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Research Article

EVALUATION OF ANTI ANXIETY ACTIVITY OF *BAMBUSA VULGARIS* EXTRACTS IN EXPERIMENTAL ANIMALS

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ABSTRACT

The objective of this study is the anxiolytic activity of petroleum ether, chloroform, ethyl acetate and hydroalcoholic extracts of *Bambusa vulgaris* was investigated by Elevated Plus Maze test, Light and Dark model and Hole Board apparatus. Swiss albino mice were used to evaluate the activity. In each experiment, six animals each were divided into different treatment groups: control group received 10% tween 80, positive control received Diazepam (2 mg/kg) as standard drug and test groups were administered Petroleum ether, Chloroform, Ethyl acetate and Hydroalcoholic extracts of *Bambusa vulgaris* (200 mg/kg and 400 mg/kg body weight i.p.). The data were subjected to ANOVA followed by tukey's post- hoc test. In Elevated plus maze model, hydroalcoholic extract (400 mg/kg) of *Bambusa vulgaris* showed increase in time spent in open arms and number of entries in open arm as compared to control; time spent and number of entries in light area was increased as compared to control in light and dark model; increase in number of head dips as compared to control was observed in Hole Board test. From the observations, it can be concluded that the hydroalcoholic extract of *Bambusa vulgaris* exhibited significant anxiolytic activity at the dose of 400 mg/kg.

Keywords: Anti-anxiety, *Bambusa vulgaris*, Elevated Plus Maze, Light and Dark model, Hole Board test.

INTRODUCTION

Anxiety is defined as an emotional state which causes generalized mood of fear, worry or uneasiness that comes from a worse feeling about something that had happened or may happen. It is an expected response to real or potential danger and can be stimulated from environmental factors[1-3]. Anxiety is affecting 16.6% of the total world population and has become an important research area in psychopharmacology in past decades[4]. There are different types of anxiety like panic disorder, agoraphobia, social anxiety, specific phobias, obsessive- compulsive disorder, post-traumatic stress disorder, generalized anxiety disorder that could be mild or severe depending on the level of disorder. Abnormal function of noradrenergic, serotonergic, GABAergic and dopaminergic neural systems as well as abnormal chemoreceptor reactivity have all been involved in the pathophysiology of anxiety[5,6].

Symptoms may also include restlessness or agitation, sweating, shortness of breath, panic attacks, having trouble concentrating or sleeping, dizziness and heart palpitations[7]. Anxiety disorders are the most common and prevalent behavioral disorders which causes significant impairment of function and quality of life[8]. The current prevalence of anxiety disorder in India is 3.6 %[9,10]. Treatments suggested for anxiety like disorders include selective serotonin reuptake inhibitors (SSRI), Serotonin-norepinephrine reuptake inhibitors (SNRI), benzodiazepines, azapirones, atypical antipsychotics and beta adrenergic antagonists[11,12]. Benzodiazepines, a major class of compounds used for treatment of anxiety having a narrow margin of safety and possesses unwanted side effects, has prompted researchers to evaluate new compounds specially plant based drugs having less undesirable side effects[13-16].

In the present study, *Bambusa vulgaris* also known as Bamboo, which belongs to family poaceae, has been evaluated for its anxiolytic potential[17]. It is a good source of different classes of bioactive compounds. In this plant, triterpenes and steroidal glycosides are the major phytoconstituents. It also contains phenolic compounds, flavonoids, terpenoids, alkaloids, phytosterols and tannins, carbohydrate, glycosides, cyanogenic glycosides, coumarins, alkaloids, triterpenoids, phenols, saponins, volatile oils, steroids, and proteins[18]. Flavonoids (vitexin and orientin) are present in the ethanolic extract.

