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Original article

Mitigation of behavioral deficits and cognitive impairment by antioxidant and neuromodulatory potential of *Mukia madrespatana* in D-galactose treated rats

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ABSTRACT

Plants and their parts have been extensively used for the therapeutic purposes such as aging due to their powerful antioxidative belongings. Presently, we intended to examine the consequence of fruit peel of *Mukia madrespatana* (M.M) on D-galactose (D-Gal) persuaded anxiety and/or depression profile, cognition and serotonin metabolism in rats. Animals were divided in to 4 groups (n = 6). (i) Water treated (ii) D-Gal treated (iii) M.M. treated (iv) D-Gal + M.M. treated. All the animals received their respective treatment for 4 weeks. D-Gal and M.M. fruit peel were given to animals with oral gavage with doses 300 mg/ml/kg/day and 2 g/kg/day respectively. After 4 weeks' behavioral analysis performed to evaluate anxiety and depression profile, cognitive function of animals. After that animals were sacrificed and whole brain removed for biochemical (redox status, degradative enzyme of acetylcholine), and neurochemical (serotonin metabolism) analysis. Results showed that administration of M.M. inhibited D-Gal-instigated anxious and depressive behaviors and improved cognition. Treatment of M.M. decreased MDA levels, AChE activity and increased antioxidant enzyme activity in D-Gal administered and control rats. Enhanced serotonin metabolism also decreased by M.M. in control and D-Gal administered rats. In conclusion, M.M. fruit peel has powerful antioxidative and neuromodulatory properties and due to this effect, it may be a good source of mitigation/treatment for aging induced behavioral and cognitive impairment.

1. Introduction

Aging, a foremost issue impairs cellular and molecular functions (de Silva et al., 2021). It gradually effects physiological dysfunctions include enhance genomic alteration, impair metabolism, and decline/loss of renewing potential. In the brain gradual decline in the functions has been characterized by synthesis of reactive oxygen species (ROS) that induced oxidative damaging and mitochondrial abnormalities (Singh et al., 2019). Mitochondria generate ROS as a byproduct of oxidative phosphorylation and hunt them by potential antioxidant mechanism. The endogenous antioxidant systems become weak gradually with aging, and leads enormous gathering of oxidative damage of macromolecules such as protein, lipid and nucleic acid (Receno et al., 2019). Aging progression is associated with many neurological problems i.e. anxiety, depression (Samad et al., 2022; 2021), cognitive impairment (Samad et al., 2020; 2019) etc. D-galactose (D-Gal) model is one of the progressive aging models, having lesser side effects and mortality rate (St-Pierre et al., 2006). Repeated administration of D-Gal enhances ROS generation which can weaken the organismal antioxidant defence system (Samad et al., 2019). In addition, intoxication of D-gal can cause alteration at central and peripheral levels (Samad et al., 2019). Administration of D-Gal causes depression

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(Samad et al., 2019; Liaquat et al., 2019), anxiety (Samad et al., 2022; Hakimizadeh et al., 2021) and impaired cognition (Ali et al., 2021 Yang et al., 2016). Previously it was observed that increased ROS contents can cause neuro-inflammation and dysregulation of apoptosis and various neural proteins such as brain derived neuro-trophic factor, which associated with memory alterations (Gao et al., 2022). It is highlighted in a study that D-Gal declined the number of neurons in brain and impaired the process of learning and memory (Prajit et al, 2020). D-Gal induced alterations in brain at molecular and cellular levels were observed in experimental models which linked with natural progression of aging (Samad et al., 2022).

Medicinal plants have a great part in the curing of variety of oxidative stress related diseases including aging and associated neurological problems. The intake of plant based natural antioxidant may be minimizing and/or reducing the risk of variety of health issues. *Mukia madrespatana*, a medicinal plant, is generally named as the Madras pea pumpkin (Thabrew et al., 1995), which is found in tropical and subtropical parts of the world (Thabrew et al., 1995). It has phytochemicals i.e. phenolic, tannins, flavonoids, alkaloids, and saponins. These phytochemicals due to presence of –OH groups are efficient reducing agent and exhibiting powerful antioxidant effects (Petrus et al., 2012). M.M. Plant has powerful antioxidant potential and has anti-inflammatory, antiarthritic (Priya et al., 2012), anti-asthmatic, anti-tussive, antihistaminic, anti-bronchitic (Ramakrishnamacharya et al., 1996), antihypertensive (Raja et al., 2007) and anti-stress (Samad et al., 2020) effects in various experimental models.

Taking the importance of M.M. plant into consideration, to evaluate anxiety and/or depression profile and cognitive behavior following D-Gal administration in rats, the present study was designed.

2. Materials and method

2.1. Animals

Due to hormonal cycle variation male rats preferred over female rats in earlier studies. Twenty–four, Male Sprague Dawley, albino rats, (180–200 g by weight; 6–7 weeks by age) were confined in transparent plastic cages. Standard rodent diet (control diet = 4.4 7 kcal/g; Borcarsly et al., 2012), 12-hrs light/dark cycle and 20 ± 5 °C temperature were given to animals. Animals were familiarized to new environment before starting the research study. All the experimental procedures were permitted by Departmental Bioethical Committee (Ref# Biochem-D/352/2021; Dated: February 17, 2021). Bio-ethical condition was firmly followed during the study period which was of international standard.

2.2. Plant material and chemicals

From the nearby areas of Multan City, Pakistan, fresh fruit of *Mukia maderaspatana* (M. M) were gathered. The plant recognized by taxonomist as reported previously (Samad et al., 2020). M.M. fruit peel were removed and shaded dry. After that grounded into powder form and stored in air tight jar. A dose of 2000 mg/kg/day of M.M. peel, which has no toxicant effect and protected, used already in an earlier study (Samad et al., 2020) and 300 mg/ml/kg/day of D-Gal (Samad et al., 2019) was given orally by gavage to animals with drinking water, once daily at 09:00–10:00 am for 28 days.

