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Original Research Paper

SCREENING OF *TEPHROSIA PURPUREA* FOR NOOTROPIC ACTIVITY

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ABSTRACT

Nootropic are class of psychotropic agent with selective facilitatory effect on integrative function of central nervous system, particularly on intellectual performance, learning capability and memory. Alzheimer disease is associated with decline in cognitive abilities. The most common causes of dementia in elderly is Alzheimer disease. The allopathic system is yet to provide satisfactory antidote, despite the severity and high prevalence of the disease. The cognitive effect of methanol extract of stem bark (100 and 200 mg/kg) was investigated using elevated plus maze apparatus by administering the extract in swiss albino wistar mice. Piracetam was taken as standard drug. The higher dose of plant extract decreases significantly the transfer latency as compared to control and exhibit the promising nootropic potential in a dose dependent way. In the present study phytochemical screening of methanol extract of *Tephrosia purpurea* confirm the presence of flavonoids, tannins and saponins, which may have been associated with central nervous system effect. Further investigations using more experimental paradigms are required for confirmation of nootropic potential cognitive disorder.

Keywords: *Tephrosia purpurea,* Piracetam, Nootropic, Acetylcholine.

INTRODUCTION

Memory is the ability of an individual to record sensory stimuli, events, information etc., retain them over short or long periods of time and recall the same at a later date when needed. Poor memory, lower retention and slow recall are common problems in today's stressful and competitive world.[8] Dementia is the name for progressive loss of memory and other aspects of thinking that are severe enough to interfere with the ability to function in daily activities. Although there are many causes of dementia, including blood vessel disease, drug or alcohol abuse, or other causes of damage to the brain the most common and familiar is Alzheimer's disease.[1] Alzheimer's disease is characterized by a progressive neurodegenerative disease that primarily affects the elderly population, and is estimated to account for 50-60% dementia cases in persons over 65 years of age.[7] The herbs acting on the brain are called Nootropic herbs and their isolated constituents are referred to as smart drugs, The term cognitive enhancer should not be confused with the word "Nootropic". Nootropics are by definition cognitive enhancers, but a cognitive enhancer is not necessarily a nootropic. A cognitive enhancer is a substance that enhances concentration and memory. Nootropics are smart drugs, memory enhances and cognitive enhancers.[5] In recent times, the use of herbal products has increased tremendously in the western world as well as in developing countries. Numerous natural products have been evaluated

as therapeutics for the treatment of variety of purposes, including poor memory. Memory is the most vital aspect for effective survival of human beings. It also differentiates humans from animals. Memory is the ability of an individual to record the information and recall it whenever needed. Traditionally herbal drugs have been used to enhance cognitive functions. A number of medicinal plants and medicines derived from these plants have shown memory enhancing properties by virtue of their medicinal constituents. Memories are stored in network of brain cells and form links between different brain cells, by interconnecting neuronal dendrites (Figure 1). The memory itself is thought to be stored by altering the structure of a molecule called RNA within brain cells. For a memory to be made it must enter the cell by seeing, hearing or doing something, which accounts for the three kinds of memory visual, auditory or kinesthetic. If a memory involves all three, it will exist in a maximum number of brain cells. The brain, particularly the hippocampus region, then decides whether it's worth storing. In Alzheimer's, the hippocampus loses its ability to file memories, resulting in an inability to store new memories. The best way to enhance your memory and mind, and protect yourself from memory decline, is to ensure you are taking in optimal levels of eight nutrients from which your body can make key brain chemicals. These natural mind and memory enhancers are:

- Acetylcholine precursors
  - Dimethylaminoethanol (DMAE)
  - Choline
- Receptor enhancers
  - Pyroglutamate
  - Phosphatidyl Serine
  - Docosahexaenoic acid (DHA)
- Fuel for brain cells
  - Glutamine
- Circulation improver
  - Gingko Biloba
- Vitamins
  - Vitamin B

