World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Potential lipid lowering effect of *Albizia procera* leaves in chronic stress induced alzheimer's disease in animal model

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World Journal of Biology Pharmacy and Health Sciences, 2025, 23(01), 381-389

Publication history: Received on 14 June 2025; revised on 20 July 2025; accepted on 22 July 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.23.1.0697

Abstract

About 70% of dementia cases have been identified to be caused by Alzheimer's disease (AD). It is a severe type of memory loss that causes problems with language, behaviour and memory. The incidence rate of sickness doubles after 65 years of age. Prolonged stress may have an impact on the body's physiological processes. The purpose of this work was to assess the antihyperlipidemic potential of *Albizia (A.) procera* leaves methanolic extract (MEt) in an animal model of AD produced by chronic stress. The plant is used as an antioxidant, analgesic, antibacterial, antidiarrheal, and antidiabetic medication. Chronic restrain stress caused learning and memory impairment over the course of 84 days with the use of MWM and EPM apparatus, hence this research aims to evaluate the impact of an oral *A. procera* leaves MEt (200 and 400 mg/kg) on learning and memory performance over an 84-days period. The profile of blood lipids was examined. The treated rats showed a decrease in entries and time spent in the closed arm and an increase in entries and time spent in the open arm in the modified EPM apparatus and in MWM, decrease in escape latency and an increase in retention time. The results demonstrated that administering *A. procera* leaves MEt enhanced learning and memory and restored abnormal lipid profile and also exhibits significant mild to moderate learning and memory enhancement activity along with antihyperlipidemic potential at both doses.

Keywords: *Albizia procera (A. procera);* Alzheimer's disease (AD); Learning and Memory enhancement activity; Hyperlipidaemia; Antihyperlipidemic activity

1. Introduction

Alzheimer's disease, or AD for short, is a long-term neurological illness. Around 70% of dementia cases have been identified to be caused by AD. Dr. Alois Alzheimer, a physiatrist and neuropathologist from Germany, is credited with being the first to describe a disorder known as memory loss. AD is a severe kind of memory loss that causes problems in language, memory, and behavior (Burns A. et.al, 2009; Indu bhusshan et. al, 2017).The buildup of tau protein and amyloid beta (Aβ) peptides cause's neurofibrillary tangles (NFTs) and the death of neuronal cells, which are hallmarks of AD. The primary reason of this aberrant protein accumulation, which damages neurons, is decreased clearance. The brain is deficient in glutamate and neuropeptides, as well as cholinergic. The human brain in a healthy state has a peptide of 39–43 amino acid residues known as Aβ. Because of cleavage from a bigger amyloid precursor protein, it exists as Aβ fibrils. The accumulation of amyloid fibrils as amyloid plaques in the extracellular space of brain cells, synaptic dysfunction, neuronal death, and inflammatory reactions are all closely linked in AD. Conversely, tau protein is widely distributed within certain spatial patterns throughout the central nervous system and is crucial for maintaining microtubule stability. The tau protein experiences severe hyper phosphorylation in AD pathology, which causes the tau protein to clump and form intracellular neurofibrillary tangles (NFTs). Axon degeneration, dendritic spinal collapse, and microtubule disintegration are caused by the intracellular creation of NFTs. A few mild symptoms of AD are mood swings, anxiety, memory loss, trouble managing finances, and poor decision-making. However,