The leaves of *Bambusa vulgaris* are used for treatment of fever, hypertension, arteriosclerosis, detoxification, respiratory diseases, chest inflammation, edema, restlessness, diarrhea, vomiting and excessive thirst[19]. Various pharmacological activities of bamboo leaves extract such as antioxidant, anti-fertility, antihypertensive, antimicrobial, anti-helminthic, anti-inflammatory, antiulcer, anti-diabetic have been reported[20-26]. The medicinal effects of bamboo leaves are mostly attributed to their antioxidant capacity. No research work on its anti-anxiety activity has been done so far. So, the aim of the present study is to evaluate the anti-anxiety activity of petroleum ether, chloroform and ethyl acetate and hydroalcoholic extracts of *Bambusa vulgaris* (Bamboo) using different animal models of anxiety.

MATERIALS AND METHODS

Collection and authentication of plant material

Leaves of *Bambusa vulgaris* were procured from the botanical garden of G. H. G. Khalsa College of Pharmacy, Gurusar Sadharand identified by Dr. Sunita Garg, Chief scientist, Raw material Herbarium and Museum (RHMD), CSIR- NISCAIR,

Delhi, India (Voucher specimen No. NISCAIR/RHMD/Consult/ 2018/3202-03). A voucher specimen was deposited in the herbarium of the institute.

Drugs and chemicals

Diazepam was obtained from Glaxo Smith Kline and petroleum ether, methanol (LR grade) were procured from S.D. Fine-Chem Ltd, Mumbai.

Preparation of extract

Bambusa vulgaris leaves (200 g) were dried in shade and powdered. They were then packed into Soxhlet apparatus and extraction was done by successive exhaustive extraction using solvents petroleum ether, chloroform, ethyl acetate and methanol: water (70:30). Extracts were dried at room temperature. The dried extracts were used for further study. Extracts were weighed and percentage yield was calculated in terms of the air-dried weight of the plant material.

Animals

Swiss albino mice (weighing 15-30 g) of either sex was procured from the animal house facility of the G. H. G. Khalsa College of Pharmacy, Gurusar Sadhar (Regd. No. GHG/ 01/ 2017). The animals were kept in polypropylene cages. Each cage contained 6 mice at 22 ± 1 °C for a 12-h light/dark cycle. Water and food were available *ad libitum*. Groups of 6 mice each were randomly assigned to different treatment groups. Control group received vehicle (10% Tween 80), standard received Diazepam (2 mg/kg i. p.) while other test groups received petroleum ether (PE), chloroform (CL), ethyl acetate (EA) and hydroalcoholic (HA) extracts at doses (200 and 400 mg/kg respectively). The effects of the drugs were estimated 45 min after the administration of the dose. In each experiment, apparatus was cleaned using 5% ethanol before introducing the next animal to preclude the possible cueing effects of odors left by previous subjects.

Phytochemical screening

A phytochemical examination was carried out for all the extracts as per standard methods[27].

Animal models for anxiety

Elevated plus maze

The plus maze apparatus consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof with a plus maze elevated (25 cm) from the floor was used to observe anxiolytic behavior of animals. The animals were fasted 18 h prior to experiment. The dose administration schedule was so adjusted that each mice was having its turn on plus maze after 45 min of administration of dose. Each animal was placed in the center of the elevated plus maze with its head facing the open arms.

During this 5 min experiment, behavior of the mice was recorded as: a) no. of entries into the open/closed arm b) average time spent by the animal in each arm. During the entire experiment each animal was allowed to socialize. Every precaution was taken to ensure that no external stimuli evoked the animal[28-30].

Light and Dark Model

The apparatus consisted of two 20 cm × 10 cm × 14 cm boxes: one dark and the other transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100 W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mice was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mice stepped into the dark box. The apparatus was cleaned thoroughly between trials[31].

Hole Board Test

The hole board apparatus consisted of a wooden box (40 × 40 × 25) with 16 holes (each of diameter 3 cm) evenly distributed on the base of box. The apparatus was elevated to a height of 25 cm. Mice were treated with test doses of the prepared extracts (200 mg/kg and 400 mg/kg i. p. respectively), diazepam (2 mg/kg, i. p.) and vehicle, 45 min prior they were placed in apparatus. The numbers of head dippings during a 5 min period were recorded[32,33].

