Original Article

Investigation of Memory Enhancing Activity of *Madhuca longifolia* Leaf Extract against Colchicine: An Experimental Study and Biochemical Alterations in Mice

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ABSTRACT

Objectives: 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. **Materials and Methods:** The analysis was conducted on a colchicine-induced model for 28 days. Morris water maze and passive avoidance paradigm were utilized for behavioral experiments, while biochemical parameters such as nitric oxide and glutathione were estimated. Swiss albino mice (48) were apportioned into eight sets, each consisting of six mice. 1 percent w/v carboxy methyl cellulose was apportioned to the first Group. The second group got 200 milligrammes/kg of Piracetam i.p. Group III received 1 mg / kg i.c.v. colchicine. The fourth and fifth set of groups was given 100 and 200 mg / kg ethanolic *Madhuca longifolia* leaf extract. Group VI has been given Piracetam (200 mg / kg, i.p.) for 28 days and Colchicine 1 mg / kg, i.p., at 60 min after 28th day piracetam injection. Group VII and VIII obtained oral extracts of *Madhuca longifolia* leaf 100 and 200 mg/kg for 28 days and injected colchicine (1 mg / kg) i.c.v for 90 mins after extract administration on the 28th day. ANOVA (one-way) was utilized and then followed by the test of Dunnett's and finally outcomes analyzed. **Results:** *Madhuca longifolia* leaf extract indicated morris water maze and had a substantial reduction in transfer latency. The transfer latency of the passive avoidance model showed a substantial increase. 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 and NO. **Conclusion:** *Madhuca longifolia* has neuroprotective effect against memory damage caused by colchicine.

Key words: *Madhuca longifolia*, Neuroprotective, Alzheimer's disease, Colchicine, HPTLC, Cognition.

INTRODUCTION

The ailment of Alzheimer 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 years. Study has revealed that globally, approximately fifteen million people suffer from the disease of Alzheimer.[1] Alzheimer's disease usually affects individuals around the age of 65 years.[2] The central nervous system 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 the disease of Alzheimer

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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. In the forebrain acetylcholine, cholinergic cell loss is responsible for the development of dementia.[5] The major risk factors for neurodegenerative disorders are toxins, stress and genetic predilection.[6] The treatment, prevention and diagnosis of a number of diseases are usually dealt with by the use of the traditional medicine. Treatment of various diseases by medicinal plants relies on findings and prior experiences contained in books or orally taught.[7] Neuroprotection includes the therapeutic techniques that can postpone or cure neuronal damage. Herbalism is effective, safer and cheaper as it is in fashion.[8]

Madhuca longifolia belongs to the family of Sapotaceae and is also known as Mahua.[9] The name Madhuca is derived from "Madhu", i.e. honey and is also referred to as the Indian butter tree. Mahua is a deciduous and medium sized tree found in India, Nepal and Srilanka.[10] In all parts of it, Mahua has many medicinal qualities. Fruit-refrigerant, aphrodisiac, tonic and antiulcerative. Leaf-healing wounds, anthelmintic, emollient and antirheumatic. Seed- Diuretic, refrigerant, liquor, hepato-protective, increased production of woman's milk and antihelmintic. Bark-tonsillitis, stomach upset, anti-venom in snake-poisoning.[11] It consists of various phytoconstituents including flavonoids, triterpenoids, glycosides, saponins and steroids.[12]

MATERIALS AND METHODS

Plant material

Madhuca longifolia L. leaves were picked from the Shri Ram Murti Smarak College Campus; a college specialized in Engineering and Technology. 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 hr. The marc obtained was then successively extracted with chloroform (50-60°C) followed by ethanol (68-78°C) for 24 hr. 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, 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 drop wise 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.[13]

Drug treatment

The extract obtained was suspended for pharmacological studies in double distilled water comprising carboxy methyl cellulose (1 percent w / v CMC) at 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 8,7,6 and 4. At the end of 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 collected from Animal Building, Department of Pharmacy, SRMS CET (Pharmacy), Bareilly, U.P. Animals have been certified by the committee responsible for the animal welfare (715 / PO / Re / S/02 / CPCSEA). The Swiss albino strains were taken in identical numbers per group (n=6) from young healthy adult mice of both sexes. At the research beginning, the mass dissimilarities of the faunas used were kept nominal and did not exceed ± 20 per cent of the average mass of individual species. Mice appeared to weigh 25-30 gm.

