Protective Effect of *Murraya koenigii* Against D-Galactose-Induced Cognitive Dysfunction in Swiss Albino Mice

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Abstract

Alzheimer's disease (AD) and age-associated cognitive deficits are primary neurodegenerative diseases and are commonly related to progressive impairment in memory, oxidative stress, neuro inflammation, and neuronal loss. Chronic D-galactose administration has been widely utilized in experimental animals to induce aging-associated deficits and cognitive decline; it has been known to induce oxidative damage and trigger inflammatory reactions in an aged brain. Natural products that are rich in antioxidant and anti-inflammatory properties are being explored extensively for the potential therapy of neurodegenerative diseases. The main goal of this investigation was to evaluate the neuroprotective efficacy of *Murraya koenigii* extract in the case of D-galactose-induced cognitive deficits in Swiss albino mice. The Swiss albino mice were separated into five groups, i.e., Group I- Normal Control, Group II- *Murraya koenigii* extract 500 mg/kg Per se, Group III- D-galactose (1000mg/kg, s.c.) + Group IV- *Murraya koenigii* extract 250mg/kg + D-galactose (1000mg/kg, s.c.), Group V- *Murraya koenigii* extract 500mg/kg + D-galactose (1000mg/kg, s.c.). The cognitive functions of Swiss albino mice were assessed by the use of the Morris Water Maze, Passive Avoidance Test, and Open Field Test. The levels of antioxidant and pro-oxidant parameters, namely the concentrations of MDA, SOD, reduced GSH, and CAT in the cerebral cortex of animals, were quantified. The levels of AChE activity and the cytokines that promote inflammation, namely TNF-α, IL-1α, and IL-10, were also quantified. Brain tissues were prepared and stained with H&E for histopathological studies.

The outcome suggests that treatment with *Murraya koenigii* may lead to the recovery of cognitive impairment by enhancing antioxidant, anti-inflammatory, and cholinergic actions to provide neuroprotection against D-galactose-mediated neurodegeneration. This study may demonstrate the plant's potential as a therapeutic remedy for age-associated cognitive disorders and Alzheimer's disease.

Keywords: *Murraya koenigii*, D-galactose, Cognitive Dysfunction, Alzheimer's Disease, Neuroprotection.

1. Introduction

Neurodegenerative disorders are rapidly becoming among the most serious and expanding public health issues in the world as people live longer due to their aging populations. Alzheimer's disease, or AD for short, is a neurodegenerative disorder. The predominant type of dementia, it entails a continuous deterioration in memory, cognition, learning, and behaviour. It involves disruption of synaptic transmission, neuronal death, amyloid-beta deposition, neurofibrillary tangles, oxidative stress, and chronic neuroinflammation in the brain. (1)(2)

Advancing Age is considered the greatest critical risk factor in the etiology of cognitive impairment. A great number of investigations have provided sufficient evidence that age-associated oxidative stress plays an essential role in neurodegeneration. In fact, an overproduction of ROS combined with the decreased defense ability of antioxidants leads to neuronal damage, including peroxidation of lipids and oxidation of proteins, mitochondrial impairment, and damage to DNA, etc. As time goes on, neuronal communication and cognitive function deteriorate gradually. (3)(4)

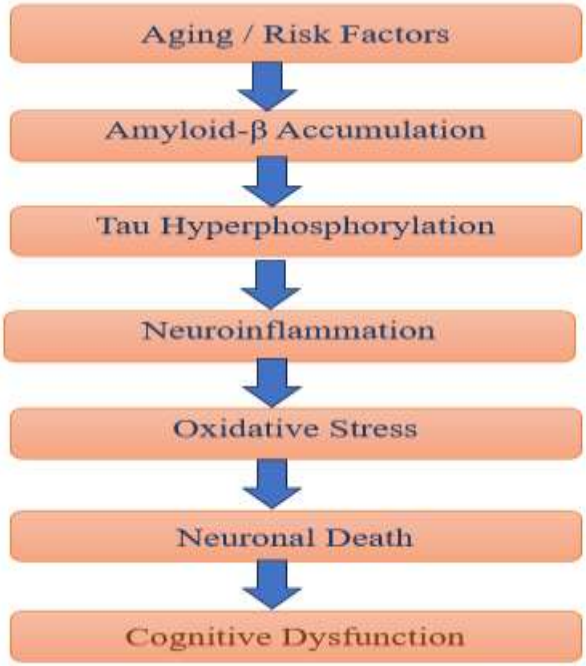

Figure 1. Pathogenesis of Alzheimer's Disease

Numerous research studies have demonstrated that the D-galactose-induced aged model is the most common experimental approach for studying age-related cognitive impairment and neurodegenerative diseases. Prolonged administration of D-galactose leads to increased production of AGEs, inflammatory processes, and oxidative stress both in vitro and in vivo, which are similar to natural aging and Alzheimer's disease both biochemically and behaviourally. Research shows that chronic administration of D-galactose caused memory deficit, hippocampal neuronal injury, mitochondrial damage, and hyper-neuroinflammation in experimental animals. (5)(6)(7)

The phenomenon of oxidative stress has become recognized as a significant process of pathogenesis in neurodegenerative conditions. Increased formation of free radicals exhausts endogenous antioxidant defenses (SOD, catalase, and GSH), producing oxidative impairment to membranes and the intracellular components of neurons. Malondialdehyde, a major product of lipid peroxidation, often increases in neurodegenerative states and is utilized as an indicator of oxidative damage.(8)(9)

Neuroinflammation also seems to contribute significantly to the progression of cognitive decline. Pro-inflammatory cytokines like TNF-alpha and interleukins alpha are released by activated micro- and astrocytes and lead to additional neuronal damage and synaptic dysfunction. However, anti-inflammatory cytokines such as IL-10 protect neurons from excessive inflammation. Such a disturbance in the equilibrium among anti-inflammatory cytokines and pro-inflammatory cytokines has been described in AD and in animal models of cognitive decline.(10)(11)

The cholinergic mechanism is still among the most well-founded mechanisms for Alzheimer's cognitive deficits. Memory impairment and the learning processes that it supports have been strongly linked to diminished acetylcholine and up-regulated acetylcholinesterase. It therefore makes logical sense to consider inhibition of acetylcholinesterase as a target programme for the symptomatic Management of AD. (12)(13)

Since the advent of neurodegenerative disease and the limitation of existing drugs, medicinal plants have shown themselves to be good therapeutic alternatives. This is because plant-based drugs have been known to exert multitarget pharmacological effects and to have fewer side effects. Plant extracts exhibit antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective activities that may attenuate disease progression and improve cognition. (14)(15)

Murraya koenigii (L.) Spreng. is an aromatic therapeutic plant with many medicinal properties, commonly named curry leaf, and is a member of the Rutaceae family. It is abundant in Bharat; it has been used extensively in traditional Indian medicine. Curry leaves contain significant carbazole alkaloids such as mahanimbine, koenimbine, girinimbine, and murrayacine, along with flavonoids, essential oils, vitamins, and phenolic substances. These phytoconstituents have numerous advantageous characteristics, containing anti-inflammatory properties, antioxidants, antibacterial, antidiabetic, and neuroprotective benefits. (16)(17)

Several experimental studies have revealed the neuroprotective effects of Murraya koenigii. It has shown memory-enhancing, antioxidant, anti-inflammatory, and neuroprotective activity in models of neuronal cell injury. Such pharmacological actions of the plant may contribute to the avoidance and alleviation of age-related neurodegenerative disorders and neurological damage.(18)(19)

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

D-galactose was acquired from CDH. Ethanolic extract of *Murraya koenigii* leaves was procured from Vital Herbs Pvt. Ltd., Add: Z-26/27 Commercial Enclave, Mohan Garden, Uttam Nagar, Delhi, 110059 (GSTIN:07CIRPD7950H1ZV). All supplementary chemicals and solvents were of the utmost analytical grade and readily obtainable in the market.

