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Full Length Research Paper

Effect of acute and sub chronic use of *Bacopa monnieri* on dopamine and serotonin turnover in mice whole brain

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Bacopa monnieri **(BM) is a perennial herb, with a historic nootropic image and utility in ayurvedic system of medicine, for the management of various central nervous system disorders like epilepsy, depression and memory deficit amongst others. We investigated the effects of acute and sub chronic (one week) treatment of BM methanolic extract (Mt-ext BM) on dopamine (DA) and serotonin (5-HT) turn over in mice whole brain. Mt-ext BM was screened on high performance liquid chromatography (HPLC) with ultraviolet (UV) detection for the quantification of BM major bioactive compound, Bacoside A, mainly comprising of Bacopasaponin C, Bacoside A3, and Bacopaside ll. For acute study, mice groups were administered single dose of 10, 20 or 30 mg/kg of Mt-ext BM orally, while in sub chronic study separate groups received single daily dose of 10, 20 or 30 mg/kg of Mt-ext BM orally for one week. Animals were killed 1 h after the dose by decapitation, and whole brains were excised and analyzed on HPLC coupled with electrochemical detector for changes in DA, 5-HT, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5 hydroxyindolacetic acid (5HIAA). Our results show that both acute and sub chronic oral administration of Mt-ext BM have no significant effect on DA and 5-HT turn over. The neurotransmitters data reflects safety of the BM in acute and sub chronic uses from DA and 5-HT modulation and subsequent pre disposition to neuropsychiatric problems. Although more studies are warranted to explore BM role in DA and 5-HT interplay in specified brain regions.**

Key words: Bacoside A, *Bacopa monnieri*, high performance liquid chromatography (HPLC), dopamine (DA), serotonin (5-HT).

INTRODUCTION

Normal behavior is an outcome of a discreet and sensitive balance of neurotransmitters in specified brain areas (Stricker and Zigmond, 2010). The maintenance of this very delicate balance is imperative as neurotransmitters modulation control developmental, behavioral, emotional and hormonal states directly or indirectly (Berridge, 2004; Stricker and Zigmond, 2010). The dopamine (DA) being major neurotransmitter is responsible for emotional balance and regulation of human cognition (Colzato et al., 2010). DA mainly controls food intake, endocrine functions, emotional states, reward and

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and sexual behavior (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999). Likewise, serotonin (5-HT) also plays an integral and pivotal role in controlling emotions, food intake, anxiety behaviors and endocrine functions (Berger et al., 2009; Charney et al., 1990; Ren-Patterson et al., 2006; Shah et al., 2003). The disturbance of this delicate balance between DA and 5-HT leads to many pathological conditions translating itself in the form of multiple neuropsychiatric disorders (Esposito, 2006; Wood and Wren, 2008). 5-HT per se is an important neurotransmitter and any disturbance of 5-HT may cause series of neurologic and psychiatric illnesses, like hallucination, anxiety, depression and migraine (Hou et al., 2006). Phytochemicals that either disrupts balance between DA and 5-HT, or modulate DA and 5-HT metabolism also leads to behavioral and endocrine disturbances and subsequent pathologies (Farias et al., 2010; Ganong, 1980; Shah et al., 2003; Verma et al., 2007). Bacopa monnieri (BM) is a perennial herb, from Scrophulariaceae family, found in marshy places in various parts of Indo-Pak Subcontinent and Europe (Qureshi and Raza Bhatti, 2008). BM has been used in ayurvedic system of medicine for the last 3000 years, for various ailments (Gohil and Patel, 2010) including insomnia, anxiety, epilepsy (Mathew et al., 2010a,b), asthma (Gohil and Patel, 2010) and also clinical management of gastric and neuropathic diseases (Gohil and Patel, 2010).

BM has many active compounds, but the major bioactive compound is Bacoside A which is in-fact a mixture of four compounds, that is, Bacoside A3 (Figure 1), Bacoside II (Figure 2), Bacopasaponin C (Figure 3) and an isomer of Bacopasaponin C (Deepak et al., 2005). BM also contains Bacoside B which is chemically an isomer of Bacoside A (Gohil and Patel, 2010). Recently, BM has been reported to have protective effect against morphine effects (Sumathy et al., 2002), antidepressant (Abbas et al., 2011), anxiolytic, mast cell stabilizing properties (Samiulla et al., 2001), antiepileptic (Mathew et al., 2010b), calcium channel inhibitory effect (Dar and Channa, 1999), antinociceptive (Subhan et al., 2010a), anti-ulcer effect (Sairam et al., 2001) and strong anti gastrointestinal (GIT) motility activity (Subhan et al., 2010b).

BM has been found to be highly effective as an adaptogen, as it has been reported to normalize acute and chronic stress induced corticosterone changes in rats (Sheikh et al., 2007). Moreover, BM has also been reported to normalize noradrenaline (NA), 5-HT, and DA in cortex and hippocampus of rats, in both acute and chronic unpredictable stress (Sheikh et al., 2007).

Keeping in view the neuropharmacological profile of BM, this study was designed to examine the contents of Bacopaside A in the methanolic extract of indigenously found BM and also to assess the effect of acute and sub chronic (one week) administration of the methanolic extract on DA and 5-HT turn over in mice whole brain.

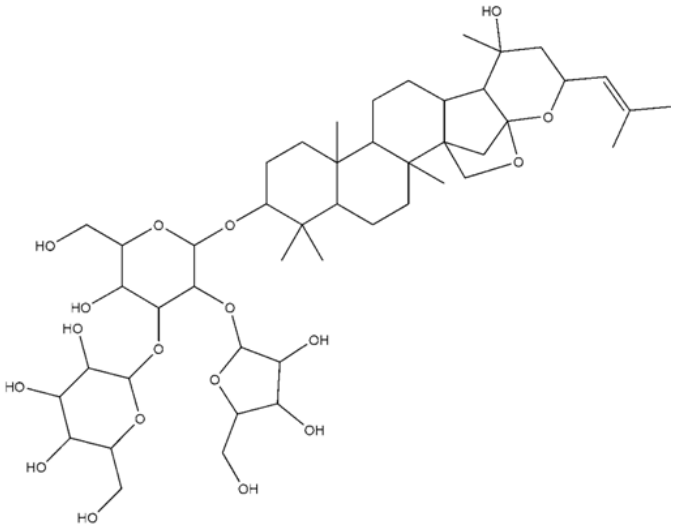

Figure 1. Bacoside A3.

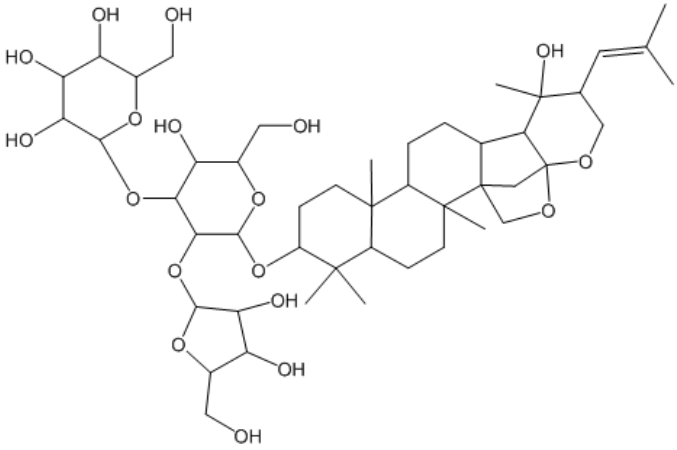

Figure 2. Bacopaside II.

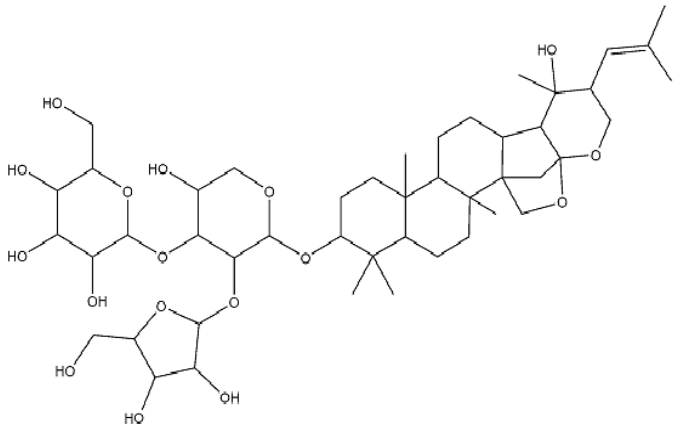

Figure 3. Bacopasaponin C.

MATERIALS AND METHODS

Animals

Mice (Balb C) weighing 23 to 28 g of either sex, bred in the Animal House Facility, Department of Pharmacy, University of Peshawar were used in the experiment. All procedures were approved by the Ethical Committee, Department of Pharmacy, University of Peshawar. Animals were kept at approved standards of temperature 22 ± 2°C, with 12 h light/12 h dark cycle and free access to food and water.

