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Evaluation of Anti-Alzheimer Activity of Ethyl Acetate Extract of Aerial Parts of *Justicia glauca* Rottler in Scopolamine Induced Memory Impairment in Swiss Albino Mice

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ABSTRACT

The aim of this study is to evaluate the *Invitro* and *invivo* anti-alzheimer activity of ethyl acetate extract of aerial parts of *Justicia glauca* Rottler (EEJG) in scopolamine induced memory impairment model in albino mice. Either sex of Swiss albino mice was separated into five groups, each consisting of n=6. Phytochemical screening of EEJG showed the presence of sterols, carbohydrates, proteins, alkaloids, cardiac glycosides, saponins, tannins, flavonoids. *Invitro* anti-alzheimer activity of EEJG was evaluated by acetylcholinesterase inhibition assay exhibited good acetylcholinesterase inhibition activity. *Invivo* anti- alzheimer activity was evaluated by scopolamine induced memory impairment model. Group I received normal feed and saline (1 ml/kg/day, 10 days, i.p) Group II received Scopolamine (1 mg/kg/day, 10 days, i.p) Group III received donepezil (2mg/kg/day, 10 days, p.o.) + Scopolamine (1 mg/kg/day, 10 days, i.p) and Group IV and V received test drug (200 mg/kg/day, 10 days, p.o.) of EEJG and (400 mg/kg/day, 10 days, p.o.) of EEJG respectively. Following treatment, the anti-alzheimer effect of EEJG was evaluated by biochemical, histopathological examination among the experimental groups. Escape latency, spontaneous alteration percentage were evaluated as behavioural parameters of Morris water maze, Y maze respectively. The Malondialdehyde (MDA) were evaluated in biochemical parameter. Escape latency were significantly decreased (***p<0.001, ****p<0.0001) in positive control and treatment group, Spontaneous alteration percentage were significantly increased in positive control and treatment group (***p<0.001, ****p<0.0001) compared with the negative control group. MDA was significantly decreased (***p<0.001) in drug-treated groups. In histopathological examination, Sections from hippocampus showed normal neurons (N), neurofilaments (NF) show mild degeneration and normal blood vessels (BV) in EEJG treated groups.

1. INTRODUCTION

Alzheimer's disease is a neurodegenerative disease with the accumulation of amyloid beta and neurofibrillary tangles as its main characteristic, it has become a major health problem that degrades neurons and leads to dementia. Dementia is a syndrome that includes amnesia and impairment of thinking, behavior and the ability to perform daily activities, and is one of the most common diseases in older adults, with more than 55 million people worldwide suffering from dementia. Amyloid beta deposition initiated microglia-activated neuroinflammation, activation of microglia and astrocytes induced the expression of various inflammatory and anti-inflammatory cytokines. **(1-4)** Age, female sex, oxidative stress, smoking, hypertension, diabetes are the risk factors of dementia. (53, 56)

In the scopolamine-induced memory impairment model, scopolamine is a non-selective muscarinic blocker that competitively inhibits the muscarinic receptor for acetylcholine, which is important for memory and learning and is used to induce cognitive impairment in experimental models because it readily penetrates the blood-brain barrier and also causes cholinergic dysfunction and increasing with amyloid-β deposition, both hallmarks of this disease. (58, 63)

There is no specific treatment for AD. Symptomatic, social and psychiatric measures and help to the family are the basis of treatment. Acetylcholinesterase inhibitors (donepezil, galantamine, rivastigmine, tacrine) or N-methyl-D-aspartate (NMDA) inhibitors (memantine) may improve cognitive function in the early stages of the disease. (52, 56)

The ethyl acetate extract of the aerial parts of *Justicia glauca* Rottler (EEJG) was subjected to GC MS has reported some very important molecules such as **gamma-Tocopherol, Stigmasterol, β-Sitosterol, β-Amyrin, Lupeol** which are found to play a role in management of Alzheimer disease (8, 11-15). Thus EEJG has been chosen as the drug to perform the research on its anti-alzheimer activity based on the literature review.

2. MATERIALS AND METHODS

2.1 Procurement of drug and extraction process

The aerial parts of ***Justicia glauca*** **Rottler** were collected from Thothukudi district, Tamil Nadu, India and were authenticated by a scientist of Xavier research foundation Dr. S. Mutheeswaran. The standard drug Donepezil and the inducing agent Scopolamine were procured from Sri Krishna medicals, Chennai, Tamil Nadu, India and then aerial parts of

Justicia glauca Rottler was extracted with ethyl acetate solvent by using a Soxhlet apparatus.

2.2 PHYTOCHEMICAL SCREENING

2.2.1 Test for Sterols

a) Salkowski Test: The sample was treated with 0.3ml of chloroform with few drops of concentrated Sulphuric acid was added, shaken well and allowed to stand for some time. Appearance of red colour will appear at the lower layer indicates the presence of steroids, formation of yellow coloured lower layer will indicate the presence of Triterpenoids.

b) Libermann Burchard Test: The sample was treated with 2ml of chloroform, small amount of acetic anhydride and 1ml of con.Sulphuric acid. The colour change from red to bluish green indicates the presence of Sterols.

