Pharmacological evidences of *Acacia Catechu*-Catechin for Anti-Alzheimer's potential

Biradar S M [a*],Ghallab Alotaibi[b], Thippeswamy B S[b],Tarak K C[c], Santhosh R. Awasthi[a], Smita D M[c].

[a]Department of Pharmacology & Doctor of Pharmacy, BLDEA's SSM College of Pharmacy and Research Centre, Vijayapur,Karnataka, India.

[b]Department of Pharmaceutical Sciences &Biomedical Science, College of Pharmacy, Shaqra University, Al-Dawadmi Campus, Kingdom of Saudi Arabia.

[C]Department of Pharmacognosy, SET's College of Pharmacy, Dharwad, Karnataka, India.

Corresponding author:

Dr. S M. Biradar. **M.Pharm. Ph.D**

Department of Pharmacology & Doctor of Pharmacy (PharmD).

BLDEA's SSM college of pharmacy and research centre,BLDE University campus, Vijayapur-586103. Karnataka, India.

Abstract

Introduction: As per regulatory bodies, National Institute of Health and World Health Organization, there will be drastic increase in neurodegenerative disorder affecting worldwide population in forth coming years. Hence an attempt been made to develop a potential role of phytochemicals in the management of Alzheimer's disease.*Methods:*In the present study, different behavioural and biochemical models have been used. *Results:Acacia catechu*-Catechin (CTN) showed significant increase in Transferlatency and Escape Latency Time in behavioural models like Elevated Plus Maze and Morris Water Maze respectively.A dose dependent CTN (40, 20 & 10mg/kg) significant ($P<0.01$) inhibition of acetylcholinesterase (AchE) activity of whole brain was seen in scopolamine and aged mice,

which were comparable to Standard Piracetam (400mg/kg). This potent neuroprotective characteristic of CTN was well supported by brain biogenic amines. In histopathology of hippocampus, the CTN treated mice were protected from the formation of pyknotic neurons and extent of cell death compared to Scopolamine group.In frontotemporal cortex of Sco + CTN (40 mg/kg) treated mice did not show internucleosomal DNA fragmentation and prominent laddering pattern.Whereas Scopolamine being a neurodegenerative inducing agent, also did not show anyinternucleosomal DNA fragmentation and prominent laddering pattern, even though it damages the nerve cells as exhibited by the histopathology study, hence it reveals the novel information, that the scopolamine damages the nerve cell, but not to an extent of DNA damage.*Conclusion:*It is worthwhile to utilise an *Acacia catechu*-Catechin in the management of Neurodegenerative disorders of a type Alzheimer's disease.

Key Words: *Acacia catechu*-Catechin,Anticholinesterase,Dopamine, Nor-adrenalin,Pyknotic neurons, DNA fragmentation.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative anarchyconstituted by dynamic loss of memory and intelligence[1]. Neurodegenerative disorders such as Alzheimer's disease, Levy-Body dementia, Parkinson's disease and cerebro-vascular dementia result in an insidious cognitive and behavioral decline culminating in the development of severe dementia. Based on current population projections, it has been estimated that by 2050 the number of individuals over 65 will increase to 1.1 million worldwide, and as a consequence, the number of individuals with dementia will reach 37 million[2]. The National Institute of Health predicts there will be not less than 8.5 million AD patients by the year 2030 in USA alone [3]. According to WHO, it is estimated that 3.7 million people are suffering from Neurodegenerative disorder in India too [4].

A various factors are being contributed to the development of learning andmemory impair, decrease in cholinergic release [5], increase in free radicals formation, oxidative stress [6] andchange in brain neurotransmitter.Memory enhancers like nootropic agents (piracetam) and cholinesterase inhibitors (Donepezil) are mainly being used to improve the memory and learning. These drugs have many adverse effects such as diarrhoea, insomnia, bronchitis, muscular cramps and various other side effects which have made their use limited [7]. A number of herbs traditionally employed in the Indian System of Medicine "Ayurveda"have yielded many positive results in the treatment of neurodegenerative disorders. Developing nations of the world, large numbers of people still rely heavily on traditional ayurvedic preparations and medicinal plants to meet their daily primary healthcare needs[8].A herbal medicines containing a phytochemicals can also modifythe brain aging [9],hence, an effort has been made to develop a potential role of phytochemical in the management of neurodegenerative disorders of a type Alzheimer's disease with scientific evidence, by measuring the level of reduced glutathione (GSH), brain thiobarbituric acid reactive substances (TBARS), brain acetylcholinesterase and neurotransmitter assay,includes Dopamine and Nor-adrenalineestimation.

*Acacia catechu*belongs to a family Mimosaceae, ethnobotanical survey reveals that heart wood of *Acacia catechu* (Linn. F.) Willd. has various uses in diarrhea, haemorrhages, relaxed conditions of gums, throat and mouth, stomatitis, irritable bowel and also used as antileprotic drug [10]. It is a good source of protein and important ingredient in the preparation of paan [paan is an Indian and South East Asian tradition of chewing betel leaf (*Piper betle*) with areca nut and slaked lime paste]. *Acacia catechu* is also widely used in food industry.Catechin is an ingredient of "MatolCatechin Drink™", which is an antioxidant drink.Another marketed product "Cocoawell" Improves blood flow and circulation, supports cognitive function and healthy immunity. Chemical structure of Catechin is been depicted in Fig 1.

Acacia catechu contains rich of catechin, epicatechin, quercetinetc[11] Catechin[12]and quercetin[13]is responsible for improvement of memory. Catechin also has potential antioxidant property which may be helpful to attenuate the AD due to oxidation [14]. Similarly, catechin and epicatechin exhibit MAO-B inhibitory activity, which is used as a part of the treatment of Parkinson's & Alzheimer's patients, and might be able to protect against neurodegeneration*in vitro*[15].Therefore molecular mechanism of *Acacia catechu* – catechin deserves further studyon neuroprotective and cognitive enhancement activity in memory deficit mice.

2. Methodology

2.1. Drugs and Chemicals

Acacia catechu-catechin(CTN), from Natural Remedies(herbal resource company) as a gift sample,Bangalore. Piracetam (Pira)as a gift sample from Elite pharmaceuticals,Gujarat. Scopolamine was obtained as a gift sample from Alkaloids Corporation of India.5, 5'-dithiobis (2-nitro benzoic acid) (DTNB), n-Heptane and Isobutanol were purchased from Spectrochem, Bangalore. RNA later solution and QIAGEN DNeasy blood and tissue kit from Karnataka University, Dharwad. Other chemicals used were of analytical grade.

2.2. Animals

All the experiments were carried out with 3 months old young Swiss Albino Mice of 22-28g and Aged 14 months old of 35-42 g after approval from the Institutional Animal Ethical Committee (SETCP/IAEC/2010-2011/454). Animals were kept in the animal house of S.E.T's College of Pharmacy, Dharwad, India, under controlled conditions of temperature (23±2ºC), humidity (50±5%) and 12 h light-dark cycle. Animals were fed with rat diet pellet and water ad libitum. All the animals were acclimatized for seven days before to start the experimental studies.

2.3.Preparation of drugs

The selected doses of CTN 40, 20, 10 mg/kg b.wwas administered orally (p.o) by dissolvingin distilled water (DW) based on literature survey.Piracetam (400 mg/kg, b.w), standard drug was dissolved in distilled water and administered per orally (p.o). Amnesia was induced by Scopolamine (3 mg/kg, b.w) intraperitoneally, (i.p.) bydissolving itin distilled water.

