Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 13 [1] December 2023 :167-172
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Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Antioxidant Potential Analysis of Myrica *esculenta* Leaves in Scopolamine –Induced Alzheimer's Disease in Mice

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ABSTRACT
Alzheimer disease (AD) is a common neurodegenerative disease in the elderly. It is characterized by psychological & behavioral deterioration that severely limits one's ability to participate in everyday life activities. Alzheimer's disease is the most common cause of dementia. The prevalence of Alzheimer's disease is growing. Roughly 24 million individuals all over the globe are affected by it. It is estimated that AD affects around one in ten adults over the age of 65 and every third person of those over the age of 85. Now a day's , phytotherapy is being used as adjunctive and alternative potential pathway for the treatment of various disorders. The current work was conducted to investigate the antioxidant effects of plant extract from Myrica esculenta in cognitive problems The antioxidant capacity of plant was tested in vivo was measured by measuring catalase (CAT) and malondialdehyde levels (MDA) and in vitro using DPPH and ABTS scavenging tests Excellent in vitro and in vivo antioxidant properties were observed with extract of Myrica esculenta obtained from ethanol. This research finding suggests that Myrica esculenta have potent anti-oxidant potential and might be useful in Alzheimer's disease.
Keywords: *Alzheimer's Disease, Myrica esculenta, Oxidative stress, antioxidant*

Received: 25.10.2023 Revised: 28.11.2023 Accepted: 08.12.2023

INTRODUCTION

Cognitive functions, reasoning, planning, language, and perception memory, emotion, and the learning of motor skills are the functions of brain. Brain consists billion of neuron which are connected by synapse. Synapses are destroyed by age-related diseases, injuries, and illnesses. As the leading cause of death worldwide, neurodegenerative diseases have received a lot of coverage recently. Thousands of individuals throughout the globe lose their lives annually neurodegenerative conditions, and it's a tragedy that no effective treatment has been found yet [1-4].
As the population ages, neurological diseases like Alzheimer's and Parkinson's are expected to become more common. The difficulty is that early detection is quite challenging. Nevertheless, a person who follows an Ayurvedic prevention plan beginning at an early age may easily avoid getting this condition or, if they already have it, keep it under control. When someone has dementia, their brain is unable to operate properly because their neurons have been damaged [5-7].
There are number of factors which may be responsible of the development of AD Cholinergic neurons in the forebrain are particularly vulnerable to degradation in Alzheimer's disease.Reactive oxygen species (ROS) and reactive nitrogen species (RNS), which may be produced both within and outside of cells, are one of the most important intermediate risk variables that begin and accelerate neurodegeneration[8-11].
Phytoconstituents provide a natural defense mechanism against various diseases. These bioactive compounds whether in the form of combination of plants or an extract from a single plant are being used by numerous traditional medical systems around the world. *M. esculenta belongs to the Myricaceae family and is more commonly known as Kaiphala or Katphala.*It has a been noticed that infusion and decoction of *M. esculenta* may be useful to successfully treat fever, cold. Gastrointestinal problems, asthma. Bronchitis and other lung disorders have also been shown to benefit from it [12-15].
The current study was conducted to investigate the ameliorative effect of *M. esculenta* against neuroinflammation anti-inflammatory activity and also to check antioxidant activity. So that it gives a scientific justification for the use of this plant in the management and treatment of Alzheimer's disease.

MATERIAL AND METHODS

Collections, identification and preparation of plant materials

The leaves of *M. esculenta were* collected from Region of Someshwar District Almora (Uttarakhnad) and the plant was authenticated by Patanjali Research Foundation Herbium Haridwar.

The leaves of the plant were washed with distilled water (DW) and dried under the shade. The plant material was then pulverized and crushed to powder for extraction.

Experimental design

In this study, Albino mice weighing between 25 and 30 g. The housing and care of the animals followed all requirements set out by the CPCSEA. Mice were kept in groups of five, with constant access to water and food, and under constant supervision at a temperature of 25 ±1°C and a relative humidity of 60±10%. The placebo group was given merely saline solution. An intraperitoneal dose of scopolamine (1 mg/kg) was given to all other groups. Donepezil (at 20 mg/kg administered orally) was the standard treatment. To induce amnesia and brain injury in mice, scopolamine (1 mg/kg, intraperitoneally) was administered. Oral (p.o.) administration of EEME utilising CMC as the vehicle was done for Groups 3 and 4. In contrast, saline was used as the delivery medium for both scopolamine and donepezil. All the chemicals used are analytical grade and purchased from SD fine chemical limited.

