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A research on screening of learning and memory enhancing activity of whole plant extract of *Tinospora cordifolia* (Willd)

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Abstract
In the present study, we examined the learning and memory effect of whole plant of *Tinospora cordifolia*. Dried and powdered whole plant of *Tinospora cordifolia* was extracted with hydro alcohol by soxhlet extraction method and the influence of plant extract on spatial recognition memory was studied using Y-maze. Spatial memory was measured by the total number of entries and duration spent in each arm of the Y-maze following administration of the extract with the reference to a standard. Transfer latency (TL) in the Ymaze was expressed as inflexion ratio (IR). The number of arm visits during the first trial and second trial period were measured as an index of locomotor activity. Diazepam (100mg/kg) was used to induce amnesia in animals. The results indicated that after 24 h, the plant extract shown significant reduction in transfer latency when compared to the reference standard.

Keywords: Y-maze, memory enhancing activity, *Tinospora cordifolia*, Transfer latency

1. Introduction
Learning is defined as the acquisition of information and skills, while subsequent retention of that information is called memory. One of the challenging tasks for neuroscientists is to elucidate the biochemical and molecular mechanisms underlying learning and memory. To assess the learning and memory paradigms in laboratory animals, mazes are used conventionally.
Tinospora cordifolia (Willd.) Miers is a climbing deciduous shrub belonging to the family Menispermaceae. It is found throughout India & also in Srilanka, Bangladesh and China. This plant is commonly referred to as *Amrita* or *Guduchi*. It requires fair moisture level and can be grown in a wide range of soil, from acid to alkaline. In Ayurveda, the drug is known for building up the immune system and body's defence against definite infecting organism. *T. cordifolia* is an endangered rasayana herb. In Ayurvedic system of medicine, it has a special place as a effective adaptogen and aphrodisiac. It is used for immunostimulant, antioxidant, anticancer and anti diabetic properties. Guduchi is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, antiperiodic, antispasmodic, anti-inflammatory, antiarthritic, anti allergic and anti diabetic properties. Earlier studies reported the anti osteoporotic, hepato protective, immunomodulatory, antihyperglycemic, anti-tumor, anti-HIV properties of *Tinospora cordifolia*. The stem of *Tinospora cordifolia* is one of the constituents of several ayurvedic preparations used in general debility, dyspepsia, fever and urinary diseases. The stem is bitter, stomachic, diuretic stimulates bile secretion, prevents from constipation, burning sensation, vomiting, enriches blood and cures jaundice.

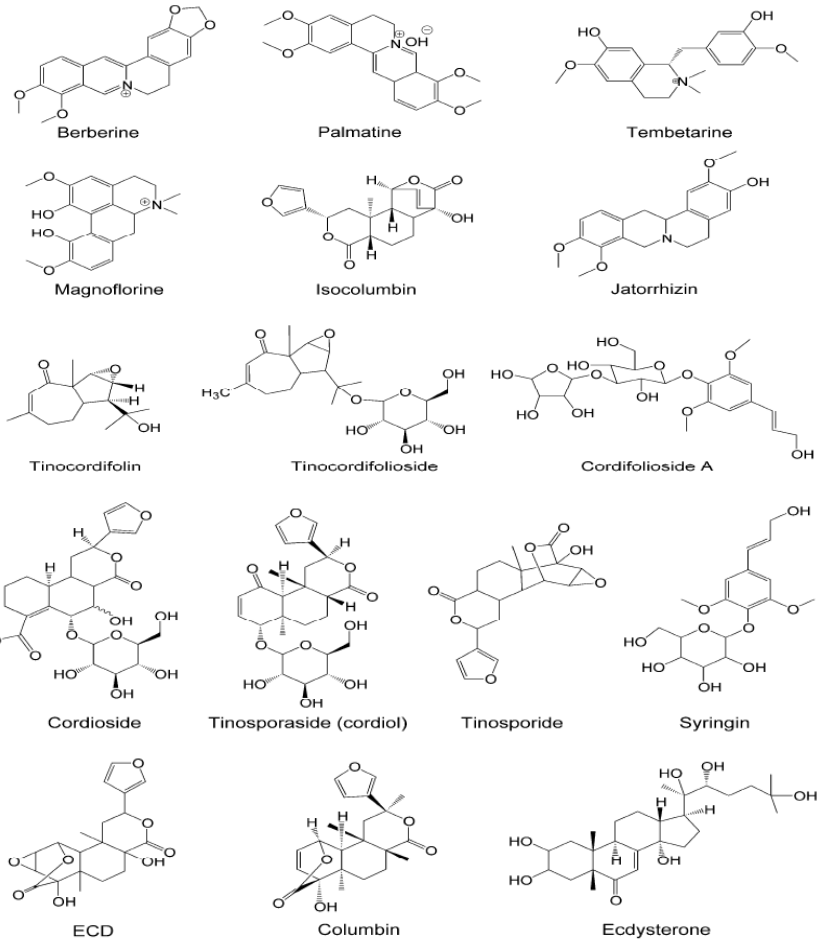
Chemical Composition
Constituents isolated from Tinospora cordifolia belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides.

Literature Review
Jitendra Mittal 1, Madan Mohan Sharma 2*, Amla Batra 3 in 2014, Traditional systems of medicine such as Ayurvedic, Uninai, Siddha and Homeopathy (AYUSH) have been in practice in a great account. Owing to population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments as witnessed by the use of folk medicines in the present scenario. This review article describes the prominence of a medicinal plant Tinospora cordifolia in therapeutics such as use of crude extract of plant for the amelioration of various

diseases, morphology, growth constraints, biochemical composition, biological activities, research work done, projects sanctioned to this plant species and the future prospects of this important neglected plant species for research in the field of plant tissue culture, natural products and nano-biotechnology.

Neha Upadhyay, Showkat Ahmad Ganie*, Rajneesh K Agnihotri, Rajendra Sharma In the present study, we examined the anti-oxidant effects of Tinospora cordifolia stem. Dried and powdered stem of Tinospora cordifolia was extracted with ethanol and methanol. Total phenolic content of different solvent extracts were determined, to find the correlation between phenols and antioxidant activity. Ascorbic acid was used as standard. Antioxidant assay was carried out by using DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical scavenging activity. Ethanolic stem extract showed the highest free radical scavenging activity (56.35%). The antioxidant activity of methanolic extract was poor when compared to the ethanolic extract. Ethanolic stem extract had the highest phenol content of 66.28 ± 0.82 mg/g. These results suggest that, Phytocompounds were better extracted in ethanol and there is direct correlation between the total polyphenols extracted and its antioxidant activity. Anju Meshram1, Sameer S. Bhagyawant 2, Sanskriti Gautam 3 and Nidhi Shrivastava 4*in 2013, Tinospora cordifolia (Giloy) is one of the most important medicinal plant and the forms an important drug of the Ayurvedic system of Medicine. It is prescribed for many diseases such as general debility, fever, diabetes, dyspepsia, urinary infections, jaundice and skin diseases. This paper presents a critical review on biological description and chemical constituents of T. cordifolia with special emphasis on its ayurvedic properties and applications in the pharmaceutical sector.

L.N. SANKHALA, R.K. SAINI, B.S. SAINI in 2012, Tinospora cordifolia is an important herb in folk and ayurvedic systems of medicine. This paper presents a critical review on chemical and biological properties of T. cordifolia. Tinospora cordifolis contain various chemical constituents belonging to different classes such as alkaloids, diterpenoid lactones, glycosides and steroids. The most important biological properties reported are antioxidant, anti-diabetic, anti inflammatory, anti-arthritic, anti-stress, hepatoprotective, immunomodulatory and anti-neoplastic activities



All the experimental studies and chemical examinations were performed in the laboratory and CPSEA registered animal house.

Chemicals used

Standard-Diazepam (100 mg/kg)- Neon laboratories Ltd.

The drug was diluted in CMC and administered intra peritoneally 30 min prior to behavioral testing.

Control-CMC

Animals: Male Wistar rats (120-150g body weight) were obtained from the animal house of the department of pharmacology. Animals were housed five to a cage and maintained at room temperature under a 12 h light/dark cycle with free access to food and water. They were acclimatized to the laboratory conditions 7 days before behavioral studies.