2.4.Experimental Design

In the present investigation the mice were divided into different groups for employing various interoceptive and exteroceptive behavioural memory models. Each group comprised of a minimum of six animals. Young Normal animals received Distilled Water (DW) in the dose of 10 ml/kg b.w orally. CTN (40, 20 and 10 mg/kg) was administered orally for 14 successive days to young and aged mice. After 90 min. of the administration of the last dose on 14th day, amnesia was induced in young animals by injecting Sco (3mg/kg, i.p) and in aged mice, naturally aging of mice was considered as amnesia. Young and aged mice were exposed to the training session after 30 min. of scopolamine injection (only young mice)using Elevated Plus Maze. After training trials, retention memory was recorded on 15th day. In Morris Water Maze, amnesia was induced on 11^{th} day, and training trials was continued for 4 days, i.e. (11^{th} day to 14^{th} day). Retention memory was recorded on 15th day. Piracetam (400 mg/kg, p.o) was used as an established nootropic agent and was injected for 14 days to positive control groups.

2.5.Elevated Plus Maze

Elevated Plus Maze (EPM) served as the exteroceptive behavioural model to evaluate short-term memory in mice. EPM for mice consisted of two open arms (16 cm x 5 cm) and two covered arms (16 cm x 5 cm x 12 cm) extended from a central platform (5 cm x 5 cm), and

the maze was elevated to a height of 25 cm from the floor. On the first day (i.e. 14th day), each mouse was placed at the end of an open arm, facing away from the central platform to measure the Transfer latency (TL) time. TL was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. The mouse was allowed to explore the maze for another 2 min. and then returned to its home cage. Retention of this learned task (memory) was examined 24 h after the acquisition trial. Significant reduction in TL value indicated improvement of memory.[16].

2.6.Morris Water Maze

Morris water maze (MWM) test was employed to assess learning and memory of the animals.MWM is a swimming-based model where the animal learns to escape on to a hidden platform. It consists of a large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1°C). The water was made opaque with white-colored non-toxic dye. The tank was divided into four equal quadrants with the help of two threads fixed at right angles to each other on the rim of the pool. A submerged platform (10 cm^2), painted in white, was placed inside the target quadrants of this pool, 1 cm below surface of the water. The position of the platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive training trials on each day with an inter trial gap of 5 min. The mouse was gently placed in the water between the quadrants, facing the wall of the pool, with the drop location changing for each trial, and allowed 120 s to locate the submerged platform. Then, it was allowed to stay on the platform for 20 s. If it failed to find the platform within 120 s, it was gently guided onto the platform and allowed to remain there for 20 s. The day 4 escape latency time (ELT) to locate the hidden platform in the water maze was noted as index of acquisition or learning. The animal was subjected to training trials for four consecutive days, the starting poison was changed with each exposure as mentioned below and the target quadrant (Q 4) remained constant throughout the training period.

Day1:Q1,Q2,Q3,Q4

Day2:Q2,Q3,Q4,Q1

Day3:Q3,Q4,Q1,Q2

Day4:Q4,Q1,Q2,Q3

On the fifth day, the platform was removed and each mouse was allowed to explore the pool for 120 s. The mean time spent in all the four quadrants was noted. The mean time spent by the animal in the target quadrant searching for the hidden platform was noted as index of retrieval[17]

2.7.Estimation of whole brain acetylcholinesterase

The estimation of whole brain acetylcholinesterase (AchE) activity is carried out based on Ellman's method with slight modifications. The animals were decapitated and brains were dissected out immediately and placed in ice-cold saline. The tissue was weighed and homogenized in 0.1M Phosphate buffer pH 8 (10% w/v), the homogenized tissue was centrifuged to 15,375 x g for 10 min. 0.4ml aliquot of the supernatant is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412 nm in a Lab India spectrophotometer. When absorbance reaches a stable value, it is recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine is added and change in absorbance is recorded for a period of 10 min. at intervals of 2 min. Change in the absorbance per min. is thus determined. AchE activity is calculated using the formula; [18]

$$R = 5.74 \times 10^{-4} \times A/CO$$

Where,

R = Rate in moles of substrate hydrolyzed /min. / gm of brain tissue

A = Change in absorbance / min.

CO = Original concentration of the tissue (mg / ml).

2.8.Biochemical estimation of brain biogenic amines.

The animals were sacrificed and whole brainwas dissected out. Weighed quantity of tissue and was homogenized in 10 ml hydrochloric acid - butanol, (0.85 ml of 37% hydrochloric acid in one litre *n*- butanol for spectroscopy) for 1 min. in a cool environment. The sample was then centrifuged for 10 min. at 3,075 x g. 0.08 ml of supernatant phase was removed and added to an eppendrof reagent tube containing 0.2 ml of n-heptane (for spectroscopy) and 0.025 ml 0.1 M hydrochloric acid. After 10 min. of vigorous shaking, the tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded and to the aqueous phase of 0.02 ml, 0.05ml 0.4M and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stored after two min. by addition of 0.01ml Na_2SO_3 in 5M NaOH. Acetic acid was added 1.5 min. later. The solution was then heated to 100 ^oC for 6 min. When the sample again reached room temperature, excitation and emission spectra were read at 395-485nm for Nor-Adrenaline and 330-375nm for Dopamine respectively. The values were expressed as fluorescent excitation spectral height of fluorescenceintensity correspondence to its concentration of biogenic amines present in the sample and percent decrease in release of NT was calculated [19].The prototype spectra of emitted height of fluorescenceintensity of Dopamine and Nor-Adrenaline are shown in Fig 2(a) and 2(b).

2.9.DNA Fragmentation studies

The animals were sacrificed; whole brain was dissected out and stored in RNA later solution at -80°C. The samples were brought to room temperature and used. The isolation of DNA was carried out using QIAGEN DNeasy Blood & Tissue kit, which isolates DNA by coloumn elution method. The samples were first treated with Qiagenlysis buffer and protease K and

incubated at 55°C until the tissue was completely lysed. Then, RNase (20 mg/mL) was added to each sample and incubated at room temperature for 2 min. Next, 180 µL of ethanol and buffer was added to each sample, and the mixture was transferred to a spin column. The columns were centrifuged at 6000 x g for 1 min. and washed twice. The DNA was then eluted from the spin column by centrifugation. Approximately 4 µl of DNA was loaded in each lane and run at 100 V on a 1 % agarose gel stained with ethidium bromide (0.5 mg/mL). 1µg of DNA standard was run in next lane.

