International Journal of Drug Development & Research | July-September 2012 | Vol. 4 | Issue 3 | ISSN 0975-9344 | Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.03 & H index 2

Evaluation of Antioxidant and Acetyl Cholinesterase inhibitory activity of *Peltophorum pterocarpum* in Scopolamine treated Rats

N. B. Sridharamurthy*[1], B. Ashok[1], R. Yogananda[2]
Department of Pharmacology, Dayananda Sagar College of Pharmacy, Bangalore, India
Department of Pharmaceutics, SJM College of Pharmacy, Chitradurga, India

Abstract

To evaluate the antioxidant and Acetyl cholinesterase activity of Methanol extract of *Peltophorum pterocarpum* (MEPP) in scopolamine treated rats. The methanol extract was subjected for phytochemical analysis to identify different phytochemical constituents. Neuroprotective activity of methanol extract was studied against accelerated ageing with D-galactose followed by scopolamine to induce memory. Behavioral studies, AChE activity, antioxidant parameters of brain homogenate, serum biochemical parameters and histopathological changes of brain were assessed in Control / Scopolamine / Standard / Extract treated animals. Phytochemical investigation of methanol revealed the presence of carbohydrates, proteins, aminoacids, glycosides, triterpinoids, flavonoids and total phenolic content. D-galactose induced oxidative stress and scopolamine induced memory impairment in rats were significantly prevented by treatment with methanol extract of *Peltophorum pterocarpum* bark. Methanol extract treated group improved memory in elevated plus maze and Y maze tests, reduction in AChE activity, increased activity of brain antioxidant enzymes such as catalase, super oxide dismutase, glutathione and reducing the increased activity of lipid peroxidation and also reduction in serum biochemical parameter glucose, total cholesterol and reverse in the degenerative changes in the histopathological study of the rat brain. Hence methanol extract of *Peltophorum pterocarpum* treated animals confirmed the protection of brain against oxidative stress, neuro-degeneration and behavioral (learning, memory) changes of extract under study.

*Corresponding author, Mailing address:
N. B. Sridharamurthy
Email: murthy.sridhara@yahoo.com

***Key words*:**
Peltophorum pterocarpum, AChE, Scopolamine, Neurodegeneration.

***How to Cite this Paper*:**
N. B. Sridharamurthy*, B. Ashok, R. Yogananda "Evaluation of Antioxidant and Acetyl Cholinesterase inhibitory activity of *Peltophorum pterocarpum* in Scopolamine treated Rats" Int. J. Drug Dev. & Res., July-September 2012, 4(3): 115-127

Article History:----------
Date of Submission: 24-05-2012
Date of Acceptance: 05-06-2012
Conflict of Interest: NIL
Source of Support: NONE

Introduction

The herbal medicines represent a valuable resource in prevention of the therapy of some CNS diseases, in association with a healthy lifestyle including correct dietary habits and moderate physical activity. As complementary and alternative therapy, herbal medicine, or simply phytotherapy, refers to the

medical use of plant organs (leaves, stems, roots, flowers, fruits and seeds) for their curative properties. Generally, herbal products contain complex mixtures of active components (phytochemicals), including phenylpropanoids, isoprenoids and alkaloids, and it is often difficult to determine which components of the herbs has biological activity .[1, 2]

Nutritional therapy is a healing system using functional foods and nutraceuticals as therapeutics. This complementary therapy is based on the assumption that food is not only a source of nutrients and energy, but can also provide health benefits. In particular, the reported health-promoting effects of plant foods and beverages can be ascribed to the numerous bioactive chemicals present in plant tissues and, consequently, occurring in foods. Consumed as part of a normal diet, plant foods are thus not only a source of nutrients and energy, but may additionally provide health benefits beyond basic nutritional functions, by virtue of their dietary therapeutics (phytochemicals).[3]

Nootropic agents such as piracetam, pramiracetam, aniracetam and cholinesterase inhibitors like donepezil, gallantamine, rivastigmine, tacrine are being primarily used to improve memory. However, the resulting adverse effects associated with these agents have limited their use [4, 5] and it is worthwhile to explore the utility of traditional medicines in the treatment of cognitive disorders.

The Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence. Plants like *Bacopa monniera,* [6] *Azadirachta indica*, [7] *Withania somnifera*, [8] as well as *Ocimum sanctum*, [9] have been investigated for their cognitive function. Although, several plant products are traditionally used to treat the age related neurodegenerative complications, still many agents are poorly studied in the literature. Hence, the present study is undertaken to investigate the antioxidant and cholinesterase inhibitory effects of *Peltophorum pterocarpum* for neuroprotection and cholinergic influenced learning and memory using interceptive and exteroceptive tests.

Neurodegenerative Disease:

Neuroprotection refers to the strategies and relative mechanisms able to defend the central nervous system (CNS) against neuronal injury due to both acute (e.g. stroke or trauma) and chronic neurodegenerative disorders.[10] Age is the single most important risk factors for degenerative disease of the CNS. As the lifespan of humans continues to increase, an increasing burden of degenerative diseases is emerging. Some signs of degeneration, such as neuronal loss or even specific pathologic changes, may also occur in ageing in the absence of disease. Healthy ageing is also characterized by changes in neurotransmitters, which could be responsible for some of the changes in cognitive (age associated memory impairment) or motor abilities in older individuals.

