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Screening of Taxillus Tomentosus Ethanolic Extract for Nootropic and Antistress Activity in Rats

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ABSTRACT: In recent years, attempts have been made to develop drugs for treatment of dementia and attention deficit disorders to improve memory and learning. Some nootropic agents (eg: Piracetam) are widely used but the resulting chemo phobia associated with them and other similar agents has made their use limited. So it is worthwhile to explore medicines from the traditional system in the treatment of these cognitive disorders.
Stress is a broad, ambiguous, and often poorly understood concept. In its most simplified Sense, stress is what one feels when life's demands exceed one's ability to meet those demands. The objective of the study is to study the effect of ethanolic extract of *Taxillus tomentosus* for nootropic and antistress activity in experimental animals. It has been documented in traditional system that *Taxillus tomentosus* plant belonging to family loranthaceae effective in nervine disorders, acts as a nervine tonic. In a survey of ethnobotanical literature, numerous plant preparations have been used in the treatment of age related cognitive disorders in human beings in European countries; so in present study is to evaluate the same on *Taxillus tomentosus* alone by using various screening models in rats.
However, this plant has not been scientifically investigated for the same. Therefore, the present study is designed to evaluate effect of whole plant extract on learning and memory.

KEYWORDS: Cognition, Memory, Stress, Ethanolic extract, Alzheimer, Nootropic.

1 INTRODUCTION

Learning is the most characteristic attributes of the man and also of higher animals. Learning is defined as the ability to alter behaviour on the basis of experience[1]. Memory is special facility of brain which retains the events developed during the process of learning and both are mediated by nervous system[2]. Once memories have been stored in the brain, it becomes the part of brain –process mechanism when it will recall in future[3]. Learning and memory are closely related, all learning involves memory but all memory not involves to learning[4].

Probably learning and memory are most evolutionary advantageous developments for human. These are interesting but ill-understood subjects. By utilizing past experience due to learning and it's storage in memory one can get success and avoid failure[5].

Alzheimer's disease is progressive loss of memory and cognitive function in middle age individuals. (Cognition: that means by which one is aware of the process of thinking and perceiving. It involves an awareness of sensation and usually its cause. Mental component consist of cognitive changes.) Symptoms of Alzheimer's disease are–memory is failed for recent events, lack of spontaneous activity and initiative with loss of intellectual functions, loss of spatial orientations.

After 2 or 3 years dementia will take place. Following symptoms are found-aphasia (speech disorder), ataxia (unable in voluntary movements) and agnosia (unable in recognizing objects). Dementia is not a disease, but rather a group of

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symptoms caused by the impact of diseased brain. 10-15% of the populations over 65 years of age and 50% of population over 85 years have some degree of dementia. Severity increases with increase in age.

The indications of cognition enhancer's are- senile dementia of Alzheimer type and multi infarct dementia - common symptoms of elderly, dizziness and memory disturbances – mental retardation in children, learning defects, attention deficit disorder[6].

Stress can be described as the sum of all the reactions of the body, which disturb the normal physiological equilibrium and results in a state of threatened homeostasis. Stress is an internationally recognized phenomenon fortified by advancement of industrialization and a demanding civilization. Modern life is full of hassles, deadlines, frustrations, and demands. For many people, stress is so common place that it has become a way of life. Thus, every person today faces stressful situations in day to day life. Stress represents reaction of body to stimuli that tend to disturb its normal physiological equilibrium or homeostasis and has been defined as non-specific response of the body to any demand imposed on it[7].

For optimal survival of the individual it is important that bodily functions are subject to homeostatic control. Hence there is a continuous effort to maintain these functions within a certain range variable to demand by a process referred to as allostasis [8]. Just about everybody men, women, children and even foetuses suffer from stress. Relationship demands, chronic health problems, pressure at workplaces, traffic snarls, meeting deadlines, growing-up tensions or a sudden bearish trend in the bourse can trigger stress conditions. Stress isn't always bad. In small doses, it can help you to perform under pressure and motivate you to do your best. But when you're constantly running in emergency mode, your mind and body pay the price. As per the observations of American Institute of stress it has been reported that forty-three percent of all adults suffers from adverse health effects due to stress and if remain untreated more than 50% suffers from lifetime emotional disorders. Stress costs American industry more than $300 billion annually.

