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Evaluation of neuroprotective activity of isolated fraction from *Biophytum sensitivum* on α-(hydroxymethyl) Benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced dementia in rats

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Abstract
Objective: To isolation of fractions, estimation of In Vitro anti oxidant and Neuroprotective effects of Fraction A from Methanolic extract of *Biophytum sensitivum on* α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced Dementia.
Methods: In this study *in vitro* antioxidant activity is estimated by DPPH assay method. Neuroprotective activity is estimated by α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced dementia method. In neuroprotective method behavioral parameters were checked by Escape latency time (Sec) by using Morris water maze, Active avoidance testing by using Elevated plus maze and nuerochemicals like glutathione, catalase, and Cholinesterase were estimated.
Results: In α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced dementia increased escape latency time, active avoidance and neurochemicals like glutathione, catalase and Cholinesterase and lipid peroxidase levels increased. Whereas Standard and Fraction A treated animals decreased in escape latency time, active avoidance and neurochemicals like glutathione, catalase are increased and Cholinesterase and lipid peroxidase levels were increased. For *in vitro* antioxidant we calculate the IC $_{50}$ value. IC $_{50}$ value of ascorbic acid is 7.501 and IC $_{50}$ value of HEMBS Fraction A is 8.315
Conclusion: The present study clearly demonstrates that HEMBS Fraction A (50 and 100 mg/kg) significantly attenuate α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester -induced dementia by improving the learning, memory, antioxidant potentiality and anti-acetylcholinesterase activity. Therefore, this Fraction A can be a potential novel therapeutic strategy for controlling neurodegenerative dementia especially AD. Yet, advance studies are needed to characterize the active compound(s) and expose the possible mechanism of action.

Keywords: Fraction, Dementia, Antioxidant, neuroprotective

Introduction

Cognitive dysfunctions are the common neurological disorder in clinical practices that may found to be associated with the Alzheimer's disease, epilepsy, depression, schizophrenia, and stroke [1, 4]. Cognitive deficits may be congenital or caused by environmental factors such as brain injuries, neurological disorders or mental illness [5, 7]. It has promoted a growing awareness that, like schizophrenia and neurological disorders, mood disorders may be associated with a distinct pattern of cognitive impairment. Dementia is characterized by loss of intellectual ability leading to disruption of multiple higher cortical functions including memory, reasoning, orientation, learning capacity, and emotional stability [8, 9]. Progressive dementia is associated with Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of neurons and is characterized by the presence of excessive amounts of neuritic plaques containing amyloid β protein and abnormal tau protein filaments in the form of neurofibrillary tangles [10, 12]. Degeneration of cholinergic neurons, particularly in the basal forebrain, has been found to be associated with loss of the neurotransmitter acetylcholine [13, 14]. Depletion of acetylcholine level in Alzheimer's disease patients appears to be a critical element in producing dementia [15]. At present, the most appropriate approaches focused on modulation of acetylcholinesterase (AChE) activity. AChE inhibitors such as tacrine, donepezil, physostigmine, galantamine and heptylphysostigmine have been tested for the symptomatic treatment of Alzheimer's disease. Studies reported that these agents increase

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the availability of acetylcholine at cholinergic synapses, slow the progression of dementia symptoms and enhance cognitive process in humans and animals [16-18]. However, these drugs suffer from drawbacks in form of their therapeutic efficacy and adverse side effects to limit their application [19, 20]. Generation of free radicals associated with enhanced oxidative stress has been found to be attributed in the pathogenesis of Alzheimer's disease resulting in aging and cell apoptosis [21]. A number of studies, including us have been suggested the involvement of reactive oxygen species (ROS) in the neurological and neurodegenerative disorders [22, 23], thus directing the role of free radicals in progression of Alzheimer's disease. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced models for memory deficits have been widely implicated for screening of anti dementia drugs [24, 25]. Enhance oxidative stress in different brain regions and increased activity of AChE in the hippocampus has also been reported following treatment with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester [26].

Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. They are also referred to as botanicals, biomedicines or herbal supplements. Herbal drugs range from parts of plants to isolated, purified active constituents. They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers [27]. They are eaten, swallowed, drunk, inhaled, or applied to the skin. During the past decade, there has been increasing acceptance and public interest in natural therapies in both developing and developed countries. Due to poverty and limited access to modern synthetic medicine, about four billion people, i.e. 80% of the world's populations, living in developing countries depend upon herbal medicine as source of their primary health care.

