOI: 10.1016/0304-3940(89)90094-3]. 1989;102(2-3):291-6.
10. Lanteri C, Salomon L, Torrens Y, Glowinski J, Tassin J. Drugs of abuse specifically sensitize noradrenergic and serotonergic neurons via a non-dopaminergic mechanism. Neuropsychopharmacology. 2007;33(7):1724-34.
11. Olson VG, Heusner CL, Bland RJ, During MJ, Weinshenker D, Palmiter RD. Role of Noradrenergic Signaling by the Nucleus Tractus Solitarius in Mediating Opiate Reward. Science. 2006 February 17, 2006;311(5763):1017-20.
12. Fadda P, Scherma M, Fresu A, Collu M, Fratta W. Dopamine and serotonin release in dorsal striatum and nucleus accumbens is differentially modulated by morphine in DBA/2J and C57BL/6J mice. Synapse. 2005;56(1):29-38.
13. Babbini M, Davis W. Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. British journal of pharmacology. 1972;46(2):213.
14. Gauchy C, Agid Y, Glowinski J, Cheramy A. Acute effects of morphine on dopamine synthesis and release and tyrosine metabolism in the rat striatum. European Journal of Pharmacology. 1973;22(3):311-9.
15. Kuschinsky K, Hornykiewicz O. Effects of morphine on striatal dopamine metabolism: possible mechanism of its opposite effect on locomotor activity in rats and mice. European Journal of Pharmacology. 1974;26(1):41-50.
16. Rethy CR, Smith CB, Villareal JE. Effects of narcotic analgesics upon the locomotor activity and brain catecholamine content of the mouse. Journal of Pharmacology and Experimental Therapeutics. 1971 February 1, 1971;176(2):472-9.

