Evaluation of Cognitive Enhancing Activity of *Piper Longum* Fruit Extract on Albino Rats

D. Bindu Madhavi, KL. Senthil Kumar and V. Palanivel

Padmavathi College of Pharmacy and Research Institute, Periyanahalli, Dharmapuri, Tamilnadu, India.

ABSTRACT
To investigate the cognitive enhancingactivity of ethanolicfruit extract of *Piper longum*two methods are used.piperlongum were subjected to preliminary phytochemical investigation and pharmacological screening of cognitive enhancing activity. The dried fruits were subjected to successive solvent extraction with 70% ofethanol.the extract revealed the presence of tannins , lignans , and volatile oils. In the pharmacological screening the extract was used for the evaluation of cognitive enhancing activity using elevated plus maze and passive avoidance task methods using Donepezil as standard by using the parameters of step down and transfer latency. Induction was carried out by Diazepam for 7 days . Alcoholic extract showed significant effect when compared to control, their was significant increase in the step down latency and decrease in the transfer latency which was as effective as that of standard drug.

Keywords: *piper longum*, donepezil, diazepam , stepdown, transfer latency.

INTRODUCTION

Herbal Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic quality. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in Ayurveda, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. Pippali consists of the dried, immature, catkin-like fruits with bracts of **Piper longum** Linn. (Fam. Piperaceae), a slender, aromatic climber with perennial woody roots, occurring in hotter parts of India from central Himalayas to Assam up to lower hills of West Bengal and ever green forests of Western ghats as wild, and also cultivated in North East and many parts of the South.Fruit greenish-black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm thick, consisting of minute sessile fruits, arranged around an axis; surface rough and composite; broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis; taste, pungent producing numbness on the tongue; odour, aromatic.

Chemical Constituents and Components

Main chemical components are piperine, rutin, beta-caryophyllenepiperyline, piperoleines, piperamine, sabinene, chavicin, pinene, phellandrene, pentadecane, beta-bisabolene, linalool and limonene.

MATERIALS AND METHODS
Collection of plant material

The dried fruits of piper longum were purchased from Munniyapa Aruvedha Krishnagiri. The plant was authenticated by Prof. P. Jayaraman. Ph.D. Director, National Institute Of Herbal Sciences Chennai.

EXTRACTION OF CRUDE DRUGS

The powdered fruit material was extracted with methanol using soxhlet apparatus and the extract obtained was then concentrated on the water bath. In successive solvent extraction, dried material was extracted with methanol.
For extraction, 150gm of powdered stem was packed in thimble containing whatmann filter paper and extracted with methanol (60˚C-70˚C) in soxhlet apparatus for the period till all the crude substances were extracted. The extract thus obtained was concentrated with the help of rotatary vacuum evaporator.

Selection of Animals for testing the cognitive enhancing activity
Animal care and handling as per CPCSEA guideline
Male Wistar albino rats of weight 150-250 grams were selected, procured from Padmavathi college animal house. The animals were acclimatized to the standard laboratory conditions in well cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 10 and 14 hours respectively for 1 week before and during the experiments. The animals were fed with standard diet and water adlibitum. The experiments were approved by CPCSEA and the institutional ethics committee. Food was withdrawn 18hours before the start of the activity.

Method for Testing Cognitive Enhancing Activity[60]
The animals were divided into Five groups of six in each group
Group-1: Normal given water daily for 7 days.
Group-2: positive control, given extract for sub group 2 and standard drug for sub group 3.
Group-3: negative control, in which Alzheimer's disease is induced by using diazepam.
Group-4: treated group in which sub group 5 was treated with extract and sub group 6 with standard drug.

Table 1: Grouping of Animals

Main Groups		Sub-groups
Group I	Normal control	1.No induction and no Treatment
Group II	Positive control	2. Only *Piper Longum* 3. Only Standard
Group III	Negative control	4. Induction with Diazepam
Group IV	Treated group: Induced with Diazepam+ Treatment with extracts	5.*Piper Longum* Fruit Extract 6. Standard

Procedure
The animals were trained on the 0(zero) day and the acquisition of memory was tested on day1, later all the animals were dosed with respective drugs and kept in their home cage. Dosing is continued for 7days and on 7th day animals were subjected to retention test 25min.after the last dose for evaluating passive avoidance task(step down latency) and elevated plus maze(transfer latency).

Screening test for memory
Model 1
passive avoidance task (step down latency)
Pole climbing apparatus chamber is used for passive avoidance response where pole is replaced by a wooden platform fixed on electrified grid floor .when rats stepped off the platform, they receive a continuous foot shock from grid floor. The normal reaction of rat was to jump back to the wooden platform. After about 4-5 trials the animals acquired the passive avoidance response and they refrained from stepping down. The criterion was reached when the animal remained on the platform for at least 60 sec. SDL is defined as time in sec taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor.

Model 2
Elevated plus maze (transfer latency)
An elevated plus maze consists of two open arms and two closed arms with an open roof. The maze was elevated to a height of 50cm. the animals were individually placed at the end of either of the open arms and the time taken for the animal to move from open to closed arm (transfer latency) was noted on zero day. The animals were allowed to explore the apparatus for 30sec. after 24h of first exposure TL was noted on day1 of the study for determining the acquisition. The criterion was reached when the animal moved in to the closed arms in a very short period keeping the cut off time of 60sec.

Statistical analysis
The SDL and TL were analysed using the students paired "t" test, twotailed. later the inflexion ration was calculated for the TL which was analysed using one way analysis of variance(ANOVA),Followed by Dunnets "t" test for individual comparision of groups with diazepam induced and controlled groups.

RESULTS

### Table 1: Mean Values of Transfer latency of various drug treated groups

GROUP	S.NO. OF SUB GROUP	SUB GROUP	TRANSFER LATENCY(in Sec)	
			Before day1	after day 7
Normal control	01	Normal control	22.36±1.634	6.492±0.5852
	02	Extract	52.82±2.263	32.45±1.140
Positive control	03	Standard	28.89±6.007	19.04±4.447
Negativecontrol	04	Diazepam	24.71±4.325	47.12±5.485
	05	Extract	37.43±1.665	14.90±1.169
Treated group	06	standard	21.30±9.572	15.48±7.693

Table 2: Mean Values of step down latency of various drug treated groups

GROUP	S.NO. OF SUB GROUP	SUB GROUP	STEP DOWN LATENCY(in Sec)	
			Before day1	after day 7
Normal control	01	Normal control	17.41±3.014	25.57±2.700
	02	Extract	14.89±1.442	27.82±0.6974
Positive control	03	Standard	16.76±1.799	60.00±0.0
Negativecontrol	04	Diazepam	27.43±3.445	11.73±2.402
Treated group	05	Extract	17.46±3.162	41.19±1.782
	06	standard	32.04±2.235	60.00±0.0
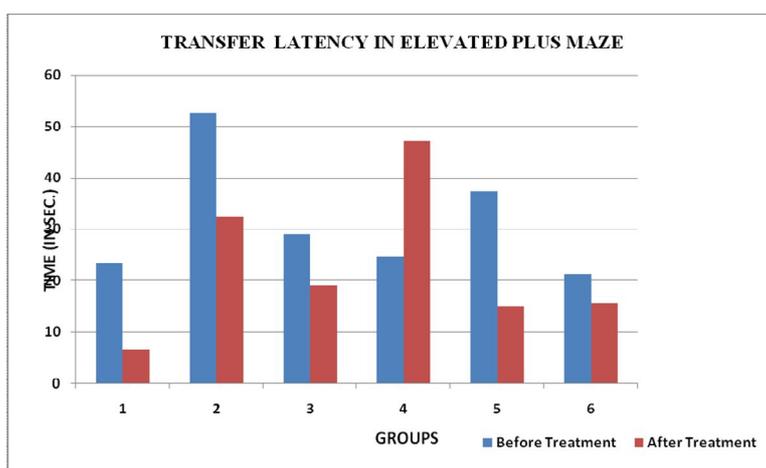
Mean Graph of Transfer Latency
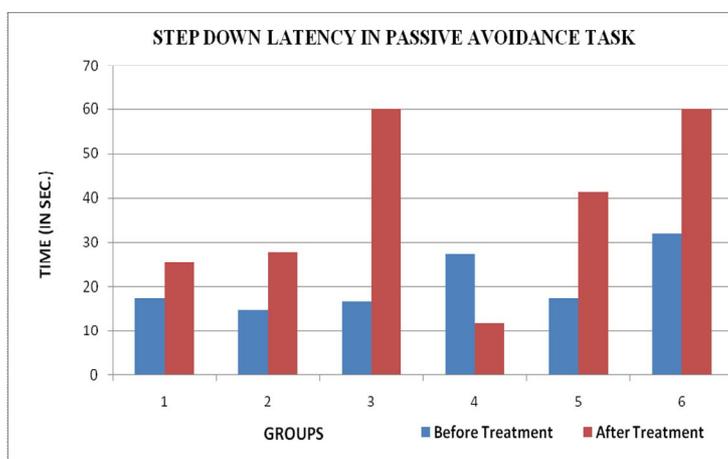
Mean Graph of Step down Latency

DISCUSSION

AD is a progressive and fatal neurodegenerative disorder manifested by cognitive and memory deterioration, progressive impairment of routine activities of living, and a variety of routine activities of living, and a variety of neuropsychiatric symptoms and behavioural disturbances. The clinical features of AD are an amnesic type of memory impairment, deterioration of language and visuo spatial deficits. Motor and sensory abnormalities, gait disturbance and seizures are uncommon until the last phase of the disease. Despite the severity and high prevalence of this disease ,allopathic system of medicine is yet to provide a satisfactory antidote. Therefore, we were motivated to explore the new approach in the Indian traditional system to manage this deadly disease (AD).

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. According to the cholinergic hypothesis, memory impairment in patients with the senile dementia are due to selective and irreversible deficiency in the cholinergic functions in the brain. This serves as a rationale for the use of AChE inhibitors for the symptomatic treatment of AD in its early stages. There are extensive evidences linking decreased brain cholinesterase activity and improvement in memory. cognitive dysfunction has been shown to be associated with impaired cholinergic function and the facilitation of central cholinergic activity with improved memory. Selective loss of cholinergic neurons and decrease in cholineacetyltransferase activity was reported to be a characteristic feature of senile dementia of Alzheimer's type.

It has been observed that elderly patients suffering from Alzheimer's disease showed reduction in symptoms up on chronic use of anti-inflammatory drugs. Epidemiological studies have already confirmed that non-steroidal anti-inflammatory reduced the incidence of AD. *piperlongum* have been proved as anti-inflammatory agents ,which might protect from the development of inflammatory lesions in brain.

In the present study, the fruits of *piper longum* were subjected to phytochemical investigation and cognitive enhancing activity. Theethanolic extract when tested for preliminary phytochemical investigation showed presence of alkaloids and amides, volatile oils , lignans, esters.

Cognitive Enhancement Activity was done by step down and transfer latency method, impairment of memory consolidation was done by diazepam. Chronic exposure to diazepam for 7 days produced a significant decrease in step down latency and increased the time of latency in elevated plusmaze. The alcoholic extracts(3mg/kg.B.W) showed significant effect when compared to control, there was significant increase instep down latency and decrease in the transfers latency but was not so effective as that of standard drug. This suggested that application of diazepam disrupts the acquisition, retention and consolidation of learning task which was reversed by alcoholic extracts and standard.

The result obtained has shown significant cognitive enhancing activity of alcoholic extract drug. Daily administration of extracts significantly attenuated the amnesic effect of diazepam.

CONCLUSION

In the present study fruits of piper longum were subjected to preliminary phytochemical investigation and pharmacological screening of cognitive enhancing activity. The dried fruits were subjected to successive solvent extraction with 70% of ethanol. The extract revealed the presence of tannins, lignans , and volatile oils. In the pharmacological screening the extract was used for the evaluation of cognitive enhancing activity using elevated plus maze and passive avoidance task methods using Donepezil as standard by using the parameters of step down and transfer latency. Induction was carried out by Diazepam for 7days . Alcoholic extract showed significant effect when compared to control, their was significant increase in the step down latency and decrease in the transfer latency which was as effective as that of standard drug. Hence it can be concluded that fruits of piper longum possess cognitive enhancing activity.

REFERENCES

1. Vaidyaratnam PS and Varier's AryaVaidyaSala. Indian Medicinal Plants, A Compendium of 500 Species, Chennai, Orient Longman. 1994;4: 62-6.
2. Maton Anthea, Jean Hopkins, Charles William McLaughlin, Susan Johnson, MaryannaQuon Warner, David La Hart and Jill D. Wright. Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall. 1993;132–144.
3. Hickman Jr, Cleveland P, Larry S Roberts, Susan L Keen, Allan Larson, Helen L'Anson and David J. Eisenhour. Integrated Princinples of Zoology: Fourteenth Edition. New York, NY, USA: McGraw-Hill Higher Education.

