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Original Article

MEMORY ENHANCING ACTIVITY OF *MADHUCA LONGIFOLIA* ETHANOLIC LEAF EXTRACT (FLAVONOID FRACTION) AND ITS HPTLC

PRAGATI KHARE[1*], NOOPUR KHARE[2]

[1]Department of Pharmacy, Shri Ram Murti Smarak, C. E. T., Bareilly, U. P., India, [2]Department of Biotechnology, Bhai Gurdas Group of Instiutions, Sangrur, Punjab, India
Email: pragatikhare10@gmail.com

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ABSTRACT

Objective: The present study aimed at investigating the protective role of *Madhuca longifolia* ethanolic leaf extract flavonoid fraction against colchicine induced cognitive dysfunction and oxidative damage in swiss albino mice and to estimate the biochemical alterations in mice brain. HPTLC, total flavonoid and total phenols were also estimated in the study.

Methods: The analysis was conducted on a colchicine-induced model for 28 d. Morris water maze and passive avoidance paradigm were used for conducting behavioral experiments, while biochemical parameters such as nitric oxide and glutathione were also estimated. Swiss albino mice (48) were apportioned into eight sets, each consisting of six mice. ANOVA (one-way) was utilized and then followed by the Dunnett's test and finally outcomes were analyzed.

Results: The total flavonoids content in *Madhuca longifolia* leaves was found to be (14.17±0.56 QE and 21.24±0.94 RE mg/g of dry material) and total phenolic compounds were 299.32±2.73 mg/g of dry material. *Madhuca longifolia* leaf extract indicated a substantial reduction in escape latency of mice in morris water maze. The transfer latency of mice in passive avoidance model showed a substantial increase.

Conclusion: The *Madhuca longifolia* leaf extract presented a substantial increase (P<0.001) in GSH intensities and a major decline (P<0.001) in total protein, NO and AChE. *Madhuca longifolia* has neuroprotective effect against memory damage caused by colchicine.

Keywords: *Madhuca longifolia*, Neuroprotective, Alzheimer's disease, Flavonoid, Acetylcholinesterase, Cognition

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INTRODUCTION

Alzheimer's disease is a prolonged and neurological condition that is progressive and has a long-lasting effect on memory loss, behavior change, reasoning capacity, personality and ability to think. Dementia in older people is primarily caused by Alzheimer's disease. Duration between symptom initiation and death takes about 8.5 y. Study has revealed that globally, approximately fifteen million people suffer from the disease of Alzheimer [1]. Elderly people of about 65 y and above are mostly affected by Alzheimer's disease [2]. The CNS is often affected by more free radical generation. Excessive free radical formation can cause neuronal damage to DNA, membrane lipids and proteins. In elderly people, there is a noticeable reduction of cholinergic neurotransmission due to reduced levels of acetylcholine in the brain [3]. Dementia is associated with the prevalence of Alzheimer's disease where neuron loss occurs in various areas of the brain [4]. AD is characterized by the development of neurotic plaque that contains amyloid β protein. The development of dementia in the forebrain is mainly caused due to acetylcholine and cholinergic cell loss [5]. Stress, toxins and genetic predilection are the crucial risk factors for the development of neurodegenerative disorders [6]. The treatment, prevention and diagnosis of a number of diseases are usually dealt with the use of traditional medicine. Treatment of various diseases by medicinal plants relies on findings and prior experiences contained in books or orally taught [7]. Neuroprotection includes therapeutic techniques that can postpone or cure neuronal damage. Herbalism is effective, safer and cheaper [8].

Madhuca longifolia belongs to the family of Sapotaceae and is also known as Mahua [9]. The name Madhuca is derived from "Madhu", meaning honey and is also called Indian butter tree. Mahua is a medium-sized and deciduous tree commonly found in Nepal, India and Srilanka [10]. In all parts of it, Mahua is full of various medicinal properties. Mahua leaf act as a wound healer, emollient, anthelmintic and antirheumatic agent. Mahua fruit is a good aphrodisiac, refrigerant, tonic and anti-ulcerative. Mahua bark is useful in the treatment of tonsillitis, stomach disorder and also treats snake poisoning. Mahua seeds are efficient diuretic, refrigerant, hepatoprotective and antihelmintic. Mahua is composed of various phytoconstituents like glycosides, flavonoids, saponins, triterpenoids and steroids [11].

MATERIALS AND METHODS

Plant material

The leaves of *Madhuca longifolia* L. were fetched from college campus of Shri Ram Murti Smarak College and verified by Prof. A. K. Jaitly, HOD Plant Science Department. The collected sample specimen was kept as a reference for future studies at the institutional herbarium (specimen number-RU/PS/2016/415).

Extracts preparation and fractionation into flavonoids fraction

Mahua leaves were washed in tap water, followed by drying in shade and then powdered. This powder was packed in Soxhlet column. The extraction was initiated with petroleum ether (60-80 °C) and was continued for 24 h. The marc obtained was then successively extracted with chloroform (50-60 °C) followed by ethanol (68-78 °C) for 24 h. The extracts were kept on water bath at 50 °C to make them concentrated. The dried powder extract was stored at room temperature after concentrating the preparation. Using a rotary evaporator, the solvent was extracted under reduced pressure to obtain dried ethanolic extract, which was subsequently dissolved in water and chloroform extraction was performed in separating funnel and 10 percent NaCl solution was applied dropwise to the aqueous layer to precipitate the tannins. The supernatant liquid was partitioned with ethyl acetate and the crude fraction of flavonoids was evaporated from the solvent. The yield of the petroleum ether extract, chloroform extract, methanol extract, ethanolic extract and water extract and were found to be 0.83 % (w/w), 1.73 % (w/w), 25.5% (w/w), 28.1 % (w/w) and 25.9% (w/w) respectively. Ethanolic extract was selected for the experiment [12].