D-Gal, Thio-barbituric acid (TBA), Acetylthiocholine Iodide, Trichloroacetic acid (TCA), Dithio-bis nitrobenzoic acid (DTNB), Hydroxylamine hydrochloride, Potassium dichromate/acetic acid, Sodium bicarbonate, Nitro blue tetrazolium (NBT), reduced glutathione and disodium hydrogen phosphate were purchased from Sigma- Aldrich Inc (St. Louis, USA). Hydrogen peroxide (H_2O_2), sodium carbonate, sodium azide and ethylene diamine tetra acetic acid (EDTA) were purchased from British Drug House (BDH, Dorset, UK).

2.3. Treatment

Twenty-four rats were divided into following sets with n = 06 as previous studies reported (Samad et al., 2022; Samad et al., 2021); (i) water + water (ii) water + M.M. (iii) water + D-Gal (iv) D-Gal + M.M. and received their particular treatment daily for 28 days. Behavioral activities i.e., Elevated Plus-Maze (EPM) and Light/dark activity (LDA) tests conducted to evaluate anxiety profile; Forced Swim Test (FST) conducted to assess depressive symptoms and Morris Water Maze (MWM) test (acquisition, short-term-memory (STM), long-term-memory (LTM) for cognitive functions. After behavioral activities rats were decapitated using guillotine (Samad et al., 2021) and their brains collected from skull as reported previously (Tabassum et al., 2017), and then frozen at −20 °C for further neurochemical and biochemical assessments (Experiment layout Fig. 1).

2.4. Behavioral tests

Anxiety and/or depression symptoms and cognition were analyzed by various behavioral tests. LDA and EPM tests were conducted to monitor the anxiety profile. Behavioral method used by Samad et al., (2022) was used. For LDA, the box was comprised of two compartments (one was transparent while other was black/dark). A central opening between the two compartments was present that used for the independent movement of animal. The movement of animal was started from the transparent box, and the activity between the two compartments was monitored for 5 min. The apparatus utilized for EPM was included of four arms/supports (two arms/supports were closed and two were opened while joint with the central part). To assess the anxiety like behavior, firstly animal was positioned in open arm/support and the time period it passed in open arm/support monitored for 5 min. To evaluate the depression-like behavior, FST was used with already reported method by Samad et al. (2019). A glass tank was used for FST. In this water tank, feet of rats did not get in contact to the floor and animal tried to escape. The immobility time during the test is recorded for 5 min. Cognitive ability of rat was assessed by MWM as reported previously (Samad et al. 2018). Acquisition, STM and LTM were monitored by MWM. Latency escape in all the three phases was monitored for 2 min. All the behavioral activities were recorded manually by a blind observer.

2.5. Estimations of biochemical

Malondialdehyde (MDA) as oxidative stress marker evaluated in the brain of rat. To estimate MDA levels in brain, tissue homogenate (3 ml) was added with TCA-TBA (2 ml) for reaction mixture preparation, which is boiled for 15 min and then cooled down at room temperature. The mixture was centrifuged for 10 min at 35,000RPM. Light blue colored supernatant was produced that read at 532 nm and absorbance was noted (Chow et al., 1972). Method of Naskar et al. (2010) was used to evaluate brain SOD activity. Tissue homogenate (0.5 ml), NaHCO3 (0.1 ml), NBT (0.4 ml) and EDTA (0.2 ml) were used to prepare reaction mixture with homogenate. Hydroxylamine-hydrochloride (0.4 ml) added in reaction mixture to start the reaction. Absorbance was recorded at 570 nm and inhibition (%) by SOD was calculated. Activity of CAT was evaluated by the method of Pari and Latha (2001). By adding tissue homogenate (0.1 ml), H2O2 (0.4 ml) and PO4 buffer (1 ml, 7.4 ml) reaction mixture was prepared. The wave length set at 570 nm to record

