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Anxiolytic and nootropic activity of *Vetiveria zizanioides* roots in mice

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ABSTRACT

Background: *Vetiveria zizanioides* (VZ) (family: Poaceae), an aromatic plant commonly known as "*Vetiver*" has been used for various ailments. Concerning the various ailments being listed as the traditional uses of VZ, no mention about anxiety and memory was found. **Objective:** The present study examined the anxiolytic and memory enhancing activity of ethanolic extract of *V. zizanioides* (EEVZ) dried roots in mice. **Materials and Methods:** Activity of EEVZ was assessed using models of anxiety (elevated plus-maze [EPM], light/dark test, hole board test, marble-burying test) and learning and memory (EPM, passive shock avoidance paradigm). **Results:** EEVZ at doses of 100, 200, and 300 mg/kg b.w. illustrated significant anxiolytic activity indicated by increase in time spent and number of entries in open arm, time spent in lightened area, number of head poking and number marble buried when compared to that of diazepam (1 mg/kg b.w.), a reference standard. The same treatment showed a significant decrease in transfer latency to reach open arm, shock-free zone, and number of mistakes when compared to that of scopolamine (0.3 mg/kg b.w.). EEVZ in all the doses (100, 200, and 300 mg/kg b.w.) significantly decreased mortality in sodium nitrite (250 mg/kg b.w.) induced hypoxia and also significantly increases contraction induced by acetylcholine on rat ileum preparation. **Conclusion:** The result emanated in the present investigation revealed EEVZ possesses significant anxiolytic and nootropic activity by possibly interplaying with neurotransmitters implicated in anxiety and learning and memory.

Key words: Acetylcholine, diazepam, dopamine, gamma-aminobutyric acid, *Vetiver*

INTRODUCTION

Anxiety is the most frequent psychiatric conditions commonly found in humans.[1] Anxiety disorders have a high impact on daily life (illness intrusiveness) and cause a great deal of suffering for the individual patient.[2] Anxiety is a condition of persistent and uncontrollable nervousness, stress, and worry that is triggered by anticipation of future events, memories of past events, or ruminations over day-to-day events, both trivial and major, with disproportionate fears of catastrophic consequences.[3] Anxiety is characterized by a diffuse, unpleasant, vague sense of apprehension, often accompanied by autonomic symptoms, such as headache, perspiration, palpitations, tightness in the chest, and mild stomach discomfort.[4] Human anxiety involves an ability, to use memory and imagination to move backward and forward in time, that animals do not appear to have. Moreover, a large portion of human anxiety is produced by anticipation of future events.[1]

Learning is the process by which we acquire knowledge about the world.[5] Learning can also be defined as the lifelong process of transforming information and experience into knowledge, skills, behaviors, and attitudes.[6] Memory, defined in psychology as storing of learned information, and the ability to recall that which has been stored.[7] Anxiolytic agents impair learning and memory in both animals and humans. This prediction might be due to the role of amygdala in memory and learning.[8,9] The interaction between the cholinergic and GABAergic systems in learning and memory has been

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shown by several studies. The amygdala and hippocampus are some of the neuronal systems taking part in memory formation and are rich in cholinergic synapses that are under the inhibitory control of the GABAergic system. Many previous studies suggest that GABAergic drugs might impair memory formation through effects on cholinergic systems. However, other investigators have shown that the GABA receptor agonist muscimol and baclofen enhance memory.[10]

Vetiveria zizanioides (VZ) (Khus or Ushira) a plant in family - Poaceae possesses antispasmodic, antihypertensive,[11] antiinflammatory,[12] antioxidant,[13] and antibacterial activities.[14] The chemical constituents present in the plant are vetiverol, vetivone, khusimone, khusimol, vetivene, khositone, terpenes, benzoic acid, tripene-4-ol, β-humulene, epizizianal, vetivenyl vetivenate, iso-khusimol, β-vetivone, and vetivazulene.[14] Ethanolic extract of VZ (EEVZ) roots contains saponins, flavonoids, tannins, and glycosides.[15] Most of the plants such as brahmhi (*Bacopa monnieri*), shankhpushpi (*Convolvulus pluricaulis*), and siris (*Albizia lebbeck*) with nootropic activity contain a high concentration of saponins. The recent research evidences suggest that flavonoids play the protective role in various neurodegenerative diseases and disorders like anxiety disorders and cognitive impairment.[16-18] Various tribes use the different parts of VZ for many of their ailments such as mouth ulcer, fever, boil, epilepsy, burn, snakebite, scorpion sting, rheumatism, fever, and headache.[19,20] Although various activities of VZ plant have been reported, there are no reports on anxiolytic, nootropic activity of VZ root in mice.[21]