Drug treatment

The extract obtained was suspended for pharmacological studies in double distilled water comprising carboxy methyl cellulose (1 percent w/v CMC) at the dosage of 100, 200 mg/kg p. o. The dosage were calculated upon the basis of acute oral toxicity studies of ethanolic extract of *Madhuca longifolia* and were given to each mice in groups 4,5,7 and 8. Throughout the study, there was no death or mortality because of the medication. *Madhuca longifolia* extract, during the period of therapy, did not result into deaths and abnormality.

Animals

Animals have been procured from Animal House, Department of Pharmacy, SRMS CET (Pharmacy), Bareilly, U. P. Animals have been certified by IAEC (Institutional Animal Ethics Commitee), the committee responsible for the animal welfare (715/PO/Re/S/02/CPCSEA). The Swiss albino strain of mice were taken in identical numbers per group (*n*=6) from young, healthy adult mice of both sexes. Mice weighed about 25-30 gm. The experimental temperature of the animals was kept 22 °C (±3 °C). Relative humidity ranged from 50% to 60%. The series had artificial lighting, 12 h of darkness and another 12 h of light. Drinking water with normal laboratory diets was supplied ad-libitum. Control, normal and treatment groups were randomly allocated to healthy young adult mice. The animals were marked at the base of the tail. Prior to the initiation of this research, the mice were acclimatized in their cages for at least 5 d.

Chemicals and drugs

Drugs: Colchicine and Piracetam were bought from Sigma Aldrich.

Chemicals: Chloroform Ethyl Acetate, Ethanol, Petroleum ether and Methanol were bought from Central Drug House Laboratory (CDH).

Vehicle

In 1 percent w/v CMC, *Madhuca longifolia* extract (MLE) was suspended and orally given to the mice. Piracetam and Colchicine were dissolved in normal saline separately and given by i. c. v. and i. p. routes, respectively. Consumption by the oral route and i. p. route was 1 ml/100 g of mice.

HPTLC study

The ethanolic leaf extract of *Madhuca longifolia* was analyzed for the presence of flavonoids by comparing with the Rf value and spectral comparison with co-chromatographic standard compounds, Quercetin [13] (Wagner, 1996). HPTLC study was performed to standardize the extract of *Madhuca longifolia* leaves for the presence of flavonoids.

Determination of total flavonoids (TF)

The method, according to Kim [14] was proceeded to estimate the total flavonoid content. 1 ml ethanolic leaf extract of 1000 µg/ml concentration was taken and 4 ml of distilled water was added, followed by the addition of 0.3 ml $NaNO_2$ and 0.3 ml $AlCl_3$ to the solution. The mixture was incubated at room temperature for 5 min. To the incubated solution, 2 ml of sodium hydroxide and 2.4 ml distilled water were added and the absorbance was measured at 510 nm using a spectrophotometer. Total Flavonoid content was estimated from standard curve. Rutin and Quercetin were used as standards and Total Flavonoid content was expressed as rutin/Quercetin equivalents (RE/QE) in mg/g of the dry sample.

Determination of total phenols

Folin-Ciocalteu (FC) reagent was used for the determination of total phenolic content spectrophotometrically according to [15] with slight modifications. 0.1 ml of leaf extract (1 mg/ml) was taken in a test tube, 1.9 ml distilled water and 1.0 ml of Folin–Ciocalteau's reagent was taken in a test tube, and then added 1.0 ml of 100 g/l Na_2CO_3 to the solution. The mixture was stored under room temperature for 2 h and the absorbance of the solution was measured at 765 nm using a spectrophotometer. Total phenolic content was estimated from the standard curve of gallic acid. The total phenolic compounds of the plant extract were expressed as gallic acid equivalents (GAE) which denoted the phenolic content equal to the gallic acid (mg/g) of the dry material.

Oral toxicity studies

Ethanolic extract of the *Madhuca longifolia* leaves was researched for severe oral harmfulness as per reviewed OECD rules No.425. The extract was free from any harm in mice when administered in dosage of up to 2000 mg/kg orally. Hence, 100 and 200 mg/kg dosage of ethanolic leaf extract were used for the experiment.

The experimental design is tabulated in table 1.

Table 1: Experimental design

Group	Treatment	Dose (mg/kg)
I	Control	Vehicle
II	Piracetam	200 mg/kg, i. p
III	Colchicine	1 mg/kg, i. p
IV	Low dose *Madhuca longifolia*	100 mg/kg, p. o.
V	High dose *Madhuca longifolia*	200 mg/kg, p. o.
VI	Piracetam+Colchicine	200 mg/kg, i. p+1 mg/kg, i. p
VII	Low dose *Madhuca longifolia*+Colchicine	100 mg/kg, p. o.+1 mg/kg, i. p
VIII	High dose *Madhuca longifolia*+Colchicine	200 mg/kg, p. o.+1 mg/kg, i. p

Group I: It represented the control group. The vehicle was given orally for 28 d. The transmission latency was measured on 28th d and again after 90 min of administration on 29th d.