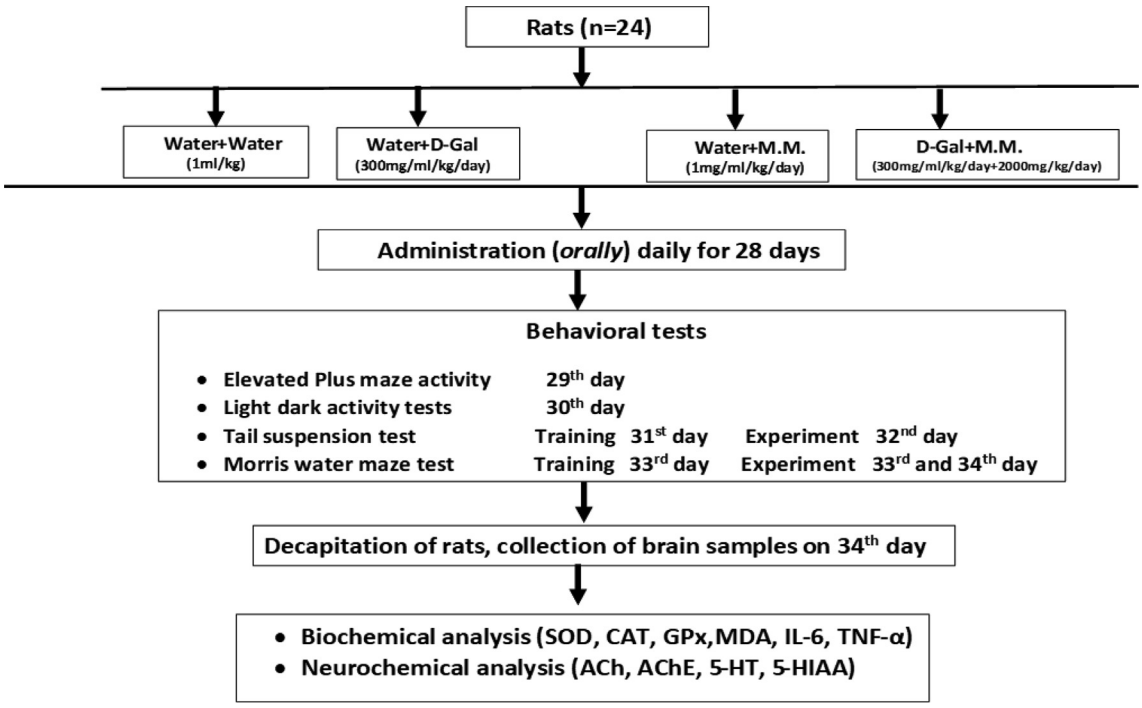

Fig. 1. Experimental Layout.

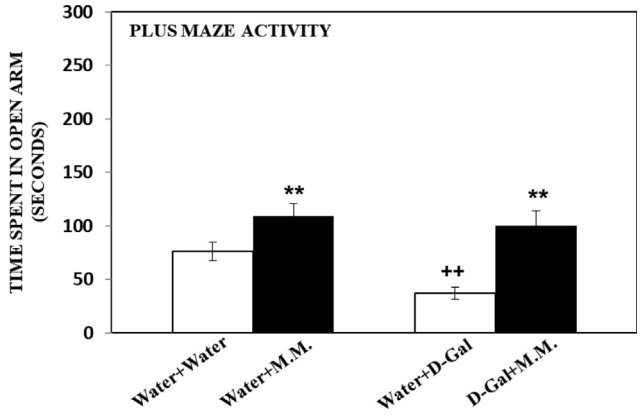

Fig. 2. Effect of M.M. on anxiety profile in water and D-Gal treated animals evaluated in EPM. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; ++p < 0.01 than water + water *vs* water + D-Gal treated animals.

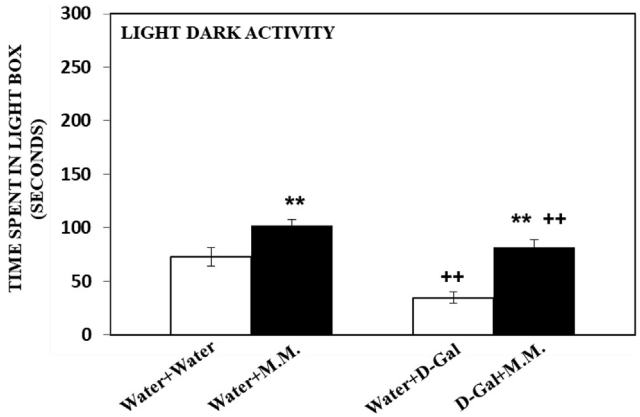

Fig. 3. Effect of M.M. on anxiety profile in water and D-Gal treated animals evaluated in LDA. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; ++p < 0.01 than water + water *vs* water + D-Gal and water + M.M. *vs* D-Gal + M.M. treated animals.

the absorbance. Brain GPx was determined by the method reported earlier (Flohe and Gunzler 1984). Brain homogenate (0.2 ml), H2O2 (0.1 ml), sodium azide (0.1 ml) and reduced glutathione (0.2 ml) were added to prepare reaction mixture. That was incubated for 15 min at 37 °C, for inhibition of reaction TCA (0.5 ml) was added in the reaction mixture. The reaction mixture was centrifuged at 35,000RPM for 5 min and in the collected supernatant (0.1 ml), Na2HPO4 (0.2 ml) and DTNB (0.7 ml) were added. 420 nm wave length was used to record the absorbance. TNF-a and IL-6 contents in the brain were evaluated by ELISA using a kit purchased from Abcam (Garabadu et al., 2020).

2.6. Estimations of neurochemical

Acetylcholine (ACh) contents were estimated by the method reported previously (Liaquat et al. 2019). By boiling, brain sample released Ach (bound) and vitiate the enzymes. 1% $FeCl_4$ solution and reaction mixture were mixed to produce a colored complex (brown) which was recorded at 540 nm. The method of Ellman (1961) was used to estimate brain acetylcholinesterase (AChE). Brain homogenate contain DTNB and phosphate buffer, was mixed with reaction mixture. Basal reading was recorded at 412 nm after stability of reaction. Enzyme reaction was started by addition of acetylthiocholine (ATC) and absorbance at 412 nm was recorded and the variance of absorbance was taken at 0 min and after 10 min. Method of Samad et al. (2019) was exercised to evaluated contents of brain 5-hydroxyindole acetic acid (5-HIAA) and 5-hydroxytryptamine (5-HT) using High-Performance Liquid Chromatography- Electrochemical detector (HPLC-EC) technique. Stationary phase comprised of octa decyl silane while phosphate buffer with octyl sodium sulphate run as mobile phase via column with 2000-3000psi pressure pump.

2.7. Statistics

All the data were evaluated by anova (2-way) using SPSS software (Ver. 20) as used previously (Samad et al., 2022) for analysis of variance. $p < 0.05$ was examined as substantial.

