IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2020 Vol.:17, Issue:3

Pharmacological Screening of Anxiolytic Activity of Methanolic Extract of *Zingiber officinale* Roscoe Rhizomes in Mice

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Prashant kumar*[1], Yogendra pal[2], Shashi Bhooshan Tiwari[3]

Department of pharmacy, M.J.P. Rohilkhand University, Bareilly-243006 (U.P.), India

Submission: 20 January 2020
Accepted: 28 January 2020
Published: 29 February 2020

Keywords: Anxiety, *Zingiber officinale*, elevated plus maze, light dark arena model

ABSTRACT

This project work study was carried out the pharmacological screening of anxiolytic activity of methanolic extract of *Zingiber officinale* (ginger) rhizomes in mice. Ginger is an ancient medicinal plant belonging to Zingiberaceae family which is officially known as *Zingiber officinale* Rosc. The behavioural test was measuring by using the two anxiolytic models such as elevated plus maze (EPM) and light-dark arena model. The extraction of ginger was carried out by soxhlet apparatus using methanol as solvent. The ginger extract dose was administered in two groups as 200 mg/kg, p.o and 400 mg/kg, p.o. which is compared with control (received vehicle) and standard groups (diazepam 1 mg/kg, i.p.) for its anxiolytic activity for 7 days. The dose at 200 mg /kg and 400 mg/kg administered orally which increase the time spent and no of entry in elevated plus maze and increase time spent in box and no of crossing between light dark compartment boxes. The results of the study work showed that the extracts exhibit anxiolytic activity.

INTRODUCTION

Anxiety is most common affective mental disorders that require an adequate medical treatment. It is characterized by uneasiness, change of mood, panic attacks, decrease in libido and various mental impairments. Anxiety disorder is among the most prevalent mental disorders in the general population. In India, prevalence rate of anxiety disorders are 18.5 per 1000 population. [1]

Anxiety disorders are the most common of all mental health problems. It is estimated that they affect approximately 1 in 10 people. The female-to-male ratio for any lifetime anxiety disorder is 3:2. Most anxiety disorders begin in childhood, adolescence and early adulthood. Panic disorder demonstrates a bimodal age of onset in the age groups of 15-24 years and 45-54 years. The age of onset for obsessive compulsive disorder appears to be the mid 20s to early 30s. Most social phobias begin before the age of 20 years (median age at illness onset is 16 years.). Most simple (specific) phobias develop during childhood (median age at illness onset is 15 years) and eventually disappear. Those that persist into adulthood rarely go away without treatment. [3, 4] Anxiety disorder involves a variety of symptoms such as fear, distractibility, muscle tension, increase blood pressure, depression, irritability and restlessness. But some other important symptoms are following (Mood symptoms, Physical symptoms, Cognitive symptoms, Motor symptoms). Anxiety disorder has a high impact on daily life (illness intrusiveness) and cause a great deal of suffering for the individual patient. [2]

Many herbal drugs were used from ancient time to treat anxiety such as *Magnolia dealbata* Zucc (Magnoliaceae), *Achillea millefolium* linn. (Asteraceae), *Bacopa monnieri* penn. (Scrophulariaceae), Azadirachta indica (Meliaceae), *Coriandrum sativum* (Apiaceae), *Citrus sinensis* (Rutaceae), *Euphorbia neriifolia* (Euphorbiaceae), *Piper methysticum* G. (Piperaceae), *Salvia rofficinalis* (Lamiaceae), *Panax ginseng* (Araliaceae), *Zingiber officinale*(Solanaceae), *Zingiber officinale* Roscoe. Commonly known as Adrak (Zingiberaceae) is the most useful traditional plant in India. Every part of the plant possesses many pharmacological properties that were used to treat many human ailments. [5]

