Original Article

TJPS
The Thai Journal of Pharmaceutical Sciences
39 (3), July-September 2015: 70-75

Evaluation of anti-anxiety activity of *Melissa parviflora* (Benth.) in rats

Kundan Singh Bora* and Aruna Dubey

Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun, Uttarakhand 248161, India

Abstract

Melissa parviflora Benth. (Family: Lamiaceae) has been traditionally used as a tranquillizer, relaxants, nervine and sleeping aids throughout the world. The plant is reported to relieve tension and stress reactions, and widely valued for its calming properties. Despite a long tradition of uses, no scientific pharmacological work has ever been carried out on this potential plant. Therefore, the present study was design to evaluate anti-anxiety activity of *M. parviflora* in rats. Various extracts viz. petroleum ether, chloroform, methanol and aqueous were prepared by successive soxhlet extraction method. Anxiolytic activity of various extracts of the plant was evaluated using elevated plus-maze apparatus and light and dark test model of anxiety in Wistar rats of either sex. The bioactive extract was standardized on the basis of total phenolic and flavonoid content estimation using colorimetric method. Results showed that only methanol extract of *M. parviflora* exhibited significant anxiolytic activity (100 and 200 mg/kg, p.o.) using elevated plus maze test and light and dark test models of anxiety with respect to vehicle treated control and diazepam (2 mg/kg, p.o.) as positive control. The total phenolic and flavonoid content in the bioactive methanol extract was estimated to be 15.21 ± 0.72 mg gallic acid equivalents, and 8.06 ± 0.58 mg rutin equivalents per gram of the extract, respectively. The present study leads to the conclusion that methanol extract of the plant showed predominantly anxiolytic activity. Therefore, *M. parviflora* could serve as a new approach for the treatment of CNS disorders like anxiety.

Keywords: Melissa parviflora, Anxiolytic activity, Elevated plus-maze, Light and dark box test

Introduction

Anxiety disorders are one of the most prevalent and highly comorbid psychiatric conditions [1]. They are among the most common mental, emotional, and behavioral problems [2, 3]. However, less than 30 % of individuals who suffer from anxiety disorders seek for treatment [4]. Anxiety is a normal, emotional, reasonable and expected response to real or potential danger [2, 3].

Mostly synthetic drugs like benzodiazepines, selective serotonin inhibitors, tetracyclic antidepressants etc. are prescribed in the treatment of anxiety disorders but these have common limitations of anti-anxiety include co-morbid psychiatric disorders and increase in dose leading to intolerable side effects such as nausea, sedation, nervousness, dry mouth, pharmacological dependence, etc. [4, 5]. Therefore, research has been conducted to identify safer, more specific medications possessing anxiolytic effect without complications. World Health Organization (WHO) estimates that 80 % of the world populations rely on herbal medicines. In past few

Correspondence to: Kundan Singh Bora, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun, Uttarakhand 248161, India
Phone: +91-135-2686246; Fax: +91-135-2686231
Email: kundanresearch1381@gmail.com

Received: 7 May 2015
Revised: 27 June 2015
Accepted: 20 July 2015

Academic Editor: Chatchai Chaotham

years, several herbal medicines have been used for the management of anxiety worldwide [6- 8].

Melissa parviflora (Family - Lamiaceae), commonly called gentle balm, is an aromatic perennial, pubescent or glabrate herb [9-11]. Traditionally, *M. parviflora* is used as tranquillizer, nervine relaxant and sleeping aids throughout the world. The plant has been used for treatment of neuralgia, anxiety-induced palpitation, insomnia and tension-relating disease such as migraine. It is widely valued for calming properties [10-13]. The major phytoconstituents of the plant *M. parviflora* are alkaloids, tannins, saponins flavonoids and phenolic compounds. Despite a long history of use of *M. parviflora* as a traditional medicine for the treatment of various ailments, especially in central nervous system (CNS) disorders, the plant has never been subjected to CNS activity studies. Thus, it was considered worthwhile to evaluate *M. parviflora* for anti-anxiety activity studies.