3. Results

3.1. Elevated plus maze test for anxiety like symptoms

Fig. 2. displays the consequence of D-Gal administration on anxiety-like behavior by EPM activity in control and test animals. The data was assessed by anova (2-way) disclosed considerable effect of M.M. ($F_{1,20}$ = 128.59), D-Gal ($F_{1,20}$ = 31.70) and M.M.*D-Gal ($F_{1,20}$ = 12.94). Analysis by Tukey's test unveiled that Administration of M.M. enhanced ($p < 0.01$) the time that rats given in open arm in control and D-Gal administered animals. D-Gal treated rats decreased ($p < 0.01$) time period given in open arm than control animals.

3.2. Light dark activity test for anxiety like symptoms

Fig. 3. displays the consequence of D-Gal administration on anxiety-like behavior by LDA in control and test animals. The data was assessed by anova (2-way) disclosed considerable effect of M. M. ($F_{1,20}$ = 192.84), D-Gal ($F_{1,20}$ = 115.05) and M.M.*D-Gal ($F_{1,20}$ = 10.18). The Administration of M.M. enhanced ($p < 0.01$) the time that rats given in transparent box in water and D-Gal administered animals. The time period given in open arm decreased ($p < 0.01$) in water + D-Gal and D-Gal + M.M. treated animals than control and M.M. treated animals.

3.3. Forced swim test for depression like symptoms

Fig. 4 displays the consequence of D-Gal on depression profile in FST in control and test animals. The data was assessed by anova (2-way) disclosed considerable effect of M.M. ($F_{1,20}$ = 219.02), D-Gal ($F_{1,20}$ = 155.92) and M.M.*D-Gal ($F_{1,20}$ = 59.19). The Administration of M.M decreased ($p < 0.01$) immobility time in control and D-Gal administered animals. The time of immobility was increased ($p < 0.01$) in water + D-Gal and D-Gal + M.M. treated animals than water + water and water + M.M. treated animals.

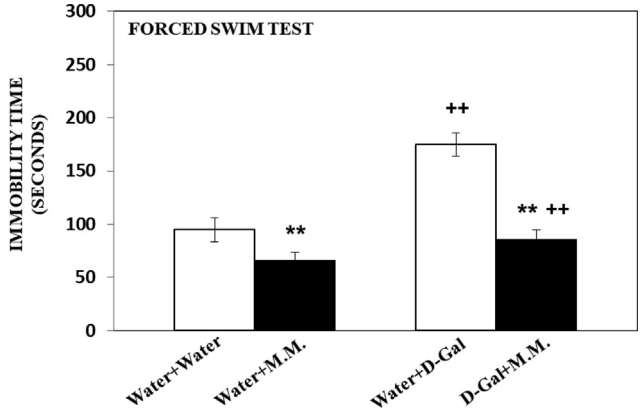

Fig. 4. Effect of M.M. on depressive symptoms in water and D-Gal treated animals evaluated in FST. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; ++p < 0.01 than water + water *vs* water + D-Gal and water + M.M. *vs* D-Gal + M.M. treated animals.

3.4. MWM test to assess cognitive functions

Fig. 5 displays the consequence of D-Gal on memory in MWM activity in control and test animals. The data of acquisition was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20}$ = 172.61), D-Gal ($F_{1,20}$ = 365.08) and M.M.*D-Gal ($F_{1,20}$ = 108.03). The administration of M.M. decreased ($p < 0.01$) the latency escape in D-Gal treated animals. An increased ($p < 0.01$) latency escape was observed in water + D-Gal and D-Gal + M.M. treated animals than control and M.M. administered rats.

The data of STM was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20}$ = 222.73), D-Gal ($F_{1,20}$ = 228.85) and M.M.*D-Gal ($F_{1,20}$ = 100.80). The intake of M.M. reduced the latency escape in water ($p < 0.05$) and D-Gal ($p < 0.01$) administered rats. An increased ($p < 0.01$) latency escape was observed in D-Gal and D-Gal + M.M. administered rats than control and M.M. treated animals.

The data of LTM was assessed by anova (2-way) d disclosed substantial effect of M.M. ($F_{1,20}$ = 135.77), D-Gal ($F_{1,20}$ = 138.08) and M.M.*D-Gal ($F_{1,20}$ = 67.17). Analysis by Tukey's test unveiled that intake of M.M. reduced the latency escape in water ($p < 0.05$) and D-Gal ($p < 0.01$) treated animals. An increased latency escape was found in D-Gal treated animals than control animals.

3.5. Determination of brain antioxidant enzymes

Fig. 6 displays the consequence of D-Gal on brain antioxidant enzymes activity in control and test animals. The data of SOD was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20}$ = 440.37), D-Gal ($F_{1,20}$ = 272.91) and M.M.*D-Gal ($F_{1,20}$ = 8.19). The administration of M.M. increased ($p < 0.01$)

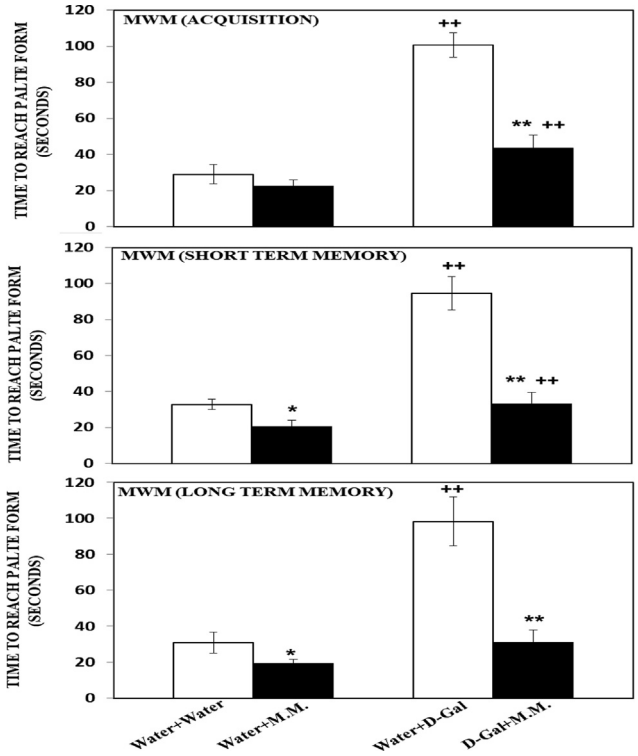

Fig. 5. Effect of M.M. on memory function in water and D-Gal treated animals evaluated in MWM. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as *p < 0.05 and **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; ++p < 0.01 than water + water *vs* water + D-Gal and water + M.M. *vs* D-Gal + M.M. treated animals.

