THE NEUROPROTECTIVE EFFECT OF LAWSONIA INERMIS SEED EXTRACT ON D-GALACTOSE INDUCED ALZHEIMER'S DISEASE IN RATS: A SPECIAL REFERENCE TO ANTIOXIDANT ENZYMES

Punabaka Jyothi, Kuna Yellamma*

Division of Neurobiology, Department of Zoology, Sri Venkateswara University, Tirupati – 517 502,

Andhra Pradesh, India

ABSTRACT

A debilitating neurodegenerative condition, Alzheimer's disease (AD), needs adequate research on successful treatment options. Plants have been shown to be a source of anti-alzheimer resources for drugs with fewer side effects than medications available today. The plant *Lawsonia inermis*, usually referred to as henna, belongs to the *Lythraceae* family, as the Telugu people call this plant Gorenta. This plant traditionally has many medicinal values against many deadly diseases. The goal of this study was to identify the phytochemical compounds present in the methanolic extract of seeds and to demonstrate their neuroprotective properties in AD rats induced by D-Galactose. Results revealed that seed extract has rich account of phenols (989.43 µg/ mg) and flavonoids (368 µg/ mg). Additionally, extract (150 mg/kg) exhibited neuroprotective property by elevating antioxidant enzymes such as SOD, CAT, GR, and GPx significantly ($p > 0.05$) in cerebral cortex of AD rats when compare to NC rats. In contrast, lipid peroxidation was significantly reduced by the MELIS when compare to AD group. In conclusion, by increasing endogenous antioxidant enzymes, MELIS is a good source of antioxidant compounds such as phenols and flavonoids that may be responsible for protecting the neuronal cells of the cerebral cortex.
Keywords: *Lawsonia inermis,* Alzheimer's, Antioxidant enzymes, Phytochemicals, phenols, flavonoids

INTRODUCTION

The most common cause of impairment in people aged > 65 years worldwide is Alzheimer's disease (AD). Neurodegeneration mediators, which are the key causative factors for Alzheimer's disease, are primarily triggered and stimulated by oxidative stress from reactive oxygen species (ROS), a mechanism that refers to an imbalance between antioxidants and oxidants (Huang *et al*., 2016). The ROS production occurs in cell at aerobic metabolism, they include hydroxyl radical ($\bullet OH$), superoxide anion ($O_2 \bullet -$), while non-radical molecules includes hydrogen peroxide (H_2O2), singlet oxygen ($^1O_2$). The production of these ROS is due to stepwise reduction of molecular oxygen (O_2) by high-energy exposure or electron-transfer reactions (Sharma *et al*., 2012). ROS can play significant role in causing neurological disorders such as Alzheimer's disease, Parkinson's disease, muscular dystrophy (Stefanis *et al*., 1997; Lobo *et al*., 2010). Hydrogen peroxide reduction creates highly reactive hydroxyl radicals, known as ROS, which are capable of reacting to lipids, proteins, nucleic acids and other molecules and may modify their structures and

functions. ROS is therefore affects tissues and organs, particularly the brain, a vulnerable organ, because of its composition (Huang *et al.*, 2016). Harmful function of ROS is depending on the delicate balance between the development and scavenging of ROS. Because of the multifunctional functions of ROS, to prevent any oxidative injury and not to remove them entirely, it is important for the cells to regulate the amount of ROS tightly (Noctor and Foyer, 1998). An effective antioxidant mechanism consisting of both non-enzymatic and enzymatic antioxidants is used to scavenge or detoxify excess ROS (Sharma *et al.*, 2012). The living 'great practises' is the Indian Ayurveda system and has important roles in the bio-prospecting of new medicines from medicinal plants that are also rich sources of antioxidants. The substances that can protect cells from damage caused by free radicals are antioxidants (Ayoub *et al.*, 2017). Though, several medications have been available to treat Alzheimer's, people looking forward to natural plants because treatment with medicinal plants is considered very effective because there are little or limited side effects. Such therapies are in tune with nature, which is the greatest gain. The golden truth is that the use of herbal therapies is independent of any age group or gender (Ravi *et al.,* 2017). Based on this phenomenon, in this study, we have selected a medicinal plant *Lawsonia inermis L* to treat Alzheimer's disease induced in rats by D-Galactose.