Materials and Methods

Plant materials: The plant material was purchased from Balkrishna & Sons Dawakhana, Dehradun 248001 (UK), India. The identity of the plant was confirmed through NISCAIR, New Delhi, India vide Ref. No. NISCAIR/RHMD/Consult/2014/2426-05.

Preparation of extracts: Dried plant material (stems) was pulverized using a mechanical grinder. Powdered material was subjected to successive soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether, chloroform and methanol and water. Before each extraction the powder material was dried in hot air-oven below 50°C. Finally, marc was digested at 50°C with distilled water for 24h to obtain the aqueous extract. The extracts was concentrated in rotary vacuum evaporator (40ºC), freeze-dried and stored at 4ºC until further use in the experiment. Extracts was weighed, and percentage was calculated in terms of the air-dried weight of the plant material.

Phytochemical analysis of extracts: The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents as per method described. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins, amino acids, lipids/fats, etc. [14, 15].

Standardization of extract (total phenol and total flavonoid content): Total phenol content was determined by Folin-Ciocalteu reagent method with modification. Whereas the total flavonoid content was estimated by aluminium chloride colorimetric method [16, 17].

Animals: Wistar rats of either sex weighing 180-200 g were used in the present study. They were maintained on standard environmental conditions. They were fed with standard laboratory feed and tap water *ad libitum*. They were housed in the departmental animal house and were exposed to natural photoperiod. The animals were fasted for 18 hours before use. The approval from the Institutional Animal Ethical Committee of SBSPGI, Dehradun, India was taken before carrying out biological studies.

Acute toxicity study: As per OECD Guideline; 2001, a limit test at one dose level of 2000 mg/kg body weight was carried out with six animals (three animals per step) [18]. Single dose (2000 mg/kg, p.o.) administration of *M. parviflora* extracts was given to adult animals. Then, the behavioral manifestations and mortality were observed up to 14 days.

Anti-anxiety study: Animals were randomly divided into fourteen groups with six animals in each group. Group 1 (vehicle treated control), the animals were administered vehicle (Distilled water + Tween 80 (5 %), 10 mL/kg) 1 h before subjecting to elevated plus maze test and light/dark test model of anxiety. Group 2 (positive control), the animals were administered diazepam (2 mg/kg, p.o.) 30 min before subjecting to anxiety models. The animals in group 3 - 14 were administered different extracts 1 h before subjecting to elevated plus maze test and light/dark test model of anxiety.

Elevated plus-maze model: Elevated plus maze (EPM) test for studying the anxiolytic effect in rodent was used. EPM consists of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm) with an open roof. After oral administration of drugs, the rat was placed in the center of the maze, facing open arm for 1 h of elevation at 50 cm. During a 5 min test period the following measures were taken: average time spent by the animal in the open arms (average time = total time spent in open arms/number of entries in open arms); and total number of entries in open arms [19, 20]. During the entire experiment, the animals were allowed to socialize. Every time before placing the animal, the arena was cleaned with 5 % alcohol to eliminate the possible bias due to the odor left by the previous animal. Every precaution was taken to ensure that no external stimuli could invoke anxiety in the animals.

Light-dark model: Two compartment exploratory models of Crawley and Goodwin has been validated pharmacologically, behaviorally and physiologically. The two compartment method titrates the natural tendency of rat to explore a novel environment, against the aversive properties of brightly light open field. The time spent in light area seems to be the most reliable parameter for assessing anxiolytic activity. The light and dark box consists of two compartments: one light area (27L × 27W × 27H cm) was illuminate by 100 W desk lamp was painted white and the other dark area (18L × 27W × 27H cm) was painted black. The two compartments were separated by partition with tunnel (7.5 × 7.5 cm) to allow passage from one compartment to other [21, 22]. Thirty min after the administration of the test drug, each animal was individually placed in the center of the light

compartment (facing away from the door). During 5-min test period, number of transitions, and time spent in light zone were noted.