SOD activity in control and D-Gal administered rats. A decreased ($p < 0.01$) activity of SOD was observed in water + D-Gal and D-Gal + M.M. treated animals than water + water and water + M.M. treated animals.

The data of CAT was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20} = 123.11$), D-Gal ($F_{1,20} = 92.29$) and M.M.*D-Gal ($F_{1,20} = 1.74$). The administration of M.M. increased ($p < 0.01$) CAT activity in control and D-Gal administered rats. Activity of CAT was reduced ($p < 0.01$) in water + D-Gal and D-Gal + M.M. treated animals than control and M.M. treated animals.

The data of GPx was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20} = 119.11$), D-Gal ($F_{1,20} = 47.81$) and M.M.*D-Gal ($F_{1,20} = 0.323$). The administration of M.M. increased ($p < 0.01$) GPx activity in control and D-Gal administered rats. A decreased ($p < 0.01$) activity of GPx was observed in water + D-Gal and D-Gal + M.M. treated animals than control and M.M. treated animals.

3.6. Determination of brain lipid peroxidation

Fig. 7 displays the consequence of D-Gal on brain lipid peroxidation (MDA contents) in control and test animals. The data was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20} = 104.96$), D-Gal ($F_{1,20} = 117.48$) and M.M.*D-Gal ($F_{1,20} = 35.43$). The administration of M.M. decreased ($p < 0.01$) contents of MDA in control and D-Gal administered rats. An increased ($p < 0.01$) in MDA levels were observed in D-Gal and M.M + D-Gal. treated animals than control and water + M.M. administered animals.

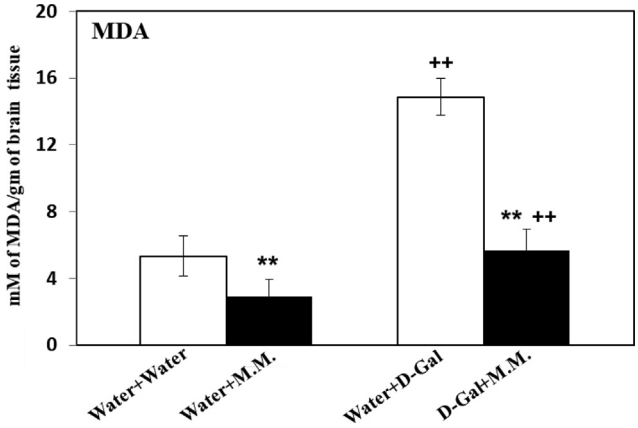

Fig. 7. Effect of M.M. on lipid peroxidation in water and D-Gal treated animals. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as and **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; ++p < 0.01 than water + water *vs* water + D-Gal and water + M.M. *vs* D-Gal + M.M. treated animals.

3.7. Determination of brain inflammatory markers

Fig. 8 displays the consequence of D-Gal on brain levels of IL-6 and TNF-α in control and test animals. The data of IL-6 levels was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20} = 504.72$), D-Gal ($F_{1,20} = 483.35$) and M.M.*D-Gal ($F_{1,20} = 304.36$). The administration of M.M. decreased ($p < 0.01$) IL-6 levels in control and D-Gal treated animals. An increased

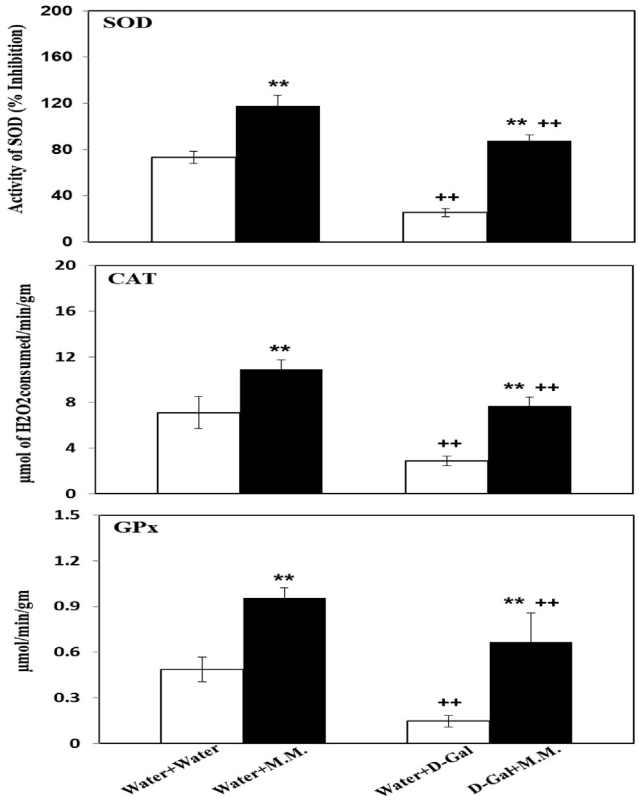

Fig. 6. Effect of M.M. on antioxidant enzymes in water and D-Gal treated animals. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as and **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; ++p < 0.01 than water + water *vs* water + D-Gal and water + M. M. *vs* D-Gal + M.M. treated animals.

