Whites Science
A Scientific Erudition

Anxiolytic and Motor Coordinaton Activity of *Alangium salvifolium* Linn. Seeds in Rats

Jasvir Kaur[1], Rajmeet Singh[1*], Jaswinder Kaur[1], Harpreet Kaur[1] and Jaspreet Kaur[2]

[*1]G.H.G. Khalsa College of Pharmacy, Gurusar Sadhar, Ludhiana, Punjab, Pin code: 141104, India
[2]Maharishi Markandeshwar University, Mullana, Ambala, Haryana, Pin code: 133203, India

ABSTRACT

The present study was undertaken to investigate the effects of methanol and aqueous extracts of *Alangium salvifolium* Linn. seeds in anxiety on rat by using elevated plus maze and light & dark models. Evaluating the motor coordination activity of *Alangium salvifolium* seeds extracts using actophotometer models. Wistar rats (either sex) were treated orally with methanol and aqueous extracts of *Alangium salvifolium* seeds (200mg/kg, 400mg/kg, 800mg/kg, 1200mg/kg, 1600mg/kg) and diazepam (2mg/kg) as a standard anxiolytic drug. The results showed that the extracts of methanol and aqueous significantly induced maximum increase in the both number of entries and time spent in open arms, compared to the closed arm in elevated plus maze model. In the light dark exploration test model, results showed that the extracts of methanol and aqueous significantly induced maximum increase in the time spent in the light compartment, compared to the dark compartment. In the actophotometer model, results showed that the methanol and aqueous extracts significantly increased motor coordination activity. Despite of these two extracts, methanol extract was found to be most effective (at dose 1200mg/kg). These results compared with control group and diazepam (2mg/kg), treated group.

Keywords: Elevated Plus Maze (EPM), Gaba Amino Butyric Acid (GABA), Noradrenaline (NA), Selective Serotonin Reuptake Inhibitors.

INTRODUCTION

Anxiety may be regarded as a particular form of behavioral inhibition that occurs as a result of exposure to a new situation. Disorders involving anxiety are the most common mental disturbances. Anxiety disorders lifetime prevalence rates ranging between 13.6% and 28.8% [1]. Anxiety is a generalized mood of fear, worry and or uneasiness that results from a bad feeling about something that happens or may happened [2]. There are different types of anxiety like panic disorder, agoraphobia, social anxiety, specific phobias, obsessive compulsive disorder, and post-traumatic stress disorder, generalized anxiety disorder that could be mild or sever depending on the level of the disorders [3]. Major drugs classes for the treatment of anxiety disorders are benzodiazepines (BZDs), barbiturates, tricyclic antidepressants (TCA's), selective serotonin reuptake inhibitors (SSRIs), beta blocker, azapirones [4,5]. These drugs classes produce various systemic side effects or exhibit tolerance upon chronic use [6]. Benzodiazepine (Diazepam, Lorazepam) is the most important group used as antianxiety, but it shows the side effects during the long term

***Corresponding Author**
rajmeetsingh80[at]yahoodotcom

Receiving Date: October 10, 2019
Acceptance Date: November 25, 2019
Publication Date: January 06, 2020

ISSN 2046-5114

therapeutic use as antianxiety [7]. India utilizes the plants in its medicinal system. Research has been conducted to identify safer, more specific medications possessing anxiolytic effect without the complications. In the past few years, several herbal medicines have been used for the management of anxiety in the world [8]. Numerous tradionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders, like anxiety disorders [9]. The plant *Alangium salvifolium* (Linn.f) Wang is one among those plant [10]**.** *Alangium salvifolium* (Alangiaceae) also called as ankola is well known in India. It is native Western Africa, Madagascar, Southern and Eastern Asia, China, Malaysia, Indonesia, India, and Philippines, tropical Australia, the western Pacific Ocean islands and New Caledonia. In India, it is throughout the Hyderabad forests and Sitamata wildlife sanctuary, Rajasthan [11]. It is a popular folk medicine to treat various disorders. The seeds of *Alangium salvifolium* are one of the most valuable in traditional system of medicine to exhibit a variety of pharmacological activities including anticancer, diuretic, ant-inflammatory, antimicrobial, laxative and antiepileptic activity etc. [12, 13]. Our study was focused on for investigate the anxiolytic and motor coordination activity of the methanol and aqueous extracts of *Alangium salvifolium* seeds in laboratory animals.

