Available online through
www.jpronline.info

Anxiolytic effects of the extracts of *Zingiber officinale* in mice

S.N.Harsha and K .R.Anilakumar*
Biochemistry and Nutrition Discipline, Defence Food Research Laboratory Mysore-570011, India

ABSTRACT

Zingiber officinale belonging to Zingiberaceae family is used worldwide as a cooking spice, condiment and herbal remedy. This study was taken up to investigate the anxiolytic activity of alcohol and water extracts of the rhizomes of *Zingiber officinale*.in mice, by behavioral tests like elevated plus maze (EPM) and open field test (OFT) which will serve as the basis for assessing anxiolytic effect. Three doses viz. 100, 200 and 400 mg/kg body wt. of 70% ethanol and water extracts were administered; i.p. to mice one hour before carrying out the tests. Diazepam (1 mg/kg body wt.) was taken as the standard anxiolytic drug. The animals administered with extracts at the levels of 200 and 400mg/kg body wt. Diazepam showed a significant increase in the time spent and total entries in the open arms of the EPM and increase in locomotor activity in OFT. However, extracts at 200 and 400 mg/kg body weight demonstrated significant increase in the time spent in the centre of the field. The results of the study suggest that the extracts possess promising anxiolytic activity and the extract may be regarded as a potent nutraceutical for treating anxiety.

Key words: anxiety, *Zingiber officinale,* elevated plus maze, open field test.

INTRODUCTION

Anxiety and anxiety-spectrum disorders are becoming increasingly prevalent in modern society, requiring new therapeutic approaches and treatments [1-3]. Anxiety induced stress activates the sympathetic nervous system, stimulating the release of catecholamines from the adrenal medulla as a response to stressors fight or flight [4-5]. Benzodiazepine, the most commonly prescribed treatment for anxiety disorders, has side effects such as sedation, myorelaxation, ataxia, amnesia and pharmacological dependence [6]. Hence various plants are used in complementary and alternative medicines for management of anxiety [7].

Zingiber officinale (ginger) belonging to *Zingiberaceae* family is used worldwide as a cooking spice, condiment and herbal remedy. As a medicinal herb, *Zingiber officinale* has long been used in China, India, Roman, and Arab for the management of various human ailments including headaches, colds, fever, nausea, and rheumatic disorders [8]. *Zingiber officinale* has received increasing attention because of its pronounced antioxidant [9], anti-inflammatory [10-11], antidiabetic [12], neuroprotective [13] and anticancer activities [14-15]. Pharmacological investigations have revealed that *Zingiber officinale* and its major pungent ingredients viz. diarylheptanoids and gingerol-related compounds [16], have chemopreventive and chemotherapeutic effects on a variety of cancer cell lines and on animal models [17-18]. A combination of *Zingiber officinale* and *Allium sepa* have been reported to produce hypoglycemic and hypolipidaemic effects [19] in albino rats. Addition of ginger (1%) to normal diet of rats prevent formation of free radicals and maintain the integrity of erythrocytes [20], probably due to its rich content of active ingredients viz. zingerone, gingerdiol, zingibrene, gingerols and shogaols, that are anti-oxidative [21] which directly act on the gastrointestinal tract [22]. Nevertheless, the studies on antianxiety properties of *Zingiber officinale* extracts are sparse. Hence this study was conducted to evaluate the antianxiety properties of the rhizome extract in mice.

*Corresponding author.
K .R.Anilakumar
Biochemistry and Nutrition Discipline,
Defence Food Research Laboratory
Mysore-570011, India
Tel.: +91-821-2473290.
E-mail:anilakumarkr@gmail.com

MATERIALS AND METHODS

Chemicals and samples

Diazepam used as standard was procured from Ranbaxy Laboratories Ltd., India (Calmpose). Fresh and healthy *Zingiber officinale* rhizomes were procured from the local market, Mysore, India. The rhizomes were washed, sliced, shade dried and powdered.