The plant *L.inermis*, usually referred to as henna, belongs to the family of *Lythraceae* and is the only species in the genus. This plant is called Gorenta by the Telugu people (Kumar *et al.*, 2005). From all parts of *L. inermis*, almost every hundred phyto constituents, representing a variety of groups, have been described. Especially prevalent in henna are phenolic compounds, including flavonoids, coumarins, and naphthoquinones. A wide range of biological activities have been attributed to henna including antioxidant, anti-inflammatory, anti-arthritic, analgesic, antipyretic, anticancer, anti-ulcer, anti-tubercular, antibacterial, antimicrobial, antifungal, antifertility, ovicidal, abortifactant, antiviral, hepatoprotective, memory enhancing, immunostimulant, antidiabetic, wound healing, thrombolytic and anticataleptic (Ahmadian and Fakhree, 2009; Sharma *et al.*, 2009; Zumrutdal and Ozaslan, 2012; Li *et al.*, 2013).

In this study we have extracted phytochemicals in to the methanol and tested for phytochemical screening (qualitative and quantitative). Further, extract was tested for neuroprotective property by supplementing to AD induced rats.

MATERIAL AND METHODS

Procurement of chemicals
All the chemicals used in the present investigation were Analar grade (AR) and Solvents obtained from the following scientific companies: Sigma (USA), Fisher (USA), Merck (India), Himedia (India), TCI (China), Molychem (India) and SRL (India).

Preparation of Seed Extract
The plant seeds have been collected in S.V. University campus, Tirupati and its surrounding areas. Further the plant seeds were confirmed by Dr. Madhava Chetty, Department of Botany S.V University Tirupati. Collected plant seeds were initially cleaned with tap water, further clean done by using distilled water. Seeds were dried under shade and made them powder with scientific grinder. The powder was soaked in methanol (100%) with 1:5 ratios. The mixture was kept in dark for 3 days and stirred occasionally. After 3 days, the methanol dissolved compounds were filtered orderly with muslin cloth, cotton and what man No 1 filter paper. The filtrate was concentrated using rotary evaporator. This methanolic extract was preserved in refrigerator for further use.

Qualitative Estimation

To find out the phytochemical compounds, we have performed the initial phytochemical screening by standard protocols described by the Abha *et al.*, (2015). In this screening, methanolic extract of *L. inermis* seeds was tested for alkaloids, anthocyanins, anthra quinones, cardiac glycosides, coumarins, flavonoids, phenolics, reducing sugars, saponins, tannins and terpenoids.

Quantitative Estimation of Phytochemicals

Total phenols

The amount of total phenolic content in the methanolic seed extract was determined using folin-ciocalteu method (Gutierrez *et al.*, 2010). Briefly, 0.2 mL of diluted folin-ciocalteu reagent (1:10%) was added to the methanolic seed extract (100 mg/ mL) and was kept for 5-8minutes at room temperature. 2 mL of sodium carbonate (Na2CO3) (7.5% v/v)solution was added to the above mixture. After incubation at 30 ^{0}C for 90 min, the absorbance was measured at 765 nm to estimate phenolic content. This result was expressed in Gallic acid equivalents.

Total flavonoids

The total flavonoids content in the extract was determined through aluminium chloride method (Jia *et al.*, 1999). To the 0.5 mL methanolic seed extract, 2 mL of distilled water was added and then 0.15 mL of $NaNO_2$(15 %) was mixed to allow for 6 minutes. 2 mL of 10% NaOH solution was added and leave it for 6 minutes again. Final volume of 5 mL reaction mixture was obtained by adding distilled water and incubated for 15 minutes. The absorbance was read at 510 nm against distilled water as a blank. The amount of flavonoids in methanolic seed extract was calculated using Rutin calibration curve.

Animal studies

Grouping of Animals:

Treatment : 60 days
Rats : Wistar strain male albino rats (160 ± 20)
Group I (Normal Control) : Rats received normal saline orally
Group II (MELIS-T 150) : Rats received extract 150 mg/kg orally
Group III (AD-I) : Rats received D-galactose (120 mg/kg) through intraperitoneal injection
Group VI (AD-I+ MELIS-T 150) : Rats received D-galactose (120 mg/kg) through intraperitoneal injection and
received extract 150 mg/kg orally.

Tissues Isolation

For biochemical estimations, all the 4 groups of rats were sacrificed on 60thdays by cervical dislocation. The experimental brain was isolated immediately and placed on a chilled glass plate. Different Cerebral cortex brain regions were separated by following standard anatomical marks (Glowinski and Iverson, 1966) and frozen in liquid nitrogen (-180°C) and then stored at -40°C until further use. At the time of biochemical analysis, the selected tissues were thawed and used. The obtained results were analyzed statistically.