MATERIALS AND METHODS

Plant material: The seeds of *Alangium salvifolium* were brought from Guru Nanak Traders, Ambala (Haryana) and were authenticated (Voucher No # 952) by Department of Botanical & Environment Sciences, Guru Nanak Dev University Amritsar, India.

Drugs and chemicals: Diazepam was manufactured by Neon Laboratories Limited Boisar Road, Palghar (Thane), M.S. Diazepam dose 2mg/kg, was used as standard drug. Methanol (Ranbaxy) was used for preparing extract.

Preparation of extracts: The dried seeds of the plant (250g, each) were comminuted to powder passing through a sieve no. 60. Then they were extracted with methanol successively using soxhlet apparatus. The marc was digested at 50°C with distilled water for 24h to obtain the aqueous extract. All the extracts were filtered through cotton wool plug and dried in vacuum on a rotary evaporator. The dried extracts were preserved in desiccators for all experimental studies.

Animals: Healthy 10 Wistar rats (either sex), weight around 165-290g were used for study. They were procured from university animal house. Animals were housed in a group of 6 per cage (polycarbonate cage size 29 ×22×14cm) under laboratory condition with alternating dark and light cycle of 12 hr each. The animals have free accessed to food and water. The animals were kept fasted over night before performing experiment. The animals were acclimatized for at least five days before behavioral experiment which were carried out between 8:00 to 12:00 hr. the experimental protocol was approved by (MMCP/IAEC/15/28) institutional animal ethical committee (IAEC) and animal care was taken as per the guidelines of committee for the purpose of control and supervision of experiment on animal (CPCSEA) Govt. of India.

ISSN 2046-5114

Experimental design

Control group: Vehicle (simple syrup IP + 5% Tween 80)
Standard: Diazepam (2mg/ kg orally)
Test groups: various seeds extracts (methanol, aqueous) at different dose (200, 400, 800, 1200, 1600 mg/kg orally) suspended in vehicle.

Anxiolytic activity: The anxiolytic activity of the various seeds extracts was evaluated using the elevated plus maze model, light dark model and actophotometer model.

Elevated plus maze model: The elevated plus maze is a widely used behavioral assay for rodents and it has been validated to assess the anti-anxiety effects of pharmacological agents [14]. The apparatus consist of two open arms (30x5cm) and two closed arms (30x5x15cm) that extend from a common central platform (5x5cm). The floor and walls of the closed arms are made of wood. The entire maze is elevated to a height of 50 cm above the ground level. Animals were treated with methanol and aqueous extracts at different dose (200, 400, 800, 1200, 1600 mg/kg orally) and diazepam (2mg/ kg orally), The animals were placed individually in the centre of the maze and head facing towards open arms .The amount of time spent on and number of entries in both open and closed arms was counted for 5 min. Arm entry was defined as all four feet in the arm. An anxiolytic response was defines as increased number of entries and time spent in the open arm of elevated plus maze [15].

Light - Dark Model: The light-dark exploration test is another commonly used murine model of anxiety. The apparatus consisted of two acrylic boxes. Two distinct chambers, a black chamber (20×30×30cm) painted black and other open chamber made up of transparent acrylic (30×30×30cm). The two chambers are connected through a small open doorway (8×8cm) situated on the floor level at the centre of the partition each. Animals were treated with methanol and aqueous extracts at different dose (200, 400, 800, 1200, 1600 mg/kg orally) and diazepam (2mg/ kg orally). Rat was individually placed in the centre of the lighted area facing the entrances to the dark compartment. Each rat place in the light compartment and observed for the next 5 minutes for the numbers of the crossing between two compartment and time spend in the light and dark compartment [16]**.**

Actophotometer model: The animal locomotor behavior was monitored using actophotometer. Six photocells are placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam. Technically, its principle is that a photocell is activated when the rays of light falling on photocells are cut off by animals crossing the beam of light. (Bhattacharyya, L., 2015). Animals were treated with methanol and aqueous extracts at different dose (200, 400, 800, 1200, 1600 mg/kg orally) and diazepam (2mg/ kg orally). The Animals were placed in actophotometer individually and basal activity score was recorded over the period of 5 min [17].

