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

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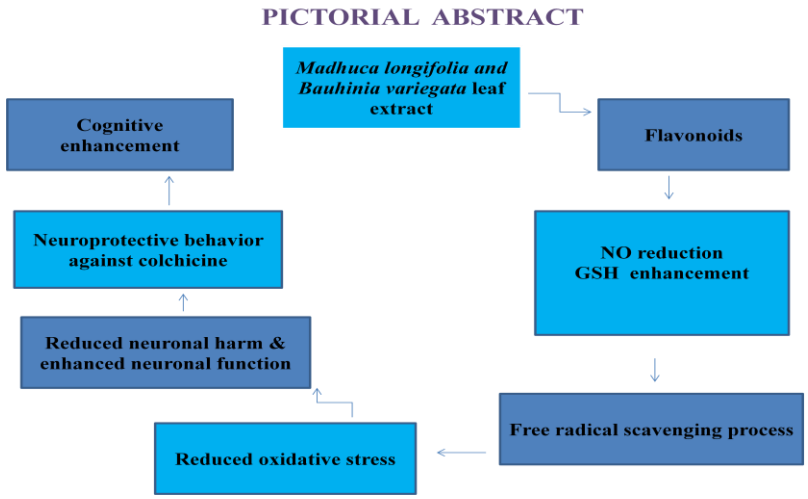
ABSTRACT

Objectives: To investigate the role of combination of *Madhuca longifolia* and *Bauhinia variegata* ethanolic leaf extracts against oxidative damage in swiss albino mice and colchicine induced cognitive dysfunction; to estimate the neuroprotective impact using *Madhuca longifolia* and *Bauhinia variegata* combination by behavioral testing and to analyze the biochemical parameters. **Materials and Methods:** The experimental analysis was done for 28 days on a colchicine-induced model. Passive avoidance paradigm and Morris water maze were used for behavioral experiments and biochemical parameters like glutathione and nitric oxide were analyzed. 36 swiss albino mice were grouped into six groups, each consisting of six mice. 1 percent w/v carboxy methyl cellulose (CMC) was administered to the Group I. The group II received ED (BV) +ED (ML) (400mg/kg+200mg/kg oral). Group III was administered ED(BV)+ED(ML)+Col(400mg/kg+200mg/kg+1mg/kg,i.c.v.). The fourth group got ED(BV)+ED(ML)+Pir(400mg/kg+200mg/kg oral+200mg/kg, i.p.). Group V has been given LDBV+LDML(200mg/kg +100 mg/kg oral) for 28 days and Colchicine 1 mg / kg, i.p., at 60 min after 28th day piracetam injection. Group VI received HDBV+HDML(400mg/kg +200 mg/kg oral). ANOVA (one-way) was followed by Tukey's test and then the results were analyzed. **Results:** *Madhuca longifolia* and *Bauhinia variegata* ethanolic leaf extracts indicated a decrease in transfer latency of mice in morris water maze. The transfer latency in passive avoidance model showed a good increment. The *Madhuca longifolia* and *Bauhinia variegata* leaf extract combination showed an increment (P<0.001) in GSH levels and a decrease (P<0.001) in NO and total protein. **Conclusion:** The combination of *Madhuca longifolia* and *Bauhinia variegata* has neuroprotective effect against memory damage caused by colchicine.

Key words: *Madhuca longifolia*, Neuroprotective, Alzheimer's disease, *Bauhinia variegata*, Colchicine, acetylcholinesterase and cognition.