Research on medicinal plants began to focus on discovery of natural products as potential active principles against various diseases [28]. Medicinal plants are very interesting, have the ability to produce remarkable chemical structures with diverse biological activities. *Biophytum sensitivum* is used as traditional medicine to cure variety of diseases [29, 30]. During the last few decades, extensive research has been carried out to elucidate the chemistry, biological activities, and medicinal applications of B. *Sensitivum* Phytochemical analysis have shown that the plant parts are rich in various beneficial compounds which include amentoflavone, cupressuflavone, and isoorientin. Extracts and its bioactive compounds have been known to possess antibacterial, anti-inflammatory, antioxidant, antitumor, radioprotective, chemoprotective, antimetastatic, antiangiogenesis, wound-healing, immunomodulation, anti-diabetic, and cardioprotective activity.

The present study is carried out the evaluation of Neuroprotective and In-vitro Anti-oxidant activities of isolated fraction A from *Biophytum sensitivum* on α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced dementia in rats.

Materials and method
Plant material
Biophytum sensitivum whole plant was collected from forest area of Rampachowdavaram, East Godavari and authenticated by Dr. P. Prasanna Kumari Department of Botany, D.N.R College, Bhimavaram A voucher specimen was kept at Department of Pharmacology, Shri Vishnu College of Pharmacy.

Extraction and isolation of the fractions
Preparation of plant extract:
The Whole plant were dried under shade at room temperature The shade dried, coarsely powdered roots (500 g) was successively extracted with petroleum ether (60-80oC) for 7 days to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95% methanol to obtain methanolic extract. The methanolic extract was evaporated under reduced pressure at low temperature (30oC) to dryness and brownish yellow colour extracts of *Biophytum sensitivum* was obtained [31].

Separation of active compounds by column chromatography
Column-chromatography A cylinder shaped glass column containing stationary phase (silica gel) is encountered slowly from the top with a liquid solvent (mobile phase) that flows down the column with the help gravity or external pressure applied. This technique is used for the purification of compounds from a mixture. Once the column is ready, the sample is loaded inside the top of the column. The mobile solvent is then allowed to flow down through the column. The compounds in mixture have different interactions ability with stationary phase (silica gel), and mobile phase, thereby will flow along the mobile phase at different time intervals or degrees. In this way, the separation of compounds from the mixture is achieved. The individual compounds are collected as fractions and analyzed further for structure elucidation.

Protocol
Isolation and purification of bioactive compounds from plant samples
1. A suitable size long cylindrical glass column (based on the amount of the sample) should be stand firm on a column-chromatography stand.
2. Completely dried plant extract sample should be mixed with silica gel to make a fine powdered form for easy distribution of sample in already packed silica gel column.
3. Sample powdered mass should be placed on the top of the pre-packed silica column and sample should be covered with a layer of cotton.
4. Then solvents of different polarities were passed through column at uniform rate under gravity to fractionate the sample extract.
5. Each fraction was collected separately in a test tube and numbered consecutively for further analysis on thin layer chromatography.
6. Thin layer chromatography (TLC) provides partial separation of both organic and inorganic materials using thin-layered chromatographic plates especially useful for checking the purity of fractions.
7. Each fraction is applied on activated TLC plates with the help of capillary tube at a 1/2 inch apart from the lower edge of TLC plate, and plate is kept in a developing chamber containing suitable solvent system for specific time until the developing solvent reaches top of the upper edge of TLC plate.
8. Plate is taken out from developing chamber, dried and solvent front is marked by lead pencil. Compound bands/spots visualized on TLC chromatoplate can be detected by visual detection, under UV light (254 nm), in iodine chamber and by using spray reagent (vanillin-sulfuric acid) for the presence of specific compounds.

9. The visualized spots of the components in the chromatoplate are marked and the Rf value of each spot is calculated by the formula: Rf = distance travelled by the sample (cm)/distance travelled by the solvent (cm). See Table. no:1

Gradient solvent system used in column chromatography for the isolation of fractions in *Biophytum sensitivum* whole plant

Table 1: Gradient solvent system used in column chromatography

Solvent system	Ratio	Fraction
Hexane	100%	-
Hexane**:** Ethyl acetate	95: 5	A
Hexane: Ethyl acetate	70: 30	B
Hexane: Ethyl acetate	50: 50	C
Only ethyl acetate	100%	D
Only methanol	100%	E

Preliminary Phytochemical Screening
Different fractions were subjected to preliminary phytochemical for the detection of various constituents.

Determination of *in vitro* Antioxidant activity Free radical scavenging (DPPH) assay
The free radical scavenging activity of The Hexane and Ethyl Acetate fraction A was measured *in vitro* by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay using the method of Blois (1958). About 0.3mM solution of DPPH in 100% ethanol was prepared and 1ml of this solution was added to 3ml of the HEMBS Fraction A dissolved in ethanol at different concentrations (10–50 _g/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC50 value of the HEMBS Fraction A was compared with that of ascorbic acid, which was used as the standard [32].