Ginger is an ancient medicinal plant belonging to Zingiberaceae family which is officially known as *Zingiber officinale* Rosc and it is indigenous to south-eastern Asia. It is one of the earliest known treasured spices esteemed for its pungency and aroma, viewed as a healing

gift from God by Indian Ayurvedic systems. Since a very long time ginger is known for its medicinal value as a digestive aid, spiritual beverage, aphrodisiac, antiemetic, anticancer, anti-platelet, anti-microbial, anti-parasitic, anti-oxidant, anti-inflammatory, analgesic, hepato-protective, and immune stimulating properties. India is the largest producer and consumer of ginger contributing about 31% of total global production followed by China, Nepal, Indonesia, Nigeria, and Thailand**.** [6] In the countries such as Canada, U.K, U.S.A. ginger is used in soft drink manufacturing industry, baking industry and meat processing industry up to a great extend but it rarely used for cooking. Ginger brine is most popular in Japan, Orissa, Kerala, Karnataka, Arunachal Pradesh, West Bengal, Sikkim and Madhya Pradesh the major ginger producing states in India. [4] Among which Kerala contribute 33 percent to the total production by which it has prove to be the largest ginger producing state in India. Cochin ginger and Calicut ginger are the popular Indian ginger varieties in the world market. India produces 683000 tons of ginger per annum that is almost 1/3rd of world's total production (FAO). 30 per cent of total production of ginger in India is transferred to dry ginger, 50 per cent is taken as fresh or green ginger and the rest part is used as seed materials. Kerala is the largest producer of dry ginger in India, which has taken a major share in export. [7]

Ginger rhizome is obtained from the underground stems that surrounded by the sheathing bases of the two-ranked leaves. It is normally an erect perennial growing plant that grown in ground from 1-3 feet in height. Rhizomes are 7-15 cm long and 1-1.5 cm broad and laterally compressed. The branches arise obliquely from the rhizome are about 1-3 cm long and terminate in depress scars or in undeveloped buds. Stem is Elongated leaves; oblong-lanceolate, clasping the stem by their sheaths. Spikes are usually radical, rarely lateral or terminal on the Leafy stem peduncle short or long; bracts persistent, usually single. [8]

The rhizome of the ginger plant is rich in inulin, alantolucton, anti-spasm and essential oils. Ginger rhizome contains also a wide variety of biologically active constituents such as 3-6% fatty oil, 9% protein, 60-70% carbohydrates,3-8% crude fiber, about 8% ash, 9-12% water and 2-3% volatile oil [10]. Some other biological active constituents including the non-volatile pungent of ginger rhizome which possesses the medicinal therapeutic property such as: gingerols, shogaol, gingerdiols, Ingenol, paradols and zingerone. One of them gingerol is the main known medicinal pharmacological active component which is chemically composed of 6-, 8- and 10- structural analogs**.** The volatile oil consists mainly of the mono- and sesquiterpenes; camphene, β-phellandrene, curcumene, cineole, geranyl acetate, terphineol,

terpenes, borneol, geraniol, limonene, β- elemene, zingiberol, linalool, α-zingiberene, β-sesquiphellandrene, β-bisabolene, zingiberenol and α- farmesene. [9]

MATERIAL AND METHODS

Experimental requirements

Animals: The albino mice (either sex) were obtained from the departmental animal house of Mahatma Jyotiba Phule Rohilkhand University Campus, Bareilly.

Chemicals: Zingiber officinale rhizome powder, Methanol, Diazepam and Distilled water.

Apparatus: Water bath apparatus, Elevated plus maze apparatus and light dark arena model.

Authentication and extraction of plant

The ginger plant rhizomes were collected from the Bareilly region Uttar Pradesh and it was authenticated and identified by the botanist at Mahatma Jyotiba Phule Rhoilkhand University Bareilly.

Firstly dried rhizomes of ginger were crushed in fine powder. Powdered rhizomes were weighed and packed in soxhlet apparatus. The mixture of methanol and water in the ration of 8:2 was used respectively. Extraction was continued at temp of 50-55^{o}C till clear solvent was observed in the round bottom flask. The extract was dried at 40^{o}C in the rotatory evaporator. Dried extract was packed in the airtight container and store 8^{o}C. Finally, we obtained a highly greenish roots extract powder with 8.11 percentage yield. [11]

Preparation of animals

Adult albino mice will be obtained from the departmental animal House of M. J. P. Rohilkhand University Campus, Bareilly. Animals will be kept (1 week) in a controlled environment (light cycle of 12 hours, temp. not exceeding to 25^{o}C) with free access to food and water *ad libitum*. Animals handling will proceed according to the Institutional Animals Ethics Committee.

Experimental design: Adult albino mice (either sex) were weighed and divided into 4 groups, each group consisting of 6 rats. They were treated once per day for 7 days as follows:

Table No. 1: Experimental design of different treated groups

Group	Treatment
Group I (control)	Administered normal saline 15 ml/kg, p.o.
Group II (Standard)	Administered Diazepam, (1mg/kg, i.p) suspended in normal saline.
Group III (test a)	Administered methanolic rhizome extract of *Zingiber officinale* (200mg/kg, p.o.) suspended in normal saline.
Group IV (Standard) (test b)	Administered methanolic extract of *Zingiber officinale* rhizomes (400mg/kg, p.o.) suspended in normal saline.

The extract of ginger rhizome was prepared by soxhlet apparatus which suspended in normal saline as vehicle and administered p.o. at dose 200 mg/kg and 400 mg/kg to mice one before observing the anxiolytic activity. Six mice were taken in each four groups. The standard group was received diazepam 1 mg/kg, i.p. suspended in vehicle. The control group was received normal saline.

Elevated plus maze apparatus

The elevated plus maze was used which is recognized as standard model for this purpose. This apparatus has two open arms (10×50 cm) and two closed arms (40×50×10 cm). Similar arms were opposite and 50 cm above ground. The animal was individually placed in to the apparatus facing towards the centre of maze after 30 minutes of treated doses of each group. During 5 min period, the four parameters were evaluated during the experiment such as: the number of entrance to open and closed arms and spent time in each arm. Significant increase in open arms entrance and spent time in this arm plus no movement activity shows anxiety reduction in this test. [12]

Light dark arena model

This model was consisting to enter connected compartment. This is different in size (2:1), colors (white and black) and light source (bright and dim). After 30 min of treatment with the each group doses animal were individually placed in the apparatus (LDAM) for the testing behavior activity by observing main parameters such as number of crossing between two compartments, time spent in each compartments. Some time rearing and grooming behavior activity are also observed. [13]

STATISTICAL ANALYSIS

Statistical data was analyzed by one way analysis of variance (ANOVA) and values expressed as mean ±SEM. The statistical analysis was performed by using graph pad prism. The results were considered statistically significant at P≤0.05.

RESULT

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 as well as an increase in the number of entries in the open arms. The methanol extracts of *Zingiber officinale* showed increase in time spent in open arm and in the number of entries into the open arms compared to control group. Though 200 and 400 mg/kg body wt. treated group of both extracts showed significant increase which is comparable to control group of mice.

Table No. 2: A comparative observation on the mean no. of entries and percentage of mean time spent in open arms of different treated groups:-

Sr. No.	Treatment	Mean no. of entries in open arms	Meantime spent in open arms (sec.)	Meantime time spent in closed arm (sec.)
1.	Control	8 ± 0.577	91.83 ± 0.654	193.66 ± 0.494
2.	Standard	18.83 ± 0.477	206.16 ± 0.477	78.16 ± 0.600
3.	Test A	11.66 ± 0.557	142.0 ± 0.749	107.5 ± 0.619
4.	Test B	14.83 ± 0.477	178.5 ± 0.500	116.33 ± 0.666

(n=6, values were expressed as mean ±SEM, statistically significant at P≤0.05 compared to control and standard group).

