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Memory-enhancing potentials of hydroalcoholic extract of *Eragrostis tremula* Hochst. exSteud. (Poaceae) in Mice

Abdullahi Balarabe Nazifi[*1], Mohammed Garba Magaji[2], Musa Aliyu[1], Nuhu Mohammed Danjuma[2]

[1]*Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria;*

Email:** ***abnazifi.pha@buk.edu.ng

[2] *Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria;*

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ABSTRACT

Background & Aim: Cognitive impairment is one of the age-related mental problems and a typical indicator of neurodegeneration. *Eragrostis tremula* Hochst. ex Steud. is a commonly used medicinal plant in Nigeria for memory enhancement. This study, therefore, aimed at evaluating the memory-enhancing potential of aqueous ethanolic extract of *E. tremula* in mice.

Experimental: Classes of phytochemicals present in the extract were determined using standard protocol while its oral median lethal dose (LD50) in mice was estimated. The effect of *E. tremula* extract (125, 250 and 500 mg/kg) on learning and memory was evaluated in mice using behavioural paradigms: elevated plus maze (EPM), novel object recognition and Barnes maze. Open field and hole-board tests were also carried out to evaluate locomotion.

Results: The phytochemical constituents of *E. tremula* were alkaloids, cardiac glycosides, flavonoids, tannins, saponins, steroids and triterpenes. Oral LD50 was estimated to be >5000 mg/kg. *E. tremula* extract significantly ($P<0.05$) decreased the transfer latency of mice during the retention phase of EPM test. In the novel object recognition test, it significantly ($P<0.05$) increased the discrimination index. In Barnes maze test, the extract significantly ($P<0.05$) decreased the mean primary errors during the acquisition trials. It also significantly ($P<0.05$) decreased the primary latency, primary error and increased the time spent in the target quadrant during the probe trial. *E. tremula* extract significantly ($P<0.05$) decreased the immobility time of mice in an open field at 250 mg/kg, while in the hole-board test, it significantly ($P<0.05$) increased the mean head-dip of mice at 125 mg/kg when compared to the negative control.

Recommended applications/industries: The ethanol extract of *E. tremula* possesses memory enhancing properties which can be utilized in the management of amnesia and cognitive deficit.

1. Introduction

Cognitive function is susceptible to a variety of pathological state including many neuropsychiatric and neurodegenerative diseases (Bhattacharjee *et al*., 2015). Cognitive impairment is thus a distressing comorbid outcome of neurodegenerative diseases that often negatively impact on daily activities and quality of life (Sandry, 2015). Alzheimer's disease is the most

common neurodegenerative disorder that shows cognitive deficit (disorientation, impairmentsin learning and memory functions) as the primary symptom (Silva *et al.*, 2014). It is estimated that currently 50 million victims of Alzheimer's disease exist worldwide and that number is expected to grow up to more than 80 million by 2050 as a result of life expectancy increase over the next decades (Thies and Bleiler, 2013; Patterson *et al.*, 2018). Notwithstanding the extensive research in Alzheimer's disease drug development, limited number of approved drugs are available for the symptomatic treatment of the disease and such drugs are frequently associated with adverse effects (Anand *et al.*, 2014). Thus, there remains an insistent need for the development of safer alternatives to treat cognitive deficits (Cummings *et al.*, 2017; Vyas *et al.*, 2019).

Research on medicinal plants has led to the discovery of bioactive compounds which serve as lead compounds for the development of drugs acting on new or known therapeutic targets (Uddin *et al.*, 2018). Indeed, there has been a focus on the search for new drugs from medicinal plants that will be useful in the management of cognitive dysfunction (Tewari *et al.*, 2018; Srivastava *et al.*, 2019; Vyas *et al.*, 2019). The genus Eragrostiscontains about 350 species which are important medicinal plants widely distributed in tropical, subtropical and warm temperate regions (Peterson and Vega, 2007). Some of the specieshave been used in ethnomedicine against various disease conditions including learning and memory related problems (Odugbemi, 2008; Soladoye *et al.*, 2010). *Eragrostis ferruginea* has been reported to possess important compounds with neuroprotective activities against amyloid beta peptide (Na *et al.*, 2010). Isoorientin, isovitexin, and caffeic acid isolated from *Eragrostis japonica* have also been reported to possess neuroprotective activities (Na *et al.*, 2018).