Therefore, it was considered worthwhile to explore the possible anxiolytic and nootropic potential of EEVZ root using various behavioral paradigms in mice.

MATERIALS AND METHODS

Animals

Albino male Swiss mice (18–22 g) were used for the study. The animals were housed in colony cages and maintained under standard environmental conditions: 25 ± 2°C temperature, 12:12 h light: dark cycle, and 45–55% relative humidity, with free access to food and water *ad libitum*. All experiments were carried out during the light period (08.00–16.00 h). The Institutional Animal Ethics Committee approved the protocol of the study (MGV/PC/XXV/02/2010-11).

Plant material and extraction

Dried roots of VZ (Poaceae) were purchased from the local market of Nasik, India, and authenticated at Botanical Survey of India, Pune, India. Herbarium specimen has been retained there (ZISHJI3). Dry roots were collected, and powdered mechanically and sieved through No. 22 mesh sieve. The finely powdered roots were kept separately in an airtight container until the time of use. About 400 g of powder was soaked with 2 L of ethanol for 12 h and macerated at room temperature using a mechanical shaker for 4 h. The extract was filtered, and the marc was again soaked with the same volume of ethanol for 12 h and further extracted for 4 h and filtered. The filtrates were combined and concentrated and then air dried to obtain the extract. The percentage yield of the EEVZ root was 7.8% w/w.

Phytochemical screening

Phytochemical investigation of the extract for the presence of phenolic compounds, flavonoids, tannins, triterpenes, and sterols was carried out.[22]

Acute toxicity test

The extract was administered orally in doses of 50, 100, 200, 300, 500, 1000, 1500, and 2000 mg/kg b.w. to different groups of mice. The mortality rate was observed and recorded for 24 h period.[23]

Chemicals and experimental design

Diazepam (Calmpose, Ranbaxy, India) and Piracetam (Nootropil, Uni-UCB, India) were used as a reference drug for anxiolytic and nootropic activity, respectively. Scopolamine (German Remedies, India) was used to induce amnesia in mice. All the chemicals were of analytical grade. On the day of the experiment, the standard group mice received diazepam (1 mg/kg b.w. i.p.) and piracetam (100 mg/kg b.w. i.p.) and control group received distilled water. Scopolamine (0.3 mg/kg b.w. i.p.) was used for producing amnesia in mice. The EEVZ at doses of 100, 200, and 300 mg/kg b.w. p.o. was administered 60 min before the animals were subjected to different behavioral tests. Sodium nitrite (250 mg/kg b.w. s.c.) was administered for respiratory arrest in mice. The rat ileum preparation was used for *in vitro* study. Based on the study by Boissier *et al*., the doses of drugs were selected.[24]

Anxiolytic study

Elevated plus-maze

This test has widely used to measure anxiety in rodents. Elevated plus-maze (EPM) consisted of two open arms (25 cm × 5 cm) crossed with two closed arms (25 cm × 5 cm × 20 cm). The arms were connected together with a central square of 5 cm × 5 cm. The maze was elevated to a height of 25 cm and placed inside a light and sound attenuated room. Groups of mice each containing five animals were treated with vehicle, diazepam (1 mg/kg, i.p.), and EEVZ (100, 200,

and 300 mg/kg, p.o) 1 h before placing individually in the EPM. Each animal was placed at the center of the maze, facing one of the enclosed arms. The time spent in open arms, entries in open, and closed arms was recorded for a period of 5 min.[25]

