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POTENTIAL DRUG INTERACTION BETWEEN *BACOPA MONNIERA* AND FLUOXETINE: A LAB STUDY

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ABSTRACT

Recently, the interactions of herbal medicine with synthetic drugs came in to focus of particular interest. Herbal medicine may cause significant toxicity with additive, synergistic or antagonistic effects when taken in combination with allopathic drugs. The potential interaction between *Bacopa monniera* extract (BME) and fluoxetine (FXT) was investigated on 2-week, chronic, oral, once daily administration in rats and mice. Healthy, male albino, Wistar rats and mice were divided in to eight groups of six each. Control group-I was administered distilled water. Group II received drug FXT 20mg/kg/day. Group III, IV and V received standardized water soluble BME containing 20% of total bacosides in doses of 20, 40 and 80 mg/kg/day respectively, while Groups VI and VII and VIII received combinations of FXT along with BME in three different doses for 2 weeks. Spontaneous motor activity (SMA), motor coordination, forced swimming test (FST), tail suspension test (TST) and chronic fatigue tests (CFT) were evaluated. Biochemical parameters were checked in blood and homogenized brain tissue samples. Compared to control groups, test groups VI, VII and VIII showed dose dependent reduction in SMA, improvement in motor coordination and a significant reduction in immobility in FST, and CFT With a significantly increased levels of serotonin and noradrenaline in homogenized brain tissue samples of animals receiving combination of BME along with fluoxetine compared to that of control and the groups treated with either BME or fluoxetine alone. There is a possibility of interaction between BME and FXT. The combination proved to be therapeutically synergistic. Precautions are advised while using this combination. Potential interactions of *Bacopa monniera* with newer antidepressants are matter of further study.

Keywords: Herbal drug interactions, Bacopa monniera, synthetic antidepressants.

INTRODUCTION

In recent times, the interest in the use of herbal products and the focus on plant research has grown dramatically in the western world as well as developed countries (Loya *et al*., 2009; Sparreboom *et al*., 2004; Vaidya, 1997). The vast majorities of currently available psychoactive drugs as herbal remedies seem to be a reflection of such a situation. The demand of herbal remedies as psychotherapeutics is greater than ever, having great growth potential in the global market (Dahanukar and Kulkarni, 2000). In the folklore of Indian medicine, several herbal plants have been used traditionally as brain or nerve tonics. One of the most popular of these herbs is *Bacopa monniera* (BM), an outstandingly important medicinal herbs, widely used in orient and becoming increasing popular in the west.

The plant is from a family *Scrophulariaceae* is a small creeping herb with numerous branches, small oblong leaves, and light purple or small and white flowers, with four or five petals. It is found in wetlands throughout the Indian subcontinent in wet, damp and marshy or sandy areas near streams in tropical regions (Barrett and Strother, 1978; Russo and Borrelli, 2005).

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The plant has been used for centuries in the Ayurveda, a holistic system of medicine originating from India, where it has been classified as under 'Medhya rasayana', i.e., medicinal plants rejuvenating intellect and memory (Rai *et al*., 2003) and useful in the treatment of a number of disorders, including neurological conditions, particularly those involving anxiety, depression and rejuvenating intellect and cognition (Husain *et al*., 2007; Singh and Dhawan, 1997).

The herb has long been used medicinally and as an aid to meditation (Kumar, 2006). The entire plant is used medicinally. The plant has been mentioned in several ancient Ayurvedic treatises including the "Charaka Samhita" since 6th century AD, in which it is recommended in formulations for the management of a range of mental conditions including anxiety, poor cognition and lack of concentration, as a diuretic and a tonic for the nervous system and heart (Mukherjee and Dey, 1966). Specific uses include the treatment of asthma, insanity and epilepsy (Chopra, 1958). It has been utilized extensively as a nootropic, digestive aid, and to improve learning, memory and respiratory function (Nadkarni, 1988; Kirtikar and Basu, 1918). The BM extracts and isolated bacosides have been extensively investigated for their neuropharmacological effects (Husain *et al*., 2007; Rastogi, 1990, Aithal and Sirsi, 1961, Malhotra and Das, 1959) namely, anxiolytic effects (Shankar and Singh, 2000; Bhattachrya and Ghosal, 1998), antidepressant activity (Sairam *et al*., 2002), anticonvulsive action (Shanmugasundaram *et al*., 1991) and antioxidant activity (Singh *et al*., 2006; Bafna and Balaraman, 2005; Rohini *et al*., 2004).

The herb is currently being marketed in Asian and western countries as a memory enhancing agent. In light of many reports showing important activities of BME or bacosides, the wide variety of neuropharmacological actions of BM opens up interesting avenues for further research and offers new perspectives in the treatment of many diseases. But there are very few studies reporting the role of BM in the functional regulation of neurotransmitters and their receptors or in herb-herb interactions or herb-drug interactions so far, despite a major caution raised for the same (Izzo, 2004). In recent years, various case reports and clinical studies in herbal drug interactions have been published which provided a consistent evidence that the interactions between herbal medicines and synthetic drugs exist and can have serious consequences (Fugh-Berman, 2000; Fugh –Berman and Ernst, 2001; Izzo and Ernst, 2001, Gohil and Patel, 2007; Patel and Gohil, 2008,Engler *et al*; 2009).

Therefore, it is necessary to consider the possibility of BM-drug interactions and the need for exercising requisite precautions while co-medicating the herb extract with synthetic medications. The present study was undertaken to investigate the possibility of interactions between BM with one of the most commonly prescribed synthetic antidepressant, fluoxetine.

MATERIALS AND METHODS

Plant material

The standardized, *aqueous* extracts of *Bacopa monniera* extracts (BME) containing 20% of bacosides were purchased from commercial supplier; Ansar Industries, Surat Gujarat, India. The extract was identified and authenticated at Bapalal Vaidh Institute, Botanical Research Center, VNSGU, Surat and the voucher specimens were deposited at department of pharmacology, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India.

Preparation of extracts

10 gm of BME was accurately weighed and extracted exhaustively with 90% methanol. The extract was concentrated and successively partitioned with petroleum ether, chloroform, diethyl ether and finally with n-butanol. The extracts were filtered, pooled and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol.

Preliminary phytochemical screening

The freshly prepared extract was subjected to preliminary phytochemical screening for the presence of an active phytoconstituent using standard methods (Rajpal, 2002). A Camag's HPTLC system with an automated TLC sampler Linomat V (Camag, Muttenz, Switzerland) controlled by WinCATS software, with twin-trough glass chambers, and a TLC scanner III was used for the HPTLC analysis.

The plates were developed in 10 x 10 cm twin-trough glass chambers. The mobile phase consisted of chloroform: methanol: water (18: 09: 06 v/v/v) in a twin trough chamber to a distance of 93 mm. The plates was scanned at 540 nm (for BME), using a Camag TLC scanner III. The peaks were recorded for the active phytoconstituents bacosides.

Chemicals

The conventional antidepressant drug used in this study Fluoxetine HCl, in form of active pharmaceutical ingredients (API) was obtained as gift sample on request from Sun Pharma Pvt. Ltd, Mumbai. All the other chemicals used were of analytical grade.

Animals

For the present study, healthy, male, Wistar rats (150-250g) and Swiss, albino mice (25–30 g) were obtained from Jai Research Foundation (JRF), Vapi, Gujarat, India.

