Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Memory enhancing potential of Euphorbia prostrata through antioxidant, anti-inflammatory, and anti acetylcholinesterase effect against Scopolamine -induced Alzheimer's disease in Wistar albino rats

Nirmala Kumari YADAV [1,2], Rakesh YADAV [3]*

[1] Department of Pharmacy, Banasthali Vidyapith Banasthali, Rajasthan-304022, India.
[2] Department of Pharmaceutical Sciences, Indra Gandhi University, Meerpur Rewari, Haryana, India
[3] School of Pharmacy, National Forensic Sciences University, Gandhinagar, Gujarat Tripura Campus, Agartala-799001, Tripura, India

* Corresponding Author. E-mail: ryadav122413@gmail.com; rakesh_pu@yahoo.co.in Tel. +91-946-764 24 20.

Received: 20 August 2022 / Revised: 26 December 2022 / Accepted: 27 December 2022

ABSTRACT: Alzheimer's disease (AD) is an irreversible multi-factorial disease that marks the most common neuro-degenerative disorder in the elderly population having cognitive decline as a primary clinical attribute. The present study pursued the evaluation of *Euphorbia prostrata* (*E. prostrata*) against scopolamine-induced Alzheimer's disease in Wistar albino rats. Total five groups of animals (having 90 rats) were included in the present study to access the therapeutic impact of *E. prostrata*. Morris water maze, radial arm maze, and elevated plus maze were evaluated for estimating learning and memory activity. The diverse parameters including oxidative stress, inflammatory cytokines, and acetylcholinesterase assay were assessed to estimate the mechanism of action. The consequences of the present investigation revealed the development of experimental dementia by administration of scopolamine. Whereas the treatment of *E. prostrata* (100 and 200 mg/kg, per oral (p.o.) and donepezil (1 mg/kg, p.o.) significantly improved the learning and memory ability in scopolamine treated rats. Incomparable to donepezil, the treatment of a higher dose of *E. prostrata* (200 mg/kg, p.o.) was more effective than compared to the low dose of *E. prostrata* (100 mg/kg, p.o.). Treatment of donepezil and a higher dose of *E. prostrata* (200 mg/kg, p.o.) produced comparable results for anti-inflammatory, anti-oxidative, and anti-acetylcholinesterase activity. The outcomes of the present investigation showed the memory-enhancing activity of *E. prostrata* (100 and 200 mg/kg, p.o.) against scopolamine-induced amnesia in rats. This effect of *E. prostrata* may be due to the inhibition of brain acetylcholinesterase activity, through the involvement of an anti-inflammatory pathway and due to its antioxidant potential.

KEYWORDS: *Euphorbia prostrata*; Euphorbiaceae; Alzheimer's Disease; Oxidative stress; Inflammatory cytokines; Acetylcholinesterase activity.

1. INTRODUCTION

Memory plays a dynamic role in creating a stable sense of self besides guiding the behaviors or facilitating social interaction. It is a psychological process that aids in preserving and recovering all information. In humans, memory loss at an older age is a characteristic feature of Alzheimer's disease (AD) [1,2]. According to an epidemiological report approximately 50 million people are affected with AD globally and are expected to rise exponentially and reach about 120 million by 2050. It has also been reported as the 6th major reason for mortality [3]. Pathology of AD is simple superficially but complex deceptively. AD is marked by degenerative variations in the neuro-transmission system that results in the accumulation of B-amyloid and neuro-fibrillary tangles and faulty mitochondrial, biochemical, and molecular abnormalities [4]. The affected population suffers from progressive cognitive disorders, the tremendous decline in memory combined with behavioral and neuro-psychiatric manifestations [5]. Although the etiology of the disease is poorly understood, age, head injuries, hypertension, depression, and vascular deficits are the most common risk factors associated with the disease [6]. Conversely, there have been many therapeutic strategies suggested that include several combination therapies and monotherapies, but all this fail to effectively combat the malignancy [2,7–9]. Numerous pro cholinergic agents have been employed for the treatment, some of them

How to cite this article: Yadav NK, Yadav R. Memory enhancing potential of Euphorbia prostrata through antioxidant, anti-inflammatory, and anti acetylcholinesterase effect against Scopolamine -induced Alzheimer's disease in Wistar albino rats. J Res Pharm. 2023; 27(3): 1004-1014.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.394

include rivastigmine, tacrine, donepezil, and metrifonate, but these also show several adverse effects [7,10]. Furthermore, herbal drugs have shown promising results in managing the prognosis of numerous fatal diseases. Diverse genera of Euphorbia have been investigated for their possible therapeutic action against different pathological conditions including neurological diseases [11]. Of which, some of them remain under the bridge such as *Euphorbia prostrata* (*E. prostrata*). *E. prostrata* is an annual herb belonging to the Euphorbiaceae family and is found lavishly in India and Africa [11,12]. The different parts of this herb have been reported to have numerous pharmaceutically active constituents including phytosterols, flavonoids, polyphenols, and phenolic compounds [11,12]. Contemporary research reveals that phytoconstituents of the plant endorse the numerous conventional therapeutic uses of *E. prostrata* against warts, gonorrhea, skin infections, migraines, parasitic infection, and viral diseases along with its previously reported therapeutic actions such as anthelmintic activity [13], anticandidal activity [14], analgesic [15], wound healing [16], antioxidant [12], insecticidal activity, antihyperglycemic activity, hypolipidemic effect [17], and antitumor activity [18]. Despite such a diverse pharmacological arena, the plausible therapeutic action in neurodegenerative disorders by endorsing traditional therapies including phytoconstituents and homeopathy, the underlying molecular mechanism is still a challenge now a days [19]. The phytoconstituents in *E. prostrata* have a massive opportunity to interact with numerous pathophysiological cascades and could lead to an advancement in the health system. However, the missing link of molecular interaction of *E. prostrata* with the biological system limits its therapeutic usage and lacks the confirmation of an accountable molecular pathway as a preferred therapeutic approach [20,21]. Hence, this study is a novel attempt to decipher the therapeutic impact with plausible molecular interaction of *E. prostrata* against AD.

2. RESULTS

2.1 Effect of *E. prostrata* on escape latency (EL) and time spent in the target quadrant (TSTQ) of rats using Morris water maze

E. prostrata (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) significantly decreased EL of rats on the 8th day and increased TSTQ by rats on the 8th day as compared to the control group, thus showed significant improvement of learning and memory. Scopolamine (1 mg/kg, i.p.) significantly increased EL and decreased TSTQ in rats, indicating their amnesic effects (Figure 1A and 1B). The low dose of *E. prostrata* (100 mg/kg, p.o.) did not significantly decrease EL or increase TSTQ as compared to Scopolamine treated group. *E. prostrata* (200 mg/kg, p.o.) significantly reversed scopolamine-induced learning and memory impairment in rats as compared to respective scopolamine-treated groups (Figure 1A and 1B).