Light/dark apparatus test

Two equal-sized boxes (27 L cm × 27 W cm × 27 H cm), one-dark, and other illuminated with 100 W desk lamp light, were connected with a tunnel (5 L cm × 7 W cm × 10 H cm). Mice in groups of five each were treated with vehicle, diazepam (1 mg/kg, i.p.), and EEVZ (100, 200, and 300 mg/kg, p.o.) 1 h before placing individually in the light area. The time spent in the light and dark box, and number of transitions was recorded for 10 min.[26]

Hole board test

The apparatus consisted of a wooden box (40 L cm × 40 W cm × 25 H cm) with nine holes (diameter 3 cm) evenly distributed on the floor. The apparatus was elevated to a height of 15 cm. Groups of mice each containing five animals were treated with vehicle, diazepam (1 mg/kg, i.p.), and EEVZ (100, 200, and 300 mg/kg, p.o.) 1 h before placing mice individually in the apparatus and number of head poking were recorded for 5 min.[24]

Marble-burying test

The apparatus consisted of a Plexiglas cage (42 cm × 26 cm × 15 cm) with a glass lid. The floor was covered with a 2 cm layer of sawdust and 25 glass marbles are distributed throughout the cage. 1 h after p.o. or 30 min after i.p. treatment with EEVZ (100, 200, and 300, mg/kg) or diazepam (1 mg/kg), respectively, male mice were individually placed in the cage for 10 min, after which they were removed, and the burying response quantified by counting the number of marbles that were more than two thirds covered with sawdust. A diminution of the burying response reveals a positive anxiolytic-like effect.[27]

Assessment of nootropic activity

Elevated plus-maze

The apparatus consisted of two open arms (25 cm × 5 cm) and two enclosed arms (25 cm × 5 cm × 20 cm) connected together with a central square of 5 cm × 5 cm. The entire maze was elevated at a height of 25 cm. The maze was placed inside a light and sound attenuated room. Animals were placed individually at the end of either of the open arms of the EPM facing away from the central platform. The time taken by each animal to move from end of open arm to either of the closed arms was recorded. This duration of time was called transfer latency (TL). If the animal does not enter into any of the enclosed arms within 90 s, it was gently pushed into any of the enclosed arms and the TL was assigned as 90 s. Later, the animal was allowed to explore the plus maze for 5 min after the measurement of TL. TL was then noted on 2nd day and 9th day. TL measured on 1st day serves as a parameter for the acquisition (learning) while TL on 2nd and 9th day indicates retention (memory). Retention after 24 h or 1 week for each mouse was expressed in terms of "inflexion ratio" (IR). The IR was calculated by the formula, IR = $(L_1 - L_0)/L_0$, where L_0 = initial TL in second, L_1 = TL on the 2nd and 9th day in second (Jaiswal and Bhattacharya, 1992). Drug (s) were administered immediately before the 1st day trial. Mice in groups of five each received EEVZ (100, 200, and 300 mg/kg, p.o.), or piracetam (100 mg/kg, i.p.) either alone or 30 min before scopolamine (0.3 mg/kg, i.p.) administration.[28]

Passive shock avoidance paradigm

The apparatus consists of an electric grid (35 cm × 35 cm) with shock-free zone (SFZ) (5 cm × 5 cm × 0.5 cm) in the center of the grid and the entire grid having a perplex enclosure. Each mouse was placed individually on the electric grid and allowed to explore the maze for 1 min. The stimulus (20 V) with AC current of 5 mA was then applied. The latency to reach the SFZ was recorded three consecutive times as basal reading. Animals that reach the SFZ in 2 min in the first trial were selected for the study. After 1 h of the first trial, each animal was put on the grid again. Latency to reach SFZ and the number of mistakes (descents) the animal made in 15 min were recorded as parameters for acquisition and retention, respectively. Drug (s) was administered on the day of the test, 30 min (i.p.) or 60 min (p.o.) prior to the first training session. Mice in groups of five each received EEVZ (100, 200, and 300 mg/kg, p.o.), or piracetam (100 mg/kg, i.p.) either alone or 30 min before scopolamine (0.3 mg/kg, i.p.) administration.[29]