The experimental temperature of the animals was held at 22°C (±3°C). Relative humidity ranged from 50% to 60%. The series had artificial lighting, 12 hr of darkness and another 12 hr of light. Drinking water with normal laboratory diets was supplied *ad-libitum*. Animals of the similar breed had been living in a single cage together. Control, normal and treatment groups were randomly allocated to healthy young adult mice. The animals were labeled at the base of the tail and acclimatized in their cages for at least 5 days before research initiation.

Chemicals and Drugs

Drugs: Piracetam and Colchicine 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. Colchicine and Piracetam were liquefied in regular saline separately and administered by i.c.v. and i.p. routes respectively. Consumption through the mouth and i.p. administration was 1ml/100 g of mice.

HPTLC Study

The ethanolic leaf extract of *Madhuca longifolia* was analyzed for the presence of flavonoids by comparing with the R*f* value and spectral comparison with co-chromatographic standard compounds, Quercetin (Wagner 1996). HPTLC study was performed to standardize the extract of *Madhuca longifolia* leaves for the presence of flavonoids.[14]

Determination of total flavonoids (TF)

The method according to Kim (Kim *et al*. 2003) 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 addition of 0.3 ml $NaNO_2$ and 0.3 ml $AlCl_3$ to 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 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.[15]

Determination of total phenols

Folin-Ciocalteu (FC) reagent was used for the determination of total phenolic content spectro-photometrically according to (Slinkard and Singleton 1977) with slight modifications. 0.1 ml of leaf extract (1mg/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 hr and the absorbance of the solution was measured at 765 nm using spectrophotometer. Total phenolic content was estimated from 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.[16]

Studies with serious toxicity

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

The experimental design is tabulated in Table 1.

Group I: It characterized the control set. The vehicle was orally given for 28 consecutive days and the transmission latency was assessed on 28th day and again on 29th day after 90 min of administration.

Group II: For young mice it exemplified positive control group. Piracetam (200 mg/kg i.p.) was inoculated into mice for 28 consecutive days and transmission latency was assessed on 28th day after 60 min of administration and again on 29th day after 24 hrs.

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

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 noticed on 28th day after 90 min of administration and after sometime on 29th day after 24 hr.

Group VI: Mice received injection of piracetam (200 mg/kg, i.p.) for 28 consecutive days. Colchicine 1 mg/kg, i.p., at 60 min after 28th day of piracetam injection was given. TL was seen after 45 min of colchicine administration and another time on the 29th day.

Group VII, VIII: MLE (100, 200 mg / kg, p.o.) was orally given successively for 28 days to the mice and colchicine (1 mg / kg) was inoculated i.c.v. to mice at 90 min following excerpt administration on 28th day. TL was seen 45 min after inoculation and 24 hr.

Table 1: Experimental Design.

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

Models Which Have Exteroceptive Behavior

Step through 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 acquisition trial and subsequently given retention trial after 24 hr 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.[17]

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 rounded shape was partitioned into four quadrants which are equal and an 8cm^2 platform 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 colored with a black dye which is not toxic 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 days with an inter-trial period of 20 min and the time latency to trace the target was little (< 10 sec). Through each test mice's escape latencies were registered. The mean for every testing session were 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 detached from the group afterwards. Mice were initially given acquisition trial and were given retention trial after 24 hr 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.[18,19]

Biochemical Analysis

On the 28th day after Colchicine injection the biochemical parameters for oxidative stress such as NO and GSH were calculated in mice's brain.

Brain tissue preparation

Using ether anaesthesia the mice were sacrificed. The brain was plucked out after cutting the skull. The brain was cleansed using regular (chilled) saline solution. 10 percent (w / v) homogeneous brain sample was obtained with 0.03 M Na_3PO_4 buffer (pH 7.4) at 10 strokes at 2000 rpm. NO and GSH were measured using homogenized preparation of brain tissue.