17. Shippenberg TS, LeFevour A, Thompson AC. Sensitization to the conditioned rewarding effects of morphine and cocaine: differential effects of the [kappa]-opioid receptor agonist U69593. European journal of pharmacology. 1998;345(1):27-34.
18. Spanagel R. Modulation of drug-induced sensitization processes by endogenous opioid systems. Behavioural Brain Research. 1995;70(1):37-49.
19. Gaiardi M, Bartoletti M, Bacchi A, Gubellini C, Costa M, Babbini M. Role of repeated exposure to morphine in determining its affective properties: place and taste conditioning studies in rats. Psychopharmacology. 1991;103(2):183-6.
20. Serrano A, Aguilar MA, Manzanedo C, Rodríguez-Arias M, Miñarro J. Effects of DA D1 and D2 antagonists on the sensitisation to the motor effects of morphine in mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2002;26(7-8):1263-71.
21. De Vries TJ, Shippenberg TS. Neural systems underlying opiate addiction. J Neurosci. 2002;22(9):3321.
22. Spanagel R, Weiss F. The dopamine hypothesis of reward: past and current status. Trends in Neurosciences. 1999;22:521-7.
23. Noble F, Cox BM. The role of dopaminergic systems in opioid receptor desensitization in nucleus accumbens and caudate putamen of rat after chronic morphine treatment. J Pharmacol Exp Ther. 1997;283(2):557-65.
24. Dhonnchadha BAN, Kantak KM. Cognitive Enhancers for Facilitating Drug Cue Extinction: Insights from Animal Models. Pharmacol Biochem Be. 2011.
25. Myers KM, Carlezon Jr WA. D-Cycloserine Facilitates Extinction of Naloxone-Induced Conditioned Place Aversion in Morphine-Dependent Rats. Biol Psychiat. [doi: DOI: 10.1016/j.biopsych.2009.08.015]. 2010;67(1):85-7.
26. Qureshi R, Raza Bhatti G. Ethnobotany of plants used by the Thari people of Nara Desert, Pakistan. Fitoterapia. 2008;79(6):468-73.
27. Russo A, Borrelli F. *Bacopa monnieri*, a reputed nootropic plant: an overview. Phytomedicine. 2005;12(4):305-17.
28. Gohil K, Patel J. A review on *Bacopa monnieri* Current research and future prospects2010.
29. Deepak M, Sangli GK, Arun PC, Amit A. Quantitative determination of the major saponin mixture bacoside A in *Bacopa monnieri* by HPLC. Phytochem Anal. 2005 Jan-Feb;16(1):24-9.
30. Qureshi M, McClure W, Arevalo N, Rabon R, Mohr B, Bose S, et al. The Dietary Supplement Protandim® Decreases Plasma Osteopontin and Improves Markers of Oxidative Stress in Muscular Dystrophy Mdx Mice. Journal of Dietary Supplements. 2010;7(2):159-78.
31. Pravina K, Ravindra K, Goudar K, Vinod D, Joshua A, Wasim P, et al. Safety evaluation of BacoMind (TM) in healthy volunteers: A phase I study. Phytomedicine. 2007;14(5):301-8.
32. Joshua Allan J, Damodaran A, Deshmukh NS, Goudar KS, Amit A. Safety evaluation of a standardized phytochemical composition extracted from *Bacopa monnieri* in Sprague-Dawley rats. Food and Chemical Toxicology. 2007;45(10):1928-37.
33. Sumathy T, Govindasamy S, Balakrishna K, Veluchamy G. Protective role of *Bacopa monnieri* on morphine-induced brain mitochondrial enzyme activity in rats. Fitoterapia. 2002;73(5):381-5.
34. Sumathy T, Subramanian S, Govindasamy S, Balakrishna K, Veluchamy G. Protective role of *Bacopa monnieri* on morphine induced hepatotoxicity in rats. Phytotherapy Research. 2001;15(7):643-5.
35. Sumathi T, Niranjali Devaraj S. Effect of *Bacopa monnieri* on liver and kidney toxicity in chronic use of opioids. Phytomedicine. 2009;16(10):897-903.
36. Rauf K, Subhan F, Abbas M, Badshah A, Ihsanullah, Samiullah. Effect of Bacopasides on acquisition and expression of morphine tolerance Phytomedicine. 2011:In press.
37. Sumathi T. Inhibitory Effect of *Bacopa monnieri* on morphine Induced Pharmacological Effects in Mice. Natural Product Sciences. 2007:46-53.
38. Sumathi T, Nayeem M, Balakrishna K, Veluchamy G, Devaraj SN. Alcoholic extract of '*Bacopa monnieri*' reduces the in vitro effects of morphine withdrawal in guinea-pig ileum. J Ethnopharmacol. 2002 Oct;82(2-3):75-81.
39. Gohil KJ, Patel JA. A review on *Bacopa monnieri*: Current research and future prospects. International Journal of Green Pharmacy. 2010;4(1):1.
40. Phrompittayarat W, Putalun W, Tanaka H, Jetiyanon K, Wittaya-areekul S, Ingkaninan K. Comparison of various extraction methods of *Bacopa monnieri*. Naresuan Univ J. 2007;15:29-34.
41. Subhan F, Karim N, Gilani AH, Sewell RDE. Terpenoid content of Valeriana wallichii extracts and antidepressant like response profiles. Phytotherapy Research. 2010;24(5):686-91.
42. Dar A, Channa S. Calcium antagonistic activity of *Bacopa monnieri* on vascular and intestinal smooth muscles of rabbit and guinea-pig. Journal of Ethnopharmacology. 1999;66(2):167-74.
43. Russo A, Izzo AA, Borrelli F, Renis M, Vanella A. Free radical scavenging capacity and protective effect of *Bacopa monnieri* L. on DNA damage. Phytotherapy Research. 2003;17(8):870-5.
44. Russo A, Borrelli F, Campisi A, Acquaviva R, Raciti G, Vanella A. Nitric oxide-related toxicity in cultured astrocytes: effect of *Bacopa monnieri*. Life Sciences. [doi: DOI: 10.1016/S0024-3205(03)00476-4]. 2003;73(12):1517-26.
45. Pogun S, Baumann M, Kuhar M. Nitric oxide inhibits [3H] dopamine uptake. Brain research. 1994;641(1):83-91.
46. Calignano A, Persico P, Mancuso F, Sorrentino L. Endogenous nitric oxide modulates morphine-induced changes in locomotion and food intake in mice. European Journal of Pharmacology. 1993;231(3):415-9.
47. Kastera MP, Budnia J, Santosb ARS, Rodrigues ALS. Pharmacological evidence for the involvement of the opioid system in the antidepressant-like effect of adenosine in the mouse forced swimming test. European Journal of Pharmacology. 2007;576(1):91-8.
48. Listos J, Talarek S, Poleszak E, Wróbel A, Fidecka S. Attenuating effect of adenosine receptor agonists on the development of behavioral sensitization induced by sporadic treatment with morphine. Pharmacol Biochem Be. [doi: 10.1016/j.pbb.2011.01.019]. 2011;98(3):356-61.
49. Sahoo PK, D. Pradhan, Behera P. Neuroprotective Effect of *Bacopa monnieri* leaf extract targeted at Adenosine Receptor In Diabetic Neuropathic Pain. Journal of Pharmacy Research. [Adenosine receptor; *Bacopa monnieri*; Diabetes; Neuropathic pain.]. 2010;3(8):1806-9.
50. Sumathi T. Inhibitory Effect of *Bacopa monnieri* on morphine Induced Pharmacological Effects in Mice. Natural Product Sciences. 2007.
51. Filip M, Alenina N, Bader M, Przegaliński E. REVIEW: Behavioral evidence for the significance of serotoninergic (5-HT) receptors in cocaine addiction. Addiction Biology. 2010;15(3):227-49.
52. Christophe L, Salomon L, YvetteTorrens, JacquesGlowinski, Jean-PolTassin. Drugs of Abuse Specifically Sensitize Noradrenergic and Serotonergic Neurons Via a Non-Dopaminergic Mechanism. Neuropsychopharmacology.