Neurodegenerative disorders such as Alzheimer's disease (AD), Lewy-Body dementia (LBD), frontal lobe dementia (FLD), Parkinson's disease (PD) and cerebrovascular dementia (CVD) result in an insidious cognitive and behavioural decline culminating in the development of severe dementia. Dementia (Latin, dementare meaning 'to drive mad') is generally defined as "a state of serious emotional and mental deterioration, of organic or functional origin".[11]

AD is a form of dementia, resulting from the degeneration of basal fore brain cholinergic neurons innervating the cortex amygdale and hippocampus. This is resulting into the Cognitive impairement, personality changes, psychotic symptoms, incontinence, gait and motor disturbance, seizures.

Degenerative disease of the brain is associated with deficits in the cholinergic system. The cholinergic system is an important modulator in the brain and is vital for conscious awareness. Acetyl choline (Ach) regulates high cognitive functions such as memory,

learning, dendrite arborization, neuronal developement and differentiation.[12] Evidently, since Alzheimer's disease is by far the most prevalent form of dementia, and will undoubtedly serve as the benchmark for any future treatment of dementia, an update of current symptomatic and disease-modifying therapeutic approaches (cholinergic, glutamatergic, nootropics and b-amyloid cascade inhibitors) should be reviewed.

Methodology

Experimental animals

Healthy Wister rats and Swiss Albino mice of sex, weighing between 150-200g and 20-30g respectively were procured from the animal house of Dayananda Sagar college of Pharmacy, Bangalore, India where the animals were kept in well ventilated spacious animal house with 12 ± 1 h day and night schedule. The animals were lodged in large and spacious hygienically maintained cages during the course of the experimental period. The room temperature was maintained at 25 ± 1°C. The animals were fed with standard rat feed (Brook Bond Lipton India Ltd., Bangalore.) and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. Col/IAEC/55/11-12).

Plant material

The fresh bark of *Peltophorum pterocarpum* was procured from the surroundings of Bangalore district in the month of July-September as the active constituents are found to be more during these months. Plant material was authenticated and certified by Dr. Shiddamallayya N, Botonist, National Ayurveda Research Institute, Ashoka pillar, Jayanagar, Bangalore–560 011. Vide ref no: Drug authentication/SMPU/NADRI/BNG/2011-12/210. A voucher specimen of the collected sample is deposited in the departmental herbarium for the future reference.

Preparation of extract

The powdered material of *Peltophorum pterocarpum* bark *was* refluxed successively with the solvents Petroleum ether (40°-60°, E- Merck Mumbai, India), Chloroform (50° – 70°, E-Merck Mumbai, India) and Methanol (E- Merck Mumbai, India) in a Soxhlet extractor for 48 hrs in batches of 350g each. Every time, before extracting with the next solvent the marc was dried. All the extracts so obtained were concentrated in vacuum using rotary flash evaporator (Buchi-Flawil, Switzerland). Finally the solvents were removed completely over the water bath and finally desiccators dried. The extracts so obtained from each of the solvents were labelled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered bark[12].

Qualitative phytochemical screening: [12]

The crude extracts of the bark *viz*., Petroleum ether, Chloroform, and Methanol extracts of *Peltophorum pterocarpum* were then subjected to the qualitative tests to detect the major chemical groups.

Estimation of Total Phenolic Content

Recent studies have shown that many dietary polyphenolics constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute to the protective effects *in vivo*. It is possible to assess the extent to which the total antioxidant potentials of plant extracts can be accounted for by the activities of the individual polyphenols. Hence, the total phenol content of the extracts was determined by using the Folin-Ciocalteu method.[13] This test is based on oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation, a green-blue complex formed was measured at 750 nm.

Acute toxicity study

12 Albino mice were procured from animal house of DSCP and were divided into six (6) groups of two (2) animals each.

Group 1: Control (1% CMC)

Group 2: MEPP 3000 mg/kg b.w. in 1 % CMC

Group 3: MEPP 2000 mg/kg b.w. in 1% CMC
Group 4: MEPP 1000 mg/kg b.w. in 1 %CMC
Group 5: MEPP 500 mg/kg b.w. in 1% CMC
Group 6: MEPP 300 mg/kg b.w. in 1 % CMC

Animals were fasted for 24 hour prior to the administration of MEPP. After 24 hrs, all the groups were treated with the respective dose of MEPP dissolved in 1 % CMC orally according to their body weight. Change in the behaviour and % mortality was noted for 24 hours and continued the observation up to 7 days.

Scopalamine model[14, 15]

Experimental Design

Group 1: vehicle Control (saline-0.5 ml/kg)
Group 2: D- galactose (0.5ml i.p)
Group 3: D- galactose (0.5ml i.p) + MEPP (300 mg/kg, p.o)
Group 4: D-galactose (0.5ml i.p) + MEPP (500 mg/kg, p.o)
Group 5: D- galactose (0.5ml i.p) + Piracetam (200 mg/kg i.p)

The experiment is designed in such a way that the effect of methanol extract of *Peltophorum pterocarpum* (MEPP) at two different doses could be evaluated after 12th day against D-galactose induced accelerated aging and scopolamine induced amnesia in rats.

