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Nootropic Effect of Celastrus paniculatus on Restraint Stress Induced Behavioral and Biochemical Changes in Wistar Albino Rats

Research Article

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Abstract

Background: Stress most certainly plays a significant role in everyday life. The natural biological balance is disrupted by stressful events, which has a negative impact on normal physiological and psychological function. In the last 20 years, there hasn't been much advancement in the creation of medications, efficient drug delivery systems, or treatments for Central Nervous System (CNS)-related issues. The ancient Ayurvedic herb *Celastrus paniculatus* (CP) has been used for millennia as a memory booster, anti-inflammatory, analgesic, sedative, and antiepileptic drug. The neuropharmacological effects of the seed extract have been thoroughly studied in a variety of laboratories, and there are numerous findings that support their nootropic function. Aim: To investigate the effects of *Celastrus paniculatus* against restraint stress, induced behavioral and biochemical changes in male Wistar albino rats. Methods: The animals were divided into five groups. Each group includes six animals. Group I: Control, Group II: Restraint stress (6hrs for 21 days), Group III: *Celastrus paniculatus* (400mg/kg, orally) and restraint Stress, Group IV: *Celastrus paniculatus* alone, Group V: Vehicle. The behavioral changes are assessed by Place Preference test, Elevated plus Maze. Animals were euthanized and the discrete regions of the brain were homogenized for biochemical estimation, such as Catalase, SOD, LPO, GPX, GSH, Vitamin C. Animal's cognitive and anxiety-related behavior and antioxidants were examined the following day after the stress procedure and treatment. Results: Following a period of 21 days of being subjected to restraint stress, the behavioral changes were reduced in stress group when compared to control group, and also the levels of Lipid peroxidase significant ($P<0.05$) increased in the restraint stress group as compared to the control group. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), Vitamin C, Glutathione experienced a significant ($P<0.05$) reduction in the restraint stress group as opposed to the control group. Conclusion: Chronic restraint stress has adverse effects on the animal's cognition, memory, and learning abilities as well as anxiety-related behavior. However, *Celastrus paniculatus* treatment shows enhanced performance in anxiety-related Behavior and antioxidant properties.

Keywords: Restraint stress, *Celastrus paniculatus,* Behavior, Anxiety, Antioxidant.

Introduction

Stress is a phenomenon of disturbed homeostasis brought on by internal or external factors either physical or psychological impulses. Due to the stimulation of the sympathoadrenal and hypothalamic-pituitary-adrenal (HPA) axes, it leads to enhanced release of catecholamines and glucocorticoids(1). Reactive oxygen species (ROS), which might eventually threaten cellular proteins, DNA, and lipids, are known to increase in response to stress and may be linked to neurodegeneration caused by glucocorticoid stress hormone. (2). Immobilization/restraint stress is reputedly a simple and practical way to provoke both psychological (escape reaction) and physical stress (muscle work), leading to restricted movement, swiftness and aggression(3). In optimal physiologic conditions, cells can protect themselves from the potential for destruction of O_2 radicals via their own antioxidant mechanisms.

When immobilized for six hours, lipid peroxidation and oxidative damage significantly increased, reduced glutathione levels and Catalase and superoxide dismutase activity deteriorate (4).The current study seeks to investigate the effects of 21 days of restraint stress exposure on the antioxidant status of rats. Similar research has shown how rats exposed to restraint stress, both acutely and chronically, respond(5). Recent studies have linked enhanced free radical generation, which results in oxidative stress, to a number of different pressures(6). The peroxidation of membrane lipids is one of the most significant impacts of production of free radicals. Furthermore, it has been hypothesized that stress lowers levels of glutathione (GSH) and vitamin C, two substances crucial for preventing oxidative damage to tissues(7).

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In humans, oxidative damage brought on by an increase in free radical production leads to the development of diseases like cancer, diabetes, cardiovascular disease, atherosclerosis, Parkinson's, and Alzheimer's as well as the degeneration of neural tissues (8). Several enzymes, including superoxide dismutase (SOD), Catalase (CAT), and glutathione peroxidase (GPx), provide a natural defensive system under normal circumstances. These enzymes are essential for the detoxification of free radicals. Stress can lead to a reduction a depletion of the glutathione-based antioxidant defense (9–11).The harmful effects of an imbalance between the generation of free radicals and the capacity of the body's antioxidant defenses, known as oxidative stress, have been linked to both stress and the etiology of a number of disease conditions. Membrane lipid peroxidation is one of the significant effects of oxidative stress. Because the tissues of the retina and brain contain high levels of polyunsaturated fatty acids, this reaction causes significant harm to the structure and functionality of cell membranes in these organs(12). Consequently, lipid peroxidation was thought to be the primary metabolic change driving oxidant-induced cell harm under stress, including a number of illnesses(13).

