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Experimental Research

Pharmacology Section

Preventive Role of Indian Black Pepper in Animal Models of Alzheimer's Disease

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ABSTRACT

Introduction: Dementia is the clinical symptom of alzheimer's disease. Brain cholinesterase levels and behavioural changes are the markers for Alzheimer's disease and aluminium chloride is one causative agent for polymerization of tau protein and amyloid plaque formation in Alzheimer's disease. Effect of piper nigrum and its role in prevention of alzhimer's disease and symptoms are well linked in this study.

Aim: To study the effect of piper nigrum for the prevention of alzheimer's associated histopathological, biochemical and behaviour changes in rat model.

Materials and Methods: Twenty four rats were taken in this study. Their baseline behavioural parameters were noted and group was separated randomly in four. Rats were pretreated with piper nigrum and Alzheimer's disease was induced. Biochemical and histopathological changes were noted at the end of experiment.

Results: There was marked decrease in cholinesterase level, amyloidal plaque formation in rats brain who were pretreated with piper nigrum. At the same time there was decrease in escape latency time (ELT) and increase in memory in piper treated rats.

Conclusion: Piper nigrum prove to be effective for prevention of Alzheimer's disease. This finding has to be confirmed with studies including larger population. Further research on cholinesterase inhibitors, role of flavonoids on prevention of neurodegeneration in Alzheimer's disease can be encouraged.

Keywords: Amyloid plaque, Cholinesterase, Dementia, Morris water maze, Piper nigrum

INTRODUCTION

Alzheimer's disease is characterized by progressive detoration of cognitive abilities, eventually leading to death. It primarily affects the elderly population and is considered to be responsible for the majority of dementia cases in people aged more than 65 y. Histopathologically AD has been characterized by the extracellular deposits of amyloid β (Aβ) protein in senile plaques in the brain parenchyma and cerebral blood vessels and neurofibrillary tangles of abnormally hyperphosphorylated tau filaments in the neocortex, especially the hippocampus [1,2]. The pathology of AD is complex and three main pathogenic pathways are believed to contribute to the progression of the disease; cholinergic deficit, senile plaque/amyloid-β peptide deposition and oxidative stress [3].

Piper nigrum (black pepper) is a monocious or decorous climbing vine native to southern India and Srilanka and is extensively cultivated in tropical regions. They have several uses such as they help in pain relief, rheumatism, chills, flu, colds, muscular aches and fever. It has antimicrobial [4], antimutagenic [5], antioxidant and radical scavenging proper and inhalation of black pepper oil increase the reflexive swallowing movement [6]. Reports indicate that memantine and donepezil are more effective drugs in improving cognitive impairment in patients with AD. However, two clinical trials have shown no improvement in the cognitive deficit or reduction in the institutionalization rate [7,8]. In view of the above shortcomings in the drugs used for the treatment of AD there has been an increased interest in herbal products as a source of treatment [9].

According to National institute of health "AD currently affects 18 million people worldwide, 5.0 million people in the United States and India has almost ¼ th of world's Alzheimer's patients. By 2025, 34 million AD patients will be worldwide. In the next 24 h another 1,000 people in the United States will learn they have Alzheimer's disease [10]. By keeping in mind that there is no successful treatment till now here is a need to find some preventive measure to delay the alzhimer's disease incident, we took this study to investigate the role of piper nigrum to prevent the Alzheimer's disease and its associated symptoms.

MATERIALS AND METHODS

Experimental Animals: After an approval from the Institutional Ethics Committee (JSSMC/IAEC/2439/18/july 2013), the experiments were conducted on in male albino rats (150-200g). The animals were maintained in colony cages, under an ambient temperature of 25 ± 2°C and 45-55% relative humidity, with a 10 h light/14 h dark cycle. They were allowed food and water ad libitum. Principles of Laboratory Animals Care and Use guidelines were followed throughout [11].