2008;33:1724-34.
53. Capasso A. Involvement of Serotonin in the Acute Dependence Produced by ì, and K Opioid Agonists Letters in Drug Design & Discovery. 2009;6:8-12.
54. Kastera MP, Santosb ARS, Rodriguesa ALS. Involvement of 5-HT1A receptors in the antidepressant-like effect of adenosine in the mouse forced swimming test. Brain Research Bulletin. 2005;67(1):53-61.
55. Shibasaki M, Kurokawa K, Ohkuma S. Role of á2/ä subunit in the development of morphine-induced rewarding effect and behavioral sensitization. Neuroscience. [doi: 10.1016/j.neuroscience.2009.07.017]. 2009;163(3):731-4.
56. Michaluk J, Karolewicz B, Antkiewicz-Michaluk L, Vetulani J. Effects of various Ca2+ channel antagonists on morphine analgesia, tolerance and dependence, and on blood pressure in the rat. European Journal of Pharmacology. 1998;352(2-3):189-97.
57. Sumathy T, Subramanian S, Govindasamy S, Balakrishna K, Veluchamy G. Protective role of *Bacopa monnieri* on morphine induced hepatotoxicity in rats. Phytother Res. 2001 Nov;15(7):643-5.
58. Sumathy T, Govindasamy S, Balakrishna K, Veluchamy G. Protective role of *Bacopa monnieri* on morphine-induced brain mitochondrial enzyme activity in rats. Fitoterapia. 2002 Aug;73(5):381-5.
59. Das A, Shanker G, Nath C, Pal R, Singh S, Singh HK. A comparative study in rodents of standardized extracts of *Bacopa monnieri* and Ginkgo biloba: Anticholinesterase and cognitive enhancing activities. Pharmacol Biochem Be. 2002;73(4):893-900.
60. Pravina K, Ravindra KR, Goudar KS, Vinod DR, Joshua AJ, Wasim P, et al. Safety evaluation of BacoMind(TM) in healthy volunteers: A phase I study. Phytomedicine. 2007;14(5):301-8.
61. Calabrese C, Gregory W, Leo M, Kraemer D, Bone K, Oken B. Effects of a standardized *Bacopa monnieri* extract on cognitive performance, anxiety, and depression in the elderly: a randomized, double-blind, placebo-controlled trial. The Journal of Alternative and Complementary Medicine. 2008;14(6):707-13.
62. Raghav S, Singh H, Dalal P, Srivastava J, Asthana O. Randomized controlled trial of standardized *Bacopa monnieri* extract in age-associated memory impairment. Indian Journal of Psychiatry. 2006;48(4):238.