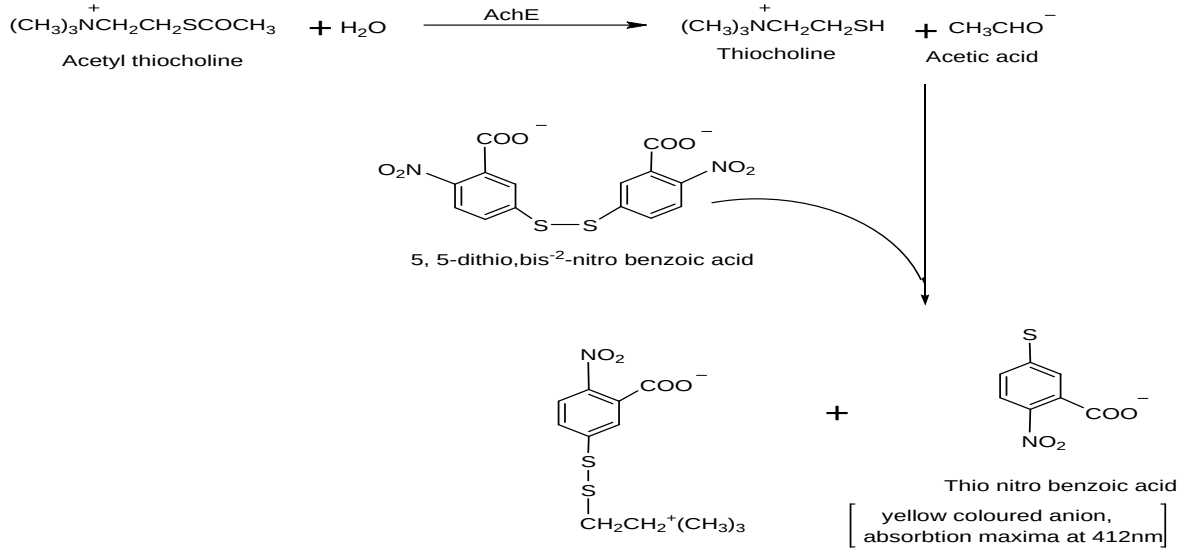
Before starting of the treatments all the groups of the animals were trained for a period of 10 days using Elevated plus maze (EPM) and Y- Maze. All the animals, except vehicle control were treated with D-galactose (0.5ml of 5% w/v i.p) and respective groups of animals were treated with MEPP (300 & 500 mg/kg p.o) and Piracetam (200mg/kg i.p) for 12 days. 60 mins after the treatment, all the animals were subjected to Scopolamine (2 mg/kg i.p) except the first group, which serves as a vehicle control.

The cognitive paradigm was evaluated 15 mins after the Scopolamine administration using Elevated plus maze (EPM) and Y- Maze. This is termed as acquisition trial (AT), which corresponds to learning. Further, the retention trial (RT) was carried out after 24 hour (on 13thday) of scopolamine administration and no drug treatment was done on 13th day. In the RT above mentioned parameters were assessed as an index of memory. On 14th day blood is withdrawn (animals fasted 18 hr. prior), serum separated and serum is used for biochemical estimation.[14]

Brain was removed and brain homogenate used for the estimation of AChE and antioxidant activity.

Estimation of Acetyl cholinesterase (AChE):[16,17]

The method of AChE activity estimation is popularly known as Ellman's method[14], developed by George Ellman in 1961.



Principle involved in AChE estimation

Thiocholine released because of the cleavage of ATC by AChE is allowed to react with the SH reagent 5, 5 - dithio- bis-(2, nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with absorption maxima at 412nm. The extinction coefficient of the thionitro benzoic acid is 1.36 × 104/ molar/ centimetre. The concentration of thionitro benzoic acid is detected using a UV spectrophotometer is then taken as a direct estimate of the AChE activity.

Reagent preparation

1. 0.1M Phosphate buffer

Solution A: 5.22g of K_2HPO_4 and 4.68g of NaH_2PO_4 are dissolved in 150 ml of distilled water.

Solution B: 6.2g NaOH is dissolved in 150ml of distilled water.

Solution **B** is added to solution **A** to get the desired pH (pH 8.0) and then finally the volume is made up to 300ml with distilled water.

2. Dissolve 10 mg of DTNB (5,5-dithiobis – 2 – nitrobenzoic acid) in 50 ml of 0.9 % sodium chloride solution and add 50 ml of 1/15 Sorensen phosphate buffer pH 8.0.

(This solution may be used for about 1 week if stored under refrigerated condition).

3. Dissolve 75 mg of the Acetylthiocholine iodide (ASCh) in 50 ml of distilled water. (Should be freshly prepared and be kept in an ice bath during a day's work).

4. Dissolve 50 mg of eserinesalicylate in 50 ml of distilled water. Place this solution in dropping bottle and store in refrigerator

Experimental Procedure

Cut open the brain, immediately transfer the brain into the chilled phosphate buffer. Separate the hippocampus and cerebrum, frontal cortex, Weigh and transfer to the glass tube of potter Elvehjem homogenizer. Add 10 volumes of 0.9 % sodium chloride solution, homogenise in an ice bath. Homogenate is then centrifuged at 3000 rpm for 10 min.

- Pipette 0.5 ml cloudy supernatant into 25 ml volumetric flask and make up to volume with freshly prepared **solution number 2**.
- From this, pipette two 4 ml portions into two test tubes (4ml each).
- Add 2 drops of **solution number 4** into one of the test tube.
- Pipette 1 ml of **substrate solution 3** into both test tubes
- Incubate both tubes for 10 min at 30° C

The solution in the tube containing eserine is used for zeroing the instrument.

Calculation:

The enzyme activity is calculated using the following formula; $\mathbf{R = 5.74x\ 10^{-4}\ x\ A\ /\ C_O}$

Where, **R** = Rate in moles of substrate hydrolyzed / minute / gm tissue

A = Change in absorbance / min

$\mathbf{C_O}$ = Original concentration of the tissue (mg / ml).

Antioxidant parmeters

The above collected supernatant was also used to estimate the antioxidant parameters like Catalase, Glutathione, Super oxidase and Lipid peroxidation.