Statistical analysis: All data were expressed as mean ± SD Statistically significant differences between groups were calculated by the application of one-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple range tests. The group treated with extract was compared with the respective control (vehicle) group. P-values < 0.05 were considered statistically significant.

Results

Phytochemical screening of plant extract

Yields of various extracts of *M. parviflora* stem with various solvents are reported in **Table 1**. The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents as per method described. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds; flavonoids, proteins, amino acids, lipids/fats etc. and results are shown in **Table 2**.

The total phenolic and flavonoid content was estimated to be 15.21 ± 0.72 mg gallic acid equivalents/g of the extract, and 8.06 ± 0.58 mg rutin equivalents/g of the extract respectively.

Acute toxicity study

In the present study, acute toxicity study revealed the non-toxic nature of all the extracts even at highest starting dose of 2,000 mg/kg body weight of animal for oral route of administration. Various extracts of *M. parviflora* did not produce any behavioral or toxic manifestations.

Anti-anxiety study

Elevated plus-maze test: The mean number of entries and mean time spent by the animal in open arms of elevated plus maze after oral administrations of 50, 100 and 200 mg/kg of various extracts of *M. parviflora*, diazepam (standard) and the control (vehicle) are shown in **Table 3** and **Figure 1**.

Table 1 Yield of various extracts of *M. parviflora*

Serial number	Extracts	Percentage yield (% w/w)
1	Petroleum ether	3.67
2	Chloroform	4.32
3	Methanol	3.81
4	Aqueous	6.60

Light-dark box test: The mean number of transitions and mean time spent by the animal in light box after oral administrations of 50, 100 and 200 mg/kg of the extracts of *M. parviflora*, diazepam and the control (vehicle) are reported in **Table 4** and **Figure 2**.

Discussion

Plants have played a very important role in drug discovery. A majority of drugs being used in modern medicine have been obtained from medicinal plants. Since the effect of central nervous system is manifested by symptoms which can be easily identified, several researchers have used behavioral parameters to discover new drugs. Some of the behaviors related to the central effect of drugs include anxiety, fear, convulsion, depression etc. In the present study elevated plus maze (EPM) test and light-dark model were used for the assessment of anxiety activity. The EPM is currently one of the most widely used models of anxiety [23], and has been validated for use with both rats and mice [24]. Therefore, we chose this test to investigate the anxiolytic potential of different extracts of the plant. 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 $GABA_A$ receptor complex, justifying the use of diazepam as a positive control (standard) in this study [23]. Amongst the various extracts viz. petroleum ether, chloroform, methanol and aqueous of *M. parviflora* tested, only methanol extract exhibited significant ($p < 0.05$) anxiolytic activity (by increasing the time spent, and number of entries in open arms of the elevated

Table 2 Phytochemical screening of different extracts of *M. parviflora*

Serial number	Phytochemical tests	Petroleum extract	Chloroform extract	Methanol extract	Aqueous extract
1	Carbohydrate	-	-	+	-
3	Fats & Oils	+	-	-	-
4	Proteins	-	-	-	-
5	Amino acids	-	-	-	-
6	Alkaloids	-	+	-	-
7	Glycosides	-	-	-	-
8	Flavonoids	-	-	++	-
9	Phytosteroids	+	-	-	-
10	Tannins	-	-	+	+
11	Phenolic compounds	-	+	++	-

plus-maze) in rats at a dose of 100 and 200 mg/kg, p.o. (**Table 3** and **Figure 1**). The light-dark exploration test was developed by Crawley et al. (25). Similar to the EPM, this animal model is based on the innate aversion of rodents to places with bright light. In rodents, this model generates an inherent conflict between their exploratory drive and their avoidance of the light compartment [25, 26]. Treatment with anxiolytic drugs such as

Table 3 Effect of various extracts *M. parviflora* on elevated plus-maze model of anxiety