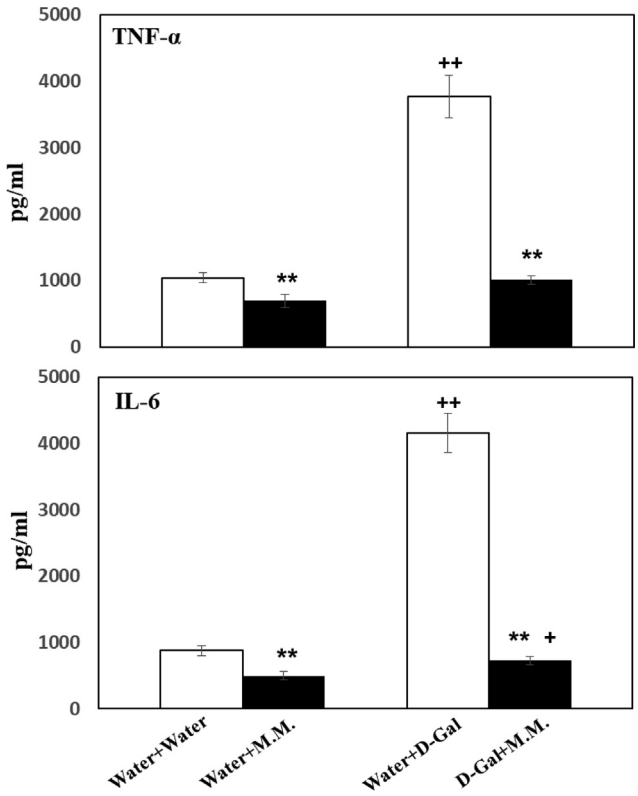

Fig. 8. Effect of M.M. on inflammatory markers in water and D-Gal treated animals. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; +p < 0.05 and ++p < 0.01 than water + water *vs* water + D-Gal and water + M.M. *vs* D-Gal + M.M. treated animals.

($p < 0.01$) levels of IL-6 observed in D-Gal administered than control rats.

The data of TNF-α levels was assessed by anova (2-way) displayed substantial consequence of M.M. ($F_{1,20}$ = 863.15), D-Gal ($F_{1,20}$ = 732.15) and M.M.*D-Gal ($F_{1,20}$ = 555.38). The administration of M.M. decreased TNF-α level in control ($p < 0.01$) and D-Gal ($p < 0.05$) treated animals. An increased levels of IL-6 observed in D-Gal treated than control rats. TNF-α levels were greater in D-Gal + M.M. than M.M. administered rats.

3.8. Determination of brain acetylcholine and acetylcholinesterase

Fig. 9 displays the consequence of D-Gal on brain levels of ACh and AChE activity in control and test animals. The data of ACh was evaluated by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20}$ = 186.11), D-Gal ($F_{1,20}$ = 9.824) and M.M.*D-Gal ($F_{1,20}$ = 3.389). The administration of M.M. increased ($p < 0.01$) ACh levels in control and D-Gal administered rats. A reduction ($p < 0.05$) in levels of ACh was observed in D-Gal treated than control animals.

The data of AChE was assessed by anova (2-way) disclosed substantial consequence of M.M. ($F_{1,20}$ = 301.91), D-Gal ($F_{1,20}$ = 49.98) and M.M.*D-Gal ($F_{1,20}$ = 85.74). The administration of M.M. reduced ($p < 0.01$) the activity of AChE in control and D-Gal treated animals. An increased ($p < 0.01$) activity of AChE was observed in D-Gal treated than control animals.

3.9. Determination of brain 5-hydoxytryptamine, its metabolite and turnover rate

Fig. 10 displays the consequence of D-Gal on brain concentration of 5-HTand its metabolite and the turnover rate 5-HIAA/5-HT in control and test animals. The data of 5-HT concentration was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20}$ = 122.03), D-Gal ($F_{1,20}$ = 185.40) and M.M.*D-Gal ($F_{1,20}$ = 66.32). Intake of M.M. decreased ($p < 0.01$) 5-HT concentration in D-Gal treated animals. An increased ($p < 0.01$) concentration of 5-HT observed in D-Gal treated than control animals.

The data of 5-HIAA was assessed by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20}$ = 118.97), D-Gal ($F_{1,20}$ = 119.07) and M.M.*D-Gal ($F_{1,20}$ = 13.60). The administration of M.M. decreased ($p < 0.01$) 5-HIAA concentration in control and D-Gal treated animals. An increased ($p < 0.01$) in 5-HIAA levels was observed in D-Gal and D-Gal + M.M. treated than control and M.M. treated animals.

The turnover rate of 5-HIAA/5-HT was evaluated by anova (2-way) disclosed substantial effect of M.M. ($F_{1,20}$ = 4.60), D-Gal ($F_{1,20}$ = 21.13) and M.M.*D-Gal ($F_{1,20}$ = 22.67). The administration of M.M. decreased ($p < 0.01$) turnover rate in control while increased ($p < 0.01$) in D-Gal administered rats. Turnover rate of D-Gal treated was reduced than control animals.