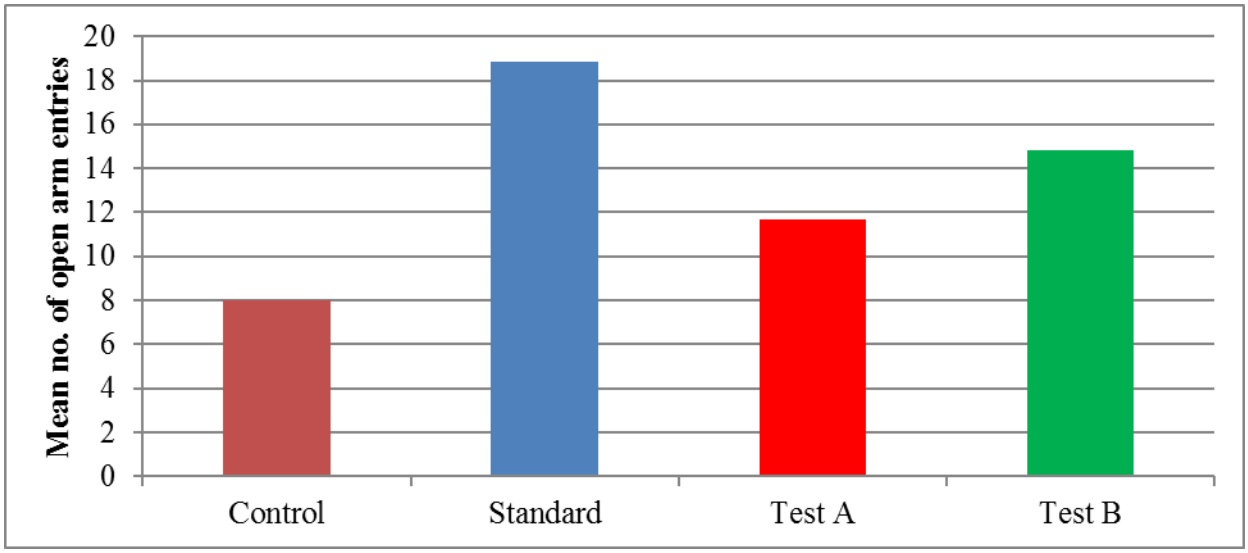


Figure No. (a)

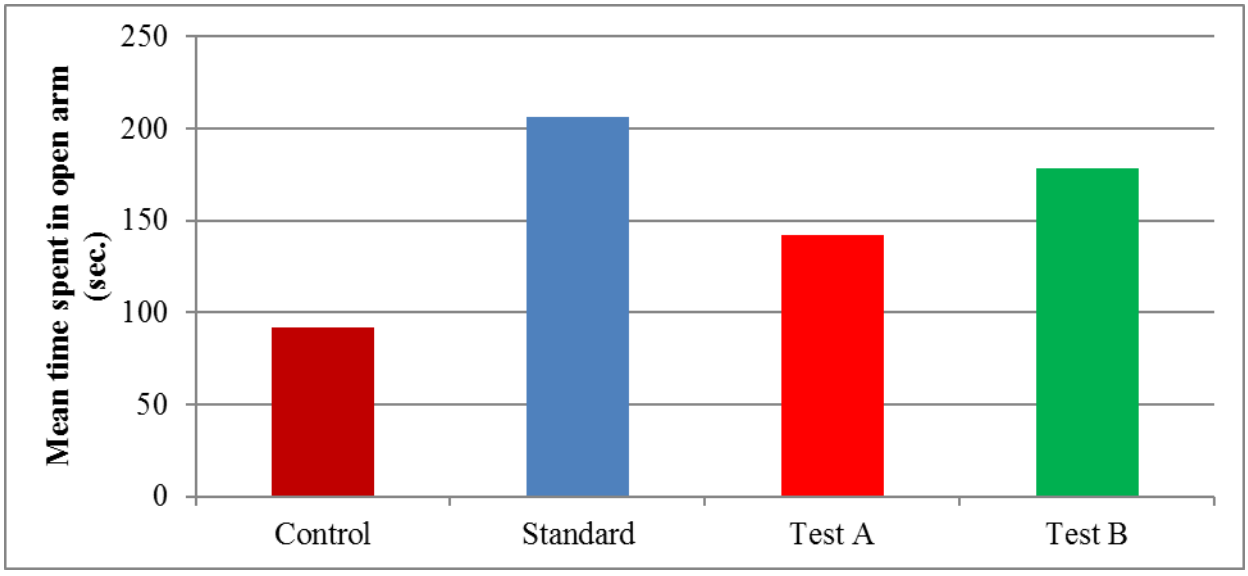


Figure No. (b)