Eragrostis tremula Hochst. ex Steud. (Family: Poaceae), is a loosely-tufted annual to short-lived perennial grass that culms 30-100 cm high with attractive trembling panicles (Burkill, 1985). It is commonly known in English as "love grass" and in local languages as "*Burburwa*" (in Hausa), "*Ariran*or *Agbado-eshin*" (in Yoruba), "*Dutaleho*" (in Fulfulde) and "*Berberinoa*" (in Nupe). *E. tremula* is used in ethnomedicine as lactation stimulant, aphrodisiac (Burkill, 1985), and as antidote to snake bites (Akobundu and Agyakwa, 1998; Poilecot *et al.*, 2007). Reports by Soladoye *et al.* (2010) showed that *E. tremula* is used traditionally in Nigeria to improve memory. Traditional medical practitioners from Northern part of Nigeria also utilize the whole plant extract of *E. tremula* as a "brain cleanser" to treat many central nervous system (CNS) disorders including learning and memory deficit (Malam Rabiu Salihu, Personal Communication, 2016). To our knowledge, reports on the memory enhancing activity of *E. tremula* are scarce in spite of its well commended efficacy and acceptability in the management of learning and memory deficit. This research therefore, aimed at evaluating the memory-enhancing potential of the whole plant extract of *E. tremula* in mice.

2. Materials and Methods

2.1. Experimental animals

Swiss Albino mice (20-24 g) of either sex were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The mice were housed in standard propylene cages under natural day and light cycle, and were given free access to standard laboratory rodent feed and water *ad libitum*. The experimental protocols were approved by the Ahmadu Bello University Committee on Animal Use and Care (Approval number: ABUCAUC/2020/68).

2.2. Reagent, drug and equipment

Ethanol (Sigma-Aldrich, U.S.A.), Piracetam tablets (Nootropil, UCB Pharma Ltd, U.K.), Digital camcorder (JVC Everio 32G HDD, Japan), Open field paradigm, hole-board and Barnes maze.

2.3. Collection, identification and extraction of plant material

The whole plant of *Eragrostis tremula* was collected in October, 2017 from Batagarawa, Katsina State. It was identified and authenticated in the Herbarium Section of Department of Botany, Ahmadu Bello University, Zaria, by comparing with existing reference voucher specimen previously deposited in the herbarium (900729). The whole plant was air-dried under shade with intermittent weighing until constant weight was obtained and then pulverized. The powdered plant material (1840 g) was extracted with

10 liters of 70% v/v aqueous ethanol (70% absolute ethanol and 30% water) by cold maceration with occasional shaking for 2 weeks. The mixture was filtered and the filtrate was concentrated using a rotary evaporator to obtain a dark brown mass subsequently referred to as ethanolic extract of *Eragrostis tremula* (EEET). Thereafter, the percentage yield was calculated and the extract was stored in a desiccator until required for further studies. Fresh solution of the extract was prepared with distilled water for each study.

2.4. Preliminary phytochemical screening

Preliminary phytochemical screening of EEET was carried out using standard procedures described by Evans (2009). The extract was screened for the presence or absence of phyto-constituents such as alkaloids, flavonoids, cardiac glycosides, tannins, anthraquinones, saponins, steroids and triterpenes.

2.5. Acute toxicity study

Acute toxicity study as described by Lorke (1983) was employed in the estimation of oral median lethal dose (LD_{50}) of EEET in mice. The test was in two phases; in phase one, three groups of mice (n=3) were administered widely differing doses of the extract (10, 100 and 1000 mg/kg, *p.o*) and observed for signs of toxicity and mortality for 24 h. In the second phase, 3 mice were administered 1600, 2900 and 5000 mg/kg of the extract orally and then observed for signs of toxicity and mortality for 24 h. The LD_{50} was then calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

2.6. Experimental design

In each experiment, five groups of seven mice each (n = 7) were used. The mice were assigned into negative control, positive control and test groups as shown below:

Group I Negative control (Distilled water, 10 ml/kg)
Group II Positive control (Piracetam, 400 mg/kg)
Group III Test group (125 mg/kg of EEET)
Group IV Test group (250 mg/kg of EEET)
Group V Test group (500 mg/kg of EEET)

All treatments were by oral gavage.