Group II: It represented the positive control group. Piracetam (200 mg/kg i. p.) was administered in mice for 28 consecutive days and the transmission latency was measured on 28th d after 60 min of administration and once again on 29th d after 24 h.

Group III: It represented the negative control group. Colchicine (1 mg/kg) was injected i. c. v to mice and transmission latency was assessed after a period of 45 min after injection and another time after 24 h (i.e. on the 29th d).

Group IV and V: MLE (100, 200 mg/kg, p. o.) were given through the mouth for 28 successive days to the mice. TL was measured after 90 min of administration on 28th d and again on 29th d after 24 h.

Group VI: Mice were administered piracetam (200 mg/kg, i. p.) for 28 d consecutively. Colchicine 1 mg/kg, was administered by i. c. v. route at 60 min after 28th d of piracetam administration. The Transfer Latency was noticed after 45 min of colchicine injection and again on the 29th d.

Group VII, VIII: MLE (100, 200 mg/kg, p. o.) was orally administered successively for 28 d to the mice and colchicine (1 mg/kg) was injected i. c. v. to mice at 90 min after administering the extract on 28th d. TL was measured 45 min later injection and also after 24 h.

Exteroceptive behavior models

Step through the passive avoidance paradigm

The long-term memory was tested using the passive avoidance model. This apparatus was composed of a small chamber connected through a guillotine door to a larger chamber. The smaller chamber, since it was lit with a 7W/12V lamp, was also called light chamber. Mice were initially given an acquisition trial and subsequently given a retention trial after 24 h followed by IInd, IIIrd and IV retention trials on successive days. In the acquisition trial, each mouse was

positioned at a maximum distance from the guillotine door in the smaller room. It noticed the time span the mouse had taken to reach the darker room. For the analysis, the mice which did not reach the door within a cut-off period (90s) were not used. The door was shut automatically after the mouse entered the dark room and an inevitable foot shock of 1 mA for 1 sec was provided. Within 10s the mouse had been removed from the dark room. This procedure has been replicated with standard, controls and test medicines. The rise in latency step-by-step was seen as learning [16].

Morris water maze

The MWM mission was utilized to test rodent spatial reminiscence and learning. It consists of a big rounded black tank with a girth of 120 cm, 50 cm height, filled with water at 26±2 °C up to a deepness of 30 cm. The pool with round shape was partitioned into four quadrants which were equal and an 8 cm^2platform was waterlogged 1 cm under the opaque superficial of one of the quadrants in the middle. The Platform's position was held steady throughout the study. The water was made colored with a black dye which is not toxic and to mask the position of the flooded platform. The mice were released one by one into the water and permitted to trace the platform for 120 sec. Animals were exposed to 2 trials per day for 4 d with an inter-trial period of 20 min and the time latency to trace the target was 10 sec. Through each test mice's escape latencies were registered. The mean for every testing session was calculated for each mouse by taking the considerations suggested. If the platform was found by the mouse it was allowed to be on it for 10 sec. If the mice didn't find the platform within 90 sec, it was positioned 10 sec upon the platform and removed from the group afterwards. Mice were initially given an acquisition trial and were given a retention trial after 24 h followed by IInd and III retention trials on successive days. Day by day in trial 1 the decrease in escape latency exemplifies lasting reminiscence or reference remembrance whereas that from trial 1 to trial 2 and 3 shows either temporary remembrance or operational memory [17].

Biochemical analysis

After administering colchicine on 28th d; the biochemical markers for the oxidative stress were examined in mice's brain like NO, GSH and AChE.

Brain tissue preparation

The mice were sacrificed by ether anaesthesia. After dissecting the skull, the brain was isolated. By using regular (chilled) saline solution, the brain was cleaned. At 10 strokes and 2000 rpm; 10 percent (w/v) homogeneous brain sample was prepared with 0.03 M Na_3PO_4 buffer (pH 7.4). NO, GSH and AChE were estimated by using homogenized brain tissue.

Scavenging action of nitric oxide

The scavenging tendency of nitric oxide was estimated using Griess reagent using the method described by Marcocci *et al.* 1994. This process dissolved 2 ml of 10 mmol of sodium nitroprusside in 0.5 ml of phosphate buffer saline (pH 7.4), which was combined with 0.5 ml of fractionated extracts of varying concentrations (50-200 µg/ml). The blend was incubated for 150 min at 25 °C. Then, 0.5 ml of the incubated solution was kept at room temperature for 5 min with 1 ml of naphthyl ethylenediamine dichloride (0.1 percent w/v) with 0.5 ml of Griess reagent [(1.0 ml of sulfanilic acid reagent (0.33 percent of 20 percent glacial acetic acid) at room temperature for 5 min with 1 ml of naphthyl ethylenediamine dichloride (0.1% w/v)]. The mixture was then incubated for thirty minutes at room temperature and its absorption was taken at 546 nm. By following this equation, the proportion inhibition of Nitric Oxide was calculated:

$$\% \text{ inhibition of NO radical} = (A0-A1)/A0 \times 100$$

In which A0 is the absorbance previous to the reaction and A1 is the absorbance afterward of the reaction occurred with Griess reagent [18].

