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EVALUATION OF IN VIVO ANTI-OXIDANT ACTIVITY OF *MENTHA ARVENSIS* LINN IN RAT BRAIN HOMOGENATES

Nazima Ameen[1] and Sabeeha Shafi*[2]

[1]Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, Kashmir, Jammu and Kashmir (India).

[2]Sr. Assistant Professor, Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, Kashmir, Jammu and Kashmir (India).

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***Corresponding Author**
Sabeeha Shafi
Sr. Assistant Professor, Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, Kashmir, Jammu and Kashmir (India).

ABSTRACT

Mentha arvensis Linn commonly called as Pudina is used in various recipes in India. It has a number of pharmacological actions like sedative-hypnotic, anti-inflammatory, anti-ulcerogenic, antifungal, anti-helminthic and hepatoprotective. But very little literature is known regarding its memory enhancing activity. In the present study, the main aim was to evaluate the in vivo anti-oxidant activity of this plant in rat brain homogenates. Memory enhancing activity is directly related to Alzheimers disease. In this the aerial parts of the plant were taken. Two extracts of this plant were taken. The phytochemical constituents of Mentha arvensis showed the presence of alkaloids, tannins, saponins, glycosides, terpenes and flavonoids. The in vivo mehods also showed positive memory enhancing activity thereby implicating that the plant can be used in curing Alzheimers disease.

KEYWORDS: Mentha arvensis, herb, in vivo study, phytochemical constituents.

INTRODUCTION

Free radicals and oxidative stress are directly linked to Alzheimers disease (Arnaiz *et al.*,2003;Backman *et al.*, 2004; Blennow *et al.*, 2006) The damage caused by free radicals is controlled by anti-oxidants like catalase and glutathione. Under pathological conditions the balance between oxidant and anti-oxidant conditions is impaired (Claiborne *et al.*, 1985; Chauhan *et al.*,2006) The damage found in Alzheimers disease includes advanced glycation end products, nitration, lipid peroxidation, adduction products, carbonyl-modified

neurofilament protein and free carbonyls. In Alzheimers disease, memory is lost, commonly called as Memory loss referred to as amnesia (Carlesimo,1992), dementia or memory impairment which is basically an abnormal degree of forgetfulness or inability to recall past events. This disorder is characterized by loss of intellectual ability which is so severe as to interfere with a person"s occupation or social activities. Alzheimers disease is a neurodegenerative disorder that destroys the cells of the brain that can lead to dementia or memory loss. This loss results in gross atrophy of the affected regions including degeneration in the temporal lobe, parietal lobe, parts of frontal cortex and other systems of the body. This disease is mainly found in people over 65 years of age. Furthermore, the disease is characterized by shrinkage of brain tissue, the grooves or furrows in the brain called sulci which are widened and there is shrinkage of gyri also. Risk factors for Alzheimers includes smoking, high blood pressure, diabetes and high lipid profile. Other diseases like Parkinsons disease, multiple sclerosis, chronic kidney disease, HIV, Down syndrome and some other learning disabilities also increase a persons risk of dementia. Number of herbal drugs have shown memory enhancing activity (nootropic activity) (Gomez., 2008;Jagdeep *et al.*,2009;Anita *et al.*,2011).

MATERIALS AND METHODS

Identification and Collection of the Plant material

Aerial parts of *Mentha arvensis* Linn. were collected from Chadoora area of Kashmir in the month of April-May. It was identified and authenticated by Dr. Anzar Khuroo, taxonomist, at Centre for Biodiversity and Taxonomy, University of Kashmir, Srinagar. A sample of the plant material was deposited in the herbarium of the Department of Taxonomy, University of Kashmir under VOUCHER SPECIMEN NUMBER 2227-KASH for future reference. The bulk collection of the plant was then proceeded after its proper identification and authentication.

Preparation of the extracts

Following extracts of the plant of *Mentha arvensis* Linn. were prepared for the current study.

- Aqueous extract of the aerial parts of *Mentha arvensis* Linn.
- 70% ethanolic extract of the aerial parts of *Mentha arvensis* Linn.

Preparation of aqueous and 70% ethanolic extracts of the aerial parts of M*entha arvensis* linn.

Aqueous and 70% ethanolic extracts of the aerial parts of *Mentha arvensis* Linn. was

prepared according to the standard method. Dried aerial parts of *Mentha arvensis* Linn were pulverized and the powdered material (600 g) was macerated in distilled water for 48 hours with occasional shaking and then it was allowed to stand for 18 hours. The contents were kept for elution and then filtered. Filtrate was concentrated on a water bath at a temperature of 40-50°C. The residue so obtained was air dried and then weighed to calculate the percentage yield. The residue obtained was then stored in a cool and dry place for further use in the experimental studies.

Preliminary Phytochemical Screening

The aqueous and 70% ethanolic extracts of the aerial parts of *Mentha arvensis* Linn. were subjected to preliminary phytochemical screening. The presence of important organic chemical constituents was determined by the standard qualitative methods.

Experimental design

A total of 54 rats were employed in the present study. They were divided into nine different groups (n=6) and the experimental study was conducted for a period of 15 days. Seven days prior to behavioral study, the rats were acclimatized to the standard laboratory conditions (Table 1).