2.2 Effect of *E. prostrata* on working memory error, reference memory error, and retrieval latency of rats using radial arm maze

A radial arm was used to access working memory error, reference memory error, and retrieval latency which estimate the therapeutic potential of *E. prostrata* (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) against scopolamine (1 mg/kg, i.p.) induced dementia. Working memory errors, reference memory errors, and retrieval latency were significantly increased in scopolamine-treated animals. Whereas the treatment of low and high doses of *E. prostrata* (100 mg/kg, p.o.) produced significant reductions in working memory error, reference memory error, and retrieval latency respectively (Figure 1C, 1D, and 1E). Although, the low dose *E. prostrata* extract (100 mg/kg, p.o.) produces a moderate reduction of working memory error, reference memory error, and retrieval latency time. Further, the consequences describe the comparable potency of *E. prostrata* extract (200 mg/kg, p.o.) with Donepezil (1 mg/kg, p.o.).

2.3 Effect of *E. prostrata* on retrieval transfer latency of rats using the elevated plus maze

E. prostrata (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) administered for 7 successive days did not significantly affect the retrieval TL of rats on the 7th day (learning) as compared to the control group. But *E. prostrata* (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) significantly decreased retrieval TL in rats on the 8th day (memory) as compared to the control group, thus showing significant memory enhancing activity (Figure 1F). The low dose of *E. prostrata* (100 mg/kg, p.o.) moderately reduced retrieval TL of rats on the 8th day as compared to Donepezil (1 mg/kg, p.o.) treated group. Scopolamine (1 mg/kg, i.p.) significantly increased retrieval TL in rats, indicating its amnesic effect (Figure 1F). *E. prostrata* (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) significantly reversed scopolamine-induced memory impairment in rats.

http://dx.doi.org/10.29228/jrp.394
J Res Pharm 2023; 27(3): 1004-1014

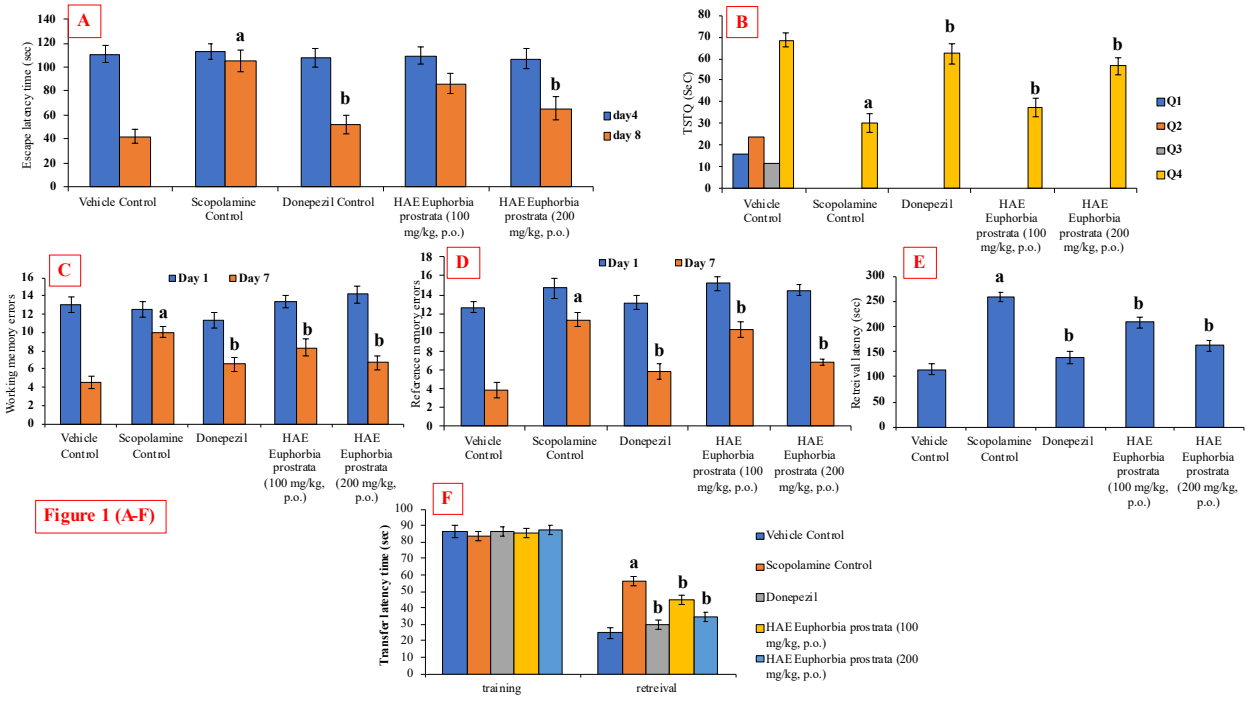


Figure 1 (A-F)

Figure 1: The figure describes the effect of *E. prostrata* on cognitive paradigms by measuring: escape latency (EL) as shown in [A]; time spent in target quadrant (TSTQ) as shown in [B] using Morris water maze; working memory error as shown in [C]; reference memory error as shown in [D]; retrieval latency of rats using radial arm maze as shown in [E]; and retrieval transfer latency of rats as shown in [F] using elevated plus maze. All values were expressed as mean ± SD whereas, 'a' represents $p < 0.001$ vs Normal Control; 'b' represents $p < 0.001$ vs disease control (Scopolamine control) group.

2.4 Effect of *E. prostrata* on oxidative stress of rats

The oxidative stress in rats having diverse interventions was measured by accessing SOD, CAT, GSH, and TBARS concentrations in brain tissue. Levels of SOD, CAT, and GSH were significantly decreased in Scopolamine (1 mg/kg, i.p.) treatment as compared to normal control. The treatment of *E. prostrata* (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) significantly upsurge SOD, CAT, and GSH levels. Although the increment in low dose E. *prostrata* (100 mg/kg, p.o.) was moderate as compared to Donepezil (1 mg/kg, p.o.), however, the consequences of *E. prostrata* (200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) were comparable that indicate the therapeutic potential of E. prostrata (200 mg/kg, p.o.) against Donepezil (1 mg/kg, p.o.) for oxidative stress level (Figure 2A, 2B and 2C). Furthermore, the increased level of TBARs was estimated in Scopolamine (1 mg/kg, i.p.) treated group as compared to the normal control. The treatment of *E. prostrata* (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) significantly reduced TBARs level as compared to the disease control group. The observation of a reduction in TBARs was dose-dependent and a higher dose of *E. prostrata* (200 mg/kg, p.o.) showed comparable TBARs level with Donepezil (1 mg/kg, p.o.) treatment (Figure 2D).