1. Catalase [18]

Principle

In U.V. range H_2O_2 shows a continual increase in absorption with decreasing wavelength. Catalase catalyses the rapid decomposition of hydrogen peroxide to water. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240nm. The difference in absorbance per unit is a measure of catalase activity.

Procedure

1) Preparation of Hydrogen peroxide solution (7.5 mM):

1.043 ml of 30% w/w H2O2 was made upto 100 ml with sodium chloride and EDTA solution (9 g of NaCl and 29.22 mg of EDTA dissolved in 1 liter distilled water).

2) Preparation of Pottassium phosphate buffer (65 mM, pH 7.8):

2.2 g potassium dihydrogen phosphate and 11.32 g of di-potassium hydrogen phosphate were dissolved in 250 ml and 1 litre of distilled water respectively, and mixed together. The pH was adjusted to 7.8 with KH_2PO_4.

3) Preparation of Sucrose Solution:

10.95g of sucrose was dissolved in 100 ml of distilled water.

4) To 2.25 ml of potassium phosphate buffer 100 µl of the tissue homogenate was added and incubated at 25° C for 30min.

5) For blank, sucrose solution was used instead of tissue homogenate.

6) Then 0.65 ml of H_2O_2 was added to initiate the reaction.

7) The change in absorbance of the reaction mixture at 240nm was measured for 2-3 min.

8) **dy/dx** for 1 min for each assay was calculated and the results are expressed at Cat units / mg of tissue (Beer and Seizer, 1952)[18].

Calculation:

$$\textbf{Cat (U)/ 100 µl of Sample} = \frac{\textbf{(dy/dx) x 0.003}}{\textbf{38.3956x10}^{-6}}$$

Where,

dy/dx - change in absorbance / minute $38.3956x10^{-6}$ – molar extinction co efficient of H_2O_2 at 240 nm

Superoxide dismutase [19]

Principle

This enzyme is necessary for survival in all oxygen metabolizing cells. It is found in the cytosol and intermembrane space of mitochondria of eukaryotic cells. It contains copper and zinc. In normal cells, this radical alone is the precursor of hydrogen peroxide.Superoxide dismutase scavenges the super oxide (O^*_2) and thus provides a first line defense against free radical damage. SOD'S are a family of metallo enzyme that catalyzes the dismutation of super oxide anion (O^*_2) to hydrogen peroxide and molecular oxygen in the following manner.

$2H_2O_2 + 2O^* \rightarrow 2H_2O + O_2$

In the erythrocytes, the super oxide anion (O^*_2) interacts with peroxides to form hydroxyl radicals (*OH), which causes heamolyses in the absence of SOD activity. SOD measurement was carried out on the ability of SOD to inhibit spontaneous oxidation of epinephrine to adrenochrome.

Procedure:

1) Preparation of Sodium carbonate buffer solution (0.05 M, pH 10.2):

5.3 g of sodium carbonate and 1.2 g of sodium bicarbonate were dissolved separately in 1 litre of distilled water, which served as a stock solution. Buffer was prepared by mixing 64 ml of sodium carbonate and 70 ml of sodium bicarconate solutions. The pH of the buffer was adjusted to 10.2 using the above stock solution accordingly.

2) Adrenaline (9 mM):

0.03 g of adrenaline was dissolved in distilled water and the final volume was made up to 10 ml with distilled water containing a drop of concentrated HCL (to bring pH down to 2). Adrenaline is light sensitive; therefore, the vial was kept covered with aluminium foil at all the times.

3) Sucrose (0.3199 M) **solution:**

10.96 g of sucrose was dissolved in distilled water and the volume was made upto to 100ml.

4) To 2.8 ml of sodium carbonate buffer, 0.1 ml of tissue homogenate was added and incubated at 30°C for 45 minutes.

5) For blank, sucrose solution was used instead of tissue homogenate.

6) Then, the absorbance obtained was adjusted to 0 for the sample.

7) Thereafter, the reaction was initiated by adding 10µl of adrenaline solution.

8) The change in absorbance was recorded at 480nm for 8-12 minutes.

Throughout the assay, the temperature was maintained at 30°C.

9) Similarly, SOD calibration curve was prepared by taking 10 units/ ml as standard solution.

10)1 units of SOD produce approximately 50% of inhibition of auto-oxidation of adrenaline. The results are expressed as unit (U) of SOD activity per mg of tissue.

Calculation:

$$\text{SOD s} = \frac{\text{C} \times \text{Total Volume} \times 1000}{50 \times \text{Sample Volume} \times \text{mg Protein per ml}}$$

Unit: Units/ mg Protein

3. Lipid Peroxidation[20]

The estimation of per oxidation of lipids has been carried out by a number of methods of which TBA-reactive substance is selected because of its high sensitivity and simplicity in operation. The TBA test is often said to measure malondialdehyde (MDA) formed in peroxidizing lipid systems. So the results are frequently expressed as micro mate malondialdehyde equivalents.

Procedure

1) Preparation of Thiobarbituric acid solution

0.8 g of thiobarbituric acid was dissolved in distilled water and volume was made up to 100ml. The pH was adjusted to 7.4 with 1 N NaOH/0.1 N HCL solutions.

2) Preparation of Acetic acid solution

20 ml of acetic acid was dissolved in distilled water and the volume was made up to 100 ml with distilled water. The PH was adjusted to 3.5 with 1 N NaOH /0.1 N HCL solution.

3) Preparation of Sodium lauryl sulfate solution

8.1 g of sodium lauryl sulfate was dissolved in distilled water and the volume was made up to 100 ml with distilled water.

4) Preparation of Mixture of n-butanol and pyridine (15.1 v/v)

15 ml of n-butanol and 1 ml of pyridine were mixed together.

5) To 1 ml of tissue homogenate, 0.2 ml of sodium lauryl sulfate solution , 1.5 ml of 20% acetic acid and 1.5 ml of thiobarbituric acid solution were added.

6) This incubation mixture was made up to 5.0 ml with double distilled water and then heated in boiling water bath for 30 min.

7) After cooling, the red chromogen was extracted with 5 ml of the mixture of n-butanol and pyridine and centrifuged at 4000 rpm for 10 min.

8) The organic layer was taken and its absorbance was measured at 532 nm.

9) The results were expressed as nM of MDA/mg of wet tissue using molar extinction co-efficient of the chromophore $1.56\times10^5 mmol^{-1}cm^{-1}$ as 99% of TBARS is MDA.

Calculation:

$$\text{LPO} = \frac{\text{Test O.D.} \times \text{Total Volume} \times 100}{1.56 \times 10^5 \times 10^{-9} \times \text{Sample Volume} \times \text{mg protein per ml}}$$

Unit: nmol MDA / min × mg protein

Glutathione (GSH) [21]

Reduced glutathione measured by the method of (Ellaman 1959)

Procedure:

Glutathione was determined by its reaction with DTNB to yield a yellow chromophore which was measured spectrophotometrically. The brain homogenate was mixed with an equal amount of 10 % Trichloro acetic acid (TCA) and centrifuged at 2000 rpm for 10 mins at 4°c the supernatant was used for GSH estimation.

To 0.1 ml of processed tissue sample, 2 ml of phosphate buffer (PH 8.4), 0.5 ml of DTNB and 0.4 ml of double distilled H_2O were added and mixture

was shaken vigorously. The absorbance was read at 412 nm within 15 min.

Results

In the preliminary studies experiments were conducted to determine the phytochemical constituents of the bark of the selected plant *Peltophorum pterocarpum*. In the present study the pharmacognostic features of all forms of extracts namely petroleum ether, chloroform and methanol and have been analyzed. Among them the percentage yield of petroleum ether is found to be least 0.3% chloroform0.5% and that of methanol is highest 3.7%. The details of the quantity of the bark powder taken for extraction and nature of the extracts are given in the table 1.

Table 1: Yield of the extract from the bark of *Peltophorum pterocarpum*

Sl. No	Extract	Wt taken	Extract yield	% yield
1	Petroleum ether (light yellowish)	1000 gm	3 gm	0.3 %
2	Chloroform(dark brown semisolid)	1000 gm	5 gm	0.5 %
3	Methanol (dark brown sticky solid)	1000 gm	37 gm	3.7 %

Qualitative phytochemical investigation

To screen the phytochemical constituents of the selected bark, qualitative phytochemical investigations were carried out. All the extracts were subjected to appropriate preliminary qualitative chemical analysis where some of the important constituents were analysed such as, carbohydrates, proteins and amino acids, glycosides, phenolics and tannins, triterpenoids, alkaloids, saponins, total phenolic content (Table -5.2). It is observed that the flavanoids, carbohydrates, triterpenoids content is found to be in higher quantity in methanol extract.

The ability of the antioxidant property of a plant extract can be determined by its total phenolic content. Hence, it is essential to determine the total phenolic contents of all the extracts of *Petophorum pterocarpum* bark quantitatively by the method of Folin-ciocalteu. The total phenolic content is found to be very high in methanol extract (29.34%).

Pharmacological investigations

An acute toxicity study was conducted for methanol extract. The maximum tolerated dose is found to be 5000mg/kg b.w when the extract was administered orally. As per the OECD (International toxicity testing) guidelines the maximum therapeutic dose is 1/10th of maximum tolerated dose, hence the therapeutic dose selected for the extract were 300mg/kg and 500mg/kg body weight. After treatment with methanol extract it is observed that the animal did not show any variations in any of the following indicators viz., body weight, behaviour, loss of appetite, hyperthermia/hypothermia, erected hair etc. The weights of the vital organs (Liver, Kidney, Brain, and Spleen) were also found to be unaltered by the treatment with test extracts. The analysis of the above parameters indicates that the dose selected will not interfere with any of the body functions while performing various pharmacological investigations.

Phytochemical investigations conducted both by qualitative and quantitative showed that methanol extracts of the bark of *peltophorum pterocarpum extract* contain polyphenols. Hence it is necessary to carry out the antioxidant property of the methanol extract of *peltophorum pterocarpum*

The methanol extracts of bark of *peltophorum pterocarpum* was subjected for in vivo free radical scavenging activity by the following methods.

a) Catalase
b) Superoxide dismutase activity
c) Lipid peroxidation inhibition activity
d) Glutathione

Acetyl cholinesterase activity

From the result scopolamine group showed very significant increase in AchE activity with mean ± SEM values 0.03717 ± 0.0021 when compared to control group 0.02283 ± 0.0024. The rats treated with Piracetam showed a moderate decrease in AChE

activity with mean ± SEM 0.02817 ± 0.0021 when compared to scopolamine group whereas a significant decrease in AChE activity is also observed in the groups treated with MEPP 300 and MEPP 500 mg/kg b.w. with mean ± SEM 0.03383 ± 0.0020 and 0.02617 ± 0.0025 when this compared to scopolamine group.