Procedure for α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced Dementia
Animals used in the study:
Healthy adult albino Wistar rats weighing 200-250grams of Wistar rats were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory ANIMAL Diet) and water ad Libitum. Animal studies had approval of IACE committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Experimental design
Animals were classified into five groups (6 rats each). Treatments were given p.o. for seven successive days. One hour after the last dose of test agents, all animals were i.p. injected with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester (5 mg/kg) except the first group (control group). Animals were treated according to the following scheme: groups I received normal saline, II received α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester 5mg/kg where as III, IV and V received donepezil hydrochloride (2 mg/kg), HEMBS Fraction A 50 mg/kg and HEMBS Fraction A100 mg/kg respectively along with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester Spatial learning, and active avoid response tests were

conducted 30 min after α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester injection [33, 35]. immediately after performing the behavioral tests, rats were sacrificed by decapitation, brains were rapidly isolated. Each brain was dissected through the midline into two hemispheres. Each hemisphere was weighed and homogenized in ice-cold 50 mM phosphate buffer (pH 7.4) to prepare 10% homogenate that was used for the biochemical parameters. Finally, brains of 2–3 rats from each group were preserved in 10% formalin and kept for histopathologic examination.
Total 30 rats were taken, each group contains 6rats.
Group I: Received normal saline
Group II: α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester 5 mg/kg (i.p.) for 7 days.
Group III: α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester 5 mg/kg (i.p.). Donepezil (2mg/kg) for 7 days.
Group IV: α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester 5 mg/ kg (i.p.) HEMBS Fraction A 50mg/kg for 7 days
Group V: α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester 5 mg/ kg (i.p.). HEMBS Fraction A 100mg/kg for 7 days

Estimation of Behavioral parameters
Estimation of escape latency time by using Morris water maze
Morris water maze test Morris water maze is one of the most widely used tasks for testing spatial learning and memory in rodents and the procedure used in the present experiment was a modification [36]. In this test, water maze consists of a circular pool (1.6 m diameter and 50 cm height) colored with black nontoxic dye filled to a depth of 44 cm with water. The temperature in pool was maintained at 25 °C ± 1 °C. Four equally spaced points around the edge of the pool were designed as North (N), East (E), South (S) and West (W). A black colored round platform of 9 cm diameter was placed 1 cm below the surface of water. The rats were trained to navigate the submerged platform. The rats were given a maximum time of 120 s (cut off time) to find the hidden platform and were allowed to stay on it for 30 s. The platform remained in the same position during the training days. Rat that failed to locate the platform within 120 s was put on platform only in the first session. The animals were given a daily session of five trials. Escape latency time to reach the platform was recorded in each trial.

Estimation of Passive avoidance paradigm
The passive avoidance test was performed according to the method as described by Yadav *et al*. [37] with slight modification. The rats were subjected to the passive avoidance test by placing in a light compartment of shuttle box. The light compartment was isolated from the dark compartment by a guillotine door. After an acclimatization period of 30 s, the guillotine door was opened and closed after entry of the rat into the dark compartment. The subject received a low intensity foot shock (0.5 mA; 10 s) in the dark compartment. The transfer of the animal from one compartment to another was recorded as transfer latency time (TLT) in seconds. The duration of a trial was 270 s. The first trial was for acquisition and retention was tested in a second trial given 24 h after the first trial. The shock was not delivered in the retention trials to avoid reacquisition. The criterion for learning was taken as an increase in the TLT on retention trials when compared with acquisition trial.

Biochemical Parameters
Estimation of Acetyl cholinesterase levels
The activity of AChE has been estimated in hippocampus using acetylthiocholine iodide as substrate following the colorimetric method [38]. Briefly, the reaction mixture in a final volume of 1.0 ml contained phosphate buffer (0.1 M, pH 7.4), post mitochondrial fraction of hippocampus containing around 15 20 µg protein, acetylthiocholine iodide and 5'dithionitrobenzoic acid (DTNB) (5 mM). The degradation of acetylthiocholine iodide was measured at 412 nm and the results are expressed as µmoles/mg protein

Estimation of Catalase activity
Assay of catalase activity The activity of catalase in hippocampus was assayed following the method of Aebi [39], using H_2O_2 as substrate. Briefly, reaction mixture in a final volume of 1.0 ml contained phosphate buffer (0.1 mM, pH 7.4), post mitochondrial fraction of sample (100 µl) and H_2O_2 (30 mM). The decrease in optical density was measured for 150 s at 240 nm using the spectrophotometer. The activity of the enzyme was calculated using the molar extinction coefficient 43.6 M/cm.