2.5 Effect of *E. prostrata* on inflammatory cytokines of rats

The anti-inflammatory potential of *E. prostrata* was measured by accessing inflammatory cytokines. The concentration of inflammatory biomarkers (IL-6 and TNF-α) was significantly raised in Scopolamine (1 mg/kg, i.p.) treated animals as compared to animals in the normal control group. Treatment of *E. prostrata* (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) significantly diminished the increased level of IL-6 and TNF-α in Scopolamine (1 mg/kg, i.p.) treated animals (Figure 3A and 3B). This reduction of inflammatory cytokines was dose-dependent as the treatment of higher dose *E. prostrata* (200 mg/kg, p.o.) was greater than the reduction of inflammatory cytokines by low dose *E. prostrata* (100 mg/kg, p.o.) as shown in figure 3A and 3B respectively.

2.6 Effect of *E. prostrata* on Brain Acetyl Cholinesterase (AChE) Activity in Rats

Scopolamine (1 mg/kg, i.p.) administration significantly increased AChE activity as compared to normal control. Treatment of *E. prostrata* (100 and 200 mg/kg, p.o.) and Donepezil (1 mg/kg, p.o.) for 7 consecutive days produced a significant decrease in brain AChE activity as compared to Scopolamine (1 mg/kg, i.p.). The

http://dx.doi.org/10.29228/jrp.394
J Res Pharm 2023; 27(3): 1004-1014

low dose of *E. prostrata* (100 mg/kg, p.o.) did not produce a significant decrease in AChE activity as compared to the standard control group (Donepezil (1 mg/kg, p.o.)) (Figure 4). Conversely, the reduction of AChE activity by *E. prostrata* (200 mg/kg, p.o.) was comparable to Donepezil (1 mg/kg, p.o.) treatment.

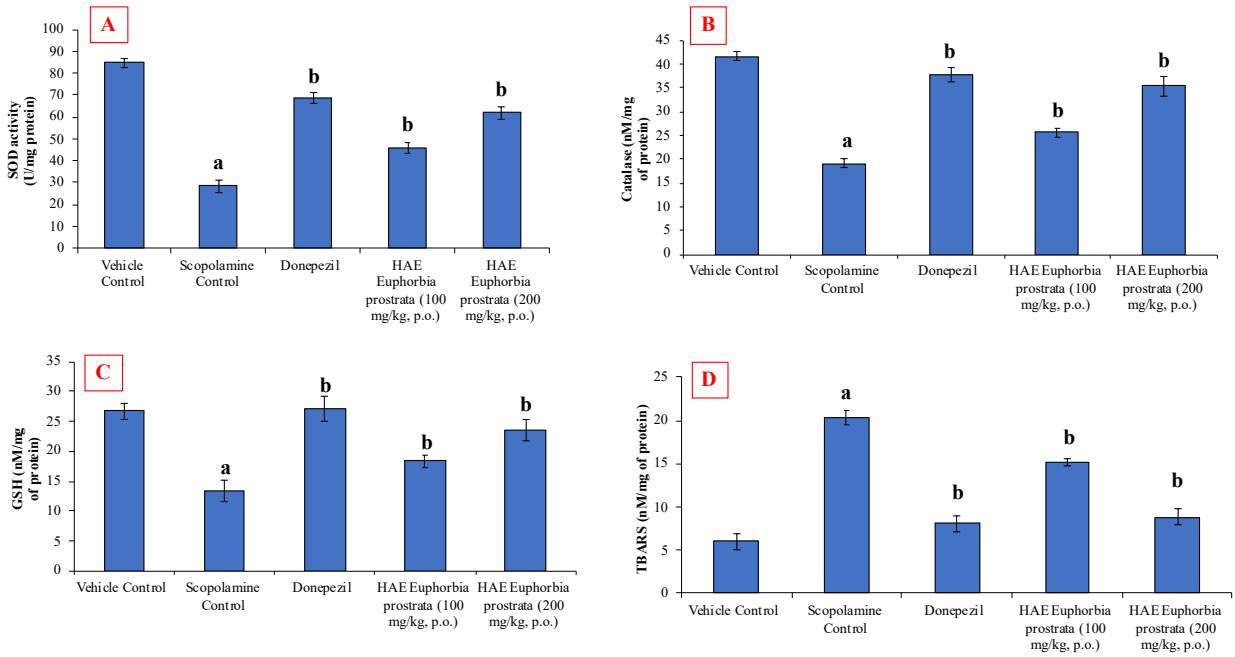


Figure 2 (A-D)

Figure 2: The figure describes the effect of *E. prostrata* on brain oxidative stress of rats by measuring: SOD activity as shown in (A), CAT activity as shown in (B), GSH level as shown in (C), and TBARS as shown in (D). All values were expressed as mean ± SD whereas, 'a' represents $p < 0.001$ vs Normal Control; 'b' represents $p < 0.001$ vs disease control (Scopolamine control) group.

2.7 Effect of *E. prostrata* on brain histology

Histological sections of brain tissue of respective groups showed diverse structural variations in the histology of small pyramidal cells of the hippocampus (CA1) region with vesicular nuclei. Section of the normal control group showed granule cells of the dentate gyrus and glial cells. The brain histology of scopolamine (1 mg/kg, i.p.) administered to rats showed vacuolization and cell death. The predominance of granule cells of the dentate gyrus and glial cells was significantly reduced in the scopolamine (1 mg/kg, i.p.) treated group. The 7 days of consecutive treatment of donepezil (1 mg/kg, p.o.), and *E. prostrata* (100 and 200 mg/kg, p.o.) significantly reversed pathological changes as compared to the histology of scopolamine (1 mg/kg, i.p.) treated group. The structural arrangements of brain tissue of animals having a higher dose of *E. prostrata* (200 mg/kg, p.o.) was comparable with the animal tissue of donepezil (1 mg/kg, p.o.) treatment (Figure 5).