Since many diseases have been linked to oxidative stress, the usage of antioxidant-rich foods and food supplements became quite popular. There is a demand for safe and efficient natural therapies due to the negative consequences of synthetic pharmaceuticals. Children with mental retardation who had cognitive deficiencies were treated with the herbal remedy *Celastrus paniculatus* (CP), also known as Jyotishmati(14). CP oil possesses antioxidant and neuroprotective properties (15). Uncertainty exists regarding the impact of CP oil treatment on cognitive problems brought on by stress. The goal of the current study was to investigate the positive effects of CP oil treatment on the impairment of spatial learning and memory caused by chronic stress as well as the enhancement of anxiety-like behavior.

Materials and Methods

Animals

Each healthy experimental animal weighed between 140 and 180 g. The rats were given free access to food and water while being reared in the Animal House of the Institute, University of Madras, Taramani, and Chennai, India. All of the animals were kept in standard laboratory conditions, including being housed three to a cage (29 cm x 22 cm x 14 cm), a constant ambient temperature, and a 12-hour dark photoperiod. Prior to the studies, this work received the necessary ethical clearance from the Institutional Animal Ethical Committee (IAEC no. 01/12/2021).

Chemicals

All analytical chemicals were obtained from Sisco Research Laboratories, Mumbai, India and Sigma-Aldrich Chemical Company, St. Louis, USA.

Experimental Design

Five groups of six rats each were formed by randomly dividing the rats into the groups. For this investigation, a hexane extract of Celastrus paniculatus was chosen. Rats in the Group I received saline (0.9%). Restraint stress was given to Group II rats for 21 days (6 hours on each day), and this group showed the effects of restraint stress. According to Latha et al.(16,17), Group III rats received 400 mg/(kg/b.w) of *Celastrus paniculatus* and also restraint stress for 21 days. Celastrus paniculatus alone (400 mg/ (kg/b.w) was administered to Group IV rats for 21 days. All of the experiments were carried out between 8:00 and 10:00 am in order to avoid alterations to findings due to the circadian rhythm.

Induction of Stress

The animal was physically restrained (immobilized) in order to create an animal model of psychological stress. Six hours a day of restraint in a plexiglass will subject the rats to stress(18). The control rats were also housed in the aforementioned cage for the corresponding period of time, but were not exposed to restraint stress stimulation, in order to eliminate the impact of handling-stress on evaluating the effects of restraint stress exposure.

Preparation and Administration of Hexane Seed Extract of CP

Hexane was used to extract *Celastrus paniculatus* seeds. The majority of the components of *C. paniculatus* seeds are soluble in the hexane solvent, hence it is employed for the extraction process. The seeds of *Celastrus paniculatus* were dried in the dark and processed into a coarse powder using an electric grinder. This powder was extracted with hexane using a Soxhlet apparatus at 60°c, and then it was kept at 4°C by freeze-drying for further experiments.

Celastrus paniculatus administration

Following a stress protocol, we used 400 mg/kg of CP oil and administered it orally once daily for 21 days. On the basis of earlier research, the CP oil doses were chosen(19). The drug mixture was freshly made after the CP was solubilized in peanuts oil. All animals underwent behavioral and biochemical examination following the treatment protocol.

Behavioral assessments

Place preference Task

The light/dark box was additionally employed to evaluate rodents anxiety-like behaviors. The box was separated into two halves: a light section measuring 18×15×15 inches (long, wide, and high) and a dark section measuring 12 ×15 ×15 inches (long, wide, and high) that was entirely enclosed. A 3×4-inch (wide, height) aperture was present in the partition separating the two compartments at floor level. This enables the animal to move freely between compartments. Each animal was initially positioned in the middle of the light container for testing. After that, five minutes of behavior were captured on camera. Initial latency to

enter the dark compartment, time spent in a bright environment, and time spent in a dark compartment were the metrics evaluated (20).