In Vivo **Antioxidant Enzymes Activities**

Preparation of tissue homogenate

We prepared cerebral cortex tissues homogenation to carry antioxidant enzyme assays (SOD, CAT, GR and GPx). The tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). Further, the homogenate was centrifuged at 10,000 rpm for 10 min; supernatant was separated and used as enzyme source.

Superoxide dismutase (SOD) (EC 1.15.1.1) estimation

SOD activity in cerebral cortex was assayed by using the standard method described by Fridovich, (1972). Based on the inhibition of Epinephrine-Adrenochrome transition by the enzyme, 880µL of carbonate buffer (0.05 M,pH 10.2, containing 0.1 mM EDTA) was added to100µL of enzyme source . To this reaction mixture, added 20 µL of 30 mM epinephrine (in 0.05 %acetic acid) and measured the optical density values in UV-Vis spectrophotometer (Scimadzu UV-1800) at 480 nm for 3 min. The SOD Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine.

Catalase (CAT) ((EC 1.11.1.6) estimation

The catalase activity in cerebral cortex was assayed by following method described by Aebi, (1984). To 100µL of tissue extract added to 10µL of 100% ethanol and then placed in ice bath for 30 min. After incubation, test tubes were cooled and mixed with triton X-100. From this reaction mixture ,50 µL was transferred in to cuvette containing 200 µL phosphate buffer and 250 µL of 0.066 M H_2O_2. The reaction was read in UV-Vis spectrophotometer for 60 seconds at 240 nm. One unit of CAT activity is equal to the moles of hydrogen peroxide degraded/mg of protein/ min.

Glutathione reductase (GR) (EC 1.6.4.2) estimation

Glutathione reductase changes in disease and treatment condition was measured by the method of Carlberg and Mannervik, (1985). 100µL of enzyme source was added to cuvette containing 50µL of GSSG (20mM) in phosphate buffer-0.1 mM EDTA (0.5M, pH 7.0) solution, 50µL of NADPH (2 mM) in 10 mM phosphate buffer (pH 7.0) and 800µL f phosphate buffer. This reaction mixture changes were observed by OD values at 340 nm for 3min. The enzyme activity was calculated in micro moles of NADPH oxidized/mg protein/min. One unit is equal to the mM of NADPH oxidized/mg of protein/min.

Glutathione peroxidase (GPx) (EC 1.11.1.9) estimation

Glutathione peroxidase activity was determined in cerebral cortex as described by Flohe and Gunzler, (1984). 100 µL of tissue extract was taken in cuvette containing 100µL of 0.01M GSH (reduced form), 500 µL of phosphate buffer, 100 µL of GR (0.24 units) and 100µL of 1.5mM NADPH. The reaction mixture was incubated at 37 ºC for 10 min and then 50µL of 12mM t-butyl hyroperoxide was added to 450µL of reaction mixture. Then reaction mixture and measured at 340 nm for 180 seconds. The enzyme activity was expressed in micro moles of NADPH oxidized/ mg protein/min. One unit of enzyme activity is equal to the mM of NADPH oxidized/mg protein/ min.

Lipid Peroxidation

Lipid peroxidation in the form of MDA was determined in cerebral cortex by following the method described by Ohkawa et al. (1979). 100 µL tissue extract was added to 50 µL of SDS (8.1 %), mixed well and incubated for10 min at normal room temperature. 375 µL of acetic acid and thiobarbituric acid (0.6%) solutions were added to above reaction mixture and then placed in boiling water bath for 1 hour. The test samples were allowed to cooling at room temperature for 1 hour. 1.25 mL of butanol: pyridine (15:1) was added to reaction mixture and subjected to centrifugation at 1000 rpm for 10 min. The upper layer color absorbance was measured at 532 nm. The OD values were expressed in µM of MDA formed/ gram wet weight of tissue.

Statistics

In triplicate, all the experiments were carried out and expressed as mean ± SD. A one-way ANOVA was used to assess the importance of variations between four distinct groups, followed by multiple range tests by Tukey and Dunnet. Using SPSS (version 20; IBM SPSS Inc., Chicago, 1L, USA), statistical analysis was performed. At $p < 0.05$, the data was found to be significantly different.

