Neuroprotective potential of *Phyllanthus niruri* on diazepam induced amnesia in mice

[1]Jayesh H. Lahoti, [2]Anjali M. Wankhade, [3]Vivek Paithankar, [4]J.V. Vyas

Department of pharmacology
Vidyabharati college of pharmacy, Amravati.

Abstract-
Phyllanthus niruri is a traditional medicinal plant used for the treatment of diseases such as kidney stone, inflammation in South east Asian countries. This study was carried out to evaluate the potential neuroprotection effect of the methanolic extract of the leaves of Phyllanthus niruri against diazepam-induced amnesia in mice. Thirty adult male mice were distributed into five groups: the two test groups received methanolic extract of leaves of Phyllanthus niruri (200, and 400 mg/kg), the normal control group received saline water, a positive control group received donepezil (5 mg/kg), and the negative control received diazepam (1.75 mg/kg). Learning and memory were evaluated using the Elevated plus maze apparatus . Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Phyllanthus niruri leaves methanolic extract decreased the transfer latency time in amnesic mice evaluated in the elevated plus maze. These results suggest that the methanolic extract of Phyllanthus niruri leaves may possess antioxidant potential and might provide an opportunity for the management of neurological abnormalities in amnesic conditions.

Keywords: Amnesia, Benzodiazepine, Bacopa monniera, Cholinergic, Diazepam, GABAergic, Elevated Plus Maze.

1. INTRODUCTION

The 'amnesic syndrome' contains a generally tall profile both within the neuropsychological writing and in prevalent culture. Typically likely due in portion to the centrality of memory in characterizing our put within the world and sense of self, in empowering compelling regular working, and of the often-striking misfortune of memory work in patients with amnesia, relative to sound people. The term is determined from the Greek a- (without) - mnesis (memory), and at a wide level, amnesia can be defined as a significant misfortune of memory. The broad impacts of this condition cruel that people with amnesia ordinarily require assistance in existence . Amnesia can be transitory [1]or have a mental root (for case, temporal worldwide amnesia and psychogenic/dissociative amnesia[2]) and may be a term utilized in reference to memory issues in different neurological conditions (for case, amnestic gentle cognitive impedance). Be that as it may, the nature of these issues implies that other utilitarian and unthinking portrayals likely apply, and so they are exterior the scope of this diagram. Instep, the center is on the amnesic condition as a long-lasting or changeless clutter, developing from a natural or neurological cause. Causes can incorporate traumatic head damage, neurosurgery (for case, to treat serious epilepsy), anoxia/hypoxia (lack of oxygen), ischemia, viral disease (Herpes simplex encephalitis), and alcoholic Korsakoff's disorder. The large larger part of cases speak to adult-acquired memory misfortune, in spite of the fact that people have been distinguished as having 'developmental amnesia', obtained at birth or in earliest stages[3] . The neurological premise of amnesia will clearly depend on the etiological nature and degree. In spite of the fact that a extend of brain zones can be included in significant memory misfortune (for illustration, the prefrontal cortex or, within the case of alcoholic Korsakoff disorder, the thalamic/diencephalic locale), neurologically determined amnesia has more commonly been related with harm to the hippocampus specifically and to the average transient projections (MTLs) more broadly. This well-established essential neurological locus implies that inquire about with patients can inform both the amnesic condition and how the hippocampus and MTL contribute to memory and cognitive work more broadly. Understanding of amnesia has been considerably driven by case thinks about of patients such as HM[4][5][6] , and in fact this approach remains enlightening given that strong methodological approaches are embraced[7] . Gather considers are too enlightening in expanding past the person, advertising more noteworthy

factual control, and permitting distinguishing proof of steady designs, in spite of the fact that care must be taken when collapsing over patients with conceivably heterogeneous profiles of harm and ability, and combining behavioral and imaging methodology can be valuable in this respect .[8]