STATISTICAL ANALYSIS

All the values were expressed as mean ± SEM. Statistically significant difference between the groups were calculated by the application of one-way analysis of variance (ANOVA) followed by Tukey's, post-hoc test.

RESULTS

Phytochemical screening

The results of phytochemical screening are summarized in Table 1.

Elevated plus Maze

The results of the number of entries and time spent in open arms are shown in Figure 1 (a) and (b). Standard drug, diazepam, increased the time spent and entries in the open arms (***P < 0.001) HA extract of *B. vulgaris* at a dose of 400 mg/kg significantly increased the time spent and number of entries in the open arms (**P < 0.01). The effect was comparable with the standard drug diazepam.

Light and dark model

There was significant increase in average time spent and mean number of entries in light box by the administration of HA extract at 400 mg/kg. The effect was comparable with the standard drug diazepam. The results of the number of entries and time spent in light compartment are shown in Figure 2 (a) and (b).

Hole board test

There was significant increase in average number of head dippings in hole board apparatus by the administration of HA extract at 400 mg/kg. The effect was comparable with the standard drug diazepam. The results of total number of head dippings are shown in Figure 3.

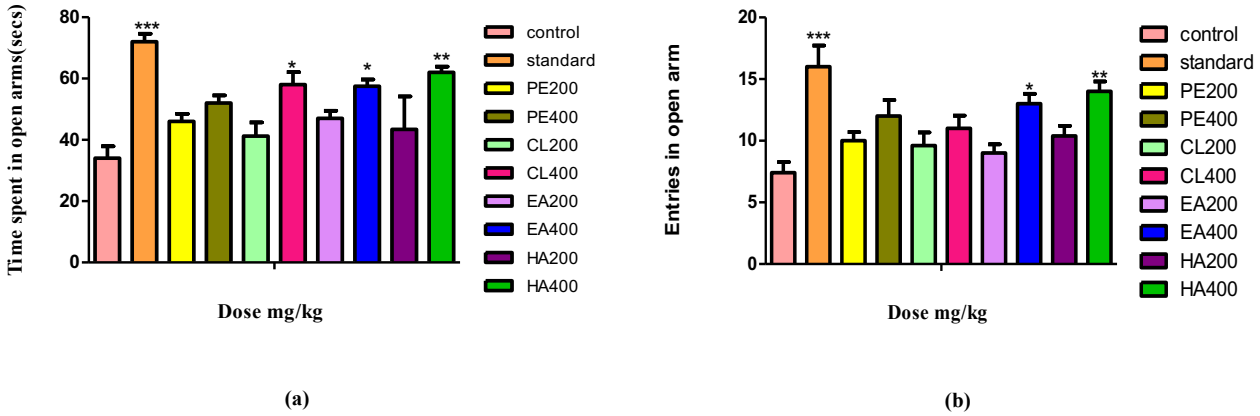

Figure 1: Relative anxiolytic profile of various extracts of BV: (a) Mean time spent in open arms by mice using EPM apparatus (b) Mean number of entries in open arms by mice using EPM apparatus

Control = 10% Tween 80, Standard = Diazepam (2 mg/kg) i.p, PE = 200 and 400 mg/kg i.p, CL = 200 and 400 mg/kg i.p, EA= 200 and 400 mg/kg i.p and HA = 200 and 400 mg/kg i.p. Results are expressed as mean ± SEM (n = 5); one way ANOVA followed by Tukey's post-hoc multiple comparison test; ***p < 0.001 and **p < 0.01 as compared to control.

