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MEMORY ENHANCING ACTIVITY OF *TAVERNIERA CUNEIFOLIA* (ROTH) ARN: A SUBSTITUTE FOR COMMERCIAL LIQUORICE

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ABSTRACT

The present study was undertaken to evaluate the potential effect of methanolic and aqueous extracts of *Taverniera cuneifolia* (Roth) Arn. for cognitive performance activity. Memory impairment was produced by administration of Scopolamine (2 mg/kg i.p) in Swiss albino mice. Elevated plus maze was used to asses learning and memory activity. *Taverniera cuneifolia* extracts treated group decreased transfer latency in elevated plus maze which is an indicative of cognition improvement. The results of HPTLC and HPLC study of *Taverniera cuneifolia* indicate the presence of Glycyrrhetinic acid as an active phytochemical constituent. Methanolic and aqueous extracts of *Taverniera cuneifolia* has been demonstrated to improve cognitive process by enhancing memory in Elevated plus maze. The present study suggests that methanolic and aqueous extracts of *Taverniera cuneifolia* might have increased brain acetylcholine level and hence it possess memory enhancing activity in Scopolamine induced amnesia model.

KEYWORDS: ***Taverniera cuneifolia*, glycyrrhizin, learning and memory, HPLC and HPTLC**

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INTRODUCTION

Memory is an unique cognitive function of brain. Central cholinergic system is considered as the most important neurotransmitter involved in regulation of cognitive functions. Cholinergic neuronal loss in hippocampal area is the major feature of AD and enhancement of central cholinergic activity by use of anti cholinesterase is presently the mainstay of the pharmacotherapy of dementia in AD. [3, 4.] Anticholinesterase from general chemical classes such as physostigmine, tacrine, galantamine and heptylphysostigmine has been tested for the symptomatic treatment of AD[2]. However, non-selectivity of these drugs, their limited efficacy, and poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges and hepatotoxicity are among the several limitations to their therapeutic success[3]. Therefore, the utility of traditional medicines for the treatment of cognitive disorder has been explored[5]. Alzheimer disease (AD) impaired cognitive function [6,7] . Centrally acting anti-muscarinic drugs (e.g. Scopolamine) impair learning and memory[8, 9]. In the recent years, several plants have been reported to possess nootropic activity [10-14]. Herbal medicine emphasizes prevention of disease rejuvenation of our body system and it extends the life span and makes life healthy [15, 16]. Medicinal plants are indispensable part of traditional medicines and extensive research is done all over the World due to easy access, low cost and lesser side effects. Plant extract may also provide a source of new compound as many synthetic drugs have been originated from herbal source. The genus *Taverniera* belonging to the family of Fabaceae, includes twelve species and is endemic to the Northeast African and Southwestern Asian countries[17]. *Taverniera cuneifolia* (Roth) Arn. popularly known as Indian liquorice is an herb and occurs along the banks of small streams. Roots of this plant taste sweet and are used by the tribals as a substitute for the commercial liquorice- *Glycyrrhiza glabra*[18]. The roots of *G. glabra* are widely used in traditional systems of medicines all over the world [19] and are rich in bioactivities like antiulcer, anti-inflammatory[21], antibacterial [22], antimalarial[23], antithrombic[24], antidiuretic [25], anti-atherosclerotic, antifungal, estrogenic[26], antiallergic[27], antidiabetic[28] and antimutagenic activities [29]. *G. glabra* extract, glycyrrhizin and its derivatives are reported to inhibit growth of viruses like HIV, [30] SARS[31] , Hepatitis B & C[32] , Influenza through the potentiation of immune system , inhibition of reverse transcriptase and induction of interferon production. The above reported pharmacological effects of plant reflects the important medicinal properties of *Taverniera cuneifolia*. The scientific literature does not found to report memory enhancing activity of *Taverniera cuneifolia.* Thus the present study was designed to investigate anti-amnesic effect of methanolic and aqueous extracts of *Taverniera cuneifolia* in scopolamine induced cognitive deficit animals.