Preparation of *Zingiber officinale* roots extracts

Bioactive compounds were extracted by soaking 100gm of *Zingiber officinale* rhizome powder in 1 litre of 70% ethanol and water respectively. The sample along with solvent was kept for 24 hours using shaker and filtered, the procedure was repeated thrice. The extracts were pooled and evaporated to dryness using flash evaporator. The lyophilised powder of these extracts were made and used for this study.

Animal experiment

Animal studies were conducted according to the institute animal ethical committee regulations approved by the committee for the purpose of the control and supervision of experiments on animals. 42 male mice weighing 25-30 g were selected from the stock colony of Defence Food Research Laboratory, Mysore, India, housed in an acryl fiber cage, temperature controlled room (temperature $25 \pm 2°C$) maintained in 12 h light/ dark cycle with free access to food and drinking water *ad libitum*.

Experimental design

The extracts of the rhizome of *Zingiber officinale* were separately suspended in a vehicle comprising 1% (w/v) Tween 20 in distilled water. The grouping of mice and the administration of the extracts were carried out as given below:
Group 1: Control
Group 2: ethanol extract, 100/kg body wt.
Group 3: ethanol extract, 200/kg body wt.s
Group 4: ethanol extract, 400 mg/kg body wt.
Group 5: water extract, 100 mg/kg body wt.
Group 6: water extract, 200 mg/kg body wt.
Group 7: water extract, 400 mg/kg body wt.
Group 8: diazepam,1mg/kg body wt.

The extracts of the rhizomes of *Zingiber officinale* were prepared by suspending the dried extracts in the vehicle and were administered, i.p. to mice one hour before carrying out the tests. Six mice were taken in each group. The doses of extracts were calculated to administer 0.25 ml of the suspension of extracts to the mice. Diazepam (1 mg/kg body wt.) suspended in the vehicle was used as standard anxiolytic drug. The suspending vehicle (0.25 ml) without any extract/drug was used as control.

Elevated plus-maze test

The test procedure and scoring methodology for the elevated plus-maze test have been described by Kulkarni *et al.,* [23]. In brief, the apparatus composed of two open (30 × 5 × 0.25 cm) and two enclosed (30 × 5 × 15 cm) arms that radiated from a central platform (5 × 5 cm) to form a plus sign. A slightly raised edge on the open arms (0.25 cm) provided an additional grip for the animals. The maze floor and the closed arms were covered with black adhesive tape. The plus-maze was elevated to a height of 40 cm above floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (7: 00–19: 00). The mice were injected with drugs or vehicle and sixty minutes later, the trial was started by placing the animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent, in each of the two types of arm, were counted during a 5 min test period. The open-arm entries and open-arm time were used as indices of anxiety. A mouse was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out with the help of ANY MAZE software.

Open Field test

Spontaneous motor activity was evaluated in open field test have been described by Bhattacharya *et al.,* [24]. The open field apparatus is made up of black plexi glass and consisted of a square 56 cm x 56 cm. The floor of apparatus was divided into 16 square of identical dimension. The entire room, except the open field was kept dark during the experiment. One hour after the treatment of vehicle/standard/extract to each animal was placed at one corner of the apparatus and the behavioral aspects were noted in the next 5min. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out using ANY MAZE software.

STATISTICAL ANALYSIS

All data are presented as mean ±SD and was analysed by one-way ANOVA. The groups treated with extracts were compared with the respective vehicle (control) group. The diazepam treated group was compared with control and P values (<0.001 and<0.05) were considered statistically significant.