2.10. Histopathology of Hippocampus of brain.

After the treatment and behavioural studies, animals were sacrificed and brains were dissected out immediately and kept in 10 % formalin solution. The brain was stained with haematoxylin-eosin stain; hippocampus region was studied under microscope (Olympus India CH 20i). The samples were examined with the help of Dr. Ammanagi (pathologist) Jawaharlal Nehru Medical College, Belgaum, Karnataka, India. Various parameters like Cell damage, pyknotic black neurons, karryorhexis and number of cell death were observed [20]

3. Statistics

The data were analyzed statistically using analysis of variance (ANOVA) followed by Tukey'spost test. Values are expressed as Mean ± Standard errors of mean (S.E.M). $P<0.05$ is considered as significant and $P>0.05$ were considered as non-significant. Statistical comparisons were performed by Tukey'spost test using Graph Pad Prism version 5.0, U.S.A

4. Results

4.1. Acacia catechu-Catechinon learning and memory of EPM and MWM models

The administration of Sco (3 mg/kg; i.p.) and natural ageing of mice in EPM and MWM induced amnesia,Sco administered mice and aged mice significantly decreased the

transfer latency (TL) and decreased the Escape latency time (ELT) compared to normal group of animals in EPM and MWM respectively. These observations suggested that Sco and aging has produced impairment in learning as well as memory. However, CTN alone did not produce significant improvement in EPM (P>0.05)and MWM (P>0.05) as indicated in table 1 and table 2 respectively. Whereas Sco and aging induced memory deficits were successfully reversed by Piracetam (P<0.001) and CTN 40mg/kg (P<0.001), 20mg/kg (P<0.01), 10mg/kg (P>0.05) as indicated by increased TL depicted in table 1. Piracetam and CTN also reversed the Sco and natural aging induced memory deficits in MWM. Significant increase in day 5 TSTQ was observed with Pre-treatment ofPiracetam (P<0.001) and CTN 40mg/kg (P<0.001), 20mg/kg (P<0.001), 10mg/kg (P<0.05) as compared to both Sco induced and aged animals as indicated in table 2.

4.2. Effect of Acacia catechu-Catechin on Brain Acetylcholineterase activity

Statistically significant differences were observed between the induced and treated groups in whole brain Acetylcholineterase(AchE) activity.Piracetam(P<0.01)*per se* decreases AchEwhen compared to control group, where asCTN alonetreated mice did not produce significant result. On the other hand, administration of Sco (3 mg/kg, i.p.) and aged animals significantly (P<0.001) increased the brain AchE activity,this was reversed by CTN in dose dependent manner 40mg/kg (P<0.001),20mg/kg (P<0.001), 10mg/kg (P<0.05), which were administered chronically for 14 days as indicated in Fig 3. Piracetam (400 mg/kg; p.o) being a standard drug, also reversed increased AchE (P<0.001) in Sco and aged animals.

4.3.Effect of Acacia catechu-Catechin on Brain biogenic amines

Animals induced with Sco(P<0.01) and natural aging (P<0.05) shows significant increase in the height of fluorescence intensity of emitted spectra of Dopamine and Nor-adrenaline as indicated in Table 3. This elevated height of fluorescence intensity is reversed by CTN

40mg/kg which shows significant (P<0.05) decrease in Nor-adrenalin, whereas insignificant decrease in Dopamine (P>0.05). Piracetamshows significant (P>0.05) reversal effect of increased height of fluorescenceintensity spectra of dopamine and significant (P<0.01) reversal effect of Nor-adrenalin compared to Sco group of animals.Piracetam also shows significant (P<0.05) decrease in height of fluorescence intensity of Dopamine and Nor-Adrenaline compared to Aged animals.The percentage content of Dopamine and Nor-Adrenaline is also depicted in Table 3.

4.4.Effect of Acacia catechu-Catechin on DNA Fragmentation Studies

The isolated DNA from frontotemporal cortex of different experimental group of animals was subjected to 1 % agarose Gel electrophoresis. In Fig4, Lane 1 shows the laddering pattern of standard Bam H fragmented DNA. Lane 2 indicates normal laddering pattern of DNA from normal group of animal. Even though Lane 3 is a dementia inducing agent(Scopolamine) there is no sign of oligointranucleosomal DNA fragmentation and found normal laddering pattern of frontotemporal cortex comparable to Hypoxic group (Fig.5 Lane 5.)of animal, in which there is clear oligointranucleosomal DNA fragmentation and distinct laddering pattern is present.Fig.4,Lane 4 also shows that there is no sign of intranucleosomal DNA fragmentation in Sco + CTN (40mg/kg) treated animals. Hence,Scopolamine being a neurodegenerative inducing agent, it damages the nerve cells(Fig.6), but not to an extent of DNA damage. Hence this DNA fragmentation study reveals the novel information regarding the effect of scopolamine on DNA fragmentation infrontotemporal cortex.

The pattern of oligointranucleosomal DNA fragmentation, which can be observed during cerebral hypoxia, is depicted in Fig 5. Lane 1 shows the laddering pattern of standard Bam H fragmented DNA. Lane 2 shows DNA fragmentation pattern of frontotemporal cortex from

normal group animal. Lane 5 shows clear laddering pattern and oligointranucleosomal fragmentation pattern from hypoxic group animal. By comparing Fig 5 with Fig 4, we can clearly predict that there is no formation of laddering pattern and oligointranucleosomal DNA fragmentation observed in Scopolamine induced group of animals.

4.5.Effect of Acacia catechu-Catechin on Pathophysiology of Hippocampus of brain tissue.

Photomicrograph of the hippocampus of brain of Normal control (A), Sco control (B), Piracetam treated (C) and CTN treated group (D) are shown inFig 6. Some of the neurons are highlighted with different arrows, solid arrow (⟶) indicates the presence of viable neuron cells, where as broken (⇢) arrow indicates the population of degenrated neurons and the detail quantification of nerve cells present in current Photomicrographs of Hippocampus are shown in Table 4.

(A) Represents normal hippocampus of mouse which shows normal cells which are intact and no neuron degeneration is observed. (B) Sco induced neuron death was observed consistently in hippocampus, as indicated by the appearance of pyknotic black neurons, karryorhexis and karyorlysis with condensed nucleus. (C) Shows mild damage to the hippocampus area Pre-treated with Piracetam 400 mg/kg, indicated by presence of less number of degenerative cells compared to Sco group. (D) Shows less damage to the hippocampus area Pre-treated with CTN 40 mg/kg, indicated by presence of less number of neurodegenerative cells compared to Sco group.

5. Discussion

Alzheimer's disease (AD) is the preferable cause of amnesia in aged persons, accounting for 3/4th of all memory impaired cases. AD-related dementias are

neurodegenerative disorders characterized by progressive brain malfunction occurring in a cascade of biological steps: from neuronal injury to synaptic failure and then finally leads to neuronal death. To improve cholinergic conveyance, differentapproaches are followed, which includes increase of Acetylcholine production, the rise in the levels of presynaptic Acetylcholine release, stimulation of post synaptic muscarinic and nicotinic receptors and the inhibition of Acetylcholine synaptic breakdown by employing anti-cholinesterase. Despite the availability of various allopathic treatments, the sternness of this disease is not yet under control. Hence, alternative medicines including traditional herbs are being utilized in the management of AD.

MWM employed in present experiment is one of the most globally used models to analyse learning and memory in mice. In water maze, a significant decrease in day 4 ELT of control animals during acquisition trials denotes normal acquisition of memory, whereas increase in TSTQ in search of absent platform during the trial on day 5 indicates retrieval of memory [17].

In the present investigation, administration of Sco and natural aged animals elucidated a significant rise in AchE, hence impaired learning and memory. Sco-induced amnesia is mediated through antagonistic effect on central muscarinic receptors. In the present study CTN antagonised Scopolamine induced amnesia, hence it appears that CTN may have produced competitive antagonism of Scopolamine from muscarinic receptors or stimulated the synthesis of acetylcholine in any other form [21].Anticholinesterase which enhance the availability of acetylcholine in synaptic cleft are able to reverse the scopolamine induce deficit indicating a neurotransmitter role of acetylcholine in learning and memory [22].

Learning and memory processes involve interactions between various neurotransmitters due to the complex brain system [23]. Nor-adrenaline and dopamine are the excitatory neurotransmitters of brain biogenic systems. Sub-chronic effects of an

environmental carcinogen show that elevated levels of excitatory neurotransmitters is related to loss of memory and amnesia [24]. Comparatively, Sco and natural aged induced animals have shown elevated levels of excitatory neurotransmitters, which is an indication of dementia. In the present study, administration of Piracetam and CTN at different test doses decreases the whole brain content of biogenic amines; hence it improves the learning and memory capabilities.