MATERIALS AND METHODS

Plant material

The roots of *Taverniera cuneifolia* were collected from Osmanabad District in the state of Maharashtra of India. The plant material was authenticated by Dr. R.M Mulani, Botany Department, S.R.T.M. University, Nanded, India. A voucher Specimen (No. SRTMU/SLS/2011-103) was deposited in the herbarium of the School of Life Sciences, SRTM University, Nanded (MS) India.

Preparation of Extract

Roots of *Taverniera cuneifolia* were washed with running tap water and distilled water to remove the dirt and soil and shade dried. The dried material was powdered and passed through a sieve. The coarsely powdered material was extracted with methanol (50% v/v) and distilled water by using Soxhlet's extraction method. The extracts were filtered and concentrated at high vacuum. (Total yield 4.5% w/w).

Drugs Treatment

Suspensions of aqueous and methanolic extracts were prepared in 0.5% carboxymethyl cellulose using tween 80 (0.2% v/v) as a suspending agent. The extracts were administered orally in a dose of 150 and 300 mg/kg respectively to mice for 10 days. Control groups were given only 0.5% carboxymethyl cellulose with tween 80 (0.2% v/v). The *Taverniera cuneifolia* drug extracts caused no abnormality or death during the course of treatment.

Animals

All the experiments were carried out using adult male Swiss Albino mice weighing about 25-30 gm. The animals had free access to food and water, and they were housed in a natural light–dark cycle (12 hrs each). The animals were acclimatized to the laboratory conditions for at least 5 days before behavioral experiments. Experiments were carried out between 0900 h and 1800 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Sudhakarrao Naik Institute of Pharmacy Pusad, District Yavatmal and the care of laboratory animals was taken according to the guidelines of CPCSEA, Ministry of Environment and Forests, Government of India (Registration number 729/02/a/ CPCSEA).

Design of Elevated plus maze

EPM was employed for measurement of transfer latency (TL). It consists of two open (16× 5 cm) and two enclosed arms (16× 5 ×12 cm) facing each other with an open roof. The maze was elevated at a height of 25 cm from the ground. The animals were placed individually 90 min after above treatment at the end of open arm facing away from central platform and the time it took to move from open arm to either enclosed arm (TL) was recorded on the 10^{th} day of treatment (training session). The TL was again recorded 24 hrs after 1^{st} exposure (i.e. on 11^{th} day). The TL measured on 1^{st} and 2^{nd} exposure served as parameter for acquisition and retrieval memory respectively [4, 20].

Mice were randomly divided into 7 groups of 5 animals each. The total treatment period was 10 days (Table-1). Group 1 and 2 were control and were received vehicle, group 3 to 6 were received methanolic and aqueous extract of *Taverniera cuneifolia* (150 and 300 mg/kg p.o.) respectively; group 7 animals received standard piracetam (400 mg/kg p.o.) for 10 days. On 10^{th} day all animals were injected with Scopolamine (2 mg/kg i.p.) 30 min after treatment and 60 min after injection. transfer latency (TL) in time (second) was recorded respectively.

Phytochemical Analysis

Phytochemical analysis was done by extracting 5 g of dried root powder with methanol for 12 hrs in soxhlet apparatus. The quantitative determination was done by HPLC (Perkin Elmer Quaternary pump Series 200). The isocratic mobile phase consists of 1% acetic acid: acetonitrile in the ratio of 80:20 v/v, flowing through the column at a constant flow rate of 1.0 ml/min. A Hypersil ODS C18 column (250mm × 4.6mm, 5 µ) was used as the stationary phase. Considering the chromatographic parameter, sensitivity, and selectivity of the method for sample, 254 nm was selected as the detection wavelength for PDA detector. The injection volume was 20 micro liter. Glycyrrhizin as 18-beta Glycyrrhetinic acid was detected at 254nm with retention time 2.7 ± 0.3 (Fig 1A) with commercially available known quantity of glycyrrhizin as reference compound.