Elevated Plus Maze

A rodent model is used to measure anxiety-related behavior using the Elevated Plus Maze (EPM) test. The wooden Perspex elevated plus maze had two opposite open arms and two opposite closed arms of the same size. It was elevated 50 cm above the ground. The equipment was placed in a completely dark space, illuminated only by a single 60 W white light bulb that was placed roughly 100 cm above the maze's center. Rats were positioned at the maze's center, facing one of the open arms. Rats were positioned in the maze's center, facing one of the arms that was wide open. Rats were taken at random from their home cages and tested in an elevated plus maze for five minutes to ensure that their anxiety levels were low. Each animal was subjected to the elevated plus maze test just once. For the first five minutes, the number of entrances into open and closed arms as well as the amount of time spent in each arm were scored. In order to prepare the area for testing, 70% alcohol was used to clean it. Animals that fell off the maze were not included in the study. The variables include the number of open and closed arm entries in addition to the number of head dips (dipping the head below the open arm of the EPM while having all four paws on an open arm(21).

Brain dissection and Biochemical assessment of antioxidants

The brain was instantly removed and washed with ice-cold phosphate-buffered saline (PBS). The foramen magnum was cut by inserting the tip of curved scissors into it, then a single lateral cut was made through the skull, extending forward on the left and right sides, to expose the brain. The olfactory bulbs were left behind as the dorsal part of the cranium was taken off with a bone cutter and the brain was placed onto the ice-cold glass plate using a blunt forceps. The entire brain removal procedure took less than two minutes. The brain was removed, blotted, and then cooled. The cold glass plate was used to perform further dissection. According to the technique developed by Glowinski and Iverso (22), the distinct parts of the brain (the cerebral cortex, cerebellum, brainstem, hippocampus, and hypothalamus) were dissected. In an OMNI TH homogenizer, the homogenates (10% w/v) of various regions were made using ice-cold Tris-HCl (100 mmol/L, pH 7.4) buffer and centrifuged within the refrigerated centrifuge at 10000 rpm for 15 min. The supernatant was collected and used for further biochemical estimation.

Estimation of Catalase

Dichromate within the ethanoic acid is reduced to chromic acetate when heated within the presence of peroxide (H_2O_2), with the formation of per chromic acid as an unstable intermediate. The chromic acetate, thus produced was measured spectrophotometrically at 570 nm. The activity of catalase within the tissue samples is expressed as µ moles of H_2O_2 consumed/min/mg protein (23).

Estimation of superoxide dismutase

Pyrogallol auto-oxidizes rapidly within the presence of EDTA in solution at a faster rate during a higher pH (8.0) to supply several intermediate products. The inhibition of Pyrogallol auto-oxidation by the enzyme SOD present within the sample is used to measure the SOD activity. The inhibition of auto-oxidation by the addition of enzyme is evaluated at the first stage as a rise in absorbance at 420 nm. The SOD activity is expressed as Units/mg of protein within the tissue and it's defined as 50% inhibition of auto – oxidation of Pyrogallol per minute by the enzyme (24) .

Estimation of lipid peroxidation

Malondialdehyde (MDA) could be a secondary product of LPO reacts with thiobarbituric acid (TBA) to create a pink chromogen ([TBA] 2-malondialdehyde adduct) and its absorbance was measured at 532nm.The thiobarbituric acid reactive substances (TBARS) thus measured was expressed as n moles of MDA formed/ mg protein(25).

Estimation of Glutathione Reductase

The NADPH present within the reaction mixture converts oxidized glutathione (GSSG) to its reduced, upon addition of enzyme results in decrease in absorbance, the change within the absorbance was measured spectrophotometrically at 340nm for two minutes at 30 seconds time intervals. Glutathione Reductase (GR) activity is expressed as µM of GSSG reduced/min/mg protein(26) .

Estimation of Reduced Glutathione

5-5'dithiobis (2-nitrobenzioc acid; DTNB) reacts with aliphatic thiol compounds at pH 8.0 to supply 1 mole of p-nitrothiophenol anion/mole of thiol. The release of p-nitro thiophenol produced an intense yellow color, this read at 412 nm. This property is employed to measure the thiol concentration of reduced glutathione. The quantity of reduced glutathione within the tissues was expressed as µg/mg protein (27).

Statistical Analysis

The GraphPad Prism version 9 (La Jolla, California, United States) was used for statistical analysis. The values were presented as mean ± SEM. One-way ANOVA was used to examine the data, and Tukey's multiple comparison tests were used to determine whether any differences were statistically significant (P 0.05, **P 0.01, and ***P 0.001). A bar chart was used to display the data.