2.2.2 Test for Carbohydrate

a) Molisch's Test: To the 0.5ml of sample, few drops of alcoholic alpha napthol and 0.2ml of concentrated sulfuric acid were added slowly through the sides of the test tube. A purple to violet colour ring at the junction indicates the presence of Carbohydrate.

b) Benedict's Test: To 1 ml of sample, few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) were added and boiled on water bath. The formation of a reddish-brown precipitate indicates the presence of Reducing Sugars.

c) Fehling's Test: To 1ml of sample, Fehling's solution A and B were added and heated for few minutes. The formation of a brick red precipitate indicates the presence of Carbohydrates.

2.2.3 Test for Proteins and Amino acids

a) Millon's Test: The sample was treated with 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid). Appearance of a white precipitate which turns red upon gentle heating indicates the presence of Proteins.

b) Biuret Test: The sample was treated with 1ml of 10% sodium hydroxide, 1ml of 1% copper sulphate solution. Appearance of Violet colour indicates the presence of Proteins.

2.2.4 Test for Alkaloids

a) Mayer's Test: To 1ml of sample, Mayer's reagent [Potassium mercuric iodide solution] was added. Formation of Cream colour precipitate indicates the presence of Alkaloids.

b) Dragendroff's Test: To 1ml of sample, Dragendroff's reagent [Potassium bismuth iodide solution] was added. Formation of Reddish-brown precipitate indicates the presence of Alkaloids.

c) Hager's Test: To 1ml of sample, Hager's reagent [Saturated solution of Picric acid] was added. Formation of yellow colour precipitate indicates the presence of Alkaloids.

2.2.5 Test for Cardiac Glycosides

Keller killani Test: The sample was added with 0.4ml of glacial acetic acid containing a trace amount of ferric chloride. It was transferred to a small test tube and then 0.5ml of con. sulphuric acid was added carefully by the side of the test tube. Appearance of blue colour in the acetic layer indicates the presence of Cardiac Glycosides.

2.2.6 Test for Flavonoids

a) Shinoda Test (Magnesium Hydrochloride reduction Test): The sample was treated with few fragments of magnesium ribbon, then few drops of concentrated hydrochloric acid was added, Appearance of magenta colour indicates the presence of flavonoids.

b) Alkaline reagent Test: The sample was treated with 1ml of sodium hydroxide. Appearance of yellow colour indicates the presence of Flavonoids.

c) Mineral acid Test: The sample was treated with few drops of concentrated sulphuric acid. Appearance of orange colour indicates the presence of flavonoids.

2.2.7 Test for Saponin

Foam froth Test: The sample was treated with 10ml of water and boiled for few mins then it was filtered. The filtrate was shaken well and noted for the stable froth. A 1 cm layer of foam indicates the presence of Saponins.

2.2.8 Test for Tannins

a) Gelatin Test: The sample was treated with 2ml of 1% gelatin and 10% sodium chloride. Formation of a white precipitate indicates the presence of Tannins.

b) Lead acetate Test: To 2ml of sample, few drops of lead acetate solution were added. Formation of a white precipitate indicates presence of tannins.

2.3 *INVITRO* METHOD

2.3.1 ACETYLCHOLINESTERASE INHIBITION ASSAY [60, 64]

PROCEDURE

The AChE inhibition of test compounds will be determined by a slightly modified Ellman's method. The final volume (4.0 ml) of the reaction mixture consists of 1.3 ml of the Tris-HCl buffer (pH 8.0; 50 mM) treated with 0.4 ml of different concentrations (2 to 256 µg/ml) of ethyl acetate extract of *J. glauca* and to it 0.1 ml of the AChE (0.28 U/ml) will be added. This mixture will be incubated for 15 minutes and to it 0.3 ml of acetylthiocholine iodide (0.023 mg/ml) and 1.9 ml (5,5'-dithiobis-(2-nitrobenzoic acid) DTNB (3 mM) solution will be added. The final reaction mixture (4 ml) will be further incubated for 30 min at room temperature and the absorbance of the reaction mixture will be taken at 405 nm. The control will be prepared by replacing the drug with the suitable solvent. The blank will be prepared by replacing all the reagents with the solvent to nullify the effect of colour of the test drug.

The percentage inhibition will be calculated using the formula:

$$\% \textbf{ Acetylcholinesterase inhibition} = (\mathbf{A0} - \mathbf{A1})/\ \mathbf{A0} \times \mathbf{100}$$

A0 = Absorbance of the control

A1 = Absorbance of the tested compound (standard/extract)

2.4 ANIMAL PROCUREMENT

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no: 1917/GO/ReBi/S/16/CPCSEA/20.09.2021 and 05/AEL/IAEC/MMC, Date: 26.12.2023. For this investigation, 33 Swiss albino mice were purchased from the Madras Medical College Animal House in Chennai, India. In a quarantine period, animals are kept apart from those already housed in the facility while their health as well as their microbiological condition are being assessed. The newly procured Swiss albino mice were quarantined for a period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop psychological, physiological and nutritional stabilization before

their use. The animals were housed in a well-ventilated animal house which was maintained at a constant temperature and relative humidity of 55 to 60%. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material. The bed material was changed twice a week. The animals were maintained on standard pellets and purified water. The animals were provided with food ad libitum except during fasting. All animal cages used in the study had proper identification i.e., labels. Each animal in the cage was marked on the tail with picric acid for their appropriate identification.

2.5 ACUTE TOXICITY

Since it is an herbal plant, limit test has been performed for the ethyl acetate extract of aerial parts of *Justicia glauca* Rotller with 3 animals at a dose of 2000 mg/kg according to OECD guidelines 423 (Acute oral toxicity) for about 14 days for dose selection. [62]

2.6 ANTI-ALZHEIMER ACTIVITY [1, 44, 47]

This activity is 15 days experimental study. Initially animals were given training/trail period of 5 days for Morris water maze, Y maze, Open field test.

Group I (Normal Control) received normal feed and saline (1 ml/kg/day, 10 days, i.p) from 6th day to 15th day

Group II (Negative control) received scopolamine (1 mg/kg/day, 10 days, i.p) from 6th day to 15th day

Group III (Positive control) received Donepezil (2 mg/kg/day 10 days, p.o.) + scopolamine (1 mg/kg/day, 10 days, i.p.) 30 minutes after donepezil administration from 6th day to 15th day

Group IV (Low dose group) received EEJG (200 mg/kg, 10 days, p.o). + Scopolamine (1 mg/kg/day, 10 days, i.p)) 30 minutes after EEJG administration from 6th day to 15th day

Group V (High dose group) received EEJG (400 mg/kg, 10 days, p.o). + Scopolamine (1 mg/kg/day, 10 days, i.p) 30 minutes after EEJG administration from 6th day to 15th day

2.7. EVALUATION OF ANTI-ALZHEIMER ACTIVITY

2.7.1 Behavioural parameters

2.7.1.1. Morris water maze [1]

In the Morris water maze test, a spherical maze 122 cm in diameter and 51 cm in height with a

circular acrylic platform 10 cm in diameter and 35 cm in height was filled with water. A platform with a diameter of about 10 cm was placed below the surface of 1 cm in the water. During training, mice were allowed to walk across the submerged platform. The maximum interruption time given to mice to explore the submerged platform is 2 minutes and they are allowed to stop on the platform for about 15 seconds. In each trial, the escape latency (time to reach the platform) was recorded and animals that failed to navigate to the platform within 120 s were placed on the platform. Animals were administered trials in four sessions per day.

2.7.1.2. Y maze Test [44]

Short-term memory will be assessed by spontaneous alternating behavior in a Y-maze task. The Y-maze used in this study consists of three arms (35 cm long, 25 cm high, and 10 cm wide) and an equilateral triangular central area. 30 minutes after the appropriate drug administration, mice are placed at the end of one arm and allowed to freely move through the maze for 8 minutes. An arm entry will be counted when the hind paws of the mouse are fully in the arm. Spontaneous switching behavior will be defined as entering all three arms based on consecutive choices. The number of maximal spontaneous alternation behaviors will then be the total number of arms entered minus two, and the percentage of spontaneous alternation is calculated as

Actual Changes / Maximum Changes × 100.

Before testing the next animal, the maze will be cleaned with a 10% ethanol solution and dried with a cloth. Spontaneous alternating behavior is thought to reflect spatial working memory, which is a form of short-term memory.

2.8. BIOCHEMICAL ESTIMATION

Collection of Brain Sample

Immediately after behavioral testing for the Morris water maze, Y maze, animals were killed by cervical dislocation. The entire brain was carefully removed from the skull. To prepare brain homogenate, fresh whole brain was weighed and transferred to a homogenizer and homogenized in an ice bath after adding phosphate buffer (pH 8, 0.1 M). The homogenate was centrifuged using a refrigerated centrifuge at 3000 rpm for 10 min at 4 °C and the resulting supernatant was used for the following assay.

2.8.1 Estimation of malondialdehyde [44]

Lipid peroxidation as demonstrated by the formation of thiobarbituric acid-reactive substances (TBARS) and hydroperoxides (HP) was measured by the method of Nieshus and Samuelsson, 1986. About 0.1 ml of homogenate (Tris HCl buffer, pH 7.4) was treated with 2 ml (ratio 1 :1:1) reagents TBA-TCA-HCL (thiobarburic acid 0.37%, 0.25N HCl and 15% TCA) and placed in a water bath for 15 minutes, cooled and centrifuged at 1000 rpm for 10 minutes. min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. Values are expressed as nanomoles MDA/min/mg protein.

2.9 HISTOPATHOLOGICAL EXAMINATION

Brain was isolated from mice in all five groups and then fixed in 10% neutral buffered formalin for 24 hours. The tissues were cleaned with methyl benzoate after drying using a graded series of alcohols and embedded in wax with paraffin. Hippocampus were sectioned from brain and stained with hematoxylin and eosin dissolved in 95 % ethanol was used to stain the counter. Hippocampus sections were observed under a microscope after dehydration and clearing.

2.10 STATISTICAL ANALYSIS

All the values were expressed as mean ± SEM. Graph Pad Prism Software version 9.5.2 was used to statistically analyze the data using one-way ANOVA and Dunnett's multiple comparison test. P values were regarded as statistically significant if they were between 0.05 and 0.0001.

3. RESULTS AND DISCUSSION

3.1.Phytochemical screening

Table 1 Results of Phytochemical screening of EEJG

S.NO.	TEST	RESULTS
1.	**TEST FOR STEROLS** a. Salkowski's test b. Lieberman burchard test	 + +
2.	**TEST FOR CARBOHYDRATES** a. Molisch's test b. Fehling's test c. Benedict's test	 + + +
3.	**TEST FOR PROTEINS** a. Millon's test b. Biuret test	 + +
4.	**TEST FOR ALKALOIDS** a. Mayer's reagent b. Dragendroff's reagent c. Hager's reagent d. Wagner's reagent	 + + + +
5.	**TEST FOR GLYCOSIDES** a. Anthraquinone glycosides i) Borntrager's test ii) Modified Borntrager's test b. Cardiac glycosides i) Keller Kiliani test	 - - +
6.	**TEST FOR SAPONINS**	+
7.	**TEST FOR TANNINS** i) $FeCl_3$	 +
8.	**TEST FOR FLAVANOIDS** i) Shinoda test ii) Alkali test iii) Acid test	 + + +
9.	**TEST FOR VOLATILE OILS**	-

3.2 *Invitro* anti-alzheimer activity

Invitro anti-alzheimer activity was evaluated by acetylcholinesterase inhibition assay. Results were presented in Table no. 1 and Figure 1 and 2, The percentage inhibition of Donepezil ranges from 21.34% to 100.18% for a concentration of 2 to 256 µg/ml respectively and IC_{50} was found to be 32.26 µg/ml. The percentage inhibition of EEJG ranges from 10.52% to 89.98% and IC_{50} was found to be 52.61 µg/ml. From the IC_{50} value of EEJG it exhibited good inhibitory activity when compared to the standard drug Donepezil which shows great inhibitory activity against AChE.

Table 2: *Invitro* enzyme inhibitory activity of Donepezil and EEJG against AChE

Concentration (µg/ml)	**Percentage Inhibition (%)**	
	Standard (Donepezil)	***Justicia glauca* Rottler**
2	21.34	10.52
4	38.71	28.13
8	40.43	42.23
16	45.31	53.19
32	61.74	56.59
64	71.4	65.52
128	89.97	75.31
256	100.18	89.98
Half maximal inhibitory concentration **IC50** (µg/ml)	32.26	52.61

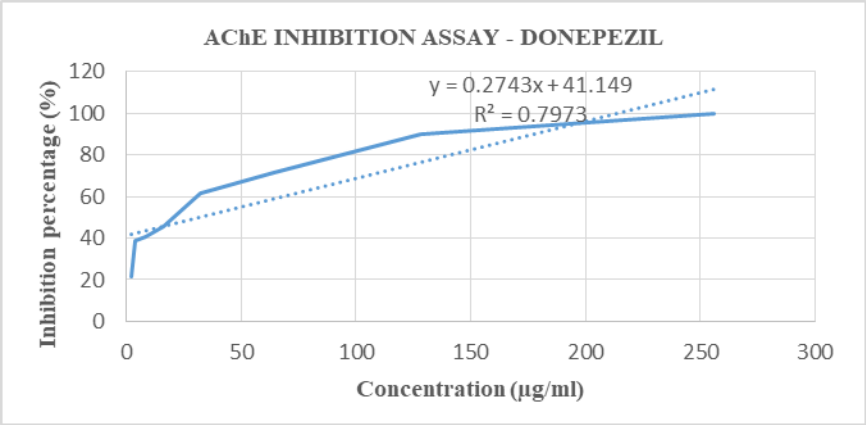

Fig 1 : Graphical representation of AChE inhibition assay for Donepezil

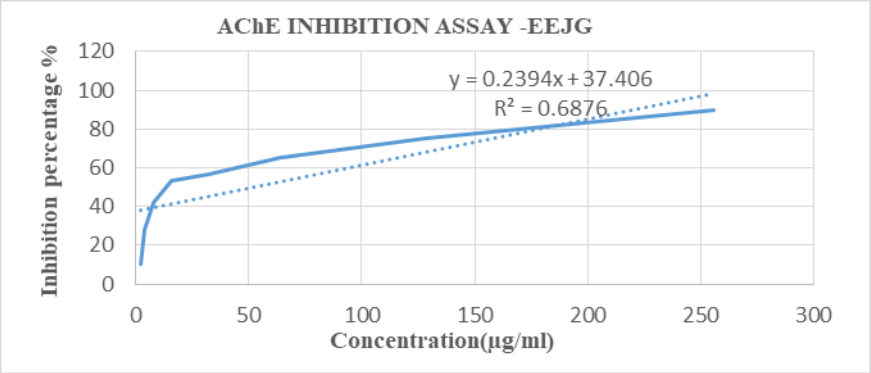

Fig 2: Graphical representation of AChE inhibition assay for EEJG

3.3 Invivo Acute Toxicity Studies

The Acute Oral toxicity is performed as per OECD guideline 423. There was no mortality and morbidity detected up to a dose of 2000 mg/kg in Swiss albino mice during a 14-day period of EEJG administration. Acute toxicity studies help to determine the safety of substances when administered in a single high dose, while the 14-day period allows for evaluation of potential sub-acute effects. Hence 1/10th and 1/5th of a dose of **200 mg/kg** and **400 mg/kg** respectively were designated as the low dose and high dose of EEJG respectively.

3.3 *Invivo* anti – Alzheimer activity by Scopolamine induced memory impairment model

3.3.1 Effect of EEJG on behavioural parameters:

3.3.1.1 Morris water maze

Escape latency time (in seconds) was taken as a parameter in Morris water maze during a period of training session (day 1 -5) and dosing and test session (day 6 – 15). There was no significant ($^{ns}p>0.05$) difference in escape latency time among all groups during training period (day 1 – 5). The escape latency time was significantly ($^{####}p<0.0001$) increased for negative control group when compared to Normal group. Positive control group also showed significant (****$p<0.001$) decrease in escape latency time when compared with Negative control group. The animals treated with EEJG 200mg/kg and 400 mg/kg showed significant (***$p<0.001$, ****$p<0.0001$) decrease in escape latency time when compared with Negative control group. (Table 3 and figure 3)

3.3.1.2 Y Maze

Spontaneous alternation in Y maze was observed on Non- drug treatment period (Day 1 -5), Dosing and test session (Day 6 – 15). There was no significant (^{ns}P>0.05) difference in spontaneous alternation percentage among all the groups during non- drug treatment period. The spontaneous alternation percentage in the Y maze was significantly ($^{####}$p<0.00001) decreased in Negative control when compared to normal control group. The animals treated with EEJG 200mg/kg and 400mg/kg showed significant (***p<0.001, ****p<0.0001) increase in spontaneous alternation percentage when compared with Negative control Spontaneous alternation percentage was also significantly (****p<0.0001) increased in positive control group when compared with negative control group. (Table 4 and figure 4)

Table 3 : Effect of EEJG on escape latency time (sec) in Morris water maze

Groups	**Induction/ Drug Treatment**	**Escape Latency Time (Seconds)**		
		Training Period (Day 1 – 5)	**Dosing and Test Period**	
			(DAY 6 – 10)	**(DAY 11 – 15)**
Group 1	Normal control Saline (1 ml/kg)	27± 0.45	27.17 ± 0.70	25.83±0.60
Group 2	Negative control Scopolamine (SCP) (1 mg/kg)	25.83 ± 0.54 ^{ns}	33.5 ± 0.56$^{##}$	38.5±0.76$^{###}$
Group 3	Positive control SCP + Donepezil (2 mg/kg)	26.17 ± 0.40 ^{ns}	20.83 ± 0.40***	15.83±0.60****
Group 4	Low dose groupSCP + EEJG (200 mg/kg)	26.33 ± 0.56 ^{ns}	26.17 ± 0.83*	25.33±0.56***
Group 5	High dose group SCP + EEJG (400 mg/kg)	26.5 ± 0.56 ^{ns}	25.83 ± 0.87**	21.17±0.70****

All values are expressed in Mean ± SEM, n = 6.

Data were analysed by one-way ANOVA followed by Multiple Dunnett's multiple comparison test.

*p<0.05, **p<0.01, ****p<0.0001 compared with Negative control

$^{###}$p<0.001, $^{####}$p<0.0001 compared with Normal control and **^{ns}p>0.05** denotes non significant.

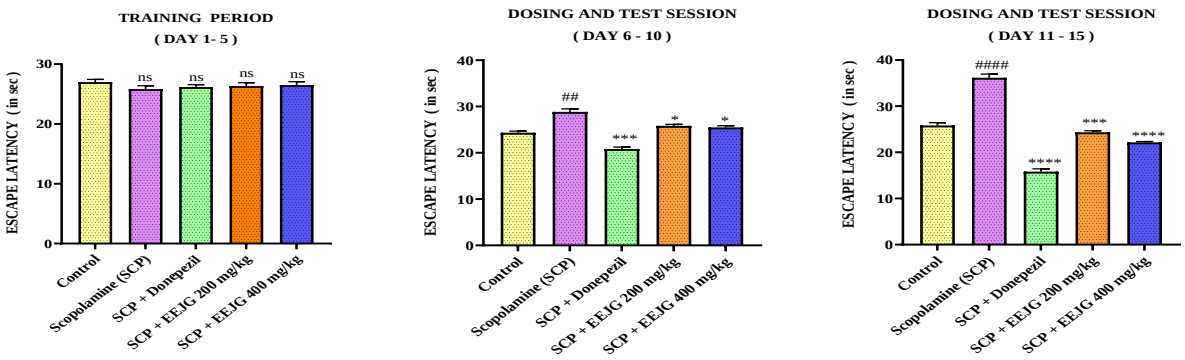

Fig 3. Graphical representation of EEJG on Escape latency time in Morris water maze

Table 4: Effect of EEJG aerial parts on Spontaneous alteration (%) in Y maze

Groups	**Induction/ Drug treatment**	**Spontaneous alteration (%)**		
		Non Dosing Period (Day 1 – 5)	**Dosing and Test session**	
			(Day 6 – 10)	**(Day 11 – 15)**
Group 1	Normal control Saline (1 ml/kg)	29.7 ± 1.81	35.1 ± 1.87	33.8 ± 1.61
Group 2	Negative control Scopolamine (SCP) (1 mg/kg)	25.8 ± 1.45 ^{ns}	16.6 ± 0.77$^{###}$	11.1 ± 0.69$^{####}$
Group 3	Positive control SCP + Donepezil (2 mg/kg)	31.1 ± 2.49 ^{ns}	41.2 ± 2.12***	54.4 ± 2.23****
Group 4	Low dose group SCP + EEJG (200 mg/kg)	27.2 ± 1.78 ^{ns}	27.1 ± 0.96**	35.1 ± 2.02***
Group 5	High dose group SCP + EEJG (400 mg/kg)	30.9 ± 1.59 ^{ns}	31.0 ± 2.4**	43.2 ± 0.8****

All values are expressed in Mean ± SEM, n = 6.

Data were analysed by one-way ANOVA followed by Multiple Dunnett's multiple comparison test.

**p<0.01, ***p<0.001, ****p<0.0001 compared with Negative control

$^{###}$p<0.001, $^{####}$p<0.0001 compared with Normal control and ^{ns}**p>0.05** denotes non significant.

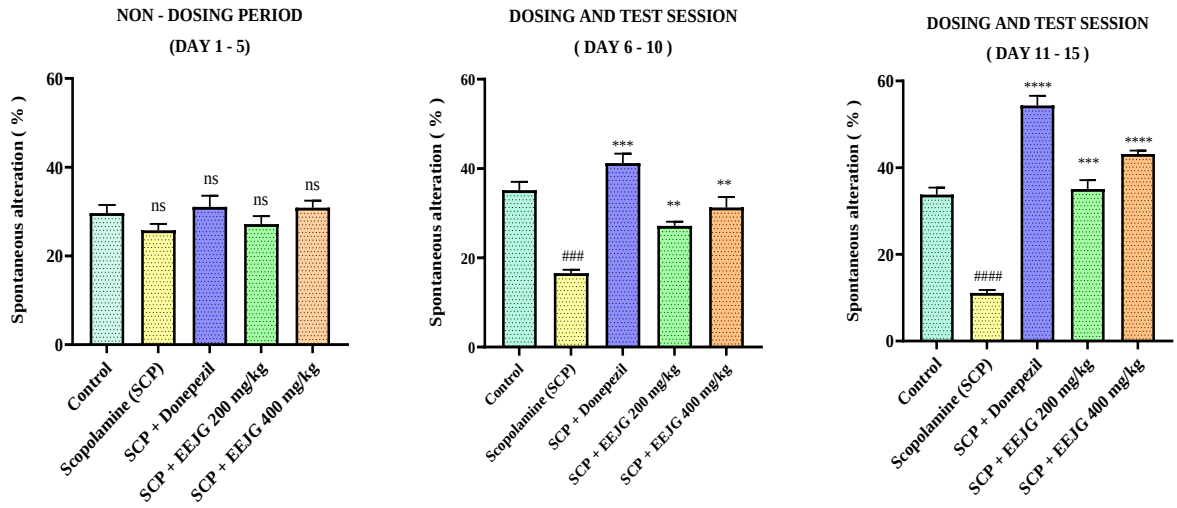

Fig. 4 Graphical Representation of effect of EEJG on spontaneous alteration (%)

3.3.2. BIOCHEMICAL ESTIMATION:

Table 5 Effect of EEJG on Malondialdehyde level

Groups	**Induction/Drug treatment**	**Malondialdehyde(nmoles/min/mg)**
Group 1	Normal control Saline (1 ml/kg)	1.14 ± 0.021
Group 2	Negative control Scopolamine (SCP)(1 mg/kg)	5.94 ± 0.03####
Group 3	Positive control SCP + Donepezil (2 mg/kg)	1.33 ± 0.02****
Group 4	Low dose groupSCP + EEJG (200 mg/kg)	3.98 ± 0.01***
Group 5	High dose groupSCP + EEJG (400 mg/kg)	3.67 ± 0.01***

All values are expressed in Mean ± SEM, n = 6.

Data were analysed by one-way ANOVA followed by Multiple Dunnett's multiple comparison test.

***p<0.001, ****p<0.0001 compared with Negative control

####p<0.0001 compared with Normal control

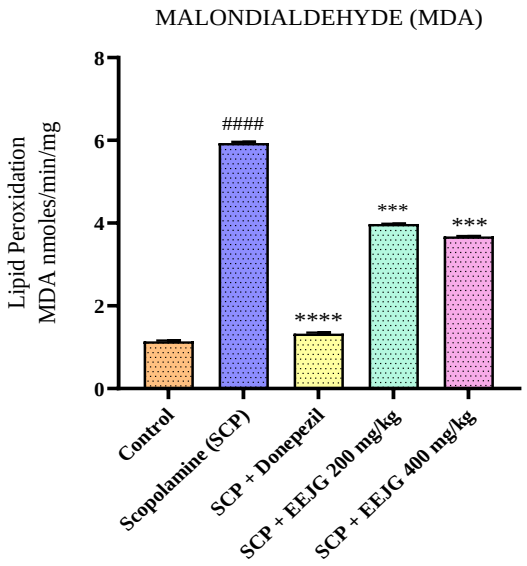

Fig. 5 Effect of EEJG on Malondialdehyde level in homogenized mice brain

3.4. HISTOPATHOLOGICAL EXAMINATION

The histopathological examination was performed in different groups using hematoxylin and eosin stain and is shown in Figure 6.

Histopathological examination of mice brain revealed sections from hippocampus showed normal neurons (N), neurofilaments (NF) with glial cells and normal blood vessels (BV) in normal control group. Sections form hippocampus showed degenerative changes in the neurons (N*) and reduction in neuronal count, neurofilament (NF) is highly fibrillary and there is mild vascular proliferation (BV) noted in negative control group. Sections from hippocampus showed normal neurons (N), neurofilaments (NF) with glial cells and normal blood vessels (BV) in positive control group. Sections from hippocampus showed normal neurons (N), and normal blood vessels (BV) and neurofilaments (NF) show mild fibrillary is noted in low dose group. Sections from hippocampus showed normal neurons (N), neurofilaments (NF) showed mild degeneration and normal blood vessels (BV) is noted in high dose group.

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Fig. 8a Normal control

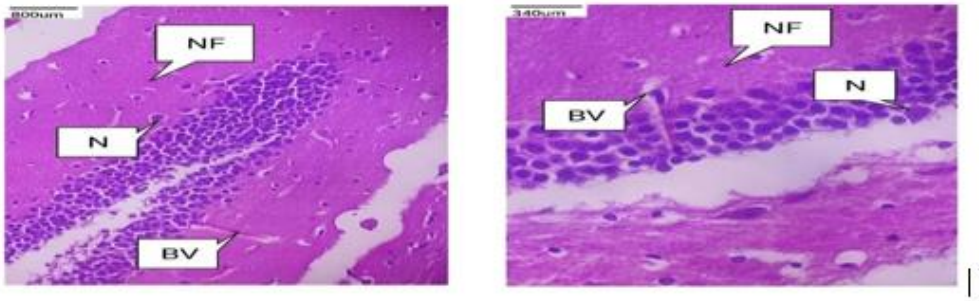
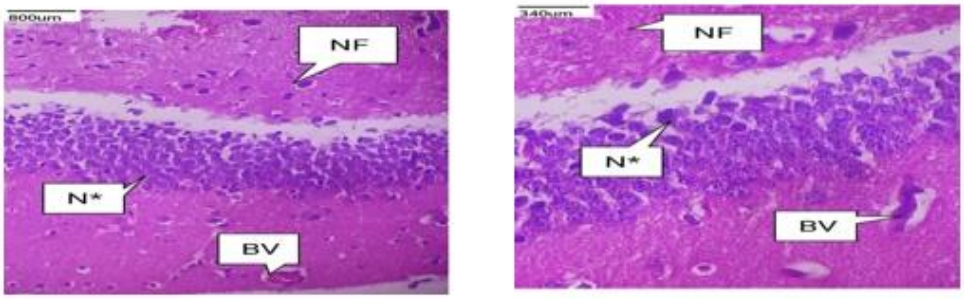