Table 1: Treatment Schedule.

Group	Treatment	Dose
I	*Normal Control-Vehicle only* *Daily single dose of 2% Acacia*	*10mg/kg: p.o*
II	*Toxic Control* *Scopolamine Hydrobromide*	*1 mg/kg b.w: i.p*
III	*Standard* *Piracetam + Scopolamine hydrobromide*	*200mg/kg: p.o + 1 mg/kg b.w: i.p*
IV	*AMA1: Aqueous Extract of the aerial parts of Mentha arvensis + Scopolamine hydrobromide.*	*100mg/kg: p.o + 1 mg/kg b.w: i.p*
V	*AMA2: Aqueous Extract of the aerial parts of Mentha arvensis + Scopolamine hydrobromide.*	*200mg/kg: p.o + 1 mg/kg b.w: i.p*
VI	*AMA3: Aqueous Extract of the aerial parts of Mentha arvensis + Scopolamine hydrobromide.*	*400mg/kg: p.o + 1 mg/kg b.w: i.p*
VII	*EMA1: Ethanolic Extract of the aerial parts of Mentha arvensis + Scopolamine Hydrobromide*	*100mg/kg: p.o + 1 mg/kg b.w: i.p*
VIII	*EMA2: Ethanolic Extract of the aerial parts of Mentha arvensis+ Scopolamine Hydrobromide*	*200mg/kg: p.o + 1 mg/kg b.w: i.p*
IX	*EMA3: Ethanolic Extract of the aerial parts of Mentha arvensis + Scopolamine Hydrobromide*	*400mg/kg: p.o + 1 mg/kg b.w: I.P*

Biochemical Evaluation: (Yogesh, 2010)

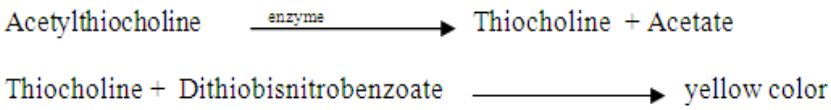
On the day 15th the biochemical estimations were carried out on brain homogenates. For preparation of homogenate, the animals were sacrificed by cervical dislocation and the brain was carefully removed and weighed. The removed brains were washed carefully with 0.9% normal saline and homogenized in ice cold 0.1M phosphate buffer (pH 7.2, 10% w/v) using a Teflon homogenizer. The clear supernatant, obtained after centrifugation at 3000 rpm for 15 min4^{o}C, was used to estimate Acetylcholinesterase (AChE) activity.

A) Estimation of Brain Acetylcholinesterase (AChE) Activity (Davles,1976; Appleyard., 1992; Ellman, 1961).

Principle

The assay is based on measurement of the change in absorbance at 412 nm. The assay uses the thiol ester acetylthiocholine instead of the oxy ester acetylcholine. AChE hydrolyses the acetyl-thiocholine to produce thiocholine and acetate. The thiocholine in turn reduces the Dithiobis-Nitrobenzoic Acid (DTNB) liberating Nitro-Benzoate, which absorbs at 412 nm.[38]

The reaction is shown below:



Reagents

- Sodium phosphate buffer (pH 7.2, 0.1M).
- Ellman's reagent (DTNB).
- Acetylthiocholine iodide.

Preparation of reagents

1. Sodium phosphate buffer (pH 7.2, 0.1M): 3.42ml of 1M Na_2HPO_4 and 1.58 ml of 1M NaH_2PO_4 were added and volume was made upto 45ml with water. pH was then adjusted by NaOH/phosphoric acid and final volume was made upto 50ml with water.
2. Ellmans reagent: 47.53 mg of Ellmans reagent were taken and added to 12 ml of distilled water.

Procedure

1. A 0.4-ml of prepared homogenate was added to a cuvette containing 2.6 ml of phosphate buffer (pH 7.2, 0.1 M).
2. 100 µl of Ellman's reagent (DTNB 0.01 M) reagent was added and taken into a photocell. The absorbance was measured at 412 nm; when this had stopped increasing, the photometer slit was opened, so that the absorbance was set to zero.
3. Of the substrate (Acetylthiocholine iodide 0.075M), 20 µl were added. Changes in absorbance were recorded and the change in absorbance per min. was calculated.
4. The rates were calculated as follows:

$$R = \frac{\Delta A}{1.36 \times 10^4} \times \frac{1}{\left(\frac{400}{3120}\right) C_0} = 5.74(10^{-4}) \frac{\Delta A}{C_0}$$

Where,

R = rate, in moles substrate hydrolyzed per min per g of tissue;

A = change in absorbance per min;

C_o = original concentration of tissue (mg/ml).