Sodium nitrite-induced respiratory arrest (acetylcholine mediated behavior)

Subcutaneous injection of sodium nitrite induces hypoxia in mice, followed by death due to respiratory arrest. Sodium nitrite reduces the oxygen-carrying capacity of the blood by converting hemoglobin to methemoglobin. This chemical-induced hypoxia is inhibited by pretreatment with drugs that increases cholinergic transmission. Mice were divided into four groups of five animals each. The first group received sodium nitrite (250 mg/kg, s.c.). The other group received EEVZ (100, 200, and 300 mg/kg, p.o.) 1 h before sodium nitrite (250 mg/kg, s.c.). The percentage mortality due to respiratory arrest was noted.[30]

In vitro activity on rat ileum

Adult rats (300–350 g) were killed by decapitation and the abdomen opened. Ileum was removed and portions 10 cm distal to the ileocecal junction discarded. A suitable

length (2–3 cm) of rat ileum was suspended in a 50 ml organ bath containing tyrode solution. The dose-response curve for acetylcholine (ACh) was taken invariant log dose concentrations. After obtaining a dose response curve of ACh on rat ileum the aqueous solution of EEVZ and same doses of ACh were repeated. Graph of the maximum percentage of the contractile response on ordinate and log dose of ACh on abscissa was plotted to record dose response curve of ACh, in absence and presence of the aqueous solution of EEVZ.[31]

Statistics

The mean ± standard error of the mean values were calculated for each group. One-way ANOVA followed by Dunnett's multiple comparison tests was used for statistical analysis. Values of $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically significant.

RESULTS

Phytochemical screening

Phytochemical screening of EEVZ revealed the presence of phenolic compounds, flavonoids, sterols, saponins, tannins, glycosides, and carbohydrates.

Acute toxicity test

Oral administration of even highest EEVZ dose, that is, 2000 mg/kg did not produce any toxic effects in mice. No mortality was observed, and EEVZ was found to be safe at the given doses (data not shown).

Anxiolytic activity

Elevated plus-maze

In EPM, EEVZ in all doses (100, 200, and 300 mg/kg, p.o.) significantly increased time spent and number of entries in open arm and decreased time spent and number of entries in a closed arm, in a dose-dependent manner. The effect is comparable to diazepam (1 mg/kg, i.p.) which also induced significant increase in the occupancy in the open arm [Table 1].

Light/dark apparatus test

In light/dark test (LDT), EEVZ in all doses (100, 200, and 300 mg/kg, p.o.) significantly increased time spent and number of transition in lightened box and decrease time spent and number of transition in the dark box in dose-dependent manner. The effect is comparable to diazepam (1 mg/kg, i.p.) which was used as a reference standard [Table 2].

Hole board test

In hole board test, EEVZ in all doses (100, 200, and 300 mg/kg, p.o.) significantly increased the number of head pokes when compared to vehicle control group. The effects are amenable to that of diazepam (1 mg/kg, i.p.), which is used as a reference standard [Table 3].

Table 1: Effect of *Vetiveria zizanioides* on exploratory behavior in elevated plus maze

Treatment (mg/kg)	Time spent (s)		Number of entries	
	Enclosed arm	Open arm	Enclosed arm	Open arm
Control	184.0±2.20	86.60±2.96	14.8±0.58	7.20±0.58
Diazepam (1)	121.0±3.31##	156.4±3.07##	9.80±0.58##	13.2±0.58##
EEVZ (100)	168.4±2.90*	99.00±2.53*	12.2±0.86*	9.80±0.58*
EEVZ (200)	159.8±3.28##	116.2±4.35##	11.4±0.51#	10.8±0.58#
EEVZ (300)	135.6±4.22##	138.2±2.56##	10.4±0.51##	11.8±0.80##

n=5, all values are shown as mean±SEM. Statistical analysis of data were carried out by one-way ANOVA followed by Dunnett's test. **P*<0.05, #*P*<0.01 and ##*P*<0.001 when compared with control. SEM=Standard error of the mean, EEVZ=Ethanolic extract of *Vetiveria zizanioides*