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individuals with late-stage AD will have a lack of environmental responsiveness, disorientation, restlessness, problems with language and thinking as well as trouble controlling their movements (Kasturba Mahalingam et.al, 2018; K. D. Tripathi 2013).The main risk factor for AD is growing older. Age affects the incidence rate. After the age of 65 incidence rate of disease become double. Incidence rate of disease differs in the sex also. Women are more proven for the disease particularly after the age of 85. (Antonio Di Carlo et. al, 2002; F. Bermejo Pareja et.al, 2007) *A. procera* is rapid-growing subtropical and tropical trees belonging to family (Fabaceae) and subfamily (Mimosoideae), and the herb is widely used in the Asian traditional medicine as antioxidant, analgesic, antibacterial, Antidiarrheal and antidiabetic drug. (S. Sivakrishnan, 2019) *A. procera* bark is brown colour and leaves is green, has characteristic odour and taste is slightly bitter. (S. Sivakrishnan, 2019) *A. procera* leaves shows the presence of saponins, steroids, tannins, glycosides and flavonoids etc. (Asolkar et al, 1992; Rastogi and Mehrotra, 1993) Flavonoids were the main chemical constituents for the learning and memory enhancement activity. (Mst Mahfuza Khatoon *et.al*, 2014) Classification of flavonoids is flavanones, anthocyanidins, flavones, flavonols, isoflavonoids, and flavanols according to their chemical makeup. While targeting multiple targets, they are helpful in the management of neurodegenerative disorders like Alzheimer's disease and delay the process of neurodegeneration. Flavonoids are studied for their antioxidant and anti-inflammatory activities; both are important in induced the pathogenesis of AD. Flavonoids have a capability of crossing the blood–brain barrier (BBB) which makes them potential agents in preventing neurodegenerative disorders. Anti-Alzheimer's disease effects of certain flavonoids, such as apigenin, catechins, rutin, fisetin, quercetin, kaempferol, and myricetin have been reported. (Haroon Khan Et.al, 2019).Thus, the present study aims to investigate the learning and memory enhancement activity and restore abnormal lipid profiles in chronic stress induced AD by administration of methanolic extract of *A. procera* leaves in animal model.

2. Materials and methods

2.1. Animals

Healthy female Sprague-dawley rats 8 weeks old, weighing between 150-250 gm were used. The rats were housed in a cage made of polypropylene wire mesh with husk bedding and maintain controlled environmental condition of light such as 12-h light and dark cycle, temperature (25±2ºC) and humidity. Rats were fed with a standard pellet diet and water-*ad libitum*, were used for the entire study. The experiments were performed during day (8.00-16.00 hrs). The rats were housed and treated according to the rules and regulations of CPCSEA and IAEC. The protocol for all the animal study was approved by Institutional Animal Ethics Committee (IAEC) constituted as per the guidelines of CPCSEA research protocol no. 650/PO/Re/S-2002/2022/CPCSEA/22

2.2. Chemicals and Instruments

From Ambika Diagnostics, diagnostic kits for measuring HDL, LDL, triglycerides, and cholesterol were acquired. Dr. Reddy's Laboratories Ltd., provided Donepezil API. Other chemicals used were of laboratory grade. EPM and MWM apparatus (K- Roy), Biochemistry Analyser AD-100 (Ambica Diagnostics, Parbhani, India), Tissue Homogenizer (Prompt), Cooling Centrifuge (RemiElektrotecnik Ltd., Vasai, India), Micro Pipette (10-100µl), anaesthetic chamber, weighing machine are used in this work.

2.3. Plant Material

Leaves of *A. procera* (family-Fabaceae) were collected in the month of September, from local area of Yavatmal district, Maharashtra, India. The plant material was identified and authenticated by Mrs. A. M. Gaharwar, Vasantrao Naik College of Agricultural Biotechnology, Yavatmal (Ref. No. VNCABT/Ytl/Hort/ 1031/2019) Leaves were dried in shade before they were ground into a coarse powder. This powder was stored in air tight container and used for extraction. Methanol and water were used as a solvent for the extraction of *A. procera* leaves in the proportion of 7:3 in glass bottle. Powdered leaves were macerated, occasionally stirred at regular intervals of time, was filtered, concentrated and dried by evaporation. (Khatoon MM, et.al, 2014) Estimation of methanolic extract for the presence of different phytoconstituents was done. (S. Siva Krishnan et.al, 2014)

2.4. Experimental design

For this study animals were divided into five groups, each group having six animals (n = 6).

