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Bioevaluation of *Albizia Procera* Leaves in the Treatment of Alzheimer's Disease in Animal Model

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Abstract

Background: 60–70% of cases of memory loss are caused by Alzheimer's disease (AD). It is a violent type of memory loss manifesting in memory, language and behavioural deficits. The primary risk of AD is advancing age. The incidence rates change with ages. After the age of 65 incidence rate of disease become double and differs in the sex also. Women are more proven for the disease particularly after the age of 85. The risk of dying from AD is higher in white non-Hispanics than in black non-Hispanics, although the Hispanic population is less likely to get AD than non-Hispanic whites. Whereas non-Hispanic whites have a higher risk of dying from AD than non-Hispanic blacks, the Hispanic population is less likely to get AD.

This study was undertaken in order to evaluate learning and memory impairment activity of the methanolic extract of *Albizia procera* R. (MEtAP) leaves in animal models. *Albizia procera* R. (AP) is a fast-growing subtropical and tropical tree belonging to the Fabaceae family's Mimosoideae subfamily. AP is a herb widely used in the Asian traditional medicine as antioxidant, analgesic, antibacterial¸ antidiarrheal and antidiabetic drug. AD is a chronic neurological disease.

Methods: The leaves of AP were collected, authenticated, dried and extracted with methanol. The effect of the MEtAP leaves (200 and 400 mg/kg, taken orally once a day for 28 days)
on learning and memory performance was assessed using Modified EPM apparatus and MWM apparatus. Learning and Memory impairment was induced by chronic restraint stress.

Results: In Modified EPM apparatus the treated rats exhibited decrease entries and time spent in the closed arm and increase entries and time spent in open arm. In MWM Apparatus the treated rats exhibited increase in the retention time and decrease in the escape latency. In calorimetric analysis the level of acetylcholinesterase decreases by the plant extract to some extent and helps in the learning and memory enhancement. Administration of MEtAP leaves significantly improved those parameters suggesting positive effects on learning and memory impairment activity.

Conclusion: The present finding indicates that the MEtAP leaves exhibits significant mild to moderate learning and memory enhancement activity at low dose (200 mg/kg) and high dose (400 mg/kg) Thus, AP is a promising herbal option in the pharmaceutical world.

Keywords: - *Albizia procera* R. (AP), Alzheimer Disease (AD), EPM apparatus, MWM apparatus, Learning and Memory enhancement activity..

Introduction

Alzheimer's disease or AD, is a long-term neurological illness. AD is the cause of 60–70% of memory loss instances. The credit for describing a memory loss condition for first time goes to German neuropathologies and physiatrist Dr. Alois Alzheimer. It is a violent