RESULTS

Elevated plus maze test

Animals treated with diazepam showed a significant increase in the time spent in the open arms and decreased time spent in closed arms (fig 1 and 2), as well as an increase in the number of entries in the open arms (fig 3). 70% ethanol and water extracts of *Zingiber officinale* showed increase in time
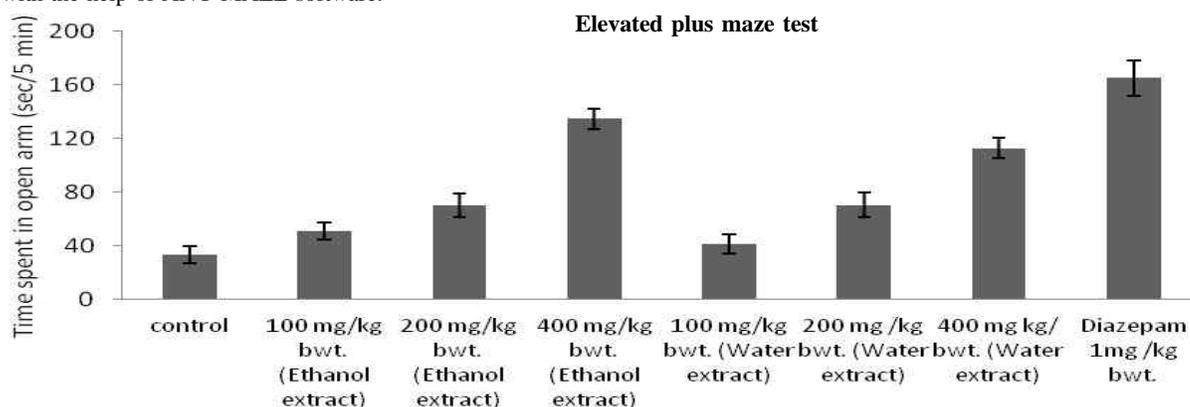