Plant Material and Standardizations of extract: The standardized ethnolic extract of the seeds was prepared as per the procedure.The seeds were bought from the local market of Mysore and authenticated by Prof. R.N. Suresh, HOD of Department of Pharmacology, JSS University, Mysore. The seeds were powdered manually and extraction was performed by using soxhlet apparatus. The procedure took two days. The extract was filtered, vacuum dried and stored in a refrigerator until further use. The yield was 10.4 %. The animals received this extract orally in dosages of 20 mg/kg/day and 200mg/kg/day suspended in propylene glycol. This particular dose was selected on the basis of our pilot studies.

Experimental Procedure Drug treatment: The animals were pretreated with the standardized extract of PN (20mg/kg/day 200mg/kg /day p.o) for two months. After two months AD was induced in the rats by oral aluminium chloride for two months. Thereafter, neurobehavioural cognitive and biochemical experiments were performed.

MODELS OF EXPERIMENT

The animals were randomly divided into four groups of six rats each. Group 1(control) - propylene glycol (1 ml/day).

Group 2 (AD control) – aluminium chloride (17mg/kg body weight for two months)

Group 3 (Test 20) – piper nigrum (20 mg/kg body weight for two months) + aluminium chloride 17mg/kg body weight for two months.

Group 4 (Test 200) – Piper nigrum (200 mg/kg body weight for two months.) + aluminium chloride 17mg/kg body weight for two months.

The dose of aluminium chloride was determined by preliminary studies. At the end of experiment all rats were sacrificed and were send for histopathological and biochemical examination.

Behavioural Procedures Morris' water maze test: Spatial learning and memory was tested in a water maze following the method of Pappas et al., [12]. The maze consisted of a black circular pool (diameter 2.14 m, height 80 cm) filled to a depth of 44 cm with water (25°C). Prior to test day, rats received habituation (exposure in water maze for one minute) in which there was no platform present. Then, a circular platform (9 cm in diameter) was kept hidden 2 cm below the water level in the centre of one of the quadrants. The platform remained in the same position during all the sessions. At the beginning of each session four starting poles were selected randomly on the perimeter of pool. Each rat was kept on pool facing the wall of starting location and allowed 90 sec to swim and find the platform. The animal was allowed a 20 sec rest on the platform. The time taken to reach the hidden platform was noted and if animal was unable to find the platform; it was guided and placed on the platform. The procedure was repeated for all the four start locations. This trial was conducted on first day. On very next day one session of four trials was conducted. After four h of this session platform was removed and same trial was conducted randomly and time spent in each quadrant was measured.

Samples Collection

At the end of the experimental period, the animals were euthanized by atlanto-occipital joint dislocation and the whole brain of each animal were rapidly removed, thoroughly washed with isotonic saline and dried on filter paper. The whole brain from each animal was sagitally divided into two halves. The whole brain and also the one half of each brain was weighed and then homogenized immediately to give 10% (w/v) homogenate in ice-cold medium contained 50 mM Tris-HC1 (pH 7.4) and 300 mM sucrose [13]. The homogenate will be centrifuged at 1800 xg for 10 min at 4°C. The supernatant (10%) was separated for biochemical analysis. The second half of each brain was then fixed in 10% buffered formalin and embedded into paraffin blocks. Histological examination was carried out on 51.1 m-thick, hematoxylin-eosin (Hand E) stained brain sections.

Biochemical Estimation

Quantitative estimation of total cholinesterase level in all rats brain hemolysate was determined by UV kinetic technique .The kit was purchased from AGAPPE Diagnostics Co.

Histopathological Examination

Paraffin block section was sectioned with microtome and was examined by Hematoxylin and Eosin Stain.

STATISTICAL ANALYSIS

In the present study, all results were expressed as mean ±S.E of the mean. Statistical Package for the Social Sciences (SPSS) program, version 11.0 was used to compare significance between each two groups. Difference was considered significant when P Percentage difference representing the percent of variation with respect to the corresponding control group was calculated using the following formula:

$$\% \text{ difference} = \frac{\text{Treated value} - \text{Control value}*100}{\text{Control value}}$$

- Analysis of Variance (ANOVA) was applied to compare the effects of the drug under study.
- Tests of significance were carried out at 5% level.

