RESEARCH PAPER

Preclinical Evaluation Of Anxiolytic And Analgesic Effect Of Vetiveria Zizanioides Root Extract With Comparison Of Diazepam And Aspirin In Wister Albino Rats

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ABSTRACT

Background

Anxiety disorders and pain are common clinical conditions requiring long term pharmacotherapy. Although benzodiazepines and non-steroidal anti-inflammatory drugs effectively manage these conditions, their prolonged use is associated with adverse effects. Consequently, there is growing interest in plant derived therapeutic agents with comparable efficacy and improved safety profiles. *Vetiveria zizanioides* possesses antioxidant and neuropharmacological properties. However, its anxiolytic and analgesic activities remain inadequately investigated. Hence this study evaluated the anxiolytic and analgesic effects of *Vetiveria zizanioides* in wistar albino rats.

Materials and Methods

Twenty four Wistar albino rats were randomly allocated into four groups (n=6 per group) for each experiment. For anxiolytic evaluation, separate groups received normal saline (control), diazepam (3 mg/kg), the alcoholic root extract of *Vetiveria zizanioides* at 200 and 400 mg/kg. The anxiolytic activity was assessed using the elevated plus maze. For analgesic evaluation, separate groups received normal saline, aspirin (100 mg/kg), the extract at 200 and 400 mg/kg. The analgesic activity was assessed by the tail flick method.

Results

Dose dependent anxiolytic and analgesic activities are noted. The 400 mg/kg dose significantly increased open arm entries compared with the control ($p<0.05$) and showed anxiolytic effects comparable to diazepam. It also significantly prolonged tail flick reaction time compared with the control and low dose groups ($p<0.05$).

Conclusion

The alcoholic root extract of *Vetiveria zizanioides* (400 mg/kg) showed significant anxiolytic and analgesic activities comparable to standard drugs, supporting its potential as a plant derived therapeutic agent. Further studies are needed to identify its bioactive phytoconstituents, the underlying mechanisms and to establish long term safety.

Keywords

Vetiveria zizanioides, Anxiolytic activity, Analgesic activity, Elevated plus maze, Tail flick method, Wistar albino rats.

How to cite this article: How to cite this article: Bhuvaneswari S, Nasrin Nisha A, Jisha Justin J, Nilofa M. Preclinical Evaluation Of Anxiolytic And Analgesic Effect Of Vetiveria Zizanioides Root Extract With Comparison Of Diazepam And Aspirin In Wister Albino Rats. Int J Drug Deliv Technol. 2026;16(71s): 613-618. DOI: 10.25258/ijddt.16.71s.73

Source of support: Nil.

Conflict of interest: Nil.

INTRODUCTION

Anxiety is a normal adaptive emotional response to recognize and respond appropriately to stressful or threatening conditions. Under physiological conditions, anxiety is transient and resolves once the perceived threat has subsided. However, excessive, persistent anxiety in the absence of an identifiable stressor leads to an anxiety disorder, resulting in significant psychological distress and impairment in occupational, social and functional activities. Anxiety disorders are among the most common psychiatric illnesses worldwide, affecting millions of people. They substantially leads to disability, reduced quality of life and increased healthcare utilization, making them a major global public health concern. [1,2] Pain is another major clinical problem frequently associated with anxiety and is considered one of the leading causes of disability worldwide. Pain may be acute or chronic and may arise from inflammatory, neuropathic or nociceptive mechanisms. Persistent pain may often coexists with anxiety disorders, with each condition capable of