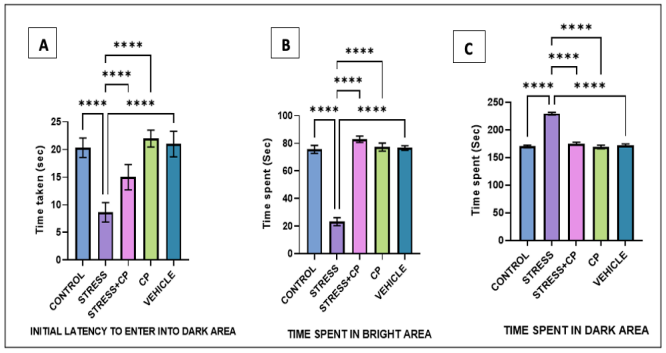
Results

Place preference test

In the light and dark test, the restraint stress group animal spent more time in the dark area while spending less time in the bright area. This was reduced in the *Celastrus paniculatus* treated group, as were the

anxiety-like behaviors as the time spent in the lighter area was increased and the latency for entering the dark area was noticeably reduced. (**Figure 1**).

Figure 1: Place preference test

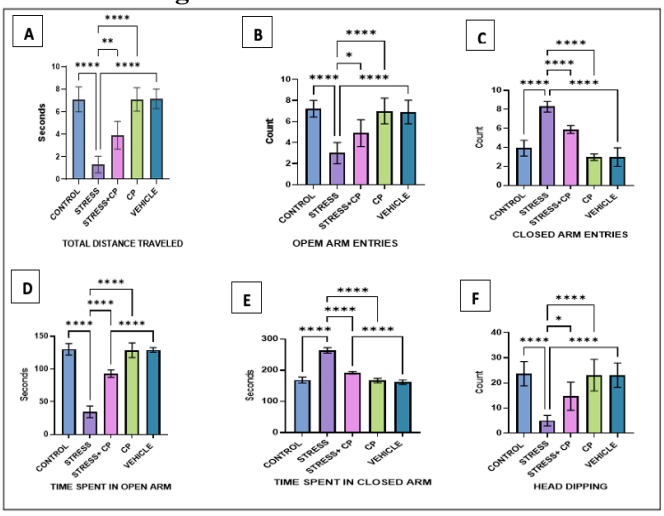


A. Initial latency to enter into dark area, **B**. Time spent in bright area, **C**. Time spent in dark area in Control, Stress group, Stress + Treated group, CP alone treated group, Vehicle alone group. The data were expressed as mean ± SEM, n = 6. The symbols represent statistical significance: *P < 0.05, **P <0.01 and ***P<0.001.

Elevated Plus Maze

An elevated plus maze test was used to assess behavior associated with anxiety. In the stress group, there was an enormous increase in the duration of time spent in closed arms when compared to the other group animals, and there was a significant decrease in head dipping, which indicates the absence of escape behavior. This demonstrates how the behavior of fear and anxiety has been significantly amplified by constraint stress. The anxiety behavior established by restraint stress has also been recovered by Celastrus paniculatus treatment to that of the control group. (**Figure 2**).

Figure 2: Elevated Plus Maze

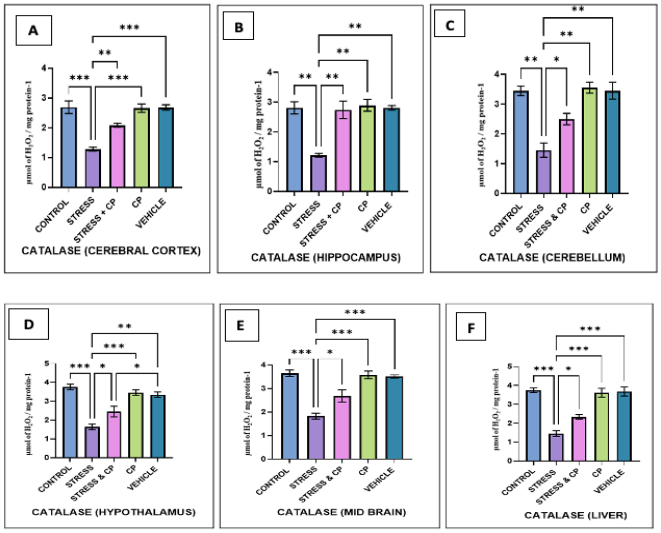


A.Total distance traveled,**B**.Open arm entreies, **C**.Closed arm entries, **D**.Time spent in open arm, **E**.Time spent in closed arm,**F**.Head dipping in Control, Stress group, Stress + Treated group, CP alone treated group, Vehicle alone group. The data were expressed as mean ± SEM, n = 6. The symbols represent statistical significance: *P < 0.05, **P <0.01 and ***P<0.001.