Figure No. 1.1: a) A comparative mean no. of entries in open arms among different treated groups

b) A comparative mean time spent in open arms (sec.) among different treated groups

Light dark arena model test: - In the light dark arena model, table shows that administered of testing drug (rhizomes extract of ginger) have anxiolytic effect.

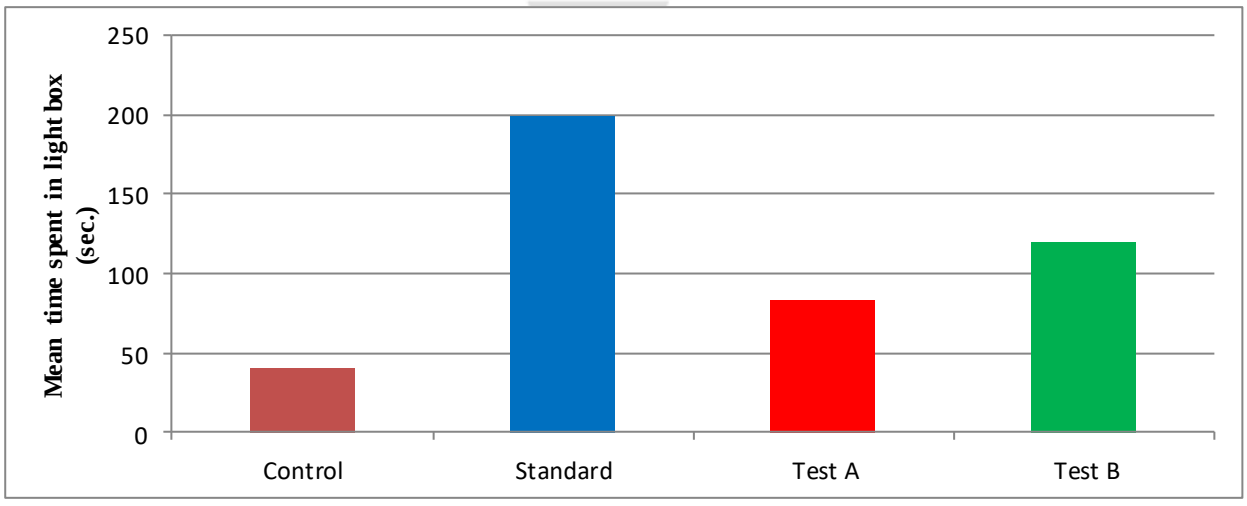
The result shows that the testing drug exhibited the statistically significant anxiolytic activity when compared with control and standard (diazepam) treated groups. However, diazepam showed a significant increases time spent in lightbox and no. of crossing between two

compartments in light dark arena model. Though 200 and 400 mg/kg body wt. treated group of both extracts showed significant increase which is comparable to control group of mice.

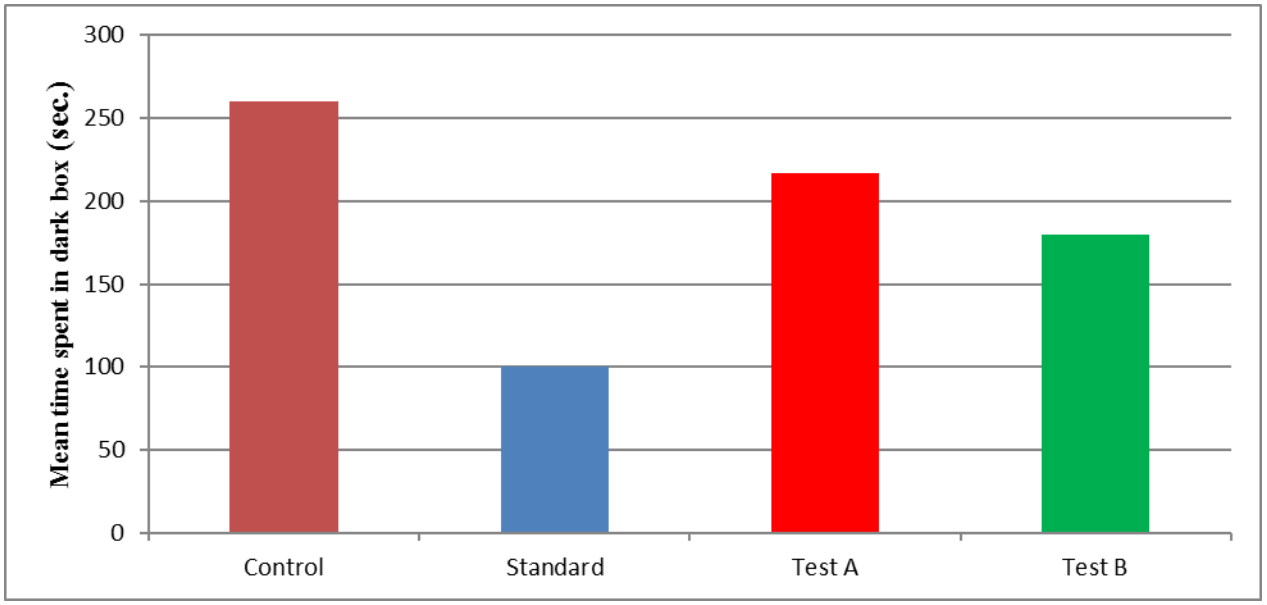
Table No. 3: A comparative observation on the mean no of latency time, rearing and mean no of no. of transmission between two compartments in light dark arena model of different treated groups:-

Sr. no.	Treatment	Mean time spent in lightbox (sec.)	Meantime spent in dark box (sec.)	Mean no of crossing between light and dark box
1.	Control	39.66 ± 0.988	260.33 ± 0.988	5.66 ± 0.494
2.	Standard	199.83 ± 1.108	100.16 ± 1.108	19.33 ± 0.421
3.	Test A	83.16 ± 0.763	216.5 ± 0.763	10.66 ± 0.950
4.	Test B	120.0 ± 1.154	180.0 ± 1.154	13.16 ± 0.703