These are becoming widely available and can be found in combination in brain boosting supplements, as well as in certain foods. Amongst the various functions of the brain, one of the most interesting is the ability to acquire new information and store it for further retrieval. Several Central Nervous System disorders (CNS) are often associated with impairment in cognitive functions. Alzheimer's disease (AD), a complex, multifactoral, progressive, neurodegenerative disease primarily affecting the elderly population is estimated to account for 50–60% of dementia cases in persons over 65 years of age. According to the World Health Organisation around 35 million people in industrialized countries suffered from AD by 2010. Alzheimer's disease has a primary impact on learning and memory. Other disorders like schizophrenia, bipolar depression are associated with secondary deficits in learning and memory functions. The mechanisms underlying learning and memory include an interaction between the various neurotransmitter systems, amongst which the central cholinergic function is known to play a prominent role. Estimation of acetylcholinesterase (AChE) activity provides a relatively easy and valuable assessment of cholinergic function.[6] Recently, the interest in the use of herbal products has grown dramatically in the western world as well as in developing countries.[4]

MATERIAL AND METHOD

MATERIAL

Collection and Identification of Plant Material

The plant specimens for the proposed study were collected from the Herbal garden of Delhi University. The plant was authenticated as *Tephrosia purpurea* by, the Department of Pharmacognosy, NIET, Greater Noida. A voucher specimen (Specimen No: NIET/M'PHARM/2014/20) is preserved in in the Herbarium section of taxonomic Department of, NBPGR, New Delhi.

Experimental Animals

Wistar albino mice of either sex weighing between 20-25 mg were used for the study. They were obtained from Central animal house of NIET, Greater Noida. They were housed in propylene cages at 25 ± 2 ºC with 12 hrs light and

12 hrs dark cycle. All the animal were fed with standard feed and water *ad libitum*. All the animals were maintained under standard laboratory condition. Study protocol was approved from the Institutional Animals Ethics Committee (IACE) and Reg. no is 1121/ac/CPCSEA/07.

Drugs and Doses

Standard Drug

Piracetam (200 mg/kg), the drug was dissolved in 10 ml distilled water and the drug is given to the rats according to the body weight.

Normal Saline Solution

0.9% w/v, the NaCl is dissolved in 100ml of distilled water and the saline is given to the rats according to body weight.

Test Drug

The extract was made suspension in the distilled water. Two doses of the extract were selected 200 mg/kg bw and 400mg/kg bw.

METHOD

Phytochemical Investigation

The coarsely powdered leaf material macerated in various solvents (Petroleum ether, Benzene, Chloroform, Methanol, Water) was subjected to phytochemical investigation. The aim of this step was to decide the best solvent among all of the above in which further extraction by soxhlet apparatus could be carried out. The best solvent would be the one in which maximum number of phytoconstituents could be detected. 10 g of powdered leaf material was soaked in 25 ml each of benzene, petroleum ether, chloroform, methanol and water for 24 hrs., filtered and subjected to various phytochemical test with respect to presence of Flavonoid, tannins, saponin, anthraquinones, reducing sugars, cardiac glycosides and fixed oils.

Tests for Tannins

- The test residue of each extract was taken separately in water, warmed and filtered. Test was carried out with the filtrate using following reagents.
  - *Ferric chloride reagent*

    Ferric chloride sol. was added to a little of the above filtrate. If dark green or deep blue colour is obtained, tannins are present.
  - *Potassium dichromate test*

    If on addition of a solution of potassium dichromate in a test filtrate, dark colour is developed, tannins are present.
- **Test for Alkaloids**
  - *Dragendroff's test*

    To the filtrate Dragendroff's Reagent (Potassium bismuth iodide) was added. A reddish brown precipitate indicate the presence of alkaloids.
  - *Mayer's test*

    To the filtrate Mayer's Reagent (potassium mercuric iodide) was added. A cream precipitate indicate the presence of alkaloid.
  - *Wagner's test*

    To the filterate Wagner's Reagent was added. A reddish brown precipitate indicate the presence of alkaloid.
- **Test for Saponins**
  - *Foam test*

    A few mg of the test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A honeycomb like froth is obtained indicates that the saponins are present.
- **Test for reducing compounds**
  - *Benedict's test*

    0.5 ml of the extract was placed in a test tube and then 5 ml benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.
  - *Fehling's Test*

    2 ml of the extract was added in 1 ml of a mixture of aqual volumes of Fehling's solutions A and B, and was boiled for few min.