DPPH radical scavenging assay

The medicines' capacity to quench free radicals was evaluated using the DPPH scavenging assay. Decolorization of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was used to evaluate the medicines' hydrogen-donating potential. In methanol solution, DPPH produces a purple/violet hue that fades to a yellowish tint when exposed to antioxidants. After making a 0.1 mM DPPH solution in methanol, 2.4 mL of it was combined with 1.6 mL of extract in methanol at concentrations ranging from 12.5% to 15%. After completely blending the solution ingredients, they were kept undisturbed for 30 minutes at room temperature and in the dark. Absorbance at 517 nm was determined using spectrophotometry. For this purpose, butylated hydroxytoluene (BHT) served as a standard [15,17]. Percentage of DPPH radical scavenging activity was calculated by the following equation:

%DPPH radical scavenging activity = $[(A_0\text{-}A_1)/A_0] \times 100$

In which the absorbance of the placebo (A0) is compared to that of the medication (A1) or the standard. Then, the concentrations were compared to the percentages of inhibition. Three separate runs of the experiment were conducted at each concentration.

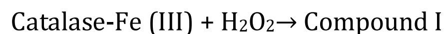
Total antioxidant capacity (ABTS assay)

The reduction of the radical cation ABTS to ABTS'(2, 2'- azino-bis(3-ethylbenzothiazolyl)succinate) provides the basis for the decolorization used in this test (3-ethylbenzthiazoline-6-sulphonic acid). A 7 mM ABTS solution in water was reacted with 2.45 mM potassium persulphate (K2O8S2) to produce the radical (1:1). The combination was kept in the dark at 27 degrees Celsius for 16 hours (time needed to obtain stable absorbance at 734 nm). Once the incubation period ended, the radical solution was further diluted with water (1 mL of ABTS reagent + 27 mL DW) until the desired absorbance at 734 nm was achieved (0.7 ± 0.005). Nine hundred and eighty microliters of ABTS.+ reagent were combined with twenty microliters of the sample or standard for the assay. After 6 minutes, the absorbance was measured at 734 nm. The first reading (0 min) and the sixth reading (6 min) were used to determine the ΔO.D. Ascorbic acid (8.8 μg/mL to 88.0 μg/mL) is used as a reference. Using the formula given, we were able to determine how effective the extract was in scavenging free radicals and compare it to ascorbic acid [15,17].

% inhibition = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$

Determination of Catalase activity

Within the body, catalase (CAT) may be found in many different tissues and organs. This enzyme has a molar weights of around 240 kDa and is composed of four identical 60-kilodalton subunits organized in a tetrahedral fashion. Each subunit includes a single ferriprotoporphyrin group. Using an iron or manganese cofactor, catalases catalyse the decomposition of hydrogen peroxide into water and oxygen. Thus, one molecule of hydrogen peroxide oxidizes its cofactor, releasing the bound oxygen, which is subsequently transferred to another molecule of substrate, regenerating the cofactor in the process.



Catalase-Fe (III) + $H_2O_2 \rightarrow$ Compound I

Compound I+$H_2O_2 \rightarrow$ Catalase-Fe (III) + $2H_2O+O_2$.

375 μL of phosphate buffer were mixed with 25 μL of tissue homogenate. Following this, 50 mM H_2O_2 was added to the test tubes, totaling 100 μL. After waiting a minute, 1 mL of 5% potassium dichromate in 1% glacial acetic acid was added to the reaction mixture. Following a 10-minute incubation period in a bath of boiling water, the tubes were cooled with running water. At 570 nm, optical densities were measured. The sample catalase concentration was calculated using a standard curve. The rate of H_2O_2 consumption per minute per milligramme of protein was used to quantify catalase activity [18].

Determination of the Level of Malodialdehyde (MDA)

Brain homogenate malondialdehyde (MDA) levels were measured using a procedure outlined by Foyet*et al.*, 2019. To recap, 2 millilitres of tissue homogenate (supernatant) was mixed with 2 millilitres of newly made 10% w/v trichloroacetic acid (TCA) and left in an ice bath for 15 minutes. The precipitate was centrifuged for 15 minutes to separate it from the clear supernatant, and then 2.0 mL of the clear supernatant was combined with 2.0 mL of newly made 0.67 percent thiobarbituric acid (TBA). The finished mixture was put in a pot of boiling water for 10 minutes. After that, it spent 5 minutes in an ice bath to chill down. The absorbance was checked against a blank reagent at 532 nm. MDA levels were reported in nanomoles per litre [18].

Statistical analysis

Mean ± S.E.M. values are used to represent data. Two groups were compared utilizing Graph Pad Prism 5's one-way analysis of variance (ANOVA) and Tukey's post hoc test for statistical significance. When the value p was less than 0.05, it was judged to be statistically significant.