* indicates significant difference as compared to control group (p<0.001). Values are given as mean ± SD of six animals.
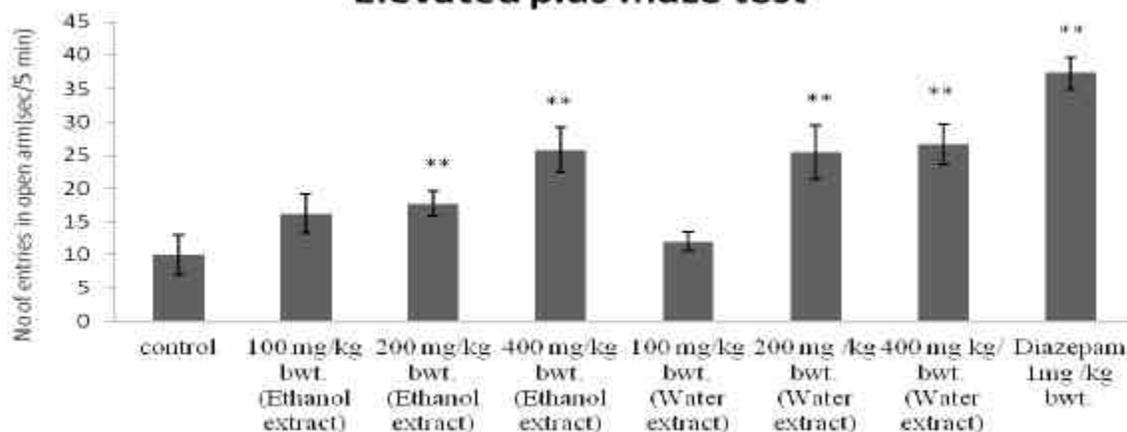
Figure1: **Elevated plus maze test**
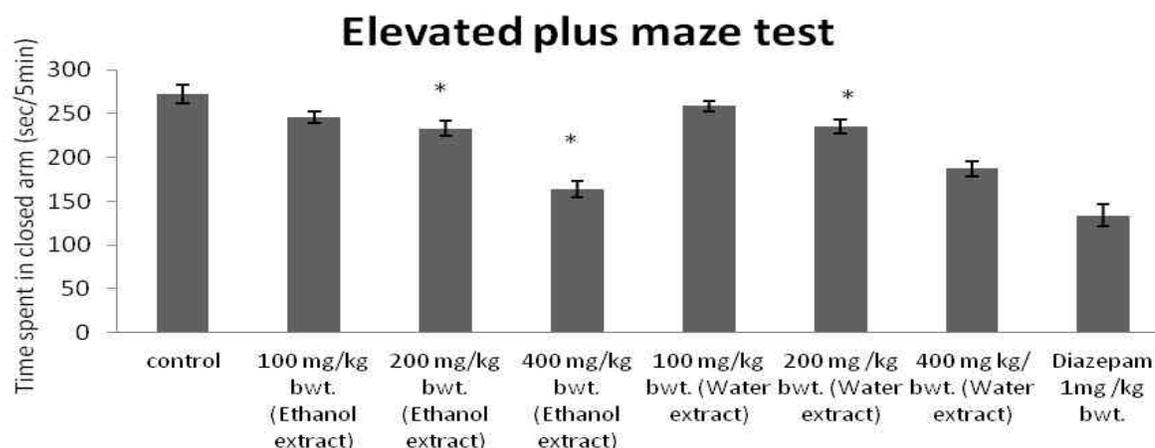
* indicates significant difference as compared to control group (p<0.001). Values are given as mean ± SD of six animals.
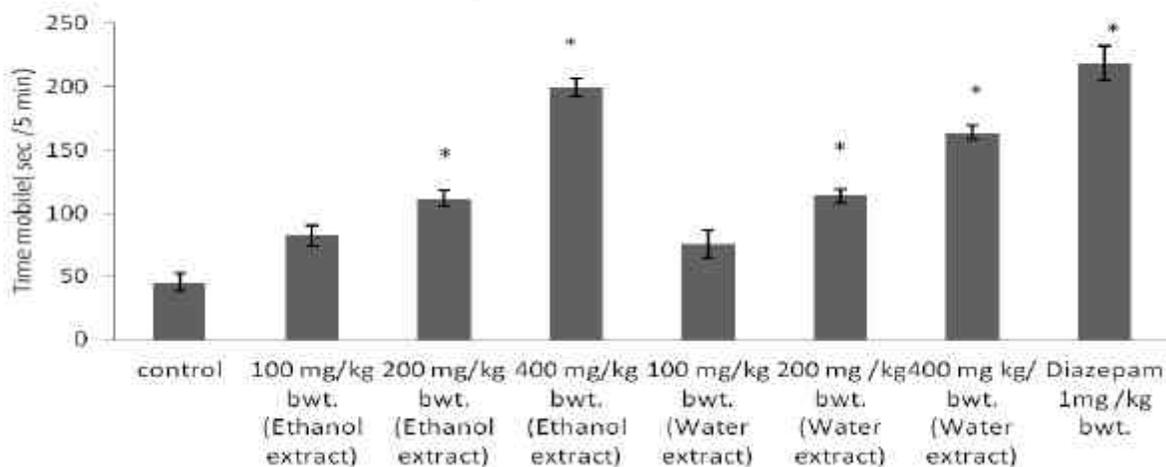
Figure2: **Elevated plus maze test**