- **Test for anthraquinones**

  Aqueous ammonia was added to the extract. After shaing, change in colour of aqueous layer was observed. Pink, red or Violet colour in aqueous layer indicated the presence of anthraquinones.

- **Test for sterols and triterpenes**

  3 ml extract was placed in a small beaker and avaporated to dryness. The residue was dissolved in 0.5 ml each of acetic anhydride and chloroform. The solution was transferred into a dry test tube and concentrated sulphuric acid was added. Brownish red or violet rings at the zone of contact with the supernatant and green or violet colour denote the presence of sterols and triterpenes.

- **Test for Flavonoids**

  - *Ammonia Test*

    Filter paper strips were dipped in alcoholic solution of the extract, ammoniated and observed for colour change from white to yellow.

  - *Shinoda / Pew Test*

    A small quantity of residue was dissolved in 5 ml of ethanol and treated with few drops concentrated hydrochloric acid and 0.5 g of magnesium turnings and observed for formation of pink colour.

Extraction Procedure

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. The powder of the bark extract of *Tephrosia purpurea* placed in a thimble and extracted with 50% methanol in a Soxhlet apparatus for 82-92 hrs. Solvents were removed in water bath. The residue (extract) of respective plant material was stored at 4°C until used. The extract yield (% w/w) from the plant material was recorded as 24.2%.

METHODOLOGY

Nootropic Activity

Elevated plus maze

Group of adult Swiss male albino mice 25-30 g, each consisting of 6 animals will be divided into Four groups Group I: Normal control, Group II: Standard drug (Piracetam), Group III: Low dose extract of *Tephrosia Purpurea,* Group IV: High dose extract of *Tephrosia Purpurea.* The mice were placed individually at the end of open arm of the elevated plus maze, facing away from the centre. The time taken by the mouse to move into the enclosed arm was noted as transfer latency (TL). On day 6th, TL was recorded after the 30 min of drugs administration for 90 sec. If the animal did not entered arms within 90 sec. It is gently pushed into one of two enclosed arms and TL is recorded at 90 s., mice were allowed to explore the maze for 10 sec. and then transferred to their home cages. The TL was again measured after 24 h *i.e.* 30 min after the administration of *drugs* on day 7 for 90 sec refers to memory period.

RESULT

Nootropic Activity

The observation of nootropic activity of *Tephrosia purpurea* is summarized in table as follows:

Table 1: Result of Methanolic extract of *Tephrosia purpurea*on Transfer latency is depicted in table

Treatment	Dose	Transfer latency	
		On the 8th day	On the 9thday
Control	0.9 ml	84.66±2.445	85.16±1.759
Standard (piracetam)	200 mg/kg	23.5±2.078*	25.33±1.994*
Low dose extract	200 mg/kg	30.33±1.520*	36.66±2.565*
High dose extract	400 mg/kg	27.33±1.745*	33±2.066*

The mean value ± SEM was calculated for each parameter, Results were statistically analyzed by ANNOVA followed by Dunnet's t- test. P< 0.01 was considered significant.

Piracetam (200 mg/kg) pretreatment for 7th days decreases the transfer latency on 8th and after 24 hours ie. On 9th days as compared to control group, indicating improvement in both learning and memory. Both dose of methanolic extract *Tephrosia purpurea* (200 mg/kg and 400 mg/kg) decreases the transfer latency on 8th and 9th day in mice when compared to control groups. Higher dose of methanolic extract *Tephrosia purpurea* (400 mg/kg) more significantly increases the learning and memory by marked decrease in transfer latency on 8th and 9th days compared to low dose of *Tephrosia purpurea* when subjected to elevated plus maze test. The present results show that the*Tephrosia purpurea* bark extract posess a significant nootropic effect and its effect is comparable to that of Piracetam (standard drug).

DISCUSSION

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities; patients also frequently have non-cognitive symptoms, such as depression, apathy and psychosis that impair daily living, Alzheimer's disease can occur at any age, even as young as 40 years, but its occurrence is much more common as the years go by . Dementia is one of the major causes of disability in late-life. Many diseases can result in dementia, the most common one being Alzheimer's disease. Although there are many causes of dementia, including blood vessel disease, drug or alcohol abuse, or other causes of damage to the brain the most common and familiar is Alzheimer's disease.[1] In a general sense, 'cognitive enhancers' are drugs that improve some aspect of brain function. Many such enhancers have been found and their actions tend to involve interactions between neurohumoral signalling and responses and the cholinergic system. Neurohumoral signalling is a type of slowacting chemical communication in the nervous system, which contrasts with the fast-acting communication of neurotransmitters. Neurohumoral signalling often occurs between the central nervous system and peripheral tissues and can influence motivational and emotional states. Cholinergic is pertaining to acetylcholine neurotransmission.[3] Memory enhancers and cognitive enhancers are drugs, supplements, nutraceuticals and functional foods that are purported to improve mental functions such as cognition, memory, intelligence, motivation, attention and concentration. Nootropics are thought to work by altering the availability of the brain's supply of neurochemicals (neurotransmitters, enzymes, and hormones), by improving the brain's oxygen supply, or by stimulating nerve growth.[2] Nootropic agents have selective facilitatory effect on integrative functions of the central nervous system particularly on intellectual performance, learning capacity and memory. Piracetam, the first representation of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. Repeated injections of piracetam had improved learning abilities and memory capacities of laboratory animals. Passive avoidance behavior is based on negative reinforcement and is used to examine long-term memory. Apart from central neurotransmitters, impairment of cerebral metabolism and cerebral blood flow are known to induce cognitive deficits, and, it is proposed that the beneficial

effect of nootropics may be the result of improvement in cerebral circulation and brain metabolism. Both piracetam and *Tephrosia purpurea* meet major criteria for nootropic activity, namely improvement of memory in absence of cognitive deficit. In the present study, *Tephrosia purpurea* significantly inhibited the AChE activity in the mice whole brain homogenate, indicating its potential in the attenuation of symptoms of cognitive deficits. Biochemical estimation of different parameter as mentioned above show the elevation of acetylcholine level by significant reduction of acetyl cholinesterase activity in brain and decreased level of serum cholesterol and glucose level of young and aged mice. Furthermore *Tephrosia purpurea* administration decreased the increase potential of MDA level, an indicator of lipid per oxidation index and increased level of reduced glutathione a potential element of free radical scavenging cycle in the brain as compared to control group of young and aged mice. Therefore, it appears that *Tephrosia purpurea* bark may possesses the memory improving capacity or useful in the treatment of the disorder related to memory deficits specially Alzheimer's disease, in the view of its (i) AChE inhibitory activity (ii) cholesterol and glucose lowering activity (iii) on the basis of its antioxidant property a significant decreased in MDA level and sharp increase in antioxidant process by increase in reduced glutathione level in mice brain. Further investigations using more experimental paradigms are required for further confirmation of nootropic potential of leaves of *Tephrosia purpurea* in the treatment of various cognitive disorders. The "Nootropic Revolution" began with the development of Piracetam in the late 1960's. The second wave of the nootropic revolution occurred in the late 1970's with the development of the Piracetam analogues Oxiracetam, Aniracetam, and Pramiracetam. The Piracetam-nootropics have been exhaustively researched; since the first scientific studies on Piracetam in the late 1960's over 1000 scientific papers on Piracetam, Oxiracetam, Pramiracetam, and Aniracetam have been published with about two thirds of them on Piracetam. Piracetam, Oxiracetam, Pramiracetam, and Aniracetam all attenuate or reverse the amnesia in mice and rats induced by electroconvulsive shock treatment in both passive and active learning conditions.Piracetam, the first representation of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. The present study suggests that *Tephrosia purpurea*is a potential anti-cholinesterase agent. It also possesses nootropic activity in view of its facilitatory effect on retention of learned task. Central cholinergic system plays an important role in learning and memory. The higher dose of plant extract decreases significantly the transfer latency as compared to control and exhibit the promising nootropic potential in a dose dependent way. In the present study phytochemical screening of methanol extract of Tephrosia purpurea confirm the presence of flavonoids, tannins and saponins, which may have been associated with central nervous system effect. Both piracetam and Tephrosia purpurea meet major criteria for nootropic activity, namely improvement of memory in absence of cognitive deficit. Further investigations using more experimental paradigms are required for further confirmation of nootropic potential of bark of Tephrosia purpurea in the treatment of various cognitive disorders.

