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Comparative evaluation of *Bacopa monniera* and *Panax quniquefolium* in experimental anxiety and depressive models in mice

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The present study was undertaken to compare medicinal plants against mixed anxiety-depressive disorder (MAD) to evaluate their potency in combating MAD disorders. Previous studies from our lab have shown that *Bacopa monniera* (BM), and *Panax quniquefolium* (PQ) have significant adaptogenic properties. Hence, we have further confirmed their activity in stress related disorders like anxiety and depression in animal model, rodents and assessed their efficacy. In our experimental protocol, gross behaviour was observed through Digiscan animal activity monitor. Anxiety was studied through light dark test, elevated plus maze test and holeboard test. Depression experiments were conducted following tail suspension test and forced swim test. Further, rotarod test was also used to study any defects in motor in-coordination in mice. It was observed that BM at the dose of 80 mg/kg (po) and PQ at 100 mg/kg (po) were effective as an anti-anxiety as well anti-depressant activity and had no motor in-coordination in mice. Hence, these extracts can be used as a potent therapeutic agent in treating mixed anxiety-depressive disorder (MAD).

Keywords: Anxiety, *Bacopa monniera*, Depression, Elevated plus maze, Forced swim test, Hole-board test, Light Dark test, Mixed anxiety-depression, *Panax quniquefolium*, Tail suspension test

Amidst all the chaos of life, a psychiatric disorder is most often neglected, which aggravates the situation and leads to an illness which requires chronic treatment. Most often people are facing continuous trials throughout their life, in the course of which they might, feel anxious or stressed out and which may also follow depression. Such feelings enter the clinical world when the symptoms start interfering in the daily activities of a person. This is when the person requires attention and care[1].

In 1992, the ICD-10 introduced the concept of mixed anxiety-depression disorder (MAD). Recent studies have reported that depression and anxiety may occur together with the association of subthreshold depressive symptoms and subthreshold depressive anxiety representing comorbid 'pure' conditions. Anxiety may also predispose depression (or *vice versa*), or symptoms of anxiety and depression may be external manifestations of one underlying cause. So drugs having properties to combat both anxiety and depression, with lesser side effects, might be useful for such clinical conditions. Drugs prescribed for neuropsychiatric disorders have more side effects than they are efficacious. In a scenario like this we need drugs with lesser side effects. Hence, ayurveda has recently become the drug of choice and investigations have been extended for the search of novel and better tolerated molecules from plant sources.

Herbal medicines play an important role in health care programme worldwide. The search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade and their therapeutic potential has been assessed in a variety of animal models[2].

Bacopa monniera (BM) (Linn.) (Scrophulariaceae) is a perennial creeping annual plant found throughout the Indian subcontinent in wet, damp and marshy areas[3,4]. Commonly known as *Brahmi*, the plant has been used by Ayurvedic medical practitioners in India for almost 3000 years and is classified as a medhyarasayana, a drug used to improve memory and intellect (medhya). Triterpenoid saponins and bacosides present in BM are considered to be responsible for enhancing cognitive function[5]. Previous studies in our lab have also reported potent adaptogenic activity of BM in stress models in rats[6].

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Panax quinquefolium (PQ) Linn (Araliaceae), commonly known as ginseng, a plant native to North America is now also cultivated and used in many countries. It has been shown that ginseng administration produces a variety of effects on the central nervous system. For example, ginseng causes behavioral changes in animals, and these changes appear to be related to the regulation of GABAergic transmission[7]. Chronic intake of ginseng stabilizes sleep and wakefulness in food-deprived rats[8] and the effects of ginseng extract on learning, memory, and physical capacities have also been reported[9].

According to the pharmacological profile of BM and PQ, it is reasonable to assume that these extracts may have some other neuroactive activities. Therefore, the present study was designed to investigate the anti-anxiolytic and antidepressant effects of these extracts by using various experimental anxiety and depression paradigms in rodents.

Materials and methods

Animals—All experimental protocols were approved by our Institutional Ethical Committee following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) which complies with International norms of Indian National Science Academy (INSA). Albino male Swiss mice weighing 20-25 g were employed in the study. Mice were housed in six per cage at constant temperature (22º±2°C) and 12 h light/dark (0800 to 2000 hour). Mice were fed standard laboratory food and water was given *ad libitum*. Each animal was used once in the behavior tests.