2.7. Exteroceptive behavioural studies

2.7.1. Elevated plus mazetest

Elevated plus maze (EPM) serves as an exteroceptive behavioral model (where in the stimulus existed outside the body) to evaluate learning and memory in mice (Itoh *et al.*, 1990). The test was conducted as described by (Komada *et al*., 2008). The paradigm consisted of two open arms (35×5 cm) and two enclosed arms of the same size with 15 cm high wooden walls. The arms are extended from central platform (5 cm × 5 cm) and elevated 60 cm above the floor. An hour after the administrations, each mouse was subjected to the EPM test. The EPM was conducted for 2 days. On the first day (acquisition test), each animal was placed at the end of one open arm, facing away from the central platform. The transfer latency (time taken for the mouse to move from the open to the enclosed arms) was recorded within 60 sec. A mouse is said to have entered an arm, when it has placed all four paws over the line separating the area and the center. Following entry into the arm, each mouse was allowed to explore the apparatus for 20 sec and then returned to the home cage. Twenty-four hours later (on the second day), the second trial (retention test) was performed and each mouse was observed for 60 sec. After each trial, the maze was wiped with a cotton wool dipped in 70% ethanol and allowed to dry to remove any olfactory cue.

2.7.2. Novel object recognitiontest (NORT)

The methods described by Ennaceur (2010) and Lueptow (2017) were adopted to assess learning and recognition memory. The apparatus consisted of a plexiglas box of 40 cm × 40 cm × 40 cm in dimension. The NORT consist of three phases: the habituation, training and testing phases. In the habituation phase (day 1), each mouse was allowed to explore the open arena without object for 2 minutes on the first day and returned to the home cage. In the training phase (day 2), two identical objects were placed in opposite quadrants of the arena 20 cm apart from each other and 5 cm away from the walls of the apparatus. Each mouse was allowed to explore the identical objects for 10 min and then returned to the holding cage. In the testing phase (24 h after training), one of the objects was replaced with another (a novel object) of different size and colour. One hour after treatment, each mouse was introduced into the arena and allowed to explore for 5

minutes. The behaviour of mice was recorded with the aid of a video camera placed above the apparatus. The exploration time (time taken for the animal's orientation towards the object) for novel and familiar objectswere taken and the discrimination and recognition indices were calculated as using the relationship below:

Discrimination index (DI) = d1/e2; Recognition index (RI) = (b/e2) × 100

Where d1 = difference between the time spent exploring the novel object and the time spent exploring the familiar object; e2 = total exploration time during testing of the familiar and the novel object; b = time spent exploring novel object

2.7.3. Barnes maze test

The method described by Attar *et al*., (2013) was adopted in this study. The Barnes maze consisted of a circular platform (95 cm in diameter) with 40 equally spaced holes (5 cm in diameter and 5 cm between holes) along the perimeter in whichrecessed goal box (10 cm × 20 cm) is located underneath one of the holes and the mazeelevated to 105 cm above the floor. A circular start box (15 cm in height and 20 cm indiameter) usually placed at the center of the maze containing the animal for 10 sec before each trial was used. The procedure consisted of three phases viz: habituation, acquisition and probe trials.

In habituation phase, each mouse underwent three habituation trials with an inter-trial interval of fifteen minutes. In the first trial, each mouse was placed directly into the recessed goal box and allowed to remainthere undisturbed for 2 min, and then returned to their home cages. In the second trial, each mouse was placed on the maze surface adjacent to the goal hole, then gently guided into thegoal box and allowed to remain there undisturbed for 1 min. Finally, each mouse wasplaced at the maze center containing a start box for 10 seconds after which the start box was lifted away, the mouse was then gently guided into the goal box and allowed to remain undisturbed for a final 1 minute.