Statistics

Results were expressed as mean ± SD of 5 mice in each group. Student's test was used to determine the statistical significance between the control group and the test groups.

RESULTS

Cognitive performance activity

Results of aqueous and methanolic (150 and 300mg/kg p.o.) extracts of *Taverniera cuneifolia* demonstrate to produce cognitive performance

activity dose dependently. The significant ($p < 0.05$) cognitive performance activity was found at higher dose level i.e. 300 mg/kg for both extracts which was comparable with standard cholinergic agent Piracetam (400 mg/kg p.o.) indicating the extracts improve the learning and memory of mice (Table 1)

Phytochemical Analysis

The results of preliminary phytochemical analysis indicate presence of saponins, flavonoids, glycosides. The HPLC and HPTLC results of the study demonstrate the presence of glycyrrhizin, which is an active ingredient in root extract of *Taverniera cunefolia*.

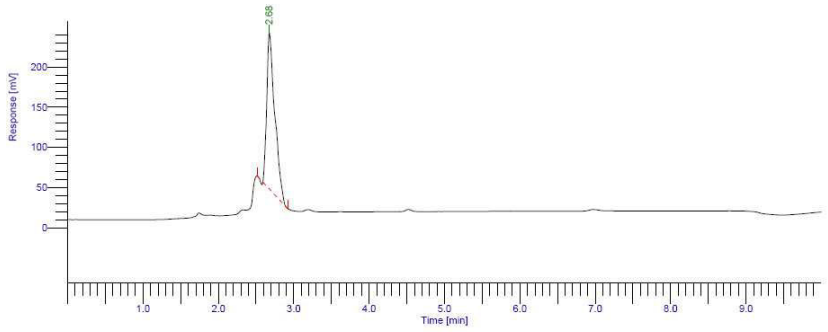
Table 1

Treatments	Acquisition memory (Sec)	Retrieval Memory (Sec)
Vehicle control	24.41± 6.92	14.03 ± 3.12[a]
Scopolamine 2mg/kg	58.09±15.05[*]	30.47±7.66 [a]
Aqueous extract (150 mg/kg)	42.08±9.92	20.8±5.34 [a]
Aqueous extract (300 mg/kg)	33.72±7.74[**]	17.95±5.5 [a]
Methanolic extract (150 mg/kg)	41.05±10.2	20.92±6.71 [a]
Methanolic extract (300 mg/kg)	36.3±10.09[**]	17.52±5.22 [a]
Paracetam (400mg/kg)	30.46±7.32	14.82±4.41 [a]

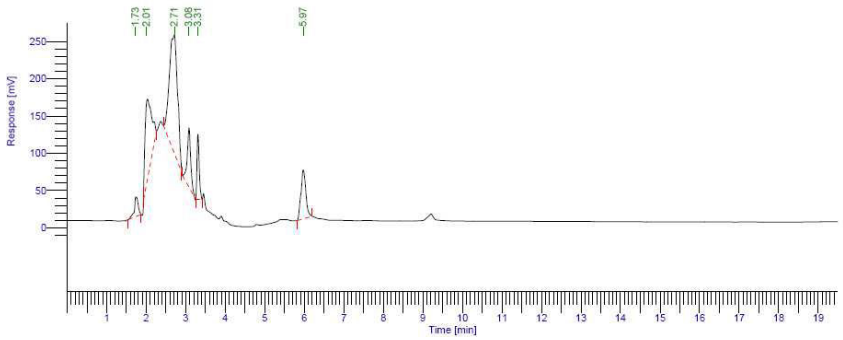
Effect of aqueous and methanolic extracts on Transfer latency (TL) in mice using Elevated Plus maze. Piracetam (400 mg/kg, p.o.) was used as standard. Values are mean ± SD (n = 5). **denotes significantly (p < 0.05) protect from amnesic effect of scopolamine. *denotes Scopolamine produced significant (p< 0.01) amnesia in mice which represent by increased in TL on day 1st compare to control. [a]denotes significant (p< 0.01) decreased in TL on 2nd day represent the retrieving the memory (Student's unpaired t-test).