Phyllanthus niruri may be a lasting tropical bush, which has been utilized for a wide run of infections in South and south-east Asian conventional pharmaceutical, counting but not constrained to jaundice, the runs, dyspepsia, genitourinary diseases and renal stones. In Brazil, where the plant is known as 'Chanca Piedra' or 'stone breaker', arrangements of P. Niruri are considered people cures for renal and vesicular calculi.[9]Traditional pharmaceutical frameworks, such as Ayurvedic and Unani pharmaceutical, have utilized the clears out and natural product, to treat gallstones and jaundice. In Malay conventional medication, P. niruri, vernacularly known as 'dukong anak', is utilized for kidney disarranges and cough.[10]In South India, where the herb is called Bhumyamalaki, the herb is accepted to treat stoppage, gonorrhea and syphilis.[11]In northern India, this herb locally known as 'pitirishi' has picked up a notoriety as a family cure for asthma, bronchitis and indeed tuberculosis.[12]The youthful shoots of this herb may at times be utilized as an implantation in cases of persistent dysentery.[13]Among conventional Chinese pharmaceutical circles, P. niruri or 'zhu zi cao'has customarily been utilized to reduce liver harm auxiliary to different hepatotoxic specialists. In truth, ever since the point of interest creature ponder by Venkates-waran and colleagues which illustrated for the primary time in vivo the potential anti-hepatitis B activity of P. niruri,[14]this herb has gotten critical logical intrigued driving to a run of considers looking at the different restorative potential of this plant species.

2. MATERIAL AND METHODS:

2.1 Collection and Authentication of Plant :

The leaves of *Phyllanthus niruri* were collected from Regional farm, Akola District, Maharashtra, India. The plants were identified and authenticated by Ms. Kajal Apale , Department of Botany, Vidyabharati Mahavidyalaya, Amravati. The leaves were cleaned, dried in shade. The leaves were kept in air tight container for further studies.

2.2 Experimental Animals :

Swiss albino mice of male sex weighing 25-35 gm was obtained from the animal house of Department of Pharmacology, Vidyabharati College of pharmacy Reg. No: 1504/PO/RE/S/11/CPCSEA, Amravati. All the animal are acclimatized to the animal house prior to use. They are kept in case in animal house with a 12 hr light: 12hr dark cycle at temperature (25°C 1°C) with 50+ 55% of relative humidity. Experiments was performed in accordance with the committee for the purpose of control and supervision of experimental animal (CPCSEA) guideline after the approval of the experimental protocol by the institutional animals ethical committee (IAEC). Animal are fed on pellets and tap water ad libitum. The care and handling of animals in accordance with the internationally accepted standard guidelines of use of animals (CPCSEA).

2.3 Drugs & Chemicals:

- Diazepam 10mg/2ml ampule (Mfg. By Sun Pharma) purchased from Sainath Medical Malegaon ,Dist. Washim ,Maharashtra , 444503
- Donepezil API was purchased from Jai Radhe Sales 309/310, Harikrupa Tower, Near Old Sharda Mandir Char Rasta, Behind Gujarat College, Ellisbridge Paldi, Ahmedabad - 380006, Gujarat, India

2.4 Preparation of Doses:

Diazepam was diluted to 5 mg/50 ml with distilled water. Two different concentrations (200 mg/kg, and 400 mg/kg) of the MEPN were prepared by dissolving the extracts in distilled water. All solutions were freshly prepared at the time of administration to the animals. Extract solution and vehicle (0.9% NaCl) were given orally and inducing drug (diazepam) intraperitoneally & standard drug (donepezil) orally.

2.5 Extraction Process:

Soxhlet Extraction Procedure: -

The leaves of *Phyllanthus niruri* was processed by washing with clean water, air-drying, pulverizing, and sieving through a 0.3 mm sieve. tool consists of several parts including a heat round bottom flask, Soxhlet extractor, and condenser. The solid coarsely powdered leaves (250g) were placed in thimble and placed in an extractor. The bottom end of the extractor was connected to a round bottom flask containing a solvent

(Methanol 1000ml was chosen as the solvent), and was connected to a reflux condenser. The bottom flask was heated to boil the solvent (Methanol), the vapor rises through the branch pipe of the extractor, was condensed and drops into the thimble and the solvent (methanol) was contacted with the solid for extraction. When the solvent (methanol) surface exceeds the highest point of the siphon, the solvent containing the extract was return back to the round bottom flask. This cycle was repeated until the all the material extracted from the solid leaves powder. The Soxhlet extractor can run continuously without any further operation, making it an excellent choice for extracting compounds over hours or even days. Filtration is not required So it save lot of time, energy and financial inputs.
The percentage yield of the extract was calculated and the extract was then subjected to different phytochemical tests.