GSH measurement

GSH was calculated by its 5, 5'-dithiobis (2-nitrobenzoic acid) reaction (Ellman, 1959), yielding a yellow chromophore that was calculated spectrophotometrically. GSH is a protein which is usually expressed in µg/mg. The homogenized brain tissue was centrifuged for 10 min at 700 g. The brain homogenate (500 µl) was combined with 10% trichloroacetic acid (500µl) and then centrifuged for protein separation at 2000 g for 10 min at 4 °C. 100 µl of supernatant was added to 2 ml of 0.1 M phosphate buffer (pH 7.4), 0.5 ml of 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double distilled water, accompanied by shaking the mixture on the vortex. The absorbance was recorded within 15 min. at 412 nm [19].

Activity of acetylcholinesterase

Acetylcholinesterase enzyme is responsible for the prolonged depletion of the brain's cholinergic system. The acetylcholinesterase levels of the brain are estimated by using Ellman's method. 0.05 ml of supernatant, 0.1 ml of DTNB (Ellman reagent), 3 ml of 0.01 M Na_3PO_4 buffer (pH 8) and 0.1 ml of acetylthiocholine iodide were used in this process. At a 30 s interval for 2 min; the absorbance shift at 412 nm was measured. The results were formulated by chromophore molar extinction coefficient (1.36 x 10^4 M^{-1} cm^{-1}) and expressed as acetylcholine hydrolysed/min/mg protein micromoles [20].

$$R=\frac{\delta \text{ OD x Capacity of Assay}}{\text{E x mg protein}} \text{x } 1000$$

Here R denotes the degree of enzyme action in 'micro' mole of acetylthiocholine iodide hydrolysed per minute per mg of protein.

δ OD denotes the change in absorbance per minute

E represents the extinction coefficient (1.36×10^4 M^{-1} cm^{-1}) [21, 22].

Protein estimation

In all brain samples, the protein was estimated by using Lowry's method, in which bovine serum albumin (BSA) (1 mg/ml) was taken as standard [23].

Reagents

1. Alkaline solution

a) 1% (w/v) $CuSO_4$.

b) 2% (w/v) Na_2 CO_4 in 0.1 M NaOH

c) 2% Sodium Potassium tartrate

Working alkaline solution: 48 ml of A+1 ml of B+1 ml of C

2. Stock standard is Bovine Serum Albumin (BSA)-1 mg/ml

3. Working standard is BSA (1000µg/ml) diluted the stock 20 times.

4. Folin-Phenol reagent (ice-cold) is diluted with equal amount of water instantly at the time of use.

Test method

0.1 ml supernatant was added to 0.9 ml DDW and 5 ml working alkaline solution. The mixture was well stirred followed by incubation at room temperature for 10 min. After this, 0.5 ml Folin-phenol reagent was incubated at room temperature. The absorbance was calculated at 750 nm against blank. A standard curve (50-1000µg) was plotted, followed by sample protein estimation as mg/ml [23].

Statistical analysis

All the findings were shown as average±SEM and evaluated by one-way ANOVA which was followed by Dunnett's posthoc test. A '*P*' value of <0.05 has been acknowledged as statistically important. Graph Pad prism software was used to analyze the data.

RESULTS

HPTLC study

The HPTLC chromatogram of Standard Quercetin (Rf value-0.46) is shown in fig. 1.

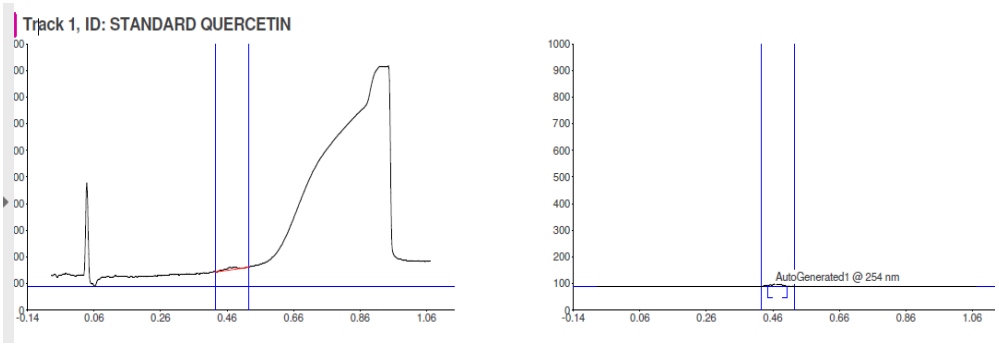

Fig. 1: HPTLC chromatogram of standard quercetin (Rf value-0.46)