Scavenging action of Nitric Oxide

The scavenging tendency of nitric oxide was probed using Griess reagent using the method described in Marcocci *et al.* 1994. This process dissolved 2 mL of 10 mM of sodium nitropruside in 0.5 mL of phosphate buffer saline (pH 7.4), which was combined with 0.5 mL of 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 put together at the temperature of the room 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 temperature of the room for 5 min with 1 mL of naphthyl ethylenediamine dichloride (0.1% w/v)]. The mix was then incubated for thirty minutes at the temperature of the room 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 afterwards of the reaction occured with Griess reagent.[20]

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 expressed in µg / mg. For 10 min homogenized brain tissue was centrifuged at 700 g. 500 µl of brain homogenate 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 mix on the vortex. The absorbance recorded within 15 min. at 412 nm.[21]

Protein estimation

In all brain samples, protein was calculated by means of Lowry 's method where bovine serum albumin (BSA) (1 mg / ml) was utilized as a standard.[22]

Reagents

1. Alkaline solution
   a) 2% (w/v) $Na_2 CO_4$ in 0.1 M NaOH.
   b) 1% (w/v) $CuSO_4$
   c) 2% Sodium Potassium tartrate

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

2. Stock std. Bovine Serum Albumin (BSA) - 1mg/ml
3. Working standard BSA (1000µg/ml) diluted the stock 20 times.
4. Folin-Phenol reagent (ice-cold) diluted with equal amount of water at the time of use.

Test Method

0.1ml of supernatant was added to 0.9ml DDW and 5ml of working alkaline reagent. The mixture was well mixed and then incubated at high temperature of the room for 10 min. Following this, 0.5 ml Folin-phenol reagent was applied and incubated at room high temperature for 30 min. The absorbance had been calculated against blank at 750 nm. Thereafter a standard curve (50-1000µg) was plotted followed by sample protein content estimate as mg / ml.[22]

Statistical Analysis

All of the findings were shown as average ± SEM and evaluated by ANOVA which is One-way method then ensued by Tukey's numerous post-hoc contrast trials. A '*P*' value of < 0.05 has been acknowledged as statistically important. Graph Pad prism software analyzed the data.

RESULTS

HPTLC Study

The HPTLC chromatogram of Standard Quercetin (R*f* value - 0.46) is shown in Figure 1.

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

The R*f* value and Maximum height is expressed in Table 2.

Quercetin was identified by HPTLC study and the R*f* value and area was found to be-

Std. Quercetin: R*f* value = 0.46, Area= 718.5

Madhuca longifolia leaves: R*f* value = 0.50, Area= 533.9

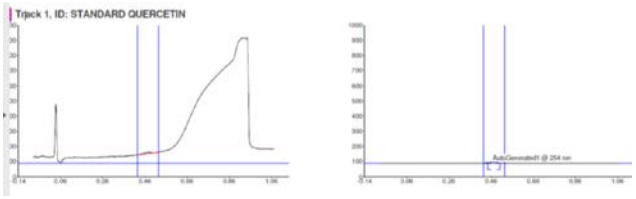

Figure 1: HPTLC chromatogram of Standard Quercetin (R_f value - 0.46).

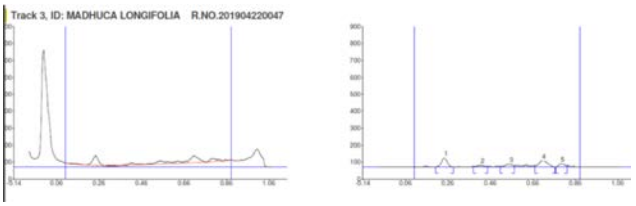

Figure 2: HPTLC chromatogram of *Madhuca longifolia.*

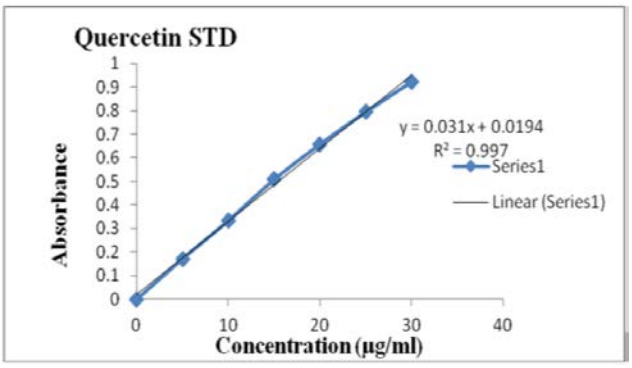

Figure 3: Standard curve of Quercetin.

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 Figure 3 and Figure 4 respectively.

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 Figure 5.