RESULTS

We have conducted the initial phytochemical screening to find out the phytochemical constituents qualitatively in the methanolic seed extract. Alkaloids, anthocyanins, anthra quinones, cardiac glycosides, coumarins, flavonoids, phenolics, reducing sugars, saponins, tannins and terpenoids were seen in the seed extract during this screening. These preliminary qualitative assays showed that phenolic compounds and flavonoids are abundant in the seed extract. Further, in quantitative estimation screening, phenolic content of the extract noticed was 989.43 µg/ mg, which was equivalent to the gallic acid, a standard phenolic compound. The flavonoid content was 368 µg/ mg, an equivalent of rutin, a standard flavanoid compound

In the present experimental study, SOD activity was measured in cerebral cortex tissues of all experimental groups includes NC, MELIS-150 mg, AD and AD+ MELIS-T 150 mg. The SOD activity was significantly decreased in AD rats over NC rats, whereas in AD+ MELIS-T 150 mg increased SOD activity was observed which was near to NC group. However, no significant changes were observed in MELIS-150 mg and NC rats (Figure 1). It was cleared that brain SOD was significantly lowered in Alzheimer's rats as compared with normal control rats. These lowered levels were significantly normalized by seed extract therapy. Several studies have reported that the activity of SOD is low AD induced rats (peera et al., 2016). Catalase (CAT) activity was decreased significantly ($P < 0.05$) in Cerebral cortex tissues under D-gal induced AD rats when compared to NC rats. In treatment of seed extract group that AD+ MELIS-T 150 mg group, CAT activity was significantly increased which was very near to the CAT activity NC group (Figure 2). Interestingly, there were no significant changes observed in the seed extract alone MELIS-150 mg and NC rats.
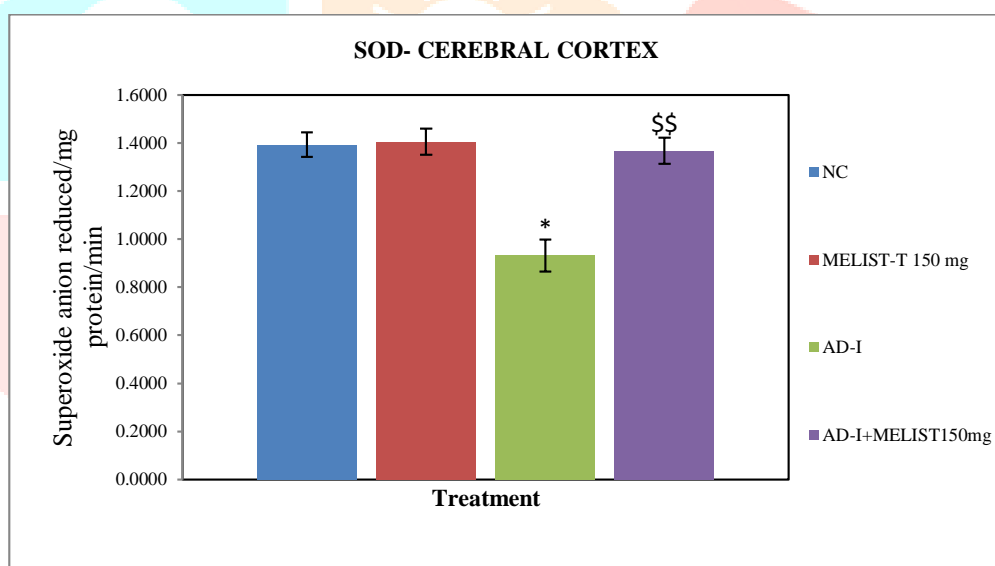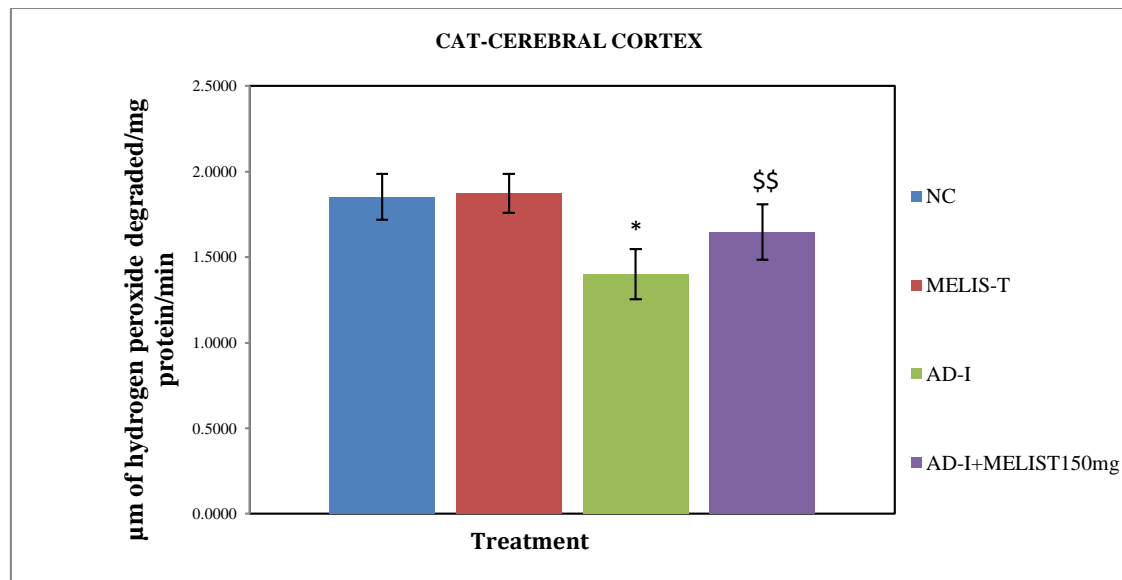

