Studies on nootropic effects of various extracts of Gmelina arborea and Cayratia trifolia

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By

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CERTIFICATE

This is to certify that the synopsis entitled "Studies on nootropic effect of various extract of Gmelina arborea and Cayratia trifolia" represents the bonafied work of Mr. Madiya Darshan Natvarlal and incorporates the results of his own research work. Studies were carried out at the Department of Pharmacology, Atmiya Institute of Pharmacy, Rajkot under my guidance and supervision. This work is up to my satisfaction. This work embodied in this synopsis is original and no part of the synopsis has been submitted previously to this university or any other university for the award of Ph.D. or any other degree or diploma.

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Abstract

Aim of the study

The aim of this study was to examine the nootropic effect of *Gmelina arborea Roxb.* and *Cayratia trifolia Linn.* by using various animal models.

Materials and Methods

The in vivo models used for the study of nootropic activity of extract of *Gmelina arborea* and *Cayratia trifolia* were step down passive avoidance, conditioned avoidance response, sodium nitrite induced amnesia, and elevated plus maze while in vitro models includes antioxidant activity by DPPH & NO and estimation of brain reduced glutathione (GSH), estimation of acetyl cholinesterase enzyme activity in mice brain, estimation of brain total protein and estimation of catalase activity. In preliminary study, we used different extracts *Gmelina arborea* & *Cayratia trifolia* like aqueous extract, methanolic extract, hydroalcoholic extract, petroleum ether extract; acetone extract and chloroform extracts were prepared. Based on effect on step down latency (SDL) using passive avoidance paradigm; we found that Aqueous Extract of *Cayratia trifolia* (AECT), Hydro-alcoholic Extract of *Cayratia trifolia* (HAECT), Chloroform Extract of *Gmelina arborea* (CEGA) and Hydro-alcoholic Extract of *Gmelina arborea* (HAEGA) has significant activity and further experiments were carried out by using these extracts only. The AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg) and HAEGA (500 mg/kg) was suspended in distilled water and administered orally to mice. Piracetam was administered orally as a standard drug. Control animals receive equivalent volume of distilled water.

Results

Effect on step down latency (SDL) using passive avoidance paradigm was measured during learning and memory trial to examine the memory formation based on negative reinforcement. SDL was defined as the time taken by the animal to step down from the shock free zone to grid floor with all its paws on the grid floor. Animals injected with scopolamine and sodium nitrate were evaluated using step down passive avoidance test in mice for the development of amnesia. Scopolamine and sodium nitrate was administered prior the training session. Scopolamine and sodium nitrate control group significantly decreased the SDL in learning and memory trials. On treatment with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) at all the doses significantly reversed scopolamine and sodium nitrate

induced spatial memory impairment as compared to negative control group. Conditioned avoidance response behavior mainly affects cognitive behavior by mesocortical pathway of dopaminergic neurons. In our study, AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) significantly reduced time taken by the rats to climb the pole. In the elevated plus-maze test, TL might be shortened if the animal had previous experience of entering the open arm and the shortened TL could be related to memory. In our study we found that mice treated with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), HAEGA (500 mg/kg) and standard drug piracetam significantly reduced TL.

In vitro study, pre-treatment with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) at all doses significantly reduced Acetylcholinesterase (AChE) and total protein levels as compared to control. While AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) treatment significantly elevate the SOD, CAT activity and GSH levels. This effect of may be attributed to its antioxidant potential. The antioxidant activity of AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) were comparable with standard vitamin C by using DPPH and nitric oxide methods.

Interpretation and Conclusion

Our study demonstrates the cognitive enhancing and/or anti-amnesic property of the plant extracts in the presence and/or the absence of amnestic agent suggests the nootropic activity.

Keywords

Nootopic; *Gmelina arborea* and *Cayratia trifolia*

Brief description on the state of the art of the research topic

The "nootropic" or simplified as a "smart drug," "brain booster," or "memory enhancing drug," is a common term that will tag along with the compound responsible for the enhancement of mental performance. By definition, nootropic is a compound that increases mental functions including memory, motivation, concentration, and attention.[1] There are two different nootropics: synthetic, a lab created compound such as Piracetam, and notable natural and herbal nootropics, such as *Ginkgo biloba* and *Panax quinquefolius* (American Ginseng).