Animals in group I are control group and received saline solution only. Group II rats were subjected to restraint stress using saline bottle for 84 days (Negative control). MEt of *A. procera* was given orally to restraint stressed rats in groups III and IV at 200 and 400 mg/kg for 84 days. Group V stressed rats were given 5 mg/kg Donepezil orally once daily for 84 days (Standard group).

2.5. Induction of memory impairment state

All groups were subjected for 84 days for restraint stress except vehicle control group which was placed in normal condition in animal house. Memory impairment was induced in female Sprague dawley rats by using saline bottle. Rats were tightly packed in saline bottle for 6 hrs. daily up to 84 days. The animal model of depression was exposing to continuous stress such as food, water deprevation and continuous tightly packed in saline bottle. (Mohamed Saleem Abdul Shukkoor, et.al.2016)

2.6. Drugs and dosing

Donepezil (5 mg/kg) was standard drug, diluted with distilled water. Two different concentrations (200 and 400 mg/kg) of the *A. procera* leaves extract were prepared by dissolving the extracts in distilled water. All solutions were prepared freshly on test days and administered orally. *A. procera* leaves extract for the dosing were calculated by the body weight of rats of different groups. Low and high dose extract group were 200mg/kg and 400mg/kg of rats.

2.7. Study of learning and memory enhancement by following model,

2.7.1. Modified Elevated plus maze apparatus (EPM): -

EPM apparatus was used for the assessment of learning and memory enhancement activity. The test was performed by putting the rat in one of the open arms of maze, typically facing opposite to closed arm. Upon release, the animal is free to explore the apparatus. One measure of memory is then recorded, the transfer latency (TL) i.e the time (in second) taken by the rat to move from the open arm into one of the closed arm with all its four legs was measured (Mani Vasudevan & Milind Parle, 2007 and Vijendar Kumar et.al, 2013).

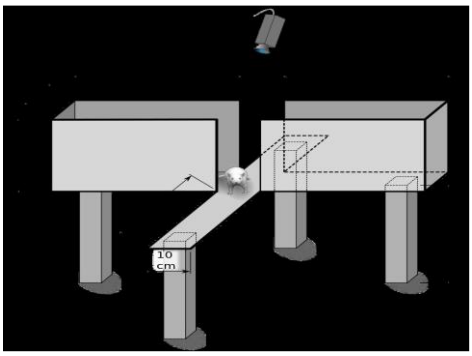

Figure 1 Elevated Plus Maze Apparatus

2.7.2. Morris Water Maze Apparatus (MWM)

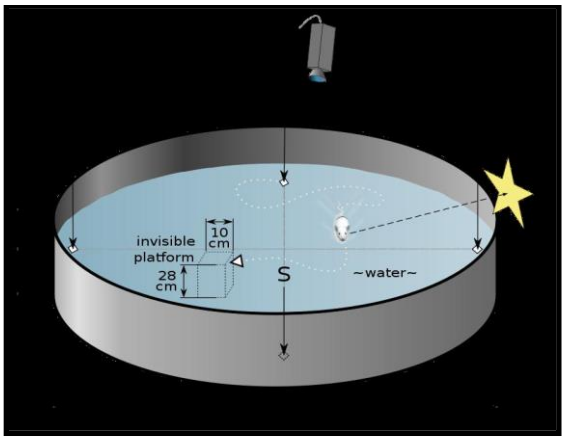

Figure 2 Morris Water Maze Apparatus

MWM apparatus is a test of learning and memory for rodents to navigate from start locations around the perimeter of an open swimming area to locate a submerged escape platform. Learning and memory is assessed across repeated trials

and reference memory is determined by preference for the platform area when the platform is absent. (Charles V., et.al, 2006)

2.8. Biochemical Estimation

2.8.1. Determination of Cholesterol

The kit utilizes the colorimetric procedure in which Cholesterol and its ester are release from lipoproteins by detergents. Cholesterol esterase hydrolysed the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 is formed. This is converted into a coloured quinoline in a reaction with 4- amino-antipyrine and phenol catalysed by peroxidation. Absorbance was measured at 505 nm. (Trinder P. etal 1969 and Kerner W. etal 2014)