Statistical analysis (ANOVA): Result is expressions as mean ± S.E.M. the statistical analysis of data have done using the one way analysis of variance (ANOVA). A probability level less than 0.05 is considered statically significant.

ISSN 2046-5114

RESULTS

Preliminary phytochemical screening test: The freshly prepared extracts were also tested. The result of phytochemical analysis indicated that presence of flavonoids, tannins, alkaloids, triterpenoids and steroids.

Elevated plus maze: The methanol extract 800, 1200, 1600mg/kg (Table 1 and Figure 1) and aqueous extract 1600mg/kg (Table 2 and Figure 2) induced significant increase in the both number of entries and time spent in open arms, compared to the closed arm in elevated plus maze model, but maximum response showed by methanolic extract at dose 1200mg/kg.

Table 1: Effect of methanol extract of seeds of *Alangium salvifolium* on behaviour of rat in Elevated Plus Maze (EPM) test

S.No	Drug	Time spent in open arm	Entries in open
1	Normal	34.2±1.7	2.4±0.31
2	Diazepam	64.8±2.3	3.6±0.56
3	200mg/kg	41.4±1.4	2.7±0.65
4	400mg/kg	49.4±2.0	2.6±0.60
5	800mg/kg	67.2±3.4*	3.8±0.74*
6	1200mg/kg	76.8±4.5*	4.1±0.87*
7	1600mg/kg	65.7±4.4*	3.6±0.78*

All values represent Mean± S.E.M (n=6), *p<0.05 vs. control group.

Table 2: Effect of aqueous extract of seeds of *Alangium salvifolium* on behaviour of rat in Elevated Plus Maze (EPM) test

S.No	Drug	Time spent in open arm	Entries in open
1	Normal	34.2±1.7	2.4±0.31
2	Diazepam	64.8±2.3	3.6±0.56
3	200mg/kg	35.7±1.8	2.3±0.30
4	400mg/kg	39.3±1.6	2.5±0.59
5	800mg/kg	44.2±1.8	2.7±0.61
6	1200mg/kg	50.1±1.9	3.1±0.67
7	1600mg/kg	54.2±2.7*	3.0±0.72*

All values represent Mean± S.E.M (n=6), *p<0.05 vs. control group.