There is increasing evidence showing the significance of apoptosis in the etiology of various neurological, behavioral, and neurocognitive dysfunctions. It is believed that the pathogenesis of each of the major neurodegenerative disorders is associated with DNA damage and apoptotic cell death[25]. In present investigation, Scopolamine did not depict the oligointranucleosomal fragmentation pattern. This may be due to various factors; recent evidence shows that genetic differences among strains of animals alter the susceptibility to excitotoxic insult. In fact, experimental evidence suggests that the stage of the cell cycle may influence apoptosis. Furthermore, the nature of a specific cell type such as a granule cell may determine its susceptibility to undergo apoptosis[26]. Further investigation of excitotoxicity may lead to a better understanding of modes of apoptosis and necrosis and to more effective neurodegenrative treatments in the future.

The hippocampus is critical for the use of spatial information to organize and guide behaviors. In addition, lesions of the hippocampus impair performance in many types of tasks that are dependent on the use of spatial information [27]. People with extensive hippocampal damage may experience amnesia, learning and memory disabilities [28].Neurodegeneration to the hippocampus starts with condensation (pyknosis) of nucleus, followed by formation of small fragments (karyorrhexis), these fragments are get dissolved (karyolysis) and disappears of nucleus will takes place followed by cell necrosis. Pre-treatment with Piracetam and CTN prevented the formation/extent of Neurodegeneration by reducing the severity of cell damage

and decrease in the count of dead cells and also reduction in the formation of pyknotic black neurons comparative to Sco hippocampus, where as in Sco group, all these parameters were increased and these results are consistent with previous findings [20] thereby, CTN further supports is role as a promising neuroprotective agent.

5. Conclusion

In the present study the*Acacia catechu*-Catechin reverses the Sco and aged induced memory deficits in EPM andMWM behavioural models. It elevated the acetylcholine level in brain by significant decrease in cholinesterase activity; hence it improves the memory of both young and aged mice. Due to its antioxidant characteristic by reducing the TBARS and increasing the GSH level it may delay the process of Neurodegeneration and natural aging (other part of the study). No intranucleosomal DNA fragmentation was observed in Sco + CTN (40 mg/kg) treated animals;moreover this potent neuroprotective characteristic is well supported by brain biogenic amines and histopathogical findings. Hence, it is worthwhile to utilise an *Acacia catechu*-Catechin as an important potential phytochemical in the management of Neurodegenerative disorders of a type Alzheimer's disease.

Acknowledgement: The authors are thankful to Principal, BLDEA's SSM college of Pharmacy & research centre vijayapur and the Pricnipal. Dr. Kulkarni VH, SET's College of pharmacy, Dharwad for providing necessary facilities to carry out the experimental study. The authors are thankful to herbal resource company; Natural Remedies, Bangalore, for providing a gift sample of *Acacia catechu*-catechin. Authors extend their thankful to Prof. Pramod B. Gai, Principal investigator and Head for Research centre for DNA diagnostics, Karnataka University, Dharwad. India.

Declarations of Conflict of interest

There is nothing to declare for conflict of interest between the authors.

References

[1]. Magdalini AP, Maria T, Spiros E, Paul C, Fotini NL, Marigoula M (2011). Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. *Behav brain res* 219:197-204.

[2]. Lokhart BP, Lestage PJ (2003). Cognition enhancing or neuroprotective compounds for treatment of cognitive disorders: why? When? Which? Expt. *Gerontology* 38:119-128.

[3]. National Institute of aging-National Institutes of Health (USA) (2000). *Progress Report on Alzheimer's disease: Taking the Next Steps.* National Institutes of Health, Washington DC 3:1154-1156.

4]. Shaji KS, Jotheeswaran AT, Girish N, Bharath S, Dias A, Pattabiraman M, Varghese M (2010). Dementia India Report: A report prepared for the Alzheimer's and Related Disorders Society of India, ARDSI-2010 New Delhi.

[5]. Zanardi A, Leo G, Bragine G, Zoli M (2002). Nicotine and neurodegeneration in aging.*ToxicolLett* 127:207–15.

[6]. Zhu X, Smith MA, Honda K. Aliev G, Moreira PI, Nunomura A (2007). Vascular oxidative stress in Alzheimer disease.*J NeurolSci* 257:240–246.

[7]. Doody RS, Stevens JC, Beck RN, Dubinsky RM, Koye JA, Gwyther L (2001). Practice parameters: Management of dementia (an evidence based review)-report of the quality standards subcommittee of the *American Academy of Neurology* 56:1154- 1166.

[8]. Lather A, Gupta V, Tyagi V, Kumar V, Garg S (2010). Phytochemistry and pharmacological activites of *Bersamaenglerianagu*erke-an Overview. Int. Res J Phar 1(1):89-94.

[9]. Hanumanthacahar J, Milind P (2006). Cholinergic basis of memory improving effects of *Foeniculmvulgare*in mice.*J Med Food* 9(3):413-417.

[10]. Khare CP (2007). Indian Medicinal Plants: an illustrated dictionary. Springer (India) private limited, New Delhi, pp. 5-6.

[11]. Li X, Wang H, Liu C, Chen R (2010).Chemical constituents of *Acacia catechu*.*ZhongguoZhong Yao ZaZhi* 35(11):1425-1427.

[12].Haque, A.M., M. Hashimoto, M. Katakura, Y. Tanabe, Y. Hara and O. Shido, 2006. Long-term administration of green tea catechins improves spatial cognition learning ability in rats. J. Nutr., 136: 1043-1047.

[13]. Tong-Un, T., P. Wannanon, J. Wattanathorn and W. Phachonpai, 2010. Cognitive-enhancing and antioxidant activities of quercetin liposomes in animal model of Alzheimer's disease.OnLine J. Biol. Sci., 10: 84-91.

[14]. Naik GH, Priyadarshini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK (2003). Comparitive antioxidant activity of individual herbal components used in Ayurvedic medicine.*Phytochemistry* 63:97-104.

[15] Hou WC, Lin RD, Chen CT, Lee MH (2005). Monoamine oxidase B (MAO-B) inhibition by active principles from *Uncariarhynchophylla*.*J Ethnopharmacol* 100(1-2):216-220.

[16] Espinola EB, Dias RF, Mattei R, Carlini EA (1997). Pharmacological activity of Guarana*(Paulliniacupana*Mart.) in laboratory animals.*J Ethanopharmacol*55:223-229.

[17]. Dalla Y, Singh N, Jaggi AS, Singh D, Ghulati P (2009). Potential of ezetimibe in memory deficits associated with dementia of Alzheimer's type in mice. *Ind J Pharmacol* 41:262-267.

[18]. Raju TR, Kutty BM, Sathyaprabha TN, ShanakranarayanaRao BS (2004). Assay of acetylcholinesterase activity in the brain. *Brain Behaviour* 142-144.

[19]. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F (1974). A fluorimetricmicromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligramamounts of brain tissue.*BiochemPharmacol*23:2337-46.

[20]. Chen Y, Lomnitski L, Michaelson DM, Shohami E (1997). Motor and cognitive deficits in apolipoprotein e-deficient mice after closed head injury. *Neuroscience* 80:1255-1262.