2008;733. ISBN 978-0-07-297004-3.

4. Campbell Neil A, Jane B Reece, Lisa A Urry, Michael L Cain, Steven A Wasserman, Peter V Minorsky and Robert B Jackson. Biology: Eighth Edition. San Francisco, CA, USA: Pearson / Benjamin Cummings. 2008;1065. ISBN 978-0-8053-6844-4.

5. Katsuhiko Mineta. "Origin and evolutionary process of the CNS elucidated by comparative genomics analysis of planarian ESTs" (pdf), 2003.

6. Romer AS. The Vertebrate Body. W.B. Saunders, Philadelphia. (2nd ed. 1955; 3rd ed. 1962; 4th ed. 1970)

7. Bear Mark F, Barry W Connors and Michael A Paradiso . Neuroscience: Exploring the Brain: Third Edition. Philadelphia, PA, USA: Lippincott Williams & Wilkins. ^ Kent, George C.; Robert K. Carr (2001). Comparative Anatomy of the Vertebrates: Ninth Edition. New York, NY, USA: McGraw-Hill Higher Education. 408. ISBN 0-07-303869-5.

8. Pelvig DP, Pakkenberg H, Stark AK and Pakkenberg B."Neocortical glial cell numbers in human brains". Neurobiology of Aging29.

9. Hippocrates (400 BCE). On the Sacred Disease. Francis Adams..

10. Rosenberger and Peter B MD, Adams, Heather R. PhD. Big Brain/Smart Brain. 18th October, 2011.

11. Shepherd GM. Neurobiology. Oxford University, 1994; Press. p. 3. ISBN 9780195088434.

12. Sporns O. Networks of the Brain. MIT Press. 143, Başar, E (2010).

13. Brain-Body-Mind in the Nebulous Cartesian System: A Holistic Approach by Oscillations. Springer. Singh, I (2006). "A brief review of the techniques used in the study of neuroanatomy". Textbook of human neuroanatomy. Jaypee Brothers Publishers. ^Principles of Neural Science p. 20

14. Principles of Neural Science, 21

15. Douglas RJ and Martin KA . "Neuronal circuits of the neocortex". Annual Review of Neuroscience^ Barnett, MW; Larkman, PM (2007). "The action potential". Practical Neurology. 2004;7(3):192–197. PMID 17515599.

16. Principles of Neural Science, Ch.10, p. 175

17. Principles of Neural Science, Ch. 10

18. Shepherd, GM . Ch. 1: Introduction to synaptic circuits. The Synaptic Organization of the Brain. Oxford University. 2004;Press US. ISBN 9780195159561.

19. Williams RW and Herrup K. The control of neuron number. Annual Review of Neuroscience11^ Heisenberg, M (2003). Mushroom body memoir: from maps to models, 1988.

20. Jacobs DK, Nakanishi N and Yuan D. "Evolution of sensory structures in basal metazoa". Integrative & Comparative Biology 47, 2007.

21. Balavoine G. "The segmented Urbilateria: A testable scenario". Integrative &Comparative Biology^ Schmidt-Rhaesa, A (2007). The Evolution of Organ Systems. Oxford University. 2003; Press. p. 110. ISBN 9780198566694.

22. Kristan Jr WB, Calabrese RL and Friesen WO. "Neuronal control of leech behavior". Prog Neurobiology. 2004;76(5): 279–327. doi:10.1016/j.pneurobio.2005.09.004. PMID 16260077.

23. Mwinyi A, Bailly X, Bourlat SJ, Jondelius U, Littlewood DT and Podsiadlowski L (. "The phylogenetic position of Acoela as revealed by the complete mitochondrial genome of Symsagittiferaroscoffensis". BMC Evolutionary Biology. 2010;10:309.

24. Barnes RD. Invertebrate Zoology (5th ed.). Saunders College Pub. 1987;1 ISBN 9780030089145.

25. Butler AB. Chordate Evolution and the Origin of Craniates: An Old Brain in a New Head. Anatomical Record261, 2000.