Preparation of extract—Whole plant of *Bacopa monniera* (BM) was collected and identified by the Division of Botany, of our Institute and preserved in the herbarium (Specimen voucher No. 380). Air dried whole plant (1 kg) was extracted with ethanol (5% aqueous) by percolation. The residue obtained after removing the solvent was dried in vacuum and macerated with acetone to give a free-flowing powder. The obtained extract was standardized to content of bacoside A, as described earlier[10]. Briefly, method involves acid hydrolysis of bacosides, which yields quantitatively a transformed aglycone-ebelin lactone containing a conjugated triene system and is estimated by UV spectrophotometer at 278 nm. The extracts of BM contained 55–60% bacosides estimated as bacoside A. Extract, thus, obtained was used within 3 months period of time.

Crude root powder of *Panax quinquefolium* (PQ) was obtained from M/s. Sigma Chemicals, USA.

Drug and treatment schedule— Imipramine (IMP), the standard antidepressant drug and diazepam (DZP), standard anti-anxiety drug were obtained from M/s. Sigma. (St. Louis, MO, USA) and Ranbaxy Laboratories Ltd. India, respectively. All compounds were dissolved in gum acacia (0.5%). The extract was administered daily for 3 days prior to the experiment. Drugs were prepared fresh daily before administration. Compounds were administered per orally (po) at a rate of 0.1 ml/10 g.

Behavioural observations

Mice were divided into three groups of 8 mice in each group. Control group mice (Gr I) were treated with vehicle (0.5% of gum acacia); mice of group (Gr II) were treated with graded doses of extract; and mice of group (Gr III) mice were treated with standard anxiety/depression drug. These mice were subjected to various tests to assess spontaneous motor activity, anxiety, depression and muscle-incoordintaion

Spontaneous motor activity

In order to study the spontaneous motor activity, mice were treated with graded doses of BM (40-120 mg/kg, po), and PQ (50-200 mg/kg, po) 1 h prior to subjecting them to the Digiscan Animal Activity Monitor.

Gross open field activity was studied using Digiscan Infrared Photocell system [Test Box model: RXYZCM (16 TAO)]; Omnitech Electronics, Columbus, Ohio] in 42 × 42 × 30 cm Plexiglass arenas, fitted into infrared beam containing metallic grid. Activity of animals was observed by the interruption of infrared beams.

Horizontal activity—The total number of beam interruptions that occurred in the horizontal sensor in the duration of 2 mins.

Total distance travelled—Distance travelled by the animal was recorded in a given sample period, (indicated in centimetres). Total distance travelled is a more accurate indicator of ambulatory activity.

Stereotypy counts—If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy, which typically involves grooming, licking, head bobbing etc.

Prior to the experiment, animals were habituated in the Test Box for 15 min. After the initial habituation process, the activity of the control and treated animals were monitored at 2 min internal for 60 min post drug treatment.

Anxiety experiments

Graded doses of BM (40-120 mg/kg, po) and PQ (50-200mg/kg, po) were administered 1h prior to subjecting mice for light/ dark test. The dose identified as effective in the light/dark model was considered as effective anxiolytic dose and used in other models of anxiety. Diazepam (1.5 mg/kg, po) was used as the standard anxiolytic drug.

Light/dark test—The apparatus consisted of a Plexiglas box with two compartments (20 cm × 20 cm each), one of which was illuminated with a white light, while the other remained dark. Each animal was placed at the junction of the light/dark, facing the illuminated compartment. The time spent in illuminated place, was recorded for 5 min[11]. After each test, the box was carefully cleaned up with a wet tissue paper (10% ethanol solution).

Elevated plus maze—This test has been widely validated to measure anxiety in rodents[12]. This apparatus was made of stainless steel and consisted of two open arms (30 × 5 cm) and two closed arms (30 × 5 cm) with 25 cm walls. The arms extended from a central platform (5 × 5 cm). The maze was elevated 38.5 cm from the room floor. All the four arms consist of infrared beams fitted at regular distance. Mice were treated with extract and DZP (1.5mg/kg p.o.) 1h prior to the experiment. Each animal was placed at the center of the maze, facing one of the open arms. The time spent in enclosed and open arms was recorded for 5 min test. The movement of animals across the arms was calculated by interruption of beams which was analyzed by Maze tracking software (M/s Columbus Instruments, USA). After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution).

Holeboard test—The apparatus was composed of a transparent Plexiglass arena (42 × 42 × 30 cm) with 16 equidistant holes 2.5 cm in diameter in the floor[13]. The centre of each hole was 10 cm from the nearest wall of the box. The floor of the box was positioned 15 cm above the ground. An animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 3 min. The number of head-dippings was recorded. A head dip was scored if both eyes disappeared into the hole.

Depression experiment

Graded doses of BM (40-120 mg/kg, po) and PQ (50-200 mg/kg, po) was administered to mice 1h prior to the tail suspension test (TST). The effective dose identified in TST was used further used in forced swim test model to confirm its antidepressant activity. Imipramine (60 mg/kg, po) was used as the standard antidepressant drug.

Tail suspension test—The tail suspension test (TST) was performed according to the method described by Steru *et al.*[14] The mice were individually suspended 60 cm above the surface of table with an adhesive tape placed 1 cm away from the tip of the tail. After 1 min acclimatization, immobility duration was recorded for 5 min. Mice were considered immobile only when they hung passively and were completely motionless.

Forced swim test—Forced swimming test (FST) in mice[15] is a behavioural despair test. The mice were placed individually in glass cylinders (20 cm height, 10 cm diameter) containing 10 cm depth of water at 25°C. After 5 min, the animals were removed from water, dried and returned back to their home cages. They were again placed in the cylinder 24 h later and after the initial 1 min acclimatization period, the total duration of immobility was measured for 5 min. Mice were considered to be immobile when they were floating motionless. The duration of swimming was measured by digital counter.

Rotarod test

The maximum dose used in the behavioural studies i.e. BM at 120 mg/kg, (po) and PQ at 200 mg/kg, (po) was used in the rotarod model for muscle in-coordination.

Rotarod test was used for evaluation of neuromuscular coordination in mice treated with various plant extracts according to the protocol as described by Dunham and Miya (1957)[16] and studied in the Rotamex 4/8 apparatus (M/s Columbus Instruments, USA). Rotarod consisted of a rod which was coated with polypropylene foam to provide friction and to prevent animals from slipping off the rod. The distance between rod and floor was kept 15 cm to avoid intentional jumping of mice. The rod was driven by a motor and the rotational speed was maintained at 8 rpm. Animals were trained on the Rotarod for 2 min per trial, with 3 trials per day for two days. On the third day, mice were given trials before and after treatment of extract. The extracts were used at the highest dose of 200 mg/kg, (po), to evaluate any defects in motor coordination.

Statistical analysis

The results were expressed as mean±SEM. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test, using Prism software version 3.0. $P < 0.05$ was considered to be statistically significant.

Results

Gross locomotor activity test

Gross locomotor activity in mice was studied in Digiscan animal activity monitor in order to determine the effect of BM and PQ on horizontal activity, total distance travelled and stereotypy counts in mice. BM extract at doses 40 and 80 mg/kg had no significant effect on the horizontal activity in comparison to control mice, however at higher doses of 120 mg/kg a significant reduction was observed in the horizontal activity (Fig. 1a). Treatment with PQ at graded doses (50-200 mg/kg) also did not significantly alter the horizontal activity counts in mice.

BM extract at 40 mg/kg had no significant effect on the total distance travelled at 60 min (Fig. 1b). The higher doses of 80 and 120 mg/kg significantly reduced the distance travelled in comparison to control mice. This effect was also seen in mice treated with the PQ extract. A significant reduction in the total distance travelled was observed at 50, 100 and 200 mg/kg doses of PQ extract.

As shown in Fig. 1c, BM, and PQ did not induce any significant alterations in the stereotypy counts in comparison to control mice at all doses observed.

Anxiety

Light/dark model—The plant extracts were tested for its anxiolytic potential in the Light/dark test model. As shown in Fig. 2, BM extract significantly increased the time spent in the light chamber by 237% at the dose of 80mg/kg, whereas there was no significant difference in the time spent in light chamber with the lower dose, 40mg/kg (10.29%) or higher dose at 120 mg/kg (0.79%).

PQ at lower dose improved the time spent in light chamber by 49.25%, but the difference was not significant in comparison to control. The dose at 100mg/kg significantly increased the time spent in light chamber by 447.76%. Further higher dose reduced the time spent in light chamber by 39.05% but the difference was not significant in comparison to control.