Serial number	Treatment	Doses (mg/kg)	Average time spent in open arm (seconds) (Mean^n ± SD)	No. of entries in open arm (Mean^n ± SD)
1	Control	Vehicle	4.7 ± 0.43^b	3.5 ± 0.47^b
2	Diazepam (standard)	2 mg/kg, i.p.	14.3 ± 0.42^a	6.8 ± 0.57^a
3	Petroleum ether extract	50 mg/kg, p.o.	4.8 ± 0.54^b	2.9 ± 0.58^b
4	Petroleum ether extract	100 mg/kg, p.o.	4.6 ± 0.64^b	3.1 ± 0.67^b
5	Petroleum ether extract	200 mg/kg, p.o.	5.0 ± 0.67^b	3.6 ± 0.56^b
6	Chloroform extract	50 mg/kg, p.o.	5.2 ± 0.72^b	2.1 ± 0.64^b
7	Chloroform extract	100 mg/kg, p.o.	5.5 ± 0.68^b	3.9 ± 0.89^b
8	Chloroform extract	200 mg/kg, p.o.	5.6 ± 0.53^b	3.8 ± 0.56^b
9	Methanol extract	50 mg/kg, p.o.	5.2 ± 0.86^b	3.8 ± 0.61^b
10	Methanol extract	100 mg/kg, p.o.	9.8 ± 0.73^a	5.1 ± 0.69^a
11	Methanol extract	200 mg/kg, p.o.	13.7 ± 0.54^a	6.2 ± 0.72^a
12	Aqueous extract	50 mg/kg, p.o.	3.1 ± 0.71^b	2.1 ± 0.51^b
13	Aqueous extract	100 mg/kg, p.o.	4.8 ± 0.84^b	3.5 ± 0.54^b
14	Aqueous extract	200 mg/kg, p.o.	2.3 ± 0.74^b	3.8 ± 0.67^b

All values are expressed as mean ± SD, n=6.
$^a p < 0.05$ when compared with control
$^b p < 0.05$ when compared with standard.