A great research is required in early diagnosis of the condition and development of newer effective drugs to prevent or halt the progression of such diseases.

2 MATERIALS AND METHODS

2.1 MATERIALS

COLLECTION AND AUTHENTIFICATION

Taxillus tomentosus plant was collected in March 2013 from S.V. University, Tirupathi, India. Verified by prof. Dr.M. Madhava Shetty Department of Botany.

DRUGS AND CHEMICALS

DRUGS

Mentat: A poly herbal preparation containing around 25 different herbs, and is a proven memory enhancing drug available in the market. It was procured from Himalaya Herbal Healthcare, Bangalore.

Scopolamine: An antimuscarinic agent for induction of loss of memory. It was purchased from Sigma Chemicals, USA.

Diazepam: Benzodiazepine (Inj. Calmpose) manufactured by Ranbaxy Laboratory Ltd.

CHEMICALS

- Ethanol (Fisher scientific)
- Diethyl ether (Fisher scientific),
- Tween 80 (Himedia)

EXPERIMENTAL ANIMALS

Wistar albino rat's 160-200 gm body weight were obtained from the Department of Pharmacology. They were housed in well ventilated cages and kept in a room where a twelve-hour light/dark cycle was maintained. They were allowed free access to water and fed commercial growers' mash (Vital feeds, Jos) *ad libitum* throughout the period of the experiment. The rats were allowed to acclimatize for two weeks. The experimental rats were all handled in strict compliance with

international guidelines as prescribed by the Canadian Council on the Care and use of Laboratory Animals in Biomedical Research. The experiments will be conducted in accordance with protocol approved by the institutional animal ethical committee (IAEC) of Sigma institute of clinical research and administration pvt ltd Hyderabad.

2.2 METHODS

PREPARATION OF PLANT EXTRACT

The collected fresh plant materials were dried in shade (2 days) and then dried in a hot air oven at 25ºC for three days and they were made in to coarse powder with the use of mixer grinder. The powder of *Taxillus tomentosus* obtained were weighed separately and transferred to a round bottomed flask and then went to continuous heat extraction with soxhlet apparatus using 90% ethanol for 24 hours. Then the extract of ethanol was concentrated. Extract obtained was dried by placing it on a big petriplate on electric water bath (70ºC) and then kept in an oven at 30ºC for 2 hour. The extract obtained was kept for drying and stored in vacuum desiccators. The percentage yield of the extract was 9.61%.

ACUTE TOXICITY STUDY

Procedure: Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). Swiss albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The plant extracts of *Taxillus tomentosus* were administered orally with maximum dose of 2000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose (Organization for economic Co-operation and development, 2001).

OBSERVATIONS

Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin, mortality and general behavioural pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. No death was observed till the end of study

QUALITATIVE CHEMICAL TEST

Preliminary phytochemical studies: Ethanolic extract of the plant of *Taxillus tomentosus* were subjected to chemical tests for the identification of their active constituents.

PREPARATION OF DRUGS AND CHEMICALS

Mentat: The tablets were crushed and used for preparing the drug suspension. Specified Quantity of Mentat powder was weighed and mixed with Tween 80, triturated well and suspended in distilled water quantity sufficient to produce a suspension of 10mg/ml and Was administered at a dose of 100mg/kg b.w.64.

Extract: ETT was weighed and triturated separately with Tween 80 (0.5%) and then was suspended separately in distilled water quantity sufficient to produce a suspension depending upon dose (200 and 400 mg/kg b.w). 120

Scopolamine: The scopolamine was administered through subcutaneous route. 20 mg of Scopolamine was weighed and mixed with distilled water in a volumetric flask to get the final volume of 100 ml with a final concentration of 0.2mg/ml and was administered at a dose of 0.4 mg/kg b.w.

SCREENING MODELS FOR NOOTROPIC ACTIVITY

The following methods will be employed to study the cognitive action of the test drugs, with the animals divided into five groups of six animals in each group. The test drug will be administered orally at concentration lower and higher doses mg/kg.