Table 2: Effect of *Vetiveria zizanioides* on exploratory behavior in light/dark box

Treatment (mg/kg)	Time spent (s)		Number of transition
	Lightbox	Dark box	
Control	199.0±3.31	326.0±5.10	10.0±1.58
Diazepam (1)	337.0±5.39##	180.6±5.50##	17.6±2.30*
EEVZ (100)	247.4±2.66*	287.4±3.84*	14.0±1.58*
EEVZ (200)	289.6±3.67##	232.6±3.84##	14.2±1.92##
EEVZ (300)	314.4±2.98##	195.4±6.99##	15.8±3.11##

n=5, all values are shown as mean±SEM. Statistical analysis of data were carried out by one-way ANOVA followed by Dunnett's test. **P*<0.05 and ##*P*<0.001 when compared with control. SEM=Standard error of the mean, EEVZ=Ethanolic extract of *Vetiveria zizanioides*

Marble-burying test

Analysis of behavior of mice in the marble-burying test revealed that the treatment of EEVZ (100, 200, and 300 mg/kg), and diazepam (1 mg/kg) produced a significant decrease in a number of marble-burying response as compared to vehicle control group [Table 3].

Nootropic activity

Elevated plus-maze

Piracetam (100 mg/kg), EEVZ (100, 200, and 300 mg/kg, p.o) significantly shortened the TL on day 1, 2, and 9 when compared to vehicle. Scopolamine (0.3 mg/kg, i.p.) significantly increased the TL on the 1, 2, and 9 day. EEVZ (300 mg/kg, p.o.) significantly antagonized the effects of scopolamine and reduced TL on the day 1, 2, and 9 [Table 4].

Passive shock avoidance paradigm

Piracetam (100 mg/kg, i.p.) significantly reduced the latency to reach SFZ and the number of mistakes when compared to vehicle treated group. EEVV (200 and 300 mg/kg, p.o.) significantly reduced the latency to reach SFZ and the number of mistakes as compared to vehicle treated group. Scopolamine (0.3 mg/kg, i.p.) significantly increased the latency to reach SFZ and the number of mistakes. The EEVZ significantly antagonized the effects of scopolamine

and significantly reduced the latency to reach SFZ and number of mistakes [Table 5].

Sodium nitrite-induced hypoxia (acetylcholine mediated behavior)

The animals receiving the vehicle showed 100% mortality after sodium nitrite (250 mg/kg, s.c.) injection while animals treated with doses that exhibited nootropic activity, that is, EEVZ (100 mg/kg, p.o.), EEVZ (200 mg/kg, p.o.), and EEVZ (300 mg/kg, p.o.) showed only 40%, 20%, and 0% mortality, respectively (data not shown).

Table 3: Effect of *Vetiveria zizanioides* on number of head poking and marble burying

Treatment (mg/kg)	Number of head poking	Number of marbles buried
Control	30.0±0.70	12.2±0.84
Diazepam (1)	62.8±0.73$^{\#\#}$	4.80±0.84$^{\#\#}$
EEVZ (100)	39.6±2.34*	10.8±0.84$^{\#}$
EEVZ (200)	42.4±2.44$^{\#\#}$	9.20±0.84$^{\#}$
EEVZ (300)	50.8±1.16$^{\#\#}$	7.00±0.70$^{\#\#}$

n=5, all values are shown as mean±SEM. Statistical analysis of data were carried out by one-way ANOVA followed by Dunnett's test. *P<0.05, $^{\#}P$<0.01 and $^{\#\#}P$<0.001 when compared with control. SEM=Standard error of the mean, EEVZ=Ethanolic extract of *Vetiveria zizanioides*

Table 4: Effect of *Vetiveria zizanioides* on transfer latency in elevated plus maze test

Treatment (mg/kg)	Transfer latency (s)		
	Day 1	Day 2	Day 9
Control	43.8±1.43	38.2±1.72	29.6±1.17
Piracetam (100)	17.8±1.43$^{\#\#}$	11.6±1.21$^{\#\#}$	6.4±0.51$^{\#\#}$
EEVZ (100)	39.2±1.16	29.2±1.07$^{\#\#}$	20.2±0.87$^{\#\#}$
EEVZ (200)	29.6±1.44$^{\#\#}$	20.8±1.16$^{\#\#}$	15.6±0.87$^{\#\#}$
EEVZ (300)	24.0±1.14$^{\#\#}$	16.6±0.51$^{\#\#}$	10.8±0.37$^{\#\#}$
Scopolamine (0.3)	50.2±0.86$^{\#}$	42.4±0.87*	32.8±1.16$^{\#\#}$
Scopolamine (0.3)+ EEVZ (300)	39.2±1.07$^{@}$	30.4±1.03$^{@\#\#}$	22.2±0.86$^{@\#\#}$

n=5, all values are shown as mean±SEM. Statistical analysis of data were carried out by one-way ANOVA followed by Dunnett's test. *P<0.05, $^{\#}P$<0.01 and $^{\#\#}P$<0.001 when compared with control and $^{@}P$<0.001 when compared with scopolamine. SEM=Standard error of the mean, EEVZ=Ethanolic extract of *Vetiveria zizanioides*

Table 5: Effect of *Vetiveria zizanioides* on passive shock avoidance test

Treatment (mg/kg)	Latency to reach SFZ	Number of mistakes
Control	23.4±1.07	17.2±1.07
Piracetam (100)	9.00±0.70$^{\#\#}$	8.80±0.86$^{\#\#}$
EEVZ (100)	19.0±0.86	14.6±0.51
EEVZ (200)	15.4±1.21$^{\#\#}$	12.0±0.71$^{\#\#}$
EEVZ (300)	11.0±0.70$^{\#\#}$	10.0±0.71$^{\#\#}$
Scopolamine (0.3)	35.0±1.52$^{\#\#}$	25.4±0.93$^{\#\#}$
Scopolamine (0.3)+EEVZ (300)	16.2±0.86$^{@\#\#}$	12.6±0.93$^{@\#\#}$

n=5, all values are shown as mean±SEM. Statistical analysis of data were carried out by one-way ANOVA followed by Dunnett's test. $^{\#\#}P$<0.001 when compared with control and $^{@}P$<0.001 when compared with scopolamine. SFZ=Shock free zone, SEM=Standard error of the mean, EEVZ=Ethanolic extract of *Vetiveria zizanioides*

Effect of ethanolic extract of Vetiveria zizanoides on isolated rat ileum

Cumulative concentration response curve (CCRC) of ACh was obtained both in absence and presence of EEVZ. All the doses of EEVZ increased the contraction induced by ACh [Figure 1].

DISCUSSION

Anxiety is a psychological and physiological state characterized by cognitive, somatic, emotional, and behavioral components. These components combine to create an unpleasant feeling that is typically associated with uneasiness, apprehension, or worry.[32] The etiology of anxiety disorders are not fully known, but various studies have shown the involvement of Serotonergic and GABAergic neurotransmission in etiology, expression, and treatment of anxiety. The dopaminergic and adrenergic systems also play a crucial role in anxiety. Rodents demonstrate anxiety, fear, and curiosity when placed in a new environment, and an overall assessment of behavior can be determined by observing freezing, grooming or rearing, head-dips (curiosity), and the number of fecal boluses.[33-35]

Dementia (loss of memory) is one of the age-related mental problems, and a characteristic symptom of various neurodegenerative disorders including Alzheimer's disease. Drugs such as diazepam, barbiturates, and alcohol disrupt the learning and memory in animals as well as in humans. However, a new class of drugs known as nootropic agents is now used specifically in situations where there is an organic disorder in learning abilities.[36]

The EPM model in rodents has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds by decreasing anxiety, increases the open arm exploration time and increases the number of entries into