Natural nootropics are proven in boosting the brain function while at the same time making the brain healthier. Nootropics act as a vasodilator against the small arteries and veins in the brain.[2] Introduction of natural nootropics in the system will increase the blood circulation to the brain and at the same time provide the important nutrient and increase energy and oxygen flow to the brain.[3] Despite the 3% weight of total body weight, the brain receives around 15% of the body's total blood supply and oxygen. In fact, the brain can only generate energy from burning the glucose, proving that neuron depends on the continuous supply of oxygen and nutrients.[4]

Definition of the Problem

While there is no cure for neurodegenerative disease, there are five prescription drugs approved by the U.S. Food and Drug Administration (FDA) to treat its symptoms. Donepezil, Galantamine, Rivastigmine, and Tacrine are called "Cholinesterase Inhibitors". These drugs prevent the breakdown of a chemical messenger in the brain important for learning and memory. The fifth drug Memantine regulates the activity of a different chemical messenger in the brain that is also important for learning and memory.[5] Moreover, these drugs are associated with number of adverse effects.

In contrast to most of other cells in the body, neuron cannot be reproduced and is irreplaceable. The neuron cells are persistently expending the converted energy to maintain the repair of the cell compartments. The energy generated from the glucose is crucial for maintenance, electrical, and neurotransmitter purposes.[6] The effect of natural nootropics is also shown to reduce the inflammation occurrence in the brain. The administration of natural origins of nootropics will protect the brain from toxins and minimising the effects of brain aging.[7]

Scope of work

Effects of natural nootropics in improving the brain function are also contributed through the stimulation of the new neuron cell. As incentive from the new neuronal cell, the activity of the brain is increased, enhancing the thinking and memory abilities, thus increasing neuroplasticity.[8] Natural nootropics alter the concentration of existing neurotransmitters. Natural nootropics have been disclosed to stimulate the release of dopamine, uptake of choline, cholinergic transmission, function of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor, turnover of phosphatidylinositol, and activity of phosphatase A2.[9] The release of neurotransmitter and the increase activity of neurotransmitter induced by natural nootropics facilitate the long-term potential (LTP) and improve synaptic transmission.[10]

Objective of study

1. To prepare the extracts of *Gmelina arborea* and *Cayratia trifolia*.
2. To evaluate the nootropic activity of *Gmelina arborea* and *Cayratia trifolia* extracts by using various in-vivo and in-vitro animal models.
3. To publish results.

Original contribution by the thesis

The contributions of our study to existence therapy/treatments includes

1. Natural nootropics *Gmelina arborea* and *Cayratia trifolia* has significant nootropics activity.
2. The natural nootropics can be used as alternative treatments with synthetic drugs.
3. The natural nootropics can be used in combinations with synthetic drugs.

Methodology of Research and Results

Animal husbandry and feeds

Specific pathogen-free male Swiss albino mice (20-30g) and Wistar rats (200-250 g) female sex were housed in a room maintained at 22 ± 1°C with a relative humidity of 55 ± 5% and a 12 h light-dark cycle. Animals had free access to standard pellet diet (certified Amrut brand rodent feed, Pranav Agro Industries, Pune, India) and filtered tap water. All experiments were carried out with strict adherence to ethical guidelines and were conducted as per protocol approved by the Institutional Animal Ethics Committee (IAEC) and as per Indian norms laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA), New Delhi. Throughout the entire study period, the animals were monitored for growth, health status, and food intake capacity to be certain that they were healthy.

Preparation of Extract

The extracts are prepared as mentioned in standard text book of as described by Khandelwal, 2004[11] and Trease and Evans, 2002.[12]

1. Preliminary study

In preliminary study different extracts like Methanolic Extract of *Gmelina arborea* (MEGA), Petroleum Ether Extract of *Gmelina arborea* (PEEGA), Chloroform Extract of *Gmelina arborea* (CEGA), Aqueous Extract of *Gmelina arborea* (AEGA) and Hydro-alcoholic Extract of *Gmelina arborea* (HAEGA), Methanolic Extract of *Cayratia trifolia* (MECT), Petroleum Ether Extract of *Cayratia trifolia* (PEECT), Chloroform Extract of *Cayratia trifolia* (CECT), Aqueous Extract of *Cayratia trifolia* (AECT), Hydro-alcoholic Extract of *Cayratia trifolia* (HAECT) were prepared and administered to different groups of animal as shown in below table.