2.8.2. Determination of Triglyceride

The kit utilizes the colorimetric procedure in which enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine, which is generated from 4- aminoantipyrine and 4- chlorophenol by hydrogen peroxide under the catalytic action of peroxidase. Absorbance was measured at 546 nm. (Trinder P. etal 1969 and Kerner W. etal 2014)

2.8.3. Determination of HDL

The kit utilizes the colorimetric procedure in which Chylomicrons, VLDL and LDL are precipitated by adding phosphotungstic acid and magnesium ions to the sample. Centrifugation leaves only the HDL in the supernatant. Their cholesterol content is determined enzymatically using Ecoline S and Cholesterol. Absorbance was measured at 505 nm.

Determination of LDL (Maruyama C., et.al 2003 and Masashi, K., et.al 2004**)**

LDL were calculated by using following formula

$$VLDL = \frac{TG}{5}$$

LDL=TC–(HDL-VLDL)

2.9. Statistical Analysis

All data were expressed as the mean ± standard deviation. For statistical Analysis of the rats, group mean were compared by one-way (ANOVA) followed by Dunnett's test, $p<0.01$ was considered as significant value.

3. Results

Table 1 Preliminary Phytochemical investigation of methanolic extract of *A. procera*

Sr. No.	Phytoconstituents	Test	MAP
1	Alkaloid	Mayer's	+
		Dragendroff's	+
2	Flavonoids	Ferric Chloride	+
		Lead Acetate	+
3	Carbohydrates	Molisch	-
		Fehling	-
		Benedict's	+
4	Steroid	Salkowski's	+
		Libermann Barchared	+
5	Tannins	Lead Acetate	+
		Gelatin	+