Figure 1: Impact of MELIS on the activity of SOD in the control and experimental rat brain region that cerebral cortex on 60 days of experiment. The values are mean ± SEM (n=6). * p<0.05 vs Control; $$ p<0.05 vs AD Model.

Figure 2: Impact of MELIS on the activity of CAT in the control and experimental rat brain region that cerebral cortex on 60 days of experiment. The values are mean ± SEM (n=6). * p<0.05 vs Control; \$\$ p<0.05 vs AD Model.

In AD rats over NC rats, GR activity was significantly reduced, while in AD+ MELIS-T 150 mg increased GR activity was observed close to the NC group. In MELIS-150 mg and NC rats, however, no major changes were observed (Figure 3). It was obvious that, relative to normal control rats, brain GR was substantially reduced in Alzheimer's rats. Similarly, as compared to NC rats, GSH-Px activity was also significantly decreased (P<0.05) in Cerebral cortex tissue in D-gal induced AD rats. GSH-Px activity was substantially increased in the treatment of the seed extract group of the AD+ MELIS-T 150 mg group, very similar to the GSH-Px activity of the NC group (Figure 4). Interestingly, no major changes have been observed in the activity of GSH-Px in MELIS-150 mg seed extract alone and in NC rats. In contrast the antioxidant enzyme activities of cerebral cortex lipid peroxidation results were observed.
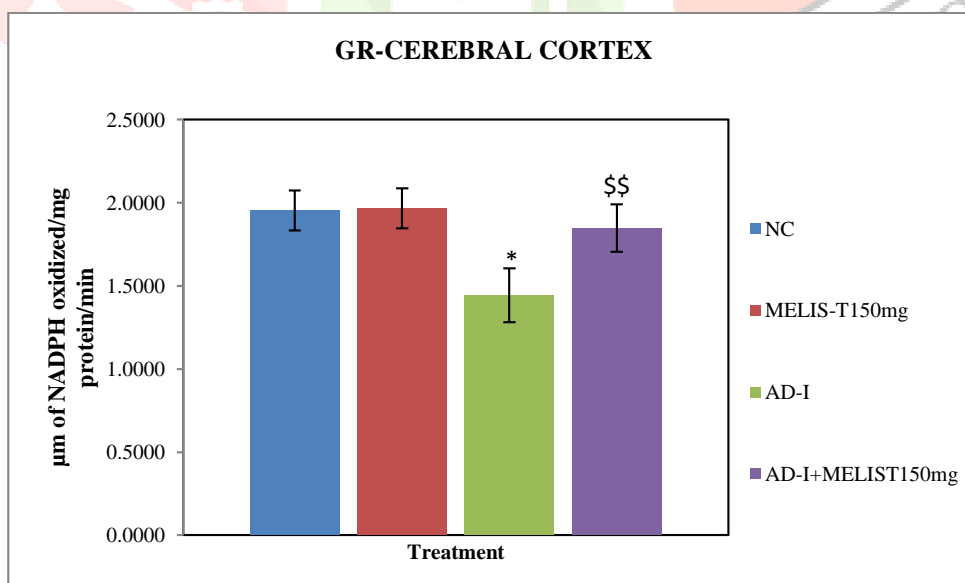