Animals were maintained in departmental animal house, grouped and housed in polyacrylic cages with not more than six animal per cage under standard environmental conditions like controlled ambient temperature (25 ± 2^{o}C), humidity (50-60%) and a 12 h light/dark cycle. All animals had free access to standard diet in form of pellets obtained from JRF, Vapi and water *ad libitum*. The animals were acclimatized to laboratory condition for 2 weeks before commencing the experiments. The study was undertaken with due approval of the study protocol by the Institutional Animal Ethical Committee and the approval number was (Ref. No. IAEC/MPC/07/0801 and Registration No. 717/02/a/CPCSEA). All the experimental procedures were conducted in accordance with the CPCSEA guidelines in accordance with 'principles of laboratory animal care'.

Preclinical toxicity tests

Acute oral toxicity studies

The preclinical tests for toxicity were conducted according to the WHO guidelines (WHO, 2002). Male rats and mice were divided in to 2 groups of each. One group was dosed 1000 mg/kg and another group was dosed 2000 mg/kg of BME. The mortality was observed for 24 hours after the dosing. Hence, additional male rats and mice were selected for the study and dosed as mentioned above and observed for 14 days for any mortality. At no sign of mortality, the main study was conducted. Healthy, male Swiss, albino mice and Wistar rats were divided randomly in to eight groups each. Animals were allowed food and water *ad libitum*. The *aqueous* BME, was administered orally in doses of 0.25, 0.5, 1.0, 1.5 and 2.0 g/kg body weight were administered once for experimental groups. The animals were observed for 24-hour period and mortality was observed.

Sub acute toxicity tests

A similar dosing was performed for BME, mentioned above in same fashion for sub acute test and animals were observed for every 24 hours up to 28 days.

Dosage administrations

Each of the extracts was refluxed with methanol. The dried extracts dissolved in distilled water were used for the combinations of herbs and drugs evaluated. The animals were randomly divided into control and experimental groups of eight consisting of 06 animals each. A total of 48 animals were divided in to eight groups consisting of 06 animals per each groups. Group I received dist water served as control, Group II was administered Fluoxetine (20 mg/kg, p.o), Group III, IV and V received BME 20, 40 and 80 mg/kg respectively and the group VI, VII and VIII was administered with the combination of BME in three different doses mentioned above along with a fixed dose of fluoxetine (20 mg/kg). All the solutions were prepared fresh, daily and were administered in animals orally for 2 weeks by intragastric gavages in morning between 8-00-10-00 hours. The administrations were continued further for one more week at the time of chronic fatigue test. In the groups receiving combinations, the herbs were administered orally, closely followed by the respective drug in the doses mentioned above.

Dose calculations

The doses of the drugs were calculated by extrapolating the therapeutic dose to rat or mice dose on the basis of body surface area ratio (conversion factor 0.18 for rats) by referring to the table of "Paget & Barns" (Paget and Barnes, 1964).

Behavioural assessments

All the experiments were performed between 9-00 am- 18-00 pm hours. On the test days, animals were transported to the dimly lit laboratory and left undisturbed for 2 h prior to the testing. Neuropsychopharmacological studies were carried out on animal models of depression for the behavioural assessments.

Gross Behaviour

The test was performed as described earlier (Morpugo, 1971). The procedure involved assignment of the scores on 0-3 point scale as per the average intensity of the phenomenon observed. The test drug was administered one hour before the experiment. There after observations were made at every hour, at 1, 2, 3 and 4 hours. The mice were placed one by one in the centre of three concentric circles drawn by chalk on a rubber sheet diameter 7cm, 9cm and 13cm. The profile measured was checked for the gross behavior changes in CNS or ANS parameters including hyperactivity, irritability, straub tail, stereotypy and tremors, at 1 hour, 2hour 3 hour and maximum up to the 4 hour.

Locomotor activity

The locomotor activity was assessed on 1 and 2-week of administration of test drugs using a photoactometer (Medicraft, Inco, India) as previously reported (Kulkarni, 1999). Each animal was placed individually in activity cage for 10 minutes at an interval of 30 minutes till maximum activity is recorded (up to 3 hours) automatically as the cumulative total counts of spontaneous motor activity of the animal.

Open field Behaviour

The test was carried out in mice using the open field apparatus described earlier (Bhattacharya *et al*., 1999). Each mouse was placed at the same bottom left hand corner, an hour after drug administration and allowed to explore the arena for 5 minutes. The

parameters noted were the number of rearing, number of faecal pellets expelled, number of squares crossed, duration of immobility (freezing time) and the time of initiation.

Motor coordination

The test for motor coordination was carried out on each group of mice as described previously (Dunham and Mija, 1957), using rota rod apparatus (Medicraft, Inco, India). The animals were placed on rotating rod for 5 minutes at the rate of 25 revolutions per minute (rpm), an hour after the administration of drugs and the "Fall off time" was noted digitally.

Forced swimming test

The test was carried out as described formerly (Porsolt et. al 1977a; 1977b). In a standard protocol, one hour after administration of a respective agents on the day preceding the test, each of the rats were placed individually for 15 minutes in a narrow plexiglass cylinder (45× 40 × 30) with 20 to 25 cm water level maintained at 250 C ± 20C. 24 hours later they were made to swim again for 10 minutes, and time of immobility was noted in last 6 minutes of total 8 minutes of the test period The animals were subjected to the test on 1st, 7th and 14th day.

Tail suspension test

The mice were individually suspended 50 cm above the floor by means of an adhesive tape, placed approximately 1 cm from the tip of the tail as per method described previously (Steru *et al*., 1985). The time of immobility was quantified during last 4 minutes of a total test period of 6 minutes.

Chronic fatigue test

A modified behavioural despair test has been employed (Kaur and Kulkarni, 2000) to assess chronic fatigue in each group of animals. The duration of immobility was noted in last 4 minutes of total test period of 6 minutes. The initial 2 minutes was given to let the animal get acclimatized to the stressed situation in jar. This procedure was followed for 7 days. The drugs were administered according to groupings, 1 hour prior to the exposure to stressful stimuli, daily for 7 days. This chronic forced swimming produced depression and fatigue resembling chronic fatigue syndrome (CFS).

Biochemical estimations

The animals were sacrificed and their brains were rapidly removed on dry ice at -20^{o} C, washed with isotonic saline, weighed and were preserved separately as per groups at - 80^{o} C for biochemical estimations.

Preparation of brain tissue

At the time of testing, the brains were thawed, rinsed with isotonic saline, weighed again and homogenized (Homogenizer, REMI, India). The 10% homogenates were prepared in 10 vol. of cold phosphate buffer (10mM, pH 7.4), mingled at 4^{o} C for 20 minutes. The mixture was centrifuged (Centrifuge, REMI, India) at 10,000 rpm for 30 minutes at 0^{o} C and the pellets were re suspended in the same buffer. Ad and NA were measured by enzyme radioimmunoassay using a kit, 2 CAT EIA (BA-10-1500, Labor diagnostics Nord (Germany) and serotonin was estimated by direct estimation method as described previously (Weissback, *et al*, 1958; Udenfriend, 1955a; 1955b).

Statistical analysis

The statistical analysis were performed using a statistical software SPSS (Statistical package for social science, SPSS 15.0 for Windows Evaluation version). Significant differences among groups were analyzed using one way analysis of variance (ANOVA) followed by post-hoc Dunnet t test. All values are presented as mean ± S.E.M (n=6) and for all the data, a probability of less than 0.05 ($P < 0.05$) was considered statistically significant.