Estimation of brain reduced glutathione (GSH) activity
Levels of reduced glutathione GSH in hippocampus was measured following the standard method [40]. Briefly, 10% homogenate was deproteinized with an equal volume of trichloroacetic acid (10%) and allowed to stand at 4 °C for 1 h. The contents were centrifuged at 3000×g for 15 min. The supernatant (0.5 ml) was added to 2 ml of Tris HCl buffer (0.4 M, pH 8.9) containing ethylenediaminetetraacetic acid

(0.02 M, pH 8.9) followed by the addition of 5, DTNB, 0.01 M. The volume was made up to 3 ml by addition of 0.5 ml of distilled water and absorbance of yellow color read on a spectrophotometer at 412 nm and the results are expressed as µg GSH/g tissue.

Assay of lipid peroxidation
As a measure of lipid peroxidation, TBARS was measured in hippocampus following the method of Ohkawa et al. [41] Briefly, homogenate of hippocampus in phosphate buffer (0.1 M, pH 7.4) was incubated with sodium dodecyl sulfate (10%, w/v) for 10 min followed by the addition of 20% acetic acid. The reaction mixture was incubated with thiobarbituric acid (0.8%) for 1 h in boiling water bath. The intensity of pink chromogen formed was read at 532 nm and the amount of TBARS was calculated using a molar extinction coefficient of 1.56×105 M/cm.

Statistical Analysis
All the values of the experimental results were expressed as mean ± standard error of mean (SEM). One-way ANOVA followed by Dunnet test. Graph Pad Prism (version 5.03) software was used for all statistical analysis and $p < 0.05$ was considered as significant.

Results
Phytochemical studies of Fraction A (Hexane: Ethyl acetate) revealed the presence of fatty acids, phenolics and flavonoids, so based on the phytochemicals presence we select the Fraction A for this study. Table. No:2

Table 2: Results of phytochemical analysis of HEMBS fractions

S.NO	Phytochemical tests	H:EA (95: 5)	H:EA (70: 30)	H:EA (50: 50)	EA only	H only	M only
1	Alkaloids	-	-	-	-	-	-
2	Glycosides	-	-	-	-	-	-
3	Tannins	-	-	-	-	-	-
4	Phenols	++	-	-	-	-	-
5	Flavonoids	++	++	-	-	-	-
6	Saponins	-	-	-	-	-	-
7	Steroids	++	++				

Where (+) Indicates presence, and (-) Indicates absence
H- Hexane, EA-Ethyl acetate, M-methanol

DPPH Assay of HEMBS Fraction A
The DPPH is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant. It has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the

antioxidative activity of plant extracts and foods. The scavenging abilities of HEMBS were concentration-dependent and expressed as IC_{50} values. Concentration of the sample necessary to decrease the initial concentration of DPPH˙ by 50% (IC_{50}) under the experimental condition was calculated. Therefore a lower IC_{50} value indicates a higher antioxidant. IC_{50} value of ascorbic acid is 7.501 and IC_{50} value of HEMBS Fraction A is 8.315 presented in Figure 1&2.
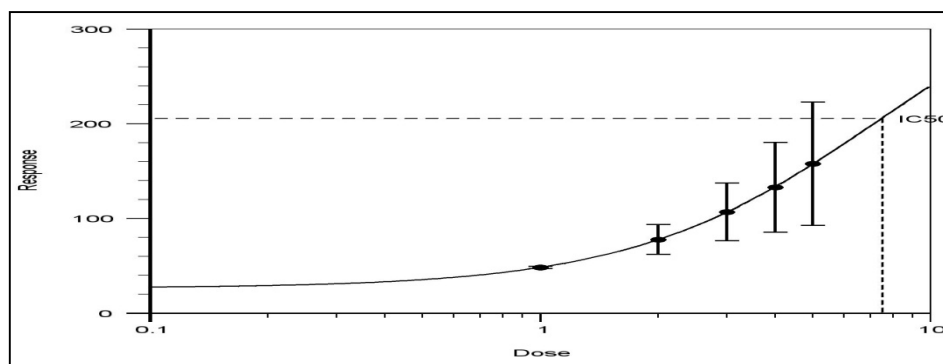

