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Research Article
Evaluation of the Anxiolytic Activity of Ethanolic Extract of *Galinsoga parviflora* (Asteraceae) in a Mice Model

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Abstract

Background and Objective: The anxiolytic drugs have an unfavourable risk/benefit ratio, as they produce anterograde amnesia, dependence, abstinence syndrome, the paradoxical reaction in humans and decay of psychomotor functions. Therefore, research has been conducted to identify safer, more specific medications possessing anxiolytic effects without complications. In the past few years, several herbal medicines have been used for the management of anxiety in the world. The present study was designed to study the anxiolytic property of ethanolic extracts of *Galinsoga parviflora*, an important and commonly used for its medicinal properties belongs to the Asteraceae family. **Materials and Methods:** The anxiolytic activity was evaluated with the adult mice by hole board test, light-dark box test and motor coordination with the rotarod test. The efficacy of the plant extract (100, 200 and 400 mg kg^{-1}) was compared with the standard anxiolytic drug diazepam (1 mg kg^{-1}) i.p. **Results:** The extract increased the time spent in the brightly-lit chamber of the light/dark box, as well as in the number of times the animal crossed from one compartment to the other. Performance on the rotarod was unaffected. In the hole board test, the extract significantly increased both head-dip counts and head-dip duration. *Galinsoga parviflora*, in contrast to diazepam, had no effect on locomotion. **Conclusion:** These results provide support for the anxiolytic activity of *Galinsoga parviflora*, in line with its medicinal traditional use and may also suggest a better side effect profile of *Galinsoga parviflora* relative to diazepam.

Key words: Anxiety, *Galinsoga parviflora*, rotarod test, hole board test, light-dark test, diazepam, behavioural changes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anxiety has consequences not only for one's subjective well-being but also for one's physical health and quality of life. Anxiety is an unpleasant state of emotional conflict that is frequently accompanied by nervous behaviour and attitude, somatic complaints and rumination[1]. When anxiety becomes excessive, it is referred to as an anxiety disorder and it can significantly decrease one's quality of life by causing a variety of psychiatric problems. Agoraphobia, specific phobia, social anxiety (social phobia), panic attack, separation anxiety disorder and mental retardation are all disorders in this category[2]. Anxiety is defined as a condition of intense apprehension, uncertainty and fear arising from the expectation of a stressful event or scenario, typically to the point where normal bodily and psychological functioning is disrupted[3]. Nearly two of patients respond to currently available treatments but the magnitude of improvement is still disappointing, furthermore, they explore a range of systemic side effects and illustrate dependence and tolerance to chronic treatment, which has become a major concern about the use of currently used medicine[4]. Anxiolytic medicines, most of which belong to the benzodiazepine family, are one of the most widely used pharmaceuticals in the world. The conventional benzodiazepines work by binding to the benzodiazepine receptors found in the GABA pentameric complex. Diazepam is the most commonly used drug (52% of the studies investigating the action of a benzodiazepine full agonist)[5].

Anxiolytic substances, which mostly belong to the group of benzodiazepines, occupy a prominent place in the ranking of the drugs most frequently used in humans. These classic benzodiazepines act through the benzodiazepine receptor, which is present in the GABA pentameric complex. The most widely used compound is diazepam (52% of studies examining the effects of a full benzodiazepine agonist)[6]. However, the clinical use of benzodiazepines is limited by side effects such as psychomotor impairment, sitting sensation, muscle relaxation, ataxia, amnesia, potentiation of other central depressant drugs and the risk of dependence with little or no tolerance and dependence[7,8]. Herbs are widely recognized medicinal sources with important roles in health programs around the world, so many traditionally used plants have pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders[9]. Also due to people's growing desire to use herbal medications, this study sought to enhance the anxiolytic effects of *Galinsoga parviflora*. *Galinsoga parviflora* comes from the Andes region. *Galinsoga parviflora* has anti-inflammatory and healing properties, it also helps to clot blood from fresh wounds. Phytochemical analysis of plant extracts revealed the presence of alkaloids, flavonoids, sterols and terpenoids. The phytochemical investigation of the ethanolic extract of the *Galinsoga parviflora* plant led to the isolation and identification of triacontanol, beta-sitosterol, stigmasterol, 3,4-dimethoxycinnamic acid, protocatechuic acid and fumaric acid[10]. Despite the widespread use of *Galinsoga parviflora* as an anxiolytic, there are no pharmacological data to support this.

Therefore, we conducted the study to evaluate the anxiolytic potential of the ethanolic extract of *Galinsoga parviflora* using several suitable rodent test models.

MATERIALS AND METHODS

Study area: The studies were carried out in the Department of Pharmacology, Aditya Bangalore Institute of Pharmacy Education and Research (IAEC Approval No: 254/1611/CPCSEA) at Central animal house in the period of April-June, 2021. The animals were kept in a room with controlled temperature (25±1°C) and lighting (light/dark 12:12 hrs in polypropylene cages with sufficient food and water), which were carried out in all series of tests.