GROUPING OF ANIMALS

Group. 1 - Normal control treated with vehicle
Group. 2 - Negative control MES induced
Group. 3 - Positive control treated with std. drug+ MES
Group. 4 - Positive control treated with extracts Lower dose+ MES
Group . 5 - Positive control treated with extracts Higher dose+ MES

i. ELECTROSHOCK INDUCED AMNESIA

Amnesia can be induced in experimental animals by electroshock stimulation. According to standard procedure, presentation of electroshock by silver corneal electrodes induced clonic-tonic seizures and impaired memory. The electroshock was applied immediately after the training trials in the task being tested. A sham electroshock was given to the control animals. Alternatively, electroconvulsive shock (ECS)-induced convulsions in animals may produce a more severe form of amnesia. In this technique, an electroshock (150mA for 0.2sec) were applied through commercially available electro stimulators. Electric shock gives very unpleasant feeling to the animal. The model offers evaluations of various nootropic agents.

ii. DRUG INDUCED (SCOPOLAMINE) AMNESIA

Scopolamine is a powerful muscarinic antagonist capable of crossing blood brain barrier, acts both peripherally by blocking the receptors for acetylcholine at the synapse. It impairs memory storage of new information (short term memory) and learning acquisition. The dose of 0.4mg/Kg is approved to produce cognitive and memory changes without causing debilitating peripheral anticholinergic effect. Though the several models for amnesia are available, but the scopolamine induced memory deficits has been proposed to have symptomatological similarities with Alzheimer's Disease and related disorders.

Group. 1 = Normal control treated with vehicle
Group. 2 = Negative control Scopolamine induced
Group. 3 = Positive control treated with std. drug+ Scopolamine
Group. 4 = Positive control treated with extracts 200mg/kg+ Scopolamine
Group. 5 = Positive control treated with extracts 400mg/kg+ Scopolamine

iii. DIAZEPAM INDUCED AMNESIA

Diazepam 1mg/kg, ip was administered to young rats and TL was noted after 45 min of injection on 8th day and after 24hrs. Extract lower and higher doses and standard mentat were administered for successive 8 days. After 60 min of administration of the last dose on 8th day, Diazepam 1mg/kg ip, was administered. TL was noted after 45 min administration of diazepam and after 24 hrs.

Group. 1 = Normal control treated with vehicle
Group. 2 = Negative control Diazepam induced
Group. 3 = Positive control treated with std. drug+ Diazepam
Group. 4 = Positive control treated with extracts 200mg/kg+ Diazepam
Group. 5 = Positive control treated with extracts 400mg/kg+ Diazepam

The following screening methods will be employed for assessment of memory:

ELEVATED PLUS MAZE

Originally it was designed to evaluate the anti-anxiety agents but now extended to measure the spatial long-term memory in animals. The transfer latency (the time in which the animal moves from open to closed arm) is shown to be related to memory processes.

SCREENING TESTS FOR MEMORY

TRANSFER LATENCY

The maze was elevated to a height of 50cm, the animals were individually placed at the end of either of the open arms and the time taken for the animal to move from open to closed arm (Transfer latency, TL) was taken as the criterion of task. The animals were allowed to explore the apparatus for 30 seconds. After 24 hours of the first exposure, TL was again noted on the day 1 of the study for determining the acquisition. Five minutes later the animals of Group 2, 3, 4 and 5 received electroshock of 150mA for 0.2 seconds through a pair of ear electrode from an Electro-convulsometer and then the animals were dosed with respective drug and kept in their home cage. Similarly, animals of Group 2, 3, 4 and 5 received scopolamine (0.4mg/kg body weight) and then were dosed with respective drug and returned to their home cage. The electroshock/scopolamine and dosing with drug continued for up to 7 days and on 7^{th} day the animals were subjected to the retention test 25min. after the last dose, for evaluating the transfer latency keeping the time period of 60 seconds as cut off criterion.