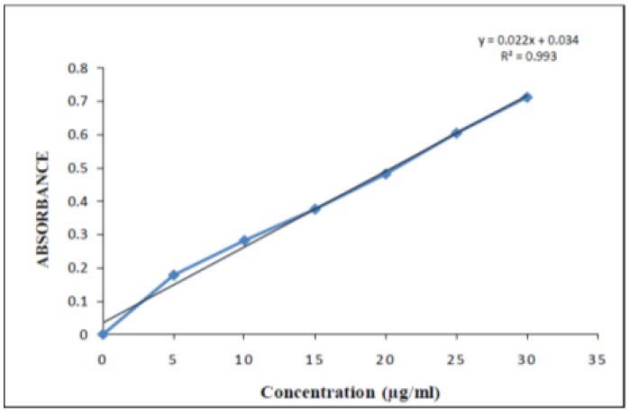

Figure 4: Standard curve of Rutin.

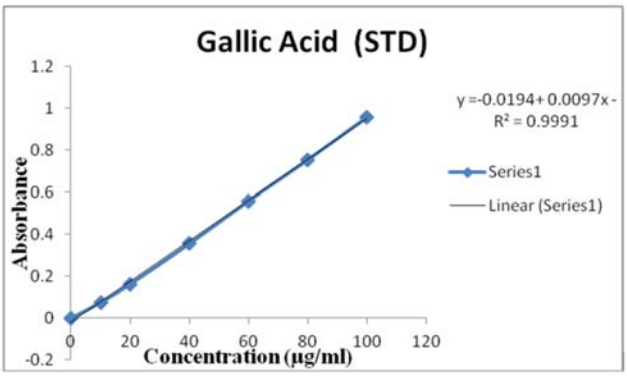

Figure 5: Standard curve of Gallic acid. Step-through Passive avoidance paradigm.

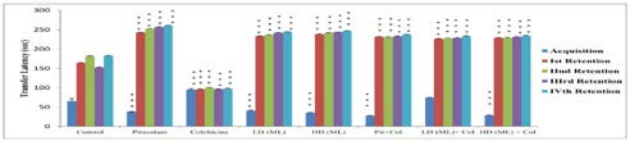

Figure 6: Effect of *Madhuca longifolia* extract on Passive avoidance paradigm.

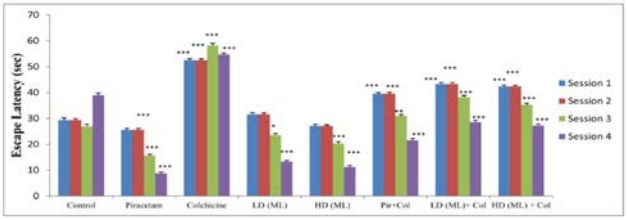

Figure 7: Effect of *Madhuca longifolia* extract on Morris water maze.

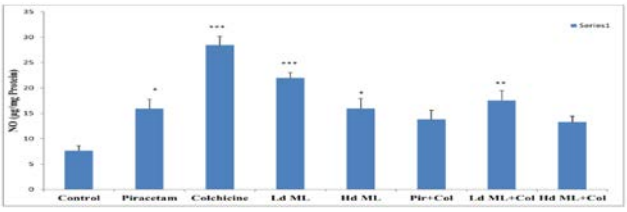

Figure 8: Effect of ethanolic extract of *Madhuca longifolia* on NO level.

The effect of *Madhuca longifolia* leaf extract on step-through passive avoidance paradigm is shown in Figure 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<0.001when ANOVA with One-way method then Tukey's tests ensues vis-a-viz control group. In the leaf extract a decrease in transfer latency was observed suggesting nootropic activity. The 200 mg / kg dosage of the *Madhuca longifolia* leaf extract showed substantial decreases in the mice's transfer latency. This shows the potential nootropic activity of leaf extract of *Madhuca longifolia*.

Morris water maze

The effect of *Madhuca longifolia* leaf extract on morris water maze is shown in Figure 7. Results are articulated as AVERAGE ± SEM (n=6), * P<0.05, * * P<0.0 1,

Table 2: R_f value and Maximum height

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.20	0.6	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

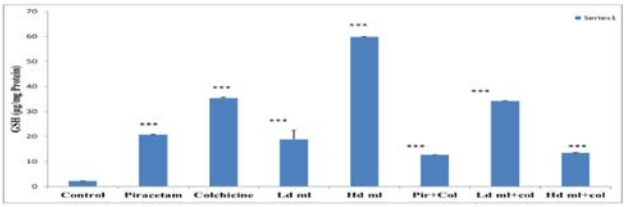

Figure 9: Effect of ethanolic extract of *Madhuca longifolia* on GSH level.

* * * *P*<0.001when with One-way ANOVA method then Tukey's tests ensues vis-a-viz to control group. In the leaf extract an increase in escape latency was observed suggesting nootropic activity. The 200 mg / kg dosage of the *Madhuca longifolia* leaf excerpt showed a substantial improvement in the mice's escape latency. This shows the potential nootropic activity of leaf extract of *Madhuca longifolia*.

Estimation of NO

The impact of *Madhuca longifolia* leaf excerpt on level of NO of mice's brain homogenate is shown in Figure 8. The mice were forfeited on the 29th day and the brain homogenate was prepared to predict NO level changes. The statistics are shown as AVERAGE ± SEM (*n*=6), * *P*<0.05, * * *P*<0.01, * * * *P*<0.001when with One-way method then Tukey's tests ensues vis-a-viz the control group. In the leaf extract a substantial decrease in the amount of NO was observed, thus confirming nootropic activity.

Estimation of GSH

The effect of *Madhuca longifolia* leaf extract on GSH level of mice's brain homogenate is shown in Figure 9. The mice were sacrificed on the 29th day and the brain homogenate was prepared to estimate GSH level changes. Statistics are articulated as AVERAGE ± SEM (*n*=6), * *P*<0.05, * * *P*<0.01, * * * *P*<0.001 when ANOVA with One-way method then Tukey's tests ensues vis-a-viz the control group. The leaf extract showed a large increase in GSH level, thus confirming nootropic activity.

DISCUSSION

The disease of Alzheimer is a gradual onset of neurodegenerative condition. A suitable treatment for full cure of Alzheimer's disease has yet to be developed in the allopathic field of medicine. So, we should look forward to treat this disease with the herbal medicines. In the current study *Madhuca longifolia* extract was given by oral route for 28 days that showed an increase in mice's learning behavior. In this study, the higher dose of *Madhuca longifolia* extract (200 mg / kg) significantly enhanced mice's memory as there was an increased Transmission Latency in a case of passive avoidance testing in vis-à-vis the control set. In the case of morris water maze, it had a decline in the Escape Latency as matched with the control set. For 28 days, pretreatment with *Madhuca longifolia* extract secured the fauna from memory insufficiencies caused by colchicine. These observations indicate that Mahua has a potential neuroprotective function.

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 ethanolic extracts of *Madhuca longifolia* at a dosage of 200mg / kg has nootropic activity that is comparable to the regular Piracetam medication. *Madhuca longifolia* ethanolic leaf extract decreased NO and increased levels of GSH. Therefore *Madhuca longifolia* ethanolic leaf extract has major nootropic activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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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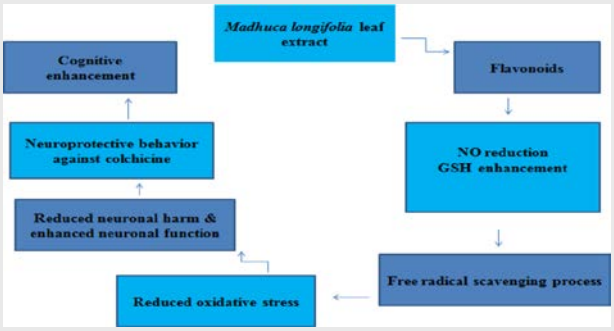
PICTORIAL ABSTRACT



SUMMARY

The neuroprotective effect of *Madhuca longifolia* leaves was tested by a colchicine-induced dementia model in swiss albino mice. Different behavioral and biochemical parameters were calculated in mice exposed to colchicine and plant extract. Chronic administration of colchicine resulted in severe memory losses, which were also biochemically endorsed as increased oxidative stress as well as elevated brain levels of acetylcholinesterase (AChE), NO and MDA. Pretreatment of *Madhuca longifolia* leaves with alcoholic extract in colchicine-exposed animals significantly improved memory deficits as well as decreased oxidative stress, AChE, NO, MDA and increased levels of GSH. As proof of an increase in the neurological score of Acetyl cholinesterase, lipid peroxidation, glutathione, nitric oxide and protein, the extract used proved to have neuroprotection. This can be due to the presence of *Madhuca longifolia* leaf flavonoids, alkaloids, steroids and tannins.

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