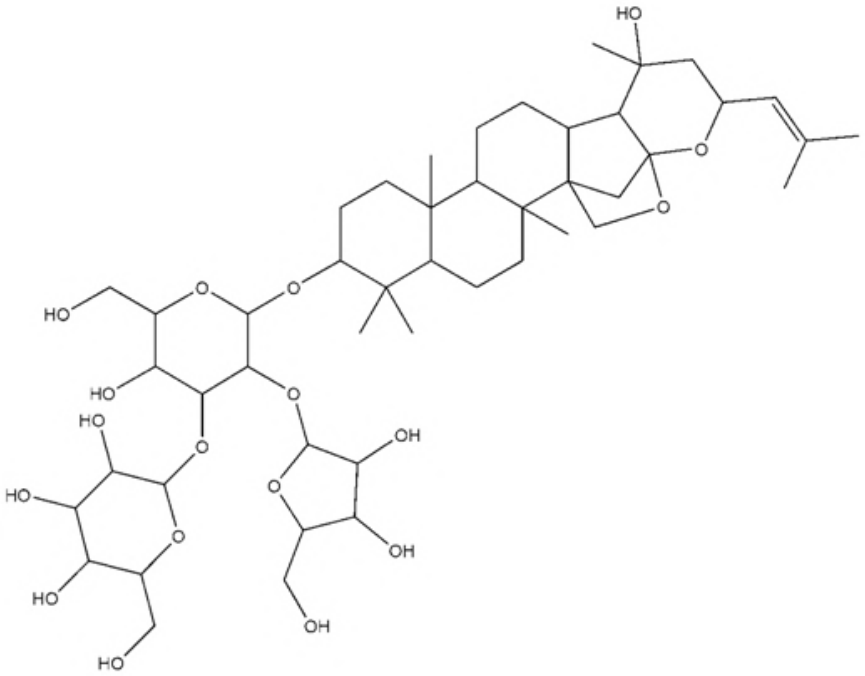

Fig 1. Bacopside A

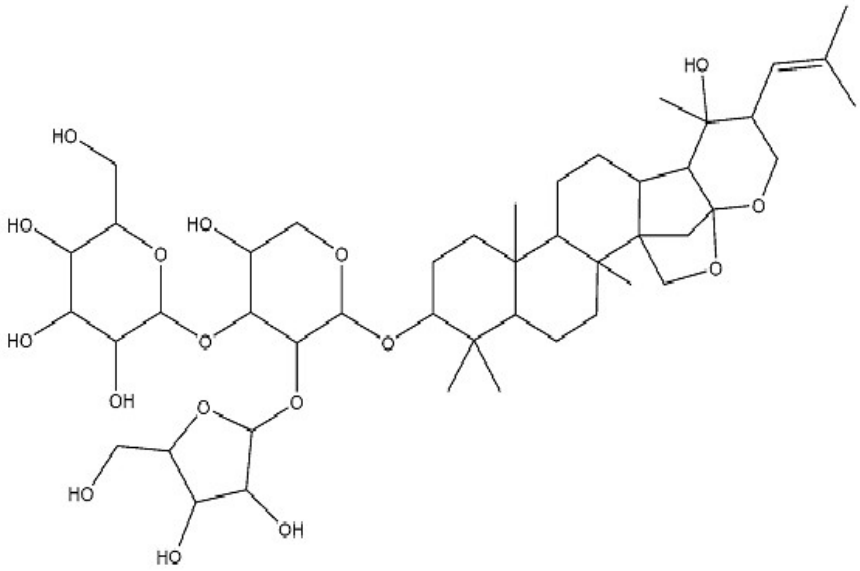

Fig 2. Bacosaponin C

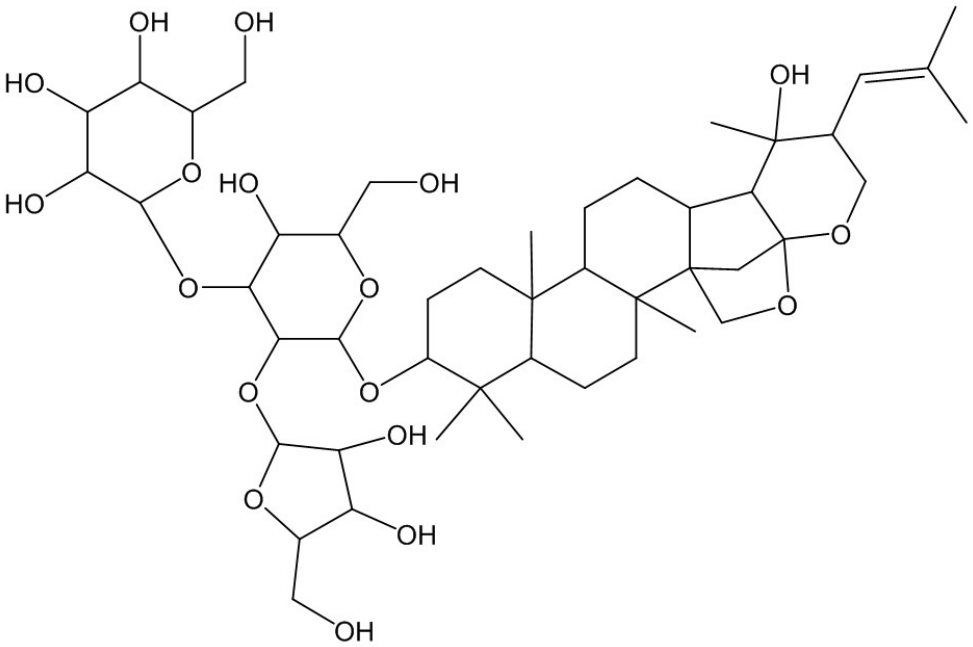

Fig 3. Bacopside II

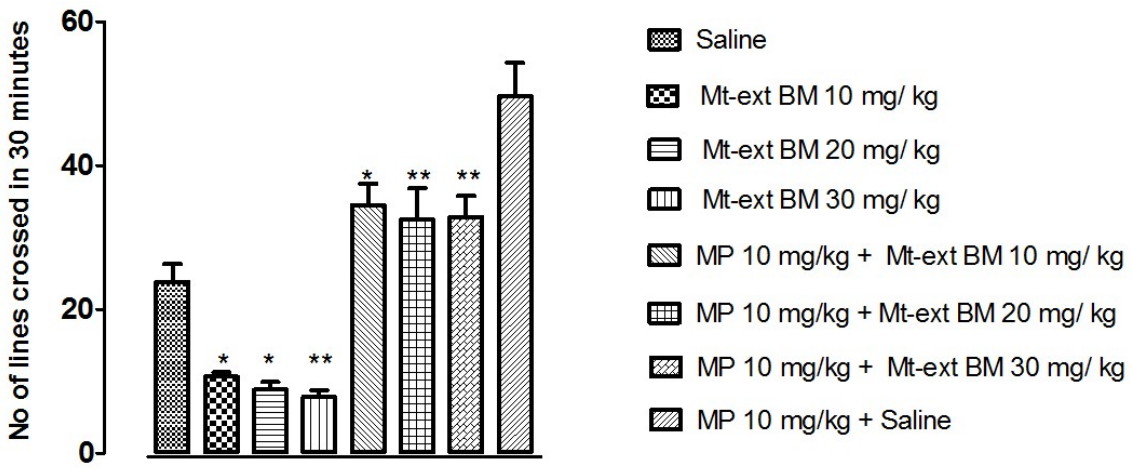

Figure 4. Effect of Mt-ext BM (10, 20 and 30 mg/kg orally) on locomotor activity in saline and morphine (10 mg/kg intraperitoneally) in mice (n=6).
Values are expressed as mean±SEM, applying ANOVA followed by Tukey's Post hoc analysis.

Treatment	Striatal tissue concentrations†					
	NA	DA	DOPAC	HVA	5HT	5HIAA
Saline	105±23	4225±235	637±42	22±7	463±46	133±23
Mt-ext BM 10 mg/kg	89±14	4496±229	368±66	17±2	281±49	120±14
Mt-ext BM 20 mg/kg	110±26	3913±321	667±14	19±2	451±14	143±35
Mt-ext BM 30 mg/kg	84±12	3592±392	847±44	18±4	431±34	169±29

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

Treatment	Striatal tissue concentrations†					
	NA	DA	DOPAC	HVA	5-HT	5-HIAA
Saline	105±23	4225±235	637±42	22±7	463±46	133±23
MP (10 mg/kg)	114±22	6180±842	1435±362	564±143	401±69	287.15
MP (10 mg/kg) + Mt-ext BM 10 mg/kg	84±28	4496±235*	561±15**	97±41***	347±51	166±04**
MP (10 mg/kg) Mt-ext BM 20 mg/kg	79±8	3913±321**	323±23***	120±25***	253±26	119±13***
MP (10 mg/kg) + Mt-ext BM 30 mg/kg	81±11	3619 ±189***	189±29***	71±18***	263±34	97±10***

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.