INTRODUCTION

Alzheimer's disease is a gradual, long-term neurological disease that causes memory loss, behavioral changes, reasoning capability, personality and the ability to think. Alzheimer's disease is the leading cause of dementia among older adults. It takes roughly 8.5 years from the onset of symptoms until death. According to a study, roughly fifteen million people worldwide suffer with Alzheimer's disease.[1] Alzheimer's disease often strikes people around the age of 65 years.[2] More free radical production frequently affects the central nervous system. Excessive free radical production can harm DNA, membrane lipids and proteins in neurons. Because acetylcholine levels in the brain are lower in the aged, there is a notable decline in cholinergic neurotransmission.[3] Dementia is linked to the prevalence of Alzheimer's disease, which causes cell loss in numerous parts of the brain.[4] The development of neurotic plaque containing amyloid protein is a hallmark of Alzheimer's disease. The loss of cholinergic cells in the forebrain is responsible for the development of dementia.[5] Toxins, stress and genetic predisposition are the main risk factors for neurodegenerative illnesses.[6] Traditional medicine is commonly used for the treatment, prevention and diagnosis of a variety of ailments. The use of medicinal plants to treat various diseases is based on findings and earlier experiences recorded in texts or taught verbally.[7] Neuroprotection refers to treatments that can prevent or reverse neuronal injury. Herbalism is not only fashionable, but it is also effective, safer and less expensive.[8] *Madhuca longifolia*, often known as Mahua, is a member of the Sapotaceae family.[9] Madhuca is also known as

the Indian butter tree and is derived from the word "Madhu," which means "honey." Mahua is a medium-sized deciduous tree native to India, Nepal, and Sri Lanka.[10] Mahua has several therapeutic properties in all of its parts. Fruit-cooling, aphrodisiac, tonic and antiulcerative properties. Leaves are anthelmintic, emollient and antirheumatic. Seeds have diuretic, refrigerant, liquor, hepatoprotective, improved milk production and antihelmintic properties. Anti-venom in snake poisoning, bark-tonsillitis, stomach discomfort.[11] Flavonoids, triterpenoids, glycosides, saponins and steroids are among the phytoconstituents found in it.[12] Mountain Ebony (English), Rakta kanchan (Marathi), and Kachnar (Hindi) are all names for *Bauhinia variegata*, which belongs to the Leguminosae family (Caesalpinioideae). It is a medium-sized deciduous tree that grows at a height of 1800 metres in the Himalayas in India. Leaflets are oval, rounded at apex, 10-15cm long, pubescent beneath when young, leaves are wider, firmly sub-coriaceous, deeply cordate with two leaflets, connate for approximately two-thirds of the way up, leaflets are ovate, rounded at apex, 10-15cm long, pubescent beneath when young. Flowers are distinct in colour, lateral, sessile, with five stamens and no staminodes, and flat fruits; rough glabrous dehiscent seeds, seeded 10-15.[13] It is spreading over India and China. It is a dependable greenhouse that grows at an altitude of 1800 metres in the Himalayas.[14] Traditional uses for *Bauhinia variegata* Linn. include bronchitis, intestinal worms, diarrhoea, bacterial infection, hepatic problems, dysentery, skin illness, leprosy, wounds, ulcers, fungal infection and tumours.[15,16]

MATERIALS AND METHODS

Plant material

Madhuca longifolia and *Bauhinia variegata* leaves were collected from Shri Ram Murti Smarak College Campus. The collected sample specimen was kept as a reference for future studies at the institutional herbarium (specimen number- RU/PS/2016/415).

Extracts Preparation

The leaves of *Madhuca longifolia* and *Bauhinia variegata* were washed with tap water, dried in the shade, and pulverised. In a Soxhlet column, this powder was packed. Petroleum ether (60-80°C) was used to start the extraction, which lasted for 24 hours. After that, the marc was extracted with chloroform (50-60°C) and then ethanol (68-78°C) for 24 hours. To concentrate the extracts, they were maintained in a water bath at 50°C. After concentrating the preparation, the dried powder extract was stored at room temperature. Solvent was extracted under decreased pressure using a rotary evaporator to obtain dried ethanolic extract, which was then diluted in water. Chloroform extraction was performed in a separating funnel and a 10% NaCl solution was added dropwise to the aqueous layer to precipitate the tannins. The crude portion of flavonoids was evaporated from the solvent after

partitioning the supernatant liquid with ethyl acetate. 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)] for *Madhuca longifolia* and [9.50 percent (w/w), 7.65 percent (w/w), 8.95 percent (w/w), 8.50 percent (w/w) and 0.30 percent (w/w)] for *Bauhinia variegata* leaf extracts respectively. Ethanolic extract was selected for the experiment.[17]