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exacerbating the other through shared neurobiological pathways involving serotonergic, noradrenergic, GABAergic and glutamatergic neurotransmission. [3] This bidirectional relationship significantly impairs physical function, emotional wellbeing and overall quality of life. Pharmacological management of anxiety primarily includes benzodiazepines, selective serotonin reuptake inhibitors, serotonin norepinephrine reuptake inhibitors and other anxiolytic agents, whereas pain is commonly treated with nonsteroidal anti-inflammatory drugs (NSAIDs), opioids and adjuvant analgesics.[4] Although these medications provide effective symptomatic relief, their prolonged use is often associated with adverse effects such as sedation, tolerance, dependence, cognitive impairment, gastrointestinal ulceration, renal toxicity and cardiovascular complications.[5,6] Furthermore, drug related adverse effects may reduce patient adherence and therapeutic outcomes. Consequently, there is growing interest in identifying safer and more effective therapeutic agents derived from natural sources. Medicinal plants have been used for centuries in traditional systems of medicine and continue to represent an important source of novel pharmacologically active compounds. [7] The scientific use of plant derived products for disease prevention and treatment, has gained considerable attention because of its potential efficacy, better tolerability, and relatively lower incidence of adverse effects. Numerous phytochemicals, including flavonoids, alkaloids, terpenoids, phenolics and essential oils, have demonstrated antioxidant, anti-inflammatory, neuroprotective, anxiolytic and analgesic activities in experimental studies, supporting their therapeutic potential. [8] *Vetiveria zizanioides*, is a perennial aromatic grass widely used in traditional Ayurvedic medicine. The roots contain biologically active constituents such as sesquiterpenes, vetiverol, vetivone, khusimol, and essential oils, which possess antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties. Traditionally, the plant has been employed in the management of nervous disorders, fever, gastrointestinal disorders and inflammatory conditions. Despite these medicinal claims, we have limited scientific evidence supporting its anxiolytic and analgesic effects.[9-11] Therefore, the present study was undertaken to evaluate the anxiolytic and analgesic activities of the alcoholic root extract of *Vetiveria zizanioides* in Wistar albino rats using standard experimental models and to compare its efficacy with diazepam and aspirin.

Materials and Methods

Study design: Preclinical experimental study
Study centre: The study was conducted in department of Pharmacology, Central Animal House, Dhanalakshmi Srinivasan Medical College and Hospital, Siruvachur, Tamil Nadu.
Study period: The study was conducted for six months

Animals

Adult Male Wistar rats weighing between 200-250 g were chosen for the study. They were housed in clean polypropylene cages and fed with rat chow and water ad libitum. The animals were maintained under standard laboratory conditions; temperature (22±2ºC), relative humidity (55±5%). The study was approved by the Institutional Animal Ethics Committee and the study was conducted in accordance with the regulations of CCSEA.

Collection of plant material

Vetiveria zizanioides roots were collected from local area of Perambalur, Tamil Nadu. The roots were authenticated by the botanist Dr V. Nandagopalan, Associate Professor, National College, Trichy, Tamil Nadu.

Preparation of alcoholic extract of *vetiveria zizanioides* root

The roots were dried and powdered and loaded into the Soxhlet apparatus in 10 batches of 200gm each and subjected to extraction for 4 days with ethanol. After that solvent was distilled and the extract was concentrated under a reduced pressure on a water bath at a temperature to a semi solid consistency then the extract was dried in a desiccator.
Procedure for anxiolytic activity (Elevated plus Maze)

Groups
Group-I: Control (Normal saline)
Group-II: Diazepam (3 mg/kg/PO)
Group-III: Alcoholic extract of *Vetiveria zizanioides* root (200 mg/kg/PO)
Group-IV: Alcoholic extract of *Vetiveria zizanioides* root (400 mg/kg/PO)

Procedure
The elevated plus maze comprised of two open arms measuring (50 × 10 × 40 cm) and two enclosed arms measuring (50 × 10 × 40 cm), with an open roof, which are arranged so that the arms of same type are opposite to each other. The height of maze is 50 cm. Thirty minutes after administration of the test drug or the standard drug to their respective groups, each rat was placed individually at the centre of the Elevated Plus Maze, facing one of the enclosed arms. The animal was allowed to explore the maze freely for 5 minutes. During the test period, the time spent in the open arms and the total number of arm entries were recorded for each animal. Behavioural assessments were performed on Days 1, 7 and 15.

Procedure for analgesic activity (Tail flick method)

Groups
Group-I: Control (Normal saline)
Group-II: Aspirin (100 mg/kg/PO)
Group-III: Alcoholic extract of *Vetiveria zizanioides* root (200 mg/kg/PO)
Group-IV: Alcoholic extract of *Vetiveria zizanioides* root (400 mg/kg/PO)

Procedure

The analgesic activity was evaluated using a tail-flick analgesia meter, which applies radiant heat as a nociceptive stimulus to determine the latency between stimulus application and the tail-flick response. A constant current of 4 A was passed through the exposed nichrome wire to generate the required radiant heat. Thirty minutes after administration of the respective test drug or standard drug, each rat was gently restrained, and the middle portion of its tail was exposed to the radiant heat source. The time taken for the animal to withdraw or flick its tail in response to the thermal stimulus, was recorded for all groups.