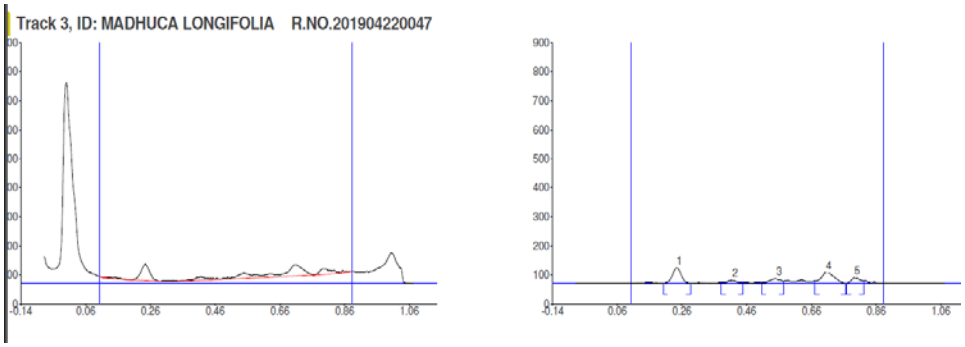

Fig. 2: HPTLC chromatogram of *Madhuca longifolia*

Table 2: HPTLC of *Madhuca longifolia* leaf extract

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	0.20	2.6	0.24	56.5	38.84	0.28	0.0	1242.3	31.57
2	0.38	2.8	0.41	11.6	8.00	0.45	4.1	328.1	8.34
3	0.50	2.9	0.55	17.9	12.29	0.57	8.5	533.9	13.57
4	0.67	8.9	0.70	38.9	26.72	0.76	0.0	1342.1	34.10
5	0.77	0.0	0.79	20.6	14.15	0.82	9.4	488.9	12.42

The HPTLC chromatogram of *Madhuca longifolia* is shown in fig. 2.

The Rf value and Maximum height is expressed in table 2.

Quercetin was identified by HPTLC study and the Rf value and area was found to be-

Std. Quercetin: Rf value = 0.46, Area= 718.5

***Madhuca longifolia* leaves:** Rf value = 0.50, Area= 533.9

Determination of total flavonoids

The total flavonoids of the leaf extract were expressed as Quercetin or rutin equivalents (QE/RE) which indicated the flavonoids content equal to the Quercetin or rutin (mg) in one gram of dry material. The total flavonoids content in *Madhuca longifolia* leaves was found to be (14.17±0.56 QE and 21.24±0.94 RE mg/g of dry material).

The standard curves of quercetin and rutin are represented in fig. 3 and fig. 4, respectively.

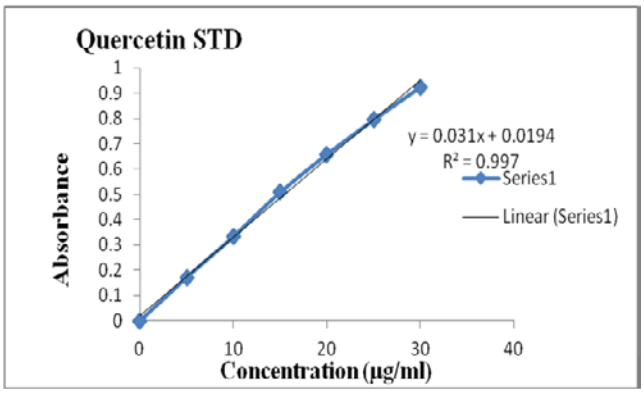

Fig. 3: Standard curve of quercetin

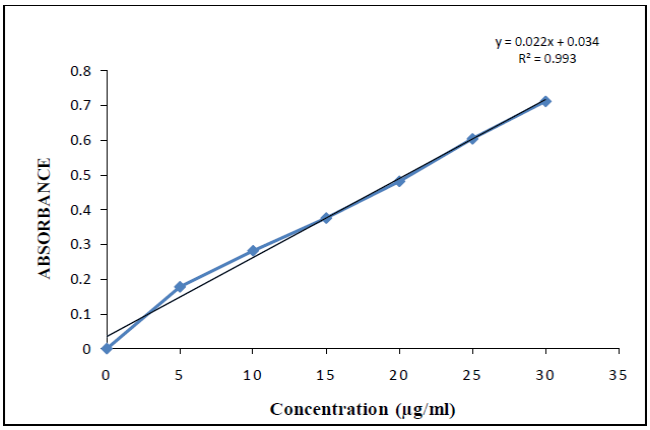

Fig. 4: Standard curve of Rutin

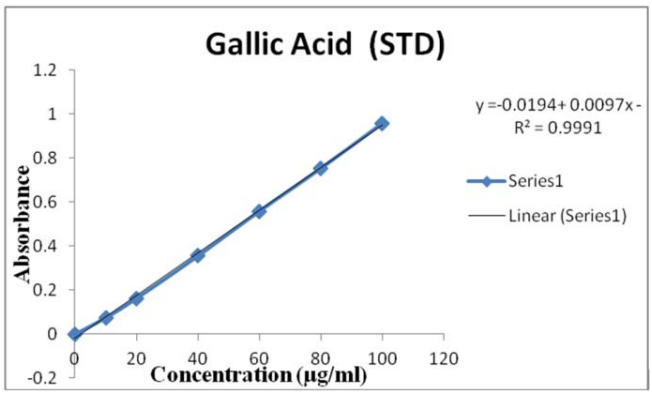

Fig. 5: Standard curve of gallic acid

Determination of total phenols

Total phenols of leaf extract were expressed in terms of gallic acid equivalents (GAE). It determines the phenolic content equal to the gallic acid (mg) in one gram of dry material. The total phenolic compounds in *Madhuca longifolia* leaves were 299.32±2.73 mg/g of dry material.

The standard curve of gallic acid is shown in fig. 5.