SCREENING MODELS FOR ANTISTRESS ACTIVITY

ELEVATED PLUS-MAZE

The elevated plus-maze comprised two open (30 cm×5 cm×0.25 cm) and two enclosed (30 cm×5 cm×15 cm) arms that radiated from a central platform (5 cm×5 cm) to form a plus sign. The maze is constructed of black painted wood. A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals. The plus-maze is elevated to a height of 40 cm above floor level by a single central support. The experiment is conducted during the dark phase of the light cycle (9:00–14:00 h). The trial is started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in, each of the two types of arm, are counted during a 5 min test period. The percentage open arm entries and percentage open arm time are used as indices of anxiety. A mouse is considered to have entered an arm when all four paws are on the arm. The apparatus is cleaned thoroughly between trials with damp and dry towels. All behavioral recordings will be carried out with the observer unaware of the treatment received by mice.

EXPERIMENTAL DESIGN

Group –I	Control treated with vehicle
Group-II	Negative Control
Group –III	Standard drug (Imipramine-10mg/kg)
Group –IV	*Taxillus tomentosus* ethanolic extract 200mg/kg
Group – V	*Taxillus tomentosus* ethanolic extract 400mg/kg

STATISTICAL ANALYSIS

All the data expressed as mean SEM will evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test using Prism Graphpad version 5 .0 and values of $P<0.05$ will considered as statistically significant.

3 RESULTS

ACUTE TOXICITY STUDY

The overall study showed the LD50 of oral toxicity of extracts ETT to be above 2000 mg/kg b.w. in mice. So, the extracts are safe for long term administration.

PHYTOCHEMICAL EVALUATION

Preliminary phytochemistry of the plant extract: The yield of ethanolic extract ETT was found to be 9.61 % W/W. Preliminary phytochemical analysis revealed that the plant possessed phyto constituent's alkaloids, tannins, Phytosterols, flavonoids.

Phytoconstituents	Presence or Absence
Carbohydrates	+
Glycosides	+
Fixed oils and fats	+
Gums & mucilage	-
Protein & amino acids	-
Saponins	+
Tannins	+
Phytosterols	+
Flavonoids	+++
Alkaloids	++

NOOTROPIC ACTIVITY

MES INDUCED AMNESIA MODEL

EFFECT ON TRANSFER LATENCY (USING ELEVATED PLUS MAZE)

The animals were subjected to transfer latency (TL) to evaluate the retrieval of memory in behavioural paradigm after a period of 7 days of acquisition trial, to know the effect of extracts on the long term memory. TL of day 1 reflects learning behaviour of the animals whereas; TL of day 7 reflects the retention of the information or memory.

The normal group animals (Table 1) have showed non-significant retrieval of memory in this behavioural paradigm. In the negative control group, the animals exposed to MES, produced significant (P<0.001) loss of memory in behavioural paradigm, which resulted in increase in TL on day 7 as compared to day 1.

The data presented in Figure 1, the animals treated with standard (Mentat) showed highly significant (P<0.001) decrease in the TL. ETT (200 and 400 mg/kg) treated animals produced significant (p<0.01 and P<0.001) decrease in TL.

Table 1. Effect of Ethanolic extract of Taxillus tomentosus on transfer latency by using Elevated Plus maze.

Groups	Subgroup	Transfer latency (In seconds) in Elevated Plus maze	
		Before	After
		1 day	7 day
I	Normal	19.79±0.8241	17.45±0.7482
II	Control (MES)	25.56±1.343	54.14±1.400a
III	Stanadard (Mentate)	26.81±0.6380	33.97±1.242***
IV	ETT 200mg/kg	21.29±0.6028	46.51±2.178**
V	ETT 400mg/kg	21.87±1.108	40.48±2.065***

*All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, **p<0.01, ***p< 0.001, as control group and [a] p<0.001, as compared to normal.*