Elevated plus maze test

*indicates significant difference as difference as compared to control group at p<0.05. **p<0.001. Values are given as mean ±SD for groups of six animals each*

Figure3: **Elevated plus maze test**

Open field test

indicates significant difference as compared to control group (p<0.001). Values are given as mean ± SD of six animals.

Figure4: Open field test

spent in open arm and in the number of entrances into the open arms compared to untreated group. Though the lower doses did not affect the number of entries and the time spent in open arms, the 200 and 400mg/kg body wt. treated group of both extracts showed significant increase which is comparable to diazepam drug adminisistered group of mice.

Open field test

Diazepam administered at the level of 1 mg/kg body wt. significantly increased (P<0.001) the locomotor activity at the centre and the total locomotion. The i.p. administration at the levels of 100, 200, 400 mg/kg body wt of 70% ethanol extract and 200 and 400 mg/kg body weight of water extract showed a significant increase (P<0.001) in the total locomotion (fig 4). However, the observed increase in total locomotion was very high in the study using the dose of 400mg/kg body wt. of both the extracts.

DISCUSSION

Anxiety is a common emotional phenomenon in humans [25], and a central nervous disorder [26]. Mechanism of anxiolytic action of plants may be by interaction with some of the natural endogenous mediators in the body [27-28]. In traditional medicine practice it has been established that there are lots of

secondary metabolites of plants that can be employed in the treatment of psychiatric disorders, especially anxiety, most of which directly or indirectly affect the central nevous system, noradrenalin, serotonin and GABA neurotransmitter activity [29-30]. There could also be a link in the interaction of the plant extract with serotonergic pathway; [31-34]. 5-HT, GABA and corticotropin-releasing hormone (CRH) have been shown to mediate anxiety and stress-related behaviors [35].

Animal behavioral models have become an indispensable tool for studying anxiety disorders and testing anxiety- modulating drugs. Several methods viz. EPM and OFT to test levels of anxiety like - behavior in mice have been developed and pharmacologically validated and is shown to be specifically responsive to agents with proven anxiolytic or anxiogenic effects. All these procedures are based on the exposure of the subject to unfamiliar aversive environment [36].

EPM is considered one of the most widely validated tests for assaying new benzadiazepine-like anxiolytic agents [37], and diazepam used in this study is one among them. In the EPM test, it was demonstrated that the preference showed for the closed arms reflects an aversion to the open arms caused by

fear or anxiety induced by open space. In our study, diazepam produced significant increase in the time spent in open arms, decreased time spent in closed arm and increased number of entries in the open arms. The extracts of the *Zingiber officinale* rhizome at the doses of 200 and 400mg/kg body wt. also demonstrated similar results. It is well known that the anxiolytic agents increase the motor activity which is measured by time spent by the animal in the open arms [38-39].

The open-field model examines anxiety-related behaviour characterized by the normal aversion of the animal to an open, brightly lit area [40-41]. In OFT, the confrontation with the situation induces anxiety behavior in rodents. The anxiety behavior is triggered by two factors, i.e., individual testing (the animal was separated from its social group) and agoraphobia (as the arena is very large, relative to the animals breeding or the natural environment). In such situations, rodents show thigmotactic behavior identified by spontaneous preference to the periphery of the apparatus and reduced ambulation. Anxiolytic treatment decreases this anxiety- induced inhibition of exploratory behavior [24]. The administration of diazepam reduced the natural aversion and promoted exploration such that the time spent in the corners is reduced significantly and the time spent in the centre is increased. Mice treated with the extracts of *Zingiber officinale* exhibited less time in the periphery and more time in centre of the field compared to the corners. However, *Zingiber officinale* extracts at 200 and 400 mg/kg body wt. demonstrated a significant increase in the time spent at the centre of the field. Nevertheless, the maximum effect was shown by the administration of 400 mg/kg body wt. A recent report states that, the ethanol and water extracts of *Zingiber officinale* contain active ingredients viz. zingerone, gingerdiol, zingibrene, gingerols 10- shogaols and 1-dehydro-6- gingerdione which might partially activate the $5HT_{1A}$ receptor [42] which leads to decrease the serotonine levels in brain. In view of these results, it is suggested that ethanol and water extracts of *Zingiber officinale* possess potent anxiolytic property.

CONCLUSION

To verify the relationship between anxiolytic effect of the extract and the locomotor activity alteration, the open field test and elevated plus maze, the classical animal models were used to assess the autonomic effects of drugs and general activity of mice. The present study indicates that acute administration of the 70% ethanol and water extracts of the rhizome of *Zingiber officinale* at the level of 400 mg/kg body wt. demonstrate anxiolytic property.