In the acquisition trial (which began 24 h after the habituation), mice were placed in the start boxlocated at the center of the maze. After 10 seconds, the start box was lifted away and a buzzer (fan) was switched on. The mice were allowed to explore the paradigm for 2 min in order to locate and enter the recess box. Upon entering the box, the fan was switched off and the mice were allowed to stay undisturbed for 20 sec before returning them to their home cage. This testing process wascarried out three timesto conclude testing day 1 with an inter-trial interval of 15 min. On testing day 2, mice were randomly selected into treatment groups (as described in the research design) for spatial learning assessment. An hour after drug administration, each mouse underwent two trials as in testing day 1 with a maximum latency of 2 min. Primary latency and total latency,primary error and total error were taken for each animal as indices of learning and working memory, respectively. Primary latency is the time taking to locate the target hole butno entering into the recessed box. Primary error is the number of head dip before firstlocating the escape hole. Total latency is time taken to locate and enter the goal box, while totalerror is the total number of head dip before entering the goal box. Animals that failed to findthe goal location within the 2 minutes trial were gently guided into it, and allowed to remainfor 20 sec then assigned a total latency and total error. The maze was cleaned after each trial with 70 % ethanol toremove olfactory cue.

The probe trial was carried out 24 hours after day 2 trials. The goal box was closed and the maze platform was divided into four quadrants viz: target quadrant, opposite quadrant, positive and negative quadranteach containing ten holes. Each mouse was placed in the start box for 10 sec after which it was lifted the mouse was allowed to explore the paradigm for 90 sec. The behaviour of the mice was recorded withthe aid of a video recorder placed above the center of the maze. The time spent in the target quadrant was taken as an index of memory. The maze was also cleaned after each trial with 70% ethanol to remove olfactory cue.

2.8. Exploratory studies

2.8.1. Open field test

Open field test (OFT) is commonly used to assess the exploratory behavior and general locomotor activity of rodents (Chen *et al.*, 2002). Gross behavioral activity was utilized to rule out any interference in locomotor activity by drugs which may affect the process of learning and memory.The open field consisted of a square arena measuring 72cm×72cm×36 cm (length× breadth × height). The floor was divided into 16 (18cm×18 cm) squares with central square (18cm×18

cm) drawn in the middleof the open field (Brown *et al.*, 1999). An hour post administrations, the mice were subjected to the open field test. Each mouse was placed at the center of the open field arena and allowed to freely move while its behaviour was observed for 5 min. After each trial, the open field arena was cleaned with 70% ethanol to remove any olfactory cue. The behaviors scored were duration of immobility (time spent in central square), centric line crossing (number of entries into the center square) and square crossings (crossing the square boundaries with both paws).

2.8.2. Hole-board test

The effect of the EEETon exploratory activity of mice was also evaluated using hole-board test. The method described by File and Wardill (1975) was adopted. The apparatus used was a wooden board (60cm x30cm) with sixteen evenly spaced holes (2 cm diameter x 2 cm depth) and elevated 105 cm above the floor. An hour post administrations, each mouse was placed singly at one corner of the board and was allowed to move about. The number of head dips (poking of the hole to the level of the eye) was counted during a five minutes period. The apparatus was cleaned after each test with 70% ethanol to remove olfactory cue.

2.9. Statistical analysis

Data analysis was carried out using SPSS software (Version 20). Differences between means were analyzed by One-Way Analysis of Variance (ANOVA) followed by Bonferroni post hoc test. Variables taken over time were analyzed using repeated measures ANOVA and Bonferroni post hoc tests. Values of $P \leq 0.05$ were considered as significant in all the statistical tests. Data obtained were expressed as mean ± standard error (S.E) and presented as charts and tables.

3. Results and discussion

The present study investigated the effect of EEET on learning and memory in mice. The learning and memory was evaluated using EPM, NORT and Barnes maze. The outcome of this study showed that EEET enhances learning and memory and thus lent credence to the use of the plant in ethnomedicine as memory enhancer.