Results

Anxiolytic activity of Alcoholic extract of *Vetiveria zizanioides* root

On Day 1, 7 and 15 the mean number of entries into the open arm was evaluated . On Day 1, Group II (diazepam) exhibited a significantly higher mean number of open arm entries than Groups I (control), III (200mg/kg), and IV (400mg/kg) ($p<0.05$). Overall comparison showed a statistically significant difference on Day 1. However, no statistically significant difference was observed between Group II and Group IV, indicating that the anxiolytic effect of the high dose extract was comparable to diazepam. In contrast, comparison between group III and group IV revealed a statistically significant difference ($p<0.05$), suggesting superior anxiolytic activity of the high dose extract. A similar pattern was noted on the 7th and 15th day. The mean number of open arm entries was significantly higher in Groups II, III, and IV than in the control group ($p<0.05$). No significant difference was observed between group II and group IV at any of these three observation periods, whereas group III and group IV differed significantly throughout the study, showing dose dependent anxiolytic effect of the alcoholic root extract of *Vetiveria zizanioides*. Overall, the high dose (400 mg/kg) extract demonstrated greater anxiolytic activity than the low dose (200 mg/kg) extract.(Table-1). Similarly, the mean time spent in the open arm differed significantly among the study groups. Compared with the control group, all treatment groups exhibited a significant increase in the meantime spent in the open arm ($p<0.05$). The group IV (400mg/kg) produced a significantly longer duration in the open arm than the group III (200mg/kg) on day 1, 7 and 15 ($p<0.05$). No statistically significant difference was observed between Group II (diazepam) and Group IV(400mg/kg), indicating that the high dose extract produced an anxiolytic effect comparable to the standard drug.(Table-2).

Table-1: Comparison of mean number of entries in open arm between the groups at different time periods

Groups	Drug/dose/route of administration	Number of entries in open arm (MEAN±SD)		
		1st day	7th day	15th day
Group-I	Normal saline/10ml/kg/PO	1.67±0.89	2.90±1.02	3.12±0.95
Group-II	Diazepam (3 mg/kg/PD)	4.98±1.43*	5.67±1.34*	6.87±2.19*
Group-III	Alcoholic extract of *Vetiveria zizanioides* root (200 mg/kg/PO)	2.89±0.75#	3.85±1.65*,#	4.93±1.05*,#
Group-IV	Alcoholic extract of *Vetiveria zizanioides* root (400 mg/kg/PO)	3.98±1.67*,$	4.18±2.05*,$	5.29±1.85*,$

(*p<0.05 significant compared with group-I, #p<0.05 significant compared group-II with other groups, $p<0.05 significant compared group-III with other groups)

Table-2: Comparison of mean number of mean time spent in open arm between the groups at different time periods

Groups	Drug/dose/route of administration	Time spent in open arm (Seconds) (MEAN±SD)		
		1st day	7th day	15th day

Group-I	Normal saline/10ml/kg/PO	40.34±1.89	41.96±2.87	41.34±1.78
Group-II	Diazepam (3 mg/kg/PD)	77.34±1.45*	76.90±2.95*	79.98±1.86*
Group-III	Alcoholic extract of *Vetiveria zizanioides* root (200 mg/kg/PO)	53.87±1.78*,#	56.45±2.95*,#	58.67±2.96*,$
Group-IV	Alcoholic extract of *Vetiveria zizanioides* root (400 mg/kg/PO)	71.56±1.54*,$	74.56±2.45*,$	76.87±1.45*,$

(*p<0.05 significant compared with group-I, #p<0.05 significant compared group-II with other groups, $p<0.05 significant compared group-III with other groups)

Anxiolytic activity of Alcoholic extract of Vetiveria zizanioides root

Analgesic activity was evaluated using the Tail Flick method by comparing the mean reaction time among the study groups. Group II (Aspirin) and group IV (400mg/kg) demonstrated a significant increase in reaction time compared with the group I (control)(p<0.05), whereas Group III (200 mg/kg) shows no significant difference from the control. Group II (Aspirin) showed the highest mean reaction time among all groups. A significant difference was observed between the low dose and high dose extract groups, indicating dose dependent analgesic activity. The absence of a significant difference between Group II and Group IV suggests that the analgesic effect of the high-dose alcoholic root extract of Vetiveria zizanioides was comparable to that of aspirin (Table-3).