Step-through passive avoidance paradigm

The efficacy of *Madhuca longifolia* leaf extract on the step-through passive avoidance paradigm is represented in fig. 6. The acquisition trial was performed and no substantial difference was identified. Outcomes are stated as AVERAGE±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 when one-way ANOVA method followed by Dunnett's tests to compare with the control group. In the group that received leaf extract; a decrease in transfer latency was observed suggesting nootropic activity. The 200 mg/kg dosage of the *Madhuca longifolia* leaf extract showed a substantial decrease in the mice's transfer latency. This showed the efficient memory-enhancing activity of the flavonoid fraction of leaf extract of *Madhuca longifolia*.

Morris water maze

The effect of *Madhuca longifolia* leaf extract on morris water maze is shown in fig. 7. Results are articulated as AVERAGE±SEM (n=6), *P<0.05, **P<0.0 1, ***P<0.001 when one-way ANOVA method is followed by Dunnett's tests and compared with the control group. An increase in escape latency was observed, suggesting nootropic activity. The 200 mg/kg dosage of the *Madhuca longifolia* leaf extract showed a substantial improvement in the mice's escape latency. This shows the potential nootropic activity of the flavonoid fraction of *Madhuca longifolia* leaf extract.

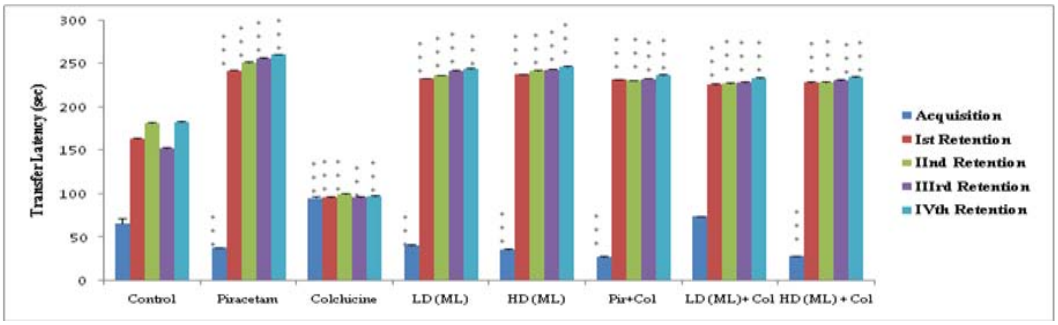

Fig. 6: Effect of *Madhuca longifolia* extract on passive avoidance paradigm

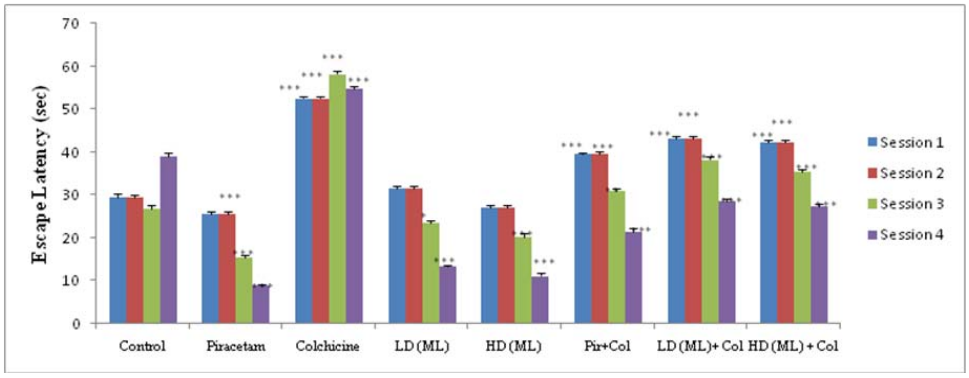

Fig. 7: Effect of *Madhuca longifolia* extract on morris water maze

Estimation of no

The impact of *Madhuca longifolia* leaf extract on the level of NO of mice's brain homogenate is represented in fig. 8. On the 29th d; the mice were sacrificed and the brain homogenate was prepared to measure changes in NO level. The statistics were represented as MEAN±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 when the one-way ANOVA test was followed by Dunnett's tests and compared with the control group. In the leaf extract (flavonoid fraction); a substantial decrease in the amount of NO was found, thus confirming its memory-enhancing property.

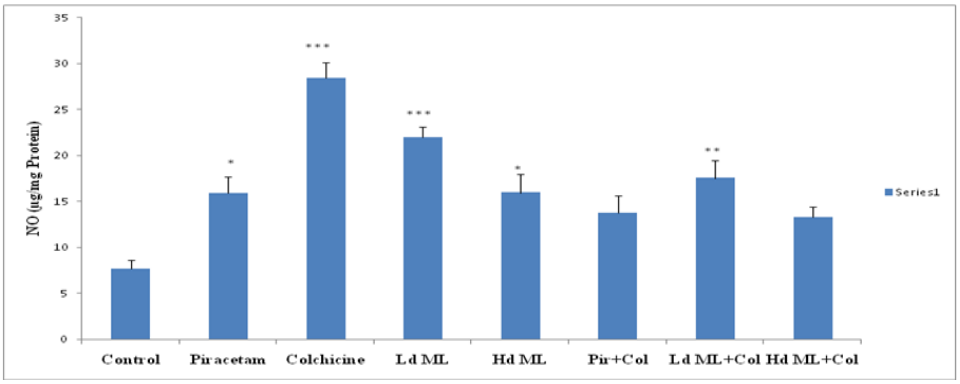

Fig. 8: Effect of ethanolic extract of *Madhuca longifolia* on NO level

Estimation of GSH

The response of *Madhuca longifolia* leaf extract on GSH level in brain homogenate of mice is represented in fig. 9. On the 29th d, the mice were sacrificed and brain homogenate was prepared to estimate the changes in GSH level. Statistics were represented in terms of MEAN±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 when the one-way ANOVA test was followed by Dunnett's tests and compared with the control group. The leaf extract (flavonoid fraction) showed a large increase in GSH level, thus confirming its nootropic property.