2.2. Experimental Animals

Male Swiss Albino Mice (25-30 g) of 6-8 weeks old were purchased from a CPCSEA- approved animal house. The mice were maintained in an animal house in normal laboratory conditions, i.e., (22 ± 2°C temperature, 55 ± 5% relative humidity, and 12 h light/dark cycle) with free access to diet and water ad libitum. Ethical clearance for the proposed study was obtained from the Institutional Animal Ethics Committee (IAEC) according to the CPCSEA guidelines. (1204/Po/ReBiBt/S/2008/ CCSEA/25-08

2.3. Induction of Cognitive Dysfunction

Cognitive dysfunction was induced by administration of D-galactose (1000 mg/kg/day, subcutaneously) for a duration of 7 weeks. Chronic exposure to D-galactose is known to produce oxidative stress, neuroinflammation, neuronal degeneration, and memory impairment resembling Age-associated cognitive deterioration and Alzheimer's disease.

2.4. Experimental Design

Mice were randomized into 5 groups, each consisting of 6 individuals (n = 6).
Group I- Normal Control
Group II- *Murraya koenigii* extract 500 mg/kg Per se
Group III- D-galactose (1000mg/kg, s.c.)
Group IV- *Murraya koenigii* extract 250mg/kg + D-galactose (1000mg/kg, s.c.)
Group V- *Murraya koenigii* extract 500mg/kg + D-galactose (1000mg/kg, s.c.)

2.5. Behavioural Assessment

Behavioural tests were conducted during the last week of treatment.

2.5.1. Morris Water Maze Assessment (MWM)

The water maze test was utilized to examine Spatial memory and cognitive processes escape delay time, duration in the designated area and crossing the platform number were tested. After being placed onto the platform, training for 60 s was started; if mice could not reach the destination within 60 s, it would be marked as 60 s. What's more, for mice that could not find the platform within the initial 4 days, the mice would be instructed to remain on the platform for 10 seconds. Mice were trained every 24 h, with results collected on the fifth day. The platform was removed on the 6^{th} day. To detect the spatial search ability of mice, mice crossing through the original placement and spending time in the target quadrant were recorded. (20)

2.5.2. Passive Avoidance Test

Memory retention and learning ability were assessed using the passive avoidance apparatus. Step-through latency was recorded during acquisition and retention trials.
Passive avoidance apparatus: The device consisted of a brightly lit white compartment and, on the other side, a dark compartment that could be opened by a movable door connecting them. An inescapable electrical shock was delivered to the animals in the dark chamber due to the fact that this chamber was electrified. Animals were first placed into the apparatus in order to acclimatize to the environment for 5 min. 24 hours after this training session, a passive avoidance test was conducted, and mice were first put in a brightly lit compartment; the movable door was opened, latency time required to move into the dark chamber was recorded as "escape latency," and the number of entries into the black box was recorded as "the number of errors". Mice who did not move into the dark chamber within 300 sec recorded a 300 sec latency time.(21)

2.5.3. Open Field Test (OFT)

The locomotion action of the animals was quantified using an open field box (50 cm × 50 cm × 38 cm; length × breadth × height). The animals were positioned in the central region of the apparatus and allowed to explore their surroundings independently for five min; movement activity was videotaped using a digital camera. Horizontal locomotor activity was evaluated as the sum of the horizontal movements in total distance. The chamber was sanitized between trials using 75% alcohol.(22)

2.6. Biochemical Assay

Mice were sacrificed at the end of behavioural studies. Brains were removed and cleaned with an isotonic saline solution. and then weighed and divided longitudinally into two halves. One-half of the brain was utilized for biochemical determinations. The remaining half was employed for further biochemistry as follows: To 10% (w/v) of homogenized tissues,0.1 M phosphate (pH 7.4) buffer was added and centrifuged at 10,000 g for 15 min at 4 °C. The supernatants were aliquoted and used for various biochemical studies using a UV spectrophotometer 1800 [Shimadzu].

2.6.1. Reduced Glutathione (GSH)

GSH was estimated as per Jollow et al. (1974). 0.25 mL of brain homogenate was combined with 0.25 mL of cold 5% TCA and centrifuged at 4000 rpm for 10 min.1.0 mL of supernatant was added to 0.25 mL phosphate buffer (0.2 M, pH 8.0) and 0.5mL of DTNB (0.6mM). The absorbance at 412nm was determined using a Shimadzu 1800. It was represented as micromoles of GSH per mg of protein. (23)

2.6.2. Malondialdehyde (MDA)

Lipid peroxidation of mouse brain was quantitatively measured using the Wills model, and calculations were performed based on the molar extinction coefficient of the chromophore (1.58 x 107 M-1 cm-1); then, values are provided in the

format of nanomole/milligram of protein in the result. The amount of malondialdehyde (MDA), a key constituent of lipid peroxidation, was further assessed using a double-beam UV-VIS spectrophotometer [UV spectrophotometer 1800 (Shimadzu)] with Thio barbituric acid interaction at 540 nm.25 e) (24)

2.6.3. Nitric Oxide (NO)
Nitric oxide (NO)synthesis was measured as the concentration of nitrite in precipitates by colorimetric determination with the Greiss reagent [2, 6% of the phosphate,1% of the sulphonamide, and 0.2% of N-[1-naphthyl] ethylenediamine dihydrochloride. An equal volume of supernatant was mixed with Greiss reagent, incubated for10 min and incubated for10 minutes at 25oc in dark then measured absorbance at 560 nm with twin beam U.V VIS spectrophotometer (UV spectrophotometer 1800(Shimadzu)). Quantity of nitrite was estimated from a standard graph of sodium nitrite, µg/mg of Protein.(25)

2.6.4. Catalase (CAT)
Activity of the catalase was estimated by the method of Luck through measurement of H2O2 degradation. The assay mixture essentially consists of 3 mL of H2O2 phosphate buffer and 0.05ml of supernatant of the tissue homogenate. Change in absorbance at 240 nm for 2 min with an interval of 30 s was measured in the UV spectrophotometer 1800 (Shimadzu). It was represented as Units per mg of protein. (26)

2.6.5. Superoxide Dismutase (SOD)
Superoxide dismutase (SOD) activity assay: SOD activity was estimated using the Kono assay method. The Assay system consists of the following solutions: EDTA (0.1 mM), sodium carbonate (50 mM), and nitro blue tetrazolium (NBT) (96 mM). The mixture (2 mL) of the solution, hydroxylamine (0.05 mL), and supernatant (0.05 mL) was put together in the cuvette, and the auto-oxidation of hydroxylamine for 2 min with 30 s intervals was observed by monitoring absorbance at 560 nm using a UV spectrophotometer 1800 (Shimadzu). The findings were represented as units per mg of protein.(27)

2.6.6. Acetylcholinesterase (AChE)
In the forebrain, AChE is a marker for loss of the cholinergic neurons. Ellman's technique was used to measure AChE activity using a mixture of 0.05 mL of supernatant, 3 mL of sodium phosphate buffer (pH 8), 0.1 mL of acetylthiocholine iodide, and 0.1 mL of DTNB (Ellman reagent). Change in absorbance was measured using a UV spectrophotometer 1800 (Shimadzu) for 2 min at 30 s intervals at 412 nm. The enzyme activity was expressed as micromoles of acetylthiocholine hydrolysed per minute per milligram of protein. (28)

2.6.7. Estimation of Interleukin-1 Alpha (IL-1α)
The concentration of the pro-inflammatory cytokine Interleukin-1 alpha (IL-1α) was quantified with a commercially available Rat IL-1α ELISA Kit sourced from Elab Science Biotechnology Co., Ltd. The concentration of IL-1α in brain tissue homogenates was quantified using the Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method as per the manufacturer's guidelines.(29)

2.6.8. Estimation of Interleukin-10 (IL-10)
The concentration of the anti-inflammatory cytokine Interleukin-10 (IL-10) was estimated using a commercially available Rat IL-10 ELISA Kit obtained from Elab Science Biotechnology Co., Ltd. The concentration of IL-10 in brain tissue homogenates was determined by the Sandwich ELISA technique following the manufacturer's protocol.(29)

2.6.9 Assessment of Tumor Necrosis Factor-Alpha (TNF-α)
The concentration of the pro-inflammatory cytokine Tumor Necrosis Factor-alpha (TNF-α) was quantified using a commercially available Rat TNF-α ELISA Kit sourced from Elab Science Biotechnology Co., Ltd. The concentration of TNF-α in brain tissue homogenates was quantified utilizing the Sandwich ELISA technique in accordance with the manufacturer's guidelines.(29)

2.7. Histopathological Examination
Brain tissue, particularly the hippocampus and cerebral cortex, was preserved in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin (H&E). The specimens were histologically analyzed for neuronal degeneration, pyknosis, vacuolization, or other neuropathological conditions.

