Pharmedico Publishers
International Journal of Pharmaceutical Science and Medicine
Volume 3, Issue 1 (2025): 1-5
DOI: 10.70199/IJPSM.3.1.1-5

RESEARCH ARTICLE

ISSN: 2584-1610 (Online)

PHARMACOLOGICAL ASSESSMENT OF MEDICINAL EXTRACT'S ANTI-ANXIETY IMPACT IN ANIMALS BY LOCOMOTIVE AND BEHAVIOURAL RESEARCH EMPLOYING LIGHT SENSITIVITY

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Article History
Received: 18 November 2024
Revised: 21 December 2024
Accepted: 09 January 2025
Published: 25 March 2025

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ABSTRACT: Background: Anxiety disorders are prevalent mental health conditions characterized by excessive fear and behavioural disturbances. Traditional herbal remedies have shown potential in managing anxiety, but their pharmacological validation remains underexplored. This study investigates the anti-anxiety effects of an herbal extract using preclinical behavioural models in mice. **Methodology:** Mice weighing 20-25g were divided into three groups (n=6 per group): Group 1 (negative control), Group 2 (Vinpocetine 10mg/kg) Herbal extract treated the anti-anxiety effects were evaluated using three models: Actophotometer and social behaviour test. **Results:** The negative control group showed higher locomotion counts, more line crossings, and less time in the centre square. Vinpocetine-treated mice displayed reduced counts, fewer line crossings, and increased central square activity. The herbal extract-treated group showed similar results to the vinpocetine group, suggesting anxiolytic effects. In the social behaviour test, the herbal extract enhanced social interaction, comparable to vinpocetine. **Conclusion:** The herbal extract demonstrated significant anxiolytic effects, comparable to diazepam, across all tested models. These findings support its potential as a natural alternative for anxiety management.

Keywords: Anxiety, Behaviour, Open Field Test, Actophotometer, Vinpocetine

INTRODUCTION

In terms of prevalence, anxiety disorders rank high, impacting millions of people around the globe. Excessive dread, concern, and behavioural abnormalities are hallmarks of these conditions, which can greatly diminish daily functioning and overall quality of life [1]. GAD, panic disorder, social anxiety disorder, and phobias are all examples of the wide range of diseases that fall under the umbrella term "anxiety disorders" [2]. A myriad of elements, including genetics, the environment, and neurobiology, interact intricately to cause these illnesses. The main kinds of drugs administered for anxiety are benzodiazepines and selective serotonin reuptake inhibitors (SSRIs) [3]. Pharmacological and psychotherapy therapies are also often used in traditional anxiety management. Drowsiness, cognitive impairment, dependency, and withdrawal symptoms are some of the negative side effects of these pharmacological medicines that restrict their usage, which in turn leads to the quest for alternative treatment techniques [4]. To better understand anxiety and assess possible treatment medicines, preclinical research has been crucial. The effectiveness of anxiolytic drugs is often evaluated in behavioural studies using rodent models, specifically rats and mice.

There are a number of popular preclinical models used to evaluate behavioural characteristics, including locomotion, exploratory activity, social interactions, and the elevated plus maze (EPM) [5]. Insights into the neurological bases of anxiety and the screening of new anxiolytic drugs are both facilitated by these models [6]. The growing demand for complementary and alternative medicine has highlighted the importance of pharmacologically validating herbal medicines that may have anxiolytic properties, even if synthetic anxiolytic medications are already on the market [7]. Ayurveda, TCM, and Unani medicine are just a few of the ancient medical systems that have long made use of herbal therapy to treat anxiety and other mental health issues [8]. The way many plant-based chemicals help reduce anxiety is by affecting neurotransmitter systems, particularly the pathways for dopamine, gamma-aminobutyric acid (GABA), and serotonin (5-HT). Among the many herbal remedies that can help alleviate anxiety are brahmi, passionflower, valerian root, chamomile, and *Withania somnifera* (ashwagandha) [10]. Preclinical research on these herbs has shown that they can reduce anxiety, with results that are on par with those of more traditional anxiolytic medications like diazepam [11].