Table-3: Comparison of mean time in tail flick test method between the groups

Groups	Drug/dose/route of administration	Reaction time in seconds (MEAN±SD)
Group-I	Normal saline/10ml/kg/PO	3.56±0.27
Group-II	Aspirin (100 mg/kg/PD)	5.34±0.98*
Group-III	Alcoholic extract of *Vetiveria zizanioides* root (200 mg/kg/PO)	3.93±0.64#
Group-IV	Alcoholic extract of *Vetiveria zizanioides* root (400 mg/kg/PO)	4.95±0.56*,$

(*p<0.05 significant compared with group-I, #p<0.05 significant compared group-II with other groups, $p<0.05 significant compared group-III with other groups)

Discussion

The present study was undertaken to evaluate the anxiolytic and analgesic activities of the alcoholic root extract of *Vetiveria zizanioides* in wistar albino male rats using the elevated plus maze and tail flick method, respectively. The findings demonstrated that the extract produced significant anxiolytic and analgesic effects in a dose-dependent manner, with the 400 mg/kg dose exhibiting greater efficacy than the 200 mg/kg dose. The effects observed with the high dose extract were comparable to those produced by the standard drugs, diazepam and aspirin.

Anxiolytic activity

The elevated plus maze is a well established experimental model for assessing anxiolytic activity based on the innate aversion of rodent models to open and elevated spaces. An increase in the number of entries in the open arm and the time spent in the open arms is considered indicative of reduced anxiety. In the present study, rats treated with the alcoholic root extract of *Vetiveria zizanioides* showed a significant increase in both parameters compared with the control group, indicating a pronounced anxiolytic effect. The 400 mg/kg dose produced a significantly greater response than the 200 mg/kg dose and demonstrated anxiolytic activity comparable to that of diazepam. In the present study Diazepam, a benzodiazepine, was used as the standard anxiolytic drug because of its established efficacy in experimental anxiety models. It exerts its anxiolytic effect by acting as a positive allosteric modulator of the GABAA receptor, enhancing influx of chloride ions and

reducing nerve excitability. The comparable efficacy of the high dose extract suggests that the anxiolytic activity of *Vetiveria zizanioides* may involve modulation of central neurotransmitter systems, although the precise mechanism remains to be established. The findings of the present study are consistent with those reported by Nirwane et al., who demonstrated significant anxiolytic activity of the ethanolic root extract of *Vetiveria zizanioides* in mice using the Elevated Plus Maze and other behavioral models. Their study also showed a dose dependent increase in anxiolytic activity, supporting the observations of the present investigation.[12]

Analgesic activity

The analgesic activity of the alcoholic root extract was evaluated using the tail flick method, a validated model for assessing nociceptive responses to thermal stimuli. In the present study, rats treated with the 400 mg/kg (High dose) exhibited a significant increase in reaction time compared with the control and 200 mg/kg (low dose) groups, indicating significant analgesic activity. Furthermore, the analgesic effect of the high dose extract was comparable to that produced by aspirin, whereas the low dose extract demonstrated only moderate activity. These findings suggest a dose dependent analgesic effect of the extract. Aspirin was selected as the standard analgesic because of its well-established peripheral analgesic property. It produces analgesia by irreversibly inhibiting cyclooxygenase (COX)-1 and COX-2 enzymes, thereby reducing prostaglandin synthesis and peripheral nociceptor sensitization. The comparable analgesic effect observed with the high dose extract shows that *Vetiveria zizanioides* may possess bioactive phytochemical constituents that are capable of modulating inflammatory mediators and nociceptive pathway. The analgesic findings of the present study are in agreement with those reported by Bharti et al., who demonstrated significant analgesic activity of *Vetiveria zizanioides* root extract in experimental animal models, with higher doses producing greater analgesic responses than lower doses.[13] Medicinal plants have been used for centuries as a valuable source of therapeutic agents because of their wide range of pharmacological activities, relatively low toxicity, and better patient acceptability. *Vetiveria zizanioides* has long been used in traditional medicine for the management of nervous disorders, pain, fever, and inflammatory conditions. The present findings provide experimental evidence supporting these traditional claims by demonstrating significant anxiolytic and analgesic activities of the alcoholic root extract in Wistar albino rats. Nevertheless, these findings are limited to preclinical animal models, and further studies are required to isolate the bioactive phytoconstituents, elucidate the underlying molecular mechanisms, evaluate chronic toxicity, and establish clinical efficacy through well-designed human studies.

Conclusion

The alcoholic root extract of *Vetiveria zizanioides* demonstrated significant dose dependent anxiolytic and analgesic activities. Further studies are needed to identify the active phytoconstituents, mechanisms of action and assess the long term safety profile of the extract.

Conflict of interest: Nil
Funding: Self

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