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Exploring the efficacy of *Aegle Marmelos* Fruit Extract as a Therapeutic Agent for Multiple Sclerosis in Animal models

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KEYWORDS

Aegle marmelos, Cuprizone, Multiple sclerosis, Neuroprotective, Morris water maze.

Abstract

Introduction: Multiple sclerosis (MS) is a neurodegenerative disease that affects the central nervous system (CNS). There has been a notable increase in reported cases worldwide, where the incidence has doubled over the past three decades. Currently, there is no effective treatment for MS that can reverse axonal damage or promote the remyelination of nerve cells. Strategies focusing on enhancing remyelination of axons are crucial in addressing MS. *Aegle marmelos*, commonly known as Bael and belonging to the Rutaceae family, is known for its bioactive compounds with studied neuroprotective properties.

Objectives: This study aims to assess the neuroprotective and remyelination effects of *Aegle marmelos* unripe fruit extract.

Methods: In MS, demyelination leads to memory loss and changes in locomotion due to the degeneration of the myelin sheath around nerve cells. Both *In vivo* and *Ex vivo* studies were conducted to evaluate various parameters, including locomotor activity, learning and memory, and *Ex vivo* assessments such as antioxidant levels and inflammatory markers.

Results: The impact of Hydro Alcoholic *Aegle Marmelos* Fruit Extract (AMFE) on mice induced with Cuprizone at high (AMHD) and low (AMLD) doses demonstrated increased neuroprotective and remyelination effects compared to the negative control groups.

Conclusions: Our findings indicate enhanced memory and spatial navigation, increased protection against oxidative stress, and improved anti-inflammatory effects. The results suggest that Aegle marmelos extract, particularly AMFE, may offer promising benefits in mitigating the effects of MS by promoting neuroprotection and remyelination.

1.Introduction

Multiple sclerosis (MS) is a progressive inflammatory, autoimmune, and chronic disease of the central system (CNS), that can cause extensive core degradation of the myelin sheath, variable axonal and neuronal injury, and disabilities in adults, mostly women (between ages 20 and 40 years)[1]. It can cause dysfunction in the spinal cord and brain[2], leading to episodes of reversible neurological deficits, followed by neurological degradation in most patients. Symptoms include numbness, memory loss, vision loss, body pain, and tremor. MS causes the nerve signals to be blocked resulting in difficulty in controlling vision, decreased muscle mobility and coordination, weakened strength, sensation, and loss of other bodily functions[3]. Neuroepidemiology studies demonstrate that the prevalence of MS has more than doubled in the past thirty

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years[3]. India has seen significant increase in multiple sclerosis due to the increased diagnosis of the disease, studies have shown that it has nearly doubled in the past decade[4].

The exact cause of MS is still largely unknown. Environmental and genetic factors have shown a significant role in causing MS. Also, studies have indicated that the prevalence of MS in siblings has instigated a role of genetic factors in the causation of MS[5,6]. Risk factors also include climate, infections, smoking, and autoimmune disease[7]. MS are classified into four types of MS, Relapsing-remitting MS, Primary progressive MS, Secondary progressive MS, and Progressive-relapsing MS[8]. The profuse diversity of MS extends beyond its symptomatic manifestations, this encompasses the variations in neuroradiologic and histologic characteristics of lesions, as well as the unique response to the therapeutic interventions[9].

The pathology of MS is controversial, and still, there is no effective treatment that stops the neuro-axonal damage or treatments that promotion of remyelination is effective. So, the goal of the treatment of MS should be to preserve the CNS architecture and function in challenging situations, this is critical because the regeneration of the CNS in adults is very poor and the treatment must be done swiftly because once the connection is lost it cannot be regenerated[10]. Promoting remyelination has emerged as a pivotal factor in the development of neuroprotective and regenerative approaches for managing the MS as Myelin is one of the most important factors that defends the axons[11,12,13].

Aegle marmelos commonly known as Bael, Bengal quince, golden apple, and apple stone; belongs to the family of Rutaceae and is widely found in the Indian subcontinent and southeast Asia. The versatile plant has been playing an important role in many traditional medicines and has shown potential clinical properties in India, Nepal, Bangladesh, Myanmar, and Sri Lanka. All the parts of the plant are being used for their therapeutic value in Ayurveda such as fruits, leaves, bark, roots, and seeds[14].

The plant possesses and has been employed in many reported activities such as gastroprotective, anti-diabetic, anti-oxidant, memory enhancing, anti-hyperlipidemic, anti-diarrhoeal, contraceptive, radioprotective, antiproliferative, analgesic, anti-inflammatory, antipyretic and antimicrobial properties[11-13,15-22].

The plant harbors a rich array of bioactive compounds that contribute to the diverse medicinal applications such as coumarins, tannins, alkaloids, pectins, flavonoids, carotenoids and terpenes that have been isolated from different parts of *A. marmelos* that play a significant role in therapeutic efficacy. Many important compounds that have been showing efficacy for therapeutics have been isolated from *Aegle marmelos* such as marmelosin, marmelin, marmelide, psoralen, alloimperatorin, skimmianine, rutaretin, scopoletin, aegelin, fagarine, anhydromarmelin. Flavoinds such as Rutin, Flavone , Flavone-3-ols , Flavone glycosides are also found which has been extensively studied for their neuroprotective activity[23,24].