Antioxidant Studies (Alam,2013)

The antioxidant study was carried out both on brain homogenates of rats. The antioxidant activity was assessed by:

***In-vivo* antioxidant activity**

The following antioxidant enzymes were evaluated to determine the degree of antioxidant activity of different selected extracts in brain homogenates:

i) Estimation of Brain Reduced Glutathione (GSH) Levels
ii) Estimation of Brain Superoxide Dismutase (SOD) Activity
iii) Estimation of Brain Glutathione Peroxidase (GSHPx) Levels
iv) Estimation of Brain Malondialdehyde (MDA/TBARS) Levels
v) Estimation of Brain Catalase (CAT) Activity
vi) Estimation of Brain Protein Levels

i) Estimation of reduced glutathione (GSH) activity (Meister, 1983; Douglas 1987, Cruz, 2003)

Principle

This is spectrophotometric procedure is based on the method of Ellman's reagent -5, 5' dithiobis-2-nitrobenzoic acid (DTNB) is reduced by SH group to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH.

Reagents

(i) Tris Buffer (0.2 M, pH 8.2).

(ii) 5, 5' dithiobis-2-nitrobenzoic acid (DTNB) (0.01 M).

(iii) Absolute methanol.

Preparation of reagents

(i) Tris Buffer (0.2 M, pH 8.2): 484.56 mg of Tris buffer was dissolved in 20 ml of distilled water, 2 ml of 0.2 M EDTA was added to it and final volume of solution was made up to 20 ml with distilled water. The pH was adjusted to 8.2 with 1 N HCl.

(ii) DTNB (0.01 M): 47.53 mg of DTNB was dissolved in 12 ml of distilled water.

Procedure (**Meister A, 1983**)

1. 100 mg of brain tissue was homogenized in 1 ml of 0.01 mM phosphate buffer.
2. 0.5ml of the homogenate was mixed with 1.5 ml of distilled water and 1.5 ml 0.2M Tris buffer. And 0.1 ml of 0.01 M Ellman's reagent and mixture was bought to 10ml by absolute methanol.
3. The tubes were then shaken intermittently for 10-15 minutes and then centrifuged at 3000 RPM for 15 minutes (R-BC DX, REMI centrifuge).
4. The absorbance was read within 5 minutes of addition of DTNB at 412 nm against reagent blank with no homogenate in spectrophotometer (UV-1601, SHIMADZU).
5. Blank.
6. Standard: Method same as that of test except std. glutathione solution (50 µg/ml) was used.

Calculation

The amount of GSH in the tissue was calculated from the following equation

$$\frac{\text{GSH}}{\text{mg Protein}} = \frac{\text{OD}_{412} \times \text{Dilution factor}}{1.36 \times 10^4} \times 100$$

Where;

OD = Optical density

1.36 x10^4 = Extinction coefficient

ii) Estimation of brain superoxide dismutase (SOD) activity (Eauchamp, 1971; Bannister 1987; Johnson, 2005)

Principle

Pyrogallol (1, 2, 3-benzenetriol) auto-oxidizes rapidly in aqueous solution, higher the pH faster is autoxidation and several intermediate products are formed. Thus the solution first becomes yellow –brown with a spectrum showing a shoulder between 400-425 nm. After a number of minutes the color begins to turn green and finally after a few hours, a yellow color appears. So autoxidation was studied essentially during the first step and the rate was taken from the linear increase in absorbance at 420 nm, which is seen for a number of minutes after an induction period of some 10 seconds. Superoxide anion radical (O_2^-) catalyses the autoxidation of pyrogallol. A simple and rapid method for assay of SOD is described, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol.

$$2\ O_2^- + 2\ H^+ \longrightarrow O_2 + H_2O_2$$

Reagents

(i) Tris HCl buffer (pH 8.5).

(ii) Potassium phosphate buffer (50 mM/l, pH 7.4).

(iii)Pyrogallol (24 mM).

Preparation of Reagents

(i) Tris HCl buffer (pH 8.5): 788 mg of Tris HCl buffer and 186 mg of EDTA were dissolved in 100 ml double distilled water and pH was adjusted to 8.5 using 1 N NaOH.

(ii) Potassium phosphate buffer (50 mM/l, pH 7.4): It was prepared by mixing KH_2PO_4 solution and $Na_2HPO_4.2H_2O$ solution of 1.1.55.

(iii)Pyrogallol (24 mM):15.1 mg of pyrogallol was dissolved in 5 ml of 10 mM HCl. The solution was prepared freshly at the time of assay.

Procedure

1. 20 mg of brain was homogenized in 2ml of 0.01mM of phosphate buffer.
2. The homogenate were centrifuged at 10,000 rpm at 4ºC in cooling centrifuge for 20 minutes.

3. 100µl of supernatant was added to 3 ml of tris HCl buffer, pH 8.5 followed by 25 µl of pyrogallol and then mixed thoroughly.
4. The change in absorbance at 420 nm was recorded at 1 minute interval for 3 minutes. The increase in absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of SOD.

Calculation

1 unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation per 3 ml of assay mixture and given by the formula:

$$\frac{\textbf{Unit of SOD}}{\textbf{ml of Sample}} = \frac{(\mathbf{A} - \mathbf{B}) \times \mathbf{100}}{\mathbf{A} \times \mathbf{50}}$$

Where, **A** = Change in absorbance per minute of Standard,

B = Change in absorbance per minute of test Sample.

iii) Estimation of brain glutathione peroxidase (GPx) activity (Brigellius,1999)

Principle

The activities of GPx are determined by quantifying the rate of oxidation of the reduced glutathione to the oxidized glutathione by hydrogen peroxide (H_2O_2) catalyzed by GSH-Px. The assay is based on the reduction of H_2O_2 by GPx through consumption of reduced glutathione (GSH) that is restored from oxidized glutathione (GSSG) in a coupled enzymatic reaction by GR. GR reduces GSSG to GSH using NADPH as a reducing agent. The decrease in absorbance at 340 nm due to NADPH consumption is then measured spectrophotometrically. One unit of GPx is defined as the amount of enzyme that catalyzes the oxidation of 1mmol of NADPH to $NADP^+$ per minute.