2.8. Statistical Analysis
Data were presented as Mean ± SEM. Statistical analysis was conducted utilizing one-way ANOVA, followed by Tukey's multiple comparison test, which was done by GraphPad Prism software. Values of p less than 0.05 were deemed statistically significant.

3. Result

3.1. Morris Water Maze Test
The impact of therapy on Learning and memory was tested over six consecutive days through the Morris Water Maze test. On days 1–5, learning ability was measured in terms of elapsed time taken to find the hidden platform (escape latency time) by each animal. The decrease in elapsed time from day 1 to day 5 indicates proper learning ability of the animal, whereas the decrease in elapsed time on day 5 when compared with day 1 indicates no significant learning impairment. On day 6, the platform was removed, and retention of memory was analysed by calculating time spent in the target

quadrant (TSTQ). More time in the target quadrant shows retention of memory, whereas time spent in the target quadrant decreases, as it shows cognitive impairment.

Control shows a significant ($p < 0.05$) elapsed time on Day 5 when compared with Day 1. Control shows significantly more time spent in the target quadrant (Q4) on Day 6 when compared with the treatment group, thereby indicating proper learning and memory retention capacity. Animals in D-galactose-treated groups had a significant elapsed time on Day 5, $F(4,25) = 9.84$, $^*p<0.05$, and time spent in the target quadrant on Day 6, $F(4,25) = 11.26$, $^*p<0.05$, which was decreased significantly, showing impairment of the learning and memory process. Treatment of MKE reversed these effects significantly and enhanced cognitive functions in a dose-dependent manner.

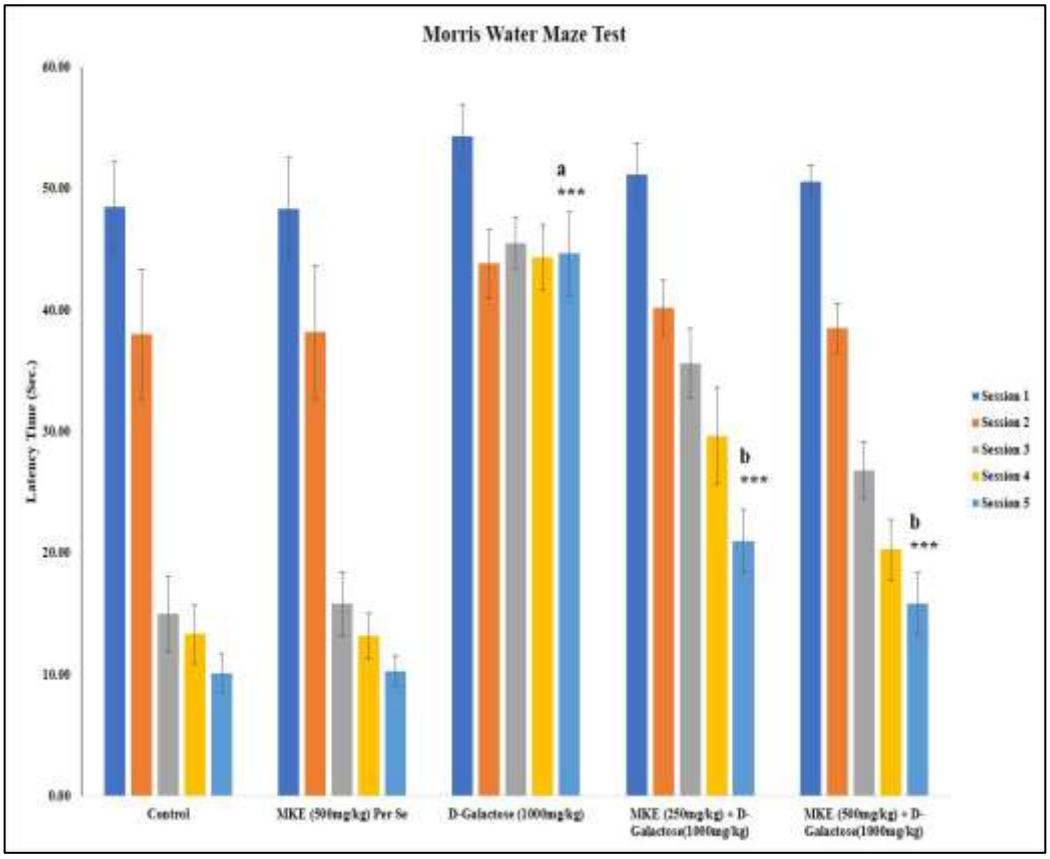

Figure 2: Effect of MKE on spatial learning and memory in D-galactose-induced experimental animals using the Morris Water Maze test.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. MKE (*Murraya koenigii Extract*).

Time Spent in Target Quadrant (TSTQ)

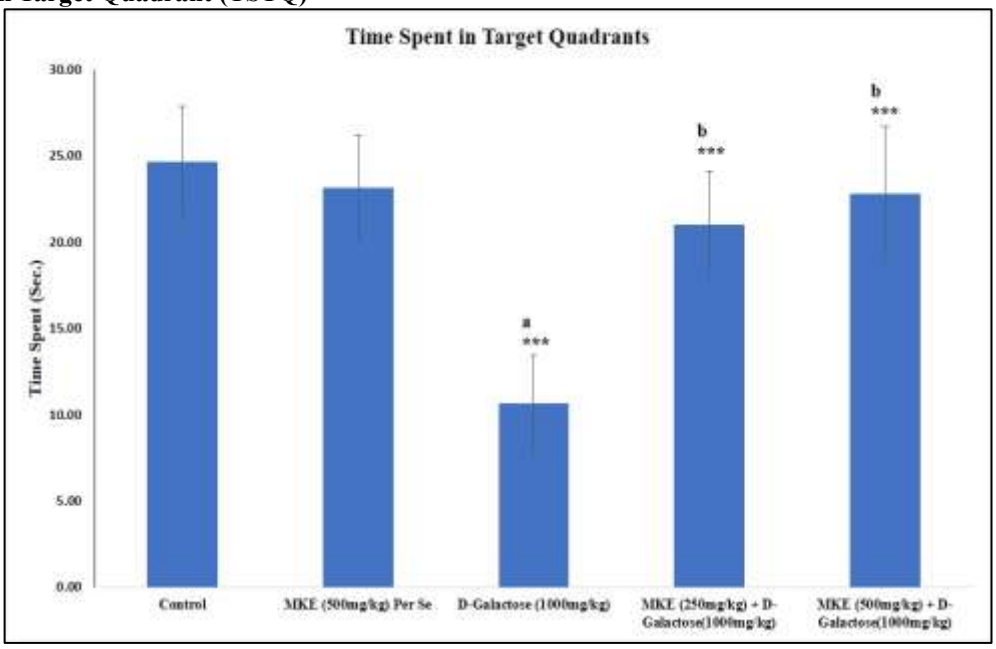

Figure 3: Effect of MKE on time spent in the target quadrant (TSTQ) in D-galactose-induced experimental animals using the Morris Water Maze test.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. TSTQ: Time spent in target quadrant; MKE (*Murraya koenigii Extract*).