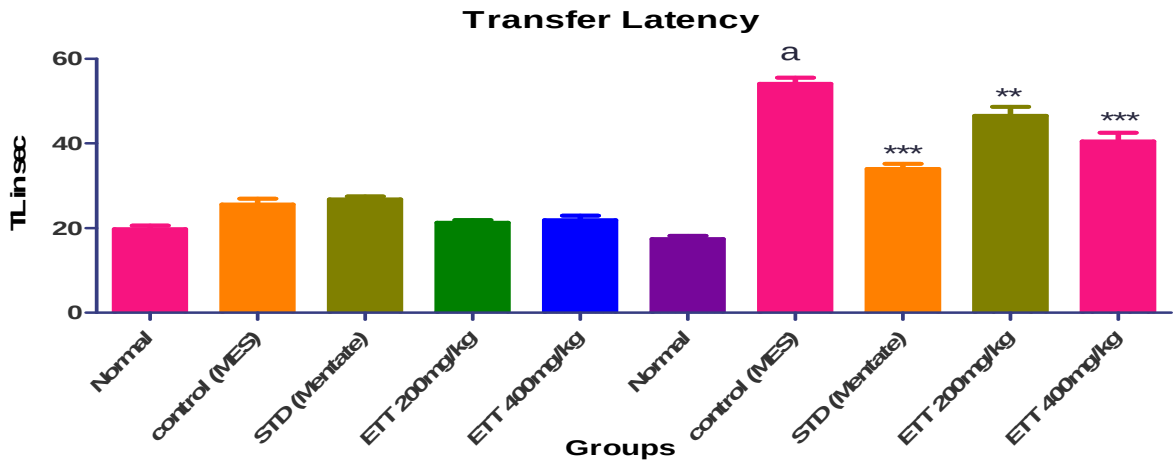


Fig. 1. Effect of Ethanolic extract of Taxillus tomentosus on transfer latency (MES Model) by using Elevated Plus maze.

*All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, **p<0.01, ***p< 0.001, as control group and [a] p<0.001, as compared to normal.*

SCOPOLAMINE INDUCED AMNESIA MODEL

The animals were subjected to transfer latency (TL) to evaluate the retrieval of memory in behavioural paradigm after a period of 7 days of acquisition trial, to know the effect of extracts on the long term memory. TL of day 1 reflects learning behaviour of the animals whereas; TL of day 7 reflects the retention of the information or memory (Table2).

The normal group animals have showed non-significant retrieval of memory in this behavioural paradigm. In the negative control group, the animals exposed to scopolamine, produced significant (P<0.001) loss of memory in behavioural paradigm, which resulted in increase in TL on day 7 as compared to day 1.

Table 2. Effect of Ethanolic extract of Taxillus tomentosus on transfer latency (Scopolamine model) by using Elevated Plus maze.

Groups	Subgroup	Transfer latency (In seconds) in Elevated Plus maze	
		Before	After
		1 day	7 day
I	Normal	24.57±1.08	16.49±1.04
II	Control (Scopolamine 0.3mg/kg)	22.99±1.55	55.51±1.48a
III	Stanadard (Mentate)	26.97±1.45	11.51±0.87***
IV	ETT 200mg/kg	29.19±1.58	34.96±1.46***
V	ETT 400mg/kg	28.64±1.82	22.71±0.61***

*All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, ***p< 0.001, as control group and [a] p<0.001, as compared to normal.*

In the treatment group, the animals exposed to Scopolamine and treated with Standard (Mentat), reduced the time taken to perform the task in elevated plus maze resulting in significant (P<0.001) retrieval of memory in behavioural paradigm. The animals exposed to Scopolamine and treated with ETT (200 and 400 mg/kg) showed significant (P<0.001) decrease in TL as compared to the negative control Figure 2.