CONCLUSION

From this study, it is clear that the herbals play a vital role against poor memory. Various herbal plants and plants extracts have significant memory improving activity in animal models. They have anti-acetylcholinesterase property and may be useful as a nootropic agent in delaying the onset and reducing the severity of Alzheimer's Disease when compared with that of reference drugs. The memory improving activity is probably due to the presence of flavonoids in almost all these plants. A variety of botanical products have been reported to possess memory improving activity; finally, it should be noted that substances such as flavonoids, and tannins that possess memory improving. These memory enhancing agents showed potential acting on

cognitive functions by maintaining the Acetylcholine (Ach) level in the brain activity are of particular therapeutic importance. The results of this study indicate that extracts of leaves and plants extracts of some medicinal plant have good potential for use in poor memory. It has been attributed with a plethora of physiological effects that could potentially benefit cognitive performance or mood. Several medicinal plants used in ayurvedic polyherbal formulations for curing the dementia and so many medicinal plants showing the memory enhancing property under several researcher studies. The methanolic extract of *Tephrosia purpurea* showed significant increase in the onset of action and decrease in duration of action and recovery of time as compared to control thus justifying its nootropic activity which may be due to presence of saponins and flavanoid as a phytoconstituent present.

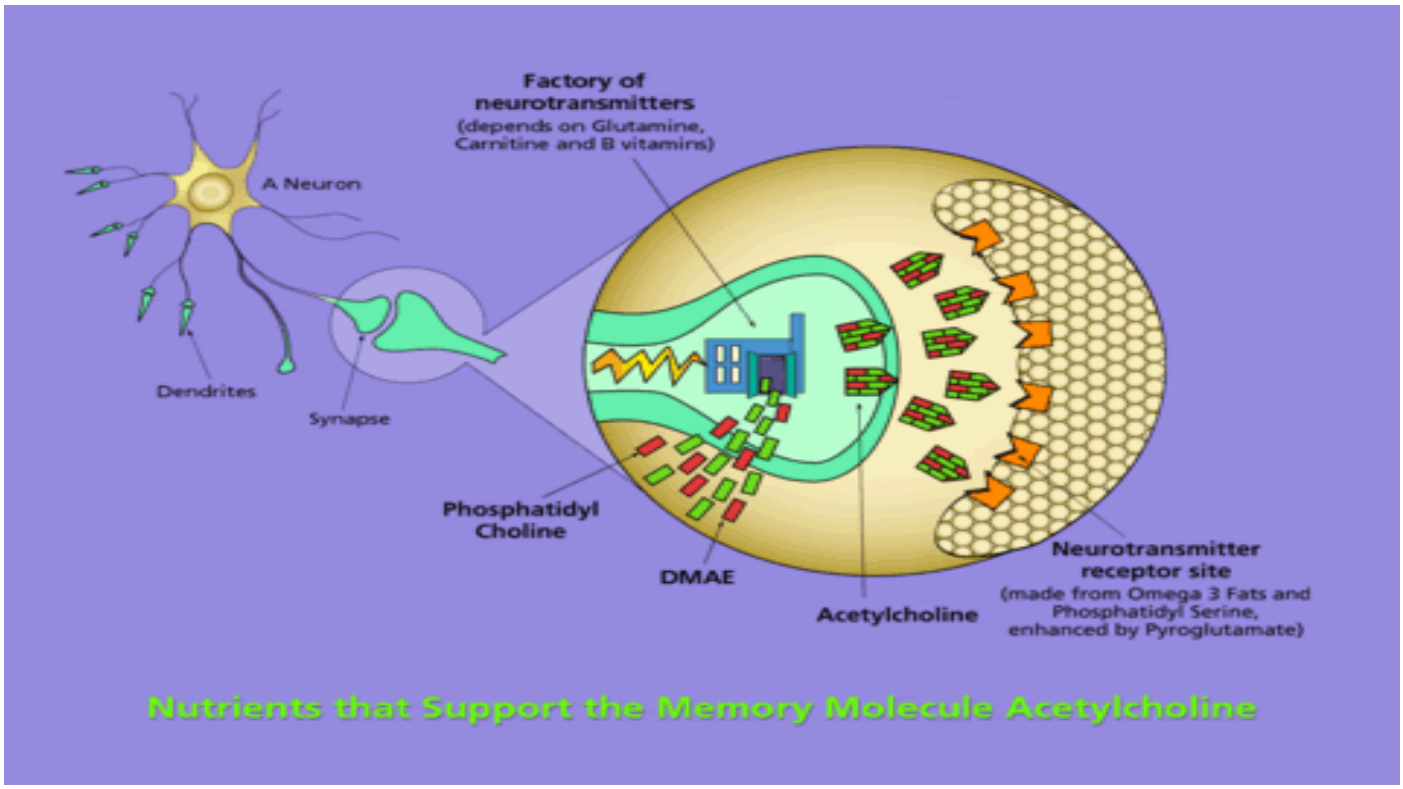

Figure 1: Network of brain cell

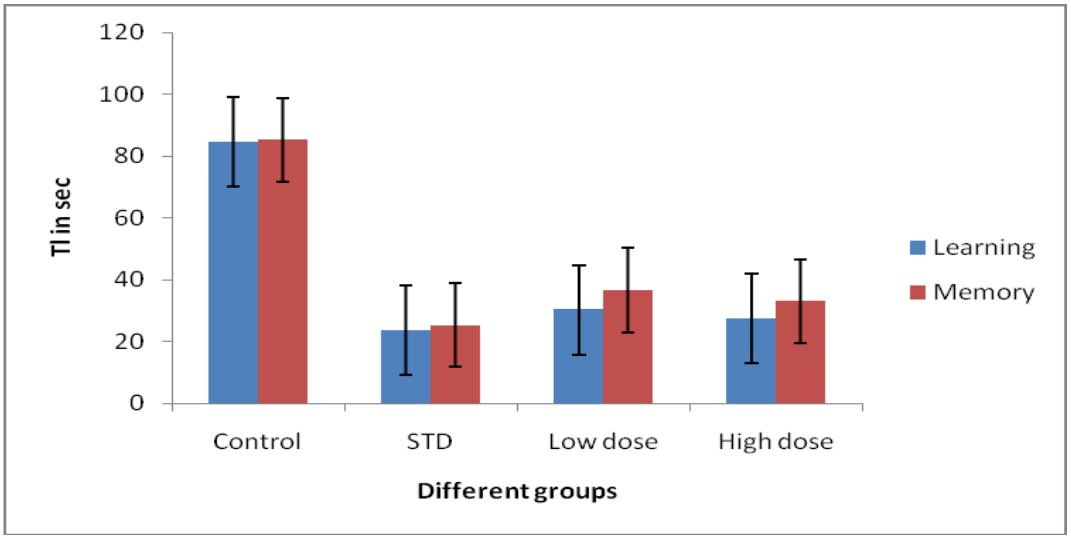

Figure 2: Effect of *Tephrosia purpurea on* TL in mice using Elevated plus maze apparatus

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