Effect of *Celastrus paniculatus* on restraint stress induced changes in anti-oxidants status

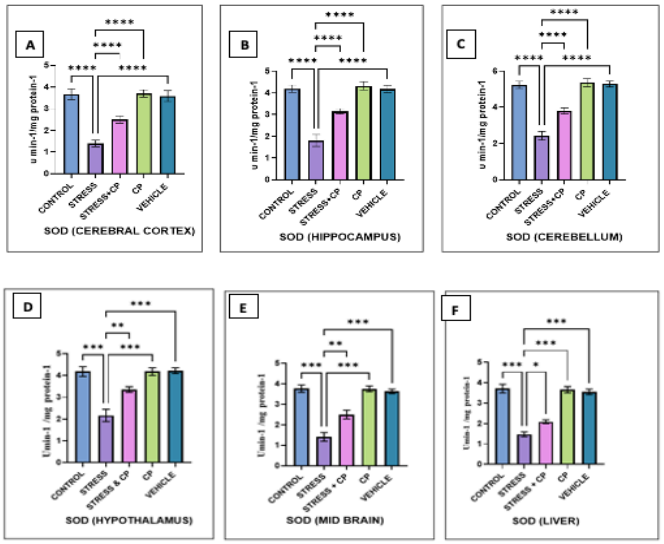
To evaluate the antioxidant activity, measurements of Catalase, SOD, GPx, GSH, and Vitamin C were made in specific areas of the brain and liver. A considerable rise in lipid peroxidation was seen in the cerebral cortex and hippocampus as a result of decreased catalase activity (Figure 3). When compared to the other groups, the stress group showed a significant decline in the activity of SOD (Figure 4), GPx (Figure 5), GSH (Figure 6), and vitamin C (Figure 7) and comparing the to all other groups, stress group there was a significant rise in lipid peroxidation (Figure 8), showing that the antioxidant system was unable to effectively combat the free radicals generated during restraint stress.

Figure 3: Effect of Celastrus *paniculatus* on Catalase activity in rat brain discrete regions and liver after 21 days of restraint stress (6 hours/day)



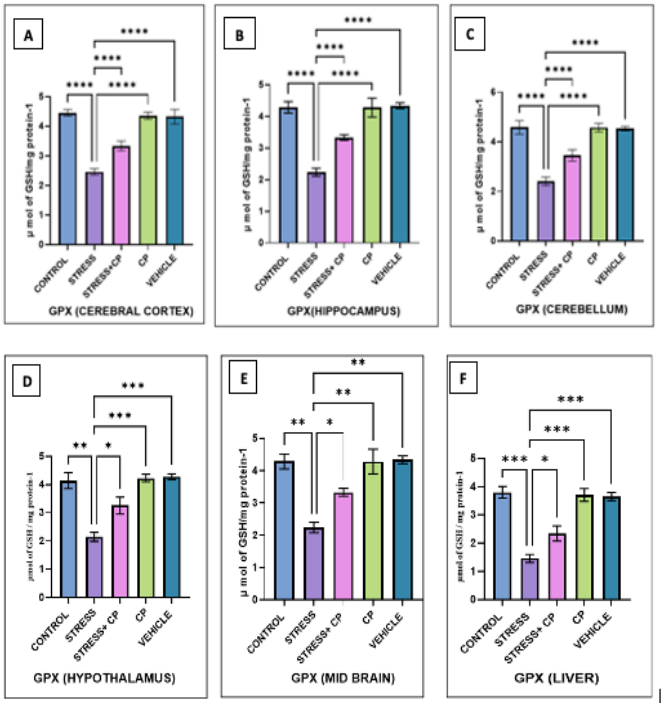
The data were expressed as mean ± SEM, n = 6. The symbols represent statistical significance: *P < 0.05, **P <0.01 and ***P<0.001.