Table 2: Effect of drug treatment on Acetyl cholinesterase activity of brain (moles of substrate hydrolysed / min / gm tissue)

Group	Treatment	AChE activity of brain
G1	Normal control (0.9 % Nacl, i.p)	0.02283 ± 0.0024
G2	Scopolamine (2mg/kg, i.p)	0.03717 ± 0.0021^{***a}
G3	Piracetam (200 mg/kg, i.p)	0.02817 ± 0.0017^{*b}
G4	MEPP 300 (300 mg/kg)	0.03383 ± 0.0020^{**b}
G5	MEPP 500 (500 mg/kg)	0.02617 ± 0.0025^{**b}

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Newman keul multiple test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001.

[a] D-galactose induced ageing followed by Scopolamine induced amnesia group was significantly different from Normal control group.

[b] Treated group were significantly different from D-galactose induced ageing followed by Scopolamine induced amnesia group.

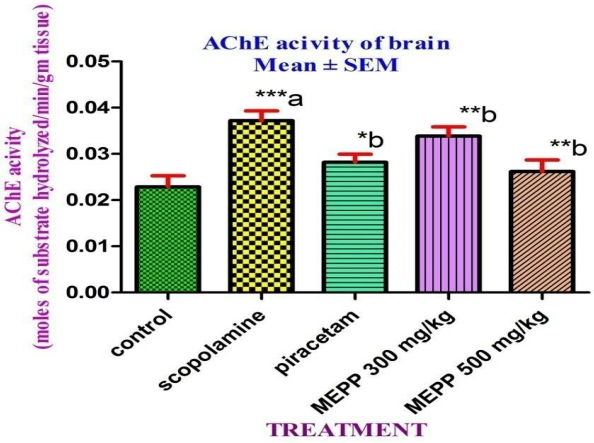


Fig 1: Effect of drug treatment on Acetyl cholinesterase activity of brain (moles of substrate hydrolyzed / min / gm tissue)

Antioxidants

From the result it is found that the scopolamine (2mg/kg, i.p) treated rats showed a very significant decrease in activities of catalase, super oxide dismutase and glutathione with mean ± SEM 1.355 ± 0.105, 1.632 ± 0.159 and 0.030 ± 0.002 when compared to normal saline treated group with mean ± SEM 2.518 ± 0.164, 3.310 ± 0.186, 0.065 ± 0.04 respectively.

In the piracetam (200mg/kg i.p) treated animals showed a significant increase in activities of catalase, SOD, glutathione with mean ± SEM 2.128 ± 0.068, 2.603 ± 0.196, 0.049 ± 0.002 when compared to scopolamine group respectively.

Whereas animals treated with MEPP 300 and MEPP 500 mg/kg b.w p.o, a significant increase in the levels of the enzymes activities of catalase, SOD, GSH were observed with mean ± SEM 1.722 ± 0.094, 2.183 ± 0.153, 0.041 ± 0.003 and 1.822 ± 0.091, 2.487 ± 0.168, 0.044 ± 0.001 respectively.

In vivo lipid per oxidation study revealed that scopolamine treated group showed significant increase in molindialdehyde (MDA) with mean ± SEM 7.798 ± 0.412 when compared to control group with mean ± SEM 4.732 ± 0.395.

Piracetam 6.205 ± 0.332 and MEPP 500 mg/kg 6.300 ± 0.292 groups were able to prevent this rise in MDA level whereas MEPP 300 mg/kg group is not significant as compared to scopolamine treated group.

Table 3: The effect of drug treatment on brain Antioxidant levels

Groups	Antioxidant level			
	Catalase (Unit/mg tissue)	SOD (Units/mg protein)	LPO (N mole MDA / min × mg protein)	GSH
Control (0.9 % NaCl, i.p)	2.518 ± 0.164	3.310 ± 0.186	4.732 ± 0.395	0.065 ± 0.04
Scopolamine (2mg/kg, i.p)	1.355 ± 0.105^{***a}	1.632 ± 0.159^{***a}	7.798 ± 0.412^{***a}	0.030 ± 0.002^{***a}
Piracetam (200 mg/kg,i.p)	2.128 ± 0.068^{***b}	2.603 ± 0.196^{**b}	6.205 ± 0.332^{**b}	0.049 ± 0.002^{**b}
MEPP 300 (300 mg/kg, p.o)	1.722 ± 0.094^{*b}	2.183 ± 0.153^{*b}	7.533 ± 0.240 ^{ns}	0.041 ± 0.003^{*b}
MEPP 500 (500 mg/kg, p.o)	1.822 ± 0.091^{*b}	2.487 ± 0.168^{*b}	6.300 ± 0.292 ^{*b}	0.044 ± 0.001^{*b}

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Newman keul multiple test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, and *** represents very significant at $p<0.001$, ^{ns} represents non significant.

[a] D-galactose induced ageing followed by Scopolamine induced group was significantly different from Normal control group.

[b] Treated grou p were significantly different from D-galactose induced ageing followed by Scopolamine induced group.