Figure 3: Impact of MELIS on the activity of GR in the control and experimental rat brain region of cerebral cortex on 60 days of experiment. The values are mean ± SEM (n=6). * p<0.05 vs Control; \$\$ p<0.05 vs AD Model.
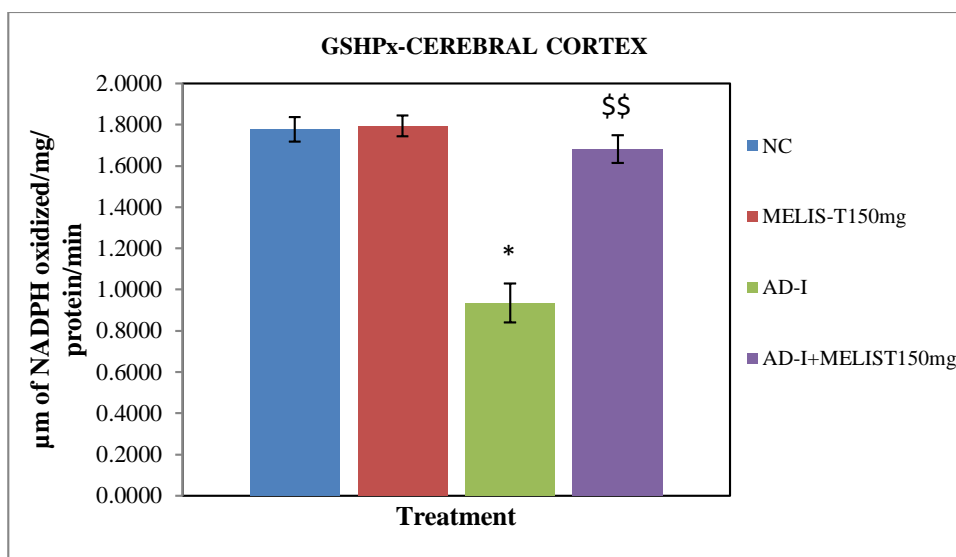
Figure 4: Impact of MELIS on the activity of GSHPx in the control and experimental rat brain region of cerebral cortex on 60 days of experiment. The values are mean ± SEM (n=6). * $p<0.05$ vs Control; \$\$ $p<0.05$ vs AD Model.

Lipid peroxidation was significantly increased in AD rats over NC rats, while decreased lipid peroxidation near to the NC group was observed in AD+ MELIS-T 150 mg. No major changes were, however, observed in MELIS-150 mg and NC rats (Figure 5). It was clear that brain cerebral cortex lipid peroxidation was significantly increased in Alzheimer's rats compared to normal control rats.
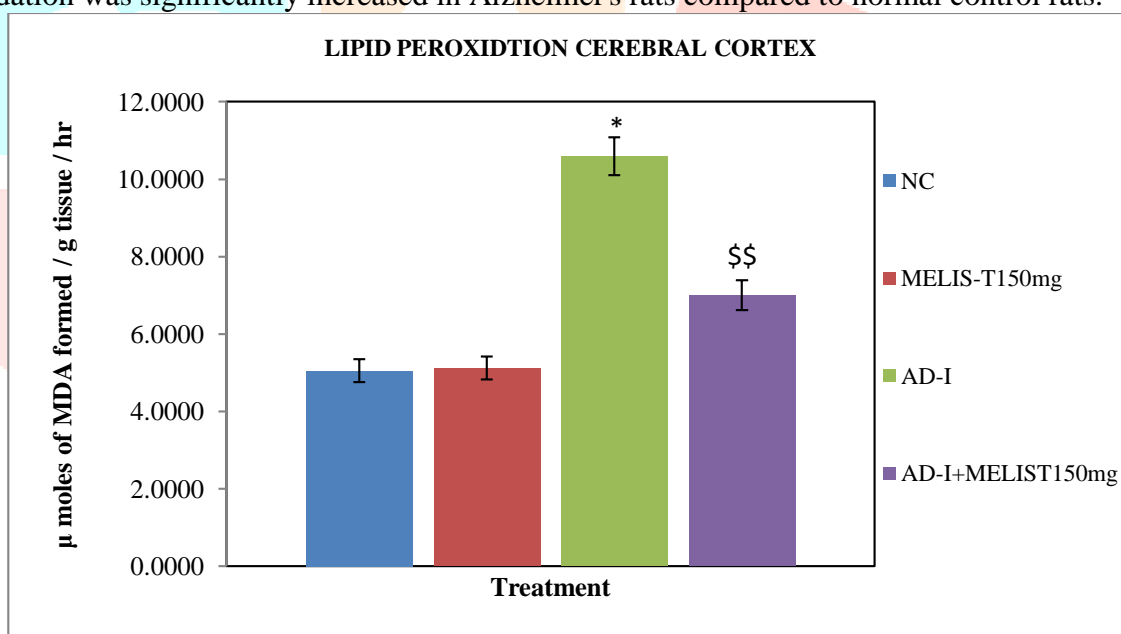

Figure 5: Impact of MELIS on the Lipid peroxidation in the control and experimental rat brain region of cerebral cortex on 60 days of experiment. The values are mean ± SEM (n=6). * $p<0.05$ vs Control; \$\$ $p<0.05$ vs AD Model.