2.6 Treatment Protocol:

Sr. No.	Group	No. of Animals	Treatment/Dose	Route of Administration
1	I (Normal) (Saline 0.9% NaCl)	6	Normal saline	Oral
2	II(Inducing drug)	6	DZPM(1.75mg/kg)	i.p
3	III(Inducing + Standard Drug)	6	DZPM (1.75mg/kg) + Donepezil(5 mg/kg)	i.p + oral
4	IV(Treatment 1)	6	DZPM(1.75mg/kg) + MEPN (200mg/kg)	i.p + Oral
5	V(Treatment 2)	6	DZPM(1.75mg/kg) + MEPN (400mg/kg)	i.p + Oral

2.7 Experimental Procedure:
Thirty mice distributed into six groups of five mice each were used. The Normal control and Negative control groups received saline (0.9 % NaCl), the positive control group received donepezil (5 mg/kg, *p. o*), and two experimental groups were treated with two doses of MEPN (200 and 400 mg/kg, *p. o*) respectively. All groups except the Normal control group were co-treated with Diazepam (1.75 mg/kg, *i. p*) daily for 14 days . To evaluate the beneficial effects of the extract on memory, the Morris Water Maze (MOR) ,Novel Object Recognition (NOR),Elevated Plus Maze tests were used. Each behavioral test was performed thirty minutes after the administration of diazepam(1.75 mg/kg) and then the III group was administered Donepezil and IV and V group were given MEPN 200 mg and 400 mg per kg respectively . At the end of behavioral studies these mice were exposed to the training session using elevated plus maze and Morris water apparatus on 14^{th} day after 90 min of the last dose. Retention (memory) of the learned task was recorded after 24 hours i.e., on 15^{th} day. Amnesia was induced in II, III, IV, V groups diazepam (1.75 mg/kg, i.p.). Donepezil (5 mg/kg p.o), an established nootropic agent was injected for fourteen days to positive control group of animals.

2.8 Behavioural Tests:
Elevated Plus Maze Model:
The elevated plus maze served as the exteroceptive behavioral model (where in stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consists of two open arms (16 cm x 5 cm) and two covered arms (16 cm x 5 cm x 12 cm). The arms extended from a central platform (5 cm x 5 cm), and maze is elevated to a height of 25 cm from the floor. On the first day of training session i.e (14^{th} day), each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs. TL was recorded

on the first day. If the animal did not enter into one of the covered arms within 90 sec, it is gently pushed into one of the two covered arms and the TL was assigned as 90 seconds. The mouse was allowed to explore the maze for 10 seconds and then returned to its home cage. Memory retention was examined on the second day i.e (15^{th} day) , 24 hours after the first day's trial.[15]

3. RESULT

3.1 Pharmacognostical Examination:

% yield = (weight of the extract / weight of powder taken) × 100

Table :1 Percentage yield of Leaves of *Phyllanthus niruri*

Drug	Leaves of *Phyllanthus niruri*
Percentage yield	7.2 % w/w

Table :2 Physical examination of extract

Extract	**Colour**	**Odour**	**Solubility**
MEPN	Brown	Faint Earthy	In water

3.2 Phytochemical Analysis of Methanolic Extract of leaves of *Phyllanthus niruri*.

Table :3 Phytochemical investigation of *Phyllanthus niruri*

Sr. No	**Chemical Constituents**	**Results**
1	**Carbohydrate**	**+**
2	**Glycosides**	**+**
3	**Alkaloids**	**-**
4	**Phytosterols**	**-**
5	**Saponins**	**+**
6	**Phenols**	**+**
7	**Tannins**	**+**
8	**Flavonoids**	**+**
9	**Protein and Amino acids**	**-**

Where (+) indicates Present & (-) indicates Absent

Phytochemical testing carried out to find out the secondary metabolite because secondary metabolic possess biological activity. Phytochemical studies of *Phyllanthus niruri* performed for the presence of Carbohydrate, Glycosides, Phytosterols, Alkaloids, Saponins, Tannins, Flavonoids, Protein and Amino acids.

3.3 Elevated Plus Maze Test(Transfer Latency)

Group	Treatment	Transfer Latency
I	Control (0.9%NaCl)	13.83 ± 0.6009
II	Diazepam 1.75 mg/kg	39.33 ± 0.7149
III	Diazepam 1.75 mg/kg + Donepezil 5 mg/kg	19.50 ± 0.5627*
IV	Diazepam 1.75 mg/kg + MEPN 200mg/kg	27.67 ± 0.6667*
V	Diazepam 1.75 mg/kg + MEPN 400 mg/kg	22.17 ± 0.5426*

Table:4 Effect of *Phyllanthus niruri* on Transfer Latency in Elevated Plus Maze Model.

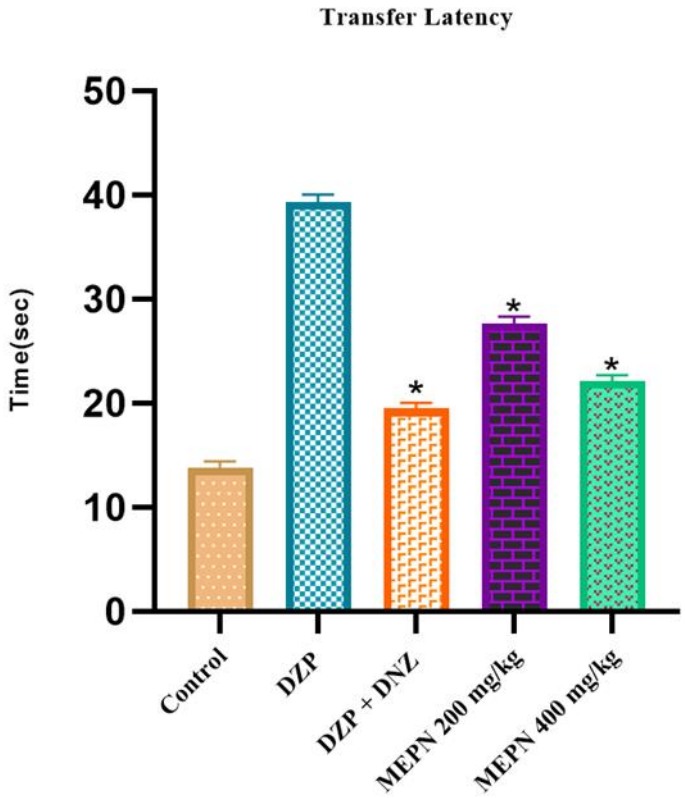

Fig:1 Effect of *Phyllanthus niruri* on Transfer Latency in Elevated Plus Maze Model.

All Values were expressed as mean ± SEM, (n=6), (*p <0.0001) compared to control, (*p <0.0001) compared to diazepam group using One Way ANOVA followed by Dunnett's test.

Table .3 and Fig.1 shows that effect of *Phyllanthus niruri* on transfer latency in Elevated plus maze model. Diazepam treatment significantly increased the latency time. There was significant decrease in transfer latency time by treatment of MEPN 200 mg/kg (*p<0.0001) & 400 mg/kg(*p < 0.0001) and Donepezil (5 mg/kg) (*p<0.0001) as compared to the diazepam (inducing) treatment group, when compared to control (*p<0.0001) using One Way ANOVA followed by Dunnett's test.

4. DISCUSSION

To investigate the neuroprotective effect of MEPN on diazepam-induced amnesia, behavioural experiments such as the Elevated plus maze model to evaluate short term memory were used. Benzodiazepines are a class

of drugs that disable memory solidification by interferometer in data exchange from short-term memory to long-term memory , giving an extra method of reasoning for conducting these tests. Numerous patients taking benzodiazepines for the treatment of different neurological clutters frequently endure from amnesia as a side impact.

Benzodiazepines are known to create anterograde amnesia in both people and creatures. They tie to benzodiazepine official position of gamma amino butyric acid A (GABAA) receptors and potentiate GABAergic neurotransmission . Improved GABA hindrance impedes the part of excitatory neural connections, which is fundamental for memory and hence causes amnesia.