3.2. Passive Avoidance Test

In comparison to the control group, the D-galactose-treated group shows a significant reduction in learning and memory, as evidenced by a reduction in step-through latency $F(4,25) = 10.76$, $^*p < 0.05$. When compared to the D-galactose group, both treatment groups show a statistically significant enhancement in step-through latency ($p < 0.05$), indicating improved memory retention. A dose-dependent recovery of cognitive performance was observed after the treatment of MKE. This result indicated that MKE showed a significant protective effect against D-galactose-induced cognitive dysfunction and memory enhancement.

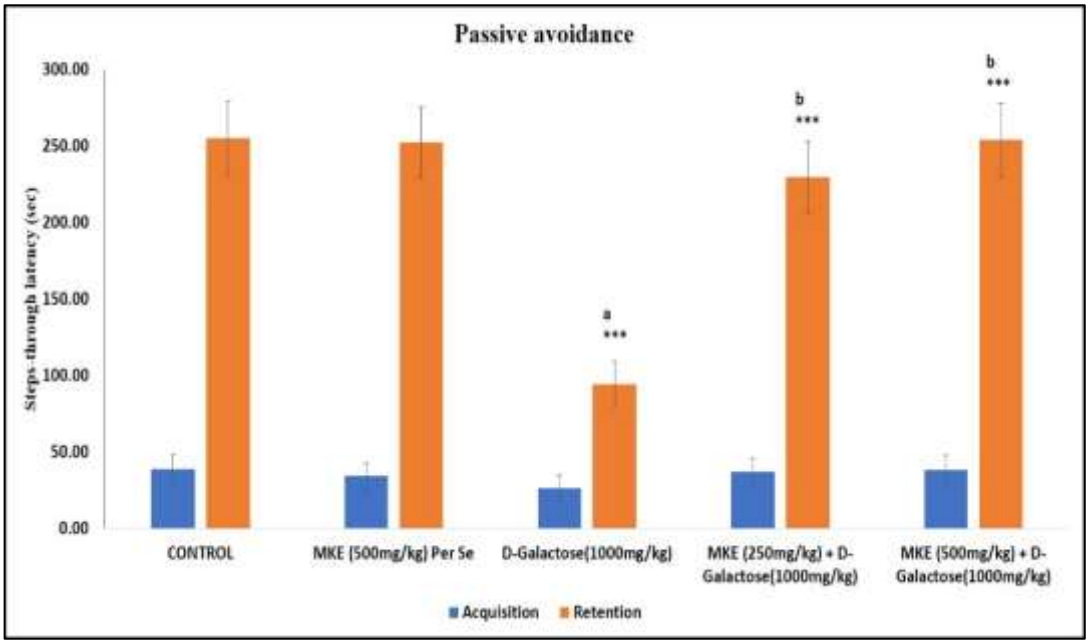

Figure 4: Effect of MKE on learning and memory in D-galactose-induced experimental animals using the Passive Avoidance test.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. MKE (*Murraya koenigii Extract*).

3.3. Open Field Test (OFT)

In comparison to the control group, the D-galactose-treated group showed no significant differences in locomotor and exploratory activity, as evidenced by line crossings, rearing, and grooming behaviour ($F(4,25) = 0.9647$, $^*p > 0.05$). Similarly, treatment with MKE (*Murraya koenigii Extract*) did not produce any significant alteration in behavioural parameters when compared with the D-galactose group. These findings suggest that neither D-galactose nor MKE treatment significantly affected general locomotor activity.

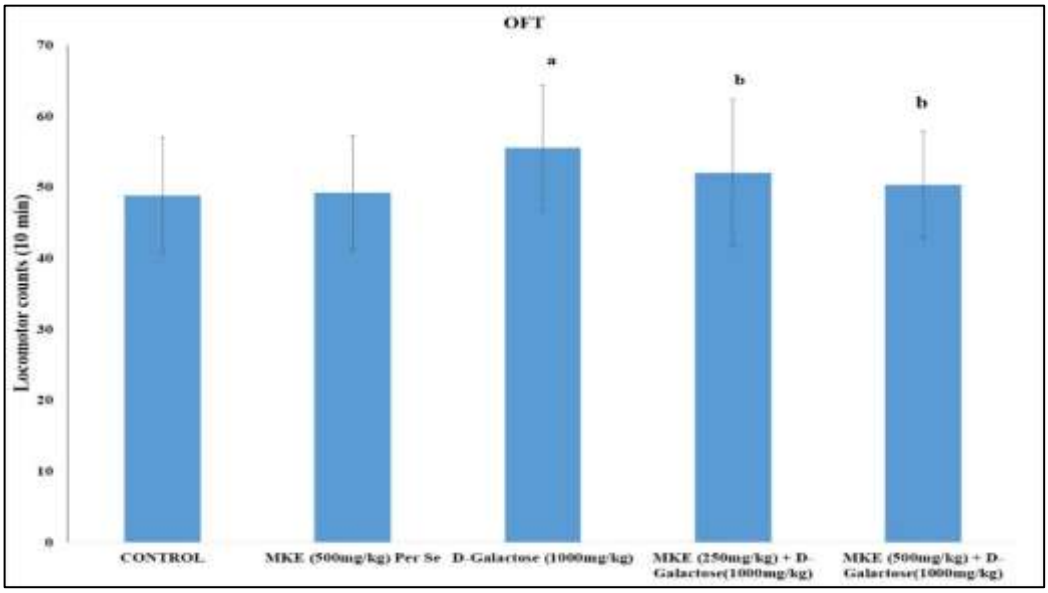

Figure 5: Effect of MKE on locomotor and exploratory behaviour in D-galactose-induced experimental animals using the Open Field Test.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. MKE (*Murraya koenigii Extract*).

3.4. Estimation of Reduced Glutathione (GSH)

In comparison to the control group, the level of GSH was significantly decreased in the D-galactose-treated group $F(4,25) = 10.51$, $^*p<0.05$. When compared with the D- galactose group, both treatment groups significantly increased the level of GSH ($p<0.05$). A dose-dependent improvement in GSH levels was observed in the following treatments with MKE (*Murraya koenigii Extract*)

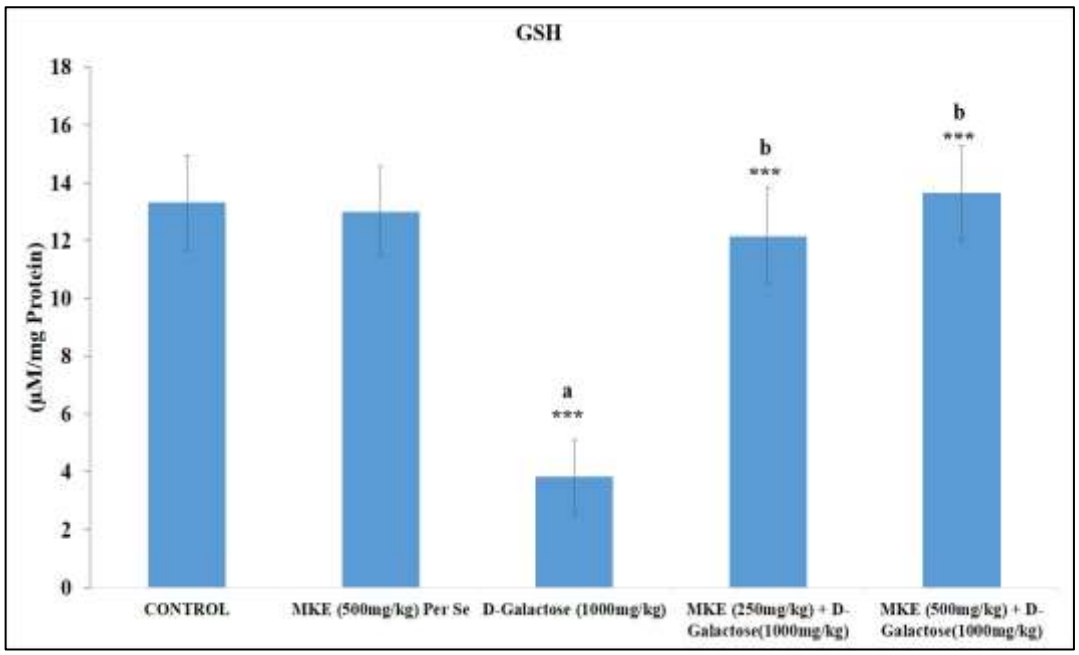

Figure 6: Effect of MKE on reduced glutathione (GSH) levels in D-galactose-induced experimental animals.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. GSH: Reduced glutathione; MKE (*Murraya koenigii Extract*).