Preliminary phytochemical screening of EEET showed the presence of alkaloids, cardiac glycosides, flavonoids, tannins, saponins, steroids and triterpenes (Table 1). Secondary metabolites from medicinal plants have been reported to play important roles in averting many diseases including cognitive deficits (Srivastava *et al.*, 2019). Some potent inhibitors of acetylcholinesterase were derived from natural sources and some of them belong to the chemical class of alkaloids, including galantamine, obtained from Amaryllidaceae species, and huperzine A, a lycopodium alkaloid (Konrath *et al.*, 2012). Phenolic compounds are also known to possess good nutritional, antioxidant and nootropic properties (George *et al.*, 2014; You *et al.*, 2018). Flavonoids isolated from *Eragrostis ferruginea* and *Eragrostis japonica* have also been reported to possess neuroprotective activities (Na *et al.*, 2010; 2018). Eclalbasaponin II is an oleanane-type triterpenoidsaponin (isolated from *Ecliptaprostrata*) reported to ameliorate cholinergic blockade-induced memory impairment (Jung, 2018). Furthermore, asiatic acid, a triterpenoid present in *Centella asiatica* has been shown to possess neuroprotective activities and modulates various pathological features of Alzheimer's disease (Rather, 2019). Thus, the observed learning and memory enhancing activity of EEET may be attributed to its phyto-constituents.

Table 1: Phytochemical constituents of hydroalcoholic extract of *Eragrostis tremula*

Constituents	Tests	Inference
Alkaloids	Wagners	+
	Mayers	+
	Dragendorrf	+
Anthraquinones	Bontragers	–
Cardiac glycosides	Salkwoski	+
Flavonoids	Shinoda	+
	Sulfuric acid	+
Saponins	Frothing	+
	Haemolysis	+
Steroids and triterpenes	Lieberman-Bucchard	+
Tannins	Ferric chloride	+

Key: + (Present), – (Absent)

Determination of LD_{50} value of medicinal plants is of utmost importance because it provides evidence regarding their margin of safety. It also serves as a

guide in dosage selection for long term toxicity studies as well as other studies that involve the use of animals (Colerangle, 2017). In the acute toxicity study, administration of varying doses of EEET did not present visible signs of toxicity and there was no mortality in the two phases of the study. The oral LD_{50} of EEET in mice was thus estimated to be >5000 mg/kg body weight. This shows that EEET is practically non-toxic in mice following acute administration (Loomis and Hayes, 1996).

In the EPM test, the mean transfer latencies of mice into the closed arms were observed during the acquisition and retention phases. In the acquisition phase, the administration of EEET (125, 250 and 500 mg/kg) and the standard drug (Piracetam, 400 mg/kg) did not produce significant (P>0.05) changes in the mean transfer latencies of mice into the enclosed arms when compared to the negative control. However, in the retention phase, the extract at all the tested doses as well as piracetam significantly (P<0.05) reduced the mean transfer latencies when compared to the negative control (Figure 1).

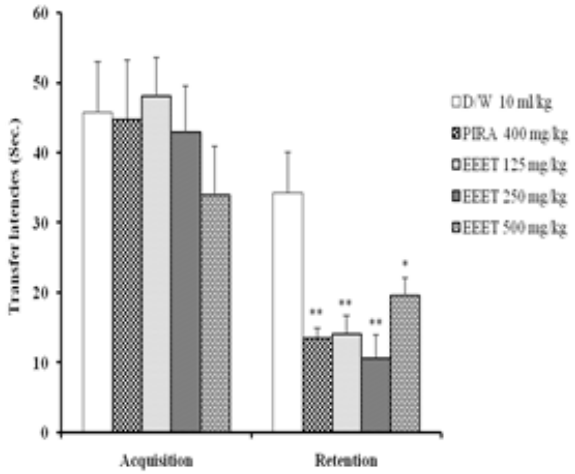

Figure 1: Effect of hydroalcoholic extract of *Eragrostis tremula* on transfer latency of mice in elevated plus maze test. Values are Mean ± S.E.M; * = P<0.05, ** = P<0.01 as compared to D/W group – One way ANOVA followed by Bonferroni post hoc test, n=7, D/W = Distilled water, PIRA = Piracetam, EEEG = Ethanolic extract of *Eragrostis tremula*

The transfer latency into the closed arms of EPM has been utilized as a parameter to assess learning and retention memory (Dhingra *et al.*, 2004). It has been demonstrated to be shorter if the animal had previously experienced entering the closed arm (learning of escape behavior) (Bhanumathy *et al.*, 2010). The significant reduction in the transfer latencies byEEET during the retention phase suggests an improvement in learning and memory. The enhancement of learning and memory by medicinal plants have also been attributed to their ability to preferentially enhance cognitive processing of information over the natural anxiety that disrupts cognitive function in EPM test (Mahmud *et al.*, 2019).