Figure 4: Effect of Celastrus *paniculatus* on SOD activity in rat brain discrete regions and liver after 21 days of restraint stress (6 hours/day)



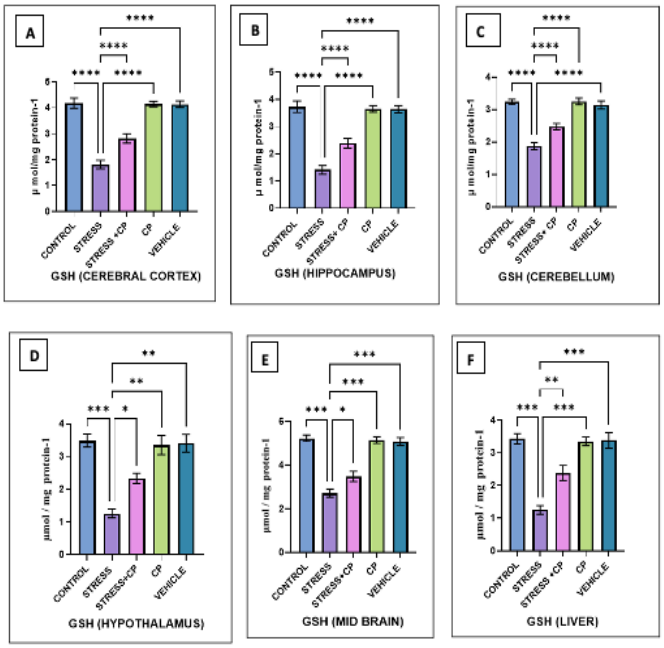
The data were expressed as mean ± SEM, n = 6. The symbols represent statistical significance: *P < 0.05, **P <0.01 and ***P<0.001.

Figure 5: Effect of *Celastrus paniculatus* on Glutathione peroxidase level in rat brain discrete regions and liver after 21 days of restraint stress (6 hours/day)



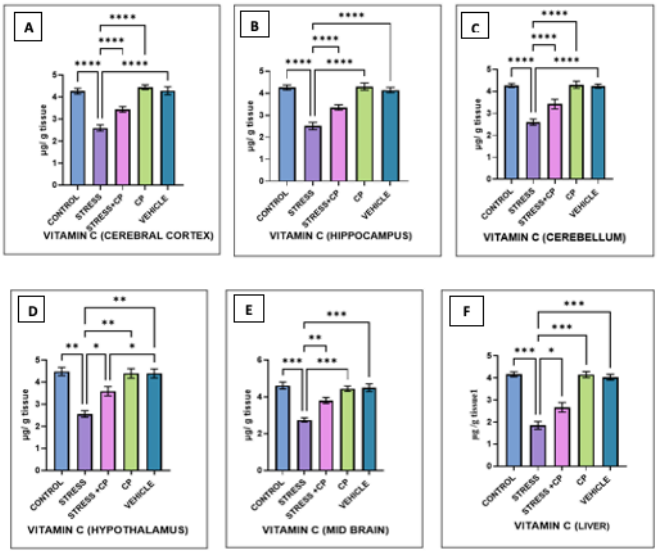
The data were expressed as mean ± SEM, n = 6. The symbols represent statistical significance: *P < 0.05, **P <0.01 and ***P<0.001

Figure 6: Effect of *Celastrus paniculatus* on Reduced Glutathione levels in rat brain discrete regions and liver after 21 days of restraint stress (6 hours/day).



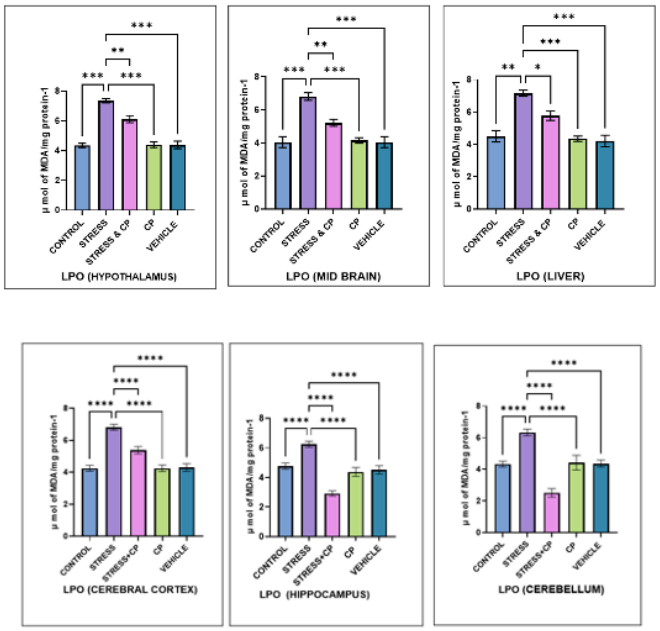
The data were expressed as mean ± SEM, n = 6. The symbols represent statistical significance: *P < 0.05, **P <0.01 and ***P<0.001.