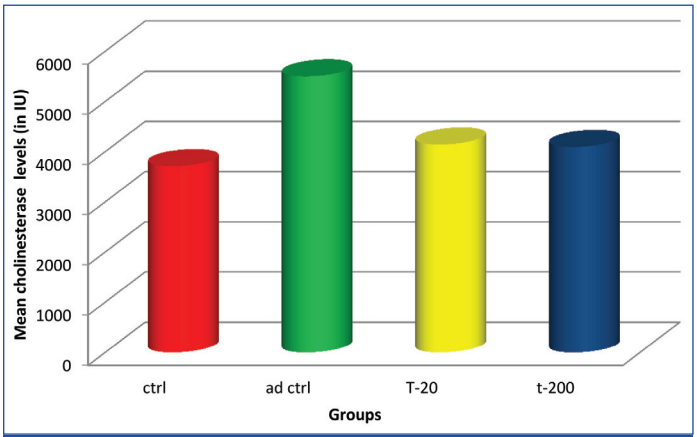
RESULTS

Effect of treatment of Piper nigrum extract on brain cholinesterase level

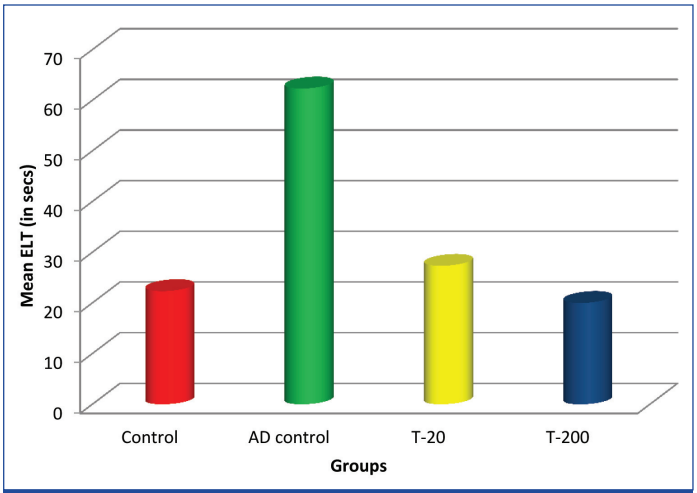
The result on the bar [Table/Fig-1] showed that piper nigrum with 20mg /kg body weight showed 24.69% less cholinesterase level than the AD control group and 11.58% more cholinesterase than the control group. On other hand with the test group with 200 mg/kg body weight it showed 25.69% less cholinesterase than AD control group and 10.29% more than the control. These results shows that piper nigrum has significant effect on preventing neurodegeneration which causes increase cholinesterase level in rat brain all results were significant at 0.001.

Effect of Piper Nigrum in Cognition and Memory

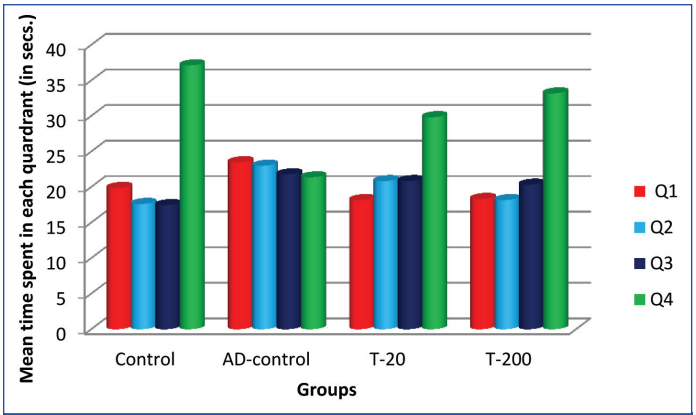
The result in [Table/Fig-2&3] showed that there is a huge effect on memory and cognition with piper nigrum extract in AD rats. In the morris water maze test the escape latency time (ELT) was remarkable less in T 20 group i.e. 56% less than AD control on same way 22% more than control group. T 200 group also showed 67% less than AD control all results are significant at 0.001 on other hand all the groups except AD groups spent more time in fourth quadrant (where previously platform was kept). These show that piper nigrum has role in cognition and memory.This is also due to the prevention of neurodegeneration and cholinesterase inhibition.