type of memory loss that causes behavioral, language, and memory problems. (Burns A. et. al, 2009; Indu bhusshan et.al, 2017). Amyloid-beta (Aβ) peptide accumulation and tau protein buildup cause neurofibrillary tangles (NFTs) and neuronal cell death in AD. Neural damage results from the abnormal proteins accumulation, which is mostly caused by decreased clearance. There is cholinergic deficiency in the brain, glutamate and neuropeptide, are also affected. The healthy human brain contains the peptide Aβ, which has 39–43 amino acid residues. A bigger amyloid precursor protein (APP) underwent cleavage to produce Aβ fibrils. The buildup of amyloid fibrils, or amyloid plaques, in the extracellular space of brain cells in AD is intimately associated with synaptic dysfunction, neuronal death, inflammatory responses, and loss of synapses. Conversely, tau protein is widely distributed throughout the central nervous system in distinct spatial patterns and is crucial for maintaining microtubule stability. The tau protein experiences severe hyperphosphorylation in Alzheimer's pathology, which causes the tau protein to clump and form intracellular NFTs. The intracellular formation of NFTs leads to microtubule disassembly, dendritic spinal collapse, and the degeneration of axons. Memory loss, mood swings, anxiety, trouble managing money, and poor judgment are some of the milder symptoms of AD. However, patients with late-stage AD may have a lack of environmental responsiveness, disorientation, restlessness, difficulties thinking and speaking, as well as problems controlling their movements.
(Kasturibai magalingam et.al,2018; K.D. Tripathi, 2013). The primary risk of AD is advancing age. The incidence rates change with ages. 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. Although the Hispanic population is less likely to have AD than non-Hispanic whites, non-Hispanic whites are more likely to die from it than non-Hispanic blacks. (Antonio Di Carlo et.al,2002; F.Bermejo Pareja et.al,2007) Within the Mimosoideae subfamily of the Fabaceae family, AP is a fast-growing subtropical and tropical tree. AP is a herb widely used in the Asian traditional medicine as antioxidant, analgesic, antibacterial, Antidiarrheal and antidiabetic drug (S. Sivakrishnan, 2019) The colour of the AP bark is brown and has characteristic odour and is slightly bitter in taste and the colour of AP leaf is green and has characteristic odour and is slightly bitter in taste. (S. Sivakrishnan, 2019) AP leaves exhibit the presence of flavonoids, glycosides, tannins, steroids, and saponins, among other substances. (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). Depending on their chemical makeup, flavonoids are categorized into six classes: flavanones, isoflavonoids, flavones, flavonols, and anthocyanidins. They help treat neurodegenerative diseases like AD and slow down the progression of neurodegeneration while focusing on several targets. The anti-inflammatory and antioxidant properties of flavonoids are being researched because they both play a significant role in the pathophysiology of AD. Because flavonoids can pass across the blood-brain barrier (BBB), they may be able to prevent neurodegenerative diseases. There has been evidence of the anti-Alzheimer's disease properties of several flavonoids, including kaempferol, myricetin, rutin, fisetin, quercetin, and apigenin. (Haroon Khan Et.al, 2019). Thus, the current work uses a rat model to evaluate the effects of administering a methanolic extract of *Albizia Procera R.* leaves on learning and memory enhancing activity in Alzheimer disease.

Methods: -

Leaves of AP belonging to family-Fabaceae were collected in the month of September from the 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 a shade and then powdered to get a course powder. This powder was stored in air tight container and used for extraction. For the extraction of AP leaves, methanol and water were used as a solvent. Methanol and water

were used in the proportion of 7:3. Glass bottle was needed for the extraction. Dried leaves of AP, methanol and water poured in glass bottle for extraction. In maceration procedure, powdered leaves were macerated; it was occasionally stirred at regular intervals of time. It was filtered and concentrated. Then it was dried by evaporation. (Khatoon MM, et.al,2014) Estimation of methanolic extract for the presence of different phytoconstituents was done. (S. Sivakrishnan et.al, 2014).

Animals

8 weeks old healthy female Sprague-Dawley rats (weighing 150-250 gm) were used for this study. Animals were housed in polypropylene cages with wire mesh top and husk bedding and maintain under control condition of light (12h-light, 12h-dark), temperature (25±2ºC), and humidity (60±5%) and fed with a standard pellet diet and water ad libitum, were used for the entire animal study. The experiments were performed during day (8 to 16 hours). 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

Experimental Design

For this study animals were divided into five groups:

Group I (Vehicle control group) –
Rats received only saline solution.

Group II (Negative control group) **-** Rats were subjected to restraint stress using saline bottle for 28 days.

Group III (Low dose group) **-** Rats were subjected to restraint stress and treated with 200mg/kg MEtAP orally for 28 days.

Group IV (High dose group) **-** Rats were subjected to restraint stress and treated with 400mg/kg MEtAP orally for 28 days.

Group V (Standard group)-
Rats were subjected to restraint stress and treate d with 5mg/kg Donepezil for 28 days.

Induction of memory impairment state

All groups received 28 days of restraint stress, with the exception of the usual control group, which was kept in an animal house under normal circumstances. A saline bottle was used to produce memory impairment in female Sprague Dawley rats. When rats were tightly packed in saline bottle for 6 hrs daily upto 28 days. The animal model of depression is kept under constant stress in saline bottles and is exposed to things like food and water deprivation. (Mohamed Saleem Abdul Shukkoor, et.al.2016)

Drugs and dosing

Standard drug was Donepezil (5mg/kg). Distilled water was used to dilute donepezil. By dissolving the extracts in distilled water, two distinct concentrations of MEtAP leaves (200 and 400 mg/kg) were produced. All solutions were prepared freshly on test days and administered orally. All solutions were prepared freshly on test days and administered orally. MEtAP leaves for the dosing were calculated by the body weight of rats of different groups. Rats in the low dose group received 200 mg/kg of extract, whereas those in the high dose group received 400 mg/kg.