1.1. Objectives:

The aim of this study is to assess the neuroprotective and remyelination potential of *Aegle Marmelos*. The focus is on investigating its impact on memory loss caused by demyelination, specifically the degeneration of the myelin sheath, which is a prominent characteristic observed in individuals with Multiple Sclerosis. The objective is to explore the potential benefits of *Aegle Marmelos* in mitigating the effects of demyelination and enhancing the process of remyelination as a possible therapeutic approach for addressing memory impairment associated with Multiple Sclerosis.

2. Methodology

2.1. Materials Collection & Authentication

The plant was collected at Lord Shiva temple from Villupuram -605602 and authenticated by SIDDHA CENTRAL RESEARCH INSTITUTE (Central Council for Research in siddha, Chennai- 600106, Ministry of AYUSH, Government of India) Certificate no.and code: 481. A07032301M.

2.2. Preparation of plant extract

The unripe fruits pulp of *Aegle Marmelos* were washed and dried in a hot air oven at 40 °C. The powdered material was extracted with Hydro-ethanol (30:70 ratio) at 70°C using a Soxhlet apparatus for 48 h. The flash evaporated and lyophilized sample yielded

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12.6% extract on a dry weight basis, and it was used for the *in vivo* studies. The hydroalcoholic extract of *Aegle Marmelos* fruit extract was screened for the presence of phytochemical constituents.

2.3. Animals

Research and animal care were approved by the **IAEC approval no: 04/321/PO/Re/S/01/CPCSEA/dated 14/03/20**, The *in vivo* experiments were performed with 6-7 weeks old Female Swiss albino mice of body weight ranging between 15 to 20 gm were used for this research study. Animals were sheltered under a temperature-controlled conditions with alternate light and dark cycles, 12 hrs each and *ad libitum* access to water and food.

2.4. Cuprizone treatment

Cuprizone was suspended and mixed in 1% Methyl cellulose stock solution of 10ml was prepared stored at 4ºC. Mice to fed daily by gavages with 10 ml/kg volume. The Cuprizone -MC suspension (40mg/ml) was prepared freshly on daily basis. Cuprizone predominantly induced oxidative stress and neurodegeneration in the experimental animals, as evidenced by histopathological changes in various brain regions, which translated into observable neurobehavioral deficits[25].

2.5. Experimental design

The mice group that 6-7 weeks age are randomly divided into four groups (n= 6 per group). The group one was fed normal diet. No induction and no treatment were done, the group was served as normal control. The second group was used as test control with induction of Cuprizone of 400 mg/kg bodyweight per day by oral gavages. The third group was induced with 400mg/kg body weight of Cuprizone and treated with 100 mg/kg AMLD (*Aegle Marmelos* low dose), And finally group four was induced with 400 mg/kg body weight of Cuprizone and treated with 200 mg/kg AMHD (*Aegle Marmelos* high dose). For 28 days of the CPZ exposure, AMFE 100mg and 200mg/kg were administered once daily from day 1 to day 28.

2.6. Assessment of Locomotor activity

Actophotometer

Quantifying horizontal movement, or locomotor activity, involved the utilization of the Actimeter Orchid Scientific Model, an Actophotometer equipped with photoelectric cells connected to a counter. The digital display registers counts when the animal disrupts the light beam reaching the photocell. Individual mice were placed in the activity cage for a 10-minute period, and locomotion counts were derived from the digital readings of the actimeter. The assessment of impulsive behavior occurred on days 7, 14, 21, and 28[26].

2.7. Assessment of Learning and Memory

Morris water maze test

The Morris water maze procedure was utilized to evaluate spatial working and reference memory. The experimental arrangement comprised a circular tank with dimensions of 120 cm in diameter and 45 cm in height. Inside the tank, an unseen platform (15 cm in diameter and 35 cm in height) was positioned 1.5 cm below the water's surface. To maintain opaqueness, a small quantity of milk was added to the water, and the temperature was kept at 21-23ºC.

The water maze was placed in a testing room where several external cues, visible from the pool, were present to help mice with spatial orientation. The positions of these cues remained consistent throughout the task. Training trials were conducted with the platform consistently placed in the middle of one quadrant, equidistant from both the center and the edge of the pool. The time taken by the mice to reach the hidden platform during each training session was recorded.

On the experimental day, the platform was removed, and the animals underwent memory testing. The duration that each animal spent in the target quadrant, searching for the absent hidden platform, was recorded as a measure of retrieval[27].

2.8. Y-Maze test

This research is based on spontaneous exploration and alternation between arms, without the need for water or food restrictions. The equipment employed is a Y-shaped maze, featuring three identical arms symmetrically arranged on an equilateral triangular

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center. The large Y-maze comprises three arms, each measuring 40×8×15 cm, while the small Y-maze consists of two arms measuring 15.24×7.62×12.7 cm and one arm measuring 20.32×7.62×12.7 cm. In a 5-minute test period, the number of entries into and time spent in each type of arm were recorded. Specifically, entries and time in the open arms, as well as entries and time in the closed arms, were documented. Scoring involved counting each arm entry, defined as the entry of all four paws into the arm[28].