Reagents

- Sodium phosphate buffer 0.1 M.
- EDTA Na 1mM.
- Sodium azide 1mM.
- NADPH 0.02 mM.
- Hydrogen peroxide 1mM.
- Reduced glutathione (GSH).

Preparation of reagents

1. **Sodium phosphate buffer (pH 7.2, 0.1M):** 3.42ml of 1M Na_2HPO_4 and 1.58 ml of 1M NaH_2PO_4 were added and volume was made upto 45ml with water. pH was then adjusted by NaOH/ phosphoric acid and final volume was made upto 50ml with water.
2. **EDTA (1mM):**7.4g 0f EDTA to be dissolved in 20ml of water.
3. **Sodium Azide (1mM):** 6mg of Sodium azide was taken and dissolved in 100 ml of water.
4. **NADPH (1mM):** 1.4mg of NADPH was taken and dissolved in 100ml of water.
5. **Reduced Glutathione (1mM):** 6.14 g of reduced glutathione was taken and dissolved in 20ml of water.

Procedure

The assay mixture consisted of 1.49 ml of sodium phosphate Buffer (0.1M), 0.1 ml EDTA (1mM), 0.1 ml Sodium azide (1mM), 0.1 ml GSH (1mM), 0.1 ml NADPH (0.021mM), 0.01ml H_2O_2 and 0.1 ml of sample in a total volume of 2ml. Oxidation of NADPH was recorded spectrophotometrically at 340 nm and the enzyme activity was calculated as nmoles NADPH oxidized/min/mg of protein, using extinction coefficient of 6.22 x 10^3 M/cm.

Calculations

$$\frac{\text{Nmol NADPH oxidized}}{\text{min/mg of protein}} = \frac{OD_{340} \times \text{Volume of assay}}{6.22 \times 10^3 \times \text{Volume of enzyme}} \times \text{mg of protein}$$

Where,

OD = Optical density at 340 nm

6.22 x 10^3 M/cm = Extinction coefficient factor

Also one unit of GPx is defined as the amount of enzyme required to oxidize 1 nmol GSH/min. The enzyme activity is expressed as Units/mg Protein.

iv) Estimation of brain malondialdehyde (MDA/TBARS) levels (Okhama,1979)

Principle

Lipid peroxidation is a free radical mediated event. The primary products of such damage are a complex mixture of peroxides which then breakdown to produce carbonyl compounds. The malondialdehyde (MDA) is one such carbonyl compound, which forms a characteristic chromogenic adduct with two molecules of thiobarbituric acid to give a pink color, the absorbance of which is determined at 540 nm. The colorimetric reaction of TBA

(Thiobarbituric acid) with MDA, a secondary product of lipid peroxidation has been widely accepted for measuring lipid peroxidation. It is also known as TBARS (Thiobarbituric acid reactive substance) estimation.

Reagents

(i) Potassium Chloride (KCl) (0.15 M).

(ii) 0.8% Thiobarbituric acid (TBA) solution.

(iii)30% Trichloro acetic acid (TCA) solution.

Preparation of reagents

(i) KCl (0.15 M): 57.5 mg KCl was dissolved in 10 ml of distilled water.

(ii) TBA (0.8%): 40 mg of TBA was dissolved in 4.95 ml of distilled water and 50 µl of glacial acetic acid.

(iii)TCA (30%): 0.30 gm of TCA was dissolved in 3 ml distilled water.

Procedure

1 ml of brain homogenate was taken and. 0.5 ml of 30% TCA was added to it, followed by 0.5 ml of 0.8% TBA reagent. The tubes were then covered with aluminium foil and kept in water bath for 30 minutes at 80°C.

1. After 30 minutes tubes were taken out and kept in ice-cold water for 30 minutes and centrifuged at 3000 rpm for 15 minutes (R-BC DX REMI centrifuge).
2. The absorbance of the supernatant was read in spectrophotometer at 540 nm against appropriate blank.
3. Blank consisted of 1 ml distilled water, 0.5 ml of 30% TCA and 0.5 ml of 0.8% TBA.

Calculation

The amount of MDA present in a sample was calculated according to the following equation and the result was expressed as nano mole of MDA/mg of protein.

$$\frac{\text{Nanomole of MDA}}{\text{mg protein}} = \frac{V \times OD_{540}}{0.156} \times \text{mg of protein}$$

Where,

V = Final volume of the test solution

OD_{540} = Optical density at 540 nm

v) Estimation of brain catalase (cat) activity (Mueller,1997; Chelikani,2004)

Principle

Catalases are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. This protein is localized to peroxisomes in most eukaryotic cells. Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate and follows a ping-pong mechanism. Its cofactor is oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate. Despite its apparent importance in hydrogen peroxide removal, humans with genetic deficiency of catalase — "acatalasemia" — or mice genetically engineered to lack catalase completely, suffer few ill effects.