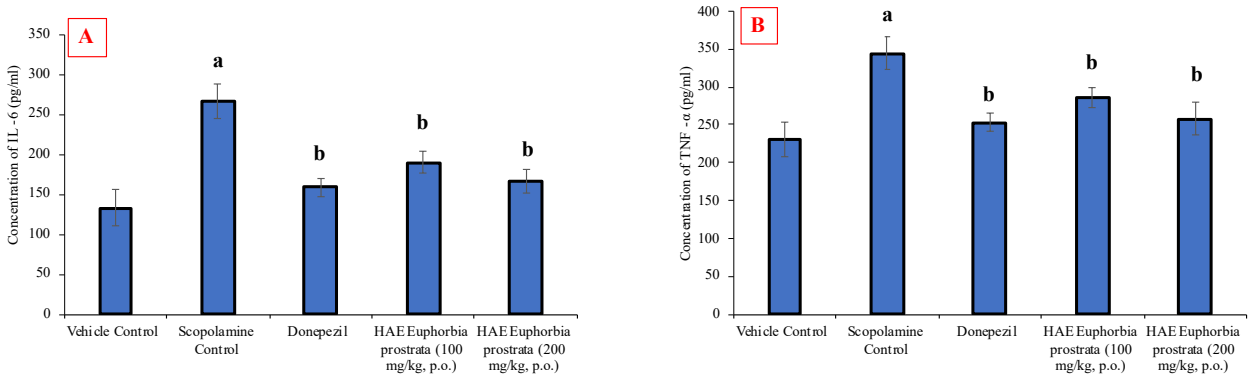


Figure 3 (A-B)

http://dx.doi.org/10.29228/jrp.394
J Res Pharm 2023; 27(3): 1004-1014

Figure 3: Figure describes the effect of *E. prostrata* on serum inflammatory cytokines of rats by measuring: IL-6 (A) and TNF-α (B). All values were expressed as mean ± SD whereas, 'a' represents $p < 0.001$ vs Normal Control; 'b' represents $p < 0.001$ vs disease control (Scopolamine control) group.

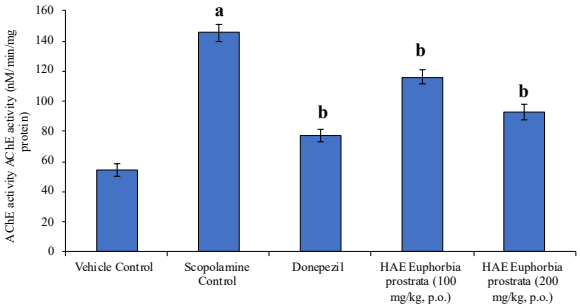


Figure 4

Figure 4: Figure describes the effect of E. prostrata on brain acetyl cholinesterase (AChE) activity in rats. All values were expressed as mean ± SD whereas, 'a' represents $p < 0.001$ vs Normal Control; 'b' represents $p < 0.001$ vs disease control (Scopolamine control) group.

3. DISCUSSION

Alzheimer's disease (AD) is a common progressive neurodegenerative disorder at an older age which gradually increased worldwide. One of the initial symptoms of AD and other dementias is frequently memory loss that interferes with everyday activities, such as getting lost in a familiar location or having trouble doing familiar duties at home, as well as diminished or poor judgement as well as mood or behaviour changes. Although numerous pharmacological interventions are available, have limited therapeutic impact [3]. *E. prostrata* is an annual herb belonging to the Euphorbiaceae family and is found lavishly in India and Africa [11]. The different parts of this herb have been described to keep the numerous pharmaceutically active constituents including phytosterols (cholesterol, stigmasterol, campesterol, beta-sitosterol), flavonoids (Apigenin, apigenin-7-glucoside, luteolin, luteolin-7-glucoside), polyphenols (ellagic acid, tannic acid), and phenolic compound (gallic acid) [11,12]. Although the growing evidence unveils the promising pharmacological influence of *E. prostrata*, the plausible therapeutic effect against AD with molecular mechanism is uncertain. In the present study, *E. prostrata* (100 and 200 mg/kg, p.o.) administered for 7 successive days showed a significant memory-improving effect in rats. This is the first report that shows memory enhancing potential of *E. prostrata* in rats. Behavioral models like Morris water maze, radial arm maze, and elevated plus maze were employed in the current study to investigate learning and memory. These models are extensively used for assessing the drug potential on learning and memory [24–26]. The consequences of the Morris water maze model revealed that treatment of *E. prostrata* leads to a significant reduction in escape latency during training and an increase in time spent in the target quadrant during retrieval showed an increase in learning and memory respectively; and vice versa. The existing evidence suggest strong correlations between the Morris water maze test and hippocampus synaptic plasticity [24]. In the radial arm maze, a considerable decrease in working memory error, reference memory error, and retrieval

http://dx.doi.org/10.29228/jrp.394
J Res Pharm 2023; 27(3): 1004-1014

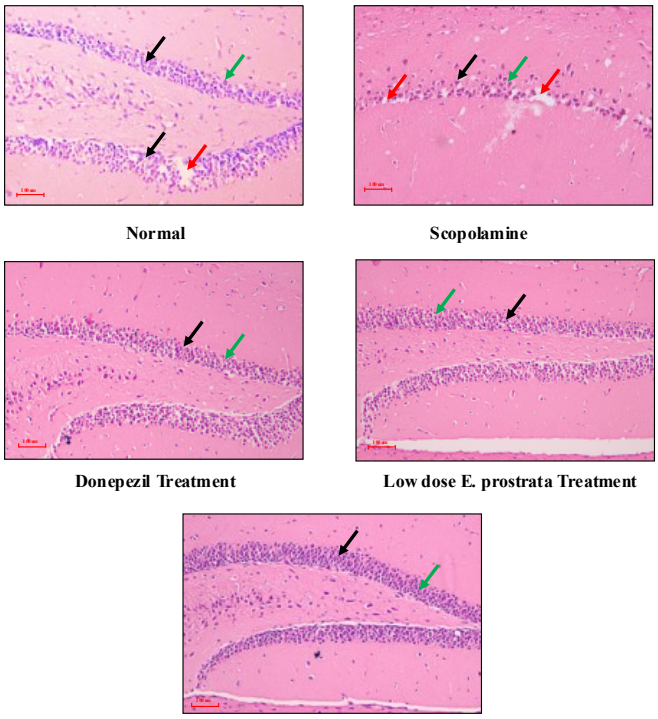


Figure 5: Figure showed the effect of E. prostrata on brain histology by identifying the small pyramidal cells of the hippocampus (CA1) region with vesicular nuclei in different groups of treatments using an inverted microscope (Cosmo Laboratory Equipment). The black arrow shows granule cells of the dentate gyrus. The red arrow shows vacuolization and cell death, and the green arrow shows glial cells.

Figure 5

latency was observed by *E. prostrata* treatment that signifies the recovery of learning and memory respectively in Scopolamine (1 mg/kg, i.p.) treated rats. These effects of the radial maze provide more proof that there may be a considerable recovery in brain regions, which may increase the outputs of these systems and increase competition for behavioural control. Moreover, the outcomes of the elevated plus maze model also confirmed a significant decrease in retrieval transfer latency which indicated improvement of memory. Although out of the two effective doses of *E. prostrata* (100 and 200 mg/kg, p.o.), the higher dose (200 mg/kg) produced significantly better memory-enhancing potential in Scopolamine (1 mg/kg, i.p.) treated rats as compared to the lower dose (100 mg/kg) treatment in all behavioral models, hence the memory enhancing the potential of *E. prostrata* probably dose dependant. AD is also described by the gradual formation of insoluble amyloid plaques and the deposition of amyloid beta-peptide in the brain [27]. Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) is a potent drug target for the treatment of AD as prolonged inhibition of BACE1 restricts the structural and functional synaptic plasticity in rats (altering the metabolism of BACE1 substrates) [28,29]. The memory-boosting action of *E. prostrata* is also supported by its BACE 1 inhibiting property. Although the over production of reactive oxygen species itself the root cause of diverse pathological conditions including neuronal diseases [26,30,31]. Oxidative stress can significantly affect amyloid-beta generation in the pathogenesis of AD through upregulation of BACE 1 gene transcription by oxidative stress [32]. Conversely, *E. prostrata* showed significant antioxidant activity as reported by the consequences of the present study. Thus, *E. prostrata* induced significantly enhancing memory in rats may be due to its antioxidant potential and resulting in the protection of vulnerable neuronal cells from oxidative stress. Moreover, the study also finds that inflammation in the brain cells leads to the progression from the presence of amyloid plaque and tau tangles to the onset of dementia and AD [33,34]. The constant stimulation of the brain's macrophages (microglia) may serve as a link in the progression of the pathogenesis of AD by exacerbating amyloid and tau proteins [34]. Some studies already revealed the anti-inflammatory potential of *E. prostrata* [35–37]. The consequences of the present study showed the significant anti-inflammatory potential of *E. prostrata* measured by the reduced level of inflammatory cytokines (IL-6 and TNF-α) in Scopolamine (1 mg/kg, i.p.) treated rats. Furthermore, the central cholinergic system also plays a foremost role in the management of the cognitive function. Acetylcholine is an important neurohumoral transmitter that regulates cognitive functions, whereas acetylcholinesterase is responsible for the breakdown the acetylcholine in the synapse. Impaired cholinergic transmission due to hyperactivation of acetylcholinesterase can leads to cognitive dysfunction and senile dementia [38,39]. Thus, the drugs which can improve cholinergic function either by diminishing

http://dx.doi.org/10.29228/jrp.394
J Res Pharm 2023; 27(3): 1004-1014

acetylcholinesterase levels or by increasing cholinergic transmission could be a promising treatment for dementia and AD. The administration of donepezil (1 mg/kg, p.o.) for 7 successive days significantly improved the memory of rats in the present study. Donepezil is widely used and reported for its memory enhancement activity. The therapeutic effect of *E. prostrata* (100 and 200 mg/kg, p.o.) was studied in comparison to donepezil. Muscarinic receptor antagonist like scopolamine diminishes cholinergic function and produce amnesia in laboratory animals [40]. The present study showed similar results and produced significant impairment in memory of rats measured by various behavioral models and increased level of AChE. The administration of *E. prostrata* (100 and 200 mg/kg, p.o.) for 7 successive days significantly reversed scopolamine-induced amnesia in rats. This reversal of scopolamine-induced amnesia by *E. prostrata* indicated the possible facilitation of cholinergic transmission. Treatment of *E. prostrata* (100 and 200 mg/kg, p.o.) also significantly lessened brain AChE activity in scopolamine (1 mg/kg, i.p.) treated rats as compared to the diseases group. These consequences suggest the memory-enhancing potential of the effect of *E. prostrata* which may be triggered by an upsurge level of brain acetylcholine through antagonizing of AChE. Additionally, the histological study confirmed the pathophysiological changes in brain tissue with or without treatment. The predominance of granule cells of the dentate gyrus and glial cells was significantly upsurged in the issue section of treated rats as compared to scopolamine (1 mg/kg, i.p.) treated rats.

4. CONCLUSION

Conclusively, the outcomes of the present investigation showed the memory-enhancing activity of *E. prostrata* (100 and 200 mg/kg, p.o.) against scopolamine-induced amnesia in rats. This effect of *E. prostrata* may be due to the inhibition of brain acetylcholinesterase activity, through the involvement of an anti-inflammatory pathway, and due to its antioxidant potential.

5. MATERIALS AND METHODS

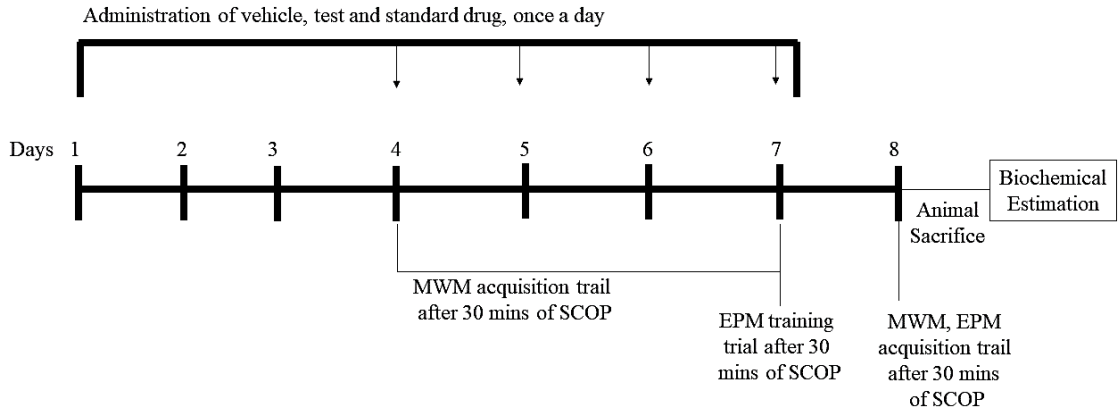
Male Wistar albino rats of 250-280 g were used in the present study. Experimentation on animals was approved by IAEC of Chitkara College of Pharmacy, Chandigarh, India, and CPCSEA, New Delhi, India (IAEC/CCP/22/01/PR-18). All animals were acclimatized for 15 days with access to food and water ad libitum, environmental conditions were maintained at temp 20 ± 2 ˚C, relative humidity 60 ± 10 %.

5.1 Experimental Design and animals

A total of 90 rats were divided into five groups including normal control, disease control, standard treatment, and drug treatment with low and high doses. All five groups were given various interventions daily for 7 days. Animals of normal control received normal saline 0.9% (10 ml/kg intraperitonially (i.p) for 7 days). Scopolamine (1 mg/kg i.p.) was given to induce dementia. Donepezil (1 mg/kg i.p.) was used as standard treatment whereas low (100 mg/kg i.p.) [22] and high doses (200 mg/kg i.p) [23] of hydroalcoholic extract of test drug (HAE *E. prostrata*) were given as test drugs against diseases and standard treatment. Scopolamine (1 mg/kg i.p.) was administered in groups II, III, IV, and V. In groups III, IV, and V, it was administered after 30 minutes of intervention administration from Day 4 to Day 7. The cognitive paradigms were evaluated 30 minutes after scopolamine administration from Day 4 to Day 7. On day 8, retention tests were performed followed by biochemical estimations.

S.No.	Group Name	Interventions
1.	Normal Control	Normal Saline 0.9% (10 ml/kg i.p.) (for 7 days)
2.	Disease Control	Scopolamine (1 mg/kg i.p.)
3.	Standard	Donepezil (1 mg/kg per oral (p.o) + Scopolamine (1 mg/kg i.p.)
4.	Treatment 1	*E. prostrata* (100 mg/kg p.o.) + Scopolamine (1 mg/kg i.p.)
5.	Treatment 2	*E. prostrata* (200 mg/kg p.o.) + Scopolamine (1 mg/kg i.p.)

http://dx.doi.org/10.29228/jrp.394
J Res Pharm 2023; 27(3): 1004-1014



5.2 Morris water maze (MWM) test

A circular pool having a radius of 110 cm and a height of 52 cm, divided into four quadrants was used. An elevated platform is placed centrally to be located by the rats. Water was made misty by using talc. A maximum time of 120 sec was considered as the cut-off time. Now, the time taken by each rat of a different group to locate the elevated platform is noted. This is escape latency time. Also, the time spent in each quadrant was recorded.

5.3 Radial arm maze (RAM) test

A set up of eight wooden arms, connected centrally with food items placed in alternate arms was used. Working memory of each animal is tested by their entry into the food-containing arms. A complete entry was considered as the one, in which the rat has entered all four paws in the arm. The time spent on each arm was also observed. % Latency time was calculated.

5.4 Elevated plus maze (EPM) test

EPM test is a tool to measure anxiety & memory in lab animals. A four-armed wooden apparatus connected centrally is used. Two arms are open and two are closed with an elevated wooden block on opposite sides. Each animal is placed on a central platform and was allowed to explore for 90 seconds. The movement of animals in the open arm from the closed arm is taken as a sign of working memory.

5.5 Estimation of oxidative stress biomarkers

Animals were sacrificed after anesthetizing with isoflurane followed by cervical dislocation. The Animal's brain was carefully isolated and treated with normal saline. A 10% w/v homogenate tissue was prepared in 0.03M phosphate buffer (pH 7.4) by centrifuging this mixture for 15 minutes at 3000 g. The supernatant was collected for further biochemical estimation.

5.6 Estimation of superoxide dismutase (SOD)

0.1 ml of brain homogenate was added to a mixture of 2.8 ml of potassium phosphate buffer (0.1M, pH 7.4) & 0.1 ml of pyrogallol solution. The formed mixture was used to measure the absorbance at 325nm with the UV spectrophotometer.

5.7 Measurement of catalase activity (CAT)

0.05ml of tissue homogenate was added to 1ml of hydrogen peroxide (H2O2) and 1.95ml of phosphate buffer (50Mm, pH 7). The absorbance was recorded at 240nM by using this formula the catalase activity was determined.

$$Catalase\ activity = \frac{\delta OD}{E \times Vol\ of\ sample\ (ml) \times mg\ of\ protien}$$

Where $^{\delta}$OD= change in absorbance per minute
E= Extinction coefficient of hydrogen peroxide (0.071mmol cm^{-1}).

5.8 Measurement of malondialdehyde (MDA)

The solution of 3ml of thiobarbituric acid (TBA) reagent (which contained 5N HCL and trichloroacetic acid; TCA) was added to 1 ml of brain homogenate to make a reaction mixture. This mixture was heated for 15 minutes at 90^{0} C. After cooling the heated solution, it was centrifuged at 3500 g for 10 minutes. The pink-colored supernatant was used to measure the absorbance at 532nm.

http://dx.doi.org/10.29228/jrp.394
J Res Pharm 2023; 27(3): 1004-1014

The MDA level was calculated by the following equation

$$conc.of\ MDA = \frac{Ab_{532} \times 100 \times V_t}{(1.56 \times 10^5) \times W_t \times V_u}$$

Where Abs_{532}= absorbance, V_t= total mixture volume, 1.56×10^5= molar extinction coefficient, W_T= weight of the brain and V_u=aliquot volume.

5.9 Measurement of reduced glutathione (GSH) Level

The 1ml brain homogenate solution was mixed with the 1ml of 10% trichloroacetic acid and centrifuged at 2000 g for 10 minutes. The resultant supernatant was separated in which 2ml of phosphate buffer (pH 8.4) and 0.5ml of DTNB reagents were added and absorbance was measured at 412nm.

The equation used to measure the GSH level are as follow:

$$GSH\ Level = \frac{Y - 0.00314}{0.0314} \times \frac{D_F}{B_T} \frac{1}{\times V_U}$$

Here, y= absorbance at 412nm, DF= dilution factor BT= brain tissue homogenate volume (1ml), and VU= volume of aliquot (1ml)

5.10 Estimation of inflammatory biomarkers

The treated animals with diverse interventions were accessed for pro-inflammatory (IL-6, TNF-α) and anti-inflammatory (IL-10) cytokines. Inflammatory cytokines were estimated from the rat serum sample at the end of the protocol by performing an enzyme-linked immunosorbent assay (sandwiched ELISA kit, Ray Biotech, USA).

5.11 Estimation of acetylcholinesterase activity

100µl of DTNB, 2.6ml of phosphate buffer; 0.4ml tissue homogenate were mixed thoroughly. The absorbance was measured at 412nm by using a UV spectrophotometer. The stable reading was recorded as the basal reading. In this mixture of 20µl, acetylthiocholine iodide was added as the substrate to change the absorbance. Further, the change in absorbance was measured for 10 minutes at an interval of 2 minutes. The following formula helps in calculating the average change in absorbance and the activity of acetylcholinesterase (AChE) was expressed as µM/min/gm of tissue.

$$R = 5.74(10^{-4}) \times \frac{\Delta A}{C_o}$$

Here, R=rate in moles substrate hydrolysed per minutes per gram of tissue, ΔA= change in absorbance per minute, and C_o= original concentration of tissue in mg/ml.

5.12 Histological Assessment

Animals from each group were sacrificed on the 8th day and brain were isolated to for histological assessment. Sections of brain sample were prepared and stained with haematoxylin and eosin staining.

5.13 Statistical analysis

Data for all biochemical markers were expressed as mean ± SD and statistically analysed by one-way ANOVA followed by post-hoc Tukey's test using Sigma Plot version 11.0 (Systat Software, Inc., San Jose California USA).