After the behavioral assessments, the animals were promptly decapitated to obtain samples for biochemical analysis. Following brain removal, a 10% (w/v) tissue homogenate was prepared using 0.1 M phosphate buffer at pH 7.4. The homogenate was then centrifuged at 10,000 rpm for fifteen minutes. Subsequently, aliquots of the supernatant were separated and used for biochemical calculations.

BIOCHEMICAL PARAMETERS

2.9. Estimation of Catalase

The catalase activity was evaluated using the Luck (1971) method, which measures the breakdown of hydrogen peroxide (H2O2) at 240 nm. The assay mixture included 0.05 ml of tissue homogenate (10% supernatant) and 3 ml of H2O2 phosphate buffer. The change in absorbance was monitored at 240 nm. The results were expressed in units of micromoles of H2O2 degraded per milligram of protein per minute[29].

2.10. Estimation of Superoxide dismutase

The evaluation of Superoxide Dismutase (SOD) activity was carried out using the Kono (1978) method, utilizing a spectrophotometer to gauge the reduction of nitrazo-bluetetrazolium (NBT), impeded by superoxide dismutase, at 560 nm. The reaction commenced with the addition of hydroxylamine hydrochloride to the mixture containing the sample and NBT. The units per milligram of protein were computed, defining one unit of enzyme as the amount required to completely inhibit the reaction rate by 100%[30].

2.11. Estimation of Glutathione reductase

The DTNB assay included the following components in the standard reaction mixture: 1.0 ml of 0.2 M potassium phosphate (pH 7.5) with 1 mM EDTA, 0.5 ml of 3 mM DTNB in 0.01 M phosphate buffer, 0.25 ml H2O, 0.1 ml of 2 mM NADPH, 0.05 ml of glutathione reductase (1 U/ml), and 0.1 ml of 20 mM GSSG. While preparing the mixture, glutathione reductase was kept in an ice bath, but upon mixing with other components at room temperature, its temperature quickly equilibrated. The components were introduced into a 4.5-ml cuvette in the specified order, and the addition of GSSG initiated the reaction.

A Beckman DU-40 spectrophotometer equipped with the DU-50 series spectrophotometer kinetics Soft-Pat module was employed to monitor the increase in absorbance at 412 nm. Although linearity was observed for only two minutes at high activity, the rate was determined from the linear portion of the curve and expressed as a rate per 5 minutes. The temperature during the assay was maintained at 24°C using an Isotemp refrigerator circulator (Fisher Scientific Model 900)[31].

2.12. Estimation of Interleukin-6 (IL-6)

Reagents: RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.4), 1 mM EDTA (Ethylene diamine tetra acetic acid), 1 mM PMSF (phenyl methyl sulfonyl fluoride), Protease inhibitor cocktail.

Procedure: One half of the brain tissue was homogenized using a disposable rotor homogenizer in 10 volumes of RIPA buffer supplemented with 1 mM EDTA, 1 mM PMSF, and a protease inhibitor cocktail to obtain a subcellular fraction. The homogenate was then centrifuged at 29,000 rpm for 20 minutes. The resulting pellet was resuspended with RIPA buffer containing 1 mM EDTA and a protease inhibitor cocktail. Concurrently, the supernatant obtained from the initial centrifugation was further centrifuged at 29,000 rpm for 45 minutes. The resulting supernatants were utilized for the determination of IL-6 using specific quantitative sandwich ELISA kits, following the manufacturer's instructions[32].

2.13. Estimation of TNF-α

The plasma level of TNF-α was evaluated using an Enzyme-Linked Immunosorbent Assay (ELISA) with a commercial TNF-α ELISA kit. The procedure was conducted in accordance with the instructions provided with the kit. Afterward, the absorbance was measured at 450 nm using a microplate photometer. A standard curve

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was constructed to determine the quantity of TNF-α present in any given sample[33].

2.14. Statistical Analysis

The results are presented as mean ± S.E.M. In order to accesses the statistical significance of the comparisons of the experiments, a through comprehensive analysis were conducted using a one-way analysis of the Variance (ANOVA) and was followed by Dunnett's 't' test. The results were interpreted based on established significance levels: non-significant (ns), *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. This rigorous statistical approach provided a robust foundation for drawing meaningful conclusions about the observed differences among the experimental groups.

3. Results and discussion

3.1. Effect of AMFE on Morris water Maze

The effect of the Morris water maze test provides an insight into the spatial learning for the mice. The test provides an validity to the measure of the hippocampally dependent spatial navigation and the mice ability to have an reference memory[34]. The data projects that the escape latency was increased for the negative control which was administered with only Cuprizone. The escape latency for the group III of AMLD was gradually deceased from 43.12±1.20** on day seven to 34.20±1.52** on day 28 this indicates the effect of AMFE on the mice. Similarly, group IV has much better effect on the mice when compared to group three. With an increased dose of the extract the escape latency steadfast decreased from 31.90±1.05**** on day 7 to 27.95±1.02**** on day 14 and finally reaching to a time of 20.15±1.16**** on day 28 this shows the effect of AMFE with a higher dose.

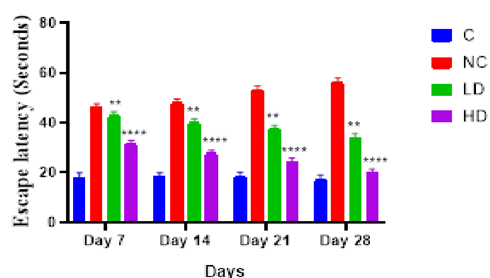

Figure 1: Shows the effect of AMFE on the Morris water maze test with respect control, Negative control, low and high dose of AM

3.2. Effect of AMFE on Y-Maze

The Y maze test can used to assess the short-term memory in mice. This test can be a measure of the mice spatial working memory. Spatial memory underlines the working efficiency of hippocampus[35]. Also, the mice intact working memory can be assessed by the behaviour of the mouse to go places previously unknown. The data listed on table 2 shows the effect of AMFE on the mice. While no significant changes are seen on control groups I, the group II shows a significant decrease in the alternations taken by the mice. Control groups I on day 7 showed an alteration of 42.17±1.70 followed by 43.20±1.20, 45.65±2.30, and 46.35±1.70 on day 14, 21, and 28 respectively.

The negative control shown an alteration of 23.80±1.05 on day 7 followed by 22.80±1.50, 20.42±1.28, and 19.50±1.15 on day 7, 14, 21 and 28 respectively.

Table 1: Effect of AMFE on Morris water Maze test on mice

S. No.	Groups	Escape latency (Seconds)			
		Day 7	Day 14	Day 21	Day 28
1	Control	17.92±1.90	18.32±1.54	18.72±1.42	17.42±1.72
2	Negative control	46.42±1.20	48.37±1.10	53.30±1.45	56.45±1.51
3	AMLD	43.12±1.20**	40.25±1.32**	37.85±1.10**	34.20±1.52**
4	AMHD	31.90±1.05****	27.95±1.02****	24.52±1.42****	20.15±1.16****

AMLD group shows 29.15±1.32*** (statistically significant compared to negative control) on day 7 followed by 32.48±1.42***, 35.84±1.05***, and 38.37±1.22*** on days 14, 21, and 28 respectively. And finally, the AMLD with a high dose of AMFE shows 38.15±1.28**** on day 7 followed by 40.55±1.18****, 43.31±1.05****, and 44.23±1.19**** on day 14, 21, and 28 respectively. The Control group shows a gradual increase in % alterations over the 28-day period. The

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Negative Control group exhibits lower % alterations, indicating potential impairment in Y-Maze performance.

The AMLD group shows a significant improvement in % alterations compared to the Negative Control groups, suggesting a positive effect of the low dose of AMFE on cognitive function. The AMHD group demonstrates a more pronounced and statistically significant enhancement in % alterations, implying a potential dose-dependent improvement in cognitive performance as a result of high AMFE dose.

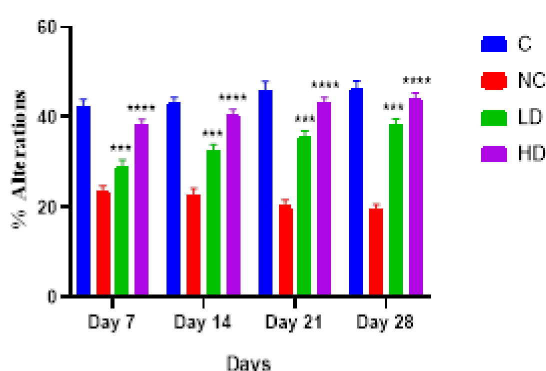

Figure 2: Shows the effect of AMFE on the mice on Y-Maze test with respect control, Negative control, low and high dose of AM

Table 2: shows the Effect of AMFE of mice on Y-Maze test

S. No.	Groups	% Alterations Y-Maze			
		Day 7	Day 14	Day 21	Day 28
1	Control	42.17± 1.70	43.20± 1.20	45.65± 2.30	46.35± 1.70
2	Negative control	23.80± 1.05	22.80± 1.50	20.42± 1.28	19.50± 1.15
3	AM LD	29.15± 1.32***	32.48± 1.42***	35.84± 1.05***	38.37± 1.22***
4	AM HD	38.15± 1.28****	40.55± 1.18****	43.31± 1.05****	44.23± 1.19****

3.3. Effect of AMFE on Actophotometer test

Actophotometer used for locomotor activity. The data is given on table 3 shows the control group with an increase in locomotor activity from day 7 with 295.18 ± 4.50 and followed by 298.70 ± 3.85, 301.17 ± 4.02, and 302.25 ± 3.50 on days 14, 21, and 28 respectively. The negative control group II is shown values of 171.5 ± 2.25 on day 7 followed by 170.13 ± 3.50, 168.70 ± 3.25, and 167.65 ± 3.10 respectively. The AMLD group III showed 223.45 ± 2.25 in day 7 and 228.40 ± 3.08, 240.48 ± 2.10, and 243.42 ± 3.08 on day 14, 21, and 28 respectively. And finally, the AMHD group IV showed values of 271.45 ± 3.72, 280.95 ± 2.98, 287.46 ± 2.65, and 292.98 ± 4.05 on day 7, 14, 21, and 28 days respectively. The control group index increases from day 7 to day 28 it suggests a natural progression and potentially an increase in the locomotor activity over the time.

The negative control group the locomotor index has been consistently shown to be lower than the control group indicating a decreasing trend from day 7 to day 28 in a potential effect on the locomotor activity in this group.

The group III the locomotor index values are shown to be higher than the negative control and this shows a potential increasing trend over the period of observation and a positive locomotor activity. And finally, the AMHD group IV shows values that are consistently higher than both the control and AMLD groups, this indicates a dose-dependent positive effect on the locomotor activity. The results show treatment groups if AMLD and AMHD have an positive effect on the locomotor activity compared to the negative control. The profound effect indicates a potential dose dependent response.

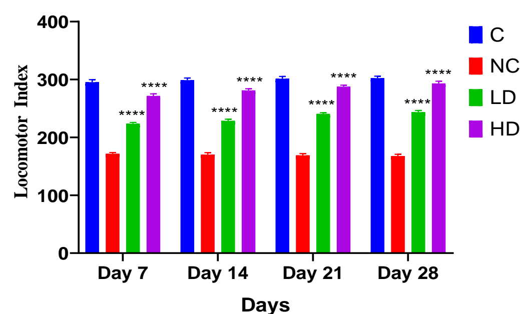

Figure 3: Shows the locomotor index with respect to days the effect of AMFE on the mice with respect control, Negative control, low and high dose of AM

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3.4. Effect of AMFE on Superoxide dismutase

The control groups activity was found to be 32.98±0.03 Units/mg Protein, the negative control group had 12.37±0.05 Units/mg Protein this lower activity indicates a negative influence of the Cuprizone administered to the mice group II. The AMLD group III showed an activity of 27.08±0.02 Units/mg Protein and this is lower than the control group but still found to be higher than the negative control.

This also shows the effect of AMFE on the mice. And finally, the AMHD group IV shows an activity level of 28.53±0.03 Units/mg Protein which is similar to the AMLD group, though this group shows a reduction in SOD activity when compared to the control, but the activity is still higher than the negative control. The diminished SOD levels was observed in the negative control. Treatment with the extract at 100mg/kg and 200mg/kg provided a higher protection of SOD.

3.5. Effect of AMFE on Catalase activity

The catalase activity for the control group I shows 23.65±0.01 Units/mg protein, this is baseline of the control group. The negative group II shows an activity of 4.95±0.04 Units/mg protein,

S. No.	Groups	Locomotor Index			
		Day 7	Day 14	Day 21	Day 28
1	Control	295.18±4.50	298.70±3.85	301.17±4.02	302.25±3.50
2	Negative control	171.5±2.25	170.13±3.50	168.70±3.25	167.65±3.10
3	AMLD	223.45±2.25****	228.40±3.08****	240.48±2.10****	243.42±3.08****
4	AMHD	271.45±3.72****	280.95±2.98****	287.46±2.65****	292.98±4.05****

Table 3: Effect of AMFE on the mice locomotor activity

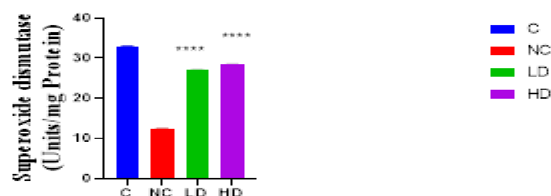

Figure 4: The effect of AMFE on the Superoxide dismutase activity of mice with respect control, Negative control, low and high dose of AM

Table 4: Shows the effect of AMFE on Superoxide dismutase of mice

S. No	Groups	Superoxide dismutase (Units/mg Protein)
1	Control	32.98±0.03
2	Negative control	12.37±0.05
3	AMLD	27.08±0.02****
4	AMHD	28.53±0.03****

this indicates that there is a low catalase activity due to the Cuprizone treatment. The catalase activity in this group III is 15.48±0.01 Units/mg protein, it indicates that there is a AMFE treatment had a moderate impact on catalase activity, and possibly inhibiting the function to some extent.

The AMHD group IV shows an activity of 19.95±0.04 Units/mg protein, the catalase activity in this group is higher than both the negative and the AMLD group III, and its interesting to note that it is very close to the control group I, this can be said that the AMHD treatment may have had an positive effect on the catalase activity, and it potentially enhances the function when compared to the negative control. There was a restoration of the enzymatic activity in mice when treated with the AMFE.