Study of learning and memory enhancement state after 28 days by following model

1] Modified EPM: -

The evaluation of learning and memory-enhancing activities was conducted using modified EPM.The test was performed by inserting the rat in one of the open arm 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 sec.) taken by the rat to move from open arm into closed arm with all its four legs was measured (Mani Vasudevan & Milind Parle, 2007 and Vijendar Kumar et.al, 2013).

2] MWM Apparatus: -

MWM tests the ability of rodents to learn and remember where to go from starting locations around the edge of an open swimming area in order to find a submerged escape platform. Repetitive trials are used to evaluate learning and memory, and preference for the platform

area in the absence of the platform determines reference memory. (Charles V., et.al, 2006)

The Brain's Acetyl Cholinesterase Activity

The colorimetric approach was used to assess the activity of brain acetylcholinesterase (AchE). A test tube holding 2.6 ml of phosphate buffer was combined with 0.4 ml of brain homogenate. Absorbance at 412 nm was measured after adding 0.1 ml of DTNB reagent to the mixture previously mentioned. Again after 15 minutes, absorbance was recorded after mixing 0.02 mL of cholinergic iodide solution. The absorbance change per minute was calculated. (Dinesh D. et.al, 2012).

The following formula was used to estimate the substrate's rate of hydrolysis:

$R = \text{change in absorbance/min} \times 5.74 \times 10^{-4}/Co.$

R= rate of hydrolysis of acetylcholine iodide/min/mg tissue,

Co = weight of tissue homogenate in mg/Ml. (Dinesh D. et.al, 2012).

RESULTS: -

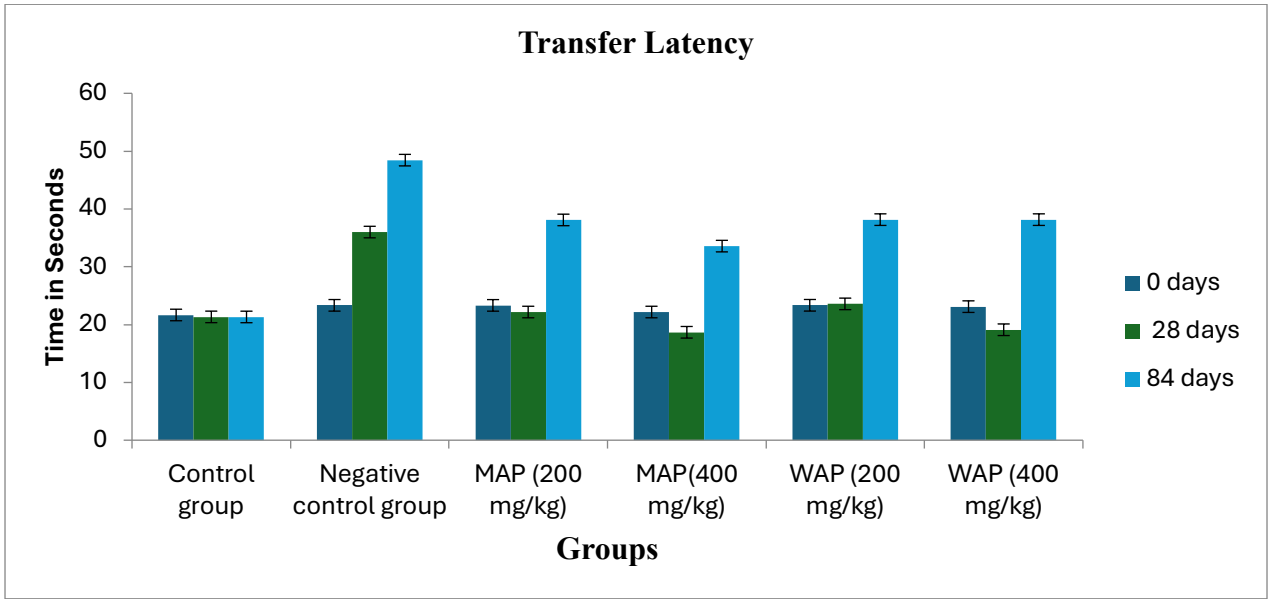

Figure 1: Effect of MEtAP leaves on transfer latency (TL) of rats in EPM apparatus.