Chemicals and drugs

Acetonitrile (HPLC grade) sodium octane sulphate (Fischer scientific), and sodium dihydrogen sulphate (Fischer scientific) were acquired from Merck local distributor in Peshawar, Pakistan. Morphine sulphate was generously gifted through legal channel by PDH Laboratories, Lahore, Pakistan. Commercial grade *n*-hexane, acetone, methanol and *n*-Butanol used for plant extraction were acquired from Haq chemicals, Peshawar, Pakistan. All drugs were dissolved in normal saline, while control group received normal saline. Bacopasaponin C, Bacoside A_3, and Bacopaside ll were generously gifted by Professor Dr. Ikhlas A. Khan, School of Pharmacy University of Mississippi, U.S.A., DA, 5 hydroxytryptamine (5-HT), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5 hydroxyindolacetic acid (5HIAA) were acquired from sigma local distributor in Peshawar.

Plant

The plant was collected from Rumalee stream near Quaide Azam University, Islamabad, Pakistan in April. The plant identity was authenticated (voucher no. 7421) by Professor Dr. Muhammad Ibrar, Department of Botany, University of Peshawar, Pakistan. The plant aerial parts were shade dried coarsely ground. Then 500 g of this coarsely ground powdered plant was extracted with *n*-Hexane, and then with acetone to remove fats and chlorophyll type pigments. The powdered plant was then further extracted with commercial grade methanol in Soxhlet apparatus. The methanolic fraction of the plant (yield 14.37 g) was used during the experiments. Extracts were dissolved in normal saline.

Chromatographic analysis of BM methanolic extract (Mt-ext BM) for Bacoside A major components

Bacopaside ll, Bacoside A3, and Bacopasaponin C were quantified in methanolic extract of the plant using high performance liquid chromatography with ultraviolet (UV) detection using Phrompitayyarat method with slight modifications (Phrompittayarat et al., 2007). The HPLC system consisted of LC-20AT double pump (Shimadzu, Japan) and SPD-20A UV Visible detector, and C18 column (250 × 4.6 mm, 5 µm particle size) a Rheodyne injector with 20 µl loop. Briefly, 50 mg of Mt-ext BM was dissolved in 10 ml methanol (HPLC Grade) and was then centrifuged for 10 min at 3000 rpm. Then, this solution was filtered through 0.45 µ filter. The mobile phase consisted of phosphoric acid (0.2%) and acetonitrile (60:40 v/v), pH adjusted to 3.0 with 3 M NaOH. The HPLC system was run at 0.6 ml/min flow rate using wavelength of 205 nm. All the peaks were secured in 22 min run time (Figure 4). The peaks were confirmed by addition of standards Bacosides to the analyzing samples.

Treatment protocol

Animals (Balb C) mice of both sexes were divided in eight groups, each having six animals. In acute treatment plan, one group received saline treatment, while the rest groups received, 10, 20, and 30 mg/kg of Mt-ext BM orally. Likewise in chronic treatment plan, one group received saline for one week, while rest three groups individually received 10, 20, and 30 mg/kg of Mt-ext BM orally for seven days.

Acute treatment groups

In these experiments, mice (23 to 27 g) groups were given single dose of 10, 20, and 30 mg/kg of Mt-ext BM orally. Control group (n = 6) received normal saline. Animals were killed one hour after the dose by decapitation, and whole brains were excised and stored at -80°C and were later analyzed on HPLC coupled with electrochemical detector for changes in DA, 5-HT, DOPAC, HVA and 5HIAA.

Sub chronic treatment groups

In this experiment, mice groups received single dose of 10, 20, and 30 mg/kg Mt-ext BM orally for seven days. On day seven, 1 h after the dose administration, all were killed 1 h after the dose by decapitation, and whole brains were excised and stored at -80°C and were later analyzed on HPLC coupled with electrochemical detector for changes in DA, 5-HT, DOPAC, HVA and 5 5HIAA. The control group (n = 6) received normal saline for seven days.

Chromatographic analysis of mice whole brain for DA and 5-HT turn over

Whole brain DA and 5-HT levels were quantified by a HPLC system coupled with electrochemical detector using the method of Rauf et al. (2011). Briefly, the system consisted of a LC-20AT double pump (Schimadzu, Japan), a communication bus module model (Model 20A), MD-150 column (3 × 150, 3 µm), a Rheodyne injection port with 20 µl injection loop and Choulchem III detector (model ESA 5300). The Choulchem III detector was coupled with a guard cell model (Model 5020) and analytical cell (model5011 A). The guard cell was run at an operating potential of 500 mv, while electrodes 1 and 2 of the analytical cell were set at +200 and -200, respectively with sensitivity of 2 µA. The mobile phase having a pH of 3, was prepared containing, 2.3 mM sodium 1 octane sulphonic acid, 94 mM sodium dihydrogen orthophosphate, 40 mM citric acid, 50 µM ethylenediaminetetraacetic acid (EDTA), and 10% acetonitrile. The mobile phase was run at a flow rate of 0.6 ml/min and all neurotransmitters peaks were obtained in 10 min (Figure 5).

Statistical analysis

The results were analyzed using ANOVA, and $p < 0.5$ was considered to be statistically significant.

RESULTS

Quantification of Bacoside A major components in Mt-ext BM

The HPLC analysis revealed that Mt-ext BM contained

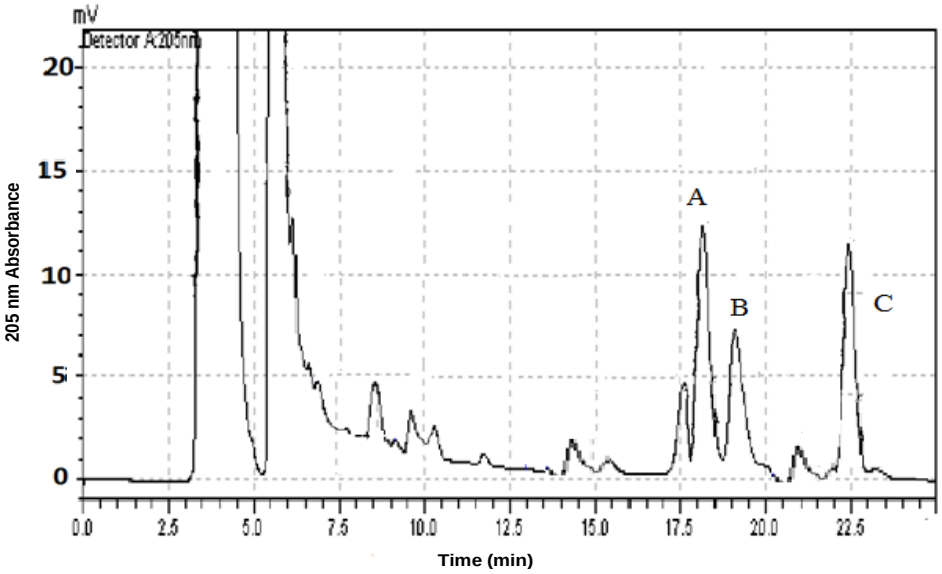

Figure 4. Chromatograms showing Bacoside A3, Bacopaside II and Bacopasaponin C as Peaks A, B and C in methanolic extract of BM. Peaks were confirmed by addition of individual standards.