6	Proteins	Xanthoprotein	+
		Biuret	-
		Lead Acetate	+
7	Glycoside	Keller Kiliani	-

+ Present, - Absent

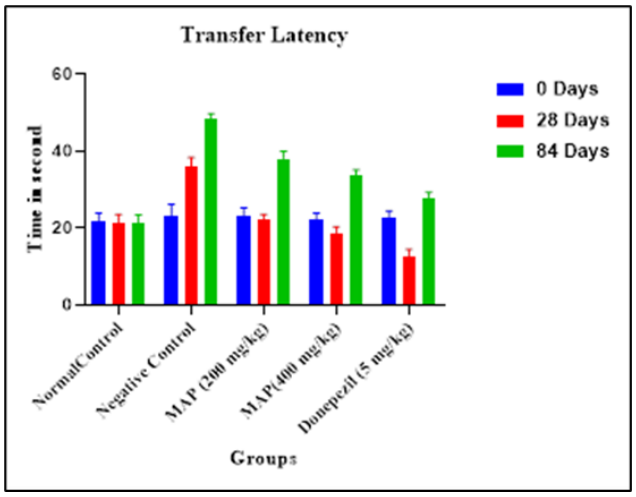

Figure 3 Effect of MEt of *A. procera*on TL in EPM in memory impairment rats

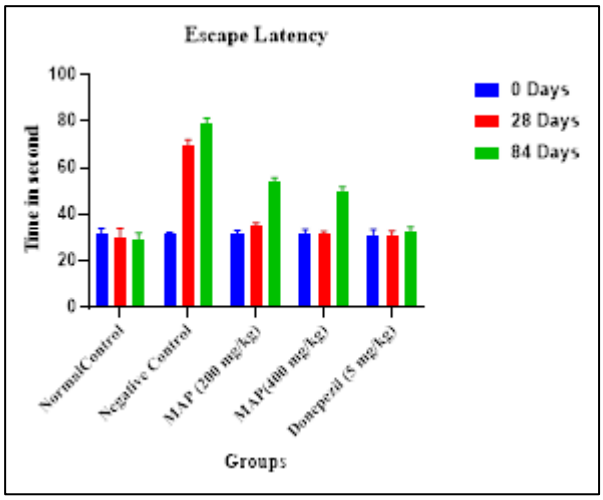

Figure 4 Effect of MEt of *A. procera* on EL in MWM

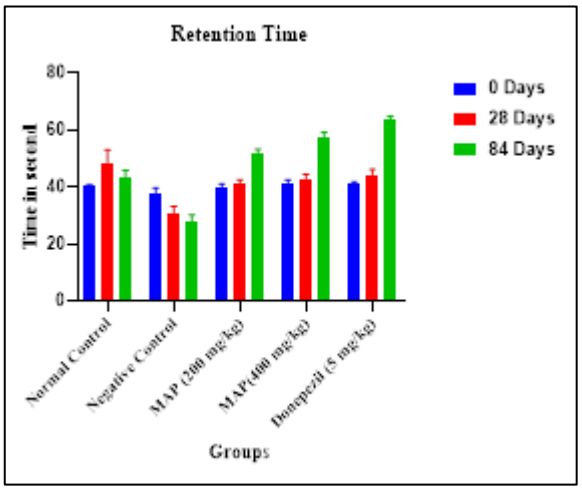

Figure 5 Effect of MEt of *A. procera* on RT in MWM

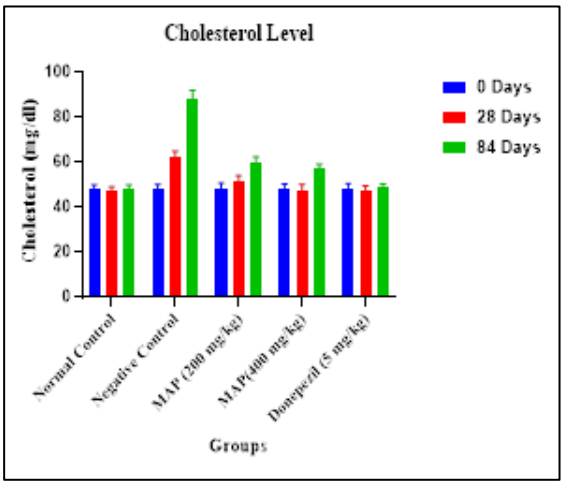

Figure 6 Effect MEt of *A. procera* on serum Cholesterol in memory impairment rats

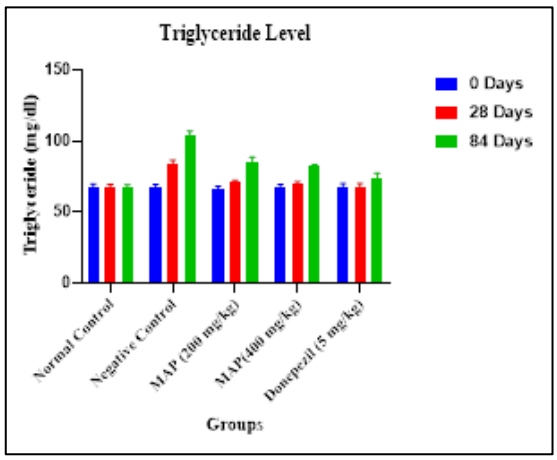

Figure 7 Effect of MEt of *A. procera* on serum Triglyceride in memory impairment rats