Drug treatment

The extracts obtained were 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. and 200, 400 mg/kg p.o. for *Madhuca longifolia* and *Bauhinia variegata* respectively. The dosage were calculated upon the basis of acute oral toxicity studies of ethanolic extracts of *Madhuca longifolia* and *Bauhinia variegata* and were given to each mice in combination. At the end of the study, there was no death or mortality because of the medication. The combination extract of *Madhuca longifolia* and *Bauhinia variegata*, during the period of therapy did not result into deaths and abnormality.

Animals

Animals were gathered from the Animal Building of the SRMS CET (Pharmacy), Bareilly, Uttar Pradesh. The committee in charge of animal welfare (715 / PO / Re / S/02 / CPCSEA) has certified the animals. The Swiss albino strains were collected from young healthy adult mice of both sexes in identical numbers per group (n=6). The mass dissimilarities of the faunas employed were kept nominal at the start of the study and did not surpass 20% of the average mass of individual species. Mice appeared to weigh between 25 and 30 grammes. The animals were kept at a constant temperature of 22°C (3°C). The relative humidity was between 50 and 60 percent. Artificial lighting, 12 hours of darkness, and another 12 hours of light were used in the series. Water was provided ad libitum along with standard laboratory meals. Animals of the same breed had been housed together in a single cage. Healthy young adult mice were randomly assigned to the control, normal and treatment groups. Before the study began, the animals were labelled at the base of their tails and acclimatized in their cages for at least 5 days.

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 (ML) and *Bauhinia variegata* (BV) were 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.

Serious Studies which are toxic

According to the reviewed OECD standards No.425, ethanolic extracts of *Madhuca longifolia* and *Bauhinia variegata* plants were investigated for severe oral toxicity. When given to mice in doses of up to 2000 mg/kg via the oral route, the extract was found to be safe. The experiment employed 100 and 200 mg/kg mahua and 200 and 400 mg/kg kachnar dosages of ethanolic leaf extract.

Table 1: Experimental Design

Group	Treatment	Dose (mg/kg)
I	Control	Vehicle
II	ED(BV)+ED(ML) (400mg/kg+200mg/kg oral)	400, 200mg/kg, i.p
III	ED (BV) +ED(ML)+Col (400mg/kg+200mg/kg+1mg/kg, i.c.v.)	400, 200, 1mg/kg, i.p
IV	ED (BV) +ED(ML)+Pir(400mg/kg+200mg/kg oral+200mg/kg , i.p.)	400, 200, 200 mg/kg, p.o.
V	LDBV+LDML (200mg/kg +100 mg/kg oral)	200, 100mg/kg, p.o.
VI	HDBV+HDML (400mg/kg +200 mg/kg oral)	400, 200mg/kg, i.p+1mg/kg, i.p

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: A combination effective dose of *Bauhinia variegata* and *Madhuca longifolia* (400mg/kg+200mg/kg oral) 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: A combination effective dose of *Bauhinia variegata* and *Madhuca longifolia* (400mg/kg+200mg/kg oral) and colchicine (1mg/kg, i.c.v.) was inoculated to mice and transmission latency was assessed after a period of 45 min after inoculation and another time after 24 hrs (i.e. on the 29th day).

Group IV: A combination effective dose of *Bauhinia variegata* and *Madhuca longifolia* (400mg/kg+200mg/kg oral) and piracetam (200 mg/kg, i.p.) was administered to mice 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 V: A combination of low doses of *Bauhinia variegata* and *Madhuca longifolia* (200mg/kg+100mg/kg oral) was administered to mice for 28 consecutive days. TL was observed after its administration on 28th day and again on the 29th day.

Group VI: A combination of high doses of *Bauhinia variegata* and *Madhuca longifolia* (400mg/kg+200mg/kg oral) was administered to mice for 28 consecutive days. TL was seen 45 min after inoculation and also after 24 hrs.