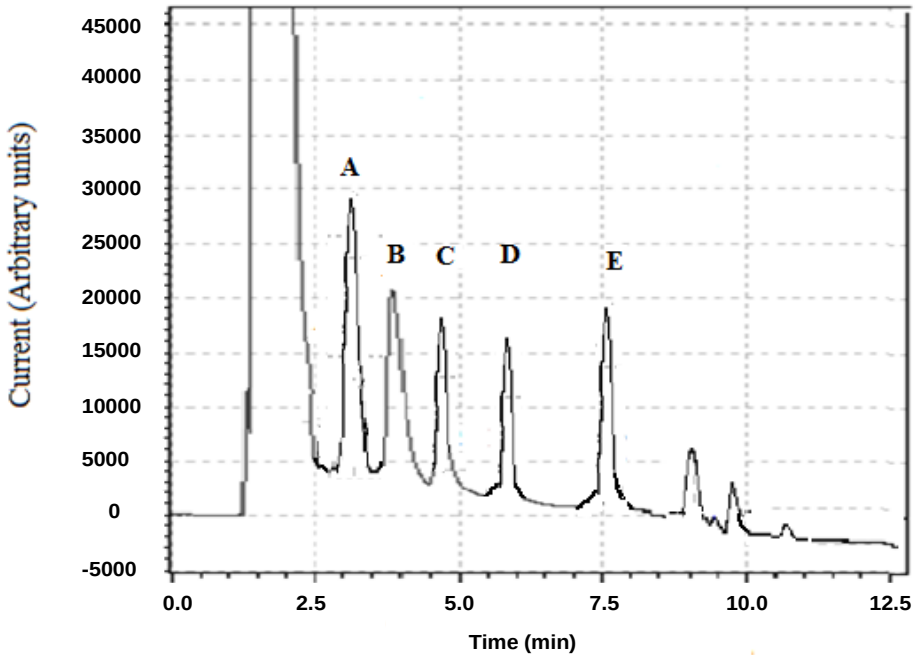

Figure 5. Chromatogram showing neurotransmitters peaks of saline treated mice whole brain. Peaks A, B, C, D, and E reflect DOPAC, DA, 5HIAA, HVA, and 5-HT.

Bacoside A, major components (Figure 1). Our results further indicated that the quantity of these Bacopasides were 1.3 µg (Bacopasaponin C), 4 µg (Bacoside A_3), and 1.3 µg (Bacopaside ll), in each milligram of Mt-ext BM.

Effect of acute administration of Mt-ext BM on whole brain neurotransmitters

As shown in the Table 1, acute administration of Mt-ext

Table 1. Effect of acute administration of BM on dopamine, serotonin and their metabolites in mice whole brain.

Neurotransmitter†	Saline	Mt-ext BM		
		10 mg/kg	20 mg/kg	30 mg/kg
DA	700 ± 73	731 ± 12	971 ± 13	1014 ± 17
DOPAC	39 ± 5	27 ± 6	46 ± 18	31 ± 8
HVA	77 ±12	51 ± 25	82 ± 16	112 ± 11
5-HT	216 ± 28	181 ± 22	300 ± 59	282 ± 45
5HIAA	75 ± 3	65 ± 11	78 ± 22	57 ± 10

†Neurotransmitter concentrations (ng/100 mg of wet tissue) are expressed as mean ± standard error of mean (SEM). Values were compared using ANOVA.

Table 2. Effect of sub chronic (one week) administration of BM, on dopamine, serotonin and their metabolites in mice whole brain.

Neurotransmitter†	Saline	Mt-ext BM		
		10 mg/kg	20 mg/kg	30 mg/kg
DA	570 ± 98	900 ± 170	*567 ± 50*	830 ± 98
DOPAC	39 ± 05	35 ± 04	58 ± 08	29 ± 04
HVA	62 ±12	91 ± 17	58 ± 15	80 ± 14
5-HT	183 ±19	220 ± 40	226 ± 37	183 ± 19
5HIAA	112 ± 29	96 ± 13	58 ± 05	46 ± 06

†Neurotransmitter concentrations (ng/100 mg of wet tissue) are expressed as mean ± standard error of mean (SEM). Values were compared using ANOVA.

Table 3. Effect of acute administration of BM on dopamine and serotonin turn over.

Neurotransmitter†	Saline	Mt-ext BM		
		10 mg/kg	20 mg/kg	30 mg/kg
HVA/DA	0.11 ± 0	0.05 ± 0	0.19 ± 0	0.12 ± 0
DOPAC/DA	0.07 ± 0	0.03 ± 0	0.06 ± 0	0.03 ± 0
HVA+DOPAC/DA	79.53 ± 14	51 ± 12	182 ± 24	113 ± 06
5HIAA/5-HT	0.47 ± 0	0.36 ± 0	0.34 ± 0.1	0.24 ± 0

†Neurotransmitter turn over as concentrations (ng/100 mg of wet tissue) are expressed as Mean ± standard error of mean (SEM). Values were compared using ANOVA.

BM had no significant effect on DA, 5-HT and their metabolites HVA, DOPAC, and 5HIAA in mice whole brain. Moreover, there was also no change in the ratios of HVA/DA, DOPAC/DA, HVA+DOPAC/DA and 5HIAA/5-HT as compared to saline treatment group (Table 3).

Effect of sub chronic administration of Mt-ext BM on whole brain neurotransmitters

Sub chronic (7 days) administration of Mt-ext BM also failed to alter DA, 5-HT and their metabolites DOPAC, HVA and 5-HIAA in mice whole brain (Table 2). Furthermore, there was also no significant effect in the ratio of HVA/DA, DOPAC/DA, HVA+DOPAC/DA and 5HIAA/5-HT as compared to saline treatment group (Table 4).