Figure 7: Effect of *Celastrus paniculatus* on Vitamin C levels in rat brain discrete regions and liver after 21 days of restraint stress (6 hours/day)



The data were expressed as mean ± SEM, n = 6. The symbols represent statistical significance: *P < 0.05, **P <0.01 and ***P<0.001.

Figure 8: Effect of *Celastrus paniculatus* on Lipid peroxidase levels in rat brain discrete regions and liver after 21 days of restraint stress (6 hours/day)



The data were expressed as mean ± SEM, n = 6. The symbols represent statistical significance: *P < 0.05, **P <0.01 and ***P<0.001.

Discussion

Free radicals, which are responsible for the formation of oxidative stress, are the key contenders for causing the neural alterations driving these behavioral abnormalities(28,29). In order to avoid oxidative damage and enhance memory, there is growing interest in the biochemical activity of natural antioxidants found in fruits, vegetables, and medicinal plants(30). Behavioral impairments of varying degrees are related to aging and age-related neurodegenerative illnesses.

One of the most prevalent techniques to induce stress-related behavioral and physiological alterations in laboratory rat is restraint stress(31,32). Animals who have been restrained increased anxiety levels in the elevated plus maze and stress-related behavioral alterations in other testing paradigms(33).As an acute (or chronic) stress paradigm, restraint stress is a well-defined concept(34–37). Figures 1 and 2 depict the elevated plus maze and place preference test behavioral assessment, respectively. After being subjected to restraint stress for 21 days, the behavioral modifications in stress group II were diminished. Our findings demonstrated that as compared to stress groups *Celastrus paniculatus* treated group (IV and V) has more effectively enhanced behavioral deficits.

Lipid peroxidation, which refers to the peroxidation of numerous significant components, including the lipid layer found in cell membranes, is a key factor in toxicity inside of cells. 4-Hydroxynonenal, a result of lipid peroxidation, may function as a secondary messenger for cell death and apoptosis(38). Restraint stress group animals indicate that increased lipid peroxidization This is a sign of the cytotoxic actions of oxygen radicals, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, which act on polyunsaturated fatty acids in the brain and propagate lipid peroxidation (39). Our findings, following a period of 21 days and also the levels of Lipid peroxidase significant increased in the restraint stress group whereas, *Celastrus paniculatus* treated groups significantly decreased lipid peroxidation level.

Immobilization-induced oxidative stress in mice brain has been established here by noting the low activities of SOD, CAT, GST, important antioxidant enzymes, which is consistent with the observation of others(40). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), Vitamin C, Glutathione experienced a significant reduction in the restraint stress group. The decrease in antioxidant enzyme activities due to immobilization might be due to their use against the free radicals destruction and/or their inhibition by free radical species(41). It is well established that SOD activity is inhibited by hydrogen peroxide that reduced Cu^{+2} to Cu^{+1} in SOD(42). The reduction of hydrogen peroxide is catalyzed by CAT that protects the tissues from highly reactive hydroxyl radicals (43). When *Celastrus paniculatus* seed oil was administered animal group, the enzymatic antioxidants such as SOD, Catalase, GPX, GSH and Vitamin C levels increased considerably, and the effect was stronger in the chronically immobilized stress-induced animals. Compared to acute immobilization, chronic immobility caused a more significant drop in levels from normal, whereas the treated group had a considerable increase in levels of these antioxidant enzymes(44).

Glutathione and vitamin C has a significant role in the detoxification of ROS species in brain cells, which is important for cognitive function. There was a decline in the level of GPx, Vitamin C, SOD, GSH, and increased lipid persistent peroxidation in specific brain areas restraint stress group, which is related to the increased ROS concentrations that indicate neurodegeneration, which is also prevented with *Celastrus paniculatus* treated group. