

POTENTIATION EFFECT OF *VETIVERIA ZIZANIOIDES* ROOT EXTRACT AND ESSENTIAL OIL ON PHENOBARBITAL INDUCED SEDATION-HYPNOSIS IN SWISS ALBINO MICE

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ABSTRACT

Vetiveria zizanioides (poaceae) is commonly known as Khas-Khas grass. The root of *vetiveria zizanioides* is used as diaphoretic, refrigerant, febrifuge etc., They are useful in vitiated conditions of *pitta* and *vata*. In the present work, the sedative-hypnotic effects of two dose levels of ethanolic extract and one dose of essential oil of *Vetiveria zizanioides* root were evaluated at 150mg/kg, 250mg/kg and 2ml/kg respectively by monitoring potentiation of phenobarbital(50mg/kg i.p) induced onset of sleep and duration methods. Oral administration of the ethanolic extract (150mg, 250mg/kg, p.o.) showed significant sedation and hypnosis by decreasing time of onset of sleep and increase in total duration of sleep in swiss albino mice($P<0.05$) as compared to control whereas essential oil(2ml/kg) showed significance as compared to control and Diazepam(3mg/kg) treated groups. Altogether, the results of this study suggest the synergistic sedative-hypnotic effect of essential oil & extract of *V.zizanioides* that could be comparable with Diazepam at sedative-hypnotic doses.

Keywords: Sedative- Hypnotic, *Vetiveria zizanioides*, Phenobarbital, Diazepam

INTRODUCTION

Disturbed sleep is amongst the most frequent health complaints physicians' encounter. Insomnia, defined as persistent difficulty in falling or staying asleep that affects daytime function, can induce significant psychological and physical disorders. Thirty years ago, many such complaints were treated with hypnotic medications without further diagnostic evaluation. Since then, a distinct class of sleep and arousal disorders has been identified. Most patients engage in long term use of benzodiazepine analogues to treat

insomnia. But these drugs have limited benefits with obvious side effects, such as impaired cognitive function, memory and affects general daytime performance. In addition, long-term administration results in tolerance and dependence.

Thus, there is a need of robust sedative-hypnotic compounds that have lesser side effects than benzodiazepines and a more immediate onset of action than currently available drugs acting on 5-HT_{1A} receptors. *Vetiveria zizanioides* has antibacterial¹, antidepressant, anti-inflammatory, antioxidant²,

anti-parasitic and antiseptic property and is regarded as the tonic for nervous system. On the basis of these considerations, this study was done to characterize sedative-hypnotic activity of ethanolic extract prepared from the roots of *Vetiveria zizanioides* L.(Gramineae,Poaceae.).

MATERIAL AND METHODS

Plant material and extraction

The plant *V. zizanioides* was purchased from a shop at Udupi, Karnataka, India in the month of January 2007. The plant was identified and authenticated by a botanist from Nehru Memorial college, Sullia, Dakshina Kannada, India and voucher specimen (No. DG-11: 28/7/2013) was kept for future reference. The underground root was separated, dried under the shade and mechanically powdered, which was then subjected to successive extraction in a Soxhlet apparatus using ethanol 70% at 80°C temperature. The yield of ethanolic extract was found and evaporated on steam boiler and it was selected for the study. The dry ethanolic extract was stored in cool and dry place, which further was used for the evaluation of hypnotic activity.

Preparation of essential oil

The essential oil was obtained from 500 g of powdered *Vetiveria* roots by subjecting to water-distillation for four hours using and solvent extraction (SDE) apparatus³.

Animals

Thirty male swiss albino mice weighing around 25-33 g were procured from authorized animal breeders and suppliers in Mangalore. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than six animals per cage

and maintained under standard laboratory conditions; temperature (22 ± 2°C), relative humidity (55 ± 5 %) with dark and light cycle (12/12 h), standard pellet diet and water *ad libitum*. The rats were allowed to acclimatize in laboratory conditions for 10 days before commencement of experiment. Current study was approved by the Institutional Animal Ethics Committee (Protocol No. 8 dated 08/07/13) and was conducted according to the regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

Drugs and treatments

Drug solutions: *Vetiveria zizanioides* extract at a concentration of 5mg/ml suspended in 2% gum acacia solution and administered as 150mg, 250mg/kg body weight, Diazepam (3mg/kg; suspended in 2% gum acacia solution) or the vehicle (2% gum acacia solution) were administered 1 hour prior to the test by oral gavage to the respective groups.

