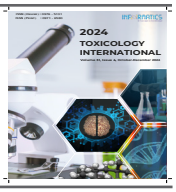
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Memory Enhancing Activity of Combination Extracts of *Bauhinia variegata* Leaf and *Madhuca longifolia* Leaf by Elevated Plus Maze and Estimation of MDA, SOD and Catalase

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Abstract

The aim is to investigate the role of a combination of *Madhuca longifolia* (ML) and *Bauhinia variegata* (BV) ethanolic leaf extracts against oxidative damage in Swiss albino mice and colchicine-induced cognitive dysfunction; to estimate the neuroprotective impact using *M. longifolia* and *B. variegata* combination by behavioral testing and to analyze the biochemical parameters. The experimental analysis was done for 28 days on a colchicine-induced model. Elevated plus maze and actophotometer were used for behavioral experiments and biochemical parameters like SOD, catalase and MDA 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 Group I. Group II received effective dose ED (BV) +ED (ML) (400mg/kg+200mg/kg oral). Group III was administered ED (BV)+ED(ML)+colchicine (400mg/kg+200mg/kg+1mg/kg,*i.c.v.*). The fourth group got ED(BV)+ED(ML)+piracetam (400mg/kg+200mg/kg oral+200mg/kg, *i.p.*). Group V has been given low dose BV+low dose ML(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 high dose BV+high dose ML (400mg/kg + 200 mg/kg oral). *M. longifolia* and *B. variegata* ethanolic leaf extracts indicated an increase in transfer latency of mice in an elevated plus maze. No significant changes were observed in the locomotor activity in actophotometer. The transfer latency in the passive avoidance model showed a good increment. The *M. longifolia* and *B. variegata* leaf extract combination showed an increment (P<0.001) in SOD and catalase levels and a decrease (P<0.001) in MDA and total protein. The combination of *M. longifolia* and *B. variegata* has a neuroprotective effect against memory damage caused by colchicine.

Keywords: Acetylcholinesterase, Alzheimer's Disease, *Bauhinia variegata*, Cognition, Colchicine, *Madhuca longifolia*, Neuroprotective

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1. 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 neuro transmission[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]. *M. 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), Raktakanchan (Marathi), and Kachnar (Hindi) are all names for *B. 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, 10-15 cm 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, 10-15 cm 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 *B. variegata* Linn. include bronchitis, intestinal worms, diarrhoea, bacterial infection, hepatic problems, dysentery, skin illness, leprosy, wounds, ulcers, fungal infection and tumours[15,16].

B. variegata possess flavonoids and antioxidant activity, CNS activity has already been reported so it can be used to as neuroprotective agent.

2. Materials and Methods

2.1 Plant Material

M. longifolia and B. 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).

2.2 Extracts Preparation

The leaves of *M. longifolia* and *B. variegata* were washed, dried and packed in soxhlet column. Extraction was initiated by petroleum ether (60-80 °C) followed by extraction with chloroform (50-60 °C), followed by ethanol (68-78 °C) for 24 hours. The extracts were concentrated on a water bath at 50°C. The solvent was extracted under decreased pressure using a rotary evaporator to obtain a dried ethanolic extract, which was then diluted in water. Chloroform extraction was performed in a separating funnel and a 10% NaCl solution was added drop wise 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 *M. 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 *B. variegata* leaf extracts respectively. Ethanolic extracts of both the plants were selected for the experiment as the yield was maximum for ethanolic extract[17].

2.3 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 *M. longifolia* and *B. variegata* respectively. The dosage were calculated upon the basis of previously done acute oral toxicity studies of ethanolic extracts of *M. longifolia* and *B. variegata* and was given to each mice in combination.

2.4 Animals

Animals were gathered from the animal building of the SRMS CET (Pharmacy), Bareilly, Uttar Pradesh. The animals were approved by IAEC of the institute (715/PO/Re/S/02/CPCSEA). The Swiss albino strains mice were procured from animal house of SRMS CET (Pharmacy), Bareilly, Uttar Pradesh. Animals of both sexes in identical numbers per group (n=6) were taken. 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 grams. 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 labeled at the base of their tails and acclimatized in their cages for at least 5 days.

2.5 Chemicals and Drugs

Drugs like piracetam and colchicine were bought from Sigma Aldrich.

Chemicals like chloroform, ethyl acetate, ethanol, petroleum ether and methanol were bought from Central Drug House (CDH) laboratory.

2.6 Vehicle

In 1 percent w / v CMC, *M. longifolia* extract (ML) and *B. variegata* extract (BV) was suspended and orally given to the mice. Colchicine and piracetam were liquefied in regular saline separatelyand administered by *i.c.v.* and *i.p.* routes, respectively.

2.7 Acute Oral Toxicity Studies

According to the reviewed OECD standards No.420, ethanolic extracts of *M. longifolia* and *B. 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 (doses being already reported in previous studies) dosages of ethanolic leaf extract.

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 *B. variegata* and *M. 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 *B. variegata* and *M. longifolia* (400mg/kg+200mg/kg, oral) and colchicine (1mg/kg, *i.c.v.*) was inoculated to mice and transmission

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, p.o.
III	ED (BV) +ED(ML)+Col (400mg/kg+200mg/kg+1mg/kg, *i.c.v.*)	400mg/kg, p.o.,200mg/kg, p.o., 1mg/kg, *i.c.v.*
IV	ED (BV) +ED(ML)+Pir(400mg/kg+200mg/kg oral+200mg/kg , *i.p.*)	400mg/kg, p.o., 200mg/kg, p.o., 200 mg/kg, *i.p.*
V	LDBV+LDML (200mg/kg +100 mg/kg oral)	200mg/kg, p.o., 100mg/kg, p.o.
VI	HDBV+HDML (400mg/kg +200 mg/kg oral)	400, 200mg/kg, p.o.+1mg/kg, p.o.

latency was assessed after a period of 45 min after inoculation and another time on the 29th day after 24 hrs.

Group IV: A combination effective dose of *B. variegata* and *M. longifolia* (400mg/kg+200mg/kg, oral) and piracetam (200 mg/kg, *i.p.*) was administered to mice for 28 successive days to the mice. Transfer Latency (TL) was noticed on 28th day after 90 min of administration and on 29th day after 24 hr.

Group V: A combination of low doses of *B. variegata* and *M. 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 *B. variegata* and *M. longifolia* (400mg/kg+200mg/kg, oral) was administered to mice for 28 consecutive days. TL was seen 45 min after inoculation andalso after 24 hrs.

2.8 Models Which have Exteroceptive Behavior

2.8.1 Elevated Plus Maze

The structure consists of a 10x10 cm-sized central platform attached to two 50x10 cm open arms and two 50x40x10 cm-sized closed arms and raised 50 cm above the floor. The research was usingmice weighing 20 to 25 g. The experiment was carried out in 2 steps. On day 14, the day of acquisition testing, each mouse was placed at the end of an open arm facing away from the center.The time consumed to enter any one of the closed arms was recorded and measured as Transfer Latency [TL]. All four legs were counted as an entry within the closed arm. For each mouse the cut-off time was 180 s. Animals who did not reach the closed arms during the time of cut-off were excluded from the analysis. Retention testing was carried out on the 15th day, and transfer latency was reported in the same manner as previously stated. Shortening of transfer latency depicted improvement of memory[6].

2.8.2 Actophotometer

Actophotometer consists of six built-in photo-sensors and 4 optical counters showing the operation of the locomotive activity. It indicated the behavior digitally. Most locomotive movements in man and animals are affected by CNS-acting drugs. The locomotive activity (horizontal activity) can be easily measured by an actophotometer that operates on photoelectric cells that are connected to a counter in circuit. When the animal cuts off the beam of light fallingon the photocell, a count is registered. An actophotometer may have either a circular or a squarearena where the animal moves.

We initially weighed and numbered the mice, then switched on the equipment and placed each mouse in the activity cage individually for 10 minutes. We noted down all the animals basal activity score[18].

2.9 Biochemical Analysis

The biochemical indices for oxidative stress, such as SOD, catalase and MDA were calculated in the mice's brains on the 28th day following colchicine administration after sacrifice.