Similar effects were also observed in animals treated with standard anxiolytic drug, diazepam that increase the time spent in light chamber by 352.44%.

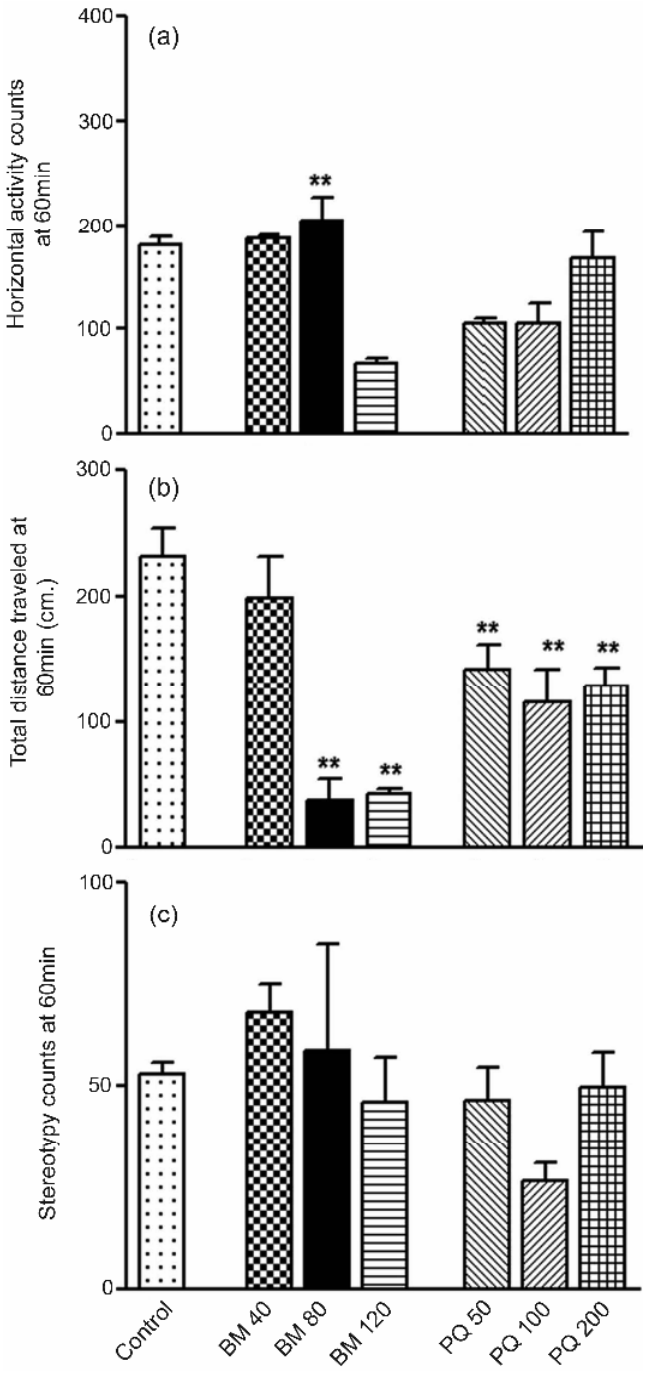

Fig. 1—Bar diagram representing (a) horizontal activity counts; (b) total distance travelled; (c) stereotypy counts of mice in Digiscan animal activity monitor. [Results are represented as mean ± SEM. with n = 8 in each group. Values are significant at P * <0.05, ***<0.001 as compared with control group.]

Elevated plus maze—As shown in Fig. 3, the oral administration of BM extract at 80 mg/kg and PQ extract at 100 mg/kg (po) significantly increased the time spent in open arms by 267.75 and 519.58%, respectively. Furthermore, the group treated with DZP also showed a similar increase in the time spent in open arms by 522.4%.

Hole-board test—Administration of different doses of BM had no significant effect on the number of hole poking in mice (Fig. 4). Similarly, PQ extract (50 mg/kg) also did not show any significant effect, whereas PQ at 100 mg/kg significantly increased the number of hole-pokings by 59.24%. The mice treated with standard drug, DZP, produced a significant increase in the no. of hole-pokings by 78.12%.