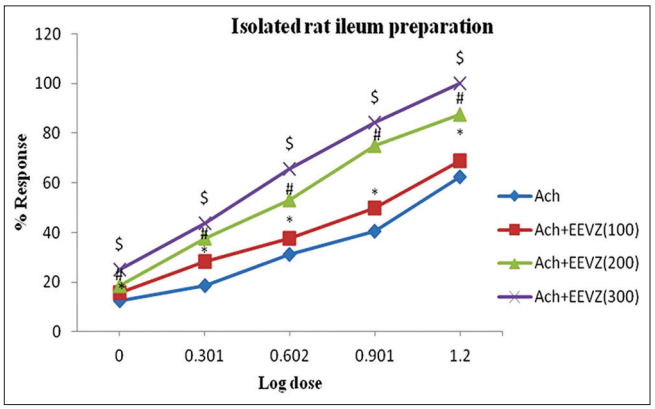

Figure 1: Effect of *Vetiveria zizanoides* on cumulative concentration response curve of acetylcholine using isolated rat ileum preparation

the open arm of the EPM.[37-39] The exploratory behavior of mice in the LDT is based on the preference of the rodents toward two-compartment boxes, where one chamber is brightly lit and the other dark. In such conditions, the rodents have a clear preference for the dark side of the box.[26,40] Head-dipping behavior is sensitive to changes in the emotional state of the animal; hence, expression of anxiolytic state in mice is reflected by an increase in head-dipping behavior.[41] The marble-burying behavior useful model for evaluating the drugs beneficial in treating obsessive-compulsive disorder. Animals, such as mice and rats, oftentimes bury objects in the bedding material of their cage. Animals that are pretreated with an antianxiety agent (e.g. diazepam or buspirone), buried significantly fewer marbles.[42] In the current investigation, we found that oral administration of EEVZ (100, 200, and 300 mg/kg) in mice shown significant anxiolytic activity as indicated by increase in time spent and number of entries in open arm, time spent in lightened area, number of head poking, and number of marble buried when compared to that of diazepam (1 mg/kg), a reference standard.

Elevated plus-maze is a widely accepted model to study learning and memory processes in rodents. It evaluates the spatial short-term and long-term memory. The impairment of learning and memory induced by scopolamine, an anticholinergic agent was reflected by prolonged TL from the open arm to the closed arm.[43] EEVZ (100, 200, and 300 mg/kg) and significantly increased the IRs and reduced the TL. EEVZ also protected the animals from scopolamine-induced impairment in learning and memory. These results implicate that EEVZ (100, 200, and 300 mg/kg,) proving its nootropic potential.

The passive shock avoidance task is the most widely used model that has given reproducible and dependable results in screening agents that affect learning and memory. Passive avoidance behavior is based on negative reinforcement and is used to examine short-term memory. The memory improving effect was manifested as a decrease in latency to reach SFZ (acquisition) and number of mistakes (descents) animal made in 15 min (retention) on passive shock avoidance task.[29] Thus, EEVZ (200 and 300 mg/kg) meets the major criteria for nootropic activity. EEVZ also protected the animals from scopolamine induced impairment in learning and memory.

Numerous studies on learning and memory reveal that the cholinergic system plays an important role. Studies have emphasized the role of neocortical ACh in spatial memory.[44] Several findings indicate that the cholinergic system in the amygdala is involved in the memory process and cholinergic neuronal activities in the amygdala change after learning a task.[45-47] In the present investigation, EEVZ (100, 200, and 300 mg/kg) significantly increased onset of sodium nitrite-induced respiratory arrest, indicating that the enhancement of cholinergic transmission in the brain, which further could be a support for their nootropic activity of the extract.[30] In addition to this, EEVZ potentiated the contraction produced by ACh on rat ileum. CCRC of ACh was obtained both in absence and presence of EEVZ. EEVZ produced contractions and increased the contractions induced by ACh. The graph was shifted to the left side indicating the cholinomimetic action of the EEVZ.

CONCLUSION

Vetiveria zizanioides is a well-known herb in Ayurveda. Results of the present study indicate that the EEVZ possesses significant anxiolytic and nootropic activity as assessed in various behavioral paradigms, mediated possibly through facilitation of neurotransmitters implicated in anxiety and learning and memory which further needs to be enlightened along with the characterization of the EEVZ active constituents responsible for the same.

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