2.9.1 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 Na_3PO_4 buffer (pH 7.4) and 10 strokes at 2000 rpm, a 10% (w/v) homogenous brain sample were produced. NO and GSH were measured in a homogenized brain tissue preparation.

2.9.2 Superoxide Dismutase Activity

Superoxide dismutase catalyzes the reduction of superoxide anions to hydrogen peroxide. It plays a crucial role in the defense of cells against the toxic effects of oxygen radicals. The SOD activity was estimated by method described by Kono, by observing the inhibitory rate of NBT (Nitroblue Tetrazolium) reduction[19].

2.9.3 Catalase Activity

The catalase enzyme is found in all the aerobic cells as endogenous antioxidant which helps in the removal of hydrogen peroxide. The method of Aebi H, was used for the estimation of catalase activity.

The rate of decomposition of H_2O_2 was measured spectrophotometrically from the changes in absorbance was recorded for 2 min at 30 s at 240 nm. The catalase activity was expressed as U/mgprotein[20].

2.9.4 MDA

MDA is a test of peroxidation of the lipids. MDA can be measured spectrophotometrically using standard 1,1,1,3,3-tetraethoxypropane, using the method described by Colado *et al.* MDA is typically expressed by nanomoles per mg protein[21].

2.9.5 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].

2.10 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.

3. Results

3.1 Elevated Plus Maze Test

Transfer Latency (TL) of the first day indicated the learning behavior of animals whereas, TL of the second day reflected the retention of information or memory. The combination of *B. variegata* and *M. longifolia* extracts ((LDBV+LDML), (HDBV+HDML), (EDBV+EDML+Pir)) introduced for 28 successive days through the mouth reasonably decreased TL on the first day as well as second days, showing definite improvement of learning and memory.

3.2 Actophotometer

The basal activity score reflected the locomotor activity of mice. The spontaneouslocomotor action never showed significant differences ($p<0.05$) among different groups.

3.3 Measurement of MDA

We have concluded that combination extracts of *B. variegata* and *M. longifolia* ((LDBV+LDML), (HDBV+HDML),(EDBV+EDML),(EDBV+EDML+col)) showed significant decrease ($p<0.05$) in MDA level when treated groups are compared with the control group.

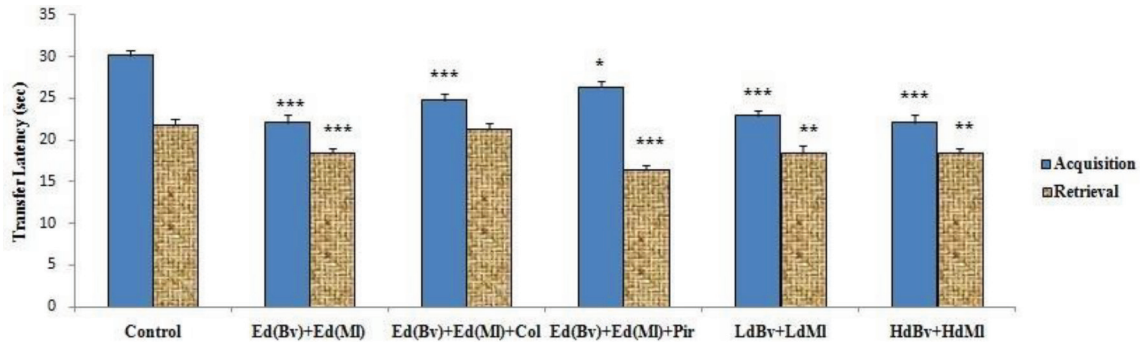


Figure 1. Effect of combination extract on transfer latency in elevated plus maze.

Findings are illustrated as Mean ± Sem (n=6), *P<0.05, **P<0.0 1, ***P<0.001 when compared to the control group by one-way ANOVA and then proceeded by Tukey's tests.