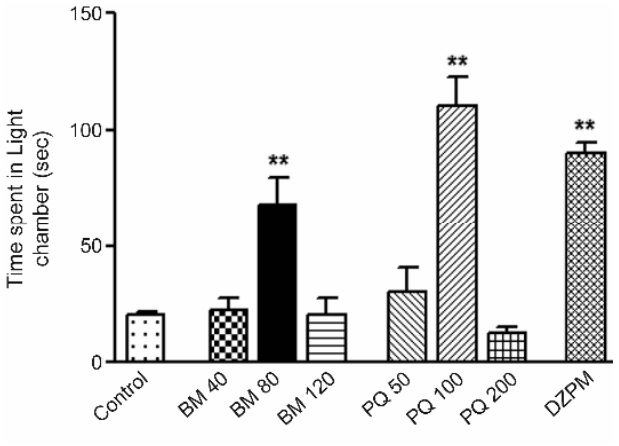

Fig. 2—Time (sec) spent in light chamber in the light/dark test. [Results are represented as mean ± SEM. with n = 8 in each group. Values are significant at *P* *< 0.05, **<0.01, *** < 0.001 vs control group.]

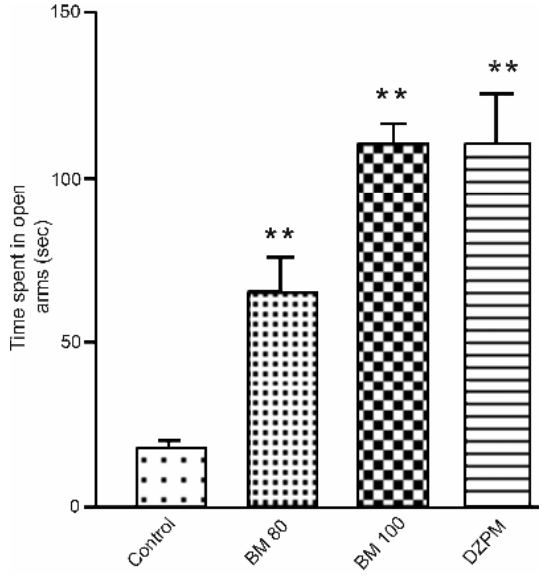

Fig. 3—Number of hole-pokings in the Hole-board test of anxiety. [Results are represented as mean ± SEM. with n = 8 in each group. Values are significant at *P* *< 0.05, **<0.01, *** < 0.001 vs control group.]

Depression models

Tail suspension test—Graded doses of BM extract (40-120 mg/kg) were studied in the tail suspension model of depression in mice. As shown in Fig 2, BM extract induced a significant reduction in the immobility duration at doses of 40 mg/kg (59.21%), 80 mg/kg (43.57%) and 120 mg/kg (27.93%), when compared to the vehicle treated groups. PQ extract also induced a significant reduction in the immobility duration at 50 mg/kg (28.49%), 100 mg/kg (56.98%) and 200 mg/kg (55.30%). IMP (60 mg/kg, po), the standard antidepressant, also produced a significant decrease (90.23%) in the immobility time (Fig. 5a).

Forced swim test—The most efficacious dose identified in the tail suspension test model was further evaluated in the forced swim test model of depression. BM at doses 40 and 80 mg/kg significantly reduced the immobility duration by 37.2 and 28.3%, respectively (Fig 5b). PQ at the lower dose of 50 mg/kg enhanced the immobility duration by 12.22%, whereas at the higher dose of 100 mg/kg it reduced the immobility duration by 88.8%. The immobility duration in IMP treated mice was reduced by 91.11%.

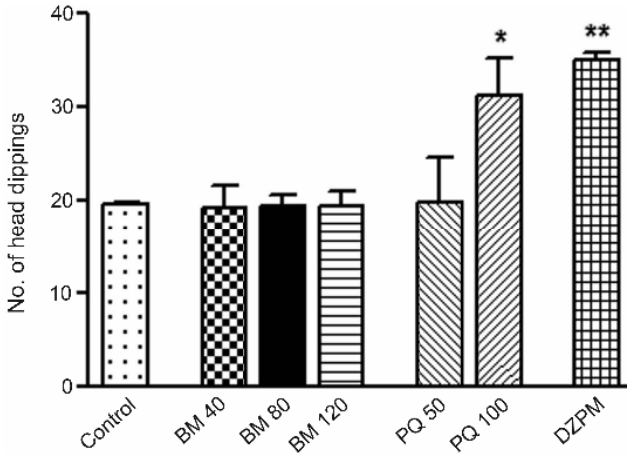

Fig. 4—Time (sec) spent in open arms in the elevated plus maze test of anxiety. [Results are represented as mean ± SEM. with n = 8 in each group. Values are significant at *P* *< 0.05, **<0.01, *** < 0.001 vs control group.]