REFERENCES

1. Murphy JM,. Trends in depression and anxiety: men and women, Acta Psychiatr Scand, 1986; 73(2):113-27
2. Twenge JM., The age of anxiety? Birth cohort change in anxiety and neuroticism, J Pers Soc Psychol, 2000; 79(6):1007-21
3. Farhath Khanum and Sakina Razack, Anxiety herbal treatment –a review, Research and reviews in Biomedicines and biotechnology, 2010; 10, 2, 71-89.
4. Koenig HG, George LK, Schneider R,. Mental health care for older adults in the year 2020: a dangerous and avoided topic, Gerontologist, 1994; 34(5):674-9.
5. Selye H. Further thoughts on "stress without distress" Med Times, 1976; 104:124-144.
6. Bisson JI., Ehlers A., Matthews R., *et al*,. Psychological treatments for chronic post-traumatic stress disorder: Systematic review and meta-analysis. Br J Psychiatry, 2007; 190(2):97-104
7. Mosoumen E., Mohammad K and Maryam FA. *Coriandrum sativum*; evaluation of its anxiolytic effect in the elevated plus maze, J Ethanopharmacol, 2005; 96**:**365-370
8. Ali BH., Blunden G., Tanira MO and Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): A review of recent research. Food and Chemical Toxicology, 2008; 46, 409–420.
9. El-Ghorab AH., Nauman M., Anjum F M., Hussain S and Nadeem M. A comparative study on chemical composition and antioxidant activity of ginger (Zingiber officinale) and cumin (Cuminum cyminum). Journal of Agricultural and Food Chemistry, 2010; .58, 8231–8237.
10. Mustafa T., Srivastava KC. Ginger (Zingiber officinale) in migraine headache. J Ethnopharmacol, 1990**; 29**:267-73
11. Minghetti P., Sosaz S., Cilurzo F., Casiraghi A., Alberti E., Tubaro A, et al. Evaluation of the topical anti-inflammatory activity of ginger dry extracts from solutions and plasters. Planta Medica, 2007; 73, 1525–1530.
12. Afshari AT., Shirpoor A., Farshid A., Saadatian R., Rasmi Y., Saboory E, et al.. The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. Food Chemistry, 2007; 101, 148–153.
13. Kondeti Ramudu Shanmugam., Korivi Mallikarjuna., Nishanth Kesireddy., Kesireddy Sathyavelu Reddy. Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats, *Food and Chemical Toxicology*, 2011; **49**, 4, 893-897
14. Shukla Y., and Singh M. Cancer preventive properties of ginger: A brief review. Food and Chemical Toxicology, 2007; 45, 683–690.
15. Xiao-Lan Cheng., Qun Liu., Yong-Bo Peng., Lian-Wen Qi., Ping Li. Steamed ginger (Zingiber officinale): Changed chemical profile and increased anticancer potential, Food Chemistry, 2011; 129, 1785–1792.
16. Policegoudra RS., Kumar MH., and Aradhya MS. Accumulation of bioactive compounds during growth and development of mango ginger (Curcuma amada Roxb.) rhizomes. Journal of Agricultural and Food Chemistry, 2007; 55, 8105–8111.
17. Chen C Y, Li Y W, and Kuo S Y.. Effect of [10]-gingerol on [Ca2+](i) and cell death in human colorectal cancer cells. Molecules, 2009; 14**,** 959–969.
18. Lee HS., Seo EY., Kang N E., & Kim WK. [6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells. The Journal of Nutritional Biochemistry, 2008; 19, 313–319.
19. Ahmed RS and Sharma SB. Biochemical studies on combined effects on garlic (Allium sativum L) and ginger (*Zingiber officinale Rose*) in albino rats. *Indian journal of Experimental Biology,* 1997; 35(8):841-3
20. Ahmed RS., Seth V., Banerjee BD. Influence of dietary ginger (*Zingiber officinale rose*) on antioxidant defence system in rat, comparison with ascorbic acid. Indian journal of experimental biology, 2000; 38: 604-606.
21. Connell D and Sutherland M. A re-examination of gingerol, shogaol and zingerone, the pungent principles of Ginger (*Zingiber officinale Roscoe*), Aust J Chem, 1969; 22:1033-43
22. Foster S and Tyler VE. Tyler's honest herbal: a sensible guide to the use of herbs and related remedies. 4th ed. New York: Haworth Herbal Press, 1999.
23. Kulkarni SK. Animals behavioral models for testing anti-anxiety agents, In hand book of experimental pharmacology, 3rd edition, Vallabh Prakashan, Delhi 2002; 27-37.
24. Bhattacharya SK., Satyan K S. Experimental methods for evaluation of psychotropic agents in rodents, Ind J Exp Biol, 1997; 35: 565-75.
25. Cle'ment Y., Calatayud F., Belzung C. Genetic basis of anxiety like behavior, a critical review, Brain Res Bull., 2002; 57**:** 57-71
26. Kjernised KD and Bleau P. Long term goals in the management of acute and chronic anxiety disorders, Can J Psychiatry, 2004; 49(1):515-655
27. Contarino A., Dellu F., Koob GF., Smith GW., Lee K., Vale W and Gold LH. Reduced anxiety like and cognitive performance in mice lacking the corticotropic releasing factor receptor 1, Brain res, 1999; 835, 9.

