Investigation of anxiolytic activity of leaf extracts of *Eclipta alba and Ziziphus jujube* in animal models

[1]Annepaka Eliya Raju, [2]Dr. R. Muthulakshmi, [3]Dr. P. Sri Lakshmi

[1]Ph.D Scholar, Meenakshi Academy of Higher Education and Research (MAHER) University, Chennai, Tamil Nadu, India
[2]Professor and Head, Department of Physiology, Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu, India
[3]Professor, Department of Biochemistry, Mamata Medical College, Khammam, Telangana, India

Corresponding Author: Annepaka Eliya Raju
Email: annepakaeliyaraju@gmail.com

Abstract

The present study deals with the investigation of alcoholic extracts of the plant *Eclipta alba* and *Ziziphus jujube* for anti-anxiety activities in rodents. The alcoholic extracts were administered in alone and in combination with a dose of 250 and 500 mg/kg, p.o. The findings indicated anxiolytic activity of the alcoholic extracts of *Ziziphus jujube* extracts and its combination when compared with standard and control groups. The results point towards the potential neuropharmacological activity of the plant *Eclipta alba* and *Ziziphus jujube* as ananxiolytic. Further neurochemical investigations can unravel the mechanism of action of the plant drug with respect to anti-anxiety activity and help to establish the plant in the armamentarium of anxiolytic agents.

Keywords: *Eclipta alba, Ziziphus jujube* and anti-anxiety

1. Introduction

The plant *Eclipta alba* (Linn.) or *Eclipta prostrata* (Linn.)] (family-Asteraceae) has been mentioned in ancient texts to be a nervine tonic (Mukhopadhyay, G *et al*., 2018; Satyavati *et al*., 1976; Uniyal *et al*., 1998) in addition to possessing hepatoprotective, hair growth promoting and anti-aging properties. The plant is reported to contain the phytoconstituents eclalbatin, alpha-amyrin, ursolic acid, oleanolic acid (Upadhyay *et al*., 2001) ecliptasaponin, daucosterol, stigmasterol-3-O-glucoside (Zhang and Chen, 1996) and coumestans as main active principles (Wagner *et al*., 1986). The plant has been extensively studied for its hepatoprotective activity and a number of herbal preparations comprising of E. alba are available for treatment of jaundice and viral hepatitis (Dalal, S *et al*., 2010; Wagner *et al*., 1986; Saxena *et al*., 1993; Singh *et al*.,

2001; Thakur, V. D., & Mengi, S. A. 2005). The aqueous and alcoholic extracts of the plant are proved to confer protection against the myotoxic effects of snake venom (Mors *et al*., 1989).

In Traditional Medicine Ziziphus jujuba, (Rhamnaceae) in the modern pharmacological studies, *Z. jujuba* possesses the hypnotic-sedative, hypotensive, antihypoxia, antihyperlipidemia and hypothermic effects (Yen, 1991). It was found to possesses the anxiolytic effect by decreasing the monoaminergic system activity (Aafi, E *et al*., 2022; Hossain, M. A. 2019; Hsieh *et al*., 1986).

In today's life of stress and strain, there is a dire need for agents having neuroprotective and neuropharmacological activity enhancing learning and memory function of the brain (Maphanga, V. B *et al*., 2020; Mukherjee and Roy, 1990). Stress involves complex biochemical, neural and immunological mechanisms and plays a crucial role in the genesis/progression of a variety of disease states ranging from psychiatric disorders like depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male impotency and cognitive dysfunctions to cardiovascular disease, hypertension, peptic ulcers, migraine, allergies, asthma, carcinoma, premature aging, rheumatic diseases and ulcerative colitis (Sen *et al*., 1992). Importantly, stress is also known to interfere with cognitive functions, tending to retard the memory engram rather than the acquisition of learning (Abubakar, A. R., & Haque, M 2019; Bhattacharya *et al*., 2000).