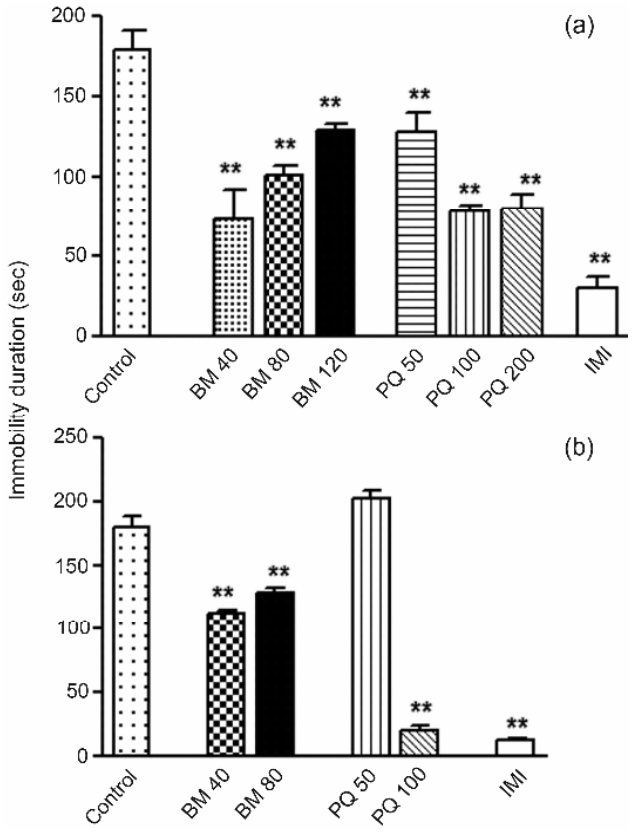

Fig. 5—Immobility duration (sec) of mice (a) Tail suspension test; (b) Forced swim test. [Results are represented as mean ± SEM. with n = 8 in each group. Values are significant at P * <0.05, *** <0.001 as compared with control group.]

Rotarod test

Both BM and PQ at the highest dose (200 mg/kg, po) did not show significant difference in the time of fall between control and extract treated groups. This confirmed the finding that the extracts had no motor impairments.

Discussion

Anxiety and depressive disorders accompanies most of the clinical conditions including cardiovascular disorder, thyroid disorders and post-partum condition. In view of this, there is an urgent need of a drug that can overcome both these symptoms. Various plant based products have been identified to possess neuropharmacological properties, which might prove useful as a therapeutic agent in these disorders.

This study was aimed to compare the behavioural effects of some medicinal plants for both anxiety and depression. In our study, it was demonstrated that BM and PQ was able to induce antidepressant as well as anxiolytic like effects without impairing the neuromuscular tone in mice.

The spontaneous locomotor activity observations indicated no significant effect of BM and PQ on the horizontal activity and stereotypy counts in comparison to the control group. However, a significant diminution was observed in the total distance travelled, which reflected its CNS depressant activity.

Further we have evaluated the anxiolytic potential of the extracts in various models of anxiety. The light/dark test has been widely used for modelling anxiety, and it has been developed for predicting the efficacy of clinically used compounds for treating this disease[11]. Administration of BM at the dose of 80 mg/kg produced a significant response in the light/dark test of anxiety, as the time spent by animals in the light chamber increase significantly as compared to the control group, indicating an anxiolytic activity of the extract. Similarly PQ at the dose of 100 mg/kg significantly increased the time spent in light chamber. The anxiolytic potential of these extracts was further confirmed by employing other models of anxiety viz., Elevated plus maze test and the holeboard test.