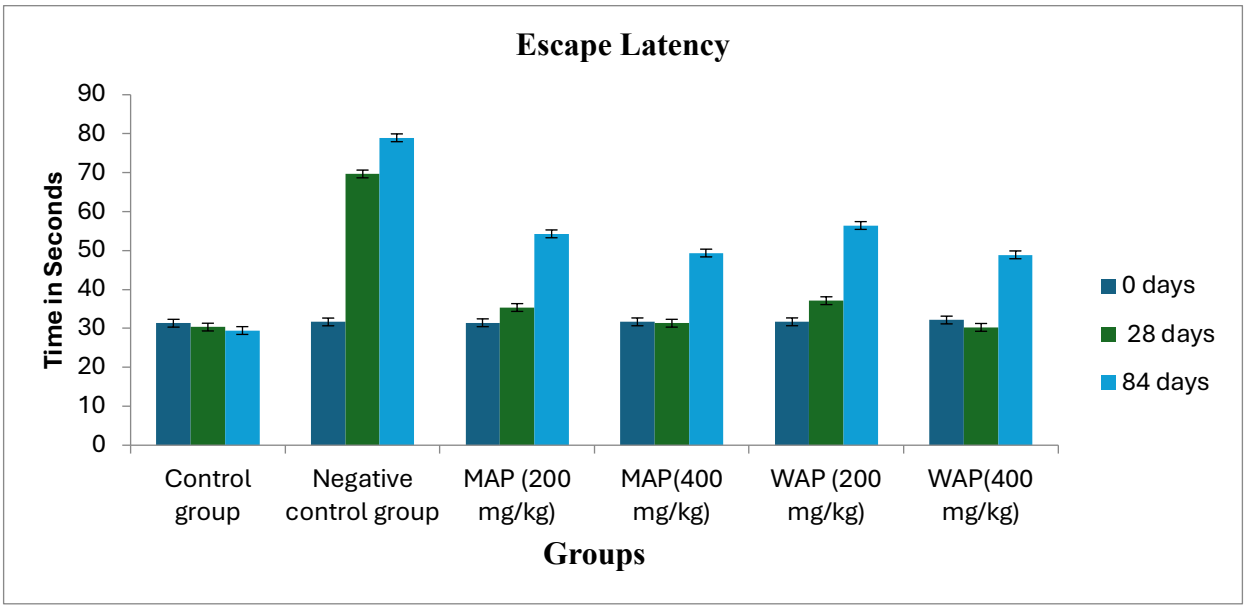

Figure 2: Effect of MEtAP leaves on Escape latency (EL) and retention time (RT) in MWM in memory impairment rats.