Research into the pharmacological validity of herbal anxiolytics is still in its early stages, despite encouraging data from both traditional use and early scientific investigations. To prove their safety and effectiveness, herbal extracts must be standardised, active phytoconstituents must be identified, action mechanisms must be explained, and extensive preclinical and clinical trials must be conducted [12]. To address this need, this study used three recognised tests—the IR Actimeter, the Open Field Test, and the Social Behaviour Test (Brightness Discrimination)—to evaluate how well a specific herbal extract can reduce anxiety. This study aims to offer empirical evidence supporting the use of the herbal extract as a natural option for anxiety treatment by comparing its effects with those of diazepam, a conventional anxiolytic medicine [14]. There may be a more accessible and safer way to manage anxiety disorders if herbal medicine is included in regular psychiatric therapy [15].

The creation of new, evidence-based natural anxiolytics is greatly assisted by preclinical research, which bridges the gap between traditional wisdom and current pharmacology [16]. This study adds to what is already known about herbal anxiolytics and shows how important it is to use rigorous scientific validation to find natural treatments that work for mental health issues. Ayurvedic practitioners typically turn to *asparagus racemosus*, or shatavari, for its curative or preventative properties against a wide range of illnesses. This plant is so powerful that it is called the "herb's queen". Vitamins, alkaloids (racemosol), saponins (especially Shatavarins I, II, III, and IV), polyphenols, flavonoids, and steroidal glycosides are among its bioactive constituents. It is frequently utilised in folk and Ayurvedic medicine because of the sapogenin present in shatavari, which is a precursor to multiple pharmacologically potent steroids. Although the entire plant is medicinal, its roots, stems, and leaves are particularly useful. An effective means of protection against disease is the "Rasayanas" crafted from shatavari.

The phytochemicals it contains make it useful for treating a wide range of illnesses. Its phytochemicals have medicinal uses for many different diseases. Among shatavari's numerous medicinal uses are its actions against spasmodic muscle spasms, diabetes, allergies, malaria, tumours, arthritic joints, inflammation, periodontal disease, ulcers, stress, diarrhoea, depression, infections, tuberculosis, and many more. There are medications available that include Shatavari extract, and research has demonstrated that they can alleviate pain, fever, infection, abortion, and leprosy.

Extracts from the roots, leaves, flowers, and stems of the shatavari plant have anti-dyspepsia, anti-psychotic, anti-bronchitis, anti-throat, and anti-reproductive system properties. on pages 17–20 *Vinpocetine* is an ethyl ester of the alkaloid apovincamine synthesised from the leaves of Vinca minor, a plant related to the Apocynaceae family; the species is more popularly known as the lesser periwinkle. Both the European and British Pharmacopoeias have given their stamp of approval. Cavinton was the authorised trade name after its discovery, improvement, and marketing by Gedeon Richter Ltd., of Budapest, Hungary. *Vinpocetine* has been widely used in several nations since it was first introduced to the market in 1978. It has been used to prevent many cerebrovascular diseases (CVDs), such as stroke, thrombosis, haemorrhage, vertebral stenosis, carotid stenosis, vertebral artery narrowing, etc. The blood-brain barrier is easily crossed by vinpocetine. Dietary supplements containing *vinpocetine* are available on the American pharmaceutical market. According to studies conducted on the extract of the periwinkle plant, it has the ability to widen blood vessels, improve blood circulation, boost oxygen utilisation, and either restrict red blood cells, making them more flexible, or limit the aggregation of platelets. The many biochemical and pharmacological effects of vinpocetine were studied. It alleviates pain, fights inflammation, enhances neural plasticity, slows down the ageing process, protects the heart, and reduces the symptoms of epilepsy, Parkinson's disease, tinnitus, and Alzheimer's and associated dementias. Notably, there have been no reported serious side effects of the vinpocetine medication. According to human studies, the upper part of the digestive system absorbs the vinpocetine medication, while the stomach absorbs its active form, apovincaminic acids. It dissolves more easily in the stomach, which has a pH of 1.2, compared to the intestines, where the pH is 6.8. Unlike in the intestines, where the pH is 6.8, it dissolves more easily in the stomach (pH 1.2). The medication is completely eliminated from the body after 8 hours, and its half-life is 1-2 hours [21-22].

Traditional Nigerian medicine makes use of *oldenandia. Oldenandia corymbosa* Linn is a blooming rubiaceous plant. India is a wonderful place to spot them. Slanted stalks allow the plant to climb higher. The leaf's breadth is about 6 mm, and its length is between 1 and 4 cm. There are four petals on a flower with a tube that is barely 1.5-2 mm across. Keralan traditional medicine is known as "parppatakapullu". There was a time when this plant's medicinal properties were widely exploited. This plant effectively disperses stagnant pee and eliminates toxins and heat from the urine. Lymphosarcoma, laryngeal tumours, and liver tumours are all regulated. This plant is useful for various skin problems, ulcers, and throat pains. Traditional medicine has long made use of this blooming plant. Studies have shown that this plant has the potential to heal malaria, neutralise free radicals, destroy cancer cells, and maintain liver function. In certain cases, this plant has shown promise in treating renal illness. Blood cell coagulation and inflammatory suppression are two effects of saponin-rich plant extracts. The haemoglobin clots because of saponins. Plants often contain phenolic chemicals. Techniques (Randox kits) for warding off or treating ageing, cancer, inflammation, atherosclerosis, coronary artery disease, protecting endothelial cell malfunction, and preventing angiogenesis and cell proliferation are all included [23-24].

Anemone latifolia Roxb. adeciduous tree of the family Combretaceae, Wall., is well-known as Axlewood or Dhawra and is highly prized for its economic, ecological, and therapeutic properties. In order to demonstrate its potential as a versatile medicinal plant, this review will attempt to give a thorough synopsis of its pharmacological characteristics,

traditional usage, phytochemical makeup, and botany. The gum, bark, and leaves of *A. latifolia* are used in traditional medicine systems like Ayurveda and Siddha to treat a wide range of conditions, including wounds, diarrhoea, diabetes, fever, and more. The ethnobotanical value of this plant is highlighted by its usage in treating syphilis, gonorrhoea, scorpion stings, and snake bites. Research into phytochemistry has uncovered bioactive substances such as glycosides, triterpenoids, phenolic compounds, flavonoids, and tannins. These chemicals have a wide range of pharmacological actions, such as protecting the liver, preventing ulcers, reducing inflammation, having antidiabetic effects, and promoting wound healing [25-26].

MATERIALS

Vinpocetine was purchased from micro labs Pvt. Ltd. *Asparagus racemosus* seeds purchased from the Vatika Agro store in Jaipur, 302020, Rajasthan, India. The herbal garden at the Maharana Pratap College of Pharmacy in Kanpur provided us with fresh leaves of, *Oldenandia corymbose and Anogeissus latifolia*. The eminent botanist confirmed the authenticity of the plant by identifying every component of it. The specimen was placed at the university's herbarium house at Janta Postgraduate College, A.P.S University, Rewa (486001), M.P. India.

METHODOLOGY

Animal Selection and Grouping

Mice weighing 20-25g were randomly divided into three groups (n=6 per group):

- Group 1: Control (Receives vehicle treatment).
- Group 2: Vinpocetine-treated (10 mg/kg, orally).
- Group 3: Test – I (*Asparagus racemosus* seeds Powder 30:75)
- Group 4: Test – II (*Oldenandia corymbose* leaves powder 45:50)
- Group 5: Test – III (*Anogeissus latifolia* Roxb leaves powder 80:25)

Every mouse was kept in a typical laboratory setting with a 12-hour light/dark cycle, a temperature maintained at 22 ± 2°C, and free access to food and drink. At least one week of acclimatisation was required prior to experimenting. Different concentrations of *Oldenandia corymbose* leaves powder, *Anogeissus latifolia Roxb* leaves powder, and *Asparagus racemosus* seeds powder were combined in three different ratios: 30:75, 45:50, and 80:25. To make sure the powders were uniform in size and composition, they were combined carefully and weighed using an analytical balance. To make oral administration easier, the combinations were suspended in a suitable vehicle, such as distilled water or a 0.5% CMC solution. An oral gavage was used to provide the extract to the mice at a constant amount per body weight. To determine the formulations' anxiolytic effects, behavioural evaluations were carried out after administration. These evaluations included social interaction, an open field test, and locomotion analysis using actophotometers [27].