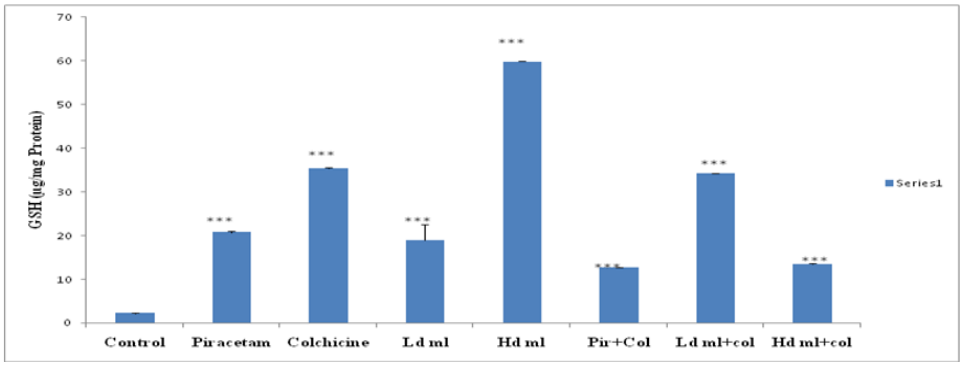

Fig. 9: Effect of ethanolic extract of *Madhuca longifolia* on GSH level

Estimation of AChE

The effect of *Madhuca longifolia* leaf flavonoid extract on AChE level in brain homogenate of mice is shown in fig. 10. On 29th d; the mice were sacrificed and brain homogenate was prepared to predict AChE levels. Statistics were calculated as MEAN±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 when the one-way ANOVA method was followed by Dunnett's tests and compared with the control group. In the leaf extract (flavonoid fraction), a reduction in AChE level was observed, proving nootropic activity.

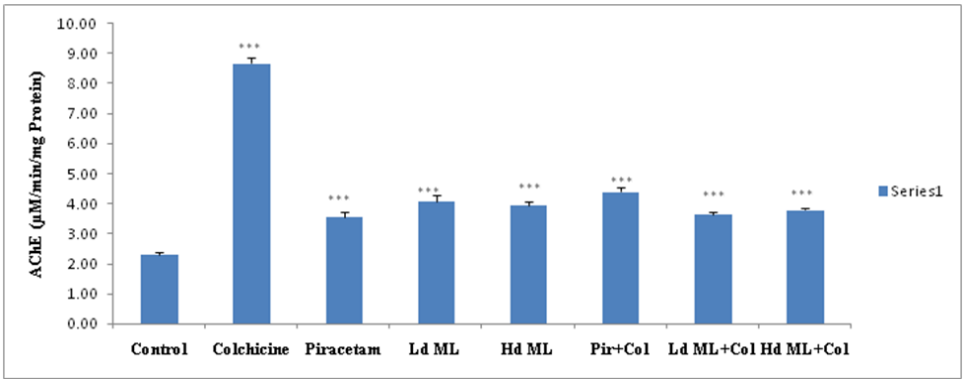

Fig. 10: Effect of ethanolic extract of *Madhuca longifolia* on AChE level

DISCUSSION

The disease of Alzheimer is a gradual onset of a neurodegenerative state. A perfect remedy for Alzheimer's disease has yet to be developed in allopathy. We can treat this disease by herbal medicines. *Madhuca longifolia* flavonoid extract was administered orally for 28 d, which showed an enhancement in mice's learning behaviour in the current study. The higher dose of *Madhuca longifolia* extract (200 mg/kg) significantly increased mice's memory due to increased transmission latency in the case of passive avoidance when compared to the control group. In morris water maze, there was a reduction in the escape latency when compared to the control group. The pretreatment with *Madhuca longifolia* flavonoid extract for 28 d protected the mice from memory dysfunction induced by colchicine. These observations indicate that Mahua has a potential neuroprotective activity.

CONCLUSION

Reactive oxygen species (ROS) are the root cause of age-related loss in cognitive ability that may be implicated in elderly people developing Alzheimer's disease. *Madhuca longifolia* has antioxidant properties too. *Madhuca longifolia* extract's neuroprotective activity is related to its antioxidant property due to which the susceptible neurons are subjected to less oxidative stress leading to reduced neuronal harm and enhanced neuronal function. From this analysis, it can be inferred that the fractionated ethanolic extracts of *Madhuca longifolia* at a dosage of 200 mg/kg has nootropic activity that is comparable to the regular Piracetam medication. *Madhuca longifolia* ethanolic leaf extract decreased NO; AChE and increased levels of GSH. Therefore *Madhuca longifolia* ethanolic leaf extract has major nootropic activity.