NORT is a popular method for evaluating compounds as potential pro-cognitive agents in preclinical drug discovery programs. It is used to assess recognition memory in rodents based on their proclivity for exploring novelty (inherent exploratory behaviour to distinguish a non-familiar object from a familiar object in the absence of externally applied rules or reinforcement (Lueptow, 2017). In the NORT, EEET produced a significant (P<0.05) increase in object discrimination at all the tested doses when compared to the negative control. Similarly, the standard drug used (Piracetam, 400 mg/kg) produced a significant (P<0.05) increase in object discrimination when compared to the negative control (Table 2).

Recognition memory is one of the domains of cognition that is often impaired in aged (non-demented) humans and Alzheimer's disease patients. Different brain regions make different contributions to recognition memory processing, including the perirhinal cortex, the medial prefrontal cortex and the hippocampus (Barker *et al.*, 2017). Lesions in these areas have been shown to produce deficits in recognitionmemory (Warburton, 2018). The significant increase in the discrimination index produced by EEET suggests it improves recognition memory of the animals. It is also possible that the extract exerted its effect on the aforementioned brain regions.

In the Barnes maze test, EEET at all tested doses did not produce significant (P>0.05) changes in the primary latencies of the mice during the 2-days acquisition trials. However, the extract at all tested doses as well as the standard drug produced significant (P<0.05) decrease in the primary errors when compared to the negative control. On comparison of the errors over time, there was significant decrease (P<0.05) in the primary errors of all the groups during the 5th trial as compared to the first trial (Table 3).

Table 2: Effect of hydroalcoholic extract of *Eragrostis tremula* on object discrimination of mice in novel object recognition test

Treatment (mg/kg)		ETN (Sec)	ETF (Sec)	DI	RI
D/W	10 ml/kg	12.50±0.56	19.33±2.80	-0.18±0.08	40.92±3.90
PIRA	400	28.33±0.53*	19.50±3.91	0.20±0.07*	59.55±3.25*
EEET	125	20.29±4.57	13.29±2.07	0.17±0.08*	58.24±3.83*
EEET	250	28.14±3.81*	17.57±1.96	0.21±0.06**	60.88±3.02**
EEET	500	16.29±2.32	10.43±1.29	0.19±0.11*	58.94±5.70*

Values are Mean ± S.E.M; *= p<0.05, **= p<0.01 as compared to D/W group – One way ANOVA followed by Bonferroni post hoc test, n=7, ETN = Exploration time on novel object, ETF = Exploration time on familiar object, DI = Discrimination index, RI = Recognition index, D/W = Distilled water, PIRA = Piracetam, EEEG = Ethanolic extract of *Eragrostis tremula*

Table 3: Effect of hydroalcoholic extract of *Eragrostis tremula* on spatial learning in mice during acquisition trial in Barnes maze

Treatment (mg/kg)	Latency (Sec.)				
	t1	t2	t3	t4	t5
D/W 10 ml/kg	98.43±11.87	96.86±13.80	103.14±10.90	94.57±11.61	88.00±12.00
PIRA 400	78.50±17.00	91.14±17.16	79.29±17.22	81.14±15.39	71.86±16.28
EEET 125	74.86±13.29	74.71±17.52	81.57±16.79	66.86±15.83	68.29±17.38
EEET 250	92.00±18.31	89.50±16.80	86.00±17.21	75.00±20.78	57.33±19.18
EEET 500	84.29±14.75	81.29±14.51	63.43±15.82	72.71±16.71	90.14±16.17
	Errors				
	t1	t2	t3	t4	t5
D/W 10 ml/kg	18.00±1.31	9.00±1.63	10.50±1.84	9.14±2.04	5.57±1.46[b]
PIRA 400	11.29±2.26	13.25±7.02	10.00±1.87	5.00±0.58	2.50±0.34*[a]
EEET 125	11.14±1.77	12.57±2.34	11.33±3.01	7.86±1.92	2.29±0.57*[a]
EEET 250	11.33±1.91	6.40±1.69	6.60±1.86	5.00±1.63	1.83±0.31*[a]
EEET 500	11.00±3.40	10.43±1.96	5.71±1.57	6.57±1.49	2.00±0.49*[a]