Acknowledgements: The authors are thankful to Banasthali Vidyapith Banasthali, Rajasthan-304022, India for providing the financial support to conduct this study.

Author contributions: Concept – N.K.Y., R.Y.; Design – N.K.Y., R.Y.; Supervision – R.K.; Resources – N.K.Y., R.Y.; Materials – N.K.Y., R.Y.; Data Collection and/or Processing – N.K.Y., R.Y.; Analysis and/or Interpretation – N.K.Y., R.Y.; Literature Search – N.K.Y., R.Y.; Writing – N.K.Y., R.Y.; Critical Reviews – N.K.Y., R.Y.

Conflict of interest statement: The authors declared no conflict of interest.

http://dx.doi.org/10.29228/jrp.394
J Res Pharm 2023; 27(3): 1004-1014

REFERENCES

[1] Vanderveren E, Bijttebier P, Hermans D. The importance of memory specificity and memory coherence for the self: Linking two characteristics of autobiographical memory. Front Psychol. 2017; 8: 2250 https://doi.org/10.3389/fpsyg.2017.02250.
[2] Rajput SK, Sharma AK, Meena CL, Pant AB, Jain R, Sharma SS. Effect of L-pGlu-(1-benzyl)-l-His-l-Pro-NH 2 against in-vitro and in-vivo models of cerebral ischemia and associated neurological disorders. Biomed Pharmacother. 2016; 84: 1256–1265. https://doi.org/10.1016/j.biopha.2016.10.059.
[3] 2022 Alzheimer's disease facts and figures. Alzheimers Dement 2022; 18: 700–789. https://doi.org/10.1002/alz.12638.
[4] Rajmohan R, Reddy PH. Amyloid-Beta and Phosphorylated Tau Accumulations cause abnormalities at synapses of Alzheimer's disease neurons. J Alzheimers Dis. 2017;57: 975–999. https://doi.org/10.3233/jad-160612.
[5] Jellinger KA. Morphological basis of Parkinson disease-associated cognitive impairment: an update. J Neural Transm (Vienna). 2022;129(8): 977-999. https://doi.org/10.1007/s00702-022-02522-4.
[6] Brown RB. Parkinson's disease etiology: Insights and associations with phosphate toxicity. Int J Mol Sci. 2022;23(15):8060. https://doi.org/10.3390/ijms23158060.
[7] Chigurupati S, Abdul Rahman Alharbi N, Sharma AK, Alhowail A, Vardharajula VR, Vijayabalan S, Das S, Kauser F, Amin E. Pharmacological and pharmacognostical valuation of Canna indica leaves extract by quantifying safety profile and neuroprotective potential. Saudi J Biol Sci. 2021; 28(10):5579–5584. https://doi.org/10.1016/j.sjbs.2021.05.072.
[8] Malhotra B, Kulkarni GT, Dhiman N, Joshi DD, Chander S, Kharkwal A, Sharma AK, Kharkawal H. Recent advances on Berberis aristata emphasizing berberine alkaloid including phytochemistry, pharmacology and drug delivery system. J Herb Med. 2021; 27: 100433. https://doi.org/10.1016/j.hermed.2021.100433.
[9] Deep A, Narasimhan B, Aggarwal S, Kaushik D, K. Sharma A. Thiophene scaffold as prospective central nervous system agent: A review. Cent Nerv Syst Agents Med Chem. 2016; 16: 158–164. https://doi.org/10.2174/1871524916666160204114424
[10] Cholinergic Medications - PubMed n.d. https://pubmed.ncbi.nlm.nih.gov/30844190/ (accessed July 29, 2022).
[11] Pharmacognostical and phytochemical investigation of Euphorbia prostrata ait. Int J Pharm Sci Res. 2012;14:1043-1048. http://dx.doi.org/10.13040/IJPSR.0975-8232.3(4).1043-48.
[12] Sundara Prabha V, Antony Rayan SB. Antimicrobial and antioxidant activity of ethanolic extract of Euphorbia prostrata AIT leaves. Int J Innov Res Technol. 2018; 5(1):575-578. http://dx.doi.org/10.13140/RG.2.2.17293.87522.
[13] Sharma SK, Singh S, Singh J. Anthelmintic effect of Euphorbia prostrata Ait. extracts. Indian J Pharmacol. 2011 ;43: 743. https://doi.org/10.4103/0253-7613.89846.
[14] Tchuenguem RT, Kuiate J-R, Dzoyem JP. In vivo anticandidal activity of Euphorbia prostrata. J Complement Altern Med Res. 2017; 4: 1–10. https://doi.org/10.9734/JOCAMR/2017/38924.
[15] Biwott T, Kiprop A, Cherutoi J, Munyendo W, Biwott G. Analgesic properties of Euphorbia prostrata crude extracts. Sci J Chem. 1015; 3(6): 100-105. https://doi.org/10.11648/j.sjc.20150306.14.
[16] Patil TR, Limaye RP. Effect of Euphorbia prostrata on the wound healing in excisional wound model in rats. Int J Pharmacog Phytochem Res. 2017; 9: 1223–1226. https://doi.org/10.25258/PHYTO.V9I09.10310.
[17] Salehi B, Iriti M, Vitalini S, Antolak H, Pawlikowska E, Kręgiel D, Sharifi-Rad J, Oyeleye SI, Ademiluyi AO, Czopek K, Staniak M, Custódio L, Coy-Barrera E, Segura-Carretero A, Cádiz-Gurrea ML, Capasso R, Cho WC, Seca AML. Euphorbia-derived natural products with potential for use in health maintenance. Biomolecules 2019; 9: 337. https://doi.org/10.3390/BIOM9080337.
[18] Wang T, Si XQ, Zhou GL, Dai R, Zhou G, Cao D, Yang C. [In vivo anti-tumor effect and in vitro anti-angiogenic effect of alcohol extract from Euphorbia prostrata]. Zhongguo Zhong Yao Za Zhi. 2017; 42(9): 1722-1729. https://doi.org/10.19540/j.cnki.cjcmm.2017.0067.
[19] Sen S, Chakraborty R. 2020. Herbal medicine in India: indigenous knowledge, practice, innovation and its value. Singapore: Springer. https://public.ebookcentral.proquest.com/choice/publicfullrecord.aspx?p=5894516.
[20] Ahmed M, Shah AS, Ali Khan R, Ullaha Khan F, Aslam Khan N, Shah MS, Rashid Khan M. Antioxidant and antibacterial activity of crude methanolic extract of Euphorbia prostrata collected from District Bannu (Pakistan). Afr J Pharm Pharmacol. 2011; 5(8): 1175-1178. https://doi.org/10.5897/AJPP11.359.
[21] Gupta PJ. The efficacy of Euphorbia prostrata in early grades of symptomatic hemorrhoids--a pilot study. Eur Rev Med Pharmacol Sci. 2011;15(2):199-203.
[22] Bakhshi G, Langade D, Desai VS. Prospective, open label study of Euphorbia Prostrata extract 100 mg in the treatment of bleeding haemorrhoids. Bombay Hospital J. 2008; 50(4): 578-583.
[23] Singla AK, Pathak K. Anti-inflammatory studies on Euphorbia prostrata. J Ethnopharmacol 1989;27:55–61. https://doi.org/10.1016/0378-8741(89)90077-9.
[24] Mutlu O, Akar F, Celikyurt IK, Tanyeri P, Ulak G, Erden F. 7-NI and ODQ disturbs memory in the elevated plus maze, morris water maze, and radial arm maze tests in mice. Drug Target Insights. 2015;9:1. https://doi.org/10.4137/DTI.S23378.
[25] Almahozi A, Radhi M, Alzayer S, Kamal A. Effects of memantine in a mouse model of postoperative cognitive dysfunction. Behav Sci (Basel). 2019 Mar 6;9(3):24. https://doi.org/10.3390/bs9030024.
[26] Deeba F, Yar MS, Haidar MR, Sharma AK, Sharma M. Synthesis, molecular docking, and pharmacological evaluation of 5-(4-(2-(5-ethyl pyridine-2-yl) ethoxy) benzyl)-3-(phenylsulfonyl) thiazolidine-2, 4-dione against HFD-induced diabesity via interaction with the CB1 receptor. Iran J Basic Med Sci. 2022;25:1028–36. https://doi.org/10.22038/IJBMS.2022.65649.14443.