ISSN 2046-5114

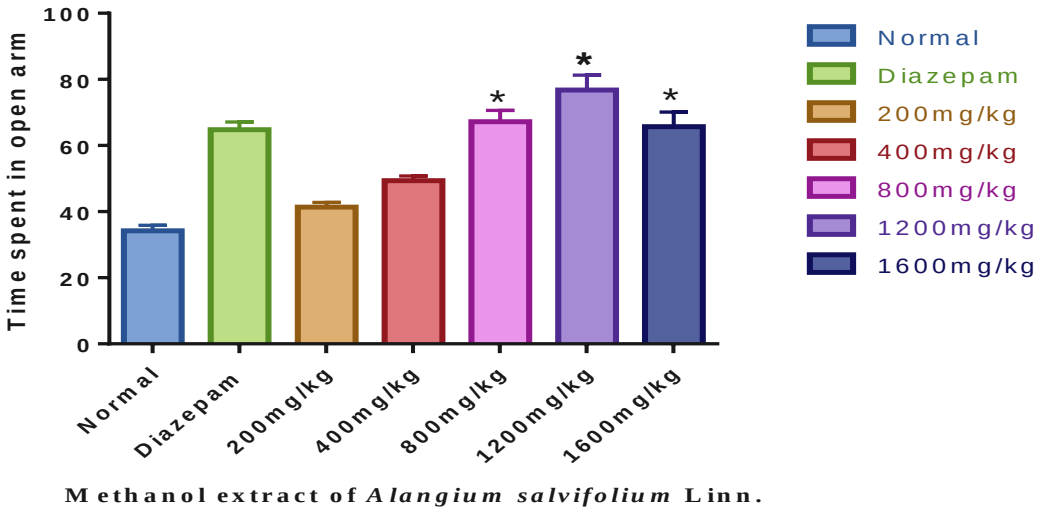

Figure 1: Effect of methanol extract of seeds of *Alangium salvifolium* on time spent in open arms of rat using Elevated Plus Maze (EPM) test

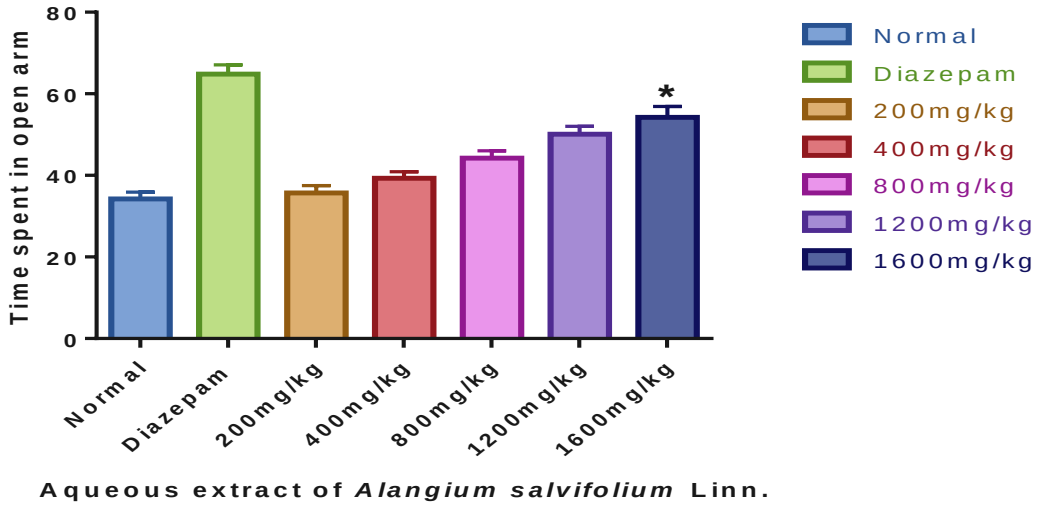

Figure 2: Effect of aqueous extract of seeds of *Alangium salvifolium* on time spent in open arms of rat using Elevated Plus Maze (EPM) test.

Light - Dark

In the light dark exploration test model, we observed that methanol extract 1200mg/kg and 1600mg/kg (Table 3 and Figure 3) and aqueous extract 1600mg/kg (Table 4 and Figure 4) induced significant increase in the time spent in the light compartment, compared to the dark compartment. In this model again methanolic extract at dose 1200mg/kg showed maximum significant effect.

ISSN 2046-5114

Table 3: Effect of methanol extract of seeds of *Alangium salvifolium* on behaviour of rat in Light and Dark Exploration test

S.No	Drug	Time spent in open area	Entries in open
1	Normal	30.4±1.3	2.8±0.5
2	Diazepam	64.4±2.9	4.4±1.1
3	200mg/kg	49.8±1.4	3.1±0.6
4	400mg/kg	51.4±1.9	3.2±0.5
5	800mg/kg	57.2±1.8	3.4±0.8
6	1200mg/kg	61.4±2.3*	3.2±0.9*
7	1600mg/kg	60.6±3.1*	3.4±0.9*

All values represent Mean± S.E.M (n=6), *p<0.05 vs. control group.