Fig. 8b Negative control



100 X 400X

Fig. 8c Positive control

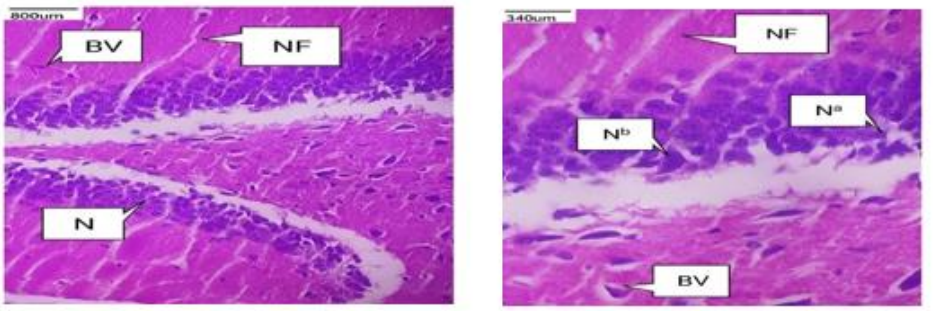


Fig. 8d Low dose group

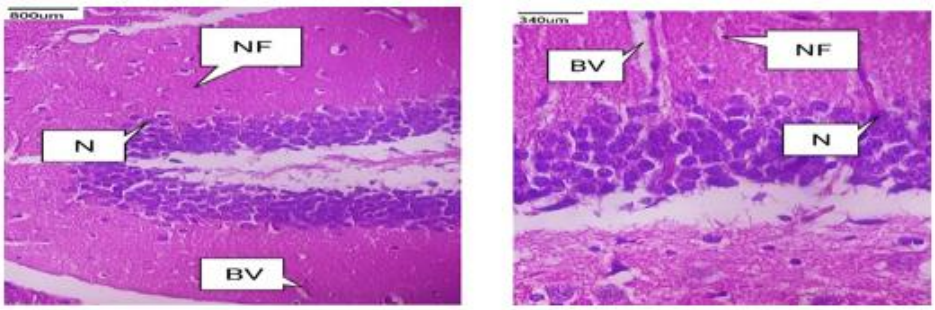
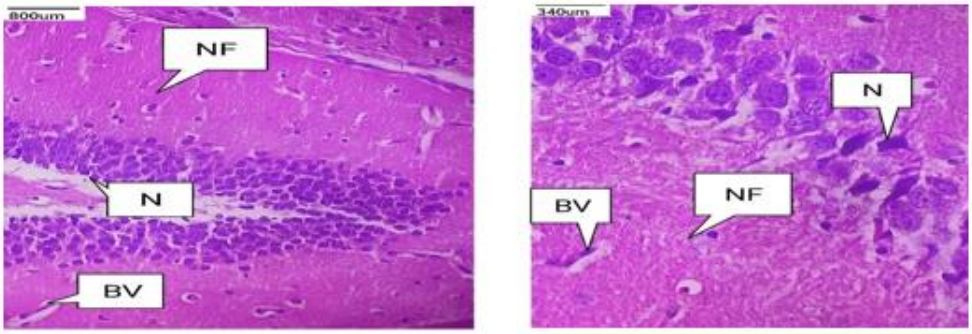


Fig. 8e High dose group



DISCUSSION

The phytochemical screening of EEJG showed important metabolites such as Flavanoids, tannins which possess anti-oxidant property which may aids in reducing oxidative stress and sterols which may play a role in reducing inflammation. *Invitro* enzyme inhibition studies (AChE inhibition assay) reported that the EEJG possessed good half maximal inhibitory concentration values for AChE was 52.61 µg/ml when compared to the standard drug Donepezil 32.26 µg/ml. The *invitro* enzyme inhibitory studies suggested that the extract has good inhibition property of inhibiting acetylcholinesterase enzyme. In order to select dose for administering extract to mice, acute oral toxicity 423 was performed and it was shown that no signs of morbidity and mortality. There was no significant difference in escape latency time among all groups during training period. The escape latency time was significantly increased for negative control group when compared to Normal group. The animals treated with EEJG showed significant decrease in escape latency time when compared with Negative control which shows that EEJG improved its memory. There was no significant difference in spontaneous alternation percentage among all the groups during non - dosing period. The animals EEJG showed significant increase in spontaneous alternation percentage when compared with Negative control group which shows EEJG improved in memory impairment. The MDA level was significantly increased in Negative control group. Groups treated with EEJG showed significant decrease in MDA levels compared to negative control group. This shows that the extract reduced the MDA level by decreasing oxidative stress due to its anti-oxidant and sterol content which was confirmed by phytochemical analysis. Then the study was further confirmed by Histopathological examination of Hippocampus of mice brain. Groups treated with EEJG showed mild neurodegeneration when compared with negative control group. This reveals that the EEJG played a role in improvement of disease.

CONCLUSION

From the study, it is concluded that the EEJG possesses a beneficial effect against scopolamine induced memory impairment proved by the valid data obtained from the *invitro* and *invivo* evaluation which includes phytochemical screening, biochemical parameters, histopathological examination. Further research and clinical trials are crucial to validate the mechanism of action, efficacy, and safety of a particular intervention or treatment in human subjects. Validating the mechanism of action involves understanding how the treatment works at a biological or physiological level. These trials will provide crucial evidence to support the adoption of new treatments, ensure patient safety and improve overall healthcare outcomes.

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