Animals: A total 30 number of animals were used. Rats were divided into 5 groups, with 6 rats in each group for this study.

Plant material: The leaves of the *Galinsoga parviflora* plant were collected in June, by the Institute of Ayurveda and Integrative Medicine, Karnataka. The plant was developed by Dr. Ganesh Babu, Bangalore, identified and authenticated.

Preparation of the ethanolic extract: The aerial part of the plant was dried and crushed at room temperature, 700 g of plant material were extracted with 6 L of ethanol and macerated at room temperature (25°C) for 48 hrs. The ethanol containing the extract was then filtered through Whatman paper and the solvent was distilled off under vacuum at 60°C on a rotary evaporator. The final extract was a dark green paste with an 11.9% dry weight. The residue was dissolved in water to appropriate final concentrations.

Medications: The ethanolic extract of *Galinsoga parviflora* was suspended in distilled water. Diazepam (10 mg/2 mL ampoule). It was diluted to the required concentration with saline before use. Benzodiazepines are known to act in higher

doses as anxiolytic and muscle relaxant effects. Therefore, we used diazepam (10 mg kg^{-1}) as a positive control for anxiolytic effects. Treatment plant experimental groups of mice were treated orally (p.o.) with ethanolic extract of *Galinsoga parviflora* at a dose of (100, 200 and 400 mg kg^{-1}), while the control groups received normal saline by the same routes. Diazepam (10 mg kg^{-1}) was administered intraperitoneally (i.p.). All drugs were freshly prepared before each experiment. The doses of the extract were calculated to give the mice 0.25 mL of the 20 g extract suspension. The tour took place 30 min after the treatments. The anxiolytic activity was examined by the light/dark box test and the hole board test and the motor coordination test was evaluated by the rotarod test.

Acute toxicity study: The procedure was followed according to OECD guidelines 423 (OECD/OECD.2002). The extract was administered orally at a dose of 2000 mg kg^{-1}) of body weight. Mice were observed for 14 days to record possible mortality, their weight was recorded and their behavioural neurological toxicity was investigated.

Light/dark test: The apparatus consisted of two 20×10×40 cm plastic boxes: One light chamber was painted white and brightly lit and the other was painted dark black and dimly lit with red light. The mice were allowed to move from one box to another through an open door between the 2 boxes. The illumination in the black zone was 50 lux, in the white zone it was increased to 1000 lux, generated by an additional light source. A mouse was placed in the light box in front of the hole. The transition between the light and dark box and the time spent in the light box was recorded for 5 min.

Hole board test: The perforated plate test was included in the test. It is made of grey plexiglass. For the study, the LETICIA plate (sign 720, LE 3333 printer) measuring 40×40 cm with 16 evenly spaced holes (3 cm in diameter and 2.2 cm deep) with built-in infrared sensors were used. The matt surface of the top plate prevents reflections that could alarm the behaviour of the animals. An animal has placed in the centre of the pegboard and allowed to freely explore the device for 5 min. The instrument automatically counted and recorded the number of times an animal plunged its head into the holes.

Rotarod test: Effect on motor coordination was evaluated with a rotarod device (Technoscientific products, Banglore, India). Rotarod consisted of a basic plant shape and an anise bar 3 cm in diameter and 30 cm in length with a non-slip surface. The rod was divided into 4 equal sections by 3 dams. The animals were preselected in a training session 24 hrs before testing based on their ability to remain on the bar for 2 min (at 12 rpm) and then four mice were preselected at 12 rpm for 30 min, they were observed 60 and 90 min. The interval between placing the animals on the spinning bar and falling off it was automatically recorded actuation time. The residence time in the apparatus was observed for 5 min (300 sec). The apparatus was flushed thoroughly with water between runs. All behaviour records were made with the observer blind to the treatment that the mice received.

Statistical analysis: All results are expressed as the Mean±Standard error of the mean. Data were statistically analyzed using a one-way variance ANOVA followed by a *post hoc* Tukey Kramer test for multiple comparisons. The $p<0.05$ was considered statistically significant.

RESULTS

Acute toxicity study: After oral administration of *Galinsoga parviflora* ethanolic extract at a dose of 2000 mg kg^{-1}), p.o., the animals were examined for signs of toxicity such as seizures, hypothermic hyperactivity and continued care for 2 hrs and mortality of up to 24 hrs and observed 1 hr after dosing. No toxicity or significant changes in body weight were observed between the treated and control groups.

Light/dark test: *Galinsoga parviflora* at the dose of 200 mg kg^{-1}) and diazepam (10 mg kg^{-1}) induces a significant increase in the time that the mice spend on the illuminated side of the device compared to the corresponding control group ($p<0.01$) without significantly influencing other parameters (Table 1).

Hole board test: The 200 mg kg^{-1} dose of the plant extract significantly increased the number of hard drives compared to control animals (Table 2).

Rotarod test: Data shows that those with 100 and 200 mg kg^{-1}) mice treated on average and 400 mg kg^{-1}) (p.o.) of the ethanolic extract of *Galinsoga parviflora* could balance on the rotating rod and remain longer without falling (Table 3). In contrast, diazepam (only at 10 mg kg^{-1}) showed a significant decrease in the locomotor score when compared to other groups.

Table 1: Anxiolytic activity of ethanolic extract of *Galinsoga parviflora* by using light/dark test

Treatments	Dose (mg kg^{-1})	Time in the lightbox	Number of transition
Saline		72.98±18.39	105±1.910
Diazepam	10	193.4±17.00**	8.5±1.928
Plant extract	100	120.6±24.03	12.33±3.383
Plant extract	200	156.8±24.23*	12.17±1.682
Plant extract	400	83.62±9.96	16.00±0.577

All values are Mean±SEM (n = 6), *p<0.01 when compared to control and one way ANOVA, Tukey Kramer *post hoc* test

Table 2: Anxiolytic activity of ethanolic extract of *Galinsoga parviflora* by using Hole board test

Treatments	Dose (mg kg^{-1})	Number of heads dipping
Saline		10.50±1.3
Diazepam	10	14.83±1.6*
Plant extract	100	14.33±3.3
Plant extract	200	31.5±2.8***
Plant extract	400	17.20±111.5*

All values are Mean±SEM (n = 6), *p<0.001 when compared to control and one way ANOVA, Tukey Kramer *post hoc* test

Table 3: Anxiolytic activity of ethanolic extract of *Galinsoga parviflora* by using Rotarod test

		Time (sec) of animals remained without falling from rod		
Treatments	Dose (mg kg^{-1})	30 min	60 min	90 min
Saline	1 mL	300	300	300
Diazepam	10	92.33±22.45***	199.8 ±35.34*	217.5±32.58
Plant extract	100	227±33.87	260,7±18.39	265.2±31.19
Plant extract	200	262.5±22.48	264.8±22.99	277.2±22.83
Plant extract	400	240.2±34.51	270.1±17.25	280.1±19.90

All values are Mean±SEM (n = 6), *p<0.001 when compared to control and one way ANOVA, Tukey Kramer *post hoc* test

DISCUSSION

Our result showed that the ethanolic extract (200 mg kg^{-1}) of *Galinsoga parviflora* increased the immersion of the head, confirming the anxiolytic effect previously shown in the light-dark test rota-rod test, a classic animal model to evaluate neuromuscular blockade. Peripheral and motor coordination deficits in motor coordination are very likely to have an impact on performance on behavioural tests. In the treatment of anxiety disorders or acute anxiety symptoms, a combination of therapeutic interventions is usually indicated. In addition to a psychotherapeutic approach, anxiolytics are part of the treatment of anxiety[11]. Dysregulation of the GABAergic, serotonergic, dopaminergic and adrenergic neuro systems has been associated with the pathophysiology of anxiety[12]. Benzodiazepines have been prescribed more frequently for the treatment of various forms of anxiety over the past 40 years, however, they have prominent side effects such as sedation, muscle relaxation, ataxia and amnesia and can cause drug dependence. Other anxiolytics are antidepressants, buspirone and beta-blockers, which are effective for many causes and also have side effects such as nausea, drowsiness, dizziness, headache, dry mouth, constipation, diarrhoea, etc. Self-administration of herbal medicines has been one of the most popular alternative therapies, there is great interest in the development of new anxiolytics, new therapies are needed for the treatment of anxiety disorders and research in medicinal plants could offer a new therapeutic option[13]. In the current work, we have the anxiolytic effect of the ethanolic extract of *Galinsoga parviflora* with the light-dark test and the pegboard and we use the Rota-rod test to examine motor coordination. In addition, the effects of *Galinsoga parviflora* and diazepam in these animal models were compared to determine if the behavioural profile of *Galinsoga parviflora* differs from an established anxiolytic In light/dark mode, anxiety before the test is caused by the conflict between the tendency to explore and the initial tendency to avoid the unknown and can be assessed by the number of transitions to the light chamber and the time spent in the light chamber[14], an increase in these parameters is considered anxiolytic properties. Our result showed that the extract (200 mg kg^{-1}) showed a longer residence time, that the light camera suggests an anxiolytic effect The hole table test is useful to model anxiety behaviour in animals when the head is submerged[15]. The final results showed that *Galinsoga parviflora* (100-200 mg kg^{-1}), in contrast to diazepam (10 mg kg^{-1}), did not have a significant influence on motor coordination. Furthermore, the extract did not affect motor coordination, evidencing more of a centrally mediated effect and not a blockage of the neuromuscular system. *Galinsoga parviflora* extract showed promising anxiolytic effects without causing the neuromuscular side effects.

CONCLUSION

The data presented here confirm the traditional use of *Galinsoga parviflora* to treat anxiety. Despite the widespread traditional use of *Galinsoga parviflora* for the treatment of various diseases, there are no reports of any scientific evaluation of its anxiolytic activity. Our study shows that *Galinsoga parviflora* extract had a clear effect on the light-dark test and the hole board test in mice. *Galinsoga parviflora* extract causes an anxiolytic behaviour comparable to the effect of diazepam. Future studies will focus on the neurobiological mechanism of action and the possible interactions of *Galinsoga parviflora* with classical neurotransmitters and the phytocomponents responsible for the observed central effects should be isolated and identified.

SIGNIFICANCE STATEMENT

This study discovers the neuronal behavioural effects of the ethanolic extract of *Galinsoga parviflora* in animals. This study will help the researcher to uncover the area of various CNS disorders that many researchers were not able to explore. Thus, a new theory on the plant extracts and possible mechanism of action may be arrived at.

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REFERENCES

1. Iverach, L., R.G. Menzies and R.E. Menzies, 2014. Death anxiety and its role in psychopathology: Reviewing the status of a transdiagnostic construct. Clin. Psychol. Rev., 34: 580-593.
2. Gutiérrez, S.L.G., R.R. Chilpa and H.B. Jaime, 2014. Medicinal plants for the treatment of ''nervios'', anxiety and depression in Mexican traditional medicine. Rev. Bras. Farmacognosia, 24: 591-608.
3. Chandrashekar, K., P. Amudha and S. Venkataraman, 2017. Anxiolytic activity of ethanolic and aqueous extract of *Ficus carica* linn fruits in Swiss albino mice. Int. J. Basic Clin. Pharmacol., 6: 2043-2050.
4. Chatterjee, M., R. Verma, V. Lakshmi, S. Sengupta, A.K. Verma, A.A. Mahdi and G. Palit, 2013. Anxiolytic effects of plumeria rubra acutifolia (Poiret) L. flower extracts in the elevated plus-maze model of anxiety in mice. Asian J. Psychiatry, 6: 113-118.
5. Elkins, G., M.H. Rajab and J. Marcus, 2005. Complementary and alternative medicine use by psychiatric inpatients. Psychol. Rep., 96: 163-166.
6. Prut, L. and C. Belzung, 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. Eur. J. Pharmacol., 463: 3-33.
7. Czobor, P., P. Skolnick, B. Beer and A. Lippa, 2010. A multicenter, placebo-controlled, double-blind, randomized study of efficacy and safety of ocinaplon (DOV 273, 547) in generalized anxiety disorder. CNS Neurosci. Ther., 16: 63-75.
8. Emamghoreishi, M., M. Khasaki and M.F. Aazam, 2005. *Coriandrum sativum*: Evaluation of its anxiolytic effect in the elevated plus-maze. J. Ethnopharmacol., 96: 365-370.
9. Verma, R., K. Hanif, D. Sasmal and R. Raghubir, 2010. Resurgence of herbal antihypertensives in management of hypertension. Curr. Hypertens. Rev., 6: 190-198.
10. Bazylko, A., K. Boruc, J. Borzym and A.K. Kiss, 2015. Aqueous and ethanolic extracts of *Galinsoga parviflora* and *Galinsoga ciliata*. investigations of caffeic acid derivatives and flavonoids by HPTLC and HPLC-DAD-MS method. Phytochem. Lett., 11: 394-398.
11. Rex, A., E. Morgenstern and H. Fink, 2002. Anxiolytic-like effects of Kava-Kava in the elevated plus maze test-a comparison with diazepam. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, 26: 855-860.
12. Kishore, R.N., N. Anjaneyulu, M.N. Ganesh and N. Sravya, 2012. Evaluation of anxiolytic activity of ethanolic extract of foeniculumvulgare in mice model. Int. J. Pharm. Pharm., 4: 584-586.
13. Faustino, T.T., R.B. de Almeida and R. Andreatini, 2010. Medicinal plants for the treatment of generalized anxiety disorder: A review of controlled clinical studies. Rev. Bras. Psiquiatria, 32: 429-436.
14. Lepicard, E.M., C. Joubert, I. Hagneau, F. Perez-Diaz and G. Chapouthier, 2000. Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6J strains of mice. Pharmacol. Biochem. Behav., 67: 739-748.
15. Thakur, P. and A. Rana, 2013. Anxiolytic potential of medicinal plants. Int. J. Nutr., Pharmacol. Neurol. Dis., 3: 325-331.