Anxiety has become a very important area of research interest in psychopharmacology this decade. This increased interest is as a result of a rapid growth of scientific studies and the discovery of new drugs that alter anxiety in animal models. Furthermore, anxiety disorders are appreciated to be very prevalent in community surveys (Regier *et al*., 1988) and the economic cost of them well justifies this increase in research interest and the development of new pharmacological approaches. Black and White test (Crawley and Goodwin, 1980), which uses the aversion of rodents to brightly lit large spaces and the elevated plus-maze, is a widely used test based on the natural aversion of rodents to heights and open spaces, which has been validated for both mice and rats (Dawson and Trickle bank, 1995; Helton *et al*., 1996).

During the last two decades, pharmacotherapy with psychoactive drugs has been increasingly recognized as most effective in the management of anxiety, stress and psychosomatic disorders. However, the prolonged use of tranquilizers and psychotropic drugs leads to a variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. Moreover, such agents primarily relieve the symptoms and offer a palliative relief of a temporary nature (Handa, 1995).

Clinically proven anxiolytics such as diazepam and buspirone are effective in the two models (Helton *et al*., 1996). However, all clinically available anxiolytics have limited clinical efficacy because of their adverse side effect of sedation. Therefore, the aims of this study were attempted to investigate the anxiolytic effects of the ethanolic extracts of *E. alba* & *Z. jujuba* in the animal models. We also profiled the secondary pharmacology of the *E. alba* & *Z. jujuba* extracts on spontaneous activity and interaction with a CNS depressant.

2. Materials and methods

2.1 Animals

Male Swiss Albino mice, weighing 15–20 g was used for the study. They were housed in groups of three per cage with a 12 L:12 D cycle and maintained on autoclaved polycarbonate cages with stainless steel top grill having facilities for holding pellet feed and drinking water in polycarbonate bottles. Animals were provided with rodent pellet feed with Aqua guard water ad libitum during acclimatization. All experiments were carried out during the light period (08:00-16:00 h). The Institutional Animal Ethical Committee approved the protocol of the study.

2.2 Preparation of the extracts

The leaves of *E. alba& Z. jujube* procured from local market was authenticated at Botany department, Osmania University, Hyderabad and a voucher specimen of the plant has been deposited (voucherno.080) The dried leaves were then coarsely powdered and the purity and quality of the crude drug was established as per the procedures laid down in Indian Pharmacopoeia 1996. The powdered material was placed in round bottom glass bottles. Three liters of 95% ethanol were added to each and reflux for 4 h. The procedure was repeated twice. The extracts were filtered and then concentrated to dryness in a Rota vapor in vacuum at 50 °C. We obtained alcoholic extracts of *E. alba & Z. jujuba* and yields were found to be 29.37% (w/w) and 31.24% (w/w), respectively.

2.3 Drugs

Diazepam (Calmpose®,5mg/tablet, Ranbaxy Laboratories, India), Vit C (sigma aldrich chemicals pvt. Ltd.) were used as reference standard drugs in this study. They were administrated in the form of suspensions using Tween 80 (0.2%, v/v) as the suspending agent. The solvents used were of analytical grade. Piracetam is a drug known to have a nootropic effect while diazepam is a renowned anxiolytic and anti-stress agents.

2.4 Administration of the extracts

Suspensions of the alcoholic extract were prepared in distilled water using Tween 80 (0.2%, v/v) as the suspending agent. The animals were divided in to Eight groups each consisting of six animals. The control group received the normal saline, where as the experimental groups received E. alba alcoholic extracts at a dose of 250 and 500 mg/kg,p.o, (EAL & EAH)., *Z. jujuba* alcoholic extracts at a dose of 250 and 500 mg/kg,p.o (ZJL&ZJH). Combination of alcoholic extracts of *E. alba* & *Z. jujuba* 250 mg/kg, p.o. (EAZJ), and the standard drug diazepam (2 mg/kg) and Vit. C 500mg/kg.