Values are Mean ± S.E.M; * = P<0.05 as compared to D/W group; a = P<0.05, b = P<0.01 as compared to first trial (t1) – Repeated measure ANOVA followed by Bonferroni post hoc test, n=7, t = trial, D/W = Distilled water, PIRA = Piracetam, EEEG = Ethanolic extract of *Eragrostis tremula*

During the one-day probe trial, the extract and the standard drug used (Piracetam, 400 mg/kg) significantly (P<0.05) decreased the primary latencies when compared to the negative control. However, there were no significant (P>0.05) changes in the primary and total errors. Furthermore, the extract and standard drug significantly (P<0.05) increased the time spent in the target quadrant when compared to the negative control (Table 4). The Barnes mazeis a non-invasive task that measures the ability of rodents to learn and remember the location of a target zone using a configuration of distal visual cues located around the testing area, and is a measure of spatial learning and memory (Harrison *et al*., 2009). Different phases of the task allow to measure spatial learning, memory retrieval and cognitive flexibility (Gawel *et al.*, 2019).

Table 4: Effect of hydroalcoholic extract of *Eragrostis tremula* on spatial learning and memory in mice during one-day probe trial in Barnes maze

Treatment (mg/kg)		PL (Sec.)	PE	TE	TSTQ (Sec.)
D/W	10 ml/kg	38.40±3.93	6.71±1.17	8.14±1.92	39.86±7.86
PIRA	400	17.25±6.79*	5.00±1.00	8.17±1.64	65.17±4.84*
EEET	125	17.80±4.32*	3.67±1.33	8.29±2.13	69.33±6.17*
EEET	250	16.40±4.52*	5.00±1.35	6.60±0.87	73.29±6.03**
EEET	500	19.50±2.47*	2.20±0.20*	3.60±0.24	71.67±8.60*

Values are Mean ± S.E.M; *= p<0.05, **= p<0.01 as compared to D/W group – One way ANOVA followed by Bonferroni post hoc test, n=7, PL = Primary latency, PE = Primary error, TE = Total error, TSTQ = Time spent in target quadrant, D/W = Distilled water, PIRA = Piracetam, EEEG = Ethanolic extract of *Eragrostis tremula*

The first part of the task, i.e., the acquisition phase allows evaluates spatial learning, whilethe acquisition probe trial evaluatesspatial memory. This part is believed to be associated with hippocampus function (Barnes 1979; Gawel *et al.*, 2019). In the Barnes maze, the escape latency has been widely used as a measure for the assessment of spatial learning with the primary latency being more sensitive than the total latency for detecting differences in learning (O'Leary and Brown, 2013). Still, studies have shown that the number of primary errors, the hole deviation score and the distance traveled are the most sensitive measure of the animal performance (O'Leary and Brown, 2013; Gawel *et al.*, 2019). The ability of EEET to significantly decrease the mean primary errors during the acquisition trials signifies an improvement in spatial learning.In addition, the extract improved working and reference memory as indicated by the significant decrease in the primary latency, primary error and an increase in time spent in the target quadrant during the probe trial.

