CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.8018274

Available online at: http://www.iajps.com *Research Article*

EVALUATION OF ANTIANXIETY EFFECTS OF *ALAFIA MULTIFLORA* LEAVES EXTRACTS IN ANIMAL MODELS

Md. Salma Sulthana[1], Gavini Saraswathi[2], Angidi Shireesha[2], Jallella Saikumar[2], Aminul Hoque[2], Abdus Samad[2]

[1]Assistant professor, Teja College of Pharmacy, kodad, suryapet, Telangana., [2]Teja College of Pharmacy, kodad, suryapet, Telangana.

Article Received: March 2023 **Accepted:** April 2023 **Published:** May 2023

Abstract:

The presant study focuses on the investigation of anxiolytic effects of methanolic extracts prepared from the mixture of bark and fruits of alafia multiflora. The plant is collected and authenticated. The prepared extract is subjected to phytochemical screening and dose selection studies through acute toxicity studies. The classic anxiolytic benzodiazepine; diazepam has long been reported for its anxiolytic activity in rat with EPM. Alafia multiflora at doses of 100 mg/kg, and 200 mg/kg showed significant anxiolytic activity by increasing the number of entries in open arms along with time spent in open arms and significant reduction in time spent in closed arms. The effect of Alafia multiflora (100, 200 mg/kg) on number of entries in closed arms was insignificant. The anxiolytic activity shown at higher doses of Alafia multiflora (200 mg/kg) was comparable with Diazepam 2 mg/kg. p.o. Alafia multiflora at doses of 100 mg/kg & 200 mg/kg significantly increased the time spent and arm entries in open arms and decreased the time spent in closed arms compared to control. The time spent in neutral zone is also reduced by both the doses compared to control group. Decreased aversion to open arms compared to control group indicates the anxiolytic activity of stem bark of Alafia multiflora and the magnitude of anxiolytic effect of 200 mg/kg and 100 mg/kg extracts of Alafia multiflora were comparable to that of standard drug diazepam 2 mg/kg p.o.

Keywords: *Alafia multiflora, diazepam, anxiety, herbal medicine etc.*

Corresponding author:

Md. Salma Sulthana,

Assistant professor,

Teja College of Pharmacy, kodad, suryapet, Telangana.

Email id: upendar.upi49@gmail.com

QR code

Please cite this article in press Md. Salma Sulthana et al, ***Evaluation Of Antianxiety Effects Of Alafia Multiflora Leaves Extracts In Animal Models.,*** *Indo Am. J. P. Sci, 2023; 10 (05).*

INTRODUCTION:

Fear and anxiety are here, respectively defined as the sense of an object to real or potential threats that impair the homeostasis [62].The principle symptoms of anxiety disorders include fear, excessive worry, nervousness and obsessions a multitude of physical symptoms also may be present. Anxiety disorders also associated with somatic symptoms like heart palpitations, gastrointestinal problems, sweating, fainting and chronic pain [1].

A subjective experience of distress with accompanying disturbances of sleep, concentration, social and/or occupational functioning are common symptoms in many of the anxiety disorders. Despite their similarities, these disorders often differ in presentation, course and treatment[4]. *The diagnostic and statistical manual of mental disorders*, Fourth Edition (DSM-IV), classifies anxiety disorders into several groups: generalized anxiety disorder (GAD); phobias; panic disorder; obsessive compulsive disorder (OCD); posttraumatic stress disorder (PTSD); acute stress disorder; and anxiety disorders due to general medical condition, induced by a substance, or not otherwise specified.

In view of the complexity of psychiatric disorders, it is possible that regulating one single target does not exert the antipsychotic effect as effectively as targeting multiple systems. Herbal medicine is commonly employed to cure mental disorders by various mechanisms of action in different systems. Keeping that in view that mental disorders such as depression, anxiety, and insomnia are frequently found together in a single patient and they share some neurological basis, the mechanisms of curing drugs for these diseases might be intertwined with each other, when some factors act on one target, an activity in another field may appear. Of course, this might influence the therapy of other related mental disorders [2,4].

Alafia multiflora is widely used in its area of distribution to treat wounds. Latex mixed with bark scrapings is applied to wounds and ulcers, and also to ulcers caused by syphilis. In Ghana the latex diluted with water is taken to cure stubborn wounds. In Cameroon fresh latex, either alone or mixed with Oncinotis glabrata (Baill.) Stapf ex Hiern, is also applied to treat yaws. Stem bark or fruits in decoction are taken to relieve abdominal pain. The seeds are an ingredient of arrow poison in DR Congo [6,7].

Drugs and chemicals: Diazepam inj. (Ranbaxy laboratories limited), All Other chemicals used for this investigation were of analytical grade from S.D Fine chemicals, Mumbai, India.

METHODOLOGY:

Collection and Authentification of Plant Material:
The Leaves powder of alafia multiflora is purchased from amazon by Shrisha Organics.