The elevated plus-maze is currently one of the most frequently used models of animal anxiety[17]. The indices of anxiety in this test, percent of open arm entries and time spent in the open arm are sensitive to agents thought to act via the GABA-A receptor complex, justifying the use of diazepam as a positive control in this study. In agreement with previously published reports, diazepam increases the percentage of open arm entries and the time spent in the open arms[18], confirming its anxiolytic effects. The extract of BM (80 mg/kg) and PQ (100 mg/kg) had similar effects on these parameters. Further, in the hole-board test, OS extract, could significantly increase the number of nose poking, signifying an anxiolytic effect of the extract. The hole-board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and/or responses to stress in animals[13]. The extract of PQ (100 mg/kg) was observed to have a significant effect on the hole-poking, which further justifies its anxiolytic potential. However the effect of BM extract was not significant in this model of anxiety. This suggested that BM extract induced its anxiolytic effects through a different mechanism altogether.

We studied the antidepressant effects of these extracts in tail suspension and forced swim models of depression which provided a rapid and reliable

behaviour screening test for antidepressants. The immobility has been expected to reflect a state of 'behavioural despair and variants' or 'failure to adapt to stress'[19]. The model is valid for a broad spectrum of antidepressants, mainly including tricyclics and monoamine oxidase inhibitors, which significantly decrease immobility time in both TST and FST.[20] It was observed that BM produces its maximal antidepressant effect at the doses of 40 and 80 mg/kg p.o. and produced a diminution of immobility time in mice exposed to both the models, comparable to that of standard antidepressant drug, imipramine. Similarly, PQ produces its maximal antidepressant effect at a dose of 100 mg/kg and the same was confirmed in the forced swim test. The comparative analysis revealed that the antidepressant potency of PQ extract was higher than BM extract.

Initial hypothesis of depression has been formulated about 40 years ago, proposing that the main symptoms of depression are due to functional deficiency of cerebral monoaminergic transmitters such as norepinephrine (NE), 5-HT, and/or dopamine (DA) located at synapses[20]. Previous studies from our lab has also shown the adaptogenic effect of these plant extracts via normalization of the various stress parameters and monoaminergic levels which may provide a clue that these extracts are bringing their possible antidepressant effect through restoration of normal monoaminergic neurotransmission[21,22]. *Bacopa monniera* extract (BM) contains a natural phytonutrients which is known as bacosides. This is responsible for improving vital neurotransmitters activities which happens in memorization and information processing. The action of the triterpinoid saponins and the bacosides A and B has resulted in the enchantment of the nerve impulse transmission. The effects of PQ on the restoration of monoamine levels can also be explained on the fact that ginsenosides belong to a family of steroids named steroidal saponins[23] which act as functional ligand of the nuclear glucocorticoid receptor[8], thus having corticosteroid-like effects on the modulation of nerve transmission by altering the availability of neurotransmitters[24].

In this regard it is also important to note that both BM and PQ have adaptogenic properties[21,22]. Adaptogens are helpful in attaining the general haemostatic response under various physiological conditions. The stress response, which has psychological and biological components, may be a common pathway leading to affective and/or anxiety reactions. Thus, these extract showing both anti-anxiety and antidepressant like activity in our study points towards such overall normalization of the system which causes these clinical conditions.

The wide variety of neuro-pharmacological actions of these plant extract opens up interesting avenues for further research[25,26]. The activity of these extract both as an anxiolytic and anti-depressant needs further evaluation. This offers new perspectives in the treatment of these diseases, as there is compelling evidence that, symptoms of anxiety and depression overlap with one another[26]. Many anti-depressants have been reported to be of use in anxiety disorders and anxiolytics in depression[27]. There are several reports pertaining to both anxiolytic and antidepressant effects of serontonergic drugs[28-30]. These reports show that anxiety and depressant may share some common etiological factors and drugs showing both anxiolytic and antidepressant activities are to be extensively studied for their therapeutic beneficial uses.

Based on our studies, we concluded that the PQ and BM demonstrated significant anxiolytic and antidepressant potential and also rules out any side effect of the extract on motor in-coordination. The anxiolytic and antidepressant effect of both PQ and BM at the same dose may be beneficial as a therapy for cases of mixed anxiety and depressive disorders. Thus, our results fortify the ethnopharmacological importance of BM and PQ in psychiatric disorders like anxiety, depression and mixed anxiety-depression disorder (MADD), however more experimentation and detailed analysis is required for a definitive conclusion.

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