[27] Upadhyay A, Vassar RJ, Savas JN. Biochemical purification and proteomic characterization of amyloid fibril cores from the brain. J Vis Exp. 2022 Apr 28;(182):10.3791/63816. https://doi.org/10.3791/63816.
[28] Hampel H, Vassar R, De Strooper B, Hardy J, Willem M, Singh N, Zhou J, Yan R, Vanmechelen E, De Vos A, Nisticò R, Corbo M, Imbimbo BP, Streffer J, Voytyuk I, Timmers M, Tahami Monfared AA, Irizarry M, Albala B, Koyama A, Watanabe N, Kimura T, Yarenis L, Lista S, Kramer L, Vergallo A. The β-Secretase BACE1 in Alzheimer's Disease. Biol Psychiatry. 2021;89(8):745-756. https://doi.org/10.1016/j.biopsych.2020.02.001.
[29] Ohno M. Accelerated long-term forgetting is a BACE1 inhibitor-reversible incipient cognitive phenotype in Alzheimer's disease model mice. Neuropsychopharmacol Rep. 2021;41:255–259. https://doi.org/10.1002/NPR2.12174.
[30] Kaur N, Sharma AK, Shakeel A, Kumar V, Singh A, Gupta A, Suhag D, Rajput SK, Mukherjee M. Therapeutic Implications of Superoxide Dismutase And Its Importance in Kinase Drug Discovery. Curr Top Med Chem. 2017;17(22):2495-2508. https://doi.org/10.2174/1568026617666170307112837
[31] Sharma AK, Taneja G, Khanna D, Rajput SK. Reactive oxygen species: friend or foe? RSC Adv 2015;5:57267–57276. https://doi.org/10.1039/C5RA07927F.
[32] Camacho-Castillo L, Phillips-Farfán B v., Rosas-Mendoza G, Baires-López A, Toral-Ríos D, Campos-Peña V, Carvajal K. Increased oxidative stress contributes to enhance brain amyloidogenesis and blunts energy metabolism in sucrose-fed rat: effect of AMPK activation. Sci Rep 2021;11 (1): 19547. https://doi.org/10.1038/S41598-021-98983-W.
[33] Bello-Medina PC, Corona-Cervantes K, Zavala Torres NG, González A, Pérez-Morales M, González-Franco DA, Gómez A, García-Mena J, Díaz-Cintra S, Pacheco-López G. Chronic-Antibiotics Induced Gut Microbiota Dysbiosis Rescues Memory Impairment and Reduces β-Amyloid Aggregation in a Preclinical Alzheimer's Disease Model. Int J Mol Sci. 2022;23(15):8209. https://doi.org/10.3390/ijms23158209.
[34] Long HZ, Zhou ZW, Cheng Y, Luo HY, Li FJ, Xu SG, Gao LC. The Role of Microglia in Alzheimer's Disease From the Perspective of Immune Inflammation and Iron Metabolism. Front Aging Neurosci. 2022;14:888989. https://doi.org/10.3389/fnagi.2022.888989.
[35] Singla AK, Pathak K. Anti-inflammatory studies on Euphorbia prostrata. J Ethnopharmacol. 1989;27:55–61. https://doi.org/10.1016/0378-8741(89)90077-9.
[36] Singla AK, Pathak K. Topical antiinflammatory effects of Euphorbia prostrata on carrageenan-induced footpad oedema in mice. J Ethnopharmacol. 1990;29:291–294. https://doi.org/10.1016/0378-8741(90)90040-Z.
[37] Hariyadi DM, Sahu VK. Euphorbia prostata exerts potent anti-ınflammatory and anti-arthritic activity in downregulating the ıncreased expression of pro-ınflammatory cytokines. Pharmaceutical Sciences 2020;26:370–378. https://doi.org/10.34172/PS.2020.51.
[38] Burzynski HE, Macht VA, Woodruff JL, Crawford JN, Erichsen JM, Piroli GG, Grillo CA, Fadel JR, Reagan LP. Pyridostigmine bromide elicits progressive and chronic impairments in the cholinergic anti-inflammatory pathway in the prefrontal cortex and hippocampus of male rats. Neurobiol Stress. 2022;18:100446. https://doi.org/10.1016/j.ynstr.2022.100446.
[39] Boukholda K, Gargouri B, Aouey B, Attaai A, Elkodous MA, Najimi M, Fiebich BL, Bouchard M, Fetoui H. Subacute silica nanoparticle exposure induced oxidative stress and inflammation in rat hippocampus combined with disruption of cholinergic system and behavioral functions. NanoImpact. 2021;24:100358. https://doi.org/10.1016/j.impact.2021.100358
[40] Konar A, Gupta R, Shukla RK, Maloney B, Khanna VK, Wadhwa R, Lahiri DK, Thakur MK. M1 muscarinic receptor is a key target of neuroprotection, neuroregeneration and memory recovery by i-Extract from Withania somnifera. Sci Rep. 2019;9(1):13990. https://doi.org/10.1038/s41598-019-48238-6.