Animal Ethics permission:
The housing of the animals were carried out in the animal house of the Teja College of pharmacy-kodad, the treatment and sample collection, analysis of samples carried out in VYAS LABS, Medchal, Malkajgiri with approved CPCSEA registration number-2085/PO/RCBIBT/S/19/CPCSEA

Preliminary Phytochemical Screening:
Preliminary phytochemical screening of the alafia multiflora extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids. As per the standard methods.

Experimental Animals and Housing of Animals:
Albino Wistar rats weighing 150 ± 25 g of either sex were used for the study in different models. The animals were procured from National institute of Nutrition (Hyderabad) at least 2 weeks prior to the study, so that animals could acclimatize to the new environment.

Animals kept in well-maintained room under standard hygienic conditions. Commercial pellet diet and water were made available ad libitum. They were housed in propylene cages (32 x 24 x 16 cm) with stainless steel grill top, bedded with rice husk.

Preparation of Extract:
The leaves of alafia multiflora were dried under shade in room temperature for 3 days and powdered and the powder was used for preparation of methanolic extract.

A 95% w/v methanolic extract was prepared by Soxhlet extraction method. The dried powder was extracted with 95% methanol for 12 h using Soxhlet apparatus. The combined extracts were concentrated at 400 C to obtain light brown residue. The yield obtained from above process was found to be 9.5%. The extract was preserved in a refrigerator

Hot Continuous Extraction (Soxhlet):
In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated,

and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

Selection of Doses and Preparation of Drug for Study:

Since the lethal dose was found at 2000mg/kg body weight, the 1/10th of the preceding dose i.e 100mg/kg body weight was taken as the testing dose for this study and the double of the dose i.e 200mg/kg body weight also tested to find out was there any dose dependent pharmacological effect or not.

Screening of Anti Anxiety activity:

Grouping: Albino Wistar rats weighing between 150-200gms were divided into 4 groups of 6 rats each; three animals being housed in labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum

Grouping:

Group I------- Animals were administered 0.1ml saline p.o

Group II------ Animals were administered standard reference Diazepam (2 mg/kg, p.o.)

Group III------Animals were administered Alafia multiflora (100 mg/kg) p.o

Group IV------Animals were administered Alafia multiflora (200 mg/kg) p.o

Elevated Plus-Maze Model:

Experimental Procedure: The plus-maze apparatus consisting of two open arms (30 x5 x 0.2cm) and two closed arms (30cm x 5cm x 15cm) extending from central platform and was elevated to a height of 45cm above the floor. The entire maze was made up of clear plexi glass. Prior to the test, animals were treated with respective drugs. One hour after the treatments, each rat was individually placed on the center of the elevated plus maze with its head facing the open arm. During the entire experiment, rats were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze could invoke maze anxiety. During the 5 min experiment, following behavior of the mouse was recorded.

- Number of entries into open arm
- Number of entries into closed arm
- Time spent in the open arm and
- Time spent in the closed arm

Every time before placing each animal, the arena was washed with 5% alcohol to eliminate the possible bias due the odor left by the previous animal.

STATISTICAL ANALYSIS:

All the data's were analyzed using One-Way ANOVA method followed by Dunnet's / Tukey's test. All values were reported as mean ± SEM. $P \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening

Investigation revealed the presence of steroid, Alkaloid, saponins, Tannins, phenols & Flavonoid in Methanolic Extract of *alafia multiflora*

Table.no. 1. Preliminary Phytochemical Screening

Phytochemical	Results
Steroid	+
Alkaloid	+
Tannin	+
Carbohydrate	-
Phenol	+
Flavonoid	+
Saponin	+

(+) Present **(-)** Absent

Acute toxicity studies:
As per (OECD) draft guidelines 423 Female albino mice were administered *alafia multiflora* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 2000 mg/kg. Hence therapeutic dose was calculated as $1/10^{th}$ and $1/20^{th}$ i.e. 100mg/kg and 200 mg/kg of the lethal dose for the purpose of antidiabetic investigations.

ANTI ANXIETY ACTIVITY:

Elevated Plus-Maze Test:

TABLE 2. Effect of *Alafia multiflora* on Elevated Plus-Maze Model

TREATMENTS	ELEVATED PLUS MAZE MODEL			
	NUMBER OF ENTRIES (COUNTS/5MIN)		TIME SPENT IN (SEC/5MIN)	
	OPEN ARM	CLOSED ARM	OPEN ARM	CLOSED ARM
Control	3.66±0.55	13.16±1.22	29.16±7.94	218.16±14.28
Diazepam (2 mg/kg)	12.16±1.04***	15.16±1.04	137.16±9.33***	142.83±7.71***
alafia multiflora (100 mg/kg)	4.16±1.0	12.17±1.90	37.17±11.81	216.33±15.71
alafia multiflora (200 mg/kg)	7.66±0.84*	12.0±0.93	89.83±12.13**	170.66±8.55*

Values are expressed as mean±SEM from 6 rats. *P*<0.05 *, <0.01 ** and <0.001 * as compared to control group**

The classic anxiolytic benzodiazepine; diazepam has long been reported for its anxiolytic activity in rat with EPM. In our study also, a significant anxiolytic effect was recorded with diazepam as it increased the number of entries in open arms and the time spent in open arms along with significant decrease in time spent in closed arms *Alafia multiflora* at doses of 100 mg/kg, and 200 mg/kg showed significant anxiolytic activity by increasing the number of entries in open arms along with time spent in open arms and significant reduction in time spent in closed arms. The effect of *Alafia multiflora* (100, 200 mg/kg) on number of entries in closed arms was insignificant. The anxiolytic activity shown at higher doses of *Alafia multiflora* (200 mg/kg) was comparable with Diazepam 2 mg/kg. p.o.