2.5 Anxiolytic activity

2.5.1 Tail suspension test (TST)

The tail suspension test (TST) is an experimental method used in scientific research to measure stress in rodents (Steru. L, Chemat. R, 1985). The total duration of immobility induced by tail suspension was measured. Tail suspension test (TST)

mechanical assembly comprised of wooden chamber (70 cm high). A bar was fitted between side dividers of load, at a tallness of 60 cm from ground or 10 cm from top of the mechanical assembly. Creatures were hung with the pole by putting sticky tape 1-in from tip of tail. Creatures were given a pretest session of 15 minutes every 1 day before definite test session. Creatures were treated with separate gathering medicines following pretest session, 360 minutes before conclusive test session and half-hour before definite test session. Half-hour after definite portion or 1 day after pretest session, every creature was separately hung with pole for conclusive test session of 300 seconds each. Term of stability was noted for every creature for 300 seconds. Mouse was viewed as stable when it inactively hung with bar without any endeavors to get away.

2.5.2 Elevated plus maze test

One of the most widely used tests for measuring anxiety-like behavior. The test is based on the natural aversion of mice for open and elevated areas, as well as on their natural spontaneous exploratory behavior in novel environments (Jaiswal and Bhattacharya, 1992; Lister, 1987). The EPM apparatus consisted of two open arms (16×5cm) and two closed arms (16×5×12cm) emanating from a common central platform (5×5cm).When exposed to the novel maze alley, the animals experience an approach-avoidance conflict, which is stronger in the open arm as compared to the enclosed arms. At the start of the session, the mouse was placed at the center of the maze, its head facing an open arm and allowed to explore them aze for 5min. The parameters noted were the percentage of time spent and percentage of arm entries in each type o farms. The plus maze was carefully wiped with a wet towel after each animal. Vit. C and Diazepam (2.0 mg/kg, i.p.) were used as the positive control.

2.5.3 Light-dark model

The apparatus consisted of two boxes (25 x25 x 25 cm) joined together. One box was made dark by covering its top with plywood, whereas a 40-W lamp illuminated the other box (Crawley and Goodwin, 1980). The light source was placed 25 cm above the open box. The mice were placed individually in the center of the light box and observed for the next 5 min for the time spent in the light and dark boxes. The mice were orally administered with extracts, diazepam (2.0 mg/kg; ip) or vehicle 30 min before being placed in the light box.

2.6 Statistics

All the results are expressed as Mean ± SEM. All the groups were analyzed using student's 't' test.

3. Results

3.1 Tail suspension test (TST)

Time frame of mobility as well as immobility in animals of standard along with the treatment groups was contrasted and that of normal control group (p<0.05). Plant

extracts likewise expanded the time span of mobility and diminished the immobility in dosage-dependent way (figure 1).