RESULTS

Gross behaviour

The data pertaining to the effect of an aqueous BME alone and in combination with fluoxetine on gross behaviour of mice are presented in Table-1-3. The groups of rats administered with the combinations of BME in 20, 40 and 80 mg/kg along with fluoxetine 20 mg/kg respectively, showed significant reductions in hyperactivity and irritability in dose dependent fashions. The effects persisted well over 4 hours after drug administrations. The straub tail response was also attenuated in the same groups of rats administered with combinations of BME and fluoxetine. These responses were quite in contrast to the group administered with fluoxetine 20mg/kg alone that showed increase in hyperactivity, irritability and straub tail phenomenon. The reductions in hyperactivity, irritability and straub tail observed in the groups treated with the combinations of BME and fluoxetine were significantly heightened, specially the last group of rats receiving the highest dose of BME-80 mg/kg along with fluoxetine 20 mg/kg, which showed prominent decrease; compared to that of group receiving BME-20, 40 and 80 mg/kg, alone. Other parameters like narcosis, ataxia, ptosis, exophthalmos lacrimation or stereotypy were not observed and marked as nil.

Locomotor activity

The locomotor activity was reduced significantly in the groups treated with the combinations of BME and fluoxetine on 1 week test and the changes were even more profound after 2 weeks, the effect observed, as early as 0

min onwards and persisting till 3 hours of the test. The results are reported in table 4 and 5.

In 01 –week test, the maximum reduction in locomotor activity, which was statistically significant was noted at 120 min in group VII (99.17 ± 3.45) administered with BME-40 mg/kg along with fluoxetine-20 mg/kg than that of control group (204.67 ± 12.69) treated with only vehicle and compared to that of group II (219.83 ± 26.43) administered with fluoxetine-20 mg/kg, alone. In the same group, reduction was again significant at 180 min (88.33 ± 4.11 compared to that of control group I (240.83 ± 6.87), to that of group II (213.5 ± 20.23) administered with fluoxetine alone and to that of group IV (151.67 ± 5.02) receiving BME-40 mg/kg alone. In group VIII receiving a combination of BME-80 mg/kg and fluoxetine-20 mg/kg, the reduction in locomotor activity was significant from 30 min (192.67 ± 5.78) onwards, the maximum reduction being noted at 120 min (58.17 ± 9.37) compared to that of control group I (204.67 ± 12.69) and that of group II (219.83 ± 26.43). The reduction was even more marked at 180 min (36.00 ±5.13) compared to that of control group I (240.83 ± 6.87).

In 2-week test, the reduction in locomotor activity was observed in all groups at 150 min which was found to be statistically significant except in group III receiving BME-20 mg/kg alone. A significant reduction in locomotor activity was observed at 150 min in group VI (137.67 ± 2.58) receiving combination of BME-20 mg/kg along with fluoxetine-20 mg/kg compared to that of control group (254.50 ± 5.72) and compared to that of group III (238.6 ± 8.34). Similarly a significant decrease was observed in group VII (77.50 ± 10.56) receiving combination of BME-40 mg/kg and fluoxetine-20 mg/kg compared to control group and compared to that of group IV (137.83 ± 9.10) treated with BME-40 mg/kg, alone and likewise in the last group VIII (48.67± 9.66) administered with combination of BME-80 mg/kg and fluoxetine-20 mg/kg compared to that of control grop I (254.50 ± 5.72), at 150 min during test.

Open field activity

Each of the six mice in eight groups of each of the three combinations was placed individually in open field apparatus containing a floor board divided into 36 equal squares and was allowed to explore the arena for 5 minutes, 2 hours after the drug administration. The five parameters noted were, ambulation to explore the area, number of rearing, freezing time, time of initiation and frequency of defecation.

In the present study, the groups of rats treated with combinations of BME and fluoxetine significantly promoted ambulation and the number of rearing, but demoted freezing time, initiation time and frequency of defecation compared to the groups treated either with the BME or fluoxetine alone. The results are reported in table 6.

Motor coordination

Each of the 6 animals in 8 groups was placed on rotating rod revolving at 25 rpm for 5 minutes and "fall off" time in second was noted on 7^{th} and 15^{th} day. In the groups treated with the combinations of BME and fluoxetine, the increase in "fall off" time in rats was noticeable on 7^{th} day in groups VI-VIII receiving combinations of BME-20, 40 and 80 mg/kg, respectively along with fluoxetine-20 mg/kg, but the data were found to be statistically non significant. The results are reported in table 7.

Forced swimming test (FST)

Each of the 48 animals was forced to swim individually for 8 min, in glass containing fresh water up to a prescribed height at room temperature (22^{o} C ± 3^{o} C) and duration of immobility were noted during the final 6 minutes of total 8 minute of the test on 1^{st}, 7^{th} and 15^{th} day, in the three combinations evaluated. The duration of immobility was reduced significantly in groups receiving combination of BME and FXT compared to that of that of control and the groups treated with either BME or fluoxetine alone. The results are reported in table 8.

On 15^{th} day, the reduction in duration of immobility was remarkably significant in group VI (67.83 ± 3.13) receiving combination of BME-20 mg/kg and fluoxetine-20 mg/kg compared to that of control group I (214.17 ± 6.90), to that of group II (34.17 ± 1.70) receiving only fluoxetine-20 mg/kg and to that of group III (98.33 ± 1.41) receiving only BME-20 mg/kg. Similarly, the reduction was significant in group VII (48.50 ± 3.71) receiving combination of BME-40 mg/kg and fluoxetine-20 mg/kg compared to that of control group I (214.17 ± 6.90) and to that of group IV (77.67 ± 0.73) receiving BME-40 mg/kg alone and in group VIII (25.33 ± 2.06) treated with the combination of BME-80 mg/kg and fluoxetine-20 mg/kg compared to that of control group I (214.17 ± 6.90) and to that of group V (62.83 ± 4.07) receiving BME-80 mg/kg alone.

In group VII (57.00 ± 1.41) treated with a combination of BME-40 mg/kg and fluoxetine-20 mg/kg, the reduction in immobility time was significant compared to that of control group I (151.33 ± 10.78), to that of group II (38.67± 1.48) and to that of group IV (81.00 ± 1.71) receiving only BME-40 mg/kg, on 7^{th} day. Similarly, for group VIII (95.33 ± 2.14) receiving BME-80 mg/kg along with fluoxetine 20 mg/kg, the changes seen in time of immobility were significant compared to that of control group I (144 ± 5.98), to that of group II (109.5 ± 2.45) and to that of group V (66.83 ± 1.89) receiving BME-80 mg/kg, alone on 1^{st} day of FST.

Table 1. Effects of *aqueous* BME alone and in combinations with fluoxetine on hyperactivity in mice, after 2 weeks

Hyperactivity				
Groups	**1 hour**	**2 hour**	**3 hour**	**4 hour**
Group I: Control (D.W)	0.48 ± 0.02	0.46 ± 0.02	0.48 ± 0.02	0.50 ± 0.00
Group II: F-20	0.51 ± 0.02	0.60 ± 0.05*	0.67 ± 0.06	0.86 ± 0.06*
Group III:BME-20	0.50 ± 0.00	0.45 ± 0.02	0.48 ± 0.02	0.50 ± 0.00
Group IV: BME-40	0.48 ± 0.02	0.43 ± 0.02	0.48 ± 0.02	0.45 ± 0.02
Group V: BME-80	0.38 ± 0.02*	0.38 ± 0.03	0.32 ± 0.02*[a]	0.32 ± 0.02*
Group VI: BME-20 + F-20	0.50 ± 0.00	0.48 ± 0.02	0.50 ± 0.00	0.50 ± 0.00[a]
Group VII: BME-40 + F-20	0.42 ± 0.02[a]	0.40 ± 0.03[a]	0.43 ± 0.02	0.40 ± 0.00*[a]
Group VIII: BME-80 + F-20	0.35 ± 0.02*[a]	0.32 ± 0.02*[a]	0.33 ± 0.02*[a]	0.25 ± 0.02*[a]

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Dunnett T3 multiple comparisons for 1-hour, 2-hour, 3-hour and 4-hour test.

* Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V; P<0.05 was considered significant for all comparisons.

D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 2. Effects of *aqueous* BME alone and in combinations with fluoxetine on irritability in mice, after 2 weeks

Irritability				
Groups	**1 hour**	**2 hour**	**3 hour**	**4 hour**
Group I: Control (D.W)	0.50 ± 0.00	0.65 ± 0.05	0.58 ± 0.02	0.55 ± 0.02
Group II: F-20	0.75 ± 0.11	0.70 ± 0.04	0.67 ± 0.04	0.61 ± 0.04
Group III:BME-20	0.51 ± 0.02	0.51 ± 0.02	0.53 ± 0.02	0.50 ± 0.00
Group IV: BME-40	0.48 ± 0.02	0.48 ± 0.02	0.46 ± 0.02*	0.46 ± 0.02
Group V: BME-80	0.38 ± 0.02*	0.38 ± 0.02	0.30 ± 0.00*	0.28 ± 0.02*
Group VI: BME-20 + F-20	0.48 ± 0.02	0.50 ± 0.00	0.50 ± 0.00*	0.46 ± 0.02
Group VII: BME-40 + F-20	0.46 ± 0.02	0.43 ± 0.02[a]	0.42 ± 0.02*	0.40 ± 0.00*[a]
Group VIII: BME-80 + F-20	0.33 ± 0.02*	0.30 ± 0.00[a]	0.25 ± 0.00*[a]	0.18 ± 0.16*[ad]

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Dunnett T3 multiple comparisons for 1-hour, 2-hour and 3-hour and 4-hour test.

D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 3. Effects of *aqueous* BME alone and in combinations with fluoxetine on straub tail phenomenon in mice, after 2 weeks

Straub tail				
Groups	**1-hour**	**2-hour**	**3-hour**	**4-hour**
Group I: Control (D.W)	2.67 ± 0.21	3.00 ± 0.00	2.33 ± 0.33	2.67 ± 0.21
Group II: F-20	2.83 ± 0.17	2.83 ± 0.17	2.67 ± 0.33	2.83 ± 0.16
Group III:BME-20	2.83 ± 0.17	2.50 ± 0.22	2.50 ± 0.22	2.67 ± 0.21
Group IV: BME-40	3.00 ± 0.00	2.67 ± 0.21	2.00 ± 0.00	2.83 ± 0.16
Group V: BME-80	2.50 ± 0.22	1.83 ± 0.31	1.16 ± 0.16	1.83 ± 0.16
Group VI: BME-20 + F-20	2.83 ± 0.17	2.16 ± 0.17	3.00 ± 0.00	2.50 ± 0.22
Group VII: BME-40 + F-20	2.00 ± 0.37	2.16 ± 0.17	2.00 ± 0.00	1.67 ± 0.21*[ab]
Group VIII: BME-80+F-20	1.33 ± 0.21*[a]	0.50 ± 0.22[a]	0.67 ± 0.21*[a]	0.16 ± 0.16*[ad]

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Dunnett T3 multiple comparisons for 1-hour, 2-hour, 3-hour and 4-hour tests.

D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 4. Effects of *aqueous* BME alone and in combinations with fluoxetine on locomotor activity in rats, after 1 week

Locomotor activity							
Groups	**0 Min**	**30 Min**	**60 Min**	**90 Min**	**120 Min**	**150 Min**	**180 Min**
Group I: Control (D.W)	254.17± 4.95	243.33± 6.96	239.33± 2.46	233.33± 11.39	204.67± 12.69	224.67± 3.12	240.83± 6.87
Group II: F-20	113.33± 8.69*	187.33± 1.82*	259.00± 51.96	150.00± 21.72	219.83± 26.43	143.50± 21.63	213.50± 20.23
Group III: BME-20	233.50± 13.97	233.00± 10.95	236.50± 10.51	239.33± 12.92	241.33± 11.97	234.50± 9.83	219.67± 14.32
Group IV: BME-40	172.83± 7.83*	110.17± 10.64*	108.67± 1.52*	120.60± 5.30*	110.50± 1.54*	94.17± 3.42*	151.67± 5.02*
Group V: BME-80	486.83± 24.85*	186.67± 17.78	143.00± 17.80	78.50± 2.38*	73.83± 3.49*	68.00± 3.09*	75.67± 3.20*
Group VI: BME-20 + F-20	263.00± 7.80[a]	262.17± 4.75 [a]	263.00± 2.50*	257.83± 2.96	248.00± 2.27	241.00± 3.24	250.17± 3.03
Group VII: BME-40 + F-20	237.17± 20.46[ac]	152.17± 15.08*	110.00± 5.86*	106.17± 6.89*	99.17± 3.45*[a]	117.00± 9.32*	88.33± 4.11*[ac]
Group VIII: BME-80 + F-20	335.17± 33.31[ad]	192.67± 5.78*	129.33± 22.32	68.33± 10.76*	58.17± 9.37*[a]	76.67± 12.98	36.00± 5.13*

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Tukey HSD T3 multiple comparisons for 0 minutes and Dunnett T3 test for 30, 60, 90, 120, 150, 180 minutes.

*Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V; P<0.05 was considered significant for all comparisons.

D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 5. Effects of *aqueous* BME alone and in combinations with fluoxetine on locomotor activity in rats, after 2 weeks

Locomotor activity							
Groups	**0 Min**	**30 Min**	**60 Min**	**90 Min**	**120 Min**	**150 Min**	**180 Min**
Group I: Control (D.W)	350.83± 11.99	250.67± 7.55	249.17± 7.98	231.50± 7.03	251.17± 7.85	254.50± 5.72	264.00± 4.06
Group II: F-20	198.67± 25.75	124.17± 28.16	152.67± 30.48	145.83± 15.31	115.83± 37.4	106.67± 11.63*	127.83± 13.82*
Group III: BME-20	248.17± 8.31*	251.83± 9.48	253.33± 9.37	256.33± 9.15	253.17± 9.51	238.6± 8.34	251.33± 7.83
Group IV: BME-40	217.50± 24.46	173.17± 18.21	176.50± 17.28	171.33± 16.29	151.17± 7.56*	137.83± 9.10 *	149.00± 10.19*
Group V: BME-80	424.83± 12.99	170.67± 17.09	161.00± 12.74*	92.67± 8.79*	74.67± 2.49*	61.17± 2.34*	103.00± 5.13*
Group VI: BME-20 + F-20	152.20± 7.46	160.83± 2.50*[b]	159.33± 3.97 *[b]	155.67± 3.42*[b]	139.67± 2.01*[b]	137.67± 2.58*[b]	150.17± 2.15*[b]
Group VII: BME-40 + F-20	168.33± 7.00*	141.67± 6.96*	81.17± 11.91*[c]	56.50± 5.60*[ba]	64.00± 5.24*[c]	77.50± 10.56*[c]	112.33± 4.75*
GroupVIII: BME-80 + F-20	323.50± 9.45[d]	79.83± 8.77*[d]	60.17± 10.84*[d]	53.83± 14.08*	67.50± 13.05*	48.67± 9.66*[a]	107.83± 11.51*

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Tukey HSD T3 multiple comparisons for 0 minutes, and Dunnett T3 multiple comparisons for 30, 60, 90, 120, 150, 180 minutes; P<0.05 was considered significant for all comparisons.
D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 6. Effects of *aqueous* BME alone and in combinations with fluoxetine in open field activity in mice, after 2 weeks