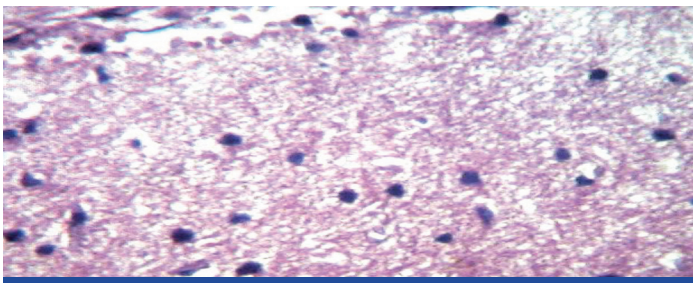
[Table/Fig-1]: Brain Cholinesterase levels



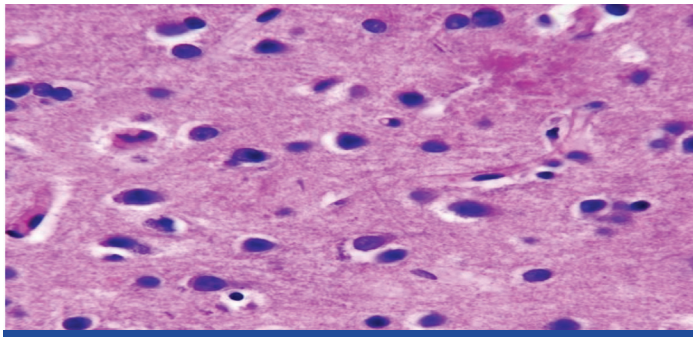
[Table/Fig-2]: Escape latency time in different groups of morri's water maze test



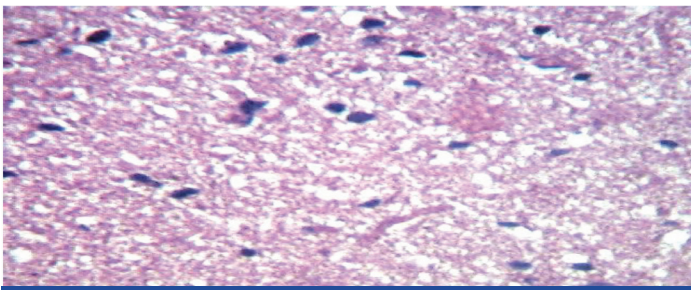
[Table/Fig-3]: Time spend in each quadrant
Q1=First quadrant,Q2=second quadrant,Q3=third quadrant,Q4=fourth quadrant



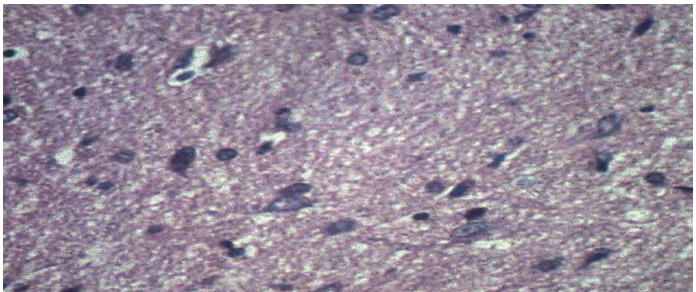
[Table/Fig-4]: Micrograph of brain section of normal control rat showing highly active nerve cells, having huge nuclei



[Table/Fig-5]: Micrograph of brain section of $AlCl_3$ administered rat showing formation of amyloid plaques in cerebral cortex



[Table/Fig-6]: Micrograph of brain section of rats taken piper nigrum 20mg/kg body weight, showing no amyloid plaques



[Table/Fig-7]: Micrograph of brain section of rats taken piper nigrum 200mg/kg body weight, showing no amyloid plaques

Histopathological Changes

Microscopic examination of brain section of control rats showed the normal morphological structure in cortex in [Table/Fig-4] while $AlCl_3$ induced showed various size of amyloid plaque in [Table/Fig-5]. In case of Piper nigrum treated rats brain there is no more amyloid plaque but there is morphological disturbance in [Table/Fig-6&7].