DISCUSSION:

The elevated plus-maze is one of the most widely used model of animal anxiety, having been employed by many research laboratories in the past 6 years and has been extensively validated for use with rats and mice. The test is principally based on the observations of Montgomery showing that exposure of animals to an elevated maze alley evokes an approach-avoidance conflict that is considerably stronger than that evoked by exposure to an open maze allay. Elevation of the maze causes greater fear and more avoidance conflict. EPM consisting of two (opposite) open and two walled allays. The animal will explore the different allays (total number of entries). The open arms are more aversive than the closed ones, as revealed by a preference of the animals to explore the closed allays. Anxiolytic drugs will help to overcome the fear induced inhibition of open allay exploration. Diazepam a standard anxiolytic used clinically and is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic-like effects, even when the compound being screened does not act via benzodiazepine receptors. As expected standard diazepam significantly increased the number entries and time spent in open arm. *Alafia multiflora* at doses of 100 mg/kg & 200 mg/kg significantly increased the time spent and arm entries in open arms and decreased the time spent in closed arms compared to

control. The time spent in neutral zone is also reduced by both the doses compared to control group. Decreased aversion to open arms compared to control group indicates the anxiolytic activity of stem bark of *Alafia multiflora* and the magnitude of anxiolytic effect of 200 mg/kg and 100 mg/kg extracts of *Alafia multiflora* were comparable to that of standard drug diazepam 2 mg/kg p.o.

CONCLUSION:

The findings in this study suggest that the *alafia multiflora* possess Anti- anxiety activity.

The results have been obtained in carefully controlled experiments with laboratory animals where psychological factors can presumably be ruled out. In all the tests the responses have been assessed by actual measurement and not by subjective comparisons which may be influenced by the observer. Therefore the statistical validity of the findings has been proved and they provide a scientific foundation for the use of the biologically active ingredients of *alafia multiflora* in anxiety conditions and explain the clinical effectiveness of the *alafia multiflora.*

Acknowledgements:

The authors are grateful to the management of TEJA COLLEGE OF PHARMACY-KODAD for the support in the utilization of laboratory facilities.

Conflicts of interest:

The authors express no conflicts of interest regarding the publication, all the authors worked and provided support equally and credited equally.

REFERENCES:

1. Mccaffery m. Nursing management of the patient with pain. Lippincott. Philadelphia. 1972.
2. Turk dc, dworkin rh. "what should be the core outcomes in chronic pain clinical trials?" arthritis research & therapy. 2004; 6 (4):151-154.
3. Lynn b, holden av, winlow w. "cutaneous nociceptors" in the neurobiology of pain.Manchester, uk: manchester university press. 1984:106.
4. Bernston gg, cacioppo jt, shah jy, gardner wl. "the neuroevolution of motivation" in handbook of motivation science. Newyork: guildford press. 2008:191.
5. Astin ja. "why patients use alternative medicine: results of a national study". Journal of the american medical association. 1998; 279 (19): 1548–1553.
6. Eisenberg dm, kessler rc, foster c, norlock fe, *et al*. "unconventional medicine in the united states. Prevalence, costs, and patterns of use". New england journal of medicine. 1993; 328 (4): 246–52.
7. Doogan dp, langdon cj. A double-blind, placebo-controlled comparison of sertraline and dothiepin in the treatment of major depression in general practice. Medical department, pfizer ltd, sandwich, kent, uk. Int clin psychopharmacol. 1988; 9 (2): 95-100.
8. Haworth o, levy bd. Endogenous lipid mediators in the resolution of airway inflammation.eur respir j 2007; 30:980-92.
9. Nathan, c., 2002. Points of control in inflammation. Nature 19-26 420, 846–852.
10. Číž m, pavelková m, gallová l, králová j, kubala l, lojek, a the influence of wine polyphenols on reactive oxygen and nitrogen species production by murine macrophages raw 264. Physiology research 2008; 57:393-402.
11. Li q, verma im. Nf-kappab regulation in the immune system. Nat rev immunol 2002;2:725-34
12. Shen sc, lee wr, lin hy, huang hc, ko ch, yang ll, chen yc. In vitro and in vivo inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin e (2) production. European journal pharmacology 2002; 446:187-94.
13. Ban ta, et.al. Therapeutic monograph on anxiolytic-sedative drugs. Cma journal 1981;124:1439-46.
14. Hwa yc, jeong hp, jin th, hwan sy, sukjil s, bang yh, et.al. Anxiolytic-like effects of gensinosides on the elevated plus-maze model. Biol pharm bull 2005; 28(9):1621-1625.