Parameters					
Groups	**No of squares crossed**	**No. of Rearing**	**Freezing time (Sec)**	**Time of initiation (Sec)**	**No. of faecal pellets expelled**
Group I: Control (D.W)	158.67± 2.74	14.83± 1.14	24.67± 1.45*	53.50± 2.16*	5.33± 0.49
Group II: F-20	165.90± 4.19	21.67± 1.20*	13.83± 0.95*	27.67± 2.26*	4.33± 0.33
Group III:BME-20	171.00± 1.63	15.17± 1.30	13.00± 0.82*	14.83± 0.98*	5.33± 0.99
Group IV: BME-40	187.33± 2.96*	22.50± 0.89*	12.50± 0.43*	13.50± 1.02*	5.83± 0.40
Group V: BME-80	187.67± 2.38*	24.00± 1.18*	10.17± 1.49*	10.33± 0.00*[a]	2.33± 0.67
Group VI: BME-20 + F-20	188.33± 6.19*	25.33± 1.67*[b]	6.33± 0.33*	9.50± 0.22*[ab]	0.67± 0.33
Group VII: BME-40 + F-20	195.30± 2.14*[a]	27.67± 1.02*	5.00± 0.78*	7.50± 0.56*[ac]	0.67± 0.33
Group VIII: BME-80 + F-20	204.00± 7.33*[a]	29.00± 1.59*[a]	3.17± 0.48*	4.00± 0.82*	0.67± 0.33

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Tukey HSD T3 multiple comparisons for number of squares crossed, number of rearing and freezing time and Dunnett T3 for time of initiation and number of faecal pellets expelled. P<0.05 was considered significant for all comparisons.
*Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V; P<0.05 was considered significant for all comparisons.
D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 7. Effects of *aqueous* BME alone and in combinations with fluoxetine on motor coordination in rats

"Fall off" time (in sec)		
Groups	**Day-7**	**Day-15**
Group I: Control (D.W)	43.66 ± 2.23	42.33 ± 1.26
Group II: F-20	36.67 ± 4.42	44.67 ± 3.92
Group III:BME-20	44.83 ± 1.82	50.50 ± 2.14
Group IV: BME-40	53.83 ± 4.24	58.50 ± 2.28*
Group V: BME-80	60.00 ± 0.52*	77.50 ± 11.25
Group VI: BME-20 + F-20	58.67 ± 5.26	58.33 ± 1.93*
Group VII: BME-40 + F-20	70.00 ± 10.01	98.50 ± 10.47*
Group VIII: BME-80 + F-20	113.67 ± 18.55[b]	100.67 ± 10.51*

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Dunnett T3 multiple comparisons for day-7 and day-15. P<0.05 was considered significant for all comparisons.
*Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V; P<0.05 was considered significant for all comparisons.
D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 8. Effects of *aqueous* BME alone and in combinations with fluoxetine on duration of immobility in rats in forced swim test (FST)

Duration of immobility (in sec)			
Groups	**Day -1**	**Day-7**	**Day-15**
Group I: Control (D.W)	144.00 ± 5.98*	151.33 ± 10.78	214.17 ± 6.90*
Group II: F-20	109.50 ± 2.45*	38.67 ± 1.48*	34.17 ± 1.70*
Group III:BME-20	116.67 ± 3.47*	101.33 ± 0.92	98.33 ± 1.41*
Group IV: BME-40	125.83 ± 2.75*	81.00 ± 1.71*	77.67 ± 0.73*
Group V: BME-80	123.83 ± 3.53*	66.83 ± 1.89*	62.83 ± 4.07*
Group VI: BME-20 + F-20	128.83 ± 3.30	95.00 ± 1.98*[a]	67.83 ± 3.13*[ab]
Group VII: BME-40 + F-20	103.00 ± 1.29*[c]	57.00 ± 1.41*[ac]	48.50 ± 3.71*[c]
Group VIII: BME-80 + F-20	95.33 ± 2.14*[a]	35.67 ± 2.06*[d]	25.33 ± 2.06*[d]

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Tukey HSD multiple comparisons for day-1. Dunnett T3 for day-7 and day-15 of forced swim test P<0.05 was considered significant for all comparisons.
*Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V; P<0.05 was considered significant for all comparisons.
D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 9. Effects of *aqueous* BME alone and in combinations with fluoxetine on duration of immobility in mice in tail suspension test (TST)

Duration of immobility (in sec)	
Groups	**Day-15**
Group I: Control (D.W)	278.83 ± 11.18*
Group II: F-20	97.33 ± 1.47*
Group III:BME-20	175.67 ± 6.10*
Group IV: BME-40	133.83 ± 6.98*
Group V: BME-80	125.33 ± 4.79*
Group VI: BME-20 + F-20	91.83 ± 4.20*[b]
Group VII: BME-40 + F-20	71.00 ± 1.84 *[ac]
Group VIII: BME-80 + F-20	49.50 ± 2.51*[ad]

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Tukey HSD multiple comparisons for time of immobility in tail suspension test. P<0.05 was considered significant for all comparisons.
*Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V; P<0.05 was considered significant for all comparisons.
D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 11. Effects of *aqueous* BME alone and in combinations with fluoxetine on adrenalin and noradrenalin concentrations in whole brain of rats

Concentrations in pg/ml		
Groups	**Adrenalin**	**Noradrenalin**
Group I: Control (D.W)	53.83 ± 0.17	38.50 ± 0.22
Group II: F-20	50.17 ± 0.40*	61.17 ± 0.40*
Group III:BME-20	50.17 ± 0.17*	182.33 ± 4.21*
Group IV: BME-40	50.17 ± 0.17*	281.50 ± 7.59*
Group V: BME-80	50.17 ± 0.17*	342.17 ± 8.45*
Group VI: BME-20 + F-20	51.17 ± 0.42*	399.00 ± 4.25*[ba]
Group VII: BME-40 + F-20	50.67 ± 0.42*	605.17 ± 15.36*[ca]
Group VIII: BME-80 + F-20	52.00 ± 0.52*[da]	806.67 ± 3.33*[da]

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Tukey HSD multiple comparisons for adrenalin and noradrenalin concentrations. P<0.05 was considered significant for all comparisons.
* Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V; P<0.05 was considered significant for all comparisons.
D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 10. Effects of *aqueous* BME alone and in combinations with fluoxetine on time of immobility in rats, after 3 weeks in chronic fatigue test (CFT)

Duration of immobility (in sec)							
Groups	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Group I: Control (D.W)	184.60 ± 3.76	229.83 ± 3.80	208 .00 ± 14.78*	218.17 ± 12.11	236.83 ± 1.58*	225.60 ± 10.99	236.83 ± 1.47
Group II: F-20	61.83 ± 2.23 *	43.67 ± 1.63*	32.67 ± 1.91*	55.67 ± 3.86*	40.83 ± 5.30*	52.67 ± 5.52*	61.17 ± 6.81*
Group III: BME-20	169.33 ± 7.50	161.17 ± 8.30*	146.83 ± 8.76*	123.17 ± 8.65*	120.67 ± 6.31*	135.33 ± 14.50*	141.17 ± 14.36*
Group IV: BME-40	166.50 ± 9.20	136.00 ± 8.45*	99.00 ± 0.53*	83.50 ± 4.17*	150.83 ± 9.02*	149.33 ± 9.13*	180.00 ± 8.48*
Group V: BME-80	97.33 ± 1.45*	95.83 ± 1.40*	83.00 ± 3.99*	142.17 ± 14.16	120.33 ± 11.44*	120.50 ± 11.47*	116.00 ± 9.83*
Group VI: BME-20 + F-20	135.83 ± 3.90*[ab]	130.67 ± 4.90*[a]	114.33 ± 5.60*[a]	113.50 ± 5.00*[a]	116.83 ± 4.37*[a]	125.00 ± 5.47*[a]	124.00 ± 7.02*[a]
Group VII: BME-40 + F-20	36.83 ± 0.61* [ac]	36.67 ± 1.54*[c]	30.50 ± 3.00*[c]	24.50 ± 4.07*[c]	19.50 ± 4.57*[c]	31.33 ± 7.09*[c]	47.33 ± 11.20*[c]
Group VIII: BME-80 + F-20	33.33 ± 5.59*[d]	35.50 ± 8.13*	36.50 ± 4.70*[d]	17.67 ± 2.25* [ad]	24.50 ± 2.26*[d]	35.16 ± 5.50*[d]	35.50 ± 3.66*[d]