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ABBREVIATIONS

AChE: Acetyl Cholinesterase; MDA: Malondialdehyde; NO: Nitric oxide; GSH: Glutathione; ANOVA: Analysis of variance; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; TL: Transfer Latency; ML: *Madhuca longifolia*; AD: Alzheimer's Disease.

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CONFLICT OF INTERESTS

Writers affirmed that they don't have a conflicting interest.

REFERENCES

1. Jivad N, Rabiei Z. A review study on medicinal plants used in the treatment of learning and memory impairments. Asian Pac J Trop Biomed. 2014;4(10):780-9. doi: 10.12980/APJTB.4.2014APJTB-2014-0412.
2. Patel VS, Jivani NP, Patel SB. Medicinal plants with potential nootropic activity: a review. Res J Pharm Biol Chem Sci. 2014;5(1):1-10.
3. Xu J, Rong S, Xie B, Sun Z, Zhang L, Wu H. Rejuvenation of antioxidant and cholinergic systems contributes to the effect of procyanidins extracted from the lotus seedpod ameliorating memory impairment in cognitively impaired aged rats. Eur Neuropsychopharmacol. 2009;19(12):851-60. doi: 10.1016/j.euroneuro.2009.07.006, PMID 19716273.
4. Dhingra D, Parle M, Kulkarni SK. Memory enhancing activity of *Glycyrrhiza glabra* in mice. J Ethnopharmacol. 2004;91(2-3):361-5. doi: 10.1016/j.jep.2004.01.016, PMID 15120462.
5. Kulkarni PD, Ghaisas MM, Chivate ND, Sankpal PS. Memory enhancing activity of *Cissampelos pariera* in mice. Int J Pharm Pharm Sci. 2011;3(2):206-11.
6. Ramadas D, Ravishankar M, Shwetha S, Srinivas L. The learning and memory enhancing properties of *Bacopa monnieri* plant leaves protein: a systematic study in wistar albino rats model system. Sch Acad J Bio Sci. 2016;4:179-84.
7. Babawale OP, Taiye FR, Adetunji OS. Ethnobotanical survey of plants used as a memory enhancer in three states of Southwestern Nigeria. J App Pharm Sci. 2016;6(09):209-14. doi: 10.7324/JAPS.2016.60931.
8. Phukan P, Bawari M, Sengupta M. Promising neuroprotective plants from north-east India. Int J Pharm Pharm Sci. 2015;7(3):28-39.
9. Dahake AP, Chakma CS, Chakma RC, Bagherwal P. Antihyperglycemic activity of methanolic extract of *Madhuca longifolia* bark. Diabetol Croat. 2010;39(1):3-8.
10. Annalakshmi R, Uma R, Chandran GS, Sahayam CS, Charles A. Evaluation of physico-chemical constants and phytochemical analysis of *Madhuca longifolia*. Int J Nat Prod Res. 2012;1(3):64-6.
11. Kumar KP, Vidyasagar G, Ramakrishna D, Reddy IM, Atyam VG, Raidu CS. Screening of *Madhuca indica* for antidiabetic activity in streptozotocin and streptozotocin–nicotinamide induced diabetic rats. Int J PharmTech Res. 2011;3(2):1073-7.
12. Akhil M, Sarma SKD, Poornachandra RGVN, Jyothi Ch VS, Kumar RD. Evaluation of the anthelminthic activity of leaves of *Madhuca longifolia*. Int J Pharmacol Toxicol. 2014;4:99-104.
13. Wagner H, Bladt S. Plant drug analysis. Springer-Verlag, Berlin; 1996. p. 359-64.
14. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 2003;81(3):321-6. doi: 10.1016/S0308-8146(02)00423-5.
15. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Vitic. 1977;28(1):49-55. doi: 10.5344/ajev.1974.28.1.49.
16. Vogel HG. Drug discovery and evaluation pharmacological assays. Berlin, Heidelberg: Springer-Verlag, Germany; 2002. p. 620.
17. Su J, Sripanidkulchai K, Wyss JM, Sripanidkulchai B. *Curcuma comosa* improves learning and memory function on ovariectomized rats in a long-term morris water maze test. J Ethnopharmacol. 2010;130(1):70-5. doi: 10.1016/j.jep.2010.04.012, PMID 20420894.

18. Marcocci I, Marguire JJ. Droy-lefaiz MT, Packer L. The nitric oxide scavenging properties of *Ginkgo biloba* extract. Biochem Biophys Res Commun. 1994;2(1):748-55.
19. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7. doi: 10.1016/0003-9861(59)90090-6, PMID 13650640.
20. Ellman GL, Courtney KD, Andres Jr V, Feather Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95. doi: 10.1016/0006-2952(61)90145-9, PMID 13726518.
21. Joshi H, Parle M. Antiamnesic effects of *Desmodium gangeticum* in mice. Yakugaku Zasshi. 2006;126(9):795-804. doi: 10.1248/yakushi.126.795, PMID 16946593.
22. Pitchaimani V, Arumugam S, Thandavarayan RA, Thiyagarajan MK, Aiyalu R, Sreedhar R. Nootropic activity of acetaminophen against colchicine induced cognitive impairment in mice. J Clin Biochem Nutr. 2012:1-4.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75. doi: 10.1016/S0021-9258(19)52451-6, PMID 14907713.