Table 4: Effect of aqueous extract of seeds of *Alangium salvifolium* on behaviour of rat in Light and Dark Exploration test

S.No	Drug	Time spent in open area	Entries in open
1	Normal	30.4±1.3	2.8±0.5
2	Diazepam	64.4±2.9	4.4±1.1
3	200mg/kg	31.3±1.5	2.9±0.6
4	400mg/kg	34.4±1.7	3.1±0.7
5	800mg/kg	37.2±1.2	3.2±0.8
6	1200mg/kg	43.4±1.9	3.2±0.9
7	1600mg/kg	51.7±2.2*	3.4±0.9*

All values represent Mean± S.E.M (n=6), *p<0.05 vs. control group.

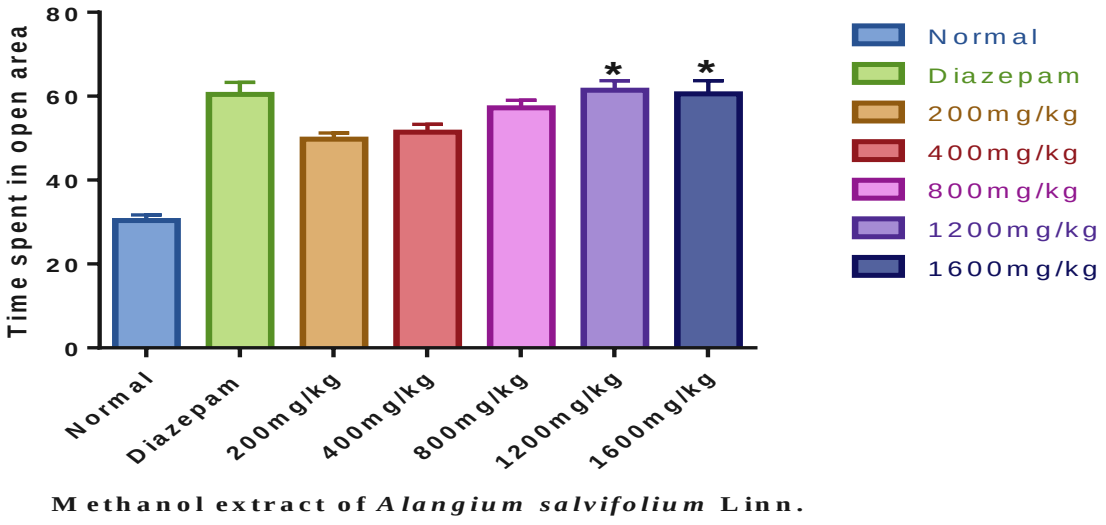

Figure 3: Effect of methanol extract of seeds of *Alangium salvifolium* on time spent in light compartment using Light and Dark Exploration test

ISSN 2046-5114

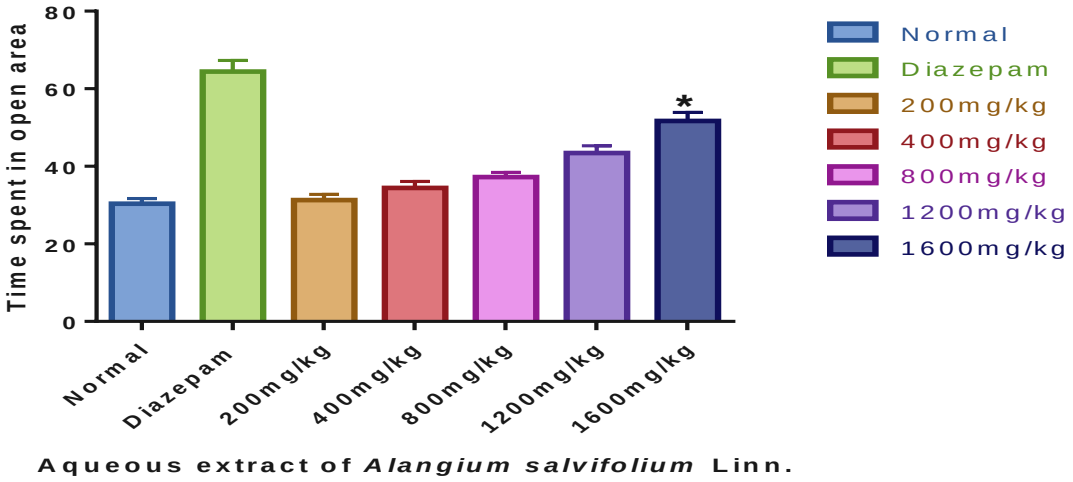

Figure 4: Effect of aqueous extract of seeds of *Alangium salvifolium* on time spent in light compartment using Light and Dark Exploration test

Actophotometer

Actophotometer model, we observed that methanol 800, 1200, 1600mg/kg (Table 5 and Figure 5) and aqueous extract 1600mg/kg (Table 6 and Figure 6) shows significant increase of locomotor activity, which means both extract showed anxiolytic activity without sedative effect.