[21].Parle M, Bansal N (2010). Antiamnesic Activity of an Ayurvedic Formulation Chyawanprash in mice.*eCAM*30:1-10.

[22]. Kulkarni SK (1999). New drug Discovery process, In: Handbook of Experimental Pharmacology. VallabhPrakashan, New Delhi, pp. 43-84.

[23].Castellano C, Cabib S, Puglisi-Allegra S (1996). Psychopharmacology of memory modulation: evidence for multiple interactions among neurotransmitters and hormones. *Behav Brain Res* 77:1–21.

[24]. Yinyin X, Shuqun C, Junlin H, Xueqing L, Yan T, Haiyan Y, Lijun H, Tao L, Baijie T, Yingxiong W (2011). Effects of subchronic exposure to benzo[a]pyrene (B[a]P) on learning and memory, and neurotransmitters in male Sprague–Dawley rat. *NeuroToxicology* 32:188-198.

[25]. Bagnyukovaa TV, Powellb CL, Pavlivc O, Tryndyaka VP, Pogribnya IP (2008). Induction of oxidative stress and DNA damage in rat brain by a folate/methyl-deficient diet.*Brain res*;1237:44-51.

[26]. Pulera MR, Adams LM, Liu H, Santos DG, Robert N (1998). Apoptosis in a Neonatal Rat Model of Cerebral Hypoxia-Ischemia.*Stroke* 29:2622-2630.

[27]. Silversa JM, Tokunagaa S, Berrya RB, Whiteb AM, Matthewsa DB (2003). Impairments in spatial learning and memory: ethanol, allopregnanolone, and the hippocampus. *Brain Res Rev* 43:275–284.

[28]. Sivanandan R, Saraswathi P, Rajendran SM (2011). Behavioral Effect of FructusPsoralea on Ethanol Induced Neurodegeneration of Hippocampus in Wistar Albino Rat. *Rec Res Sci Tech* 3(10):98-102.

Figure 1: Structure of Catechin

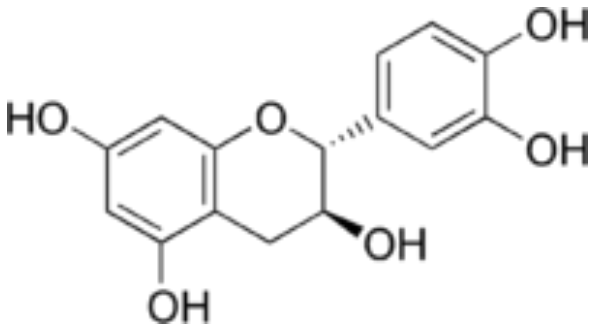
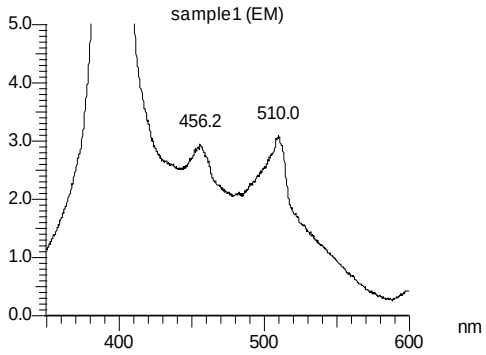
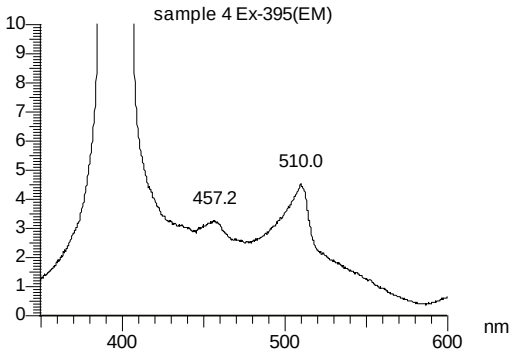


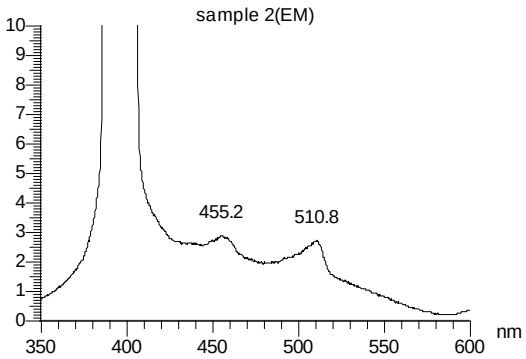
Figure 2(a): Prototype spectra of height intensity of Dopamine of Normal young mice, Sco group and Aged mice



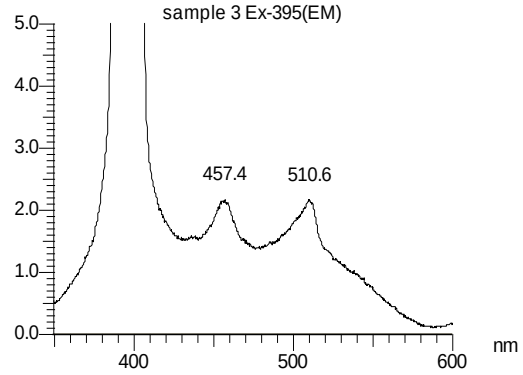
(1) Normal



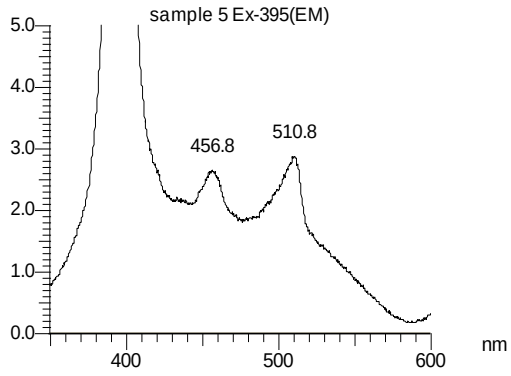
(2) Sco



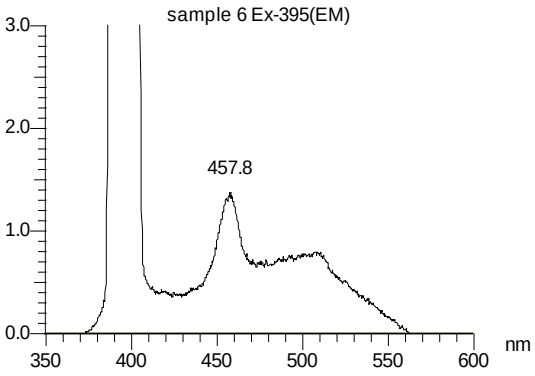
(3) Sco +Pira



(4) Sco + CTN (40)

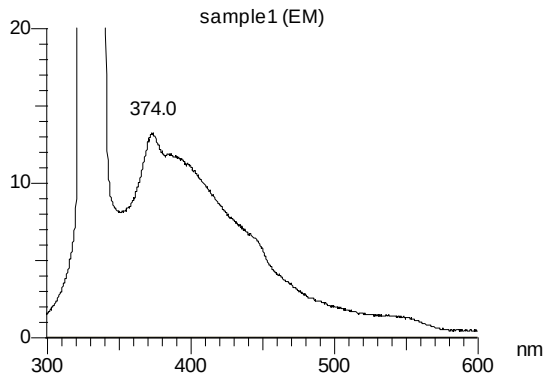


(5) Aged

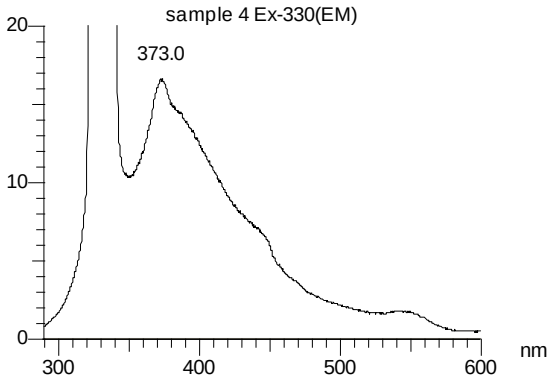


(6) Aged + CTN (40)