Reagents

- Phosphate buffer(0.05 M, pH- 7)
- H_2O_2 (0.019 M)

Preparation of reagents

- **Phosphate buffer (0.05 M, pH- 7):** 400ml of 0.1M phosphate dibasic is taken in a beaker and desired pH is adjusted by adding as much as 0.1M sodium phosphate monobasic.

Procedure

1. The mixture consists of 1.95 ml of phosphate buffer (0.05 M, pH- 7), 1 ml of H_2O_2 (0.019 M)
2. And 0.05 ml sample (10 % w/v) in a final volume of 3 ml.
3. Control cuvette contains all the components except substrate.
4. The Change in absorbance is then recorded at 240 nm and the catalase activity is calculated.

Calculations

$$\frac{\text{CAT U}}{\text{mg Protein}} = \frac{\text{OD}_{240} \times \text{Volume of assay}}{0.081 \times \text{Volume of enzyme}} \times \text{mg of protein}$$

Where;

OD = Optical density at 240 nm

0.81 = Extinction coefficient

vi) Estimation of total protein levels (Doumas,1981)

Protein estimation was done for determining the levels and activity of various antioxidant enzymes in brain. Total protein content was estimated by Biuret method.

Principle

Colorimetric determination of the total protein based on the principles of Biuret reaction (Copper salts in an alkaline medium). Protein in plasma or serum forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of blue colour is proportional to the protein concentration.

$$\text{Protein} + Cu^{++} \longrightarrow \text{Blue violet colored complex}$$

Contents

Biuret reagent (R1) (2 x 50 ml).

Total protein standard (8g/dl).

Stability

The reagents and standard are stable upto expiry date indicated on the vial label.

Assay

Wavelength	520-570nm
Reaction Temperature	37^{o}C
Measurement	Against blank

Sample

Supernatant of the centrifuged homogenate.

Manual Procedure

Pipette into test tubes	Blank	Standard	Test
Biuret reagent (R1)	1000 µl	1000 µl	1000 µl
Total protein standard (8g/dl)	-	10 µl	-
Supernatant (Brain homogenate)	-	-	10 µl

Mix well and incubate at room temperature for 5 minutes. Measure the absorbance of standard (Abs. S) and test sample (Abs. T) against blank, at 555 nm (520-570) within 60 minutes.

Calculation

Total protein (g/dl) = Abs of T/Abs of S x **8**

Performance Characteristics

Linearity: The procedure is linear upto 15 g/dl. If the values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

RESULTS

Acetylcholinesterase (AChE) Activity

Both Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of ***Mentha arvensis*** **Linn.** showed dose-dependent decrease in AChE activity in the brains of animals, which were administered Aqueous and hydro-alcoholic extracts at different doses for 14-days along with Scopolamine Hydrobromide at 14th day after last dose of extracts when compared to the rats of toxic control group (Group II) that received scopolamine Hydrobromide only. The results are shown in Fig 1.

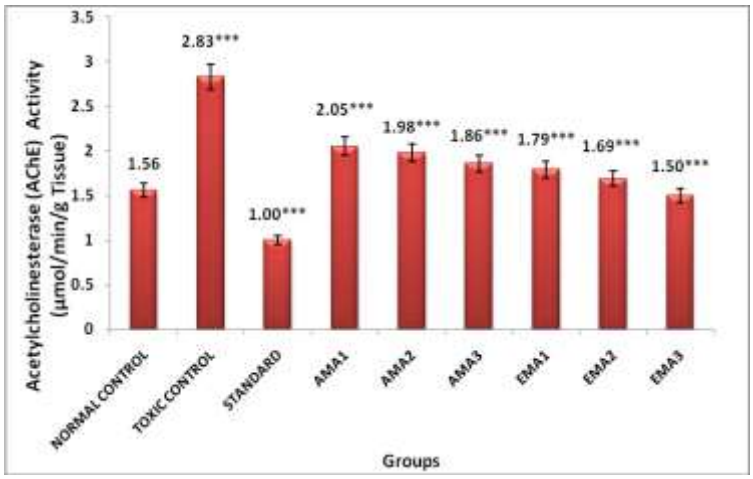


Fig. 1: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Acetylcholinesterase (AChE) activity against Scopolamine Hydrobromide induced memory impairment.

In Vivo Antioxidant Study

Reduced Glutathione (GSH) Levels

Both Aqueous and 70% ethanolic extracts of the aerial parts of ***Mentha arvensis*** **Linn.** showed dose-dependent increase in Reduced Glutathione levels (GSH) in the brain homogenates of rats which were administered Aqueous and Ethanolic extracts at different

doses for 14-days along with Scopolamine Hydrobromide when compared to the rats of Group II (toxic control) that received vehicle 2% Acacia (along with Scopolamine Hydrobromide (1mg/kg b.w, i.p once at 14th day).The results are shown in Fig 2.