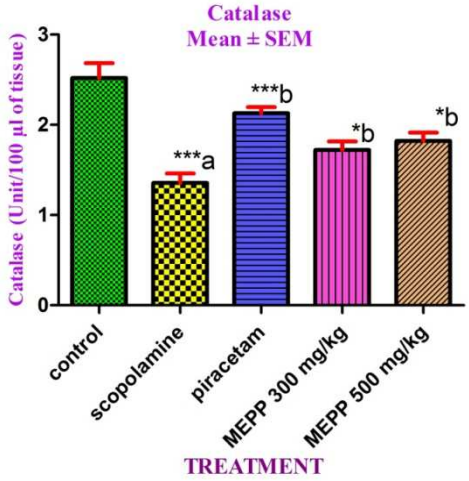


Fig 2: The effect of drug treatment on Catalase

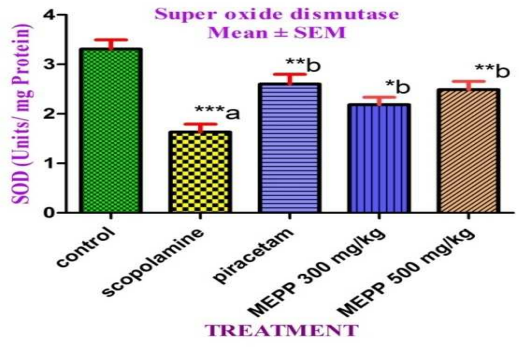


Fig 3: The effect of drug treatment on SOD

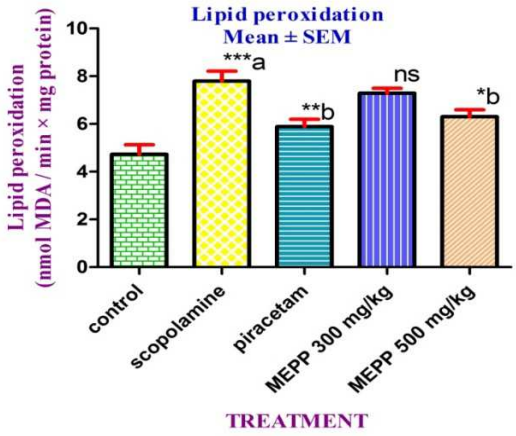


Fig 4: The effect of drug treatment on LPO

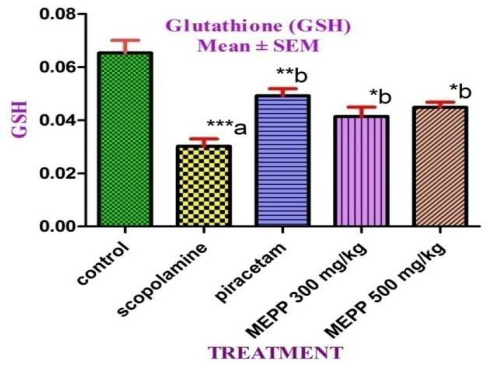


Fig 5: The effect of drug treatment on LPO

DISCUSSION

Alzheimer's disease is characterised by degenerative changes in the brain accompanied by loss of memory, especially for recent events. Evidently it is accepted, the learning and memory is closely associated with the functional status of the central cholinergic system.

The levels of acetylcholine, a chemical that acts as a messenger in the brain, fall dramatically. At the same time, nerve endings and brain cells begin to die. The areas commonly affected are those involved in receiving and storing information. Aging seems to be the biggest factor implicated in the development of Alzheimer's disease, but some people are more likely to develop the disease because of particular genes they have inherited.

The most widely used nootropic drugs acts as modulators of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors, NMDA receptors, Kainate receptor subtypes and the cAMP-dependent signalling cascade. AMPA receptor modulators, including AMPAkinase, enhance LTP induction by prolonging the depolarization produced by synaptically released glutamate and thus facilitating activation of the NMDA receptor.[22]

The drugs donepezil, galantamine and rivastigmine are known as anticholinesterase and appear to work by maintaining levels of the neurotransmitter acetylcholine in the brain by preventing its breakdown. NMDA-antagonism by memantine is thought to exert a nootropic effect in the long term as a result of protection from glutamate-induced excitotoxicity. Rolipram, an inhibitor of phosphodiesterases that increases the availability of cAMP and thereby increases the activity of PKA, enhances LTP and memory.[23]

Compounds which prevent mitochondrial damage and improves mitochondrial function such as coenzyme Q10 (CoQ10; ubiquinone) or nicotinamide have been shown to be neuroprotective.

Estimation of Brain Acetyl cholinesterase activity

AD is associated with intellectual malfunction and subsequent decline in cognitive, behavioral and motor function. Increased levels of AChE in AD patient has lead to the hypothesis that cognitive decline is related to cholinergic degeneration. Therefore promising approach for treating AD is to enhance Acetylcholine concentration in the brain.

In the present study there was a very significant rise in AChE in the scopolamine treated rats. This rise in enzyme level leads to rapid cleavage of Acetylcholine and thereby reduces concentration and turnover of Ach. A significant inhibition of AChE activity has been found in the rats treated with MEPP 300 and 500mg/kg b.w. for 12 days. Thus, the plant extract is found to inhibit the rise in AChE activity.

Antioxidant parameters:

Catalase (CAT), Super oxide dismutase (SOD) and Glutathione (GSH):

Our further study revealed that the oxidative stress is induced by administration of D – galactose for 12 days and further administration scopolamine on 12th day in rats depletes the catalase, super oxide dismutase and glutathione. D-galactose is a reducing sugar that readily reacts with the free amine group of neuronal proteins and neuropeptides to form advanced glycation end product (AGEs). These AGEs undergo chemical oxidation and degradation via AGE receptor binding and activation of signalling pathways to form free radicals and causes oxidative stress. In the present study, it reveals that treatment with MEPP could restore the activity of both these antioxidant enzymes and possibly could reduce generation of free radicals and neuronal damage.

Lipid Peroxidation:

Increase in the level of lipid peroxides in the brain reflects the neuronal damage. The depletion of antioxidant defences and/or raise in free radical production deteriorates the pro-oxidant antioxidant balance, leading to oxidative stress and cell death. D-galactose followed by scopolamine induced oxidative stress has been associated with increased amount of lipid peroxidation. Indeed, MEPP supplementation in our study was potentially effective in blunting lipid peroxidation, suggesting that MEPP possibly has antioxidant property to reduce oxidative stress induced membrane lipid peroxidation. However in our result Piracetam is found to be insignificant against the lipid peroxidation.