Models Which Have Exteroceptive Behavior

Step through Passive avoidance paradigm

The passive avoidance paradigm was used to assess long-term memory. This device consisted of a tiny room connected to a bigger chamber by a guillotine door. The smaller chamber was also known as the light chamber since it was lighted by a 7W/12V bulb. Mice were given an acquisition trial first, then a retention trial after 24 hours, followed by a second, third, and fourth retention trial on successive days. In the acquisition trial, each mouse was placed as close to the guillotine door as possible in the smaller room. It took notice of how long the mouse took to go to the darker room. The mice that did not reach the door within a 90-second time limit were not used in the study. After the mouse entered the dark chamber, the door closed automatically, and the mouse received an unavoidable 1 mA for 1 sec foot shock. The mouse had been evacuated from the dark room in less than ten seconds. The technique was repeated using standard, control, and test medications. The gradual increase in delay was interpreted as learning.[18]

Morris Water maze

Rodent spatial recollection and learning were tested using the MWM mission. It consists of a large spherical black tank with a girth of 120 cm and a height of 50 cm, filled to a depth of 30 cm with water at 262°C. The pool was divided into four equal quadrants, each with an 8cm2 platform buried 1 cm beneath the opaque superficial of one of the quadrants in the centre. Throughout the investigation, the Platform's position remained constant. To hide the site of the flooded platform, the water was tinted with a non-toxic black dye. The mice were put into the water one by one and given 120 seconds to trace the platform. Animals were given two trials per day for four days, with a 20-minute inter-trial interval and a short time latency to trace the target (less than 10 seconds). The escape latencies of the mice in each test were recorded. Using the stated considerations, the mean for each testing session was computed for each mouse. If the mouse discovered the platform, it was allowed to stay on it for 10

seconds. If the mice didn't discover the platform within 90 seconds, it was placed on the platform for 10 seconds and then disconnected from the group. Mice were given an acquisition trial first, then a retention trial after 24 hours, followed by a second and third retention trial on subsequent days. The decrease in escape latency day by day in trial 1 illustrates permanent reminiscence or reference remembrance, but the decrease in escape latency from trial 1 to trial 2 and 3 exemplifies either momentary remembrance or operational memory.[19,20]

Biochemical Analysis

The biochemical indices for oxidative stress, such as NO and GSH, were calculated in the mice's brain on the 28th day following Colchicine administration.

Brain tissue preparation

The mice were slaughtered under ether anaesthesia. After cutting the skull open, the brain was taken out. Regular (chilled) saline solution was used to cleanse the brain. With 0.03 M Na3PO4 buffer (pH 7.4) and 10 strokes at 2000 rpm, a 10% (w/v) homogenous brain sample was produced. NO and GSH were measured in a homogenised brain tissue preparation.

Scavenging action of Nitric Oxide

Using Griess reagent and the method proposed by Marcocci et al. 1994, the scavenging propensity of nitric oxide was investigated. 2 mL of 10 mM sodium nitropruside was mixed in 0.5 mL of phosphate buffer saline (pH 7.4), which was then combined with 0.5 mL of extracts of different concentrations (50-200 g / mL) in this process. At 25°C, the mixture was incubated for 150 minutes. Then, at room temperature for 5 minutes, 0.5 mL of the incubated solution was mixed with 1 mL of naphthylethylenediamine dichloride (0.1 percent w/v) and 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 minutes with 1 mL of naphthylethylenedichloride (0.1% w/v)]. After thirty minutes of incubation at room temperature, the mixture's absorbance was measured at 546 nm. The proportion inhibition of Nitric Oxide was estimated using this equation:

% inhibition of NO radical = (A0 - A1)/ A0 x 100

In which A0 is the absorbance previous to the reaction and A1 is the absorbance afterwards of the reaction occurred with Griess reagent.[21]

GSH Measurement

GSH was estimated using the 5, 5'-dithiobis (2-nitrobenzoic acid) reaction (Ellman, 1959), which produced a yellow chromophore that was spectrophotometrically calculated. GSH is a protein that is measured in milligrammes per gramme. The homogenized brain tissue was centrifuged at 700 g for 10

minutes. 500 l of brain homogenate was mixed with 500 l of 10% trichloroacetic acid and centrifuged at 2000 g for 10 minutes at 4°C for protein separation. 100 l of supernatant was combined with 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 in a vortex. At 412 nm, the absorbance was measured in 15 minutes.[22]

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.[23]

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

0.1 mL supernatant was added to 0.9 mL DDW and 5 mL working alkaline reagent in a mixture of 0.9 mL DDW and 5 mL working alkaline reagent. The mixture was thoroughly mixed before being incubated for 10 minutes at room temperature. After that, 0.5 mL Folin-phenol reagent was added and incubated for 30 minutes at room temperature. At 750 nm, the absorbance was determined against a blank. Following that, a standard curve (50-1000g) was constructed, followed by an estimate of sample protein concentration in mg/ml.[23]

Analysis of Statistics

All of the results were presented as average SEM, and they were analyzed using One-way ANOVA, followed by Tukey's many post-hoc contrast trials. A statistically significant 'P' value of less than 0.05 has been established. The data was examined using Graph Pad prism software.

RESULTS AND DISCUSSION

Step-through Passive avoidance paradigm

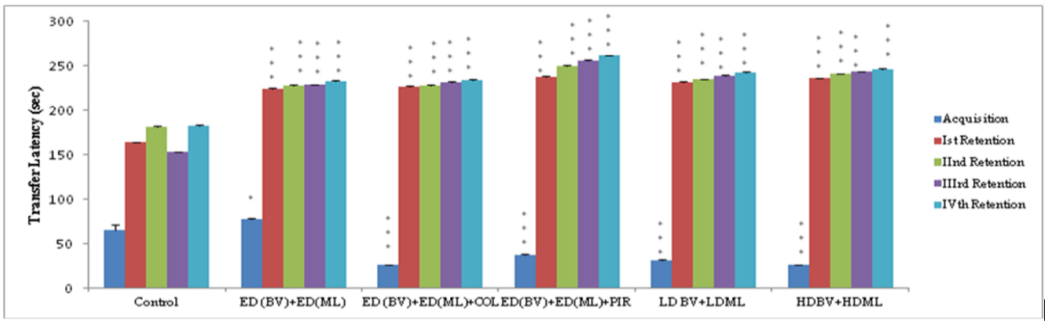


Figure. 1 Effect of Combination extract on transfer latency in Passive avoidance paradigm

The effect of Combination leaf extract on step-through passive avoidance paradigm is shown in Figure 1. 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* and 400 mg / kg dosage of the *Bauhinia variegata* leaf extract combination showed substantial decrease in the mice's transfer latency. This shows the potential nootropic activity of combination leaf extract of *Madhuca longifolia* and *Bauhinia variegata*.