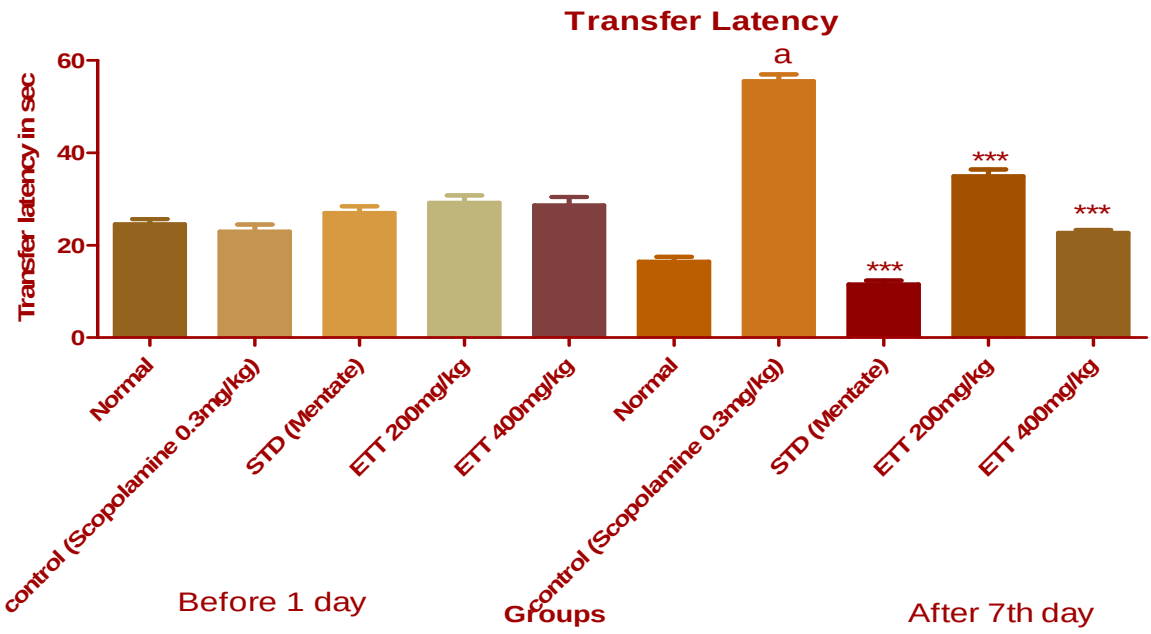


Fig. 2. Effect of Ethanolic extract of Taxillus tomentosus on transfer latency (Scopolamine model) by using Elevated Plus maze.

*All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, ***p< 0.001, as control group and [a] p<0.001, as compared to normal.*

DIAZEPAM INDUCED AMNESIA MODEL

The animals were subjected to transfer latency (TL) to evaluate the retrieval of memory in behavioural paradigm after a period of 8 days of acquisition trial, to know the effect of extracts on the long term memory. TL of day 9 reflects learning behaviour of the animals whereas; TL of day 8 reflects the retention of the information or memory.

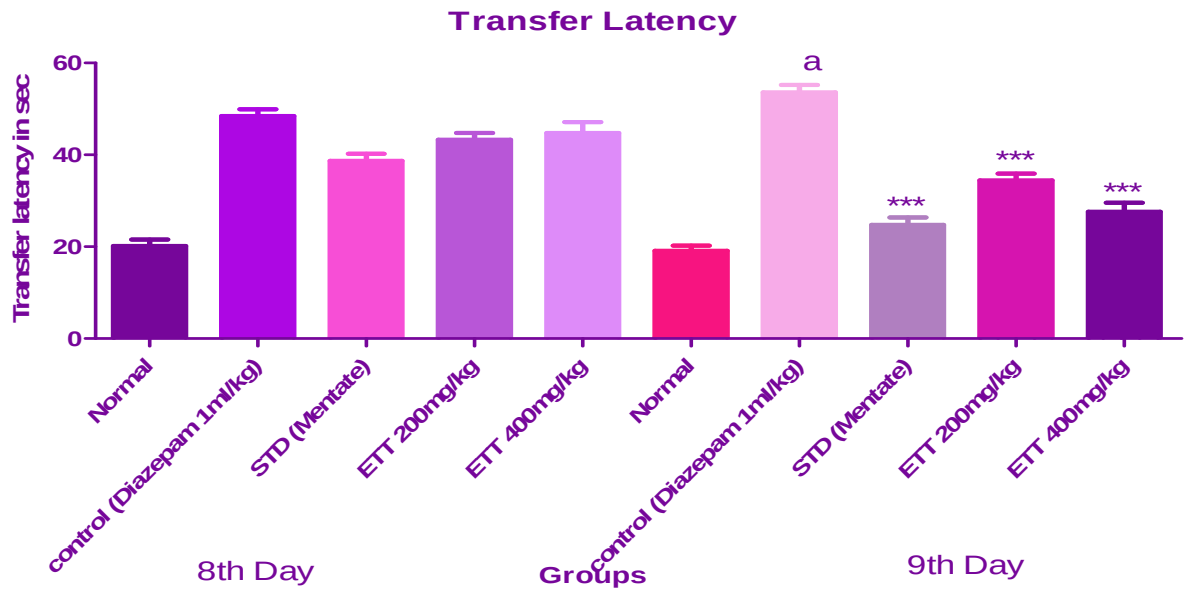
The normal group animals have showed non-significant retrieval of memory in this behavioural paradigm. In the negative control group, the animals exposed to diazepam, produced significant (P<0.001) loss of memory in behavioural paradigm, which resulted in increase in TL on day 8 as compared to day 9 (Table 3).

Table 3. Effect of Ethanolic extract of Taxillus tomentosus on transfer latency Diazepam model) by using Elevated Plus maze.

Groups	Subgroups	Transfer latency (In seconds) in Elevated Plus maze	
		8^{th} day	9^{th} day
I	Normal	20.18±1.35	19.14±1.09
II	Control (Diazepam 1mg/kg)	48.42±1.49	53.59±1.64
III	Stanadard (Mentat)	38.67±1.53	24.74±1.65***
IV	ETT 200mg/kg	43.26±1.49	34.44±1.42***
V	ETT 400mg/kg	44.80±2.33	27.59±1.92***

*All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, ***p< 0.001, as control group and [a] p<0.001, as compared to normal.*

The data represented in Figure 3, the animals treated with standard (Mentat) showed highly significant (P<0.001) decrease in the TL. ETT (200 and 400 mg/kg) treated animals produced significant (P<0.001) decrease in TL.



Effect of Ethanolic extract of *Taxillus tomentosus* on transfer latency (Diazepam model) by using Elevated Plus maze.

*All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, ***p< 0.001, as control group and [a] p<0.001, as compared to normal.*

ANTISTRESS ACTIVITY MODEL

ELEVATED PLUS MAZE MODEL

The results showed that the number of open arm entries and time spent in the open arms were increased (Table 4) and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard Diazepam and the data is represented in Figure 4

Table 4. Effect of ethanolic extract of Taxillus tomentosus on EPM model.

Treatment Groups		Elevated Plus Maze			
		Open Arm		Closed Arm	
		Time Spent	No of Entries	Time Spent	No of Entries
I	Normal	50.50±9.87	1.500±0.22	239.5±6.49	4.00±0.51
II	Control (Diazepam 2mg/kg)	161.8±1.85***	4.500±0.67***	66.50±2.50***	2.00±0.25**
III	ETT 200mg/kg	114.2±4.15***	2.333±0.42**	112.5±4.42***	3.26±0.21*
IV	ETT 400mg/kg	96.50±1.76***	1.667±0.33***	97.83±6.11***	3.66±0.42*

*All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, *p<0.05, **p<0.01, ***p< 0.001 as control group.*