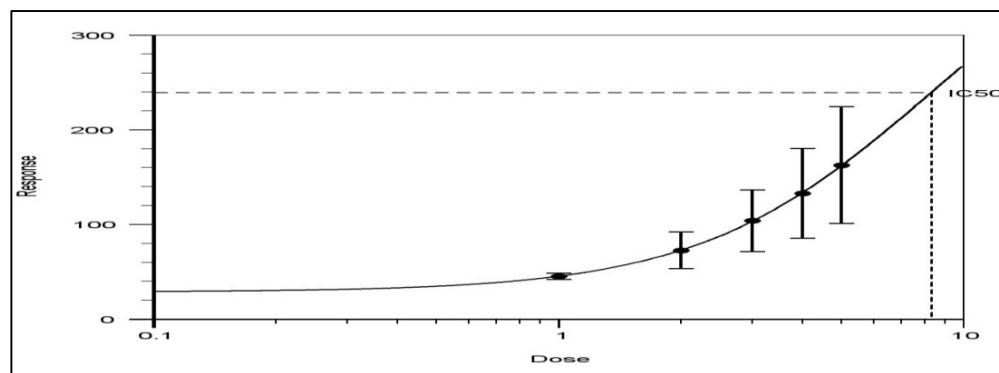**Fig 1:** Represents IC_{50} calculation of Ascorbic acid

Fig 2: Represents IC_{50} calculation of HEMBS Fraction A

Effect of HEMBS Fraction A on escape latency using Morris water maze

Morris water maze task training for 7 days leads to progressive improvement in the ability of rats to explore the hidden platform in the target quadrant. The α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals exhibited longer escape latencies (time taken to find platform) throughout training days than vehicle treated controls indicating impairment of memory. The saline treated control group rapidly learned the location of the platform. HEMBS fraction A treated groups significantly attenuated the effects of α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester on escape latency as did donepezil treated groups presented in Figure 3&4.
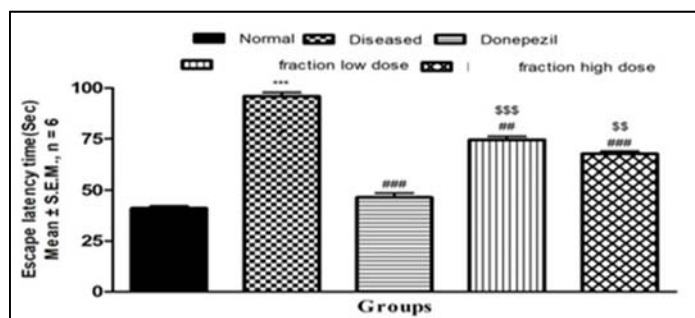

Fig 3: Effect of HEMBS fraction A on escape latency. All values are expressed in mean ± SEM. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated group animals show increase in escape latency time when compared to normal control group of animals, whereas Donepezil treated animals decrease in escape latency time($***p<0.001$) when compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals and HEMBS fraction A(100mg/kg)treated animals significantly decrease in escape latency time($\#\#\# \ p<0.001$) compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals.

Group I control

Group II Toxic control (Scopolamine 5mg/kg)

Group III Standard control (Donepezil 2mg/kg)

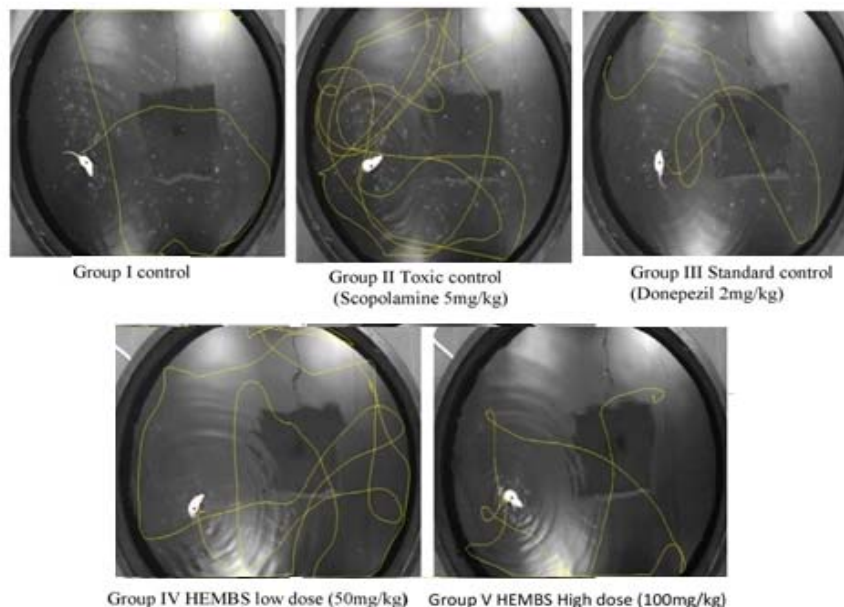Group IV HEMBS low dose (50mg/kg)   Group V HEMBS High dose (100mg/kg)