Morris water maze

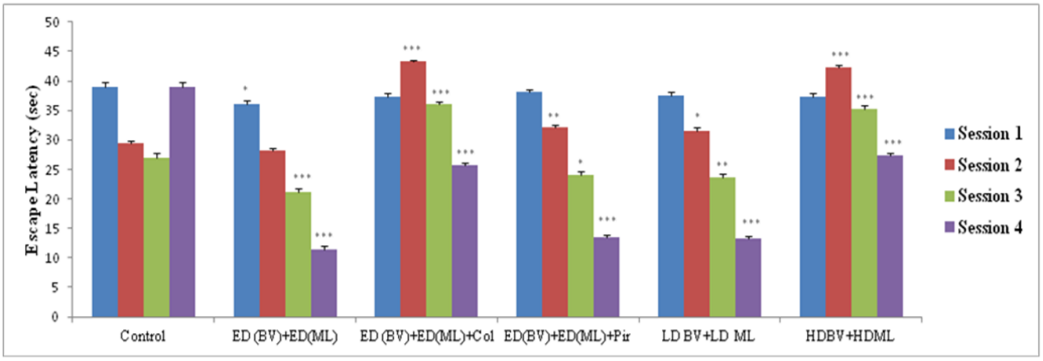


Figure. 2 Effect of Combination extract on escape latency in Morris water maze

Escape latency (EL) of first day reflected learning behavior of animals whereas, EL of following days indicated retention of information or memory. The combination extracts of *Bauhinia variegata* and *Madhuca longifolia* ((LDBV+LDML), (HDBV+HDML), (EDBV+EDML+Pir)) introduced for 28 successive days through the mouth, intra-peritoneally reasonably decreased EL on first day as well as second days, showing valuable improvement of learning and memory. Ethanolic extracts of *Bauhinia*

variegata and *Madhuca longifolia* and Piracetam introduced through the mouth for 28 days protected the animals from colchicine-induced learning and memory failure.

Estimation of NO

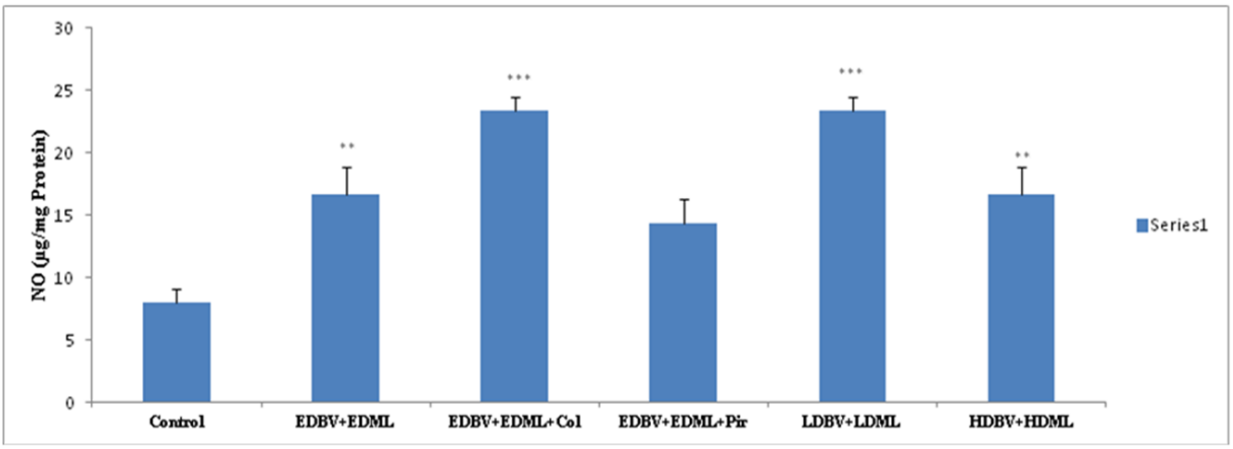


Figure. 3 Effect of combination extract on NO level

From Fig. 3 we have concluded that combination extracts of *Bauhinia variegata* and *Madhuca longifolia* ((LDBV+LDML), (HDBV+HDML), (EDBV+EDML), (EDBV+EDML+Col)) showed significant decrease in NO level.