Evaluation of locomotory activities in mice

We used a digital actophotometer to measure the level of mental alertness in mice, following a method similar to that of Sugumaran et al. (2008) with a few tweaks. The basic design of an actophotometer is a square or circular chamber with five or six horizontal light beams that illuminate photocells on the other side. The apparatus also includes a floor that is embedded with rod grid lines that allow different electric currents ranging from zero to one hundred volts to flow through, and a glass cover that allows the experimental animals to be brought into the chamber. Each animal wanders freely or is assisted by the non-lethal electric currents in the floor rod grid lines of the enclosed room to disrupt the horizontal light beams, and a digital counter is connected to the photocells so that it can keep track of the count. The whole experiment made use of a current of 20 V (Fig. 1).

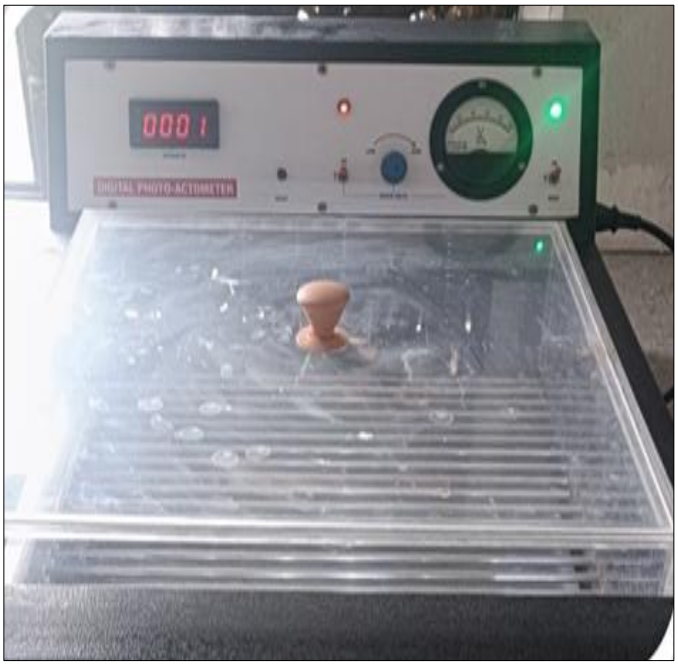

FIG. 1: DEPARTMENT OF PHARMACOLOGY MAHARANA PRATAP COLLEGE OF PHARMACY DIGITAL ACTOPHOTOMETER

Open Filed Test

A popular behavioural test for anxiety-related reactions focused on movement and exploration is the Open Field Test (OFT). It is possible to estimate the degree of anxiety by observing that mice tend to avoid the centre of an open field and instead choose the edges.

Social Behaviour Test

The Social Behaviour Test evaluates anxiety-related responses based on the tendency of mice to explore a brightly lit area versus a dark chamber, assessing their social interactions and willingness to enter an aversive environment [28].

RESULTS

TABLE 1: COMPARISON OF PRE- AND POST-TREATMENT LEARNING OUTCOMES SCORES (IN NUMERICAL FORM) BETWEEN GROUPS

S. No.	*Treatment Groups*	*Locomotory Scores*	
		Before Treatment	*After Treatment*
1	Control (Receives vehicle treatment)	130	117
2	Vinpocetine (10/mg/kg orally)	132	78
3	Test – I *Asparagus racemosus* seeds Powder 30:75	140	111
4	Test – II (*Oldenandia corymbose leaves* powder 45:50)	145	82
5	Test – III (*Anogeissus latifolia* Roxb leaves powder 80:25)	130	90