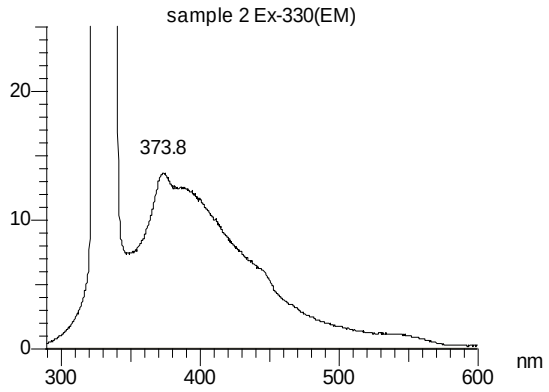
Figure 2(b): Prototype spectra of height intensity of Nor-Adrenaline of Normal young mice, Sco group and Aged mice



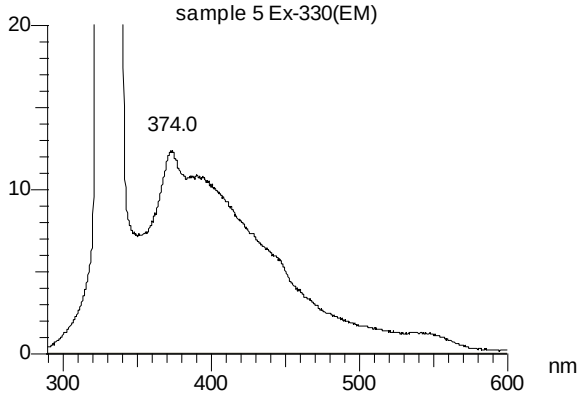
(1) Normal



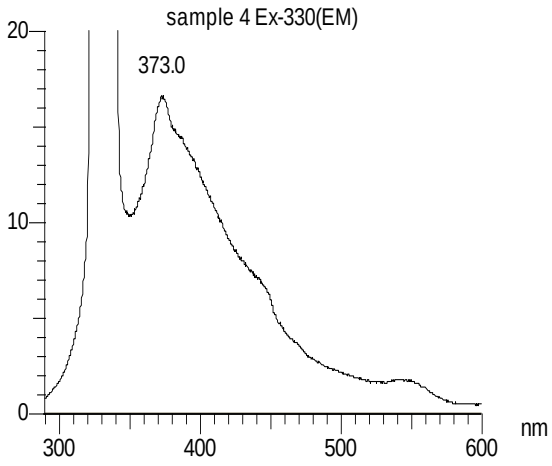
(2) Sco



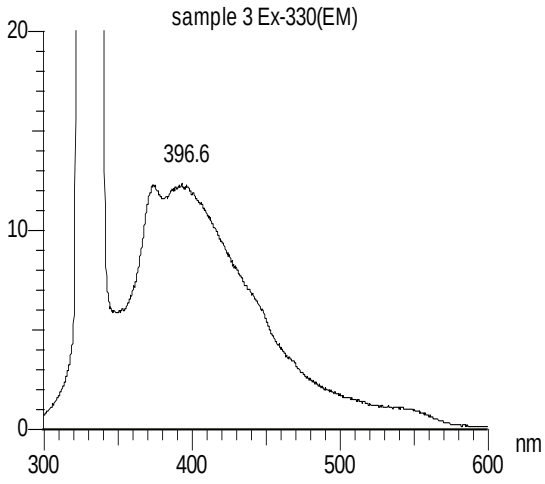
(3) Sco +Pira



(4) Sco + CTN (40)



(5) Aged



(6) Aged + CTN (40)

Figure 3: Effect of Acacia catechu-Catechin on whole brain Acetylcholinesterase (AchE) of Normal young mice, Sco group and Aged mice

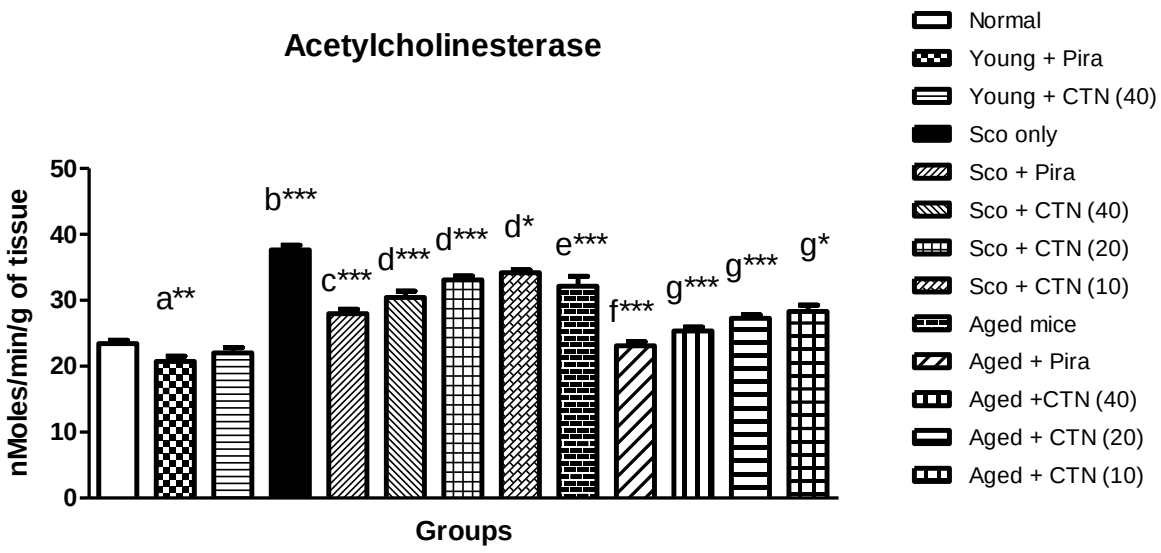
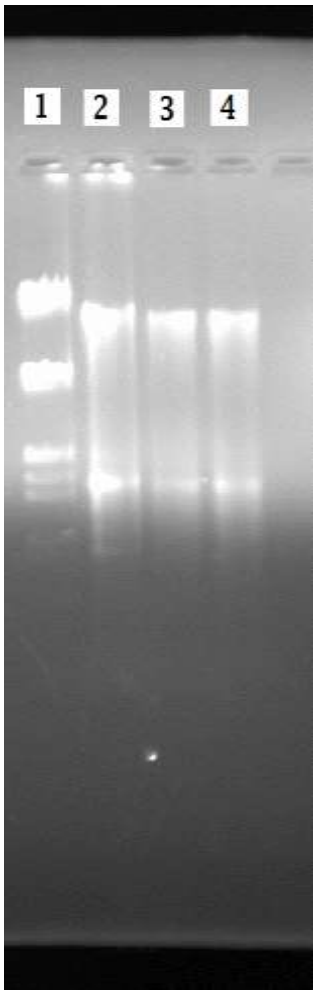


Fig 3: Pira- Piracetam; CTN- Acacia catechu- Catechin; Sco- Scopolamine. Each group consists of 6 animals (n=6). Values are Mean±S.E.M. P<0.05 is considered as significant, ***P<0.001, **P<0.01, *P<0.05. Asterisks with P value 'a' indicate significant difference of Young group vs. Normal group, 'b' indicates Sco induced group vs Normal group, 'c' indicates Pira treated groups' vs Sco group , 'd' indicates CTN treated groups' vs Sco group, 'e' indicates Aged group vs Normal group, 'f' indicates Pira treated groups' vs Aged group and 'g' indicates CTN treated groups vs Aged group.