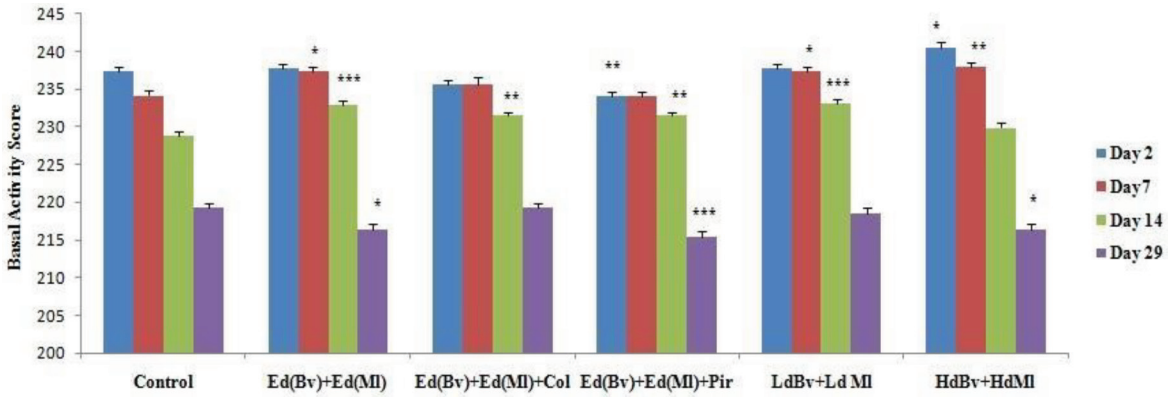


Figure 2. Effect of combination extract on basal activity score in actophotometer.

The findings are illustrated as Mean ± Sem (n=6), *P<0.05, **P<0.0 1, ***P<0.001 when put under comparison to the control group by one-way ANOVA then proceeded by Tukey's tests.

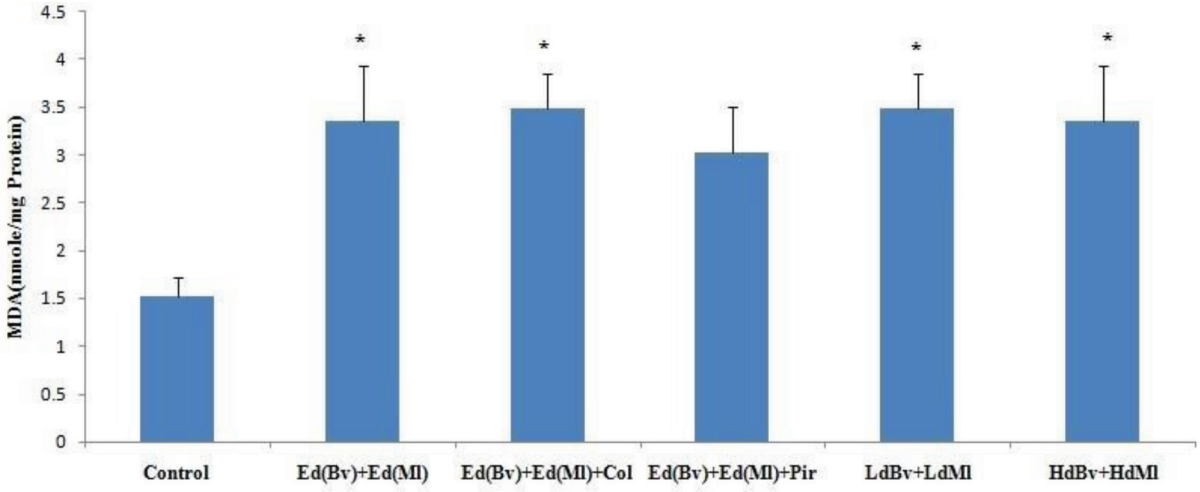


Figure 3. Effect of combination extract on MDA level.

The findings are illustrated as mean ± sem, (n=6), evaluated using one-way ANOVA and then proceeded by Tukey's test. *P<0.05, **P<0.01, ***P<0.001.