Figure 1

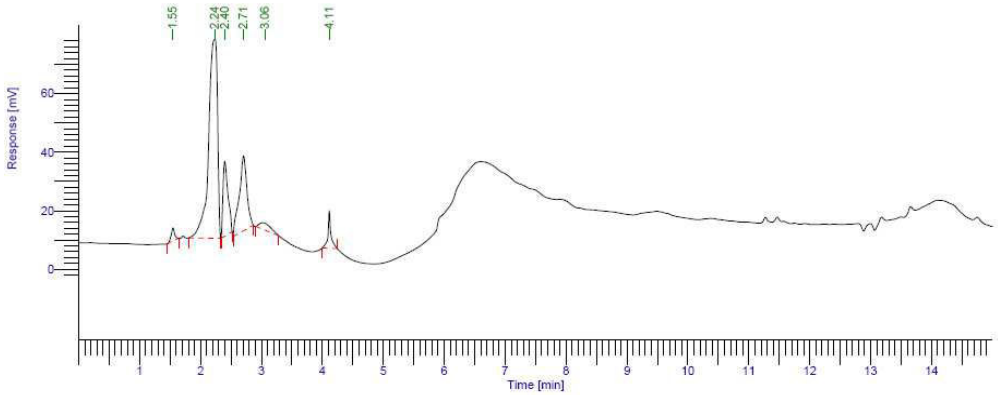
HPLC Profile of G .glabra, Taverniera cuneifolia and Standard Glycyrrhizin



1.1 Standard – Glycyrrhizin



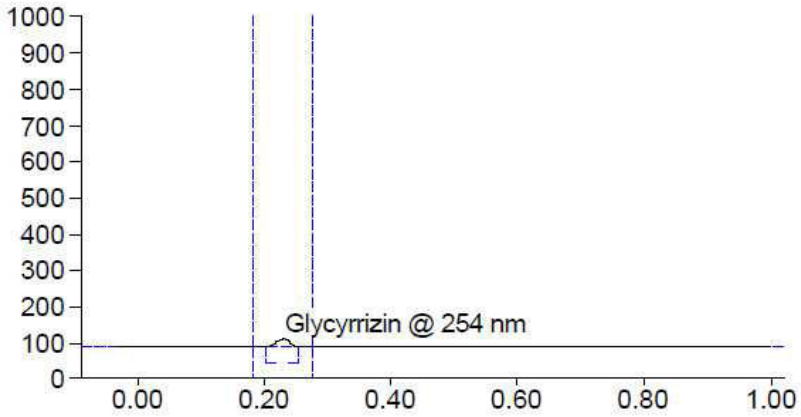
1.2 .Roots G .glabra contain glycyrrhizin



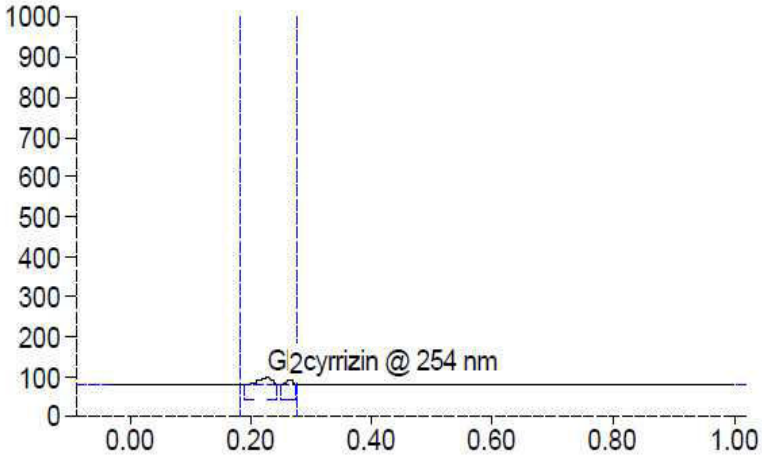
1.3. Roots of Taverniera cuneifolia contain glycyrrhizin