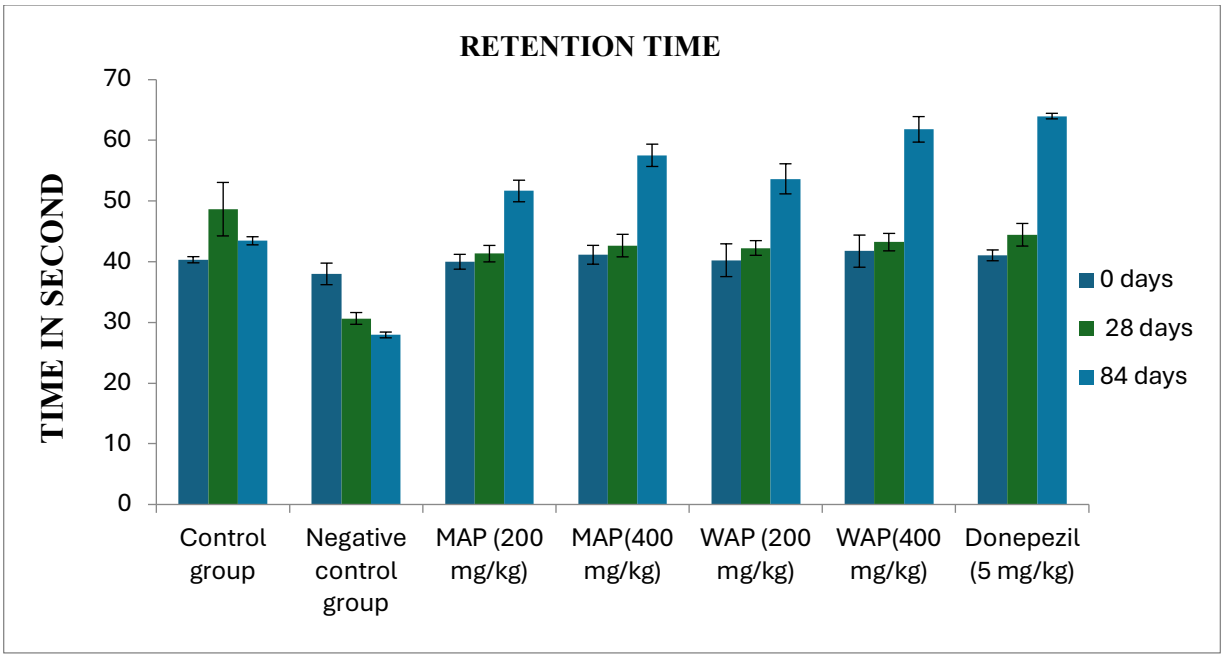

Figure 3: Effect of MEtAP leaves on retention time in MWM in memory impairment rats.

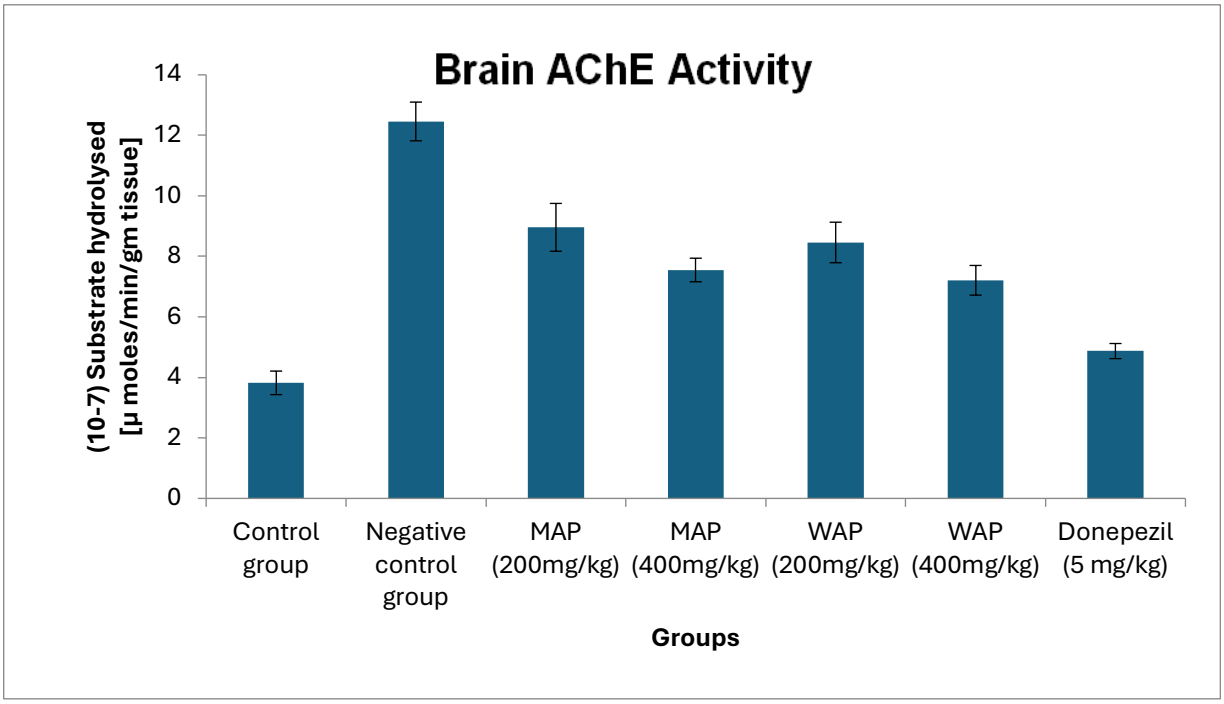

Figure 4: Effect of MEtAP leaves on Brain AChE activity in memory impairment rats:-

Figure 1 shows effect of MEtAP leaves on TL of rats on EPM apparatus in stressed rats. On the 28th day, the negative control group's TL increased significantly ($p<0.01$) in comparison to the normal control group. When treated with 200 and 400 mg/kg of MEtAP leaves and 5 mg/kg of donepezil, there was a significant ($P<0.01$) decrease in the TL as compared to the negative control group.