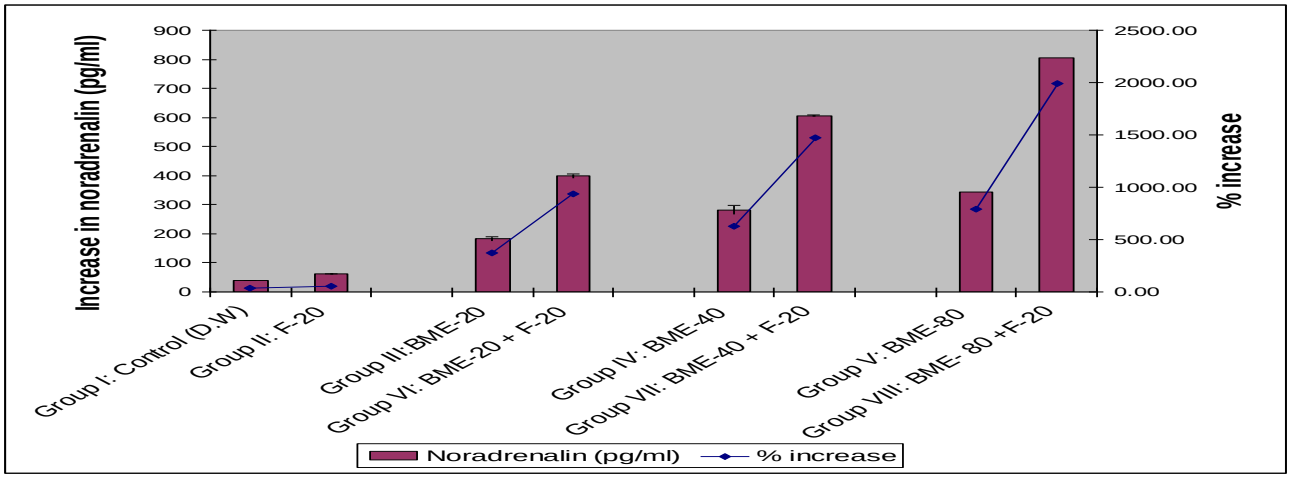
Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Dunnett T3 multiple comparisons for day 1, day 2, day 4, day 5, day 6. Day 7 of chronic fatigue test. Tukey HSD multiple comparisons for day 3 of chronic fatigue test. P<0.05 was considered significant for all comparisons.
* Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V; P<0.05 was considered significant for all comparisons.
D.W stands for distilled water as vehicle, F-20 stands for fluoxetine 20 mg/kg; p.o; BME-20, 40 and 80 represents *Bacopa monniera* extracts, 20 mg/kg, 40 mg/kg and 80 mg/kg respectively; while last three groups VI-VIII represents combinations of BME-20 mg/kg, 40 mg/kg and 80 mg/kg along with fluoxetine 20 mg/kg respectively, p.o.

Table 12. Effects of *aqueous* BME alone and in combinations with fluoxetine on serotonin concentrations in whole brain of rats

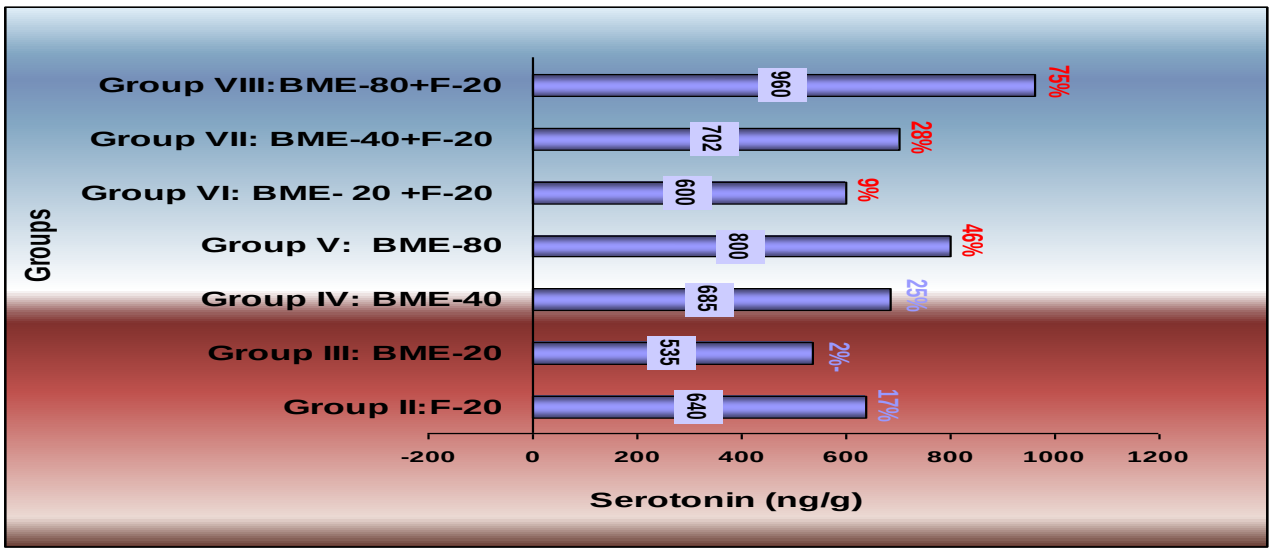
Concentrations in ng/g	
Groups	**Serotonin**
Group I: Control (D.W)	548.33 ± 0.28
Group II: F-20	667.00 ± 3.07*
Group III: BME-20	535.00 ± 2.30
Group IV: BME-40	684.50 ± 1.93*
Group V: BME-80	800.00 ± 2.30*
Group VI: BME-20 + F-20	600.00 ± 2.11*[ba]
Group VII: BME-40 + F-20	701.50 ± 1.63*[a]
Group VIII:BME-80 + F-20	960.00 ± 6.17*[da]

Values are mean ± SEM (n=6), Data analysed by one way ANOVA followed by Tukey HSD multiple comparisons for adrenaline and noradrenaline concentrations; P<0.05 was considered significant for all comparisons. * Significantly different when compared to control; [a] when compared to group II; [b] when compared to group III; [c] when compared to group IV and [d] when compared to group V.

Graph 1. The % increase in noradrenalin in brain of rats administered with BME alone and in combinations with fluoxetine compared to the control group



Graph 2. Effects of *aqueous* BME alone and in combinations with fluoxetine on serotonin concentrations in whole brain of rats



Tail suspension test (TST)

Each of the mice was individually suspended 50 cm above the floor by means of an adhesive tape, placed approximately 1 cm from the tip of the tail. The duration of immobility was assessed during last 4 minute of total 6 minutes of the test. The results are shown in table 9.