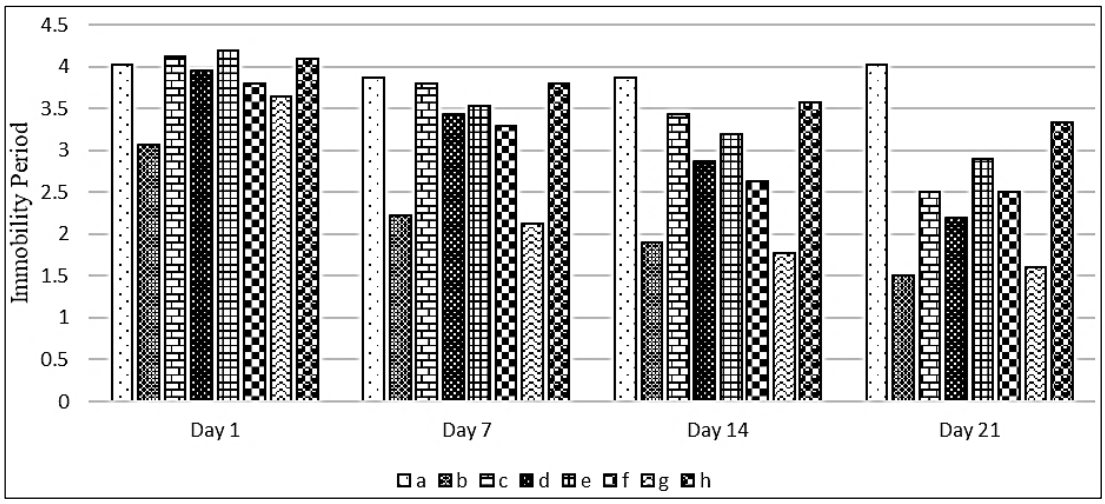

Fig 1: Tail Suspension Test

N=6; a-Control; b-Diazepam; c-EAL (*E. alba* extract 250mg/kg); d-EAH (*E. alba* extract 500mg/kg); e-ZJL (*Z. jujube* extract 250mg/kg); f-ZJH (*Z. jujube* extract 500mg/kg); g-EAZJ (*E. alba* + *Z. jujube* extract 250mg/kg); h-Vit. C.

3.2 Elevated plus maze apparatus

The elevated plus maze comprising of two open and two enclosed arms, produced a novel environment which helped in inducing anxiety in animals because of the open nature of the arms and elevation from the floor. When the animals were placed on the maze, they showed a preference for the enclosed (dark) arms and showed anxiety and fear like movements characterized by immobility, freezing and defecation on entering the open arms.

As shown in Fig. 2a & 2b, extracts in combination increased the number of arm entries and time spent in the open arms, decreased the number of arm entries and time spent in the closed arms. The alcoholic extract in combination produce significant increase in percent preference for open arm as first entry, in total number of entries in the open arm, as well as in the duration of stay in the open arm, when compared to the control group.