The exchange idleness into the closed arms of EPM has been utilized as a parameter to evaluate maintenance memory . It has been built up to be shorter on the off chance that the creature had already entered the closed arms. Within the show think about, diazepam expanded the cruel exchange inactivity into the closed arms of the EPM which signifies memory impedance. In any case, pretreatment with MEPN(200 mg/kg & 400 mg/kg) essentially diminished the exchange latencies within the maintenance stage which shows an upgrade in maintenance memory. Subsequently, the capacity of MEPN to avoid diazepam-induced amnesia within the EPM test infers that its cognitive improvement may well be mediated through tweak of GABAergic neurotransmission among other components.

The phytochemical constituents available in the sedate were found to be Carbohydrate, Glycosides, Alkaloids, Phytosterols, Saponins, Phenols, Tannins, Flavonoids. The chemical components isolated from *Phyllanthus niruri* are:

Rutin, Quercetin, Astragalin, Catechin, Limocene, P-cymene, Methyl brevifolincarboxylate, Niranthin, nirtetralin, phyltetralin and lintetralin.

Acetylcholine is considered as the foremost important neurotransmitter included within the direction of cognitive capacities. There is extensive evidence linking the central cholinergic framework to memory. Cognitive brokenness has been shown to be associated with decreased cholinergic transmission and the assistance of central cholinergic transmission with made strides memory . Specific misfortune of cholinergic neurons and diminish in cholinacetyltransferase movement was detailed to be a characteristic highlight of amnesia. In this consider, MEPN when managed for 14 days to mice may have appeared critical diminishment of brain acetylcholinesterase action subsequently likely encouraging cholinergic transmission and improving memory of creatures. Oxygen free-radicals are embroiled within the handle of age-related decay in cognitive execution and may be mindful for the improvement of amnesia in elderly people . Oxygen-free radicals and other byproducts of oxidative metabolism have been appeared to be neurotoxic , and antioxidant wealthy diets made strides cerebellar physiology and engine learning in mice . Anti-oxidant constituents of MEPN may be favorably contributing to the memory improving effect seen in the present study. In this way, the protective effect of MEPN may be ascribed to its antioxidant property by ethicalness of which helpless brain cells get uncovered to less oxidative push coming about in reduced brain harm and improved neuronal work.

Benzodiazepenes have too been utilized for investigating the anti-amnesic impact of candidate drugs subsequently empowering recognizable proof of novel targets for sedate improvement that has specificity for GABA receptors . As the instrument of BZ activity is known to happen through the GABA receptors, it is enticing to credit the watched anti-amnesic activity of MEPN to the inclusion of the GABA framework. GABA, an inhibitory neurotransmitter, diminishes the capacity of other neurotransmitters to work. The moo level of GABA leads to disabled neuronal communication, which causes overstimulation of the neuronal circuit and is related with epilepsy, bipolar clutter, and mania, while a tall level of GABA actuates unwinding and sedation, amnesia . It is conceivable that MEPN anticipates the diazepam-induced help of GABAergic inhibitory reactions by fortifying the locomotory reactions or interceding GABA shift of work from inhibitory to excitatory receptor activity. Advance thinks about that can assess the GABA receptor upon treatment can give coordinate prove.

We conclude that MEPN has capacity to evoke antiamnesic impact against diazepam-induced amnesia and advance clinical thinks about can help in discovering the foremost ideal measurements in people. It is reasonable to hypothesize that this impact is being mediated by the GABAergic framework and amply supplemented by its known antioxidant and antiapoptotic impacts. The atomic events underlying the antiamnesic effect of MEPN got to be observed to not as it were screen the dynamic constituents of MEPN but to explore the inclusion of downstream molecular pathways of LTP such as protein kinase–cyclic adenosine monophosphate reaction component official protein and retrograde messenger-mediated pathway.

Understanding the atomic signaling pathway interceding such antiamnesic impact can encourage advancement of novel targets to upgrade memory.

5. CONCLUSION

This study aimed to evaluate the neuroprotective effects of the Methanolic leaf extract of MEPN on diazepam-induced amnesia in mice. The results obtained strongly showed that the methanolic leaf extract of *Phyllanthus niruri* (200, and 400 mg kg) effectively protected memory processes from the diazepam-induced damage in mice. It emerges that this extract may have effects that prove to be neuroprotective against the amnesia induced by diazepam. More precisely, this extract may have improved long-term and short- memory; and may have antioxidant properties and could have protected the hippocampus from the neurotoxic effect of diazepam. These beneficial effects of the MEPN extract could justify the use of this extract to manage nervous system diseases in Indian traditional medicine.