Table 5: Effect of AMFE on mice Catalase

S. No	Groups	Catalase (Units/mgProtein)
1	Control	23.65±0.01
2	Negative control	4.95±0.04
3	AMLD	15.48±0.01***
4	AMHD	19.95±0.04****

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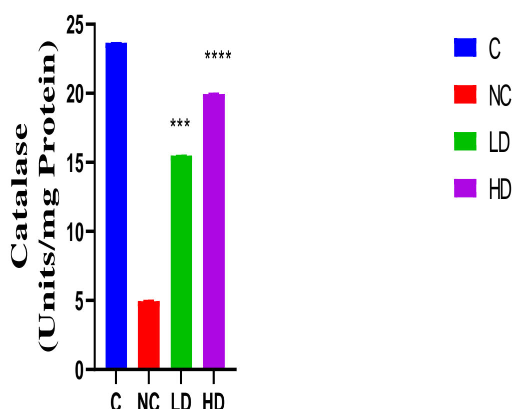


Figure 5: Shows the catalase activity with respect to control, Negative control, low and high dose of AM

3.6. Effect of AMFE on Glutathione reductase

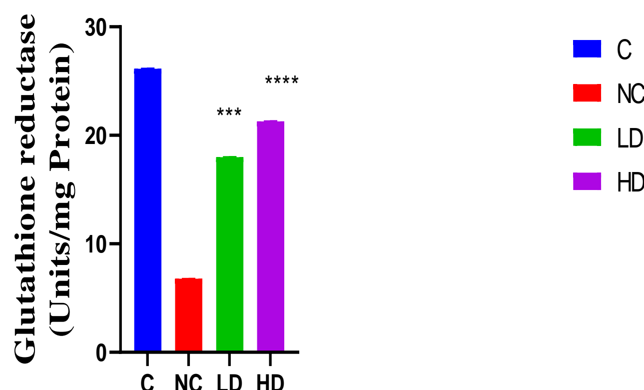
The control group had a baseline activity of 26.15±0.02 Units/mg Protein. The negative control had an activity range of 6.78±0.01 Units/mg Protein this shows a drastic decrease in the activity when compared to the control. This is due to the treatment of Cuprizone. The AMLD Group III showed an activity scale of 17.98±0.03 Units/mg Protein this is a significant difference when compared to the negative control this suggests that the treatment with AMFE has a restorative effect on the Glutathione reductase activity.

AMHD Group IV shows an activity of 21.28±0.02 Units/mg Protein this is with an higher dose of AMFE this is higher than the other two groups indicating a dose dependent response, this increase in the dose of AMFE might lead to pronounced level elevation in glutathione reductase activity.

Table 6: Effect of AMFE on Glutathione reductase

S. No	Groups	Glutathione reductase (Units/mg Protein)
1	Control	26.15±0.02
2	Negative control	6.78±0.01
3	AMLD	17.98±0.03***
4	AMHD	21.28±0.02****

Figure 6: Shows the Glutathione reductase activity with respect control, Negative control, low and high dose of AM



3.7. Effect of AMFE on Interleukin-6

The levels of IL-6 9.702 ± 0.02 pg/ml in Group I followed by Negative control (Group II) level of IL-6 that is 35.586 ± 0.03 pg/ml. Group III and IV has IL-6 levels of 16.205 ± 0.02 pg/ml and 13.105 ± 0.03 pg/ml respectively. The IL-6 levels in group II are significantly higher this indicates an inflammatory response when compared to group III, similarly in group III the IL-6 levels is significantly higher than group IV, shows the effect of AMFE doses.

Both low and high doses (AMLD and AMHD), has a significant impact on reducing the elevated IL-6 levels observed in the negative control group. Lower IL-6 levels are generally associated with a less inflammatory response, and this could indicate a potential anti-inflammatory effect of AMFE.

Table 7: Effect of AMFE on Interleukin-6

S. No	Groups	Interleukin-6 (Pg/ml)
1	Control	9.702±0.02
2	Negative control	35.586±0.03
3	AMLD	16.205±0.02***
4	AMHD	13.105±0.03****

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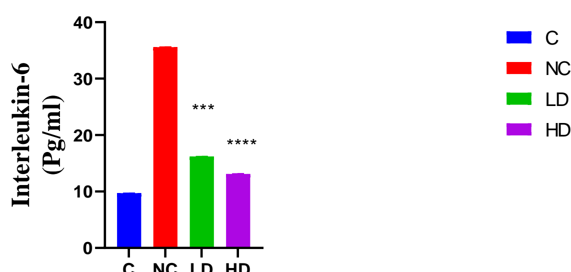
Figure 7: Shows the levels of IL-6 with respect control, Negative control, low and high dose of AM



3.8. Effect of AMFE on TNF-α

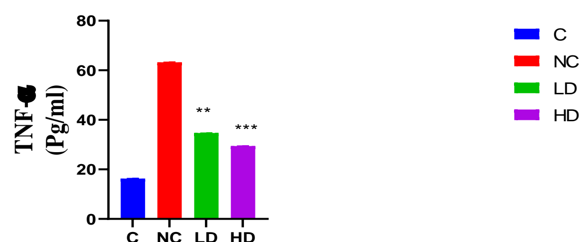
Tumor necrosis factor (TNF)-alpha helps in regulating the immune response and acts as a proinflammatory cytokine, Also, TNF-α is implicated in the immune pathogenesis of various autoimmune, inflammatory diseases. TNF-α levels of group I was found to be 16.32±0.03 Pg/ml. The negative control group II, the levels are 63.25±0.02 Pg/ml. which is significantly higher when compared to the control group.

This suggests the role of Cuprizone. The AMLD group III has increased levels of TNF-α 34.68±0.02 Pg/ml. This suggests that the modulating effect of TNF-α, possibly reducing its levels compared to the negative control. The final group IV is with TNF-α levels are 29.46±0.03 Pg/ml. This suggests higher dose of the treatment might have stronger effect on TNF-α levels compared to the lower dose group III.

Table 8: Effect of AMFE on TNF-α

S. No	Groups	TNF-α (Pg/ml)
1	Control	16.32±0.03
2	Negative control	63.25±0.02
3	AMLD	34.68±0.02**
4	AMHD	29.46±0.03***

Figure 8: Shows the levels of TNF-α with respect control, Negative control, low and high dose of AM

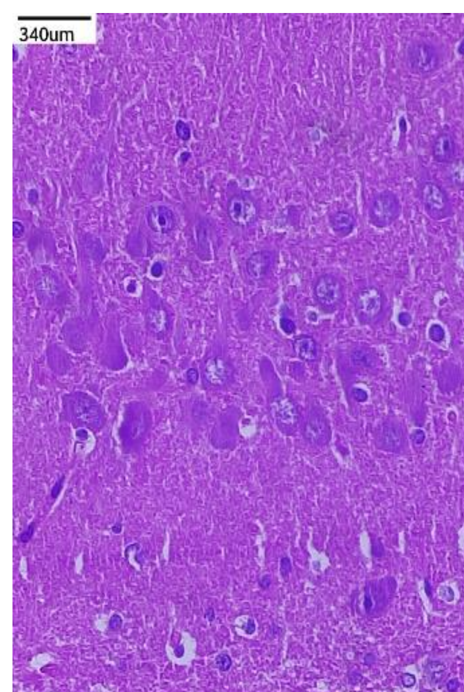


The herbal fruit extract of *Aegle marmelos* has shown remarkable potential in enhancing cognitive function, spatial learning, and motor activity in mice, as demonstrated by both Morris water maze and Y-maze tests. Notably, higher doses of the extract yielded superior results in improving performance. Additionally, AMFE exhibited antioxidant properties, evidenced by increased activity of Superoxide Dismutase and Catalase, suggesting potential protection against oxidative stress. The extract also displayed anti-inflammatory effects by reducing levels of Interleukin-6 and Tumor Necrosis Factor-alpha.

3.9. Methods for histopathological study

One mouse from each experimental group was euthanized using chloroform. Following euthanasia, the brain was carefully extracted without causing any damage, following the opening of the skull. The extracted organs were rinsed with ice-cold normal saline and subsequently fixed in 10% buffered neutral formalin. The tissues were then subjected to routine paraffin embedding, and 5-micron sections were stained with Maye's Hematoxylin Eosin stain.

Group-1 Control



Group -2 Negative control

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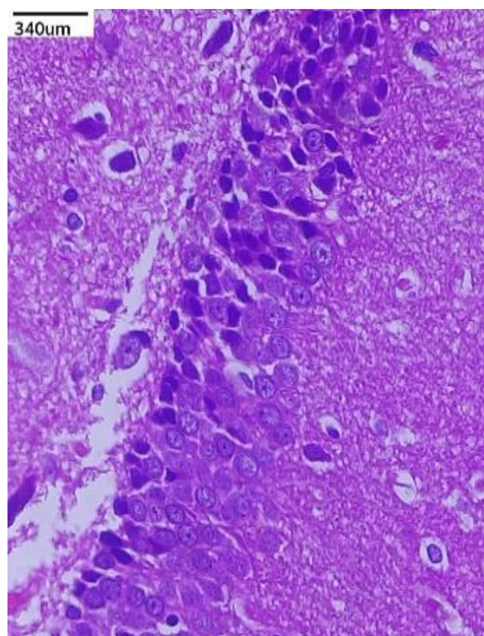
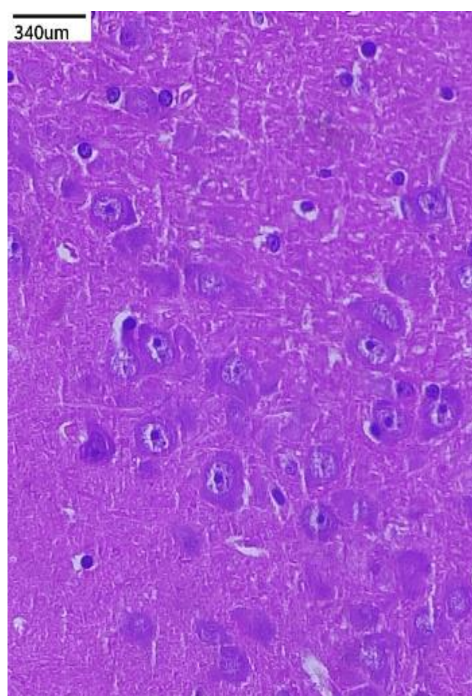