DISCUSSION

BM has a reputed image as nootropic herb with long history of clinical usage in ayurvedic system of medicine (Russo and Borrelli, 2005). Currently, herbal products containing BM are available in east and west, for various cognitive disorders (Morgan and Stevens, 2010; Pravina

Table 4. Effect of sub chronic administration of BM on dopamine and serotonin turn over.

Neurotransmitter†	Saline	Mt-ext BM		
		10 mg/kg	20 mg/kg	30 mg/kg
HVA/DA	0.12 ± 0	0.13 ± 0	0.08 ± 0	0.14 ± 0
DOPAC/DA	0.10 ± 0	0.05 ± 0	0.07 ± 0	0.05 ± 0
HVA+DOPAC/DA	72 ± 08	91 ± 17	57 ± 14	80 ± 1
5HIAA/5-HT	0.34 ± 0	0.48 ± 0	0.46 ± 0	0.29 ± 0

†Neurotransmitters turn over as concentration (ng/100 mg of wet tissue) are expressed as Mean ± standard error of mean (SEM). Values were compared using ANOVA.

et al., 2007) attributed mainly to its bioactive component, that is, Bacoside A.

In this study, HPLC analysis of locally available BM plant showed the presence of all the major components of Bacoside A, that is, Bacopasaponin C, Bacoside A3, and Bacopaside ll calculated as 1.3, 1.4, and 1.3 µg respectively in each milligram of Mt-ext BM.

Assessment of DA and 5-HT turnover can be judged from the rates of accumulation of their metabolites such as DOPAC, HVA and 5-HIAA. In this respect, it has been reported that the ratios of metabolites to neuro-transmitters are more sensitive measure as compared to steady state levels of neurotransmitters (Baldessarini et al., 1992). Agents that increase DA and its metabolites concentration have abuse potential like opiates, cocaine, and compounds that lower DA induce cognitive, behavioral and motor coordination defects (Berridge and Robinson, 1998; Esposito, 2006).

In this study, we found that acute and sub chronic administration of Mt-ext BM did not significantly change DA, DOPAC, HVA and ratios of DOPAC/DA, HVA/DA, in mice whole brain. It reflects the safety and subsequent tolerability of BM in preclinical models. Apparently based on these findings, it can be concluded that BM is free from such untoward and toxic effects.

Since compounds having psychoactive potential modulate the balance of brain 5-HT and DA in mice whole brain at both acute and chronic administration (Ahtee and Attila, 1987; Babbini and Davis, 1972; Fadda et al., 2005; Kuschinsky and Hornykiewicz, 1974; Rethy et al., 1971; Sulzer, 2011; Tejada et al., 2011). In this study, the balance between DA, and its metabolites and 5-HT and its metabolites portray an image that BM maintained monoamines homeostasis and did not induce DA and 5-HT imbalance which contributes to various neurologic, behavioral and hormonal anomalies in both acute and sub chronic use.

Additionally, both acute and sub chronic administration of Mt-ext BM did not significantly alter 5-HT, 5HIAA, or ratio of 5HIAA/5-HT in mice, which further strengthen nootropic action of BM as drugs increasing 5-HT metabo-lism are associated with development and augmentation of retrograde amnesia and cognition problems (Semba et al., 2005). Additionally, drugs lowering 5-HT synthesis induce emotional, behavioral and neurologic abnormalities (Hou et al., 2006). Administration of BM did not alter dopaminergic and sero-tonergic systems in mice, these findings further validate the safety and tolerability of BM usage in ayurvedic system of medicine for various neuropsychiatric disor-ders. Furthermore, BM has been reported to have an adaptogenic effect (Rai et al., 2003), and restores NA, DA and 5-HT modulations induced by acute unpredic-table stress and chronic stress in rats striatum (Sheikh et al., 2007). There are clinical trials that have reported the safety and tolerability of BM in human, thus signifying the safety and lack of central untoward effects that could be attributed to influence neurotransmitters like DA or 5-HT or their metabolism (Calabrese et al., 2008; Nathan et al., 2001; Raghav et al., 2010; Stough et al., 2008; Stough et al., 2001).

Conclusively, acute and sub chronic administration of methanolic extract of BM containing Bacopasides A components, that is, Bacopasaponin C, Bacoside A_3, and Bacopaside ll, have no significant effect on the levels of DA, 5-HT and their metabolites in mice whole brain. It can be implied that BM may not have DA and 5-HT modulation effect in healthy preclinical models, although further long term treatment studies are warranted to assess DA and 5-HT turn over in discrete brain areas through microdialysis.

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