28. Shih JC., Ridd MJ., Chen K., Meehan WP., Kung M., Self I and Demaeyer E. Cetanserin and tetrabenzene abolish aggression in mice lacking mono amino oxidase A, Brain Res, 1999; 835**:**112.

29. Wolfman C., Viola H., Paladini A., Dajas F and Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea.* Pharmacol Biochem Behav*,* 1994; 47**:** 4.

30. Paladini A., Marder M., Viola H., Wolfman C., Wasowski C and Medina JH. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds*,* J Pharm Pharmacol, 1999; 51:526.

31. Kadaba BKA. A safe herbal treatment for anxiety, Brit J Phyto Phar, 1994; 3**:** 1500.

32. Griebel G,. 5-hydroxytryptamine pathways in anxiety and its treatment, Pharmacol Ther*,* 1995; 66: 103-148.

33. Graeff FG., Guimares FS., de Andrade TG and deakin JF. Role of 5-HT in stress, anxiety and depression, Pharmacol Biochem Behav, 1996; 54: 129-141.

34. Unis AS., Cook H., Vincent JG., Gderde DK., Perry BD., Matson C and Mitchell J. Platelet serotonin measures in adolescents with conduct disorder, Biol Psych, 1997; 42:559.

35. Wood WM., Ocran KW., Gordon DF., et al. Isolation and characterization of mouse complementary DNAs encoding alpha and beta thyroid hormone receptors from thyrotrope cells: themouse pituitary-specific beta 2 isoform differs at the amino terminus from the corresponding species from rat pituitary tumor cells. Mol Endocrinol*,* 1991; 5 (8):1049-61.

36. Belzung C and Griebel G. Measuring normal and pathological anxiety like behavior in mice a review, Behav Brain Res, 2001; 125:141-149.

37. Pellow S., Chopin P., File SE., Briley M. Validation of open closed arm entries in an elevated plus maze as a measure of anxiolytic in the rat, J Neuroscience methods, 1985; 14**:**149-167.

38. Hui KM, Huen MS, Wang HY, Zheng H, Sigel E, Baur R, *et al..* Anxiolytic effect of wogonin, a benzodiazepine receptor ligand isolated from *Scutellaria baicalensis* Georgi. Biochem Pharmacol, 2001; 64:1415-24.

39. Melo CTV., Monterio AP., Leite CP et al. Anxiolytic like effects of (o methyl)-N 2,6 dihydroxybenzoyl-tyramine(Riparin IIII) from Aniba rip aria (neem)MEZ Lauraceae in mice, Biol Pharm Bull, 2006; 29**:**451-454.

40. Choleris E., Thomas AW., Kavaliers M., Prato FS. A detailed ethological analysis of the mouse open field test: Effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neuroscience Behavioral Review*,* 2001; 25: 235-260.

41. Mechan A.O., Moran P.M., Elliott M., Young A.J., Joseph M.H., Green R., A comparison between Dark Agouti and Sprague-Dawley rats in their behaviour on the elevated plus-maze, open-field apparatus and activity meters and their response to diazepam. Psychopharmacology. 2002; 159: 188-195.

42. Andreas Nievergett., Peter Huonker., Roland Schoop., Karl Heinz Altmann., Jury Gertsch. Identification of serotonin $5HT_{1A}$ receptor partial agonists in ginger, Bioorganic and Medicinal chemistry, 2010; **18**: 9, 3345-3351.