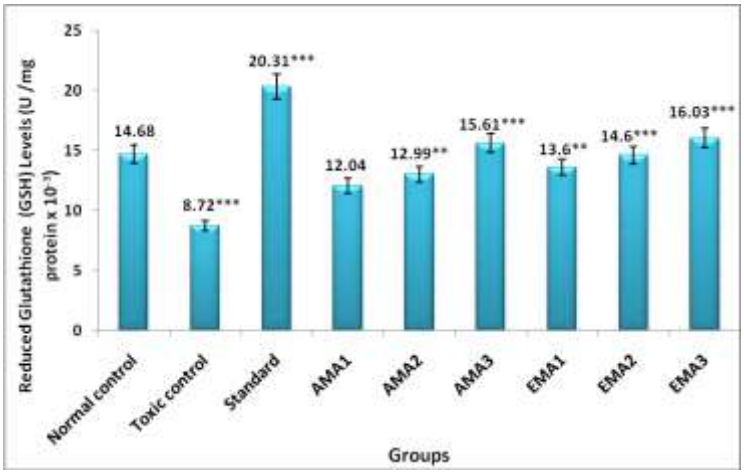


Fig 2: Effect of aqueous and 70% ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Reduced Glutathione (GSH) levels against Scopolamine Hydrobromide induced memory impairment.

A) Malondialdehyde (MDA/TBARS) Levels

Both Aqueous and 70% Ethanolic extracts of the aerial parts of ***Mentha arvensis* Linn.** showed dose-dependent decrease in MDA levels in the brain homogenates of rats administered extracts for 14-days along with Scopolamine Hydrobromide when compared to the rats of Group II (toxic control) which received vehicle 2% Acacia (along with Scopolamine Hydrobromide (1mg/kg b.w, i.p once at 14th day). Results are shown in Fig 3.

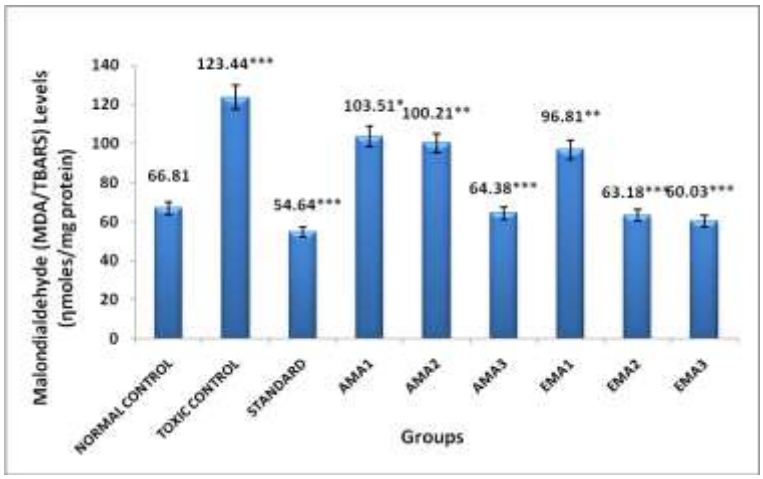


Fig. 3: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Malondialdehyde (MDA / TBARS) levels against Scopolamine Hydrobromide induced memory impairment.

B) Glutathione Peroxidase (GPx) Levels

Effects of Aqueous (AMA) and 70% Ethanolic (EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Glutathione Peroxidase (GPx) levels.

Both Aqueous and 70% Ethanolic extracts of aerial parts of ***Mentha arvensis* Linn.** (AMA and EMA) showed dose-dependent increase in Glutathione Peroxidase (GPx) levels in the brain homogenates of rats administered extracts for 14-days along with Scopolamine Hydrobromide when compared to the rats of Group II (toxic control) which received vehicle 2% Acacia (along with Scopolamine Hydrobromide 1mg/kg b.w, i.p once at 14th day).Results are shown in Fig 4.

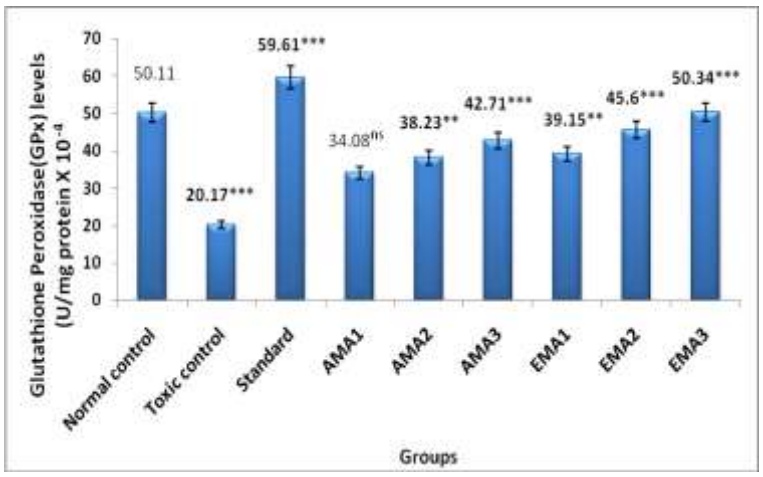


Fig. 4: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Glutathione Peroxidase (GPx) levels against Scopolamine Hydrobromide induced memory impairment.

D) Catalase Activity

Both Aqueous and 70% Ethanolic extracts of the aerial parts of ***Mentha arvensis* Linn.** showed dose-dependent increase in Catalase activity (CAT) in the brain homogenates of rats administered extracts for 14-days along with Scopolamine Hydrobromide when compared to the rats of Group II (toxic control) which received vehicle 2% acacia (along with Scopolamine Hydrobromide 1mg/kg b.w, i.p once at 14th day).Results are shown in Fig 5.

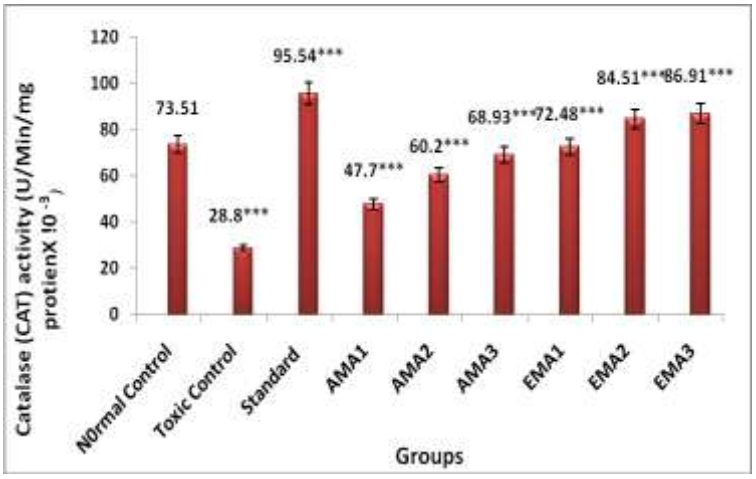


Fig.5: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Catalase Activity (CAT) against Scopolamine Hydrobromide induced memory impairment.

E) Superoxide Dismutase (SOD) Levels

Both Aqueous and 70% Ethanolic extracts of the aerial parts of ***Mentha arvensis* Linn..** showed dose-dependent increase in Superoxide Dismutase (SOD) levels in the brain homogenates of rats administered extracts for 14-days along with Scopolamine Hydrobromide when compared to the rats of Group II (toxic control) received vehicle 2% Acacia (along with Scopolamine Hydrobromide 1mg/kg b.w, i.p once at 14th day). Results are shown in Fig 6.

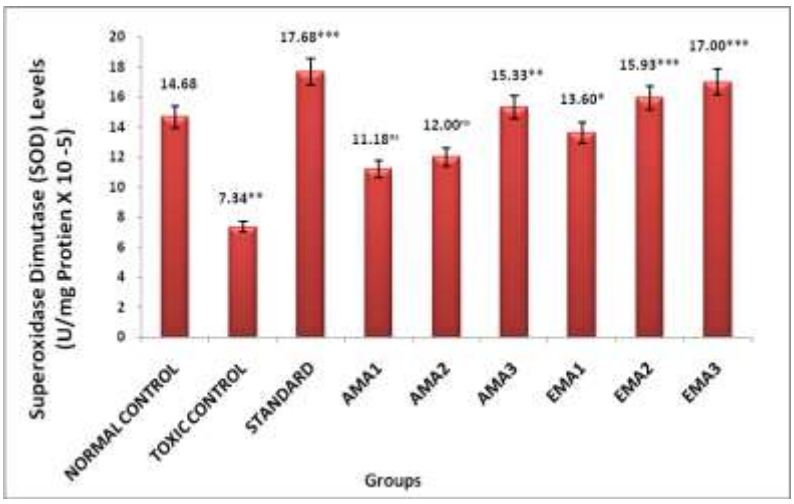


Fig.6: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Superoxide Dismutase (SOD) levels against Scopolamine Hydrobromide induced memory impairment.

F) Protein Levels

Both Aqueous and 70% Ethanolic extracts of the aerial parts of ***Mentha arvensis* Linn.** showed dose-dependent increase in Protein levels in the brain homogenates of rats administered extracts for 14-days along with Scopolamine Hydrobromide when compared to the rats of Group II (toxic control) which received vehicle 2% Acacia (along with Scopolamine Hydrobromide 1mg/kg b.w, i.p once at 14th day). A very highly significant decrease ($p< 0.001$) in the Protein levels of the rats of Group II (9.22 ± 0.475 mg/ml) when compared to the rats of Group I (15.76 ± 0.824 mg/ml) which received 2% Acacia (10 mg/kg p.o) only. Results are shown in Fig 7.