Results

DDPH and ABTS radical scavenging assay of *Myrica esculenta*

Several chemicals found in *M. esculenta*, including flavonoids, diterpenes, triterpenes, and phenolics, contributed to the plant's impressive antioxidant activity . Percent inhibition of DPPH action was revealed to be higher in Ethanolic extract as against methanolic extract, and lowest in aqueous extract. The IC_{50} value for *M. esculenta* ethanolic extract was 41.81 µg/mL, while the values for methanolic extract and aqueous extract were 43.70 µg/mL and 96.73 µg/mL, correspondingly.

Positive findings for antioxidant activity were observed in the ABTS experiment using preparations of *M. esculenta* in all three solvents tested: ethanol, methanol, and water. The IC_{50} value for the ethanolic extract was 42.10 µg/mL, while the methanolic and water extracts were 45.45 and 95.47 µg/mL, correspondingly.

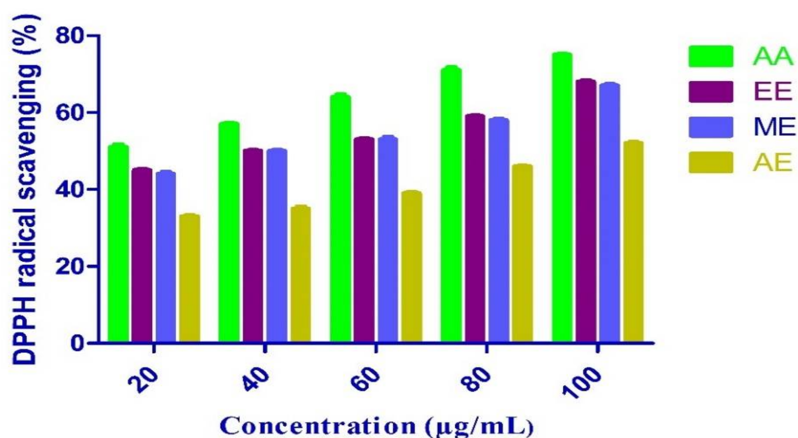


Figure 3.1: DPPH radical scavenging activity of *Myrica esculenta* leaf extract

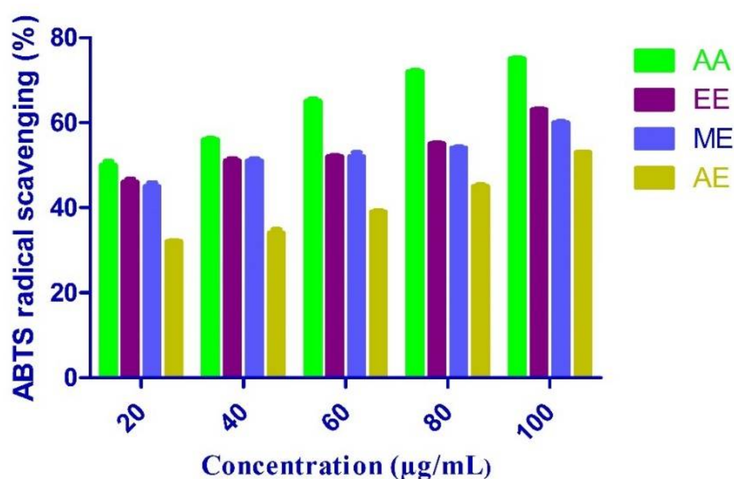


Figure 3.2 : ABTS radical scavenging activity of *Myrica esculenta* leaf extract

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Effect of EEME on antioxidant parameters

Scopolamine-treated group had significantly lower levels of brain catalase than control group ($p < 0.001$). The levels of catalase were raised after therapy with EEME 200, EEME 400 and donepezil ($p < 0.05$ $p < 0.001$, and $p < 0.001$, respectively). Scopolamine caused a significant rise in brain MDA levels ($p < 0.001$) compared to the control group. The increased levels of MDA were reduced after dosing of EEME 400, 200, and donepezil relative to the scopolamine group ($p < 0.001$).