The Future prospects of these study will be

- Continued research into the molecular mechanisms underlying the antiamnesic effects of MEPN can lead to the discovery of new drug targets and pathways for intervention.
- Conducting well-designed clinical trials to evaluate the efficacy and safety of MEPN in humans with amnesia or cognitive impairment can provide valuable evidence for their use in clinical practice.
- Developing strategies to improve the bioavailability and pharmacokinetics of active compounds in MEPN can enhance their therapeutic potential.
- Seeking regulatory approval for MEPN as treatments for amnesia and other cognitive disorders can facilitate their integration into mainstream healthcare.
- Educating the public and healthcare professionals about the benefits and safety of MEPN can increase their acceptance and use as alternative or adjunct therapies.
- Carrying out Atomic absorption and atomic emission spectroscopies for the qualitative and quantitative analyses of mineral elements in herbal drugs, often in the search for contaminants.

Therefore, it would be worthwhile to explore the therapeutic potential of MEPN in the management of Amnesia.

REFERENCES:

1. Quinette P, Guillery-Girard B, Dayan J, De La Sayette V, Viader F, Desgranges B, Eustache F. What does transient global amnesia really mean? Review of the literature and thorough study of 142 cases. Brain. 2006;129(Pt 7):1640–58. doi: 10.1093/brain/awl105.
2. Kopelman MD. Psychogenic amnesia. In: Baddeley AD, Kopelman MD, Wilson BA, editors. The handbook of memory disorders. 2nd ed. John Wiley & Sons; 2003.
3. Vargha-Khadem F, Gadian DG, Watkins KE, Connelly A, Van Paesschen W, Mishkin M. Differential effects of early hippocampal pathology on episodic and semantic memory. Science. 1997;277(5324):376–80. doi: 10.1126/science.277.5324.376.
4. Augustinack JC, van der Kouwe AJ, Salat DH, Benner T, Stevens AA, Annese J, Fischl B, Frosch MP, Corkin S, Dickerson BC. H.M.'s contributions to neuroscience: a review and autopsy studies. Hippocampus. 2014;24(11):1267–86. doi: 10.1002/hipo.22354.
5. Eichenbaum H. What H.M. taught us. J Cogn Neurosci. 2013;25(1):14–21. doi: 10.1162/jocn_a_00285.
6. Milner B, Corkin S, Teuber HL. Further analysis of the hippocampal amnesic syndrome: 14-year follow-up study of H.M. Neuropsychologia. 1968;6(3):215–34. doi: 10.1016/0028-3932(68)90021-3.
7. Rosenbaum RS, Gilboa A, Moscovitch M. Case studies continue to illuminate the cognitive neuroscience of memory. Ann N Y Acad Sci. 2014;1316:105–33. doi: 10.1111/nyas.12467.
8. Squire LR, Wixted JT. The cognitive neuroscience of human memory since H.M. Annu Rev Neurosci. 2011;34:259–88. doi: 10.1146/annurev-neuro-061010-113720.
9. Calixto JB, Santos AR, Cechinel Filho V, Yunes RA. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential. Med Res Rev. 1998 Mar;18(4):225-58.

10. Burkill IH, Haniff M. A dictionary of the economic products of the Malay peninsula. Kuala Lumpur, Malaysia: Published on behalf of the governments of Malaysia and Singapore by the Ministry of Agriculture and cooperatives; 1966.
11. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. Ranchi: Catholic Press; 1986.
12. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants for biological activity: I. Indian J Exp Biol. 1968 Oct;6(4):232-47.
13. Nadkarni NK. India Materia Medica. Bombay: Popular Prakashan Private Ltd.; 1993.
14. Venkateswaran PS, Millman I, Blumberg BS. Effects of an extract from Phyllanthus niruri on hepatitis B and woodchuck hepatitis viruses: in vitro and in vivo studies. Proc Natl Acad Sci USA. 1987 Jan 1;84(1):274-8.
15. Dhingra D, Parle M, Kulkarni SK. Memory enhancing activity of Glycyrrhiza glabra in mice. Journal of ethnopharmacology. 2004 Apr 1;91(2-3):361-5.