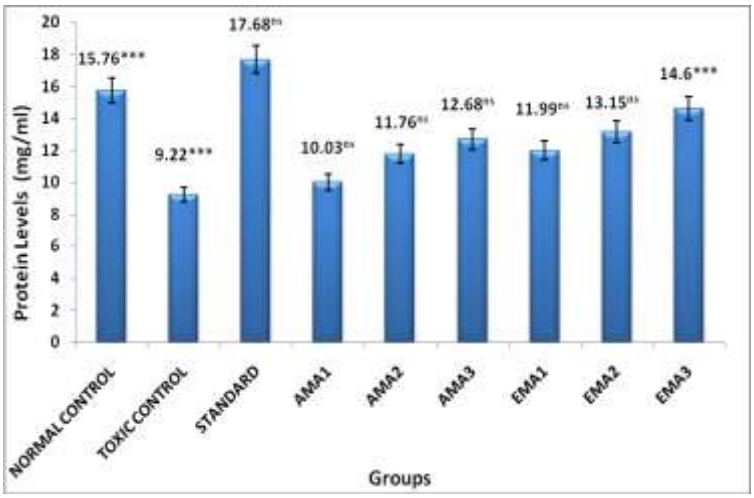


Fig. 7: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Protein levels against Scopolamine Hydrobromide induced memory impairment.

DISCUSSION

Alzheimers disease is a neurodegenerative disorder that destroys cells in the brain, leading cause of dementia, a condition that involves gradual memory loss, decline in the ability to perform routine tasks, disorientation, difficulty in learning, loss of language skills, impairment of judgment and personality changes. As the disease progress, people with Alzheimer's disease fail to care for themselves and the loss of brain cells eventually lead to the failure of other systems of the body (Iverson 1998;Halliwal 2001) The administration of Antimuscarnic agent scopolamine produces transient memory deficit. Scopolamine Hydrobromide amnesia test is widely used as primary screening test for anti-Alzheimer drug. Acetylcholine is considered as the most important Neurotransmitter involved in the regulation of Cognitive functions. Cholinergic neurons play an important role in Cognitive

deficit associated with Alzheimer's disease and Neurodegenerative diseases(Dean W,1990;Dhingra 2005; Malik, 2007;Salomon 2012; Mudasir, 2014).

Scopolamine is a centrally acting cholinergic agent which causes impairment in learning. The treatment with drugs which increase Cholinergic Neurotransmission causes an improvement in Cognitive deficits in AD.

Mentha arvensis has shown a number of pharmacological activities (Ganesh,2002; Verma,2003; Rachel 2011;Mali et al., 2012; Farnaz, 2012; Rajneesh, 2014; Saima, 2014; Shah,2014; Vikas, 2014) but not much literature has shown its memory enhancing activity.

The present study was carried at lower dose levels of 100 and 200 and 400 mg/kg b.w/day of each extract (1/20, 1/10, 1/5th of 2000mg). Various Biochemical Parameters, which include Acetylcholinesterase (AChE) activity, Malonaldehyde (MDA/TBARS) levels, Catalase (CAT) levels, Reduced glutathione (GSH) levels, Glutathione peroxidase (GPx) levels, Superoxide Dismutase (SOD) levels, and Protein levels were evaluated.

In the present study two extracts (Aqueous and 70% Ethanolic) of *Mentha arvensis* Linn., each at three different doses (100, 200, 400 mg/kg b.w) were given to different groups of albino rats, orally for 14 days followed by induction of Memory impairment by administering Scopolamine Hydrobromide (1mg/kg, i.p) only once on 15th day of study. Both Aqueous and 70% Ethanolic extract of *Mentha arvensis* Linn. inhibited Acetylcholinestrease enzyme in a dose dependent manner, there by elevating Acetylcholine concentration in the brain homogenate and ultimately improved memory in rats. Both the extracts at the dose of 400 mg/kg b.w showed highest inhibition of Acetyl cholinesterase enzyme than the other two respective doses (200, 100mg/kg b.w).

Biochemical parameters like Malondialehyde (MDA/TBARS) levels, Catalase level (CAT), Reduced Glutathione (GSH) levels, Glutathione Peroxidase (GPx) levels, Superoxide Dismutase (SOD) levels, and Protein levels were also evaluated as they are found to be associated with complications of Alzheimer's disease.

The levels of Malondialehyde (MDA) of both Aqueous and 70% Ethanolic extracts of *Mentha arvensis* Linn showed a significant dose dependent decrease. Reduced Glutathione (GSH) levels showed a significant dose dependent increase, Catalase (CAT) levels were also increased very highly significantly, Glutathione Peroxidase (Gpx) levels also showed highly

significant increase, the levels of Superoxide Dismutase (SOD) were also increased in highly significant manner, and total Protein levels were also increased in a dose dependent manner.

The results of the present study indicate that out of the two extracts (Aqueous and 70% Ethanolic), 70% Ethanolic extract showed very highly significant increase in the GSH levels, Catalase levels, GPx levels, SOD levels and total Protein levels and highly significant decrease in Anticholinesterase and MDA levels as compared to Aqueous extract which may be due to the presence of high content of Flavonoids and Phenolic compounds in Ethanolic extract.

Furthermore the 70% Ethanolic extract also showed high Antioxidant activity as compared to Aqueous extract.

CONCLUSION

All the measured Parameters demonstrate Protective role of *Mentha arvensis* Linn. against Scopolamine Hydrobromide induced Memory Impairment in rats. These findings suggest the possible Neuroprotective role for Aqueous and Ethanolic extracts of *Mentha arvensis* Linn., therefore it seems that extracts of *Mentha arvensis* Linn. may prove to be useful anti Alzheimer agent in view of its Memory Enhancing Property as observed in the present study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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