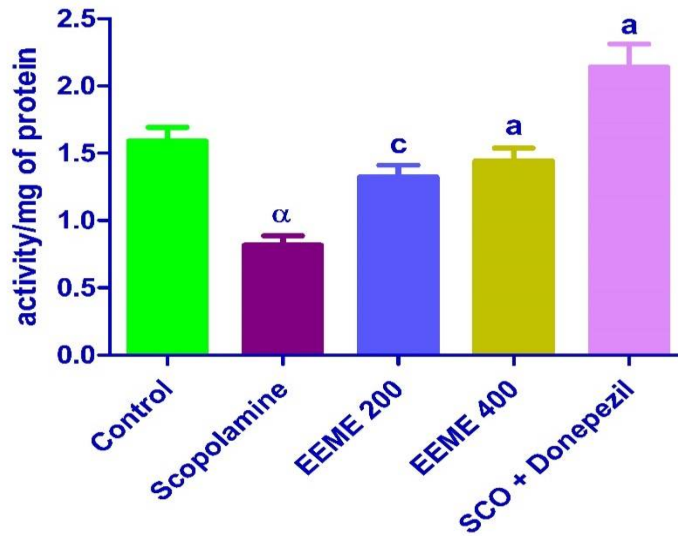

Figure 3.3: Effect of EEME on catalase levels

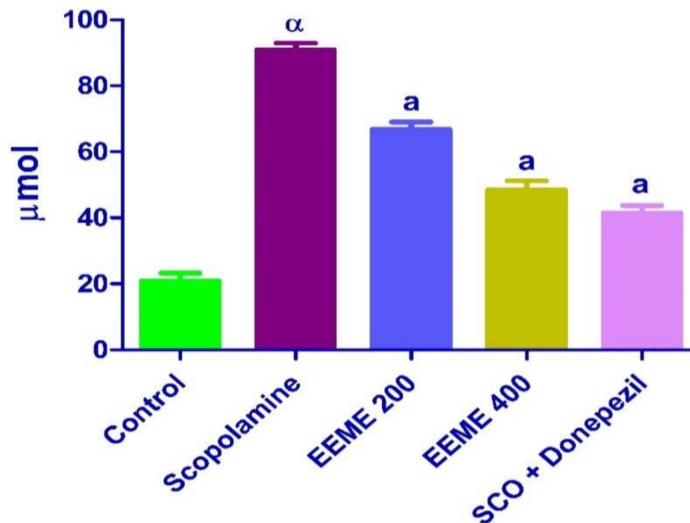

Figure 3.4: Effect of EEME on MDA levels

DISCUSSION

Millions suffer from AD, a prevalent dementia. Alzheimer's disease causes neuron death, protein buildup, and amyloid beta (Aβ) plaques and tau tangles. The causes of this illness are complex. Several hypotheses, including as the cholinergic hypothesis, Aβ hypothesis, tau hypothesis, oxidative stress hypothesis, and inflammation hypothesis, have been proposed to explain the complex causes of Alzheimer's disease [19].

In Alzheimer's, antioxidant enzyme failure promotes uncontrolled oxidative damage. DNA oxidation products, hydroxyl radical-DNA base pairing, and lipid peroxidation may damage AD pathology's cellular structures. Too many free radicals harm cells.. Research suggests that mitochondrial failure leads to increased ROS generation before Aβ pathology and symptoms appear. An imbalance between antioxidants and oxidants, common in Alzheimer's disease, may damage neurons. AD is linked to ageing and brain oxidative damage. Numerous plant extracts quenched DPPH and ABTS-produced oxidative stress-free radicals in experiments. [20].

Ethanolic extracts of *Myrica esculenta (*EEME) neutralised free radicals best. The plant extract's antioxidant ability was assessed by how quickly DPPH free radicals in purple methanol converted to yellow complex.

Higher doses (20-100µg/mL) result in higher IC50 and % inhibitory effect. Dose increased ABTS inhibition %. Ethanolic extract reduced ABTS activity more than methanolic or aqueous. In this work, we discovered that MDA levels rose while CAT levels fell in the brains of AD rats. This finding agrees with those of prior research [217]. The antioxidative capability of the plant extracts was shown by their ability to counteract these alterations when they were administered. The body's CAT enzyme helps rid itself of free radicals, which have been linked to accelerated ageing and mortality. MDA may be used as a proxy for lipid oxidation and, by extension, the severity of reactive oxygen species (ROS) attack on the organism [219]. Because of their ability to reduce oxidative stress, EEME seem to be beneficial for AD rats [21, 22]. The findings of the research make it abundantly evident that ethanolic extracts of *Myrica esculenta* leaves are much more powerful than methanolic and aqueous extracts with regard to their antioxidant, capabilities

CONCLUSION

According to the findings of this study, it is possible to draw the conclusion that *Myrica esculenta* may contain the components that have potent anti-oxidant potential and could be used as a natural antioxidant. It might be useful in the treatment of cognitive disorders i.e. AD which is due to oxidative stress in brain .

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CITATION OF THIS ARTICLE

Navin Chandra P, D.K.Sharma. Antioxidant Potential Analysis of Myrica *esculenta* Leaves in Scopolamine –Induced Alzheimer's Disease in Mice. Bull. Env.Pharmacol. Life Sci., Vol 13 [1] December 2023: 167-172