4. Discussion

We are reporting the first time, the consequence of fruit peel of M.M. on D-Gal-instigated behavioral, biochemical and neurochemical alterations in male rats. This experimental study exhibited that D-Gal-induced anxiety and depression profile in behavioral tests analysis (LDA, EPM and FST). Altered 5-HT metabolism can be associated with behavioral deficits (anxiety/ depression). Cognitive deficits were also analyzed in MWM test, and it appeared that D-gal altered the cognitive ability. Increased AChE activity and decreased ACh levels may also be concomitant with memory dysfunction. Previously, in experimental studies, D-Gal-induced oxidative stress (Samad et al., 2022b) and enhanced inflammatory markers (Qian et al., 2021), which were also observed by elevated lipid peroxidation (MDA contents), enhanced IL-6 and TNF-α, and decreased antioxidant enzymes activity. On the other hand, M.M. Fruit peels administration produced anxiolytic, antidepressant and cognitive

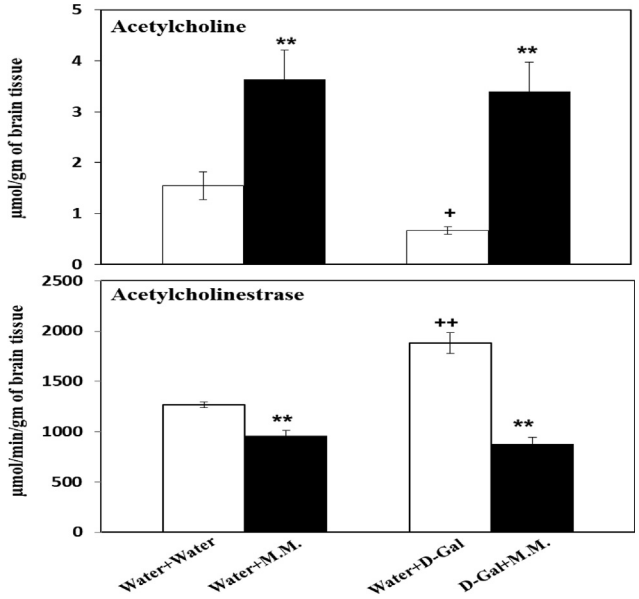

Fig. 9. Effect of M.M. Acetylcholine levels and acetylcholinesterase activity in water and D-Gal treated animals. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as and **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; +p < 0.05 and ++p < 0.01 than water + water *vs* water + D-Gal and water + M.M. *vs* D-Gal + M.M. treated animals.

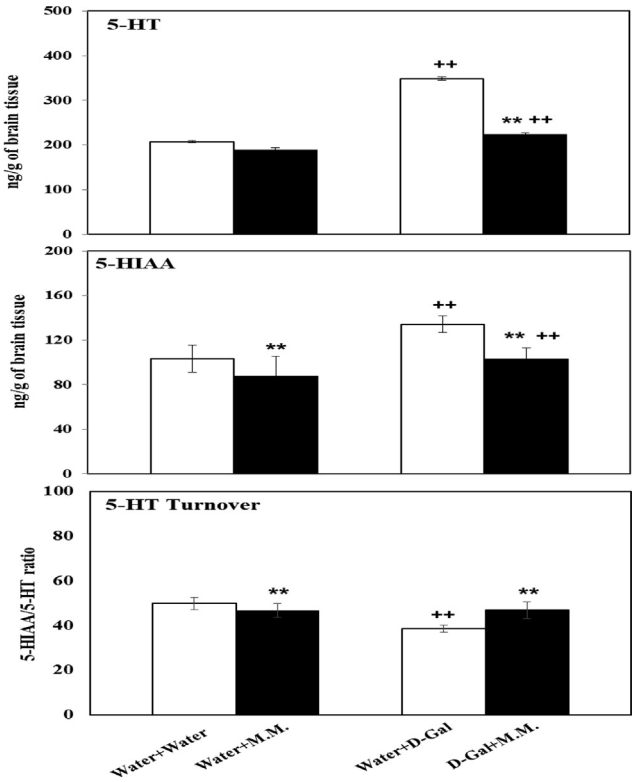

Fig. 10. Effect of M.M. on 5-HT metabolism and 5-HIAA/5-HT turnover ratio in water and D-Gal treated animals. Data was analyzed by anova (2-way) and followed by Tukey's test showed statistical difference as and **p < 0.01 water + water *vs* water + M.M. and water + D-Gal *vs* D-Gal + M.M.; ++p < 0.01 than water + water *vs* water + D-Gal and water + M.M. *vs* D-Gal + M.M. treated animals.

enhancement with normalization of serotonin metabolism and acetylcholine levels.

D-Gal intoxication is extensively used to develop aging-model and correlated therapeutics (Kaviani et al., 2017). Exogenous intake of D-Gal can alter physiological processes of various organs by inducing oxidative deterioration, which leads aging (Azman et al., 2019). It can induce behavioral deficits, cognitive impairment; alter redox status, neurochemical changes in animal models (Samad et al., 2022b). The finding showed that, repeated intake of D-Gal enhanced tine of immobility in FST (Fig. 4) which used for assessing depression like behavior with decreased 5-HT metabolism (Zhu et al., 2020) may be due to malfunction of somatodendritic 5-HT1A receptors. D-Gal reduce time given to transparent box and open arm of LDA (Fig. 3) and EPM (Fig. 2) respectively and imposed anxiety like action that could not be discussed in the same line with decreased 5-HT and its metabolite concentration. It is reported that in anxiety, levels of 5-HT become enhanced, so the up-regulation of 5-HT-2C receptor known for anxiogenesis could be involved in increased transmission of 5-HT in the brain. Secondly, oxidative stress induced by D-Gal probably a cause of anxiety like behavior because in the present work lipid peroxidation and inflammatory markers are elevated while enzymatic antioxidant status is declined. Apart from that M.M. fruit peel contains antioxidant (Petrus et al., 2012) showed anxiolytic and antidepressant effect in control and D-Gal treated rats.