3.5. Estimation of Malondialdehyde (MDA)

In comparison to the control group, the level of MDA was significantly increased in the D-galactose-treated group $F(4,25) = 19.42$, $^*p<0.05$. When compared with the D- galactose group, both treatment groups significantly reduced the level of MDA ($p<0.05$). A dose-dependent reduction in MDA levels was observed in the following treatments with MKE (*Murraya koenigii Extract*).

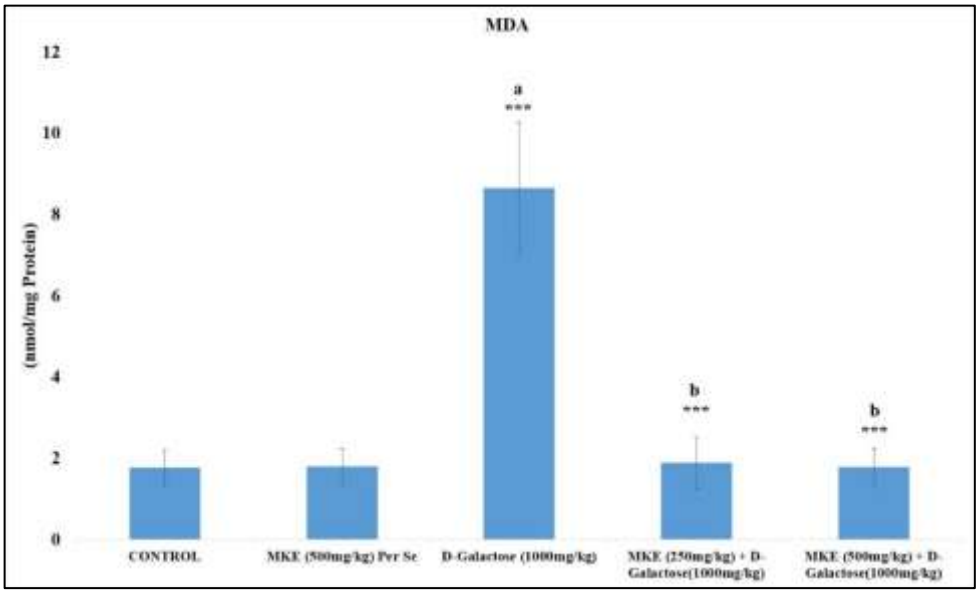

Figure 7: Effect of MKE on malondialdehyde (MDA) levels in D-galactose-induced experimental animals.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. MDA: Malondialdehyde; MKE (*Murraya koenigii Extract*).

3.6. Estimation of Nitric Oxide (NO)

In comparison to the control group, the level of nitric oxide (NO) was significantly increased in the D-galactose-treated group F (4,25) =25.59 *p<0.05. When compared with the D- galactose group, both treatment groups significantly reduced the level of NO (p<0.05). And a dose- dependent reduction in NO levels was observed in the following treatments with MKE (*Murraya koenigii Extract*).

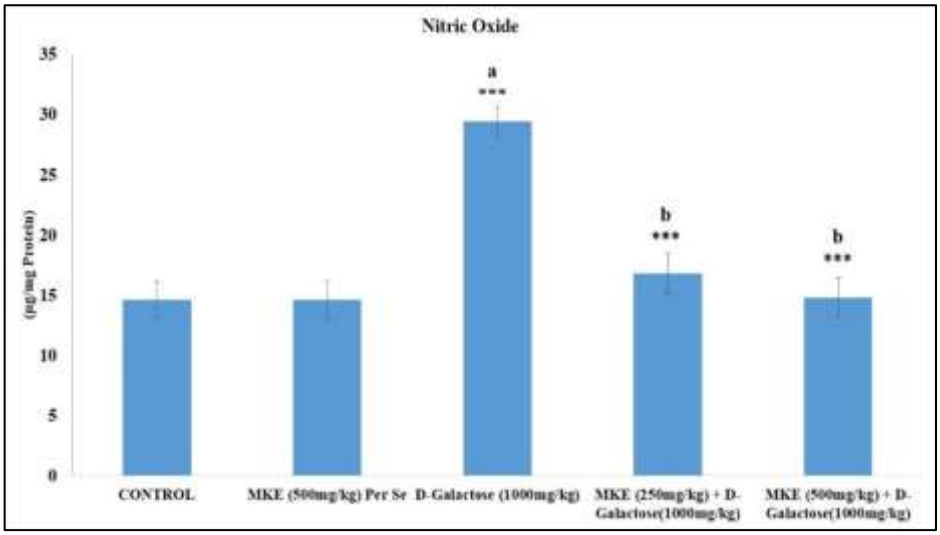

Figure 8: Effect of MKE on nitric oxide (NO) levels in D-galactose-induced experimental animals.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. p < 0.05 was considered statistically significant. NO: Nitric oxide; MKE (*Murraya koenigii Extract*).

3.7. Estimation of Catalase (CAT)

In comparison to the control group, the level of catalase (CAT) was significantly decreased in the D-galactose-treated group F (4,25) =16.64 *p<0.05. When compared with the D- galactose group, both treatment groups significantly increased the level of catalase (CAT) (p<0.05). And a dose- dependent improvement in CAT levels was observed in the following treatments with MKE (*Murraya koenigii Extract*)

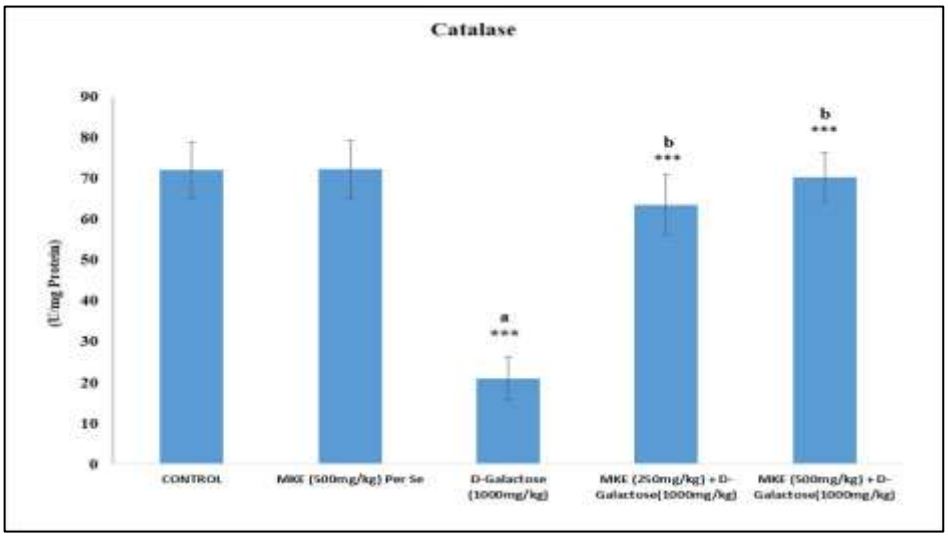

Figure 9: Effect of MKE on catalase (CAT) levels in D-galactose-induced experimental animals.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. p < 0.05 was considered statistically significant. CAT: Catalase; MKE (*Murraya koenigii Extract*).