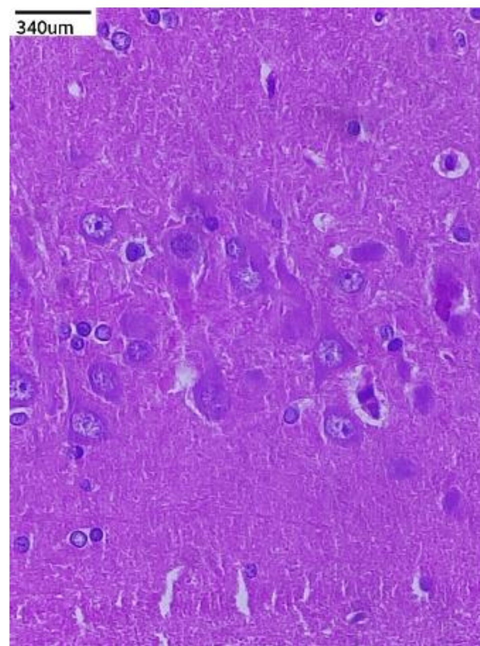
3.10. Histopathology of Brain

Figure 9:

Group-3 Test Drug LD



Group-4 Test Drug HD



The examination of the hippocampus in mice brains aimed to observe cellular changes. In Group I animals (control group), normal, healthy, and normochromic neurons were observed, displaying well-outlined cell bodies with an absence of intercellular spaces. Additionally, there was a lower concentration of activated glial cells. In Group II animals (induced group), significant and severe neuronal degeneration was noted, accompanied by congested blood vessels, perivascular edema, neuronophagia, pyknotic cells, and a higher concentration of glial cells.

Conversely, groups treated with the test drug in low dose (LD) and high dose (HD) did not display any notable changes in brain architecture. The brain sections from these groups showed no visible activated glial cells, suggesting a potential protective effect of the test drug on neuronal health and overall brain structure.

Discussion

In summary, AMFE holds promise for enhancing cognitive function, mitigating oxidative stress, and suppressing inflammation in mice. However, further research, including clinical studies, is essential to validate these findings for potential human applications.

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Animals treated with Cuprizone+200mg/kg AMFE outperformed the negative control, as evidenced by *in vivo* and histopathological studies. Group IV demonstrated superior results compared to Group III and the negative control, highlighting the enhanced benefits of AMFE in this experimental setting.

The present study focused on investigating the potential ameliorative effects of orally administered AMFE on cuprizone-induced oxidative stress and neurodegenerative accompanied by memory decline. Cuprizone predominantly induced oxidative stress and neurodegeneration in the experimental animals, as evidenced by histopathological changes in various brain regions, translating into observable neurobehavioral deficits.

Neurobehavioral tests in this study revealed that animals treated with AMFE had a decreased escape latency period compared to the negative control group animals. Cuprizone treatment significantly depleted long and short-term memory in animals, as indicated by the Morris water maze. Animals treated with Cuprizone showed an increased escape latency period, a marker for long-term memory loss, while AMFE-treated animals exhibited a decreased escape latency.

Furthermore, AMFE treatment normalized the levels of Superoxide Dismutase, Catalase and Glutathione reductase, suggesting a strengthening of the antioxidant system. This treatment contributed to boosting intrinsically produced antioxidants, thereby providing potential therapeutic benefits.

4. Conclusion

In conclusion, the administration of the herbal extract from *Aegle marmelos* (AMFE) demonstrated significant positive effects on cognitive function, spatial learning and motor activity in mice. The Morris water maze and Y-maze tests revealed improved performance, particularly with higher doses of AMFE, suggesting a potential role in enhancing memory and spatial navigation.

Moreover, AMFE exhibited antioxidant properties, as evidenced by increased Superoxide Dismutase and Catalase activity, indicating a protective effect against oxidative stress. The extract also demonstrated anti-inflammatory effects by reducing Interleukin-6 and Tumor Necrosis Factor-alpha levels. This dual action on oxidative stress and inflammation suggests a comprehensive neuroprotective potential of AMFE.

The observed dose-dependent responses in both behavioural and biochemical parameters indicate that the efficacy of AMFE may be influenced by dosage. Higher doses, as represented by the AMHD group, consistently outperformed lower doses (AMLD) in various aspects, emphasizing a potential dose-response relationship.

It is crucial to note that the negative control group, treated with only Cuprizone, exhibited detrimental effects on cognitive function, locomotor activity and antioxidant enzyme levels. In contrast, AMFE-treated groups showed protective effects, highlighting the potential therapeutic benefits of the herbal extract in mitigating the adverse effects induced by Cuprizone.

These study results provide valuable insights into the multifaceted effects of AMFE on neurological and biochemical parameters. However, further research, including clinical trials, is necessary to extrapolate these findings to human applications. Additionally, histopathological studies and further exploration of the underlying molecular mechanisms would contribute to a more comprehensive understanding of AMFE's therapeutic potential.

In summary, the findings suggest that AMFE holds promise as a natural remedy for cognitive enhancement, protection against oxidative stress and anti-inflammatory effects. The observed benefits, coupled with its herbal origin, position AMFE as a potential candidate for further investigation in the development of neuroprotective interventions.

The present experimental results indicate that *Aegle marmelos* fruit extract has neuroprotective activity in Cuprizone-induced Multiple Sclerosis. This widely available and cost-effective herbal plant serves as a complementary medicine with potential benefits in MS, offering a safer alternative to current therapies that are either partially effective or pose long-term safety concerns.

5. Conflict of interest

The authors confirm that this article content has no conflict of interest.

6. Acknowledgements

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