Fig 4: Video tracking images of Morris water maze during experiment

Effect of HEMBS Fraction A on passive avoidance paradigm in response to assess learning and memory in rats

Exposure of rats to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester caused a significant decrease in TLT of the retention trials as compared to the acquisition trial indicating decrease in the learning and memory activity of rats in comparison to controls. The TLT in rats in the control and in simultaneous treatment with donepezil and α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester group were significantly increased in the retention trial as compared to acquisition trial. Furthermore, simultaneous treatment of HEMBS fraction A with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester separately in rats caused a significant increase in the in the retention trial as compared to acquisition trial. The treatment of HEMBS and α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester in rats caused a significant increase in the retention trial as compared to acquisition trial indicating improved learning and memory in rats presented in [Figure 5.]
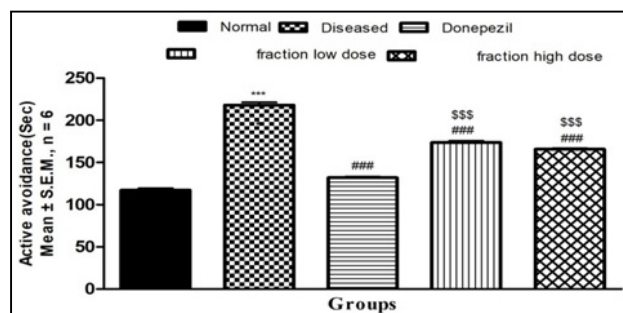

Fig 5: Effect of HEMBS fraction A on passive avoidance response. All values are expressed in mean ± SEM. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated group animals show increase in active avoidance response when compared to normal control group of animals, whereas Donepzil treated animals decrease in active avoidance response (***$p<0.001$) when compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals and HEMBS fraction A(100mg/kg) treated animals significantly decrease in active avoidance response (### $p<0.001$) compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals.

Effect of HEMBS Fraction A on neurochemical parameters
Effect of HEMBS Fraction A on acetylcholinesterase levels

Exposure of rats to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester caused a significant increase in the activity of AChE, an enzyme involve in the metabolism of acetylcholine in hippocampus (*P*<0.001) as compared to control. Simultaneous treatment of donepezil and α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester in rats caused a significant decrease in the activity of AChE in hippocampus as compared to those treated with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester alone. Furthermore, simultaneous treatment of HEMBS Fraction A with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester separately in rats caused a significant decrease in the activity of AChE in hippocampus as compared to those treated with α-(hydroxymethyl)

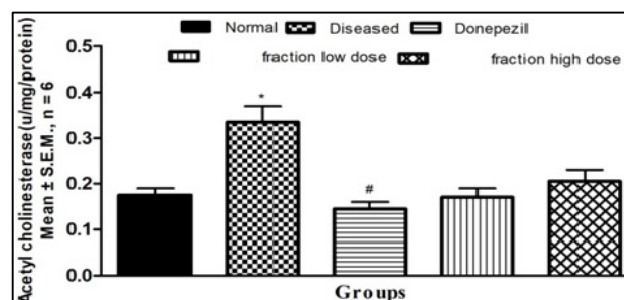benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester alone presented in Figure 6.

Fig 6: Effect of HEMBS fraction A on acetyl cholinesterase. All values are expressed in mean ± SEM. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated group animals show increase in acetyl cholinesterase levels when compared to normal control group of animals, whereas Donepzil treated animals decrease in acetyl cholinesterase levels (***$p<0.001$) when compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals and HEMBS fraction A treated animals significantly decrease in acetyl cholinesterase levels (### $p<0.001$) compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals.

Effect of HEMBS Fraction A on lipid peroxidation levels

Exposure of rats to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester caused a significant increase in the level of lipid peroxidation in hippocampus as compared to controls. Simultaneous treatment of donepezil and α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester in rats caused a significant decrease in the level of lipid per oxidation in hippocampus (P < 0.001) as compared to those treated with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester alone. Furthermore, simultaneous treatment of HEMBS Fraction A with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester separately in rats caused a significant decrease in the level of lipid per oxidation in hippocampus as compared to those treated with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester alone presented in Figure 7.
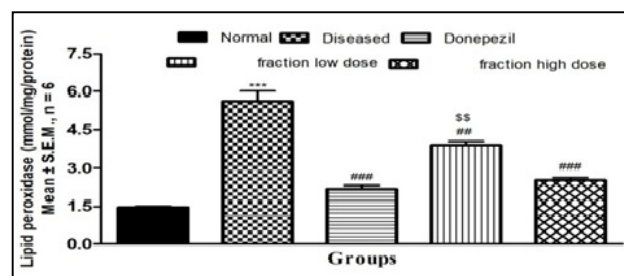

Fig 7: Effect of HEMBS Fraction A on lipid peroxidase levels. All values are expressed in mean ± SEM. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated group animals increase in lipid peroxidase levels when compared to normal control group of animals, whereas Donepzil treated animals decrease in lipid peroxidase levels (***$p<0.001$) when compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals and HEMBS fraction A(100 mg/kg) treated animals significantly decreases in lipid peroxidase levels (### $p<0.001$) compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals.

Effect of HEMBS Fraction A on reduced glutathione levels

Exposure of rats to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester caused a significant decrease in the level of reduced GSH in hippocampus as compared to controls. Simultaneous treatment of donepezil and α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester in rats caused a significant increase in the level of reduced GSH in hippocampus as compared to those treated with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester alone. Also, simultaneous treatment of HEMBS Fraction A with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester separately in rats caused a significant increase in the level of reduced GSH in hippocampus as compared to those treated with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester alone presented in Figure 8.
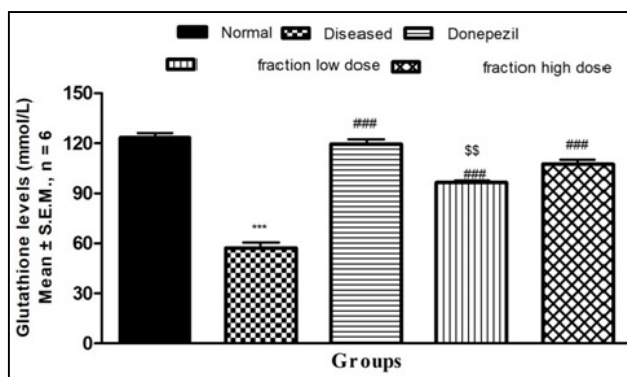

Fig 8: Effect of HEMBS Fraction A on reduced glutathione. All values are expressed in mean ± SEM. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated group animals show decrease in reduced glutathione levels when compared to normal control group of animals, whereas Donepzil treated animals increase in glutathione levels ($***p<0.001$) when compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals and HEMBS fraction A(100mg/kg) treated animals significantly increase in reduced glutathione levels ($### p<0.001$) compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals.

Effect of HEMBS Fraction A on catalase levels

Exposure of rats to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester caused a significant decrease in the activity of catalase in hippocampus as compared to controls. Simultaneous treatment of donepezil and α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester in rats caused a significant increase in the activity of catalase in hippocampus as compared to those treated with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester alone, also simultaneous treatment of HEMBS Fraction A with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester separately in rats caused an increase in the activity of catalase in hippocampus as compared to those treated with α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester alone while the activity of catalase remained decreased as compared to rats in the control group presented in Figure 9.
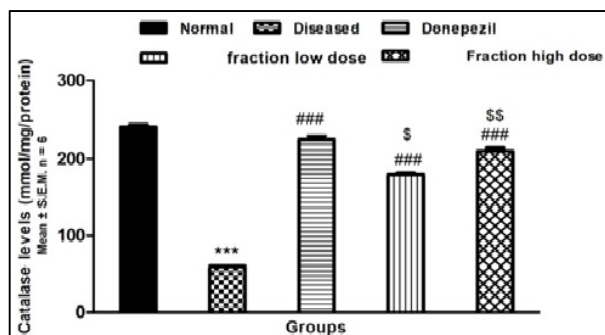

Fig 9: Effect of HEMBS Fraction A on catalase. All values are expressed in mean ± SEM. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated group animals show decrease in catalase levels when compared to normal control group of animals, where as Donepzil treated animals decrease in catalase levels ($***p<0.001$) when compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals and HEMBS fraction A(100mg/kg) treated animals significantly increase in catalase levels ($### p<0.001$) compared to α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated animals
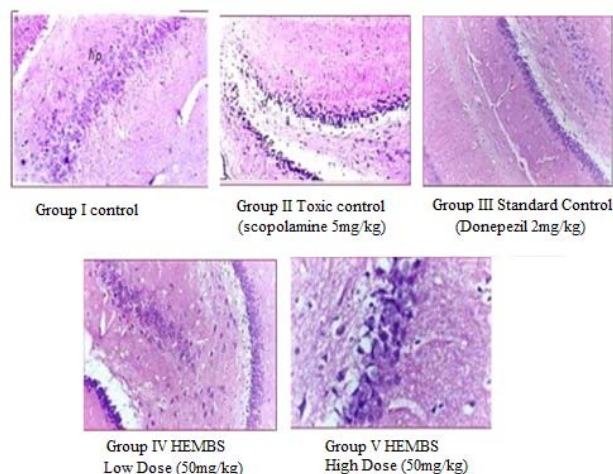