In the open field test, the administration of EEET (125, 250 and 500 mg/kg) decreased the mean immobility time in a non-dose-dependent manner. The decrease was significant (P<0.05) at 250 mg/kg when compared to the negative control group. The extract at all the tested doses as well as the standard drug (Piracetam, 400 mg/kg) increased the number of center square entry and the number of square entry of the mice. The increase was however not statistically (P>0.05) significant when compared to the negative control group (Figure 2). The open field test is useful in assessing exploratory behavior, spontaneous locomotor activity and anxiety levels (Szentes *et al.*, 2019). Locomotor activity has been shown to influence cognitive performances and in most cases, a decrease in activity is associated with impaired motor function and vice versa (Liu *et al.*, 2014). The hippocampus has been shown to play a critical role in the cognitive performances in the open field (Liu *et al.*, 2014). In this study, EEET significantly decreased the immobility time which signifies anxiolytic-like activity. However, the insignificant changes in other variables like center square crossings, peripheral line crossing or rearing shows that the extract may not have CNS stimulating or depressant effect.

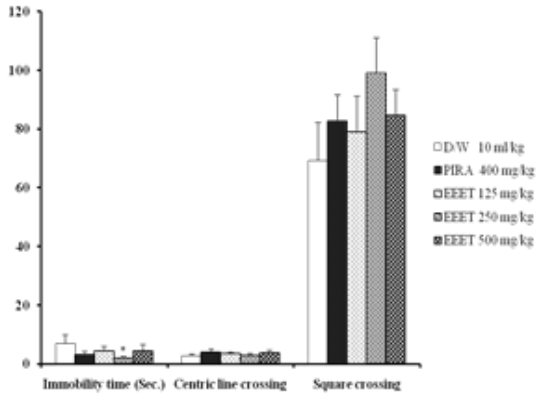

Figure 2: Effect of hydroalcoholic extract of *Eragrostis tremula* on exploratory behavior of mice in Open field test. Values are Mean ± S.E.M; * = p<0.05 as compared to D/W Group – One way ANOVA followed by Bonferroni post hoc test, n=7, D/W = Distilled water, EEEG = Ethanolic extract of *Eragrostis tremula*

The hole-board testis used to assess exploratory behaviour of rodents (File and Wardill, 1975). The head-dipping behaviour is a sensitive measure of emotional changes; an increase in head dipping behaviour has been described as anxiolysis, while a decrease in this parameter signifies sedation (Yaro *et al.*, 2015). EEET produced a significant (P<0.05) increase in the exploratory behaviour as indicated by the increase in the mean number of head-dips (Figure 3).

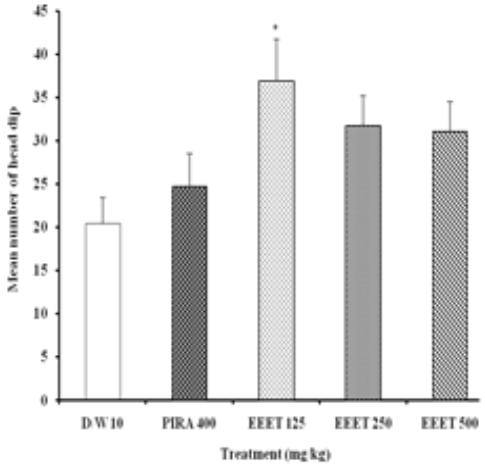

Figure 3: Effect of hydroalcoholic extract of *Eragrostis tremula* on head-dip exploratory behavior of mice in Hole-board test. Values are Mean ± S.E.M; * = p<0.05 as compared to D/W group – One way ANOVA followed by Bonferroni post hoc test, n=7, D/W = Distilled water, PIRA = Piracetam, EEEG = Ethanolic extract of *Eragrostis tremula*

This supports the anxiolytic-like effect of the extract as observed in the open field test. Anxiety is a serious and common neuropsychiatric symptom in dementia which has been shown to impact negatively on not only the demented patients but also to their caregivers (Kwak *et al.*, 2017). Anxiety has been shown to disrupt cognitive performance including working memory (Lukasik *et al*., 2019); and according to Gulpers *et al*. (2017), anxiety is associated with higher risk for incident cognitive impairment and most likely for dementia. Therefore, the anxiolytic-like actions of EEET could serve as a synergy towards preventing cognitive deficits and anxiety related dementia.

4. Conclusion

The ethanol extract of *Eragrostis tremula* possesses learning and memory-enhancing activities. This could lend scientific rationale to the ethnomedical use of the plant extract as memory enhancer.

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