DISCUSSION

Polyphenols are the one of the major secondary metabolites in plants, among them; half of the numbers are occupied by flavonoids. Polyphenols have one or more hydroxyl groups on the aromatic ring. Phenols and flavonoids having large spectrum of biological importance such as cardio vascular diseases, anti-inflammatory, antioxidant, anti-ulcer, effect on neuronal diseases and anti-cancer (Gulcin *et al*., 2004; Piacente *et al*., 2005; Vijayalaxmi *et al*.,2015). Based on the biological importance of phenols and flavonoids in the plants, which are also observed rich in phytochemical screening parameter in this study, we attempted to estimate those phytochemicals quantitatively in methanolic seed extract. In this screening, phenolic content of the extract noticed was huge than flavonoid content.

Enhanced free radical production is associated with mitochondrial respiratory chain destruction, which contributes to increased free radical generation and leads inevitably to cellular damage (Jain *et al*., 2012). In the pathophysiology of ageing, D-galactose performs a vital part. Several theories have been put forth to understand the mechanism of action of D-galactose in ageing, particularly free radical injury (Kumar *et al*., 2011). It was well recognised that free radicals generated could cause several negative functions, such as protein degradation and lipid peroxidation. As all enzymes are proteins under the enzyme law, free radicals may cause degradation of the enzyme in this manner (Sun, 1990). In this study, oxidative stress generated under D-galactose induced AD, which causes antioxidant enzyme degradation and lipid peroxidation. SOD provides the first line of protection after the symptoms of oxidative stress, followed by the actions of CAT and GPx. SOD antioxidant activity is only effective when it is preceded by GPx and CAT antioxidant activity (Haider *et al*., 2020). We observed that in AD rats, SOD and CAT activities were substantially diminished and MELIS therapy improved both activities in the brain. Similarly, GPx activity and GSH enrichment were significantly reduced in AD rats, which is consistent with Liaquat *et al* (2017)'s published work whereas, their increment were observed in MELIS treatment. In contrast, lipid peroxidation was increased under AD condition but it was depleted by MELIS treatment. Under normal conditions, free radicals are typically formed in a cell, but through the catalytic activity of antioxidant enzymes to maintain a homeostatic equilibrium, they are constantly and effectively removed from the cell but under disease conditions these free radicals shows many adverse effects like depleting antioxidant enzymes as discussed elsewhere in this manuscript (Khan *et al*., 2012). Hence, antioxidant supplementation from outside should be needed to escape the pressure of depleting antioxidant enzymes. Plant sources are the best choice as outside antioxidant supplement because they doesn't shows any adverse effects (Ravi *et al*., 2017). On this scenario, in this study, we have supplemented MELIS as the antioxidant source. As we expected it was exhibited good antioxidant property by inducing antioxidant enzymes and depleting lipid peroxidation. As we discussed earlier, this antioxidant property may be attributed to the high phenols and flavonoid content in MELIS.

CONCLUSION

This study suggests that MELIS, a potent source of antioxidants, has the ability to prevent oxidative stress by increasing the activity of antioxidant enzymes and decreasing lipid peroxidation in the cerebral cortex neurons under AD condition. This neuroprotective property of MELIS may be due to the presence of huge amount of phenols and flavonoids.

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CONFLICT OF INTEREST:

All authors declare that there is no conflict of interest.

REFERENCES

1. Abha S, Swati V, Shukla RK. Phytochemical screening, proximate analysis and antioxidant activity of Dracaena reflexa Lam. leaves. Indian J Pharm Sci. 2015; 77 (5): 640–644.

2. Aebi H. Catalase. Methods Enzymol. 1984;105:125-126.

3. Ahmadian S, Fakhree M A. Henna (Lawsonia inermis) might be used to prevent mycotic infection . Med Hypotheses .2009;73 (4):629-30.

4. Ayoub Z, Mehta A, Mishra SK, Ahirwal L. Medicinal plants as natural antioxidants: a review. Journal of Botanical Society, University of Saugor.2017; 4:1-16.

5. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol .1985; 113:484-49.

6. Flohe L,Gunzler WA. Glutathione peroxidase. Methods Enzymol.1984; 105:115–121.

7. Foyer CH, Noctor G. Redox homeostasis and antioxidant signalling: a metabolic interface between stress perception and physiological responses. Plant cell. 2005; 17 :1866-1876

8. Glowinski J, Iversion LL. Regional studies on catecholamines in rat brain. J Neurochem. 1966; 13: 655-69.

9. Gulcin I, Mshvildadze V, Gepdiremen A, Elias R. Antioxidant activity of saponins isolated from ivy: alpha-hederin, hederasaponin- C, hederacolchiside-E and hederacolchiside-F. Planta Med.2004; 70(6):561–563.

10. Gutierrez RM, Navarro YT. Antioxidant and hepatoprotective effects of the methanol extract of the leaves of Satrejamacrostema. Pharmacogn Mag. 2010; 6:125–131

11. Haider S, Liaquat L, Ahmad S, Batool Z, Siddiqui RA, Tabassum S. Naringenin protects AlCl3/D-galactose induced neurotoxicity in rat model of AD via attenuation of acetylcholinesterase levels and inhibition of oxidative stress. PLoS ONE.2020; 15(1): 1-30.

12. Huang SS, Su SY, Chang JS. Antioxidants, anti-inflammatory, and antidiabetic effects of the aqueous extracts from Glycine species and its bioactive compounds. Bot Stud. 2016; 57(1):38.

13. Jain A, Dwivedi N, Bhargava R, Flora SJ. Silymarin and naringenin protects nicotine induced oxidative stress in young rats. Oxid Antioxid Med Sci. 2012; 1:41–9.

14. Khan MB, Khan MM, Khan A, Ahmed ME, Ishrat T, Tabassum R. Naringenin ameliorates Alzheimer's disease (AD)-type neurodegeneration with cognitive impairment (AD-TNDCI) caused by the intracerebroventricular-streptozotocin in rat model. Neurochem Int. 2012; 61:1081–93.

15. Kumar A, Prakash A, Dogra S. "Centella asiatica Attenuates D-Galactose-Induced Cognitive Impairment, Oxidative and Mitochondrial Dysfunction in Mice", International Journal of Alzheimer's Disease. 2011: 1- 9.

16. Li Q, Gao WQ, Zhao YQ. Advances in studies on chemical constituents and biological activities of Lawsonia inermis]. Zhongguo Zhong Yao Za Zhi. 2013: 38(6):795-9.

17. Lobo V, Patil A, Phatak A, Chandra N.Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews. 2010; 4(8): 118-126.

18. Misra HP, Fridovich I.The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247: 3170-3175

19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-358.

20. Piacente S, Pizza C, Oleszek W. Saponins and Phenolics of Yucca schidigera Roezl: chemistry and bioactivity. Phytochem Rev .2005;4(2):177–190.

21. Ravi S, Shanmugam B, Subbaiah GV, Prasad SH, Reddy KS. Identification of food preservative, stress relief compounds by GC-MS and HR-LC/Q-TOF/MS; evaluation of antioxidant activity of Acalypha indica leaves methanolic extract (in vitro) and polyphenolic fraction (in vivo). J Food Sci Technol. 2017;54(6):1585-1596.

22. Sharma A, Rathore M, Sharma N, Kumari J, Sharma K. Phytochemical Evaluation of Eucalyptus Citriodora Hook.and Lawsonia Inermis Linn. Biosci Biotechnol Res Asia 2008; 6(2): 639-645.

23. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive Oxygen Species,Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany. 2012; 1-26.

24. Stefanis L, Burke RE, Greene LA. Apoptosis in neurodegenerative disorders. Curr Opin Neurol. 1997; 10:299–305.

25. Vijayalaxmi S, Jayalakshmi SK &Sreeramulu K (2015) Polyphenols from different agricultural residues: extraction, identification and their antioxidant properties. J Food Sci Technol 52(5):2761–2769.

26. Yi Sun, Free radicals, antioxidant enzymes, and carcinogenesis,Free Radical Biology and Medicine. 1990;8(6): 583-599.

27. Zumrutdal E , Ozaslan M. A Miracle Plant for the Herbal Pharmacy; Henna (Lawsonia inermis). International Journal of Pharmacology. 2012; 8: 483-489.