3.8. Estimation of Superoxide Dismutase (SOD)

In comparison to the control group, the level of superoxide dismutase (SOD) was significantly decreased in the D-galactose-treated group F (4,25) =11.12, *p<0.05. When compared with the D- galactose group, both treatment groups significantly increased the level of SOD (p<0.05). A dose-dependent improvement in SOD levels was observed in the following treatments with MKE (*Murraya koenigii Extract*)

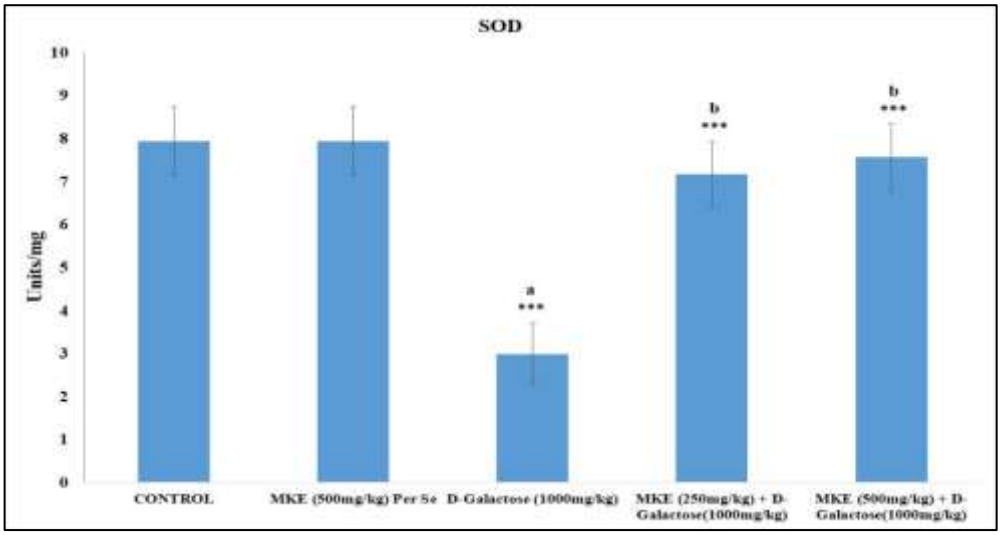

Figure 10: Effect of MKE on superoxide dismutase (SOD) levels in D-galactose-induced experimental animals

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. SOD: Superoxide dismutase; MKE (*Murraya koenigii Extract*).

3.9. Estimation of Acetylcholinesterase (AChE)

In comparison to the control group, the level of acetylcholinesterase (AChE) was significantly increased in the D-galactose-treated group $F(4,25) = 5.677$, $^*p<0.05$. When compared with the D- galactose group, both treatment groups significantly reduced the level of AChE ($p<0.05$). A dose-dependent reduction in AChE levels was observed in the following treatments with MKE (*Murraya koenigii Extract*).

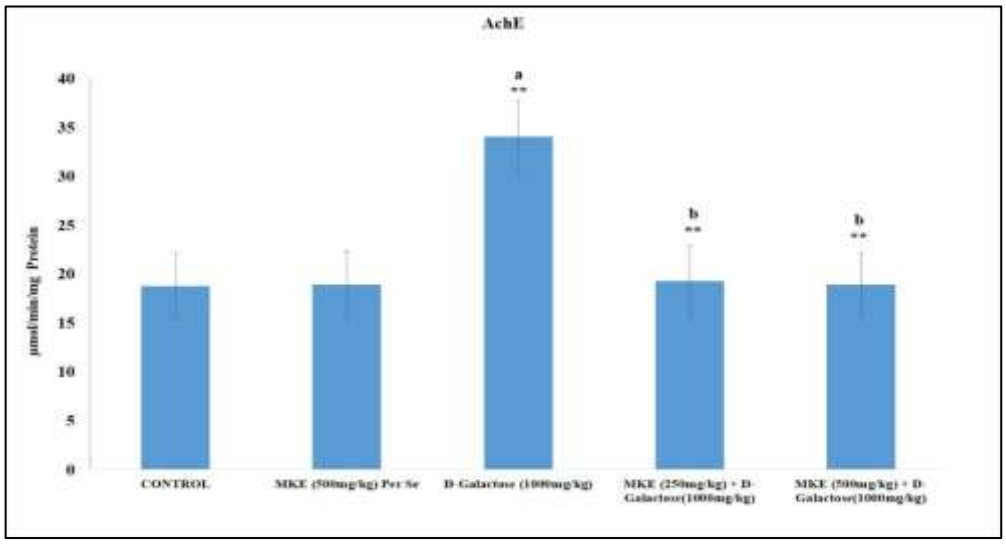

Figure 11: Effect of MKE on acetylcholinesterase (AChE) levels in D-galactose-induced experimental animals.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. AChE: Acetylcholinesterase; MKE (*Murraya koenigii Extract*).

3.10. Estimation of Tumor Necrosis Factor-Alpha (TNF-α)

In comparison to the control group, the level of TNF-α was significantly increased in the D-galactose-treated group $F(4,25) = 15.39$, $^*p<0.05$. When compared with the D- galactose group, both treatment groups significantly reduced the level of TNF-α ($p<0.05$). And a dose- dependent reduction in TNF-α levels was observed in the following treatments with MKE (*Murraya koenigii Extract*).

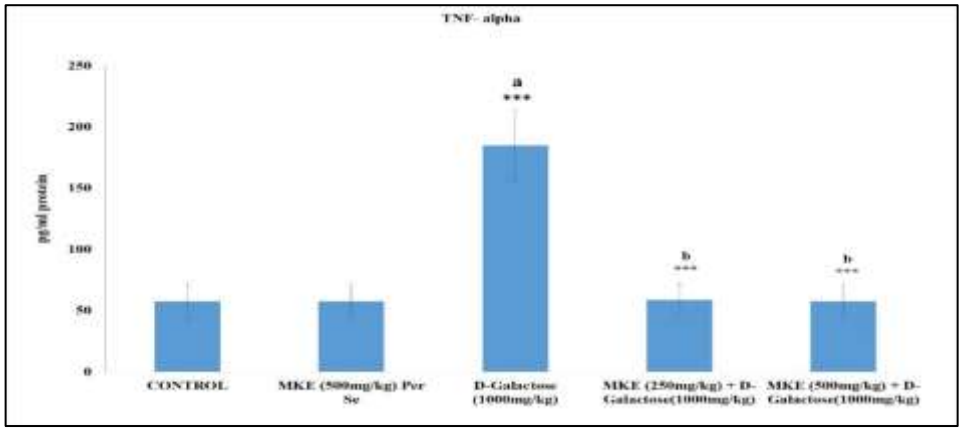

Figure 12: Effect of MKE on tumor necrosis factor-alpha (TNF-α) levels in D-galactose-induced experimental animals. Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. TNF-α: Tumor necrosis factor-alpha; MKE (*Murraya koenigii Extract*).

3.11. Estimation of Interleukin-1 Alpha (IL-1α)

In comparison to the control group, the level of IL-1α was significantly increased in the D-galactose-treated group $F(4,25) = 71.31$, $^*p<0.05$. When compared with the D- galactose group, both treatment groups significantly reduced the level of IL-1α ($p<0.05$). And a dose- dependent reduction in IL-1α levels was observed in the following treatments with MKE (*Murraya koenigii Extract*).

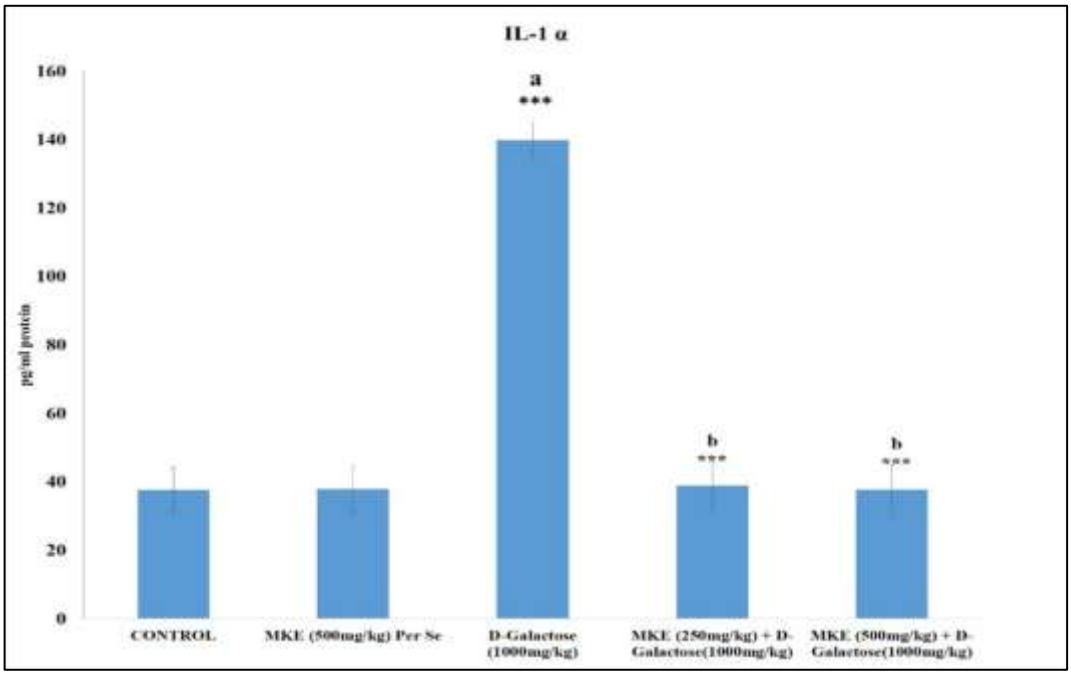

Figure 13: Effect of MKE on interleukin-1 alpha (IL-1α) levels in D-galactose-induced experimental animals.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. IL-1α: Interleukin-1 alpha; MKE (*Murraya koenigii Extract*).

3.12. Estimation of Interleukin-10 (IL-10)

In comparison to the control group, the level of IL-10 was significantly decreased in the D-galactose-treated group $F(4,25) = 7.529$, $^*p < 0.05$. In comparison with the D-galactose group, both treatment groups showed a significant increase ($p < 0.05$) in IL-10 levels. Furthermore, a dose-dependent improvement in IL-10 levels was observed following treatment with MKE (*Murraya koenigii Extract*).

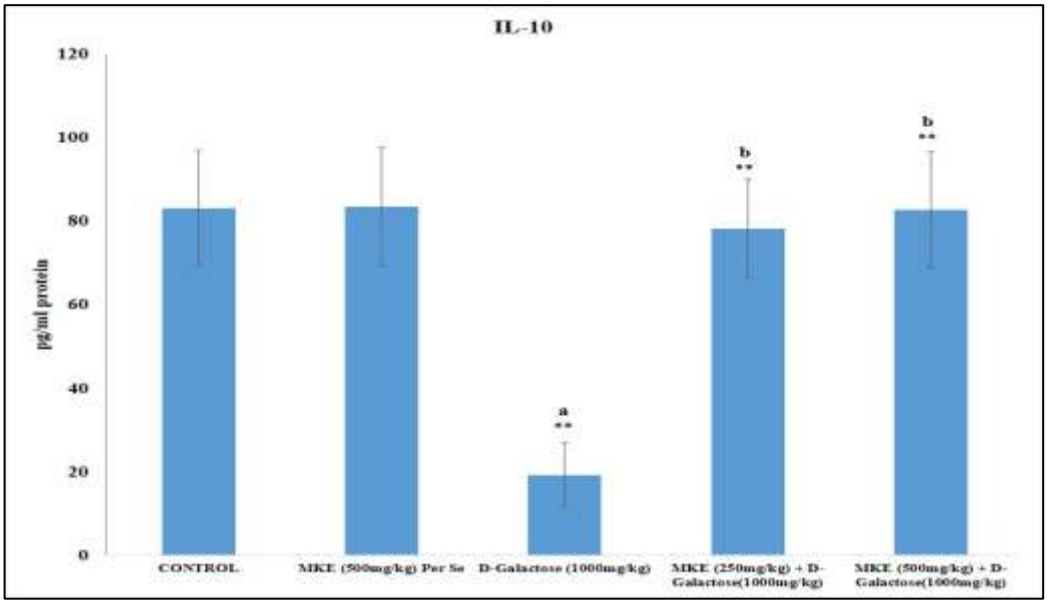

Figure 14: Effect of MKE on interleukin-10 (IL-10) levels in D-galactose-induced experimental animals.

Results are presented as mean ± SEM (n = 6). Statistical analysis was performed utilizing one-way ANOVA, followed by Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant. IL-10: Interleukin-10; MKE (*Murraya koenigii Extract*).

3.13. Histopathological Observations of Brain Tissue

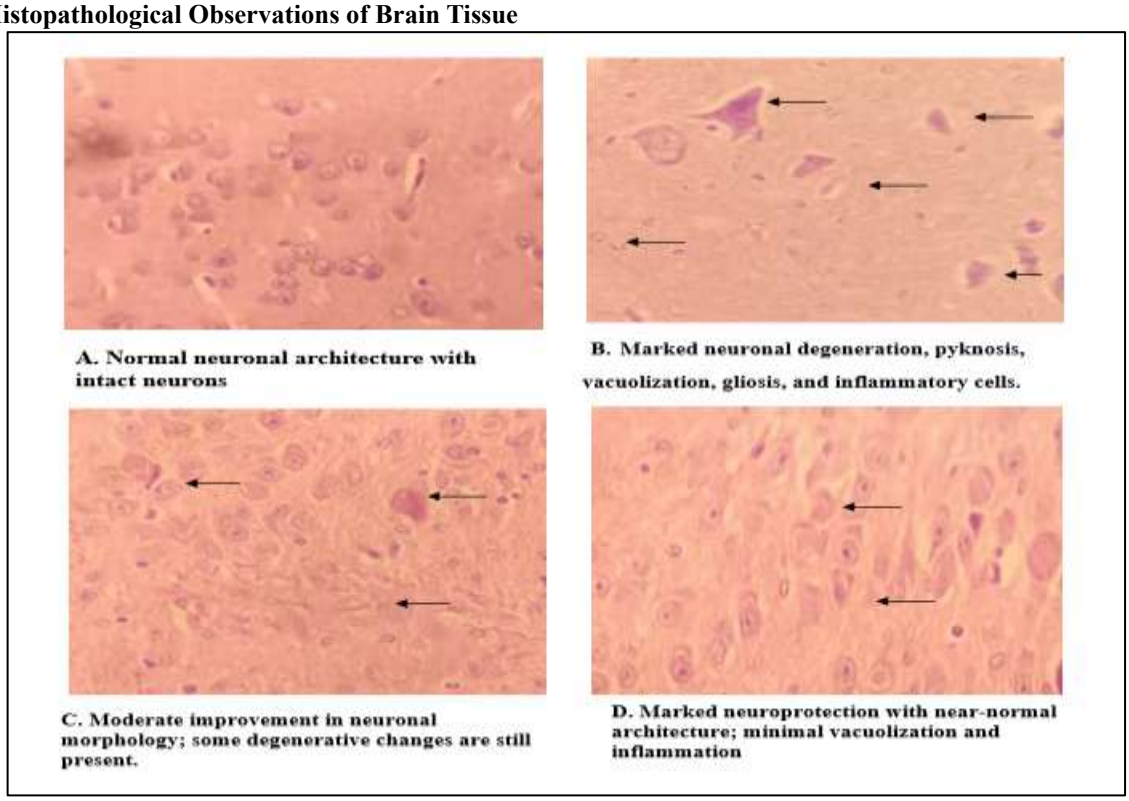

Figure 15: Illustrative pictures of the H&E-stained (Magnification x 400) in the Cortex area

Group A: Normal Control

In brain slices obtained from the normal control group, neurons showed normal histological appearance with a normal cellular structure in both cortical and hippocampal regions. Neuronal membranes are intact with clear nuclei and normal cytoplasmic characteristics, and there was no evidence of neuronal degenerative changes, vacuolization, pyknosis, inflammatory cellular infiltration, and tissue edema.

Group B: D-Galactose (1000mg/kg)

Histology of D-galactose treated group indicates a marked neuronal degenerative change where neurons appear degenerate with cytoplasmic vacuolization, pyknotic nuclei, neuronal shrinking, disordered neurons and hippocampus neurons disorganization. Also, presence of gliosis, inflammatory cellular infiltration, intercellular edema observed indicating high neuronal oxidative stress and neuronal inflammation due to chronic D-galactose treatment.

Group C: *Murraya koenigii* (250 mg/kg) + D-Galactose (1000mg/kg)
Histology of rat brain treated with *Murraya koenigii* (250 mg/kg) indicates moderate neuronal restoration compare to D-galactose treated group. Neuronal degenerative changes such as neuronal loss, cytoplasmic vacuolization and pyknotic cells were reduced and partial neuronal organization and hippocampal architecture restoration noted. Mild neuronal inflammation, and moderate degenerated neuron detected. Thus, *Murraya koenigii* provides partial protection from the D-galactose induced neurodegeneration.

Group D: *Murraya koenigii* (500 mg/kg) + D-Galactose (1000mg/kg) Histology of brain tissues obtained from the rat treated with *Murraya koenigii* (500 mg/kg) illustrates that majority of the neuronal elements is almost normal and neuronal cell structure well preserved with clear nuclei, there is a great degree of reduction in neuronal loss, neuronal degenerative changes like vacuolization, pyknosis and neuronal infiltration of inflammatory cells. The hippocampus and cortex architecture is almost restored and shows significant recovery from neuronal damage that demonstrates a dose-dependent effect *of Murraya koenigii* on neuroprotection.

4. Discussion

Chronic D-galactose administration serves as an established accelerated aging model leading to neurodegeneration, characterized by oxidative stress, neuroinflammation, cholinergic dysfunction, and cognitive deficits resembling Alzheimer's features. The data obtained in this study indicated that administration of M. Koenigii prevented the D-galactose- induced behavioural impairment and biochemical deficit; hence, M. Koenigii could act as a neuroprotective agent.(30)(7)
This investigation was conducted to determine the neuroprotective effects of *Murraya koenigii* against D-galactose-mediated biochemical, behavioural, and inflammatory alterations associated with cognitive impairment in Swiss albino mice.
D-galactose increased the escape latency and decreased the time spent in the target quadrant in **MWM test**, which means spatial learning and memory was disturbed. This result supports the former statement that D-galactose-induced oxidative stress and neuron damage disturb hippocampal functions. Meanwhile, *Murraya koenigii* obviously reduced escape latency and enhanced retention memory compared with the disease control. This spatial memory betterment perhaps owes to the existence of carbazole alkaloids mahanimbine together with flavonoid and phenolic components having significant antioxidant and neuroprotective activity.(31)
Moreover, in the Passive Avoidance Test, a D-galactose-treated group exhibits significantly decreased step-through latency, indicating long-term memory retention impairment. However, in the group treated with *Murraya koenigii* extract, latency time was substantially increased, suggesting memory consolidation and long-term memory retention was increased. Cognitive improvements could correlate to how the extract maintains neuronal integrity and reduces oxidative damages in brain tissues.(32)
Locomotor activity and exploration did not show any significance differences between control group and D-galactose-treated group in terms of their locomotion activity, exploration frequency and rearing. Oxidative damage: Oxidative damage leads to neurodegenerative diseases and dementia. The results have indicated the role of oxidative damage in the underlying causes of dementia due to D-galactose injection. In this study, endogenous antioxidant enzymes; like Sod, catalase and GSH content decreased significantly after d-galactose treatment and the expression indicates that oxidative damage can caused by excess of reactive oxygen species production. By comparison with the disease group, *Murraya koenigii* ameliorated levels of antioxidant enzymes, namely SOD, catalase and GSH; the improvement of defense mechanisms can be attributed to the scavenging of free radical and protections against injury to the neuronal cells by the extracts of the same herb. In previous studies, research indicated the presence of many components that exert antioxidant effect and can reduce lipid peroxidation in vitro.(33)
The cholinergic mechanism plays a key role in cognitive functions, memory, and learning. The acetylcholinesterase (AChE) activity in the D-galactose-treated animals were significantly high, suggesting an increase in breakdown of acetylcholine and deficiency of cholinergic transmission. High AChE levels are a typical symptom of most neurodegenerative disease such as Alzheimer's disease.
Administration of the *Murraya koenigii* resulted in a dose-dependent decrease of the elevated levels of AChE in the brain and helped in normalizing acetylcholine and learning function.
These data indicate that the extract modulates cholinergic neurotransmission and can lead to enhancement of learning, retention, and recall processes.(34)
Inflammatory response: Neuroinflammation is another mechanism by which cognitive impairment happens. It was observed in the study that administration of D-galactose has led to marked elevation of pro-inflammatory mediators like IL-1 alpha and TNF- α, and reduction of anti-inflammatory mediator IL-10. Higher levels of cytokines such as IL-1 and TNF- α are correlated with microglia activation, damage of neurons, and progression of neurodegeneration. Treatment with M koenigii led to a significant reduction in the IL-1 and TNF- α levels and the elevation of IL-10 levels in treated animals compared to diseased control animals, and this suggests that the potent anti-inflammatory property of the extract protects from the damage caused by cytokines.(35)
The observed therapeutic effect in the present study could be due to the combined effect of the different bioactive compounds present in Murraya koenigii, such as carbazole alkaloids, flavonoids, phenolic compounds, and essential oils. Different phytochemical constituents showed anti-inflammatory, antioxidant, anti-apoptotic, and cholinesterase inhibitory effects, and these contributed to the neuroprotective effect observed.

5. Conclusion

Our results indicate that *Murraya koenigii* produced significant neuroprotective effects on the D-galactose-induced cognitive impairment in Swiss albino mice. *Murraya koenigii* reversed learning and memory deficits induced by D-galactose on the Morris Water Maze and Passive Avoidance test, respectively, as well as corrected explorer-type behaviour in the Open Field Test. The extract substantially increased the endogenous antioxidant defenses by significantly elevating GSH, SOD, and catalase levels and decreasing the level of acetylcholinesterase.

In addition, *Murraya koenigii* suppressed neuroinflammation and reduced the levels of IL-1α and TNF- α and increased the level of IL-10.

The data indicate that the neuroprotective action of *Murraya koenigii* is facilitated by antioxidant, anti-inflammatory, and cholinergic modulatory mechanisms. Consequently, *Murraya koenigii* may be proposed as a therapeutic agent for the prevention and treatment of age-related cognitive impairments and neurodegenerative diseases, such as Alzheimer's disease. Research is required to find active chemicals and elucidate molecular mechanisms of action, while clinical trials are necessary to determine the therapeutic significance of this herb.

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Conflict Of Interest

The authors declare the denial of any conflict of interest.

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