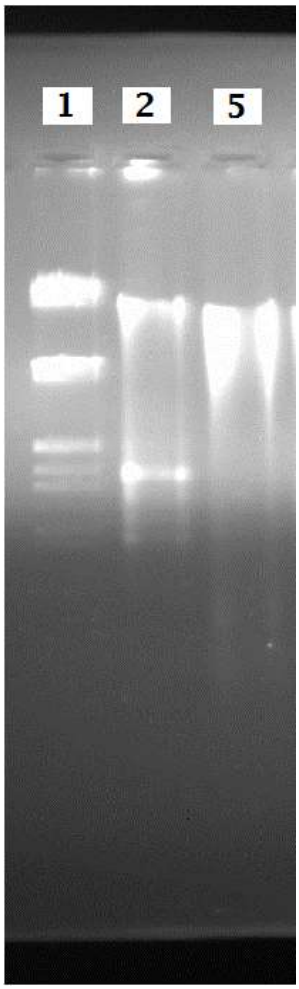


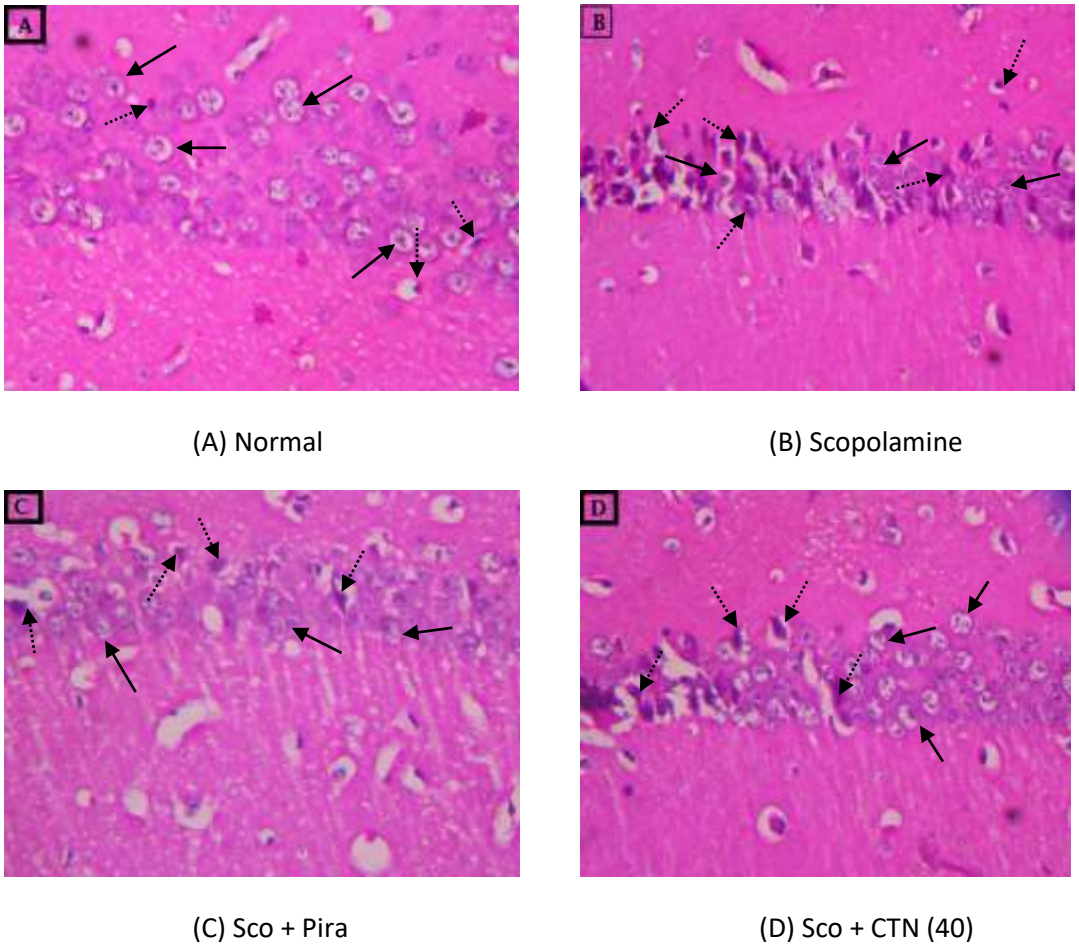
Fig 4 **Fig 5**

Fig 4: Gel electrophoresis of isolated DNA of Frontotemporal cortex from various experimental groups of animals. Lane 1, Standard DNA ladder, Lane 2, Normal frontotemporal cortex; lane 3, frontotemporal cortex of the amnesic brain; lane 4, frontotemporal cortex of the amnesic brain treated with CTN (40mg/kg, b.w). There is no display of prominent laddering pattern, and hence no indicative of oligointranucleosomal DNA fragmentation between the groups.

Fig 5: Gel electrophoresis of isolated DNA of hypoxic Frontotemporal cortex from various experimental groups of animals. Lane 1 shows standard DNA fragments. Lane 2, Normal

frontotemporal cortex shows normal laddering of DNA. Lane 5, cerebral ischemia induced frontotemporal cortex shows oligointrenucleosomal DNA fragmentation pattern and distinct laddering pattern.

Figure 6: Histopathology



(A) Normal (B) Scopolamine

(C) Sco + Pira (D) Sco + CTN (40)

Fig 6: Key words:

→ Solid arrow indicates presence of viable neuron cells.

⇢ Broken arrow indicates presence of degenerated neuron cells.

Photomicrograph of hippocampus of brain (Magnification 40x), (A) Normal control, (B) Scopolamine, (C) Scopolamine + Piracetam pre-treated and (D) Scopolamine + CTN pre-treated group are shown.

(A) Represents Normal hippocampus of mice which shows normal cells which are intact and less neuron degeneration is observed. (B) Scopolamine induced neuron death was observed consistently in hippocampus, as indicated by the appearance of pyknotic black neurons, karryorhexis and karyorlysis with condensed nucleus. (C) Shows mild damage to the hippocampus area pre-treated with Piracetam 400 mg/kg, indicated by presence of less number of degenerative cells compared to Scopolamine group. (D) Shows moderate damage to the hippocampus area pre-treated with CTN 40 mg/kg, indicated by presence of medium number of degenerative cells compared to Scopolamine group.