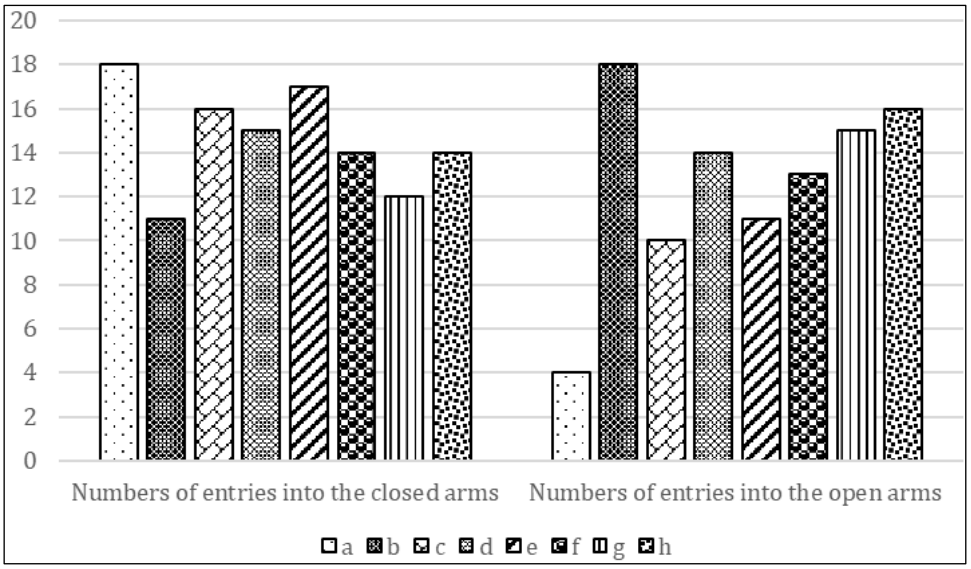

Fig 2a: Elevated Plus Maze Test

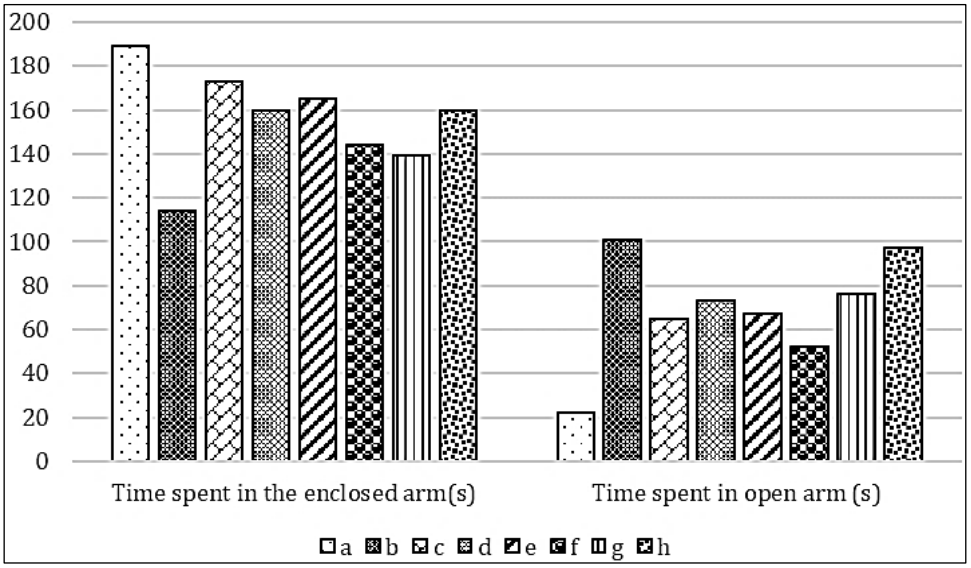

Fig 2b: Elevated Plus Maze Test

N=6; a-Control; b-Diazepam; c-EAL (E.alba extract 250mg/kg); d-EAH (E.alba extract 500mg/kg); e-ZJL (Z.jujube extract 250mg/kg); f-ZJH (Z.jujube extract 500mg/kg); g-EAZJ (E.alba+Z.jujube extract 250mg/kg); h-Vit.C.

Diazepam (2 mg/kg, p.o.) significantly increased (P<0.001) the percent preference for open arm, the number of entries as well as the duration of stay in the open arms, indicating anxiolytic activity.

3.3 Light-dark model

Extracts of ZJ at 500mg and EAZE at 250mg and diazepam (1mg/kg) induced a significant increment of the time spent by mice on the illuminated side of the apparatus (p<0.05, p<0.01), without significantly affecting other parameters (Figure 3a & 3b).

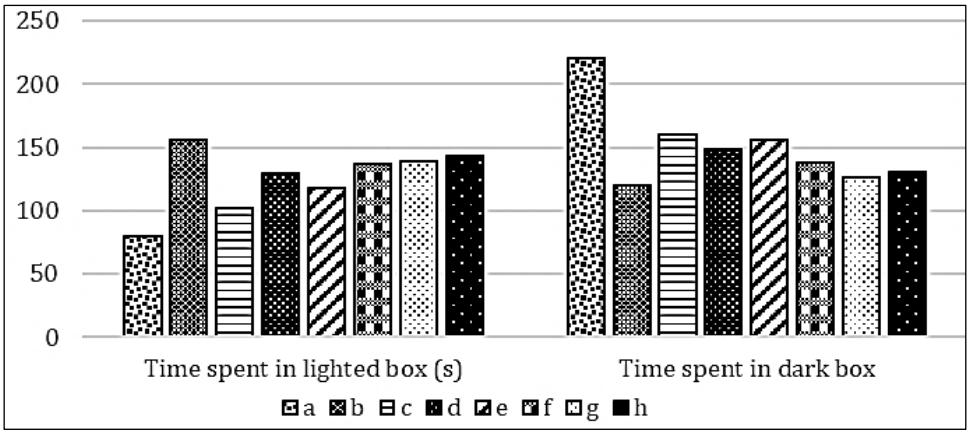

Fig 3a: Light Dark Test

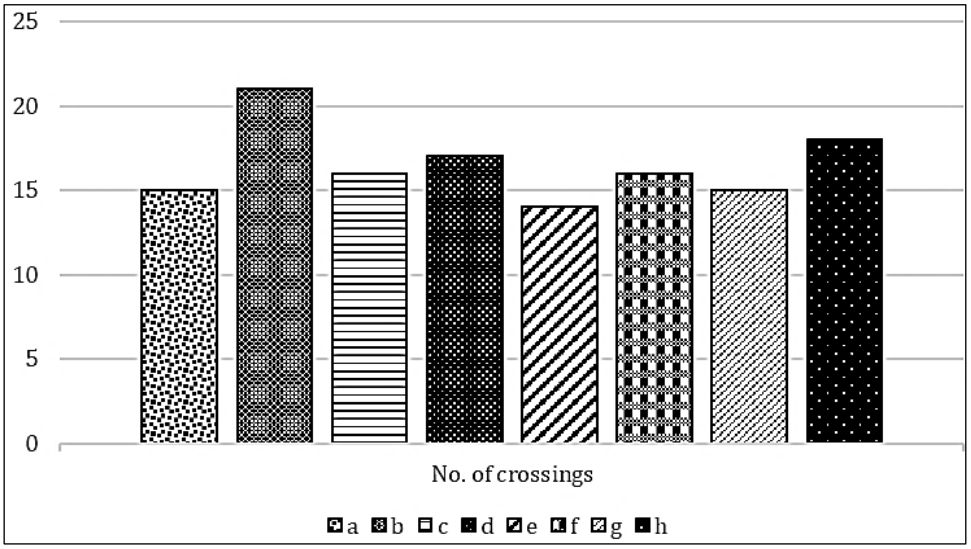

Fig 3b: Light Dark Test

N=6; a-Control; b-Diazepam; c-EAL (*E. alba* extract 250mg/kg); d-EAH (*E. alba* extract 500mg/kg); e-ZJL (*Z. jujube* extract 250mg/kg); f-ZJH (*Z. jujube* extract 500mg/kg); g-EAZJ (*E. alba* + *Z. jujube* extract 250mg/kg); h-Vit. C.

4. Discussion

Anxiety may be regarded as a particular form of behavioral inhibition that occurs in response to environmental events that are novel. It has been established that there are lots of plant secondary metabolites being employed in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system, noradrenaline, serotonin, GABA and BZD neurotransmitters activities.

In our investigation, the extracts produced significant change in the exploratory activity of the rats in the elevated plus maze model. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time as well as the number of entries into the open arm. The extracts of the plant showed such effects in the rodents and hence we can conclude that extracts possess anxiolytic activity.

Generally, most of the anxiolytic agents have an adverse effect on memory as seen with the benzodiazepines, commonly used as anxiolytics (Mrugnandam *et al*., 2000). An important point to be noted is that recently the plus maze model is also being used to study learning and memory processes in rodents. The impairment of learning and memory induced by scopolamine, an anti-cholinergic agent, is reflected by prolonged transfer latency from the open arm to the closed arm (Iyer *et al*., 1998). With respect to our findings, in contrast to that of diazepam, Combination of extracts showed significant values in entries in to the open arm and time spent in open arms, in the same way reduction in entries as well time spent in in to the closed arm.

Tail suspension tests (TSTs) were utilized in test groups and results reported that 250 mg/kg dosage of alone extracts as well in combination indicated potent anxiolytic effect that was significant to that of diazepam. The outcomes of forced swim as well as TST affirm considerable anxiolytic capability of both plants extracts. Promising mechanisms for antidepressant effect of crude extracts of the plant accounted may be due to inhibitory action on monoamine oxidase A. Additionally, the extracts also showed to increase exploratory activity of the animals in the light dark model.

The light-dark test may be useful to predict the anxiolytic like activity of drugs. Transitions have been reported to be an index of activity exploration because of habituation over time and the time spent in each compartment to reflect aversion. The administered extracts showed dose dependent and significant increase in the time spent in lit box, number of crossings and the time of latency with decrease in time spent in the dark box.

In the light/dark test, anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar and can be evaluated according to the number of transitions in to and the time spent in the light chamber where in increase in these parameters is considered to reflect anxiolytic-like properties. Our results showed that the extracts alone and in combination (250 mg/kg) increased time spent in the light chamber, suggesting anxiolytic action.

Mechanism of anxiolytic action of plants may be by interaction with some of the natural endogenous mediators in the body as reported by various workers. There could also be a linkage in the interaction of the plant extract with serotonergic pathway. Effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. Thus, the present study showed that extracts possessed potent anxiolytic activity which was evidenced by all the models as described above. Effects were dose dependent, optimum effect was observed at 250 mg/kg (po) which was significantly higher than vehicle treated control group. Our findings indicated both the extracts at a dose of 250 mg/kg, respectively; in the entire test models demonstrated a significant improvement in the anxiolytic, thus demonstrating anxiety activity.

In summary, the both leaf extracts exhibit anxiolytic effect at 250 mg/kg dose. The major active components and precise anxiolytic mechanisms need to be identified, but they indicate the value of further investigation of extracts with known and possible future clinical application.

References

1. Aafi E, Reza M, Mirabzadeh M. Jujube (*Ziziphus jujuba* Mill. (Rhamnaceae)): A review on its pharmacological properties and phytochemistry. Tradit. Med. Res. 2022;7(4):1-9.
2. Abubakar AR, Haque M. Medicinal plants with reported anxiolytic and sedative activities in Nigeria: A systematic review. İstanbul Journal of Pharmacy. 2019;49(2):92-104.
3. Bhattacharya SK, Bhattacharya A, Chakrabarti A. Adaptogenic activity of Siotone, a polyherbal formulation of Ayurvedic rasayanas. Indian Journal of Experimental Biology. 2000;38:119-128.
4. Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacology, Biochemistry and Behavior. 1980;13(2):167-170.
5. Dalal S, Kataria SK, Sastry KV, Rana SVS. Phytochemical screening of methanolic extract and antibacterial activity of active principles of hepatoprotective herb, Eclipta alba. Ethnobotanical leaflets. 2010;(3):3.
6. Dawson GR, Tricklebank MD. Use of the elevated plus maze in the search for novel anxiolytic agents. Trends in Pharmacological Sciences. 1995;16(2):33-36.
7. Handa SS. Plants and plant products for mental health. Decade of the brain. Rockville. MD: US Department of Health and Human Services, 1995, 163-Á171.
8. Helton DR, Berger JE, Czachura JF, Rasmussen K, Kallman MJ. Central nervous system characterization of the new cholecystokin in Bantagonist LY288513. Pharmacology Biochemistry and Behavior. 1996;53(3):493-502.
9. Hossain MA. A phytopharmacological review on the Omani medicinal plant: Ziziphus jujube. Journal of King Saud University-Science. 2019;31(4):1352-1357.
10. Hsieh MT, Chen HC, Kao HC, Shibuya T. Suanzaorentang and anxiolytic Chinese medicine, affects the central adrenergic and serotonergic systems in rats. Proceedings of the National Science Council, Republic of China-Part B, Life Sciences. 1986;10(4):263-268.
11. Iyer MR, Pal SC, Kasture VS, Kasture SB. Effect of *Law-soniainermis* on memory and behavior mediated via monoamine neurotransmitters. Indian Journal of Pharmacology. 1998;30:181-185.
12. Jaiswal AK, Bhattacharya SK. Effects of Shilajit on memory, anxiety and brain monoamines in rats. Indian Journal of Pharmacology. 1992;24:12-17.
13. Lister RG. The use of a plus-mazetome as ureanxiety in the mouse. Psychopharmacology. 1987;92(2):180-185.
14. Maphanga VB, Skalicka-Woźniak K, Budzynska B, Enslin GM, Viljoen AM. Screening selected medicinal plants for potential anxiolytic activity using an *in vivo* zebrafish model. Psychopharmacology. 2020;237:3641-3652.
15. Mors WB, Nascimento MC, Parente JP, Silva MH, Melo PO, Suarez-Kurtz G. Neutralization of lethal and myotoxic activi-ties of South American rattlesnake venom by extracts and constituents of the plant *Eclipta prostrata* (Asteraceae). Toxicon. 1989;27:1003-1009.

16. Mrugnandam AV, Kumar V, Bhattacharya SK. Status report on neuropharmacology. Indian Journal of Pharmacology. 2000;32:S119-S133.
17. Mukherjee P, Roy U. Neuropharmacological profile of a herbal medicine formulation 'Trasina' with special reference to antistress activity. Indian Journal of Medical. Research. 1990;84,227-232.
18. Mukhopadhyay G, Kundu S, Sarkar A, Sarkar P, Sengupta R, Kumar C. A review on physicochemical & pharmacological activity of Eclipta alba. The Pharma Innovation Journal. 2018;7(9):78-83.
19. Regier DA, Boyd JH, Burke JDJr, Rae DS, Myers JK, Kramer M, *et al*. One-month prevalence of mental disorders in the United States. Based on five Epidemiologic Catchment Area sites. Archives of General Psychiatry. 1988;45(11):977-986.
20. Satyavati GV, Rana MK, Sharma M. In: Medicinal Plants of India. Cambridge Printing Works, Delhi. 1976;I:370-372.
21. Saxena AK, Singh B, Anand KK. Hepatoprotective effects of *Eclipta alba* on subcellular levels in rats. J Ethno pharmacol. 1993;40:155-161.
22. Sen P, Mediratta PK, Ray A. Effects of *Azadirachta indica* A Juss on some biochemical, immunological and visceral parameters in normal and stressed rats. Indian Journal of Experimental Biology. 1992;30:1170-1175.
23. Singh B, Saxena AK, Chandan BK, Agarwal SG, Anand KK. *In vivo* hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves. Indian Journal of Physiology and Pharmacology. 2001;45:435-441.
24. Soliman GF, Khattab AA, Habil MR. Experimental Comparative Study of potential anxiolytic effect of Vitamin C and Buspirone in rats. Functional Foods in Health and Disease. 2018;8(2):91-106.
25. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology. 1985;85:367-370.
26. Thakur VD, Mengi SA. Neuro pharmacological profile of *Eclipta alba* (Linn.) Hassk. Journal of Ethnopharmacology. 2005;102(1):23-31.
27. Uniyal RC, Sandhu S, Chandok JK. Herbology. In: The Ayurvedic Encyclopedia. Sri Satguru Publications, India, 1998, 77.
28. Upadhyay RK, Pandey MB, Jha RN, Pandey VB. Eclalbatin, atriterpinesaponins from *Ecliptaalba*. J Asian Nat Prod Res. 2001;3:213-217.
29. Wagner H, Geyer B, Kiso Y, Hikino H, Rao GS. Coumestansas main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulaceae*. Planta Medica. 1986;5:370-373.
30. Yen ZH. Chinese Material Medica (II). Zu-Ing, Taipei, 1991, 671-673.
31. Zhang M, Chen Y. Chemical constituents of *Eclipta alba* (L.) Hassak. Zhongguo Zhong Yao Za Zhi. 1996;21:480-481, 510.