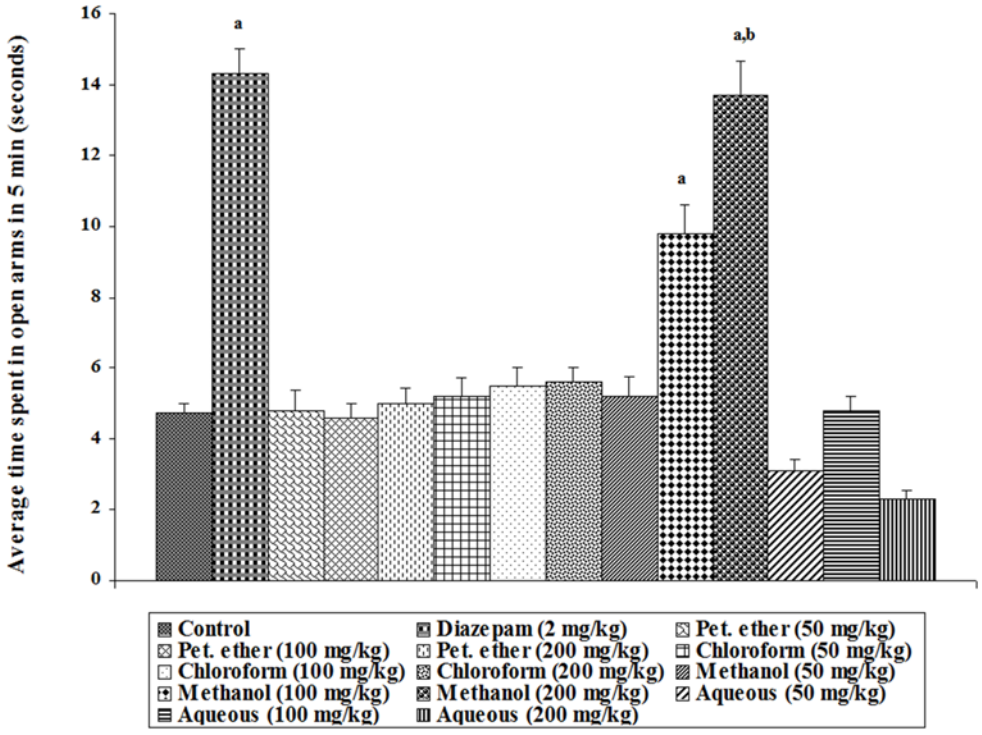

Figure 1 Effect of *M. parviflora* extracts on elevated plus-maze model. All values are expressed as mean ± SD, n=6. Superscript: Superscript: $^a p < 0.05$ compared with control; $^b p < 0.05$ compared with 100 mg/kg dose of methanol extract. Statistical analysis was done by one way ANOVA followed by Tukey's test.

benzodiazepines increases the time spent in the light compartment as well as the number of transitions between the two areas. In the present study, only methanol extract of *M. parviflora* significantly increased the time spent and number of transitions in light box of light-dark box model by the rats at the doses of 100 mg/kg and 200 mg/kg, p.o. (**Table 4** and **Figure 2**).

The plant extract was standardized on the basis of total phenolic and flavonoid content. The total phenolic and flavonoid content was estimated to be 15.21 ± 0.72 mg gallic acid equivalents/g of the extract, and 8.06 ± 0.58 mg rutin equivalents/g of the extract, respectively.

Table 4 Effect of *M. parviflora* extracts on light and dark model

Serial number	Treatment	Doses (mg/kg)	Average time spent in light box in (seconds) (Mean^n ± SD)	No of transitions (Mean^n ± SD)
1	Control	Vehicle	7.0±0.52^b	4.1±0.42^b
2	Diazepam (standard)	2 mg/kg, i.p.	16.9±0.47^a	7.8±0.45^a
3	Petroleum ether extract	50 mg/kg, p.o.	6.1±0.51^b	3.0±0.49^b
4	Petroleum ether extract	100 mg/kg, p.o.	6.9±0.64^b	3.6±0.53^b
5	Petroleum ether extract	200 mg/kg, p.o.	7.0±0.55^b	2.9±0.56^b
6	Chloroform extract	50 mg/kg, p.o.	7.3±0.89^b	3.4±0.67^b
7	Chloroform extract	100 mg/kg, p.o.	8.0±0.56^b	3.8±0.54^b
8	Chloroform extract	200 mg/kg, p.o.	8.5±0.63^b	4.5±0.34^b
9	Methanol extract	50 mg/kg, p.o.	8.1±0.56^b	4.9±0.67^b
10	Methanol extract	100 mg/kg, p.o.	13.7±0.89^a	5.9±0.56^b
11	Methanol extract	200 mg/kg, p.o.	16.8±0.87^a	7.5±0.69^a
12	Aqueous extract	50 mg/kg, p.o.	4.2±0.78^b	3.2±0.67^b
13	Aqueous extract	100 mg/kg, p.o.	5.8±0.81^b	4.3±0.87^b
14	Aqueous extract	200 mg/kg, p.o.	6.1±0.78^b	3.2±0.56^b

All values are expressed as mean ± SD, n=6.
$^a p < 0.05$ when compared with control
$^b p < 0.05$ when compared with standard.

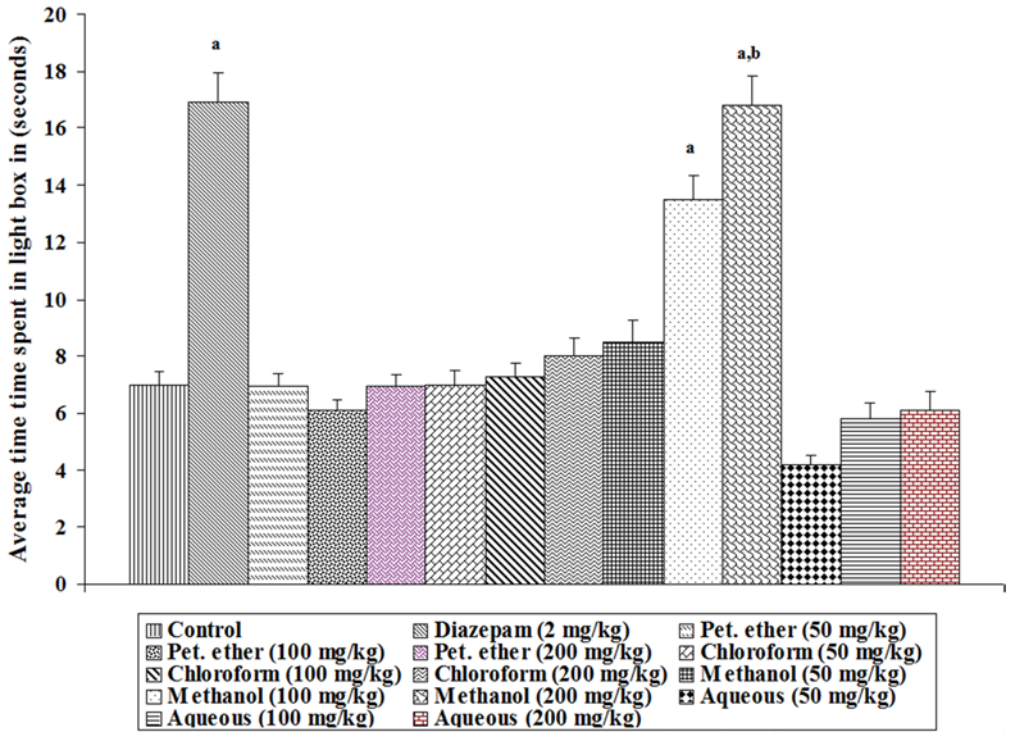

Figure 2 Effect of *M. parviflora* extract on light and dark box Model. All values are expressed as mean ± SD, n = 6. Superscript: $^a p < 0.05$ compared with control; $^b p < 0.05$ compared with 100 mg/kg dose of methanol extract. Statistical analysis was done by one way ANOVA followed by Tukey's test.

Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine [27]. Therefore, the anxiolytic effect of *M. parviflora* could be due to the presence of flavonoids in the plant extract.

Conclusion

The methanol extract of *M. parviflora* stem possess anti-anxiety properties. However, further studies are required to identify the phytoconstituents responsible for the observed anxiolytic effect of the plant, and to explain its anxiolytic mechanism involved in the brain. Currently, the authors are involved in anxiolytic-directed-fractionation of bioactive methanol extract of *M. parviflora* with a view to characterize the bioactive constituent(s).control analysis of RAM and LOS in their pharmaceutical formulation.

References

[1] R. C. Kessler and P. S. Wang. The descriptive epidemiology of commonly occurring mental disorders in the United States, *Ann. Rev. Pub. Health*. 29: 115-129 (2008).
[2] R. C. Kessler, P. Berglund, O. Demler, R. Jin, K. R. Merikangas, and E. E. Walters. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National comorbidity survey replication, *Arch. Gen. Psych*. 62: 593-602 (2005a).
[3] B. O. Olatunji, J. M. Cisler, and D. F. Tolin. Quality of life in anxiety disorders: a meta- analytic review, *Clin. Psychol. Rev*. 27: 572-581 (2007).
[4] J. P. Lepine. The epidemiology of anxiety disorders: prevalence and societal costs, *J. Clin. Psych*. 63: 14-18 (2002).
[5] K. Rickels and E. Schweizer. The clinical presentation of generalized anxiety in primarycare setting: practical concepts of classification and management, *J. Clin. Psych*. 58: 4-9 (1997).
[6] L. Y. Chung, S. H. Goh, and Z. Imiyabir. Central nervous system receptor activities of some Malaysian plant species, *Pharmaceut. Biol*, 43: 280-288 (2005).
[7] A. S. Kumar and R. Gandhimathi. Effect of *Guettarda speciosa* extracts on biogenic amines concentrations in rat brain after induction of seizure, *Int. J. Pharm. Pharmaceut. Sci*. 1: 237-243 (2009).
[8] A. Rex, E. Morgenstern, and H. Fink. Anxiolytic-like effects of Kava-Kava in the elevated plus maze test-a comparison with diazepam, *Prog. Neuropsychopharmacol. Biol. Psych*. 26: 855-860 (2002).
[9] G. Elkins, M. H. Rajab, and J. Marcus. Complementary and alternative medicine use by psychiatric inpatients, *Psychol. Rep*. 96: 163-166 (2005).
[10] R. N. Chopra, I. C. Chopra, K. L. Handa, and L. D. Kapur. *Chopra's Indigenous drugs of India*, Academic publishers, Calcutta, 1958.
[11] K. R. Kirtikar and B. D. Basu. *Indian Medicinal Plants Vol. I & II*, Dehradun, Bishen Singh Mahendra Pal Singh, 1975.
[12] P. Scartezzini and E. Speroni. Review on some plants of Indian traditional medicine with antioxidant activity, *J. Ethnopharmacol*. 71: 23-43 (2000).
[13] D. O. Kennedy, W. Little, and A. B. Scholey. Attenuation of laboratory-induced stress in humans after acute administration of *Melissa officinalis* (lemon balm), *Psychosomat. Med*. 66: 607-613 (2004).
[14] H. Moradkhani, E. Sargsyan, and H. Bibak. *Melissa officinalis* L., a valuable medicine plant, *J. Med. Plants Res*. 4: 2753-2759 (2010).
[15] G. E. Trease, W. C. Evans, and D. Evans. *Trease and Evans' Pharmacognosy*. 16th ed., Saunders, Edinburgh, 2009.
[16] K. R. Khandelwal. *Practical Pharmacognosy*. 19th ed., Pune, Nirali Prakashan, 2009.
[17] J. Y. Lin and C. Y. Tang. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effect on mouse splenocyte proliferation, *Food Chem*. 101: 140-148 (2007).
[18] M. A. Hossain, M. D. Muhammad, G. Charles, and I. Muhammad. *In vitro* total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetrastigma from Sabah, *Asian Pac. J. Trop. Med*. 4(9): 717-721 (2011).
[19] Organization of Economic Co-operation and Development (OECD) [online]. The OECD Guidelines for Testing of Chemical: 423 Acute Oral Toxicity, France. 2001 [cited 2014 August 10]; Available from:http://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl420.pdf.
[20] S. Pellow, P. H. Chopin, S. E. File, and M. Briley. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat, *J. Neurosci. Meth*. 14(3):149-167 (1985).
[21] A. C. Sharma and S. K. Kulkarni. MK-801 produces antianxiety effect in elevated plus-maze in mice, *Drug Dev. Res*. 22: 251-258 (1991).
[22] M. Hascoet and M. Bourin. A new approach to the light or dark test procedure in mice, *Pharmacol. Biochem. Behav*. 60: 645-653 (1998).
[23] M. Bourin and M. Hascoet. The mouse light/dark test, *Eur. J. Pharmacol*. 463: 55-65 (2003).
[24] S. A. Hoggs. Review of the validity and variability of the elevated plus maze as an animal model of anxiety, *Pharmacol. Biochem. Behav*. 54: 21-30 (1996).
[25] S. Pellow, P. H. Chopin, S. E. File, and M. Briley. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat, *J. Neurosci. Meth*. 14: 149-167 (1985).
[26] J. Crawley and F. K. Goodwin. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines, *Pharmacol. Biochem. Behav*. 13: 167-170 (1980).
[27] J. N. Crawley, P. J. Marangos, S. M. Paul, P. Skolnick, and F. K. Goodwin. Interaction between purine and benzodiazepine: inosine reverses diazepam-induced stimulation of mouse exploratory behavior, *Science*. 211: 725-727 (1981).
[28] H. Nishikava, T. Hata, and Y. Funakami. A role for corticotrophin releasing factor in repeated cold stress-induced anxiety-like behavior during forced swimming and elevated plus-maze test in mice, *Biol. Pharm. Bull*. 27: 352-356 (2004).