M.M. fruit peel has powerful antioxidant which were observed many *In-vivo* (Srilanth and Ananda, 2014) and *In-vitro* (Priya et al., 2012) studies. It appears in the present work that M.M. peel reduced contents of 5-HT and its metabolite in control and D-Gal while enhanced turnover rate of 5-HIAA/5-HT turnover in D-Gal but not in M.M. treated rats (Fig. 10) with reduced time of immobility in FST in control and D-Gal administered rats (Fig. 4) indicating antidepressive effect of M.M. fruit peel. It has been early reported that M.M. produced anti-depressant activity in a helplessness model with decreased 5-HT metabolism (Samad et al., 2018) same effect observed in animal model of aging in the present study. It is reported that M.M. has potential bioactive compounds due to all these it has powerful antioxidant ability which can impede D-Gal prompted oxidative deterioration and normalized neurochemical mechanism.

The cognitive ability can be altered by aging (Upright and Baxter, 2021). Alteration in redox status has vital role in inducing aging and associated memory loss/decline (Olesen et al., 2020). In the present study memory impairment was observed by D-Gal treatment with increased escape of latency by MWM test (Fig. 5). Acetylcholine is one of the neurotransmitters which are very much associated with memory function (Bostancikliogl, 2020). AChE, degradative enzyme of ACh is a potential marker for determining cholinergic function (Han and Wang, 2019). Previously in an aging model activity of AChE found increased with cognitive decline (Easton et al., 2020). The current study showed a decrease in ACh levels with increased AChE activity (Fig. 9) which is in agreement with previous works and indicating that D-Gal administration can cause memory impairment (Fig. 5). As reported earlier plants are a big source of antioxidants because they contain phytochemical/bioactive compounds (Diniz do Nascimento et al., 2020). M.M. contains phenolic acid, flavonoids, tannins etc. which possess ROS scavenging activity (Parameswari et al., 2013). Most of the bioactive compound such as gallic acid (Mori et al. 2020), thymoquinone (Hajipour et al., 2021), curcumin (Voulgaropoulou et al., 2019), quercetin (Khan et al., 2019) decreased AChE activity and increased Ach transmission and improved cognitive functions. In a previous study it has also been reported that M.M. fruit peel extract can improve cognitive ability by decreasing AChE activity and increasing ACh levels (Fig. 9). It is recommended that M.M. fruit peel can also improve memory function in an animal model of aging due to its antioxidant potential.

Various neurological disorders i.e. stress, cognitive disability etc. induced due to enhanced oxidative stress (Hassan et al., 2022). It is extensively published that repeated administration of D-Gal instigated oxidative stress (Qian et al., 2021) by increased lipid peroxidation of cell membrane which led damage in biomolecules with reduced antioxidant mechanism (Anand et al., 2012). An antioxidant enzyme SOD, which is reported earlier as first line of defence can neutralize superoxide radicals into H_2O_2, simultaneously other subsequent enzyme such as CAT and GPx converts H_2O_2 into H_2O and O_2. The findings are also in agreement with former reports indicating that D-Gal can enhance lipid peroxidation and increased MDA levels (an oxidative stress marker) (Fig. 7) and decreased the activity of antioxidant enzymes (Fig. 6) An enhanced oxidative stress can activate the process of inflammation and secrete inflammatory intermediaries (markers) (Luc et al., 2019). Aging can mimic the apoptotic pathway by activation of inflammatory markers and oxidative stress which lead death of neuronal cells (Jiang et al., 2020). Previously it was mentioned in a study that increased ROS generation, oxidative damages and activation in inflammatory mediators were observed by D-Gal intake (Singh et al., 2021). An increased contents of IL-6 and TNF-α (inflammatory markers) (Fig. 8) are observed in D-Gal treated animal. The present data agrees with the previous study as mentioned above and suggesting the impact of neuro-inflammation and neuro-toxicity following D-Gal i.e. brain areas such as hippocampus deterioration which involves in anxiety and depression-like behaviors (Samad et al., 2022b) and cognitive disability (Shwe et al., 2020). The recent research work proved that neuro-inflammation and neuro-degenerative conduits have a crucial role in depression/anxiety/dementia by increased contents of inflammatory intermediaries (Cheng et al., 2018). Anxiolytic (Samad et al., 2020; Sarvanan et al., 2012), antidepressant (Samad et al., 2020), anti-inflammatory (Petrus et al., 2012) and antioxidant (Srilantha and Ananda, 2014) effects of leaf extract of M.M. have been reported. Antioxidative potential of M.M extensively scavenge free radicals and made more effective the antioxidant enzyme system (Srilantha and Ananda, 2014; Sarvanan et al., 2012). The finding displayed that M.M. improves antioxidant enzymes by increasing enzymatic antioxidant (Fig. 6) and mitigating the oxidative deterioration (Fig. 7) and inflammatory markers (IL-6, TNF-α). It is indicating that D-Gal-prompted oxidative deterioration is mitigated/prevented by M. M. possibly via its antioxidant and neuromodulatory effects.

In conclusion, powerful antioxidant potential of M.M. involves in attenuation of D-Gal-induced anxiety/depression-like symptoms and progresses cognitive ability. Modulation of serotonin and acetylcholine mechanism via potential antioxidant system of M.M. produces anti-stress and cognitive improving effects. It is suggested that dietary habit of M.M. can help out to reduce aging and related neurological problems. This study is limited to an extent, however in the future more studies should be performed to evaluate/compare the effect of M.M. on female and male rats with developed aging model and normal aging model with associated neurological disorders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors Contribution

NS conceived and design research. M.A.B.H.A conducted experiments. II provided laboratory facility for the conduction of experiments. FA provided funding support for experiments. NS wrote the manuscript. NS, TA review the manuscript. All author read and approved the manuscript.

Ethical Approval

Institutional Bio-ethical committee (Ref# D/352/2021/Biochem; Dated: February 17, 2021) was received for the animal experiment from the Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan.

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