Table 5: Effect of methanol extract of seeds of *Alangium salvifolium* on behaviour of rat on Actophotometer test

S.No	Drug	Reading/Counting
1	Normal	107± 6.8
2	Diazepam	274± 9.3
3	200mg/kg	180± 7.5
4	400mg/kg	225± 10.1
5	800mg/kg	311±11.6*
6	1200mg/kg	369±14.2*
7	1600mg/kg	249± 8.7*

All values represent Mean± S.E.M (n=6), *p<0.05 vs. control group.

Table 6: Effect of aqueous extract of seeds of *Alangium salvifolium* on behaviour of rat on Actophotometer test

S.No	Drug	Reading/Counting
1	Normal	107± 6.8
2	Diazepam	274± 9.3

ISSN 2046-5114

3	200mg/kg	111± 7.2
4	400mg/kg	119± 8.0
5	800mg/kg	130±10.6
6	1200mg/kg	167±10.5
7	1600mg/kg	196± 11.2*

All values represent Mean± S.E.M (n=6), *p<0.05 vs. control group.

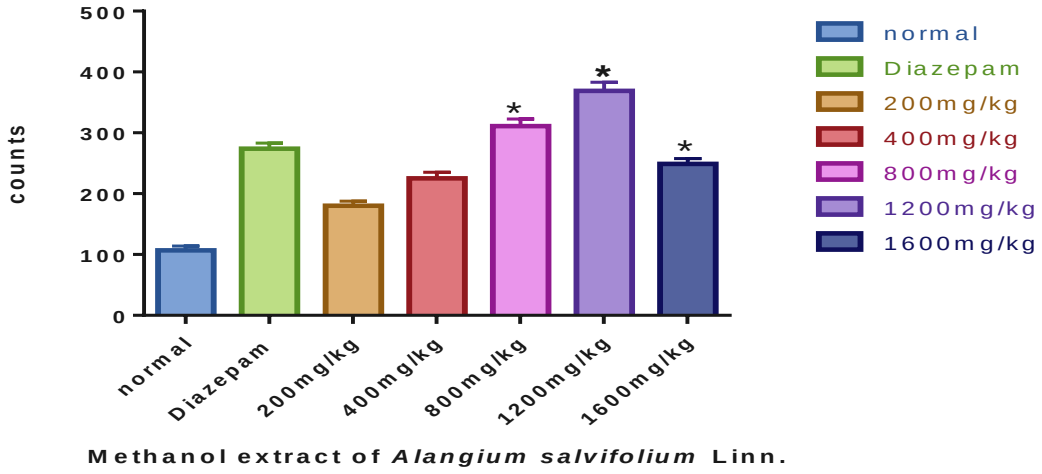

Figure 5: Effect of methanol seeds extract of *Alangium salvifolium* on counts/ reading by Rat using Actophotometer test

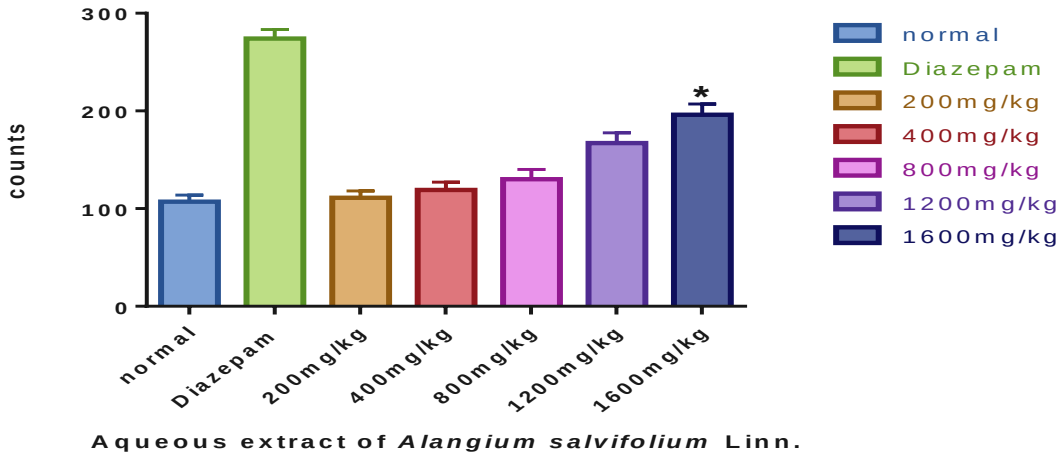

Figure 6: Effect of aqueous extract of seeds of *Alangium salvifolium* no. of counts/ readings by rat using Actophotometer Test

ISSN 2046-5114

DISCUSSION

The present work demonstrating the anti-anxiety potential of methanol and aqueous extracts of the *Alangium salvifolium* seeds on rats by employing three experimental models *i.e*. Elevated plus Maze, Light & Dark Model and Actophotometer. These models are chosen because they are validated as an animal model of anxiety on pharmacological, physiological and behavioral grounds [18]. In this study, we observed that the methanol extract (800, 1200, 1600mg/kg) and aqueous In extract (1600mg/kg) induced significant increase in the both number of entries and time spent in open arms, compared to the closed arm in elevated plus maze model but maximum response showed by methanolic extract at dose 1200mg/kg. In the light dark exploration test model, we observed that methanol extract (1200mg/kg and 1600mg/kg) and aqueous extract (1600mg/kg) induced significant increase in the time spent in the light compartment, compared to the dark compartment. In this model again methanolic extract at dose 1200mg/kg showed maximum significant effect. In the actophotometer model, we observed that methanol (800, 1200, 1600mg/kg) and aqueous extract (1600mg/kg) shows significant increase of locomotor activity, which means both extract showed anxiolytic activity without sedative effect. These results compared with control group and diazepam (2mg/kg), treated group, which is the standard anxiolytic drug. Despite of these two extracts, methanolic extract was found to be most effective. The methanol extract (at dose 1200mg/kg) significantly induced maximum response in elevated plus maze model, light dark exploration test model and actophotometer model. It may be due to the present of flavonoids in the methanolic extract. Phytochemical screening of methanolic seed extract of *Alangium salvifolium* Linn showed presence of flavonoids and Tannins. Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine such as *Passiflora coerulea* [19]. The anxiolytic effect of flavonoids has been attributed to its effect on central nervous system. Different type of flavonoids was found to be ligands for the GABA receptors in the central nervous system [20]. Gamma-amino butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different type of anxiolytic drugs is shown their action through GABA. The anxiolytic effect of methanolic seed extract of *Alangium salvifolium* may be due to the presence of flavonoids, that action on GABA with other excitatory neurotransmitter in CNS. The results of this study showed that the methanol extract of *Alangium salvifolium* Linn possess anxiolytic effects without sedation at therapeutic acceptable doses. Further study is required to find out the exact mechanism responsible for its anti-anxiety activity.

ACKNOWLEDGEMENTS

The authors are thankful to Principal, G.H.G Khalsa College of Pharmacy, Gurusar Sadhar for providing all the facilities and Green Chem. Bangalore for providing chemicals for experimental work.

CONFLICT OF INTEREST

The authors confirm that this research article content has no conflict of interest.

ISSN 2046-5114

REFERENCES

1. Michael T, Zetsche U, Margraf J. Epidemiology of Anxiety Disorders. Epidemiology and Psychopharmacology. 2007; 6(4):134-142.
2. Alramadhan E, Mirna S. Hanna, Mena S. Hanna, Todd A. Goldstein, Samantha M. Avila, Benjamin, S. Weeks. Dietary and Botanical Anxiolytics. Med. Sci. Monit. 2012; 18(4):40-48.
3. Thakur P, Rana AC. Anxiolytic Potential of Medicinal Plants. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2013; 3 (4):325-331.
4. Bhattacharyya L. Evaluation of Anti-Anxiety Activity of Silica Gel Entrapped Traditional Medicine. Asian J. Pharm. Clin. Res. 2015; 8(2):316-319.
5. Shafeen S, Reddy ST, Arafath S, Nagarjuna S, Reddy PY. Evaluation of Antianxiety Activity of Cassla Occidentalis Leaves. Asian J. Pharm. Clin. Res. 2012; 5(3):47-50
6. Pari L, Maheshwari JU. Hypoglycaemic Effects of Musa sapientum L in Alloxan Induced Diabetic Rats. J. Ethnopharmacol. 1999; 38:1-5.
7. Chakraborty A, Amudha P, Geetha M, Singh SN. Evaluation of Anxiolytic Activity of Methanolic Extract of Sapindus Mukorossi Gaertn in Mice. International Journal of Pharma and Bio Sciences. 2010; 1(3):1-10.
8. Tanwer BS, Vijayvergia R. Biological Evaluation of Alangium salviifolium (L.F.) Wangerin. J. Chem. Pharm. Res. 2014; 6(12):611-618.
9. [9] Sousa AD. Herbal Medicines and Anxiety Disorders: an overview. Journal of Medicinal Plants Studies. 2013; 6(1):18 -23.
10. Prakash NKU, Bhuvaneswar S, Preethy S, Rajalakshmi N, Saranya M, Anto JR, Arokiyaraj S. Studies on Antimicrobial, Antioxidant, Larvicidal, Pesticidal Activity and Phytochemistry of Leaves of Alangium salvifolium Linn Wang. Int. J. Pharm. Sci. 2013; 5(2):86-89.
11. Anurag M, Prasad GG. Antidiabetic Activity of Alangium salvifolium in Alloxan Induced Diabetic Rats. IRJP. 2011; 6:101-105.
12. Sharma AK, Agarwali V, Kumar R, Balasubramaniam A, Mishra A, Gupta R. Pharmacological Studies on Seeds of Alangium salvifolium Linn. Drug Research. 2011; 68(6):897-904.
13. Yoganarasimhan SN, Medicinal Plant of India, Karnataka. Interline Publishing Pvt. Ltd Bangalore. 1994 1; 21-22.
14. Bourin M, Demouliere BP, Dhonnchadha BN, Hascoet M. Animal Models of Anxiety in Mice. Fundamental & Clinical Pharmacology. 20078; 21:567–574.
15. Walf AA, Frye CA. The Use of the Elevated Plus Maze as an Assay of Anxiety-Related Behavior in Rodents. Nat. Protoc.2007; 2(2):322–328.
16. Bourin M, Hascoet M. The Mouse Light/Dark Box Test. European Journal of Pharmacology. 2003; 463:55 – 65.
17. Bhosale UA, Yegnanarayan R, Pophale PD, Zambare MR, Somani RS. Study of Central Nervous System Depressant and Behavioral Activity of an Ethanol Extract of Achyranthes aspera (Agadha) in different Animal Models. International Journal of Applied and Basic Medical Research. 2011; 1(2):104-108.
18. Fabiana CV, Roseli S, Alexandre GP. Anxiolytic- like Effect of Sonchus oleraceus L. in Mice. Journal of Ethnopharmacology. 2009; 124:325-327.
19. Sharma AK, Agarwal V, Sharma S, Chauhan B, Sharma AD, Punia R. Antidiabetic Effect of Bark of Alangium salvifolium in Alloxan-Induced Diabetic Rats. Journal of Global Pharma. Technology. 2011; 3(4), 26-32.
20. Kalueff, DJ Nutt. Role of GABA in Memory and Anxiety. A Review of Depression and Anxiety. 1997; 4:100–110.

ISSN 2046-5114