Figure 2

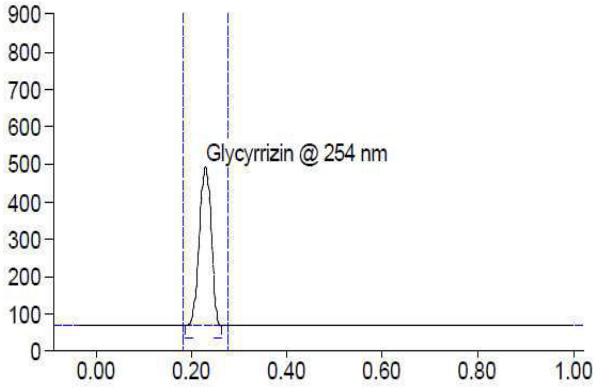
HPTLC fingerprinting of G .glabra, Taverniera cuneifolia and Standard Glycyrrhizin



2.1 Roots G .glabra contain glycyrrhizin



2.2 Roots of Taverniera cuneifolia contain glycyrrhizin



2.3 . Standard - Glycyrrhizin

DISCUSSION

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions [4, 20]. In the present study, cholinergic muscarinic antagonist scopolamine, the drug most widely used to induce amnesia in experimental model was used. Anticholinesterase which enhances the availability of acetylcholine in synaptic cleft and is able to reverse the scopolamine induced deficit indicating a neurotransmitter role of acetylcholine in learning and memory [20]. The present study was undertaken to investigate the anti-amnestic effect of methanolic and aqueous extracts. To assess efficacy, scopolamine was used to induce memory impairment in mice, and impairment was evaluated using elevated plus maze method [36, 37]. Elevated plus maze was used to measure the anxiety state in animals. However, transfer latency was markedly decreased if the animal had previous experience in entering open and closed arms and this shortened transfer latency has been shown to related with memory process. Recent studies of several nootropic and amnestic agents on elevated plus maze is a widely accepted to study learning and memory process in mice [38, 41] In the present study of *Taverniera cunefolia* methanolic and aqueous extracts (150 mg/kg, 300mg/kg) were administered orally for 10 days. The results of study demonstrate that the scopolamine produced a significant (p <0.01) increase in transfer latency on day as compared to control indicating impairment of memory. Scopolamine induced increase in transfer latency was however reversed by dose dependent on 10th day prior to the administration with aqueous and methanolic (150 and 300mg/kg p. o.) extracts respectively while the significant (P < 0.05) effect was observed with higher dose of both extracts which were comparable to standard cholinergic agent piracetam (400mg/kg p. o.) indicating the extracts improve the learning and memory of mice. The phytochemical tests of methanolic and aqueous extracts of *Taverniera cunefolia* demonstrate the presence of various phytoconstituents such as saponins, flavonoids and glycosides. It is known that carbenoxolone, one of the oleandane derivatives prepared from *G. glabra* possess considerable mineralocorticoid activity[42] which may be due to the similarity in structure of glycyrrhetic acid to the structure of hormones secreted by adrenal cortex [43]. The roots and rhizomes of *G. glabra* have been studied with respect to spatial learning and passive avoidance [44] in rats and reported to produce memory enhancement activity [43]. The results of chromatographic and spectral analysis of root extract of *T. cuneifolia* and *G. glabra* has exhibited similarity in chemical profile both in HPLC as well as in HPTLC studies (Fig 1 and 2). The similar

chromatophores included the sweetening principle, glycyrrhizin. The saponin compounds have also been reported to show nootropic activities [44]. From these findings it suggests that *Taverniera cunefolia* extracts containing glycyrrhizin may be responsible for memory enhancing activity in the present study. The memory enhancing activity in the present study may be contributed by inhibiting the cholinestrarese enzyme there by elevating acetylcholine concentration in the brain. These findings suggest that there is neuroprotective role of *Taverniera cunefolia* for memory enhancement activity.

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