In groups receiving the combinations of BME and fluoxetine, mice in groups (II- VIII) showed moderate to high decrease in duration of immobility compared to control group I which were statistically significant. The observed changes were significantly marked in group VI (91.83 ± 4.20) receiving combination of BME-40 mg/kg and fluoxetine-20 mg/kg compared to that of control group I (278.83 ± 11.18) receiving only vehicle, to that of group II (97.33 ± 1.47) receiving only fluoxetine-20 mg/kg and to that of group III (175.67 ± 6.10) receiving only BME-40 mg/kg. Similarly, significant reduction in time of immobility was noted in group VIII (49.50 ± 2.51) receiving combination of BME-80 mg/kg and fluoxetine-20 mg/kg compared to that of control group I (278.83 ± 11.18), to that of group II (97.33 ± 1.47) and to that of group V (125.33 ± 4.79) receiving BME-80 mg/kg alone.

Chronic fatigue test

In groups treated with the combinations of BME and fluoxetine, a moderate to high decrease in the duration of immobility was observed in all groups of rats on all 1 to 7th days of the test compared to that of control group I, as can be viewed from the data presented in table 10.

Adrenalin and noradrenalin estimation

The central catecholamines, Ad and NA in animal brain were estimated by enzyme-linked immunosorbent assay (ELISA). The results are shown in table 11.

In groups receiving the combinations of BME and fluoxetine, a consecutive increase in NA concentrations in rat brains was observed in all groups which were statistically significant compared to that of control. The increase was even more marked and statistically significant for the last three groups receiving combinations of BME and fluoxetine-20 mg/kg, namely, group VI (399.00 ± 4.25) receiving BME-20 mg/kg along with fluoxetine-20 mg/kg compared to that of control group (38.50 ± 0.22) receiving only vehicle, to that of group II (61.17 ± 0.40) receiving only fluoxetine and group III (182.33 ± 4.21) receiving BME-20 mg/kg, alone. The increase was also evident in group VI (605.17 ± 15.36) receiving combination of BME-40 mg/kg and fluoxetine-20 mg/kg compared to that of control (38.50 ± 0.22) receiving only vehicle, to that of group II (61.17 ± 0.40) receiving only fluoxetine-20 mg/kg and also compared to that of group IV (281.50 ± 7.59) receiving BME-40 mg/kg alone. Similarly, the increased was marked in the last group VIII (806.67 ± 3.33) receiving combination of BME-80 mg/kg and fluoxetine-20 mg/kg compared to that of control (38.50 ± 0.22), to that of group II (61.17 ± 0.40) and to that of group V (342.17 ± 8.45) receiving BME-80 mg/kg alone. A marginal decrease was observed in Ad concentrations in all groups of rat brains which were found to be statistically significant for each groups compared to that of control group I of rats. A mild but statistically significant reduction was observed in Ad concentration in the last group VII (52.00 ± 0.52) receiving combination of BME-80 mg/kg and fluoxetine-20 mg/kg compared to that of control group I (53.83 ± 0.17) receiving vehicle alone, to that of group II (50.17 ± 0.40) receiving fluoxetine-20 mg/kg alone and to that of group V (50.17 ± 0.17) receiving BME-80 mg/kg alone. The % increase in the NA concentrations in the groups of rats treated with the combinations BME plus fluoxetine and the groups treated with either BME or fluoxetine alone compared to control is also depicted in graph 1.

The serotonin concentrations in brain in groups of rats receiving combinations of BME and fluoxetine were also estimated by direct estimation as per methods described previously (Weissback *et al*., 1958, Udenfriend, 1955a; 1955b). A successive increase in serotonin concentrations (ng/g) was observed in all groups of rats which was statistical significant compared to that of control group as can be seen from the data presented in table 12.

A marked increase seen in the brain serotonin concentrations was highly significant in the last three groups receiving combinations of BME and fluoxetine, as in group VI (600.00 ± 2.11) receiving BME-20 mg/kg along with fluoxetine-20 mg/kg compared to that of control group (548.33 ± 0.28) receiving only vehicle, to that of group II (667.00 ± 3.07) receiving only fluoxetine-20 mg/kg and to that of group III (535.00 ± 2.30) receiving only BME-20 mg/kg. In terms of % increase in serotonin in brain, the combinations of BME in doses of 20, 40 and 80 mg/kg, respectively along with fluoxetine 20 mg/kg showed about 9%, 28% and 75% increase in the serotonin turn over correspondingly compared to that of control group and also compared to that of groups treated with either fluoxetine or BME alone in the doses mentiond above. The data is depicted in graph 2.

DISCUSSION

The present study revealed the possibility of interactions between BME and one of the conventional antidepressants fluoxetine utilized in the study. Recently, interactions of herbal medicines with synthetic drugs came into focus of particular interest. Contrary to popular belief that nature is always safe, herbal medicines may cause significant toxic effects and even death as these are often combinations of botanical extracts that are assumed

to have additive or synergistic effects (Fugh-Berman, 2000). The use of complementary and alternative medicine in treating neurologic disorders has increased in popularity in response to advances in human alternative and integrative therapies and implied the obvious use of alternative techniques coupled with conventional medicine (Kline, 2002).

The synthetic drugs chosen to study the interactions with BME is the widely prescribed and the most commonly utilised conventional antidepressants available in the market today, namely fluoxetine, It made for an easy choice because, the drugs of most concern for interactions with the herbs were mostly those that people take continuously, especially, for the chronic illness such as depression, a common and disabling disorder which is ranked fourth in a list of the most urgent health problems world wide, having major effects on economic productivity, individual well being and social functioning around the globe, turning out to be a huge burden on individuals, families, and society (Rao and Chen, 2008; Norman and Burrows, 2007).

The reasons for choosing behavioural models like FST, TST and CFT to study the interaction of the herbs with fluoxetine, imipramine and reboxetine and swim stress on NA levels were manifold. First, the plants used in the study were thought to play an important role in behavioural processes related to cognition, mood control, attention and motor performance (Husain *et al*., 2007). Second, the brain region receives a strong modulatory input from monoaminergic neurotransmitters, and many affective disorders were thought to reflect disruption of the regulation of these processes (Arnsten, 1997; Le Moal and Simon, 1991). Furthermore, from a clinical perspective, it has been postulated that abnormal function in the prefrontal cortex in brain is associated with affective disorders. Imaging studies have revealed differences in this brain region between depressed patients and normal controls (Gillin *et al*., 2001; Drevets, 2000). The simultaneous monitoring of neurotransmitters in brain alongwith behavioural assessment allows convergent observations on the neurochemical correlates of antidepressant drug treatment during exposure to stress.

In the study, BM emerged as a tranquillizer or sedative antidepressant which potentiated the antidepressant action of conventional drug fluoxetine as that was apparent from a significant reductions in time of immobility observed in rats in forced swim test (FST), tail suspension test (TST) and chronic fatigue test (CFT). The same was corroborated with the increased serotonin concentrations as estimated in rat brain homogenates of the groups of rats treated with the combinations of BME and fluoxetine compared to that of control group treated with vehicle alone and the groups administered with either of the BME or fluoxetine alone. The combinations were also proved to be therapeutically synergistic in improving chronic fatigue syndrome (CFS).

The doses of BME-40 mg/kg and 80 mg/kg in combinations with fluoxetine were proved to be more potent than the highest dose of BME-80 utilized in the study. While the effects on general motor learning and motor coordination were improved in the groups receiving the combinations of BME and fluoxetine, the sedation was very apparent in the same groups. The effect on locomotor activity was found to be independent from motor coordination or from the effects observed on time of immobility in animals in both FST and TST. The neurotransmitters like serotonin (5-HT), noradrenalin (NA) and γ-amino butyric acid (GABA) were implicated majorly in mediating the actions.

The CNS effects, seen in gross behaviour test were stronger in the groups receiving combinations of the herbal extracts along with the respective drugs than the ones observed in control group or to that of groups administered with the BME extracts or the groups treated with the conventional antidepressant, fluoxetine, The effects of comparatively higher intensity in the groups administered with combinations of herbal extracts with the synthetic antidepressants are suggestive of some interaction at discrete site, at pharmacodynamic level.