Group no.	**Treatment Groups (Dose)**
I	Control
II	Scopolamine (0.4 mg/kg)
III	Piracetam (140 mg/kg)+Scopolamine (0.4 mg/kg)
IV	MECT (500 mg/kg))+Scopolamine (0.4 mg/kg)
V	PEECT (500 mg/kg))+Scopolamine (0.4 mg/kg)
VI	CECT (500 mg/kg))+Scopolamine (0.4 mg/kg)
VII	AECT (500 mg/kg))+Scopolamine (0.4 mg/kg)
VIII	HAECT (500 mg/kg))+Scopolamine (0.4 mg/kg)
IX	MEGA (500 mg/kg))+Scopolamine (0.4 mg/kg)
X	PEEGA (500 mg/kg))+Scopolamine (0.4 mg/kg)
XI	CEGA (500 mg/kg))+Scopolamine (0.4 mg/kg)
XII	AEGA (500 mg/kg))+Scopolamine (0.4 mg/kg)
XII	HAEGA (500 mg/kg))+Scopolamine (0.4 mg/kg)

Based on effect on step down latency (SDL) using passive avoidance paradigm; we found that Aqueous Extract of *Cayratia trifolia* (AECT), Hydro-alcoholic Extract of *Cayratia trifolia* (HAECT), Chloroform Extract of *Gmelina arborea* (CEGA) and Hydro-alcoholic Extract of *Gmelina arborea* (HAEGA) has significant activity and further experiments were carried out by using these extracts only.

2. Efficacy Study

Sodium nitrite induced amnesia

Sodium nitrite induced amnesia test was carried out as described by Bhattacharya, 1994.[13] Sodium nitrate control group significantly decreased the SDL in learning and memory trials. On treatment with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) at all the doses significantly reversed sodium nitrate induced spatial memory impairment as compared to negative control group.

Pole Climbing Test

Cook‘s Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning and its retention. The experiment was carried out as described by Cook, 1957. [14] In our study, we found that AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) significantly reduced time taken by the rats to climb the pole.

Elevated plus maze

The experiment was carried out as described by Kulkarni and Verma, 1993.[15] In the Plus-maze test, TL (transfer latency) might be shortened if the animal had previous experience of entering the open arm and the shortened TL could be related to memory. In our study we found that mice treated with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), HAEGA (500 mg/kg) and standard drug piracetam significantly reduced TL.

In Vitro Antioxidant Activity

The antioxidant activity of AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) were comparable with standard vitamin C by using DPPH and nitric oxide methods.

Estimation of Brain AChE, total protein, SOD, CAT and GSH

Pre-treatment with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) at all doses significantly reduced Acetylcholinesterase (AChE) and total protein levels as compared to control. While AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) treatment significantly elevate the SOD, CAT activity and GSH levels.

Achievements with respect to objectives

1. *Gmelina arborea* and *Cayratia trifolia* has significant nootropics activity.
2. *Gmelina arborea* and *Cayratia trifolia* nootropics activity was comparable with standard drug piracetam.

Conclusion

Our study demonstrates the cognitive enhancing and/or nootropic activity of the plant extracts *Gmelina arborea* and *Cayratia trifolia* in the presence and/or the absence of amnestic agent suggests the memory enhancing activity. The observed nootropic activity supported by enhancement of cholinergic and up regulation of antioxidant defence systems.

Publications

1. Evaluation of nootropic activty of Ca*yratia trifolia* in experimental animal models published in World Journal of Pharmacy and Pharmaceuticals Sciences. 2018:7(6);1558-68.
2. Evaluation of nootropic activty of *Gmelina arborea* in experimental animal models published in Inventi Rapid Ethanopharmacology 2018(3):1-5.

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