Extraction procedure Soxhelet extraction procedure

- ✓ Whole plant were collected and shade dried for 16 days

- ✓ Allowed to crush in an electric grinder and powdered
- ✓ Then the powdered material is subjected to soxhelet extraction method for 72 hours
- ✓ Solvent used- Ethanol(70%)- 200 ml
- ✓ Powder quantity- 50g

Experimental procedure(Y-maze test)

Thirty minutes before the first trial (training), the animals received test- of the plant extract in a volume of 0.1ml/100g body weight. Control group- were injected (i.p.) with CMC. Standard group-served as the positive control received diazepam (100 mg/kg). For the first trial, rats were placed inside the start arm while the novel arm was blocked with a block. Therefore, rats were able to explore the start and other arms, but not the novel arm during a 20min period. Memory retrieval (second trial) was evaluated in a test session carried out 24 h after the first trial. For this trial, trained animals were placed back in the maze in the same starting arm, with free access to all three arms for 20min. TL was recorded for each animal in each trial and expressed as inflexion ratio (IR). IR was calculated by the formula described by Jaiswal and Bhattacharya (1992):

Inflexion ratio= (L1-L0)/L0

Where L1 is the initial TL (sec) and L0 is the TL (sec) after 24 h.

To measure spatial recognition memory, the number of entries and time spent in each arm of the maze by each rat was recorded and novelty versus familiarity was analyzed by comparing behavior in all three arms. The number of arms visited was taken as an indicator of locomotor and exploratory activity.

Acute Toxicity Test

Acute toxicity studies of the synthesized compounds were carried out using OECD/OCED guideline 423. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxic**.**

Procedure

Healthy young adult albino rats of either sex (normally females) were used for this study. Females should be nulliparous and non- pregnant. Each animal was 8 to 12 weeks old at the commencement of dosing. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound- related mortality of animals dosed at one step will determine the next step. Animals were fasted prior to dosing (food but not water should be withheld overnight). Following the period of fasting, the animals were weighed and the test substance administered orally. After the substance has been administered, food was withheld for a further 3-4 hours. The dose level used as the starting dose is selected from one of four fixed levels, 5, 50,300 and 2000 mg/kg body weight. As there is no information on the substance to be tested, the starting dose is 300mg/kg. The animals are observed individually after dosing, at least once during the first 30 minutes, periodically during the first four hours and thereafter for a total of 14 days. Body weight of the rat before and after treatment will be noted. Any change in skin colour, fur, eyes, locomotor activity and behavioral pattern will be observed and also signs of tremors, convulsions, diarrhoea, lethargy and sleep were noted.

Results and Discussion

The rats showed a significant decrease in transfer latency in all groups (including control) on the second day. The analysis revealed significant differences in transfer latency in EPM performance between plant extract (500 mg/kg) and vehicle-treated animals on both the days.

Discussion

The two-trial Y-maze is a specific and sensitive test of spatial recognition memory in rodents. The test relies on an innate tendency of rats to explore a novel environment but not on learning a new behavior or rule. Some studies have also used the number of arm visits as an index of locomotor activity as well. The Y- maze used in this study involves no aversive stimuli and was considered suitable for evaluating memory. The experiment described in this communication, to the best of our knowledge, is the first to report that pre-training administration of whole plant extract of T.cordifolia caused deficit in the rat's acquisition of spatial recognition memory.

Treatment	Dose (g/kg)	Inflexion ratio(Mean±SEM)
Control	0.5	0.2±0.01**
Test	0.5	0.5±0.01**
Standard	0.023	0.1±0.002**

Conclusion

The Y- maze is used to measure the anxiety state in animals; however transfer latency *i.e.* the time elapsed between the movements of the animal from an open to an enclosed arm was markedly shortened if the animal had previously experienced entering open and closed arms, and this shortened transfer latency has been shown to be related with memory processes. Recent studies of several nootropics and amnestic agents on EPM made this model a widely accepted paradigm to study learning and memory processes in rodents. In EPM, acquisition (learning) can be considered as transfer latency on first day trials and the retention/consolidation (memory) is examined 24 h later. The animals treated with plant extract showed a significant decrease in transfer latency as compared with the control group, which is an indication of the cognitive enhancer effect of *T.cordifolia* in rodents.

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