Fig 9: Histopathological studies of Hippocampus of rat brain tissue

(A) Control rat showing the normal histological structure of the hippocampus (hp).
(B) α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester -induced demented rat showing severe congestion in the blood capillaries with perivascular edema (arrow) in encephalomalacia in the hippocampus.
(C) α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced demented rat treated with donepezil showing diffuse gliosis shrinkage with pyknotic nuclei in some pyramidal cells in the hippocampus.
(D) α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester -induced demented rat treated with MEBS (50 mg/kg) showing focal gliosis and pyramidal cells in the hippocampus separated away from each other with irregular outline.
(E) α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced demented rat treated with MBS (100 mg/kg) showing the normal histologic structure of pyknotic nuclei in some pyramidal cells and vacuolated cytoplasm in the hippocampus.

Discussion

Acetylcholine is main neurotransmitter in cholinergic nervous system used for functional behavior like learning and memory by involving synaptic transmission in brains of both human beings and animals [42, 43]. Deficits in learning and memory by alter in acetylcholine levels and imbalance in acetyl cholinesterase [44, 45]. The levels of acetyl cholinesterase is increases in brain leads to decreased levels of acetylcholine by increasing metabolism of acetylcholine that implicates alter in learning and memory [46, 47]. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester(scopolamine)act as a non-selective antagonist for acetylcholine receptors like muscarinic receptors it blocks the signaling pathway of cholinergic system leads in alter in action of acetylcholine resulting in dysfunction of cognition including short and long term cognition [48, 49].

Elevated oxidative stress play a major key role in pathogenesis if neurodegenerative disorders like Alzheimer's disease. In oxidative stress, elevated levels of oxygen free radicals cause the oxidative damage of cells leads to age related decline in cognitive performance [50, 51]. α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester cause a significant increase in levels of acetyl cholinesterase leads to alter in levels of acetylcholine that results alter in learning and memory process and also increase in levels of lipid peroxidase, decrease in catalase and GSH levels in hippocampus of rat brain. The decreased levels of GSH causes the oxidative damage and per oxidative damage to cells leads to brain gaining and neuronal death which is progress to neurodegerative disorders like Alzheimer's and parkinsonism. In present study, dementia is induced by using α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester which is administered by intraperitoneally to rats leads to alter in cognitive functions like learning and memory these are assessed by estimation behavioral parameters like escape latency time and active avoidance response by using Morris water maze and elevated plus maze and also estimated the biochemical parameters like acetyl cholinesterase, lipid peroxidase, reduced glutathione and catalase levels in hippocampus part of brain. In α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester treated rats show alter in behavioral parameters like escape latency time and active avoidance time and increase in levels of acetyl cholinesterase lipid peroxidase, decrease in catalase and GSH levels in hippocampus of rat brain so it indicates α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester causes the changes in cognition.

Simultaneous treatment of Fraction isolated from methanolic extract of *B. sensitivum*(HEMBS) and α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester caused a significant protection against α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced learning and memory impairment which is associated with increase in behavioral parameters like escape latency time and active avoidance response and also decrease in acetyl cholinesterase and lipid peroxidase and increased levels of GSH and catalase in rat brain it indicates decline the oxidative stress in brain. In the present study, fraction of plant potentially exhibit potency as a memory enhancer in α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester induced amnesia. Treatment fraction from plant extract preferred a salutary effect on rodent model of memory deficit commonly used to screen as an anti dementia drug such as cholinesterase inhibitor like donepezil and nootropics agent like piracetam.

Conclusion

The present study clearly demonstrates that MEBS fraction A contain phenols, flavonoids. HEMBS fraction A (50 and 100 mg/kg) significantly attenuate α-(hydroxymethyl) benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo non-7-yl ester -induced dementia by improving the learning, memory, antioxidant potentiality and anti-cholinesterase activity. Therefore, this Fraction can be a potential novel therapeutic strategy for controlling neurodegenerative dementia especially AD. Further advance studies are needed to expose the possible mechanism of action.

Acknowledgment

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