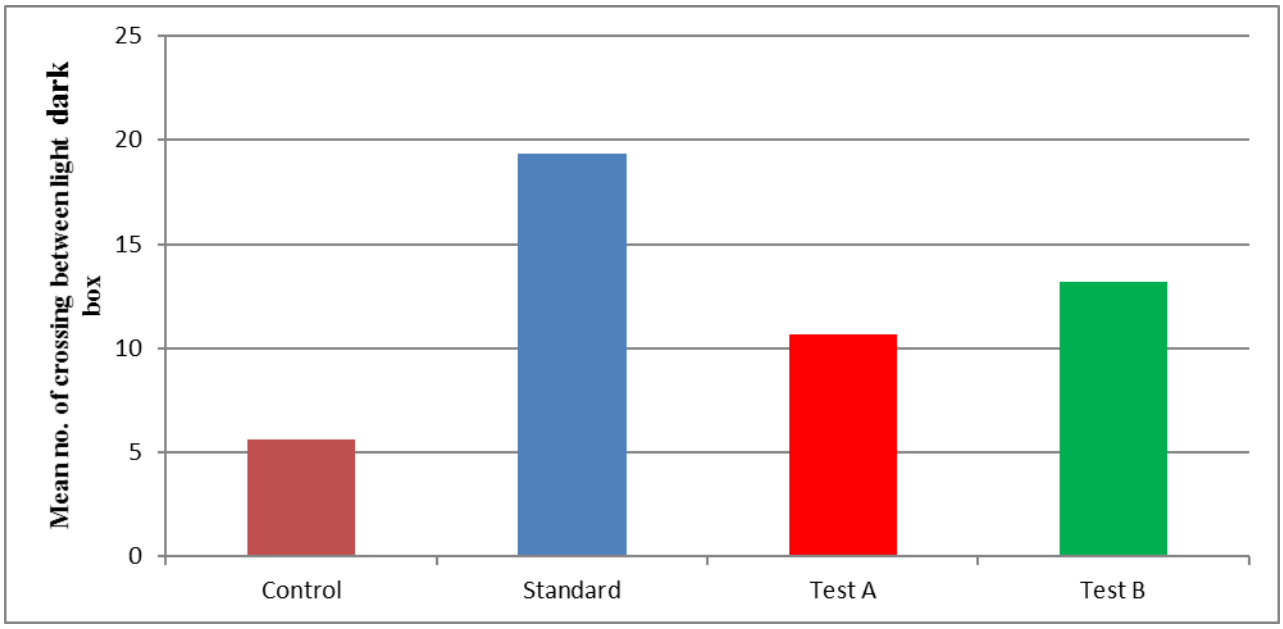
(n=6, values were expressed as mean ±SEM, statistically significant at P≤0.05 compared to control and standard group).



(a)



(b)



(c)

Figure No. 1.2 (a) A comparative mean time spent in light box (sec.) among different treated groups

(b) A comparative mean time spent in dark box (sec.) among different treated groups

(c) A comparative mean no. of crossing between light and dark among different treated groups

DISCUSSION

The screening of anxiolytic effect in this study was carried out through the treatment of methanolic rhizome extract of *Zingiber officinale* roscoe on albino mice using two different models such as elevated plus maze and light-dark arena model. These are compared with the control and standard (diazepam) groups.

Elevated plus maze (EPM) is considered one of the most widely validated tests for assaying new benzodiazepine-like anxiolytic agents 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 this 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 (both 200 mg/kg and 400 mg/kg p.o.) 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.

In light and dark arena test, the ginger extract demonstrates the same anxiolytic effect. In this study, it was demonstrate that the preference showed for 5 main parameters such as:- the latency time, for first passé from light compartment to dark, movement in each compartments, number of transmissions between two compartments, time spent in each compartments. Sometime rearing and grooming behavior activity are also observed. In this study, diazepam produced significant decrease the latency time, rearing and decrease the number of transmissions between two compartments. The extracts of the *Zingiber officinale* rhizome at the doses of (both 200 mg/kg and 400 mg/kg p.o.) also demonstrated similar results.

CONCLUSION

Results showed that extract in all doses increased time spent in the open arms significantly comparing to diazepam and control groups which indicates anxiety reduction. Also, 200 mg/kg, p.o and 400 mg/kg, p.o. both doses increased movement activity of mice in proportion to diazepam significantly. So, we can conclude that ginger extract can reduce anxiety reactions dose dependently.

Further investigations are suggested in future research to isolate and confirm the main active constituents which are responsible for possessing its anxiolytic therapeutic action.

REFERENCES

[1]. Hiremath S. B. *et al.* Effect of calcium on anxiolytic activity of diazepam and verapamil in rats, Indian journal of pharmacology, 2010, 42 (6), 406-408.
[2]. Vihang N. Vahia, Diagnostic and statistical manual of mental disorders: **Text Revision (DSM-IV-TR), 4th Edition,** American Psychiatric Association, 01-Jun-2000.
[3]. Hawke PC *et al.* Anxiety and its features in parkinsons disease, SM Journal of neurology and nruro science, 2017, 3 (1),1-5.
[4]. Rector A N *et al*. Anxiety disorder and information disorder guide, library and archives Canada cataloguing in publication, 2005.
[5]. Singh Rudra Pratap, Jain Ritesh, Mishra Rahul and Tiwari Prashant, Antidepressant activity of HydroAlcoholic Extract of *Zingiber Officinale*, IRJP, 2012, 3(2), 149-151.
[6]. Prasad Chandan *et al.* Natural, chemical and pharmacological property of *Zingiber officinale*, world journal of pharmaceutical science, 2016.
[7]. Jyotsna Dhanik, Neelam Arya and Viveka N, A Review on *Zingiber officinale*, Journal of Pharmacognosy and Phytochemistry, 2017, 6(3), 174-184.
[8]. Abdallah M. Elmakki *et al*. Effects Of dietary ground Ginger (*Zingiber officinale*) root additive on broiler performance, Global Journal of Animal Scientific Research, 2013, 1(1), 76-83.
[9]. Fadaki F *et al.* The Effects of Ginger Extract and Diazepam on Anxiety Reduction in Animal Model, Indian Journal of Pharmaceutical Education and Research, Jul-Sep, 2017, 51, 3,159-162.
[10]. Singh Anita, Nutritional benefits and pharmacological effects of ginger, Indian journal of basic and applied medical research, 2015, 4(4)377-383
[11]. Nour A. H. *et al*. Extraction and chemical composition of ginger essential oils as cockroaches repellent, Australian journal of basic and applied science, 2017, 11(3),1-8
[12]. Rohini Singh, A. Metha, P. Metha, K. Shukla, Anthelmintic Activity of Rhizome extract of *Curcuma Longa* And *Zingiber officinale* (Zingiberaceae), International Journal of Pharmacy and Pharmaceutical Sciences, 2011, 3, 2.
[13]. Bourin Michel, Animals models for screening anxiolytic like drugs: a prospective, translational research, 2015,295-303.
[14]. L**ydiard R B**, The role of GABA in anxiety disorders, Journal of Clinical Psychiatry, 2003, 64, 3:2,1-7.
[15]. Hoehn-Saric R *et al*. Somatic symptoms and physiologic responses in generalized anxiety disorder and panic disorder: an ambulatory monitor study, 2004 , 61(9),913-921.
[16]. Abdallah M. Elmakki *et al*. Effects of Dietary Ground Ginger (*Zingiber officinale*) Root Additive on Broiler performance, Global Journal Of Animal Scientific Research, 2013, 1(1),76-83
[17]. Hoehn-Saric R *et al*. Somatic symptoms and physiologic responses in generalized anxiety disorder and panic disorder: an ambulatory monitor study, Arch Gen Psychiatry, 2004, 61(9), 913-921.
[18]. Kessler RC *et al*. Prevalence and treatment of mental disorders, N Engl J Med 16; 2005 352(24),2515-2523.
[19]. Bandelow B *et al*. Guidelines for the pharmacological treatment of anxiety doxspsorder , obsessive-compulsive disorder and post treamatic disorder in primary care, IJPCP, 2012, 16, 77.
[20]. Antony M. M. *et al.* Anxiety disorder and their treatment a critical review of the evidence based literature, health Canada, 1996, 13-122.
[21]. Malhotra S and Singh A P, Medicinal property of ginger (*Zingiber officinale* rocs.), NPR, 2003, 2 (6), 296-301.

Image *Author -1*	**Prashant Kumar** M.J.P.Rohilkhand University Village Gadanpura , Post Heempur deepa, District Bijnor 246725/ M.J.P.Rohilkhand University,Pilibhit Bypass Road , Bareilly UP 243006
Image *Author -2*	**Yogendra Pal** M.J.P.Rohilkhand University Village Mujhaina Santosh , Post Paiga, District Bareilly/ M.J.P.Rohilkhand University,Pilibhit Bypass Road , Bareilly UP 243006
Image *Author -3*	**Dr. Shashi Bhooshan Tiwari** M.J.P.Rohilkhand University M.J.P. Rohilkhand University Campus Bareilly 243006/ M.J.P.Rohilkhand University,Pilibhit Bypass Road , Bareilly UP 243006