Table 1
Effect of Acacia catechu-Catechin on Transfer latencies (TL) of Normal young mice, Sco group and Aged mice on Elevated Plus Maze

Sl.no	Groups	Dose	14th day (in sec)	15th day (in sec)
1.	Normal group	10 ml/kg, p.o	29.83 ± 0.9458	25.83 ± 1.447
2.	Young + Pira	400 mg/kg, p.o	24.83 ± 1.447	21.33 ± 0.6146 ^{a*}
3.	Young + CTN	40 mg/kg, i.p	21.83 ± 1.167	16.17 ± 1.558
4.	Sco only	3 mg/kg, i.p	32.17 ± 1.905	55.83 ± 2.535 ^{b***}
5.	Sco + Pira	400 mg/kg, p.o	31.83 ± 1.887	29.67 ± 1.453 ^{c***}
6.	Sco + CTN	40 mg/kg, i.p	31.50 ± 1.803	31.33 ± 1.406 ^{d***}
7.	Sco + CTN	20 mg/kg, i.p	33.33 ± 3.602	44.17 ± 2.701 ^{d**}
8.	Sco + CTN	10 mg/kg, i.p	34.33 ± 2.186	47.33 ± 3.393
9.	Aged mice	10 ml/kg, p.o	30.50 ± 1.607	51.33 ± 2.728 ^{e***}
10.	Aged + Pira	400 mg/kg, p.o	30.17 ± 0.9458	26.67 ± 1.453 ^{f***}
11.	Aged +CTN	40 mg/kg, i.p	28.00 ± 0.8944	28.33 ± 1.406 ^{g***}
12.	Aged + CTN	20 mg/kg, i.p	29.50 ± 2.029	36.83 ± 1.740 ^{g**}
13.	Aged + CTN	10 mg/kg, i.p	29.33 ± 2.486	44.33 ± 3.393

Pira- Piracetam; CTN- Acacia catechu- Catechin; Sco- Scopolamine. Each group consists of 6 animals (n=6). Values are Mean±S.E.M. P<0.05 is considered as significant, ***P<0.001, **P<0.01, *P<0.05. Asterisks with P value 'a' indicate significant difference of Young group vs. Normal group, 'b' indicates Sco induced group vs Normal group, 'c' indicates Pira treated groups' vs Sco group , 'd' indicates CTN treated groups' vs Sco group, 'e' indicates Aged group vs Normal group, 'f' indicates Pira treated groups' vs Aged group and 'g' indicates CTN treated groups vs Aged group.

Table 2
Effect of Acacia catechu-Catechin on Escape Latency time (ELT) of Normal young mice, Sco group and Aged mice on Morris Water Maze (MWM)

Sl.no	Groups	Dose	Day 1 (in sec)	Day 4 (in sec)	Day 5 TSTQ (in sec)
1.	Normal group	10 ml/kg, p.o	78.64 ± 1.66	35.98 ± 1.39	75.05 ± 0.42
2.	Young + Pira	400 mg/kg, p.o	75.95 ± 1.02	25.82 ± 1.12 ^{a***}	99.88 ± 2.04 ^{a***}
3.	Young + CTN	40 mg/kg, i.p	77.75 ± 0.551	29.85 ± 1.02	81.45 ± 1.08
4.	Sco only	3 mg/kg, i.p	77.93 ± 1.09	68.18 ± 0.95 ^{b***}	42.28 ± 0.92 ^{b***}
5.	Sco + Pira	400 mg/kg, p.o	73.55 ± 1.04	35.68 ± 1.30 ^{c***}	76.38 ± 0.89 ^{c***}
6.	Sco + CTN	40 mg/kg, i.p	74.75 ± 1.39	41.90 ± 1.10 ^{d***}	73.62 ± 1.04 ^{d***}
7.	Sco + CTN	20 mg/kg, i.p	76.07 ± 0.88	58.35 ± 3.03 ^{d**}	54.90 ± 3.83 ^{d***}
8.	Sco + CTN	10 mg/kg, i.p	78.92 ± 0.98	60.70 ± 1.20 ^{d*}	51.47 ± 2.33 ^{d*}
9.	Aged mice	10 ml/kg, p.o	82.15 ± 1.11	71.18 ± 0.95 ^{e***}	45.28 ± 0.92 ^{e***}
10.	Aged + Pira	400 mg/kg, p.o	77.93 ± 1.09	28.52 ± 0.88 ^{f***}	91.05 ± 2.61 ^{f***}
11.	Aged +CTN	40 mg/kg, i.p	78.95 ± 1.08	38.18 ± 0.95 ^{g***}	80.88 ± 2.04 ^{g***}
12.	Aged + CTN	20 mg/kg, i.p	79.45 ± 1.06	61.13 ± 3.29 ^{g**}	65.15 ± 4.03 ^{g***}
13.	Aged + CTN	10 mg/kg, i.p	81.12 ± 1.35	63.38 ± 1.60 ^{g*}	57.33 ± 3.36 ^{g*}

Pira- Piracetam; CTN- Acacia catechu- Catechin; Sco- Scopolamine. Each group consists of 6 animals (n=6). Values are Mean±S.E.M. P<0.05 is considered as significant, ***P<0.001, **P<0.01, *P<0.05. Asterisks with P value 'a' indicate significant difference of Young group vs. Normal group, 'b' indicates Sco induced group vs Normal group, 'c' indicates Pira treated groups' vs Sco group , 'd' indicates CTN treated groups' vs Sco group, 'e' indicates Aged group vs Normal group, 'f' indicates Pira treated groups' vs Aged group and 'g' indicates CTN treated groups vs Aged group.

Table 3
Effect of Acacia catechu-Catechin on brain Biogenic amines in Normal young mice, Sco group and Aged mice

Sl.no	Groups	Dose	Dopamine (Flu. Intensity)	Nor-Adrenaline (Flu. Intensity)	Dopamine content (%)	NorAdrenaline content (%)
1.	Normal group	10 ml/kg, p.o	10.93 ± 0.49	3.0 ± 0.24	100	100
2.	Sco only	3 mg/kg, i.p	14.00 ± 0.6 ^{a**}	5.7 ± 0.40 ^{a**}	128.1	190
3.	Sco + Pira	400 mg/kg, p.o	11.45 ± 0.33 ^{b*}	3.3 ± 0.3 ^{b**}	104.7	110
4.	Sco + CTN	40 mg/kg, i.p	11.42 ± 0.61 ^{c*}	4.6 ± 0.21	104.4	153.3
5.	Aged mice	10 ml/kg, p.o	13.67 ± 0.40 ^{d*}	4.8 ± 0.26 ^{d*}	125.1	160.0
6.	Aged + Pira	400 mg/kg, p.o	11.36 ± 0.47 ^{e*}	3.2 ± 0.36 ^{e*}	103.9	106.6
7.	Aged +CTN	40 mg/kg, i.p	12.04 ± 0.51	3.7 ± 0.42	110.1	123.3

Flu.= Fluorescence.

Pira- Piracetam; CTN- Acacia catechu- Catechin; Sco- Scopolamine. Each group consists of 6 animals (n=6). Values are Mean±S.E.M. P<0.05 is considered as significant, ***P<0.001, **P<0.01, *P<0.05. Asterisks with P value 'a' indicate significant difference of Sco group vs. Normal group, 'b' indicates Pira treated group vs Sco induced group, 'c' indicates CTN treated groups' vs Sco group , 'd' indicates Aged group vs Normal group and 'e' indicates Pira treated groups vs Aged group.

Table 4
The quantification of nerve cells present in current Photomicrographs of Hippocampus.

Sl. No	Groups	Dose	Viable cells	% of Viable cells	Degenerative cells	% of Degenerative cells
1	Normal + DW	10 ml/kg, p.o	68	100	09	0
2	Scopolamine	3 mg/kg, i.p	21	30.88	64	85.93
3	Scopolamine + Piracetam	400 mg/kg, p.o	59	86.76	17	47.05
4	Scopolamine + CTN	40 mg/kg, p.o	52	76.47	29	68.96