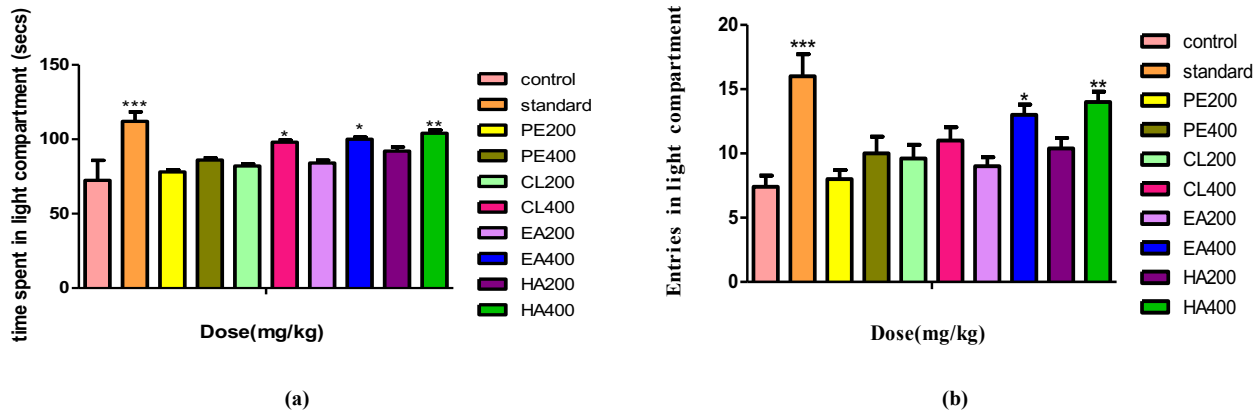

Figure 2: Relative anxiolytic profile of various extracts of BV: (a) Mean time spent in light area by mice using light and dark model (b) Mean number of entries in light area by mice using light and dark model

Control = 10% Tween 80, Standard = Diazepam (2 mg/kg) i.p, PE = 200 and 400 mg/kg i.p, CL = 200 and 400 mg/kg i.p, EA= 200 and 400 mg/kg i.p and HA = 200 and 400 mg/kg i.p. Results are expressed as mean ± SEM (n = 5); one way ANOVA followed by Tukey's post- hoc multiple comparison test; ***p < 0.001 and **p < 0.01 as compared to control.

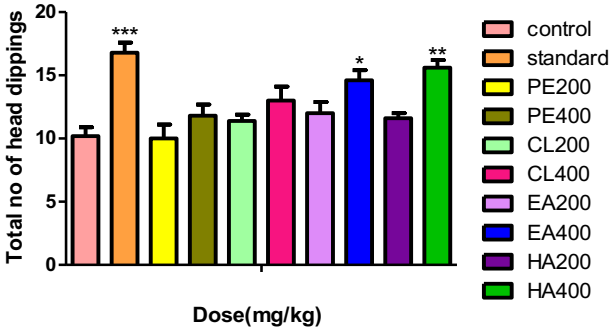

Figure 3: Relative anxiolytic profile of various extracts of BV– number of head dips in hole board test

Control = 10% Tween 80, Standard = Diazepam (2 mg/kg) i.p, PE = 200 and 400 mg/kg i.p, CL = 200 and 400 mg/kg i.p, EA= 200 and 400 mg/kg i.p and HA = 200 and 400 mg/kg i.p. Results are expressed as mean ± SEM (n = 5); one way ANOVA followed by Tukey's post- hoc multiple comparison test; ***p < 0.001 and **p < 0.01 as compared to control.

Table 1: Results of Preliminary Phytochemical Screening of *Bambusa vulgaris* leaves

Phytochemical Constituents	Tests	Pet ether extract	Chloroform extract	Ethyl acetate extract	Hydroalcoholic extract
Alkaloids	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Dragendroff's test	+	+	+	+
Flavonoids	Shinoda test	+	+	+	+
	Lead acetate test	+	+	+	+
	Alkaline reagent	+	+	+	+
Steroids	Salkowski test	_	_	_	_
	Sulphur powder test	_	_	_	_
Tannins	Ferric chloride test	+	+	+	+
	Gelatin solution	+	+	+	+
	Chlorogenic acid test	+	+	+	+
Saponin Glycosides	Froth formation	+	+	+	+
	Foam test	+	+	+	+
Carbohydrates	Molish's Test	+	+	+	+
	Benedict's Test	+	+	+	+
	Barfoed's Test	+	+	+	+
Reducing sugar	Fehling' test	+	+	+	+
Free Amino Test	Biuret test	+	+	+	+
	Ninhydrin test	+	+	+	+
Fixed oils	Oily spot test	_	_	_	_
Proteins	Heat coagulation test	+	_	+	_

Present = (+); Absent = (-)

DISCUSSION

From the past few years, anxiety has become a very important area of research in psychopharmacology. Plants play a very important role in drug discovery. Majority of drugs used in modern medicine have been obtained from medicinal plants. The effect of central nervous system is manifested by symptoms which can be easily identified so researchers used behavioral parameters to discover new drugs. Some of the behaviors related to the central effect of drugs include anxiety, fear, convulsion, depression etc[34]. *Bambusa vulgaris* leaves have shown to possess antioxidant, antimicrobial, haemolytic, anti-inflammatory, antiulcer, anti-helminthic, anti-diabetic, anti-fertility activities, however there are no reports available on the anxiolytic effect of *Bambusa vulgaris* leaves. The present work has demonstrated the anti-anxiety potential of Petroleum ether, Chloroform, Ethyl acetate and Hydroalcoholic extracts of *Bambusa vulgaris* leaves on mice by employing three experimental models i.e. Elevated Plus Maze, Light and Dark Model and Hole board test.

The elevated plus maze is considered to be a valid animal model of anxiety because it uses natural stimulus that is the fear of a new, brightly light open space and fear of balancing on a narrow-raised platform. Moreover, it is known that anxiolytic agents increase the frequency of entries and time spent in open arms of elevated plus maze model. In the present study, hydro alcoholic extract (400 mg/kg) increased the number of entries and time spent by the animal in open arms.

Animals such as mice display a natural aversion to brightly lit areas. They also have a drive to explore a perceived threatening stimulus. Because of these two conflicting drives, the result is anxiety. Decreased anxiety means increase in exploratory behaviour. In light and dark model anxiolytic agents showed

increased exposure to light area. Hydroalcoholic extract (400 mg/kg) showed increase in time spent and number of entries in light area.

The hole board test provides a simple method for measuring the response of an animal to an unfamiliar environment. It has been showed that head dipping behavior was sensitive to changes in the emotional state of the animal and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head dipping behavior. In the present study, hydroalcoholic extract (400 mg/kg) increased head dip counts.

In elevated plus maze, light and dark model, and hole board test, results showed that hydroalcoholic extract (400 mg/kg) induces significant anxiolytic activity however all the extracts produced no effect at lower dose (200 mg/kg). Chloroform and ethyl acetate extract at dose of 400 mg/kg showed slight anxiolytic activity.

Many herbal products used to treat anxiety, may contain a constituent that influence the function of ionotropic receptors for the brain like major inhibitory neurotransmitters GABA, Serotonin and BZD[35-37]. Phytochemical screening of *Bambusa vulgaris* leaves extracts revealed the presence of flavonoids, tannins, alkaloids, saponins that may be the possible reason for the anxiolytic mechanism of action of *Bambusa vulgaris* extracts, as these constituents may bind to the $GABA_A$ - BZD complex. In support to this, it has been found that flavonoids bind with $GABA_A$ - BZD complex with high affinity. So, flavonoids may possess the reason for anxiolytic activity of BV extracts[38]. Presence of alkaloids in *Bambusa vulgaris* extract may also be responsible for anxiolytic activity as alkaloids have also proven to be responsible for exhibiting anxiolytic activity[39,40]. However, further studies are needed to explore the constituent responsible for the anti-anxiety activity[41].

CONCLUSION

From our research studies it can be concluded that Hydroalcoholic extract of *Bambusa vulgaris* possesses significant anxiolytic activity at the dose of 400 mg/kg in elevated plus maze, light and dark model and hole board apparatus. Further studies can be conducted for exploring the bioactive constituent and to ascertain an exact mechanism of action.

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REFERENCES

1. Lang PJ, McTeague LM. The anxiety disorder spectrum: Fear imagery, physiological reactivity, and differential diagnosis. Anxiety, Stress, and Coping 2009; 22(1): 5-25.
2. Shri R. Anxiety: causes and management. International Journal of Behavioral Science 2010; 5(1): 100-18.
3. Wolitzky-Taylor KB, Castriotta N, Lenze EJ, Stanley MA, Craske MG. Anxiety disorders in older adults: a comprehensive review. Depression and anxiety 2010; 27(2): 190-211.
4. Kheirbek MA, Klemenhagen KC, Sahay A, Hen R. Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. Nature neuroscience 2012; 15(12): 1613.
5. Levitt EE. The psychology of anxiety. Routledge; 2015.
6. Sylvester CM, Corbetta M, Raichle ME, Rodebaugh TL, Schlaggar BL, Sheline YI, Zorumski CF, Lenze EJ. Functional network dysfunction in anxiety and anxiety disorders. Trends in neurosciences 2012; 35(9): 527-35.
7. Alwahhabi F. Anxiety symptoms and generalized anxiety disorder in the elderly: a review. Harvard Review of Psychiatry 2003; 11(4): 180-93.
8. Baxter AJ, Charlson FJ, Cheng HG, Shidhaye R, Ferrari AJ, Whiteford HA. Prevalence of mental, neurological, and substance use disorders in China and India: a systematic analysis. Lancet Psychiatry 2016; 3(9): 832-41.
9. Miller BR, Hen R. The current state of the neurogenic theory of depression and anxiety. Current opinion in neurobiology 2015; 30: 51-58.
10. Murthy RS. National mental health survey of India 2015–2016. Indian Journal of Psychiatry 2017; 59(1): 21.
11. Das S, Sarma PH. A study on the anticonvulsant and antianxiety activity of ethanolic extract of *Punica granatum* Linn. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(2): 389-92.
12. Vikander B, Koechling UM, Borg S, Tönne U, Hiltunen AJ. Benzodiazepine tapering: a prospective study. Nordic Journal of Psychiatry 2010; 64(4): 273-82.
13. Balkrishna A, Misra L. Chemo-botanical and Neurological Accounts of Some Ayurvedic Plants Useful in Mental Health. The Natural Products Journal 2018; 8(1): 14-31.
14. Neal JA, Edelmann RJ, Glachan M. Behavioral inhibition and symptoms of anxiety and depression: Is there a specific relationship with social phobia? British Journal of Clinical Psychology 2002; 41(4): 361-74.
15. Ravindran LN, Stein MB. The pharmacologic treatment of anxiety disorders: a review of progress. The Journal of Clinical Psychiatry 2010; 71(7): 839-54.
16. Farach FJ, Pruitt LD, Jun JJ, Jerud AB, Zoellner LA, Roy Byrne PP. Pharmacological treatment of anxiety disorders: Current treatments and future directions. Journal of anxiety disorders 2012; 26(8): 833-43.
17. Clark LG, Zhang W, Wendel JF. A phylogeny of the grass family (Poaceae) based on ndh F sequence data. Systematic Botany; 1995. p. 436-60.
18. Kumar M, Krishnamurth S, Suseel S. Phytochemical investigation and acute toxicity study of methanol extract of *Bambusa vulgaris* leaves. Journal of Pharmacy Research 2011; 4(2): 403.
19. Hossain MF, Islam MA, Numan SM. Multipurpose Uses of Bamboo Plants: A Review. International Research Journal of Biological Sciences 2015; 4(12): 57-60.
20. Yakubu MT, Bukoye BB. Abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. Contraception 2009; 80(3): 308-13.
21. Yakubu MT, Bukoye BB, Oladiji AT, Akanji MA. Toxicological implications of aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. Human and experimental toxicology 2009; 28(9): 591-98.
22. N'guessan K, Zihiri GN, Etien DT. Hypotensive effect of aqueous extract of *Bambusa vulgaris* sheets on the arterial pressure of rabbits. American Journal of Scientific Research 2009; 2: 60-72.
23. Rajeshwari E. Evaluation of anti-microbial activity of *Bambusa vulgaris* leaves. International Journal of Phytotherapy Research 2012; 2(2): 36-9.
24. .Owokotomo IA, Owoeye G. Proximate analysis and antimicrobial activities of *Bambusa vulgaris* L. leaves' beverage. African Journal of Agricultural Research 2011; 6(21): 5030-32.
25. Lodhi S, Jain AP, Rai G, Yadav AK. Preliminary investigation for wound healing and anti-inflammatory effects of *Bambusa vulgaris* leaves in rats. Journal of Ayurveda and Integrative Medicine 2016; 7(1): 14-22.

26. Senthilkumar MK, Sivakumar P, Changanakkattil F, Rajesh V, Perumal P. Evaluation of anti-diabetic activity of *Bambusa vulgaris* leaves in streptozotocin induced diabetic rats. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(3): 208-10.
27. Farnsworth NR, Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences 1966; 55: 225-276.
28. Kulkarni SK, Reddy DS. Animal behavioral models for testing anti-anxiety agents. Methods and findings in experimental and clinical pharmacology 1996; 18(3): 219.
29. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature protocols 2007; 2(2): 322.
30. Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neuroscience and Bio behavioral Reviews 2005; 29(8): 1193-205.
31. Arrant AE, Schramm-Sapyta NL, Kuhn CM. Use of the light/dark test for anxiety in adult and adolescent male rats. Behavioral Brain Research 2013; 256: 119–127.
32. Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. European Journal of Pharmacology 1998; 350(1): 21-9.
33. Brown GR, Nemes C. The exploratory behavior of rats in the hole-board apparatus: Is head-dipping a valid measure of neophilia? Behavioral processes 2008; 78(3): 442-8.
34. Chandrashekar R, Manohar VR, Rao SN. Acute anxiolytic activity of aqueous extract of *Terminalia chebula* fruit pulp in rats. International Journal of Research in Ayurveda and Pharmacy 2013; 4(1): 112-5.
35. Griebel G, Perrault G, Tan S, Schoemaker H, Sanger DJ. Pharmacological studies on synthetic flavonoids: comparison with diazepam. Neuropharmacology 1999; 38(7): 965-77.
36. Paladini AC, Marder M, Viola H, Wolfman C, Wasowski C, Medina JH. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. Journal of Pharmacy and Pharmacology 1999; 51(5): 519-26.
37. Singh N, Kaur S, Bedi PM, Kaur D. Anxiolytic effects of *Equisetum arvense* Linn. extracts in mice.Indian Journal of Experimental Biology 2011; 49(5): 352-56.
38. Kumar S, Sharma A. Anti-anxiety activity studies of various extracts of *Turnera aphrodisiaca* Ward.Journal of Herbal Pharmacotherapy 2005; 5(4): 13-21.
39. Chandrashekar R, Manohar VR, Rao SN. Acute anxiolytic activity of aqueous extract of *Terminalia chebula* fruit pulp in rats. International Journal of Research in Ayurveda and Pharmacy 2013; 4(1): 112-15.
40. Sarris J. Herbal medicines in the treatment of psychiatric disorders: a systematic review. Phytotherapy Research 2007; 21(8): 703-16.
41. .Marder M, Paladini AC. GABA (A)-receptor ligands of flavonoid structure. Current Topics in Medicinal Chemistry2002;2(8):853-67.

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