Conclusion

Antioxidant and acetyl cholinestrase inhibitory activity of *Peltophorum pterocarpum* in scopolamine treated rats was confirmed by the following parameters and considerations.

At firstly the phytochemical constituents in the methanol extract was found to be carbohydrates, amino acids (proteins), glycosides, triterpenoids, phenolics and tannins, flavonoids. Scopolamine treated rats showed a significant increase in the AChE activity whereas, increased activity is reversed by the MEPP treatment. Reduced activity of the AChE would increase the availability of Ach by decreasing its breakdown. From this decreased activity of AChE by MEPP may directly or indirectly influence on cholinergic based learning and memory. In case of D galactose induced oxidative stress and scopolamine treated groups there will be decrease in ROS scavenging enzyme activities such as catalase, super oxide dismutase and glutathione. Treatment with MEPP restored the activity of these enzymes possibly by reducing the generation of free radicals and neuronal damage.In the present study, treatment with MEPP reduced the lipid peroxidation indicated by decrease in the toxicant elevated levels of thiobarbituric acid reactive substances (TBARS) like Molindaldehyde. Hence, it was concluded that methanol extract of *Peltophorum pterocarpum* bark possess antioxidant activity in the dose used.

Bibilography

1) McClatchey WC, Mahady G, Bennett BC, Sheils L, Savo V. Ethnobotany as a pharmacological research tool and recent developments in CNS active natural products from ethnobotonical sources. *Pharmacol. Therapeut* 2009; 12; 239-254.
2) Ho YS, So KF, Chang RC. Anti-aging herbal medicine how and why can they be used in anti-associated neurodegenerative disease? *Ageing Res.Rev* 2010.
3) Esposito E, Rotilio D, Di matteo V, Di Guilio C, Cacchio M, Algeria S. A review of specify dietary antioxidant and the effect on biochemical mechanisms related to neurodegenerative process. *Neurobiol Aging* 2002; 544: 203-215.
4) Roger SH, Farlow MR, Doody RS, Mohs R, Friedhoff L.I. A 24 week double blind placebo controlled trial of Donepezil in patients with Alzheimer's disease neurology 1998; 50:136-45.
5) Nabeshima.T. behavioral aspects of cholinergic transmission: role of basal fore brain cholinergic system in learning and memory. *Progr Brain Res* 1993; 98:405-11.
6) Sing HK, Dhawan BN. Drugs affecting learning & memory. Lectures in neurobiology. New Delhi: *wiley Eastern* 1992; 189-202.
7) Jaiswal AK, Bhattacharya SK, Acharya SB. Anxiolytic activity of *azadirachata indica* leaf extract in rats. *Indian J Exp Biol* 1994; 32:489-91
8) Bhattacharya K, Kumar A, Ghoshal S. Effects of glycowithanolides from *withania somnifera* an animal model of Alzheimer's disease and pertirbate cholinergic markers of cognition in rats. *Phyto Res* 1995; 9:110-3.
9) Joshi H, Parle M. Evaluation of nootropic potential of *Ocimum sanctum* Linn. In mice. *Ind J Exp Biol* 2006; 44:133-6.
10) Marcello Iriti, Sara Vitalini, Gelsomina Fico, Franco Faoro. Neuroprotective herbs and foods from different traditional medicines and diets. *Molecules* 2010; 15:3517-3555.
11) Goedert M. Neurofibrillary pathology of Alzheimer's disease and other tauopathies. *Prog Brain Res* 1998; 117: 287–306.
12) Khandelwal KR. Practical pharmacognosy. Neerali prakashan ; 2000
13) Sadasivam S, Manikam A. A Biohemical Methods for Agriculture Sciences. New Delhi; Wiley Eastern Limited 1992: 187.
14) Kirti.SK, Kasture SB, Mengi SA. Efficacy study of *Prunus amygdalus* (almond nuts) in Scopalamine induced amnesia in Rats. *Indian J pharmacol* 2010; 42(3): 168-173.
15) Deepika S, Munish P, Ashok KT, Nirmal S, Amteshwar SJ. Antiamnesic effect of Stevioside in Scopalamine treated Rats. *Ind J pharmacol* 2010; 42 (3): 164-167
16) Raju TR, Kutty BM, Sathyaprabha TN and Shanakranarayana Rao BS. Assay of acetylcholinesterase activity in the brain. *Brain and Behavior*. National Institute of Mental Health and Neuro Sciences, Bangalore, India. 2004:142-144.

17) Guenther Voss, Klaus Sachsse. Red cell and plasma cholinesterase activities in icrosamples of human and animal blood determined simultaneously by a modified Acetylthiocholine/DTNB procedure. *Toxicol and applied pharmacol* 1970; 16: 764-772.
18) Beers RF, Sizer IW. A spectrophotometric method for measuring breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952; 195: 133
19) Paoletti F, Aldinucci D, Mocali A, Caparrini A. A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. *Anal Biochem* 1986; 154: 536-541.
20) Ohkawa H, Ohishi N, Yogi K. Assay for lipid peroxide in animal tissue by thiobarbituric acid reaction. *Anal Biochem* 1979:95.
21) Ellman GL. Tissue sulphydryl groups. *Arch Biochem Biophys* 1959; 82: 70-77.
22) Cooke SF, Bliss TVP. Plasticity in the human central nervous system: *Brain* 2006; 129: 1659–1673.
23) Based on information supplied by: Alzheimer's Society, Helpline 0845 300 0336 www.alzheimers.org.uk. Reviewed on 22/10/2009.