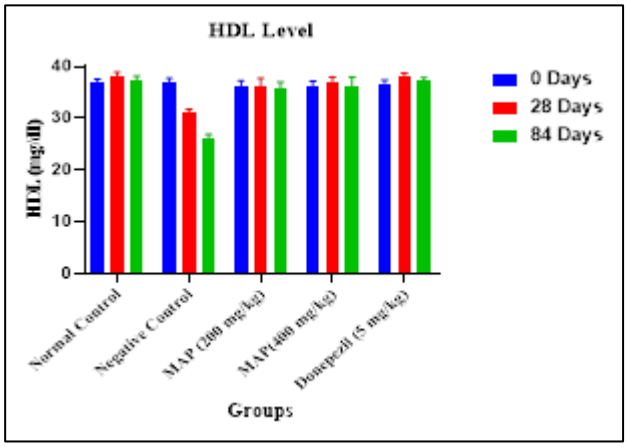

Figure 8 Effect of MEt of *A. procera* on Serum HDL in memory impairment rats

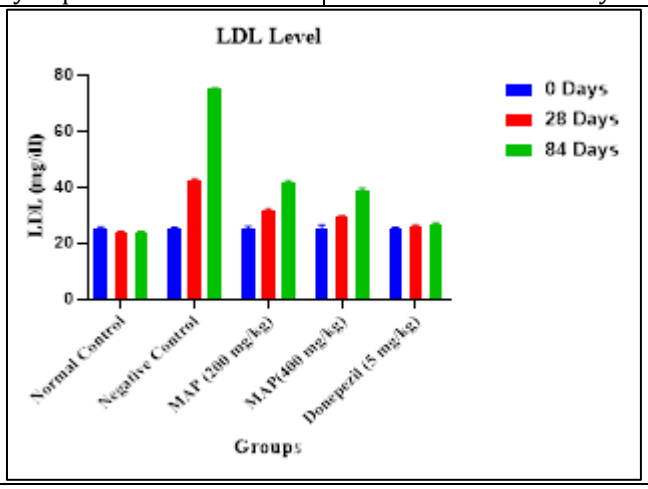

Figure 9 Effect of MEt of *A. procera* on serum LDL in memory impairment rats

Figure1 shows the effect of MEt of *A. procera* on TL on EPM in stressed rats. On the 84thday, the negative control group's TL significantly higher ($p<0.01$) in comparison to the normal control group. In comparison to the negative control group, there was a substantial ($P<0.01$) decrease in the TL when treated with MEt of *A. procera* at 200 mg/kg and 400 mg/kg and 5 mg/kg of Donepezil, on 84th day. Figure 2 shows the effect of MEt of *A. procera* on EL in MWM in memory impairment rats. In contrast to the control group, the negative control group's EL increased significantly ($P<0.01$) on 84th day. Treatment with MEt of *A. procera* at 200 mg/kg and 400 mg/kg and Donepezil (5 mg/kg) resulted in a significant ($P<0.01$) reduction in EL when compared to the negative control group on 84thday.Effect of MEt of *A. procera* leaves on RT in MWM in memory impairment rats is shown in figure 3. On 84thday, the RT was expressively increased in the groups treated with MEt of *A. procera*at 200 and 400 mg/kg and donepezil (5 mg/kg) than in the negative control group. As shown in figure4, there was expressively increased ($p<0.01$) in the Cholesterol level in negative control group compared to normal control group on 84th day. After treatment with MEt of *A. procera* significant ($p<0.01$) reduction was observed in the Cholesterol level on 84th day compared to negative control group at 200 mg/kg and 400mg/kg. Figure 5 shows the effect of MEt of *A. procera* on Triglyceride level. When compared to the normal control group, the negative control group's triglyceride level increased expressively ($p<0.01$) on day 84th.Triglyceride levels were significantly ($p<0.01$) lower in MEt of *A. procera* treated group than in the negative control group at 200 mg/kg and 400 mg/kg on 84thday. As shown in figure 6, there was substantially decrease ($p<0.01$) in the HDL level in negative control group compared to normal control group on 84th day. At 200 mg/kg and 400 mg/kg on the 84th day following treatment with MEt of *A. procera*, a substantial ($p<0.01$) decrease in HDL levels was noted in comparison to the negative control group. Effect of MEt of *A. procera* on LDL level is shown figure 7. There was substantial rise ($p<0.01$) in the LDL level in negative control group on 84th day compared to normal control group. After administering Met of plant *A. procera,* a significant ($p<0.01$) reduction was observed in the LDL level compared to negative control group at 200 mg/kg and 400mg/kg on day 84th.