Figure 2 shows that MEtAP leaves on EL in MWM in memory impairment rats. The EL in the negative control group was significantly ($P<0.01$) higher than in the control group. When treated with 200 and 400 mg/kg of MEtAP leaves and 5 mg/kg of donepezil, there was a significant ($P<0.01$) decrease in EL when compared to the negative control group.

Figure 3 that MEtAP leaves on RT in MWM in memory impairment rats. When compared to the negative control group, there was a

discernible decrease in RT after receiving 200 and 400 mg/kg of MEtAP leaves and 5 mg/kg of Donepezil.

Figure 4 shows that MEtAP leaves on brain AChE activity in rats. When compared to the normal control group, the negative control group's brain AChE level was noticeably higher. When treated with MEtAP leaves at 200 and 400 mg/kg and donepezil (5 mg/kg), there was a discernible rise in the amount of AChE in the brain in comparison to the negative control group.

Discussion

Stress is a global issue that has been made more potent by industrialization's growth and can be triggered by a wide range of things, including environmental, social, or psychological phenomena. A significant body of research, released in the past ten years, has concentrated on a set of biochemical, neurochemical, and molecular impacts of stress on the immune system, endocrine system, and central nervous system. (D Rai et al., 2003). Numerous studies have demonstrated a connection between immunological network changes, disease progression, and stress exposure, especially in neurodegenerative illnesses like AD. As of right now, the only approved pharmacological classes for treating AD are antagonists to N-methyl d-aspartate NMDA and cholinesterase enzyme inhibitors. These medications are only useful for treating the symptoms of AD; they do not prevent or cure the condition. (Zeinab Breijyeh*et al*., 2020) A number of substances, including alcohol, streptozotocin, scopolamine, and dysregulation of heavy metals like lead, aluminum, copper, zinc, and reducing sugar (D-galactose), are frequently used to imitate AD. (Onesimus Mahdi *et al*.,2019) The most prevalent and dispersed class of phytochemicals in higher plants, flavonoids have significant medicinal potential.The six kinds of flavonoids are isoflavonoids, anthocyanidins, flavanols, flavanones, and flavonols based on their chemical structure. It has been shown to be beneficial in preventing neurodegenerative diseases and, by concentrating on many targets, can halt the progression of neurodegeneration. Due to their anti-inflammatory and antioxidant characteristics, which are vital to the pathophysiology of AD, flavonoids have been studied in great detail. Research has demonstrated that flavonoids possess the capacity to cross the blood-brain barrier (BBB), which suggests that they may have applications in the defense against 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 existence of alkaloids, carbohydrates, tannins, phenolic compounds, flavonoids, anthraquinones, saponins. A number of models, including the elevated plus maze apparatus, the Morris water maze apparatus, the light and dark apparatus, the elevated T maze, the elevated zero maze, the open field test, and the white lack box, are used to screen learning and memory-enhancing activities. In this study for the assessment of learning and memory enhancing activity we have used EPM and MWM due to their economic, easily available, popularity, accuracy, specificity and shows good results. (Md. Sahab Uddin*et al*.,2016) In EPM apparatus, when compared to the normal control group, the TL in the negative control group increased significantly. In contrast, the group that received daily treatment with MEtAP leaves (at 200 and 400 mg/kg) and donepezil (5 mg/kg) for 28 days shown a substantial decrease in TL when compared to the negative control group. In MWM apparatus, when compared to the normal control group, the EL in the negative control group increased significantly. On the other hand, at 28 days, the groups treated with MEtAP leaves (at 200 and 400 mg/kg) and donepezil (5 mg/kg) had a substantial decrease in EL when compared to the negative control group. When compared to the normal control group in MWM, the RT in the negative control group decreased significantly. At 28 days, the groups treated with MEtAP leaves at 200 and 400 mg/kg and 5 mg/kg of donepezil exhibited a noteworthy rise in TL in contrast to the negative control group.

Conclusion

The current study's results show that at both low and high doses (200 and 400 mg/kg), the methanolic extract of *Albizia Procera R.* leaves significantly improves learning and memory in a mild to moderate way.

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