DISCUSSION

AD is the most common neurodegenerative disease affecting nearly 10% of the population above 70-year-old. AD patients lose memory, language skills, and the ability to regulate emotion and eventually become completely dependent upon others for care. Although there have been extensive research into the causes and potential treatments for AD, the results so far have been disappointing. The initiating molecular event and pathophysiology of AD is highly complex. ROS generated due to the accumulation of Aβ compromises the function of ion-motive ATPases, glucose, glutamate transporters, GTP-binding proteins, mitochondria, and also disrupts cellular ion-homeostasis causing cytosolic and mitochondrial Ca^{+2} overload [14]. Anti-oxidants attenuate oxidative stress caused by oxidants, and keep the fine-tuned balance between the physiological production of ROS and their detoxification.

The present study showed that aluminium chloride was able to induce accumulation of Aβ in rat brain, Rats treated with PN showed shorter swimming latencies to the goal platform than the AD group indicating improved reference, that is spatial memory performance. PN -treated rats also showed enhanced working memory in probe trials, indicating consolidation of memory. The ability to find the new platform kept in a different opposite quadrant was significantly compromised in AD groups. PN treatment reversed the above memory inability and thereby increased reversal learning. This shows that the experimental animals are able to learn a new task with better performances. PN also decreased the latency time significantly to that of AD control rats. Hence PN dose not only improves the cognition but also the acquisition of learned new information and influence CNS disability associated with learning and memory.

Cholinergic hypothesis-which essentially states that a loss of cholinergic function in the central nervous system contributes significantly to the cognitive decline associated with advanced age [15-17]. The cholinergic loss in AD is a major component of neuropathology, which has been strongly demonstrated by the fact that cholinesterase inhibitors are effective in alleviating the symptoms of AD [18]. Cholinergic neurotransmission with the acetylcholinesterase inhibitor, physostigmine, reverses scopolamine-induced deficits in nondemented subjects and has been reported to improve the performance of AD patients in tasks that require long-term memory [19]. In our experiment, treatment with aluminium chloride significantly reduced acetylcholine esterase activity. More over PN has shown significant anticholinesterase activity. Current therapeutic strategies for the symptomatic treatment of AD and other related disorders such as vascular dementia, dementia with Lewy bodies, senile dementia and Parkinson's disease are aimed at enhancing the associated cholinergic deficit by inhibiting AChE [20-22], resulting in a boost in endogenous level of ACh in the brain and an improvement of cognitive function [23] this is due to regeneration of nerve and removal of the nerve plaque by cholinesterase inhibitor drugs.

Amyloid accumulation is reduced by the anticholinesterase therapy [24] the histopathological examination of cortical area had clearly showed that the group which has taken PN as preventive therapy has no amyloid plaque. This is due to the antioxidant role of piper nigrum [25,26]which prevents the nerve degeneration.

CONCLUSION

The standardized extract of PN significantly improved learning and memory deficits associated with aluminium chloride and also showed

the anticholinesterase activity with prevention of nerve degeneration. Further, PN alleviated the neuropsychological symptoms associated with animal models of AD. The beneficial effect observed with PN can be attributed to its anticholiesterase and anti-oxidant activity and its inhibitory mechanism for the formation of amyloidal plaque and oligomerisation of tau protein. So, black papper can be used as preventive measure to reduce the incident of alzheimer's disease. However, further researches are still essential to understand the precise mechanism of piper nigrum.

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