The reduced locomotor activity could be attributed to sedative and tranquillizing properties of the BME. The combinations of BME along with fluoxetine have shown clear additive effect, which could be attributed to GABA as the BM was said to affect the GABA-ergic system which involves the nerves and synapses of the central nervous system where memory originates and is stored (Shukla *et al*., 1987). The GABA agonists have already been shown to block the augmented locomotor activity and stereotyped behaviour resulting from dopaminergic stimulation (Agmo *et al*., 1996; Sandoval and Palermo-Neto, 1995; Cott and Engel, 1977). The possible involvement of GABA and PKC in mediating the action of the herb and the additive effect observed along with synthetic antidepressant fluoxetine is an interesting speculation to be explored further.

In the present study, the groups of rats treated with combinations of BME and fluoxetine significantly promoted ambulation and the number of rearing, but demoted freezing time, initiation time and frequency of defecation compared to the groups treated either with the BME or fluoxetine alone. The ambulation in open field test was shown to have some relation to fear and anxiety and rearing was attributed to the curiosity in a rodent (Archer, 1973). From the behavioural observations in the present study, it may be stated that combinations of BME and fluoxetine showed diminished fear, anxiety anxiety and curiosity, in the groups of rats.

In many open field studies, it was commonly assumed that emotionality or fears were inversely related

and that the high emotionality inhibits exploration and low emotionality facilitates it. In one study, it was stated that low and high fear states were associated with low exploration whereas the intermediate fear states were linked with very high exploration (Lester, 1968). In another study, it was suggested that high fear facilitates exploration in an elevated maze (Halliday, 1967). On the other hand, a different study mentioned both the possibilities that fear energized responses competing with exploration (for example, freezing) and that it energized ambulation itself (Broadhurst and Eysenck, 1964). The probable explanation for the effects observed in groups of animals treated with the respective combinations of herbal extracts and drugs could be the low fear state or reduced anxiety exhibited by the actions of herbal extracts the effects which were noticeably augmented with the simultaneous administrations of the respective conventional antidepressants like fluoxetine in the animals.

The increased muscle tone is a common feature of anxiety states in humans. Thus the groups were tested for the effect on muscle coordination and balance in the rota-rod test, on 7th and 15th day after administrations of herbal extracts and drug combinations. The groups of rats receiving BME along with drug fluoxetine showed improved muscle tone compared to the control and rest of the groups compared to that of groups treated with either fluoxetine or BME alone. The effect observed on motor coordination was evidently independent of the locomotor activity which was seen to be reduced in the groups of rats receiving the same combinations. It is possible that the increased muscle coordination though a specific response to antianxiety or antidepressant activities does not correlate with the reduced locomotor activity observed in the animals. But the response was notably enhanced in the groups treated with the combinations of BME and fluoxetine, nonthless.

It has been shown previously that behavioural studies play an important role in the evaluation of antidepressant drugs. The FST and the TST are the non-escapable stressful situations which are widely used to predict the clinical efficacy of many types of antidepressant treatments (Karolewicz and Paul, 2001; Porsolt *et al*., 1978a; 1978b; 1987). The FST was a significant selection because it evokes stress-indiced behavioural depression that is sensitive to modification by antidepressant drug treatments. In the combinations evaluated, that of BME and fluoxetine, the duration of immobility were significantly reduced in animal models of depression. It was reported previously that, although all antidepressant compounds reduce behavioural immobility in FST, specific active behaviours elicited in rats by antidepressant compounds could be attributed to pharmacological drug classes; for example, SSRIs increase swimming, and NA-enhancing drugs increase climbing behaviour in rats (Page *et al*., 1999; Lucki, 1997). Previous studies have also demonstrated complex interactions between the neurochemical effects of forced swimming and the behavioural responses to antidepressant drug treatments and it was reported that fluoxetine treatment altered adaptation of the serotonin response in the lateral septum; changes in extracellular serotonin output were positively correlated with immobility and negatively correlated with swimming but not climbing (Kirby and Lucki, 1997) and that, increased extracellular NA elicited by the FST was negatively correlated with immobility and positively correlated with climbing but not swimming behaviour (Arunrut *et al*., 2009). These observations supported the mediation of these active behavioural responses to antidepressant drugs in the FST by distinct neurotransmitter systems. The groups of rats treated with combinations of BME and fluoxetine showed intense swimming behaviour compared to that of groups treated with respective herbs, drugs or vehicle alone. The co-relation between behaviour of mice in porsolt's test and in test of anxiety, locomotion and exploration has also been studied in mice (Hilakavi and Lister, 1990), which suggested that these effects need not be correlated and that the locomotor stimulant may increase swimming behaviour simply by virtue of its motor activating effect rather than a specific effect on behaviour despair and likewise for the locomotor depressants. Chronic fatigue syndrome (CFS) is a heterogeneous disorder of unknown etiology characterized by fatigue, neuropsychiatry symptoms and related somatic complaints (Kaur and Kulkarni, 2000). Modified behavioral despair test has been employed to assess the efficacy of the test drugs in this disease condition.

In the present study, the chronic, concurrent administrations of BME and fluoxetine in combinations in rats, decreased the duration of immobility significantly and more potently than that of other groups receiving either of the drug or BME alone. In this test from day-1 to day-7, of CFT, following 2 weeks of test drug administrations, groups treated with the aforementioned combinations produced superior effects in attenuation of time of immobility in rats in comparison to the control group and the rest of the groups. This clearly indicated the additive effects of BME to the antidepressant effects of fluoxetine in the treatment of chronic fatigue syndrome in rats. In groups of rats receiving BME and fluoxetine combinations, whilst the concentrations of adrenaline (Ad) were significantly decreased, the noradrenaline (NA) and serotonin concentrations were significantly increased in dose dependent fashion in all the groups treated with the parallel administrations of abovementioned combinations.

The simultaneous administrations of the combinations of BME and fluoxetine in groups of rats in

the present study have shown elevated levels of serotonin as well as NA in brains. The possibility of herbal extracts and test drugs (SSRI) alone and in combinations, acting on 5-HT receptors cannot be ruled out. The groups of animals administered with the combinations of BME and fluoxetine also showed a significant reduction in Ad concentration which may be responsible for its antistress effect that required further probing. The combinations may help further in dose reduction of the fluoxetine lessening the risk related to SSRIs concerning risk linked with over dosage, though a sedation effect was also found to be prominent in the groups receiving combinations of the herbal extracts BME and the fluoxetine than the groups treated with either the BME or the conventional drug fluoxetine alone.

CONCLUSION

BME utilized in present study showed significant antidepressant-like activity through interaction with adrenergic and serotonergic systems in CNS in animals. The antidepressant-like action of the herbal extract utilized in the present studies was clearly augmented with the simultaneous use of fluoxetine. Hence, it was contemplated that the respective combinations may have potential therapeutic value for the management of depressive and anxiety disorders as well as in management of a chronic fatigue syndrome. This could be important in reducing the dose of the drugs to achieve enhanced therapeutic effects with minimal adverse effects. Consequently, it is tempting to point out that there may be a possibility of such herbal drug interaction that of herb utilized in present study along with the newer conventional antidepressant drugs in the same categories like fluoxetine and further research in this area seem prudent.

It is very important also for pharmacists working in all settings, to be aware of such potentially dangerous herbal drug interactions and other supplement-drug interactions so that any harm to consumers can be minimized. In the last two decades, information regarding the interactions between herb and drug has been piling up and the complexities of these interactions are remarkable. Future studies will probably divulge even more complex associations. The clinically most relevant advance will be the investigations of these interactions in humans.

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