Phenobarbital-induced hypnotic test

Potentiation of Phenobarbital induced sleeping time method was implied to study the sedative-hypnotic activity of *V.zizanioides* extract & essential oil. After 1 hour of administration of extracts, essential oil and vehicle to the respective groups, Phenobarbital (50 mg/kg, i.p) was administered to induce sleep. The interval between loss and recovery of righting reflex (return to upright position) was used as index of sedative-hypnotic effect. Once a mouse righted itself, it was placed on its back once more and allowed to right a second time for confirmation. The time interval between injection of Phenobarbital

and start of sleep was recorded as latency time.^{3,4}

Rota rod assembly apparatus

Rota-rod apparatus was used to determine motor coordination in ethanolic extract & essential oil groups. It is a rotating rod; grip of mice on rotating rod is due to the muscle grip strength. A sedative-hypnotic drug decreases the grip strength & the mice may fall from the rotating rod due to the effect of drug. Loss of grip strength is measured as motor in coordination or muscle relaxant effect of drug. After 1 hour of drug dosing, animals were placed on rotating rod (13-15 rpm) for 1 min. Number of animals that fall from rod in this period were counted as the loss of grip strength. To measure motor in-coordination (muscle relaxant activity) of the test drug, number of animals that fall from rod in 1 min in 3 repeated cycles was compared between groups.^{4,5}

STATISTICAL ANALYSIS

The sleep duration and onset data was analyzed by one-way analysis of variance (ANOVA) for independent samples followed by the post-hoc Duncan Test; spontaneous loco motor activity data was analyzed by two-way ANOVA followed by the post-hoc Duncan Test. Statistical significance was set at 5% level and all the values are expressed as mean±SD.

RESULTS

Phenobarbital-induced hypnotic test

The effects of the different doses of *V.zizanioides* on sleep duration and latency

time induced by Phenobarbital are shown in **figure 2&3**. *V.zizanioides* at the doses of 150 and 250 mg/kg significantly increased sleeping time compared to the control ($p < 0.05$) and this effect is less than standard drug diazepam (3mg/kg), while essential oil (2ml/kg) significantly increased sleep duration compared to all groups and it is non-significant compared to diazepam group ($P>0.05$) (Table 2).

The latency time to induce sleep in *V.zizanioides* (150mg,250mg/kg) is significant compared to control whereas, it is non-significant compared to diazepam and *V.zizanioides* essential oil ($P>0.05$) (Table2), and in terms of latency time and total sleep duration, *V.zizanioides* essential oil has shown comparable efficacy with standard sedative-hypnotic drug diazepam.

Motor coordination test by rota-rod assembly:

The mean of number of times the animals fall from rota-rod for group those received *v.zizanioides* extract (150mg, 250mg/kg) was 3.2, 4.0 (figure 1) which was significant compared to control($p<0.05$) whereas, standard drug diazepam(5mg/kg) and essential oil have been shown maximum number of falls which is statistically significant compared to *v.zizanioides* extract treated and control groups, While essential oil (2ml/kg) significantly increased motor in-coordination effect compared to all groups and it is non-significant compared to diazepam ($P>0.05$) (Table 1)

Table-1: Motor in-coordination effect of ethanolic extract & essential oil of *vetiveria zizanioides*

Group	Dose/kg body wt	No. of times animals failed Rota rod test in 1min/3 cycles. (Mean±SD)
Control	Vehicle	2.4±0.2 times
Diazepam	3mg	5.5±0.7times
Ethanolic extract	150mg	3.2±0.6 times ^{*abe}
Ethanolic extract	250mg	4.0±0.2 times ^{*ade}
Essential oil	2 ml	5.7±0.5 times ^{*acd}

*Significant at 5% level, P<0.05, a=Control, b=Diazepam group, c=Ethanolic extract 150mg/kg, d=Ethanolic extract 250mg/kg, e=Essential oil 2ml/kg

Figure.1

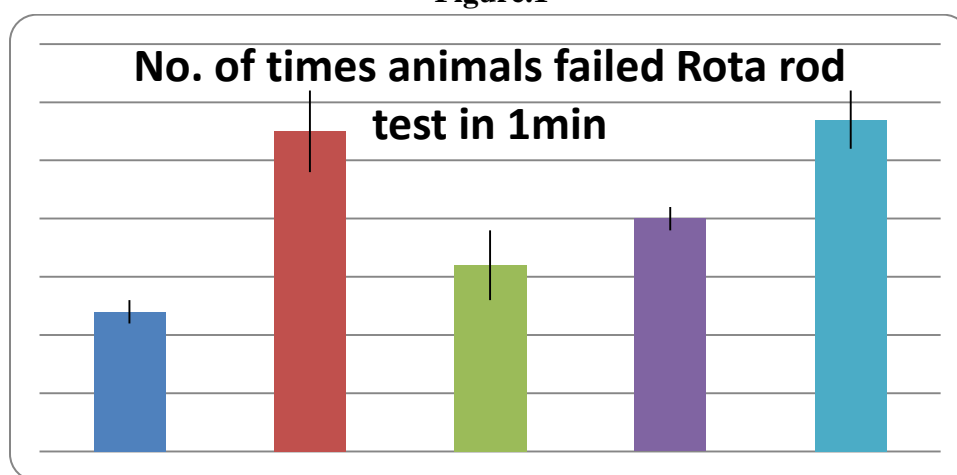


Table-2: Sedative-hypnotic effect of ethanolic extract & essential oil of *vetiveria zizanioides*

Group	Dose/kg body wt	Onset of sleep Mean±SD (min)	Sleep duration Mean±SD (min)
Control	Vehicle	13.3±1.2	21.4±3.6
Diazepam	3mg	7.7±0.8	43.8±4.1
Ethanolic extract	150mg	9.4±1.0 ^{*abde}	27.2 ±2.0 ^{*bd}
Ethanolic extract	250mg	8.2±0.4 ^{*abce}	31.3±3.3 ^{*abe}
Essential oil	2 ml	7.9±0.5 ^{*acd}	39.5±4.5 ^{*acd}

*Significant at 5% level, P<0.05, a=Control, b=Diazepam group, c=Ethanolic extract 150mg/kg, d=Ethanolic extract 250mg/kg, e=Essential oil 2ml/kg

Figure.2

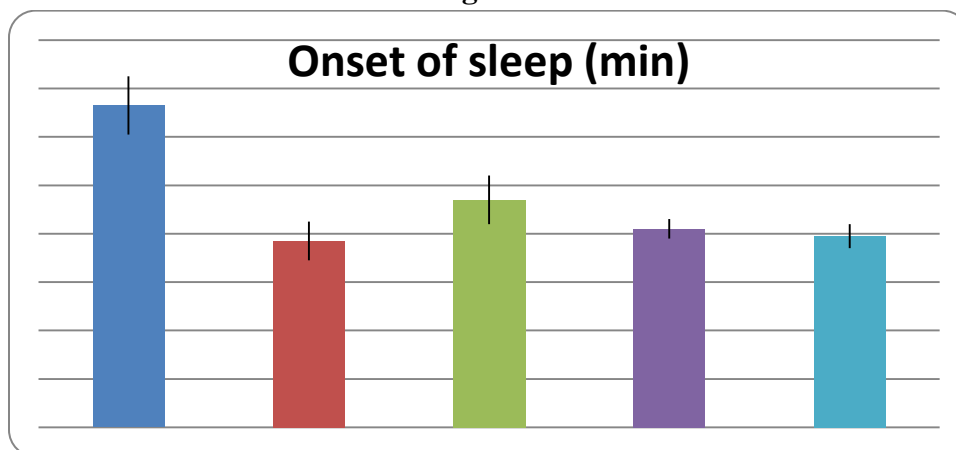
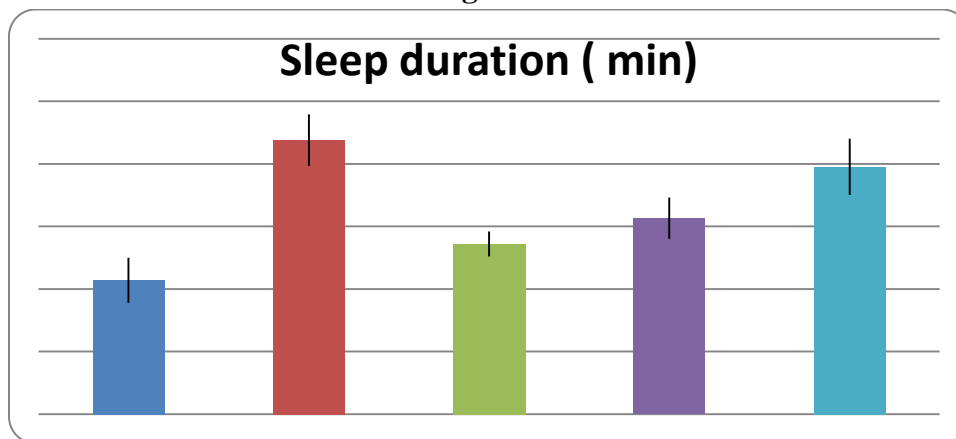


Figure.3



DISCUSSION

As per ancient ayurvedic literature, *vetiveria zizanioides* was believed to possess anticonvulsive, sedative, hypnotic and anxiolytic effects and to be useful for nervousness and insomnia.^{6,7,8} In the present study, we observed the sedative and hypnotic properties of ethanolic extract of *vetiveria zizanioides* in mice. In order to study the comprehensive effect of *V.zizanioides*, the following parameters were observed: sleep latency, total duration of sleep and loss of motor coordination in mice.

Diazepam is a central nervous system depressant used in the management of sleep disorders such as insomnia; these compounds have a binding site on GABA receptor type-A ionophore complex.^{9,10} It decreases activity, moderates excitement, and calms the recipient. Substances like diazepam reduce the onset and increase duration of barbiturate-induced sleep and reduce exploratory activity possessing potentials as sedative.^{11,12}

V.zizanioides essential oil increased the time of total sleep duration in mice (Table 2), after oral administration of 2ml/kg dosages, producing sedative-hypnotic effect similar to that observed with 5 mg/kg

diazepam. Diazepam is a very well-known anxiolytic benzodiazepine (BZD) which produces not only anxiolytic-like effect but also sedative-hypnotic action. In this respect, *V.zizanioides* essential oil produced reduction in latency of sleep onset. It is generally believed that loco motor activity results from brain activation, which is manifested as an excitation of central neurons involving different neuro-chemical mechanism and plays a important role in motor coordination. As per above mechanism, It is possible that a sedative-hypnotic agent also induces the loss of motor coordination which was well observed with *V.zizanioides* essential oil and alcoholic extract of *V.zizanioides* and it is believed that GABAergic pathway and GABAergic transmission can produce such type of profound sedation in mice.¹³ The inhibitory action of GABA consists in the opening of chloride channels. This finding suggests that some constituents in *V.zizanioides* extract produce facilitation of this inhibitory system.

Glutamate and GABA are quantitatively the most important excitatory and inhibitory neurotransmitters respectively in the mammalian brain.¹⁴ Thus, receptors for these two neurotransmitters are regarded as important targets for psychotropic drugs. In the test of Phenobarbital induced sleep in mice, the potentiated effect of *V.zizanioides* extract and oil in mice were represented. It not only prolonged the sleeping time but also decreased the latency of falling asleep and increases the sleep duration. The *V.zizanioides* extract has produced hypnosis at doses of 150mg, 250mg/kg and essential oil 2ml/kg. Since the effect of Phenobarbital

on the CNS involves the activation of the inhibitory GABAergic system.^{15,16} This finding suggests that some constituents in *V.zizanioides* might produce facilitation of this inhibitory system. Phytochemical studies have identified active components in this plant such as Vetiverol, Vetivone, Khusimone, Khusimol, Vetivene, Khositone, Terpenes, Benzoic acid, Tripene-4-ol, β -Humulene, Epizizianal, vetivenyl vetivenate, iso khusimol, β -vetivone, vetivazulene are mainly responsible for sedative and hypnotic activities.¹⁷ Further chemical and pharmacological analysis of the extract need to be conducted to isolate and characterize the active principles responsible for the sedative and hypnotic effect.

CONCLUSION

In conclusion, oral administration of essential oil of *V.zizanioides* induces similar sedative effects, supporting its use in ayurvedic medicine. Given that the LD₅₀ value for these extracts and oil was around 3000 mg/kg for oral administration, as determined by Kaushik D and Thripati R¹⁸ the extract and oil of *V.zizanioides* have good tolerance at sedative hypnotic doses. To sum up, this work represents that the ethanolic extract of *Vetiveria zizanioides* have obvious sedative and hypnotic activity; these findings provide may provide pharmacological basis and comparable therapeutic efficacy with Diazepam in insomnia.

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