Open Field Test

TABLE 2: OPEN FIELD TEST PARAMETERS FOR EVALUATING DIFFERENT TREATMENT GROUPS

S. No.	*Treatment Groups*	*No of Line Crossings*	*Number of Centre Square entries*	*Time Spent in Centre Square (Sec)*
1	Control (Receives vehicle treatment)	60	1	4
2	Vinpocetine (10/mg/kg orally)	30	3	30
3	Test – I *Asparagus racemosus* seeds Powder 30:75	50	1	14
4	Test – II (*Oldenandia corymbose* leaves powder 45:50)	48	1	20
5	Test – III (*Anogeissus latifolia* Roxb leaves powder 80:25)	35	2	21

DISCUSSION

A decrease in counts indicates less anxiety, according to actophotometer data, which compares locomotory activity before and after treatment. Habituation, not anxiolytic effects, probably explained the little decrease in the control group. The standard anxiolytic vinpocetine was found to effectively reduce locomotion. Test I (30:75) demonstrated a decrease in activity and modest anxiolytic effects across the polyherbal formulations. Test II's (45:50) highest reduction suggested a strong anxiolytic potential, surpassing that of diazepam. There was a decrease in Test III (80:25), suggesting that it was effective.

These findings point to the possibility of polyherbal formulations as an alternative to traditional methods of anxiety treatment, with Test II (45:50) showing the most promise. To validate their effectiveness and mechanisms, further research on biochemical and behavioural markers is required.

Open Field Test

The Open Field Test uses centre-square exploration and locomotion to measure anxiety. Anxiolytic effects are shown by fewer line crossings, more centre-square entries, and more time spent in the centre. High anxiety was indicated by the control group's limited centre exploration (1 entry, 4 seconds) and greatest line crossings (60). The anxiolytic impact of vinpocetine was confirmed by the fact that animals treated with it spent much longer time in the middle (30 seconds) and had fewer line crossings (3). Test III (80:25) had the largest anxiolytic effect among the polyherbal treatments (35 crossings, 2 entries, 21 seconds in the centre), followed by Test II (45:50) (48 crossings, 1 entrance, 28 seconds).

Moderate effects were seen in Test I (25:75) (48 crossings, 1 entry, 13 sec). According to these results, Test II (45:50) and Test III (80:25) could be strong anxiolytic formulations with effects that are comparable to those of vinpocetine. Additional verification is necessary.

Social Behaviour

Higher peaks in the oscilloscope readings represented closer proximity between mice in a particular chamber, reflecting their interactions. The pictures demonstrate that oscilloscope peaks were noticeable when the mice remained in the same chamber, indicating high levels of social interaction. On the other hand, the peaks decreased as they were scattered. According to this study, mice's behaviour is influenced by their surroundings, and darkness may promote clustering. Kdenlive's oscilloscope efficiently measures social interactions, providing information on social affinity and anxiety in animal models.

CONCLUSION

Using locomotory activity, open field testing, and oscilloscope-based movement monitoring, the study successfully examined mice's social behaviour and anxiety. In the open-field test, anxiolytic effects were validated by decreased movement and enhanced centre-square exploration; the polyherbal formulations in Test II (45:50) and Test III (80:25) showed high promise. Higher oscilloscope peaks in the two-chamber arrangement were associated with social closeness, suggesting that social interactions are influenced by the environment.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

SOURCE OF FUNDING: None.

DATA AVAILABILITY: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL: The college has reviewed all of the experiments and given its permission.

ACKNOWLEDGMENT: The authors are grateful to Maharana Pratap college of Pharmacy Kanpur Uttar Pradesh, India for technical and administrative support.

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How to Cite this article:
Dubey A, Kumari M, Tiwari V, Sonker A, Kumar V, Nyarko RO. Pharmacological assessment of medicinal extract's anti-anxiety impact in animals by locomotive and behavioural research employing light sensitivity. International Journal of Pharmaceutical Science and Medicine 2025; 3(1): 1-5.