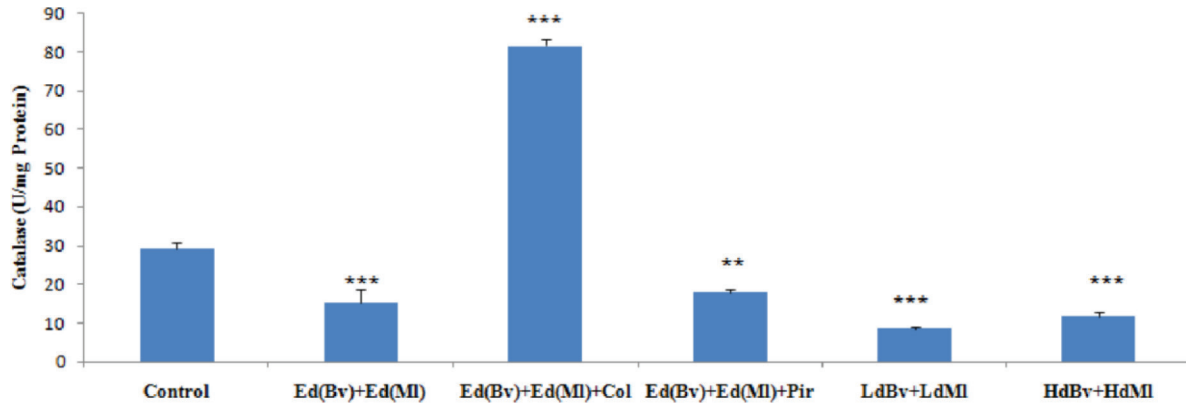


Figure 4. Effect of combination extract on catalase level.

The findings are illustrated as mean ± sem, (n=6), evaluated using one-way ANOVA and then proceeded by Tukey's test. *P<0.05, **P<0.01, ***P<0.001.

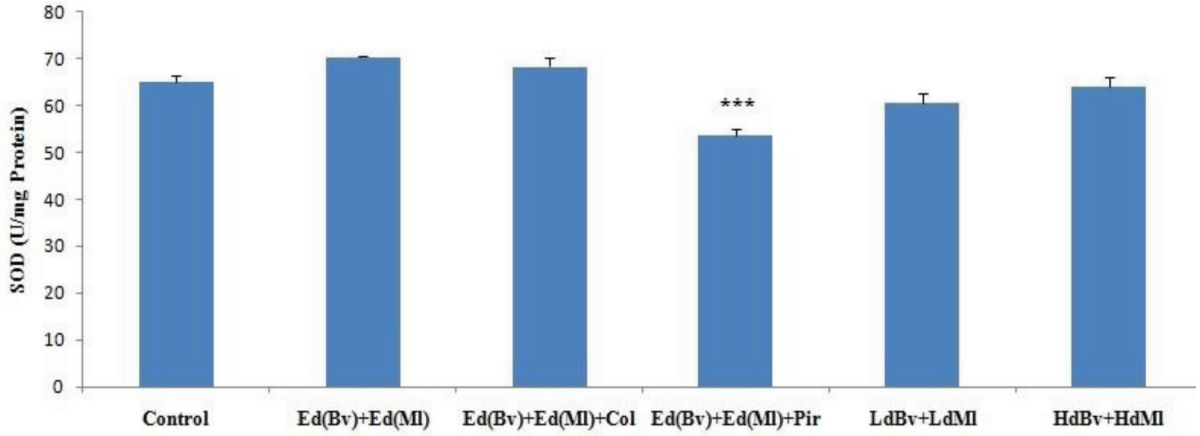


Figure 5. Effect of combination extract on SOD level.

The findings are illustrated as mean ± sem, (n=6), evaluated by the use of one-way ANOVA immediately proceeded by Tukey's test. *P<0.05, **P<0.01, ***P<0.001.

3.4 Measurement of Catalase

We have concluded that the combination extracts of *B. variegata* and *M. longifolia* ((LDBV+LDML), (HDBV+HDML), (EDBV+EDML), (EDBV+EDML+col)) showed much significant increase ($p<0.05$) in catalase level when treated groups are compared with the control group.

3.5 Measurement of SOD

We have concluded that combination extracts of *B. variegata* and *M. longifolia* (EdBv+EdMl+Pir) showed a significant increase ($p<0.05$) in SOD level when treated groups are put under comparison with the control category.

4. Discussion

Alzheimer's disease is a gradual onset neurodegenerative condition. A satisfactory drug for the complete cure of Alzheimer's disease is yet to be developed in the allopathic system of medicine.

Now we should look forward to treating this illness with herbal medicines. Neurodegenerative diseases pose a significant health challenge globally, with conditions such as Alzheimer's, Parkinson's and Huntington's diseases affecting millions of individuals world wide. The quest foreffective treatments has led researchers to explore various avenues, including the potential of plant extracts as neuroprotective agents. In recent years, considerable attention has been drawn to the neuroprotective properties exhibited by certain plant extracts, offering promising avenues for therapeutic intervention.

Neuroprotection refers to the preservation of neuronal structure and function, ultimately aimed atpreventing or slowing the progression of neurodegenerative disorders. Several factors, including oxidative stress, inflammation, mitochondrial dysfunction, and protein misfolding, contribute to neuronal damage characteristic of these diseases. Plant extracts containing bioactive compounds have shown the potential to target these mechanisms and exert neuroprotective effects.

Plant extracts exert their neuroprotective effects through various mechanisms. For instance, antioxidants present in these extracts scavenge free radicals and reduce oxidative stress, there by preserving neuronal integrity. Additionally, anti-inflammatory compounds inhibit the production of pro-inflammatory cytokines and attenuate neuroinflammation, a common feature of neurodegenerative diseases. Furthermore, certain plant-derived compounds modulate signaling pathways involved in cell survival and apoptosis, promoting neuronal resilience against pathological insults.

In the current research, *B. variegata* and *M. longifolia* extract combination for 28 days was given orally which showed an improvement in mice's learning behavior. Throughout this analysis, the higher dose of *B. variegata* extract (400 mg/kg) and *M. longifolia* extract (200 mg/kg) significantly enhanced mice's memory as there was a decrease throughout transfer latency in the case of elevated plus maze system and there was no significant effect on locomotor activity in case of actophotometer in comparison with the control group. The locomotive operation indicated no symptoms of CNS distress. *B. variegata* and *M. longifolia* extract pretreatment for 28 days protected the animals from memory deficits produced by colchicine due to presence of flavonoids. These findings indicate the possible role of *B. variegata* and *M. longifolia* extract in neuroprotection.

Despite the promising findings, several challenges need to be addressed before plant-derived neuroprotective agents can be translated into clinical practice. These include issues related to bioavailability, dose optimization, and standardization of plant extracts. Additionally, rigorous clinical trials are warranted to evaluate the safety and efficacy of these compounds in human subjects.

5. Conclusion

In conclusion, the neuroprotective potential of plant extracts presents a promising avenue for the development of novel therapeutic interventions for various neurological disorders. Through extensive research and experimentation, it has become evident that certain plant extracts harbor bioactive compounds capable of safeguarding neurons from damage caused by oxidative stress, inflammation, and other pathological mechanisms implicated in neurodegeneration.

From the present analysis it can be inferred that the *B. variegata* and *M. longifolia* ethanolic extract at a dose of 400mg/kg and 200mg/kg respectively possesses nootropic activity comparable to the regular piracetam medication. Reactive Oxygen Species (ROS) are the root cause of age-related decline in cognitive performance that could be involved in elderly people developing Alzheimer's disease. *B. variegata* and *M. longifolia* have antioxidant properties. *B. variegata* and *M. longifolia* extract's neuroprotective

activity is related to its antioxidant properties, due to which the susceptible neurons are subjected to less oxidative stress leading to reduced neuronal harm and enhanced neuronal function. The symptoms of dementia are impairment in neuronal transmission, decline in brain acetylcholine levels and neuronal circuit degeneration in the affected brain areas. Pretreatment with *B. variegata* and *M. longifolia* substantially reduced transfer latency in the elevated plus maze. The *B. variegata* and *M. longifolia* ethanolic leaf extract decreased MDA, and increased the level of SOD and catalase. Hence, the *B. variegata* and *M. longifolia* ethanolic leaf extract combination has significant neuroprotective effect.

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