Estimation of GSH

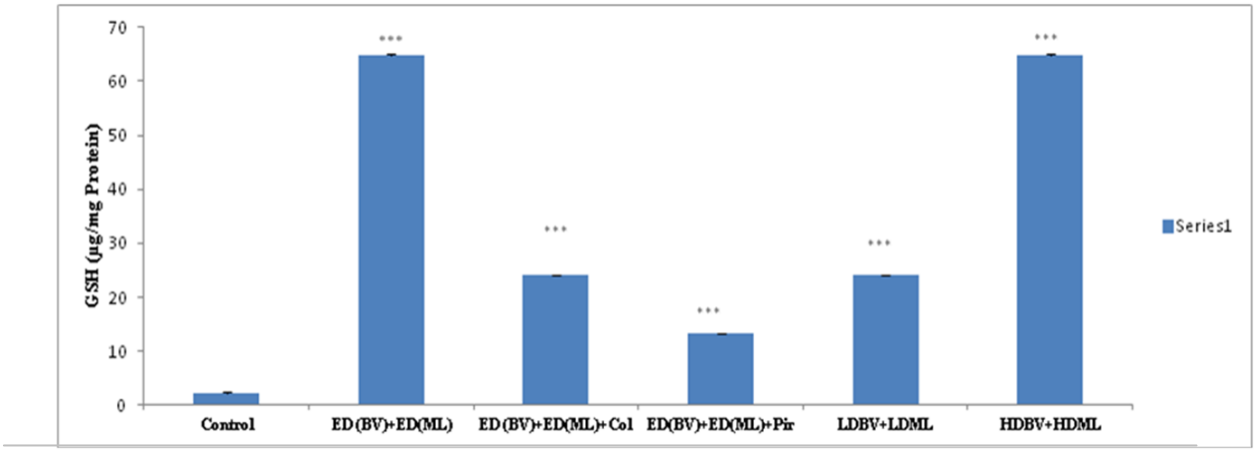


Figure. 4 Effect of combination extract on GSH level

From Fig. 4 we have concluded that combination extracts of *Bauhinia variegata* and *Madhuca longifolia* ((LDBV+LDML), (HDBV+HDML), (EDBV+EDML+Pir), (EDBV+EDML+col),

(EDBV+EDML)) showed significant increase in GSH level. The effect of combination of *Madhuca longifolia* and *Bauhinia variegata* leaf extract on GSH level of mice's brain homogenate is shown in Figure 4. The mice were sacrificed on the 29^{th} 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<0.001when ANOVA with One-way method then Tukey's tests ensues vis-a-viz the control group. The combination leaf extracts showed a large increase in GSH level, thus confirming nootropic activity.

Alzheimer's disease is a neurological illness that develops over time. In the allopathic branch of medicine, a suitable medication for full cure of Alzheimer's disease has yet to be identified. As a result, we should anticipate using herbal remedies to treat this ailment. In the current study, mice were given a mixture of *Madhuca longifolia* and *Bauhinia variegata* extracts by mouth for 28 days, and their learning behavior improved. The combination of larger doses of Madhuca longifolia and Bauhinia variegata extract (200 mg/kg and 400 mg/kg) considerably improved mice's memory in this study, as evidenced by an increased Transmission Latency in passive avoidance testing compared to the control group. In the instance of the Morris Water Maze, the Escape Latency decreased as compared to the control configuration. Pretreatment with a mixture of *Madhuca longifolia* and *Bauhinia variegata* extract for 28 days protected the animals from colchicine-induced memory loss. These findings suggest that combining Mahua and Kachnar leaf extracts may have neuroprotective properties. Reactive oxygen species (ROS) are the root cause of age-related cognitive decline, which may be linked to the development of Alzheimer's disease in the elderly. Antioxidant capabilities are also found in *Madhuca longifolia* and *Bauhinia variegata*. The neuroprotective efficacy of *Madhuca longifolia* and *Bauhinia variegata* extract is linked to its antioxidant properties, which cause sensitive neurons to be exposed to less oxidative stress, resulting in less neuronal injury and improved neuronal function. The ethanolic extracts of *Madhuca longifolia* and *Bauhinia variegata*, at dosages of 200 mg/kg and 400 mg/kg, respectively, show nootropic effect comparable to normal Piracetam medicine, according to this investigation. The ethanolic leaf extracts of *Madhuca longifolia* and *Bauhinia variegata* were combined to reduce NO and raise GSH levels. As a result, the ethanolic leaf extract of *Madhuca longifolia* and *Bauhinia variegata* displays significant nootropic action.

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CONFLICT OF INTEREST

Writers affirmed that they don't have conflicting interest.

ABBREVIATIONS

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*; **BV:** *Bauhinia variegata*; **AD:** Alzheimer's disease.

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