4. Discussion

Stress is a universal threat fortified by the advancement of industrialization and elicited by a variety of factors, viz environmental, social or pathological phenomenon of life. A considerable fact published in the last decade has focused on a group of neurochemicals, biochemical and molecular effects caused by stress in the CNS, endocrine system, and immune system (D Rai et al., 2003). Numerous researches have shown a link between stress exposures, alterations in the immune network, and the progression of disease, particularly in neurodegenerative disorders like Alzheimer's disease. (AD) Presenly, there are only two classes of approved drugs to treat AD, including inhibitors to cholinesterase enzyme and antagonists to N-methyl d-aspartate (NMDA), which are effective only in treating the symptoms of AD, but do not cure or prevent the disease. (Zeinab Breijyeh *et al*., 2020) Some of the common chemicals used for modelling AD are scopolamine, streptozotocin, and alcohol, as well as dysregulation of heavy metals, such as aluminum (Al), copper (Cu), zinc (Zn), lead (Pb), and reducing sugar (D-galactose), among others. (Onesimus Mahdi *et al*., 2019). The most prevalent and extensively dispersed class of phytochemicals in higher plants, flavonoids has significant medicinal potential. Flavonoids are divided into six groups according to their chemical makeup: isoflavonoids, anthocyanidins, flavanols, flavanones, flavonols, and flavonols. It has been demonstrated to be helpful in avoiding neurodegenerative illnesses and can slow the progression of neurodegeneration by focusing on several targets. Since flavonoids have anti-inflammatory and antioxidant properties that are crucial to the pathophysiology of AD, they are extensively researched. Research has demonstrated that flavonoids possess the ability to penetrate the blood-brain barrier (BBB), which suggests that they may have applications in the prevention of neurodegenerative diseases. Nevertheless, the BBB-crossing capacity of distinct flavonoid subgroups varies (Haroon Khan *et al*., 2020).Literature shows that *Albizia procera* contains carbohydrates, phenols, flavonoids, steroids, alkaloids, anthraquinones and amino acids. Current study confirms the presence of alkaloids, carbohydrates, tannins, phenolic compounds, flavonoids, anthraquinones, and saponins. There are various models for the screening of learning and memory enhancing activity like EPM apparatus, MWM apparatus, light and dark apparatus, elevated T maze, elevated zero maze, open field test and white lack box. In this study for the assessment of learning and memory enhancing activity we have used EPM apparatus and MWM apparatus due to their economic, easily available, popularity, accuracy, specificity and shows good results. (Md. Sahab Uddin*et al*., 2016) In EPM apparatus, there was expressively increased in the TL in negative control group as compared to the normal control group. Whereas MEt of *A. procera* (at 200 mg/kg and 400 mg/kg) and Donepezil (5 mg/kg) daily for 84 days treated group showed significant decrease in TL as compared to negative control group. In MWM apparatus, there was expressively increased in the EL in negative control group as compared to the normal control group. Whereas of MEt of *A. procera*(at 200 mg/kg and 400 mg/kg) and Donepezil (5 mg/kg) treated group showed significant decrease in EL as compared to negative control group at 84 days. In MWM, there was substantially decrease in the RT in negative control group as compared to the normal control group. Whereas of MEt of *A. procera* (at 200 mg/kg and 400 mg/kg) and Donepezil (5 mg/kg) treated group showed substantial rise in TL as compared to negative control group at 84 days. In this study a variety of biochemical markers, including cholesterol, triglycerides, HDL and LDL were examined. Our finding shown that, there was significant increase in cholesterol, triglycerides, LDL and notable depletion in the HDL level in negative control group as compared to the normal control group. Whereas of MEt of *A. procera* (at 200 mg/kg and 400 mg/kg) and Donepezil (5 mg/kg) treated group showed substantial reduction in cholesterol, triglycerides, LDL and expressively higher in the HDL level as compared to negative control group at 84thday.

5. Conclusion

The present finding indicates that the methanolic extract of *Albizia procera* leaves exhibits significant mild to moderate learning and memory enhancement activity along with antihyperlipidemic potential at low dose (200 mg/kg) and high dose (400 mg/kg).

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclsoed.

Statement of ethical approval

Ethical approval was obtained.

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