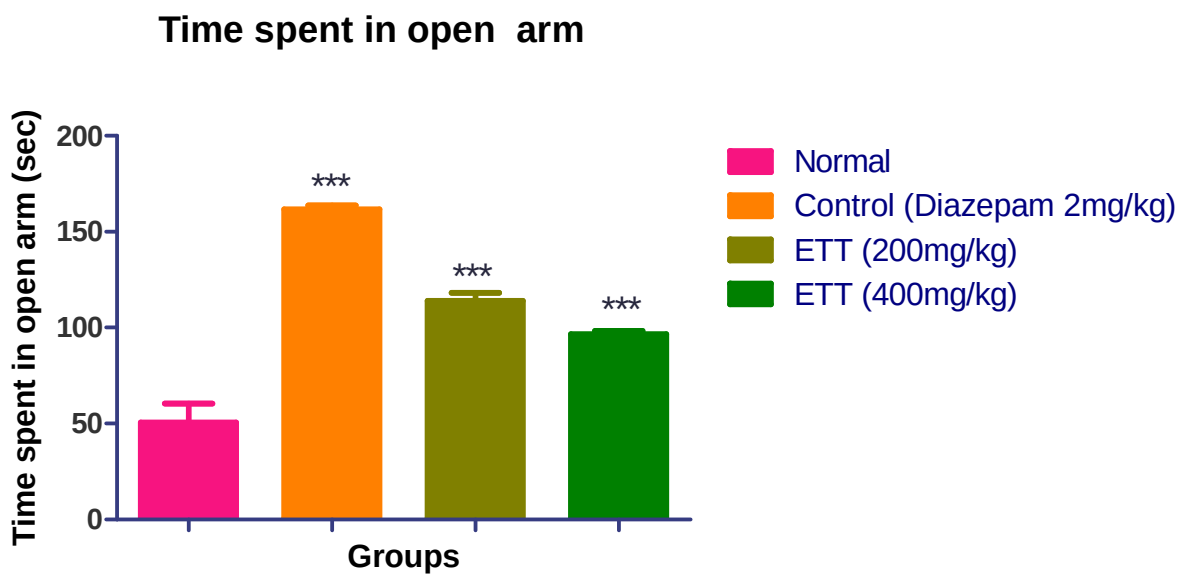


Fig. 3. Effect of ethanolic extract of Taxillus tomentosus on time spent in open arm in EPM model

*All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, ***p< 0.001 as control group.*

4 DISCUSSION AND CONCLUSION

Dementia is generally defined as "a state of serious emotional and mental deterioration, of organic or functional origin". Neurodegenerative disorders such as Alzheimer's disease, Lewy-Body dementia, Parkinson's disease and cerebrovascular dementia result in an insidious cognitive and behavioral decline culminating in the development of severe dementia.

Stress can be described as the sum of the all the reactions of the body, which disturb the normal physiological equilibrium and result in a state of threatened homeostasis. Stress is an internationally recognized phenomenon fortified by advancement of industrialization and a demanding civilization.

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.[27]

The plant taxillus tomentosus used in traditional system of medicine for nervine tonic. Preliminary phytochemistry of the plant extract revealed that the plant possessed phytoconstituents alkaloids, tannins, Phytosterols, flavonoids.

On the basis of this traditional claimed and phytochemical analysis, the present study was designed for screening of Nootropic and antistress activity of ETT.

For inducing amnesia, Scopolamine, a muscarinic antagonist was used which resulted in cognitive impairment. [28]

In the present study, Mentat was used as the standard drug. Mentat improves mental functions by modulation of the cholinergic and GABAergic neurotransmissions. By restoring the frontal cortical muscarinic and cholinergic receptor activities it improves the mental quotient, memory span, concentration ability and stress threshold. It helps in reducing the level of tribulin (endogenous monoamine oxidase inhibitor). It also ameliorates attention fluctuation and behavioral disorders. Mentat exhibits significant anti-parkinsonian activity by enhancing dopamine post synaptic receptor activity. Its sedative and tranquilizing effects offer protection against convulsions which are beneficial in insomnia. It improves articulation and corrects speech defects. [30]

Stress induced effects mainly depend on duration and type of stressors. Diazepam has been the ideal choice for the induction of stress responses in animals and more specifically, for the investigation of drug effects, on typical stress-related neuroendocrine, and immunological pathology. The distinct advantage of using diazepam as a stressor lies in the fact that it produces both physical as well as inescapable psychological stress.[31]

Exposure to stress results in adrenal hypertrophy and gastric ulceration, indicating the active involvement of the hypothalamic-pituitary-adrenal (HPA) axis. The hyper-activation of the paraventricular nucleus (PVN) of the hypothalamus during stress causes a decrease in mucosal blood flow and hyper-contractility through descending projections that induce pathogenesis of gastric ulcers.

Diazepam is reported to possess non-specific anti-stress activity involving the mesocortical dopamine system and the nor-epinephrine and 5-HT levels of whole brain and hypothalamus. The mesocortical dopamine system is thought to play an important role in the etiology of the stress response. Diazepam, an anxiolytic benzodiazepine, can reverse the effects of stress on cortical DA. Though Diazepam does not affect the brain and hypothalamic 5-HT and plasma corticosterone, it attenuates stress induced elevation of brain and hypothalamic 5-HT and also simultaneously diminishes the stress induced enhancement of plasma corticosterone levels.

Data obtained from the study shows significant dose dependent neuro-protection, memory enhancement activity of ETT which might also be useful as supportive adjuvant in treatment of stress and stress related disorders, but to establish its therapeutic value in treatment of amnesia, stress and stress related disorders, further investigations are required to characterize the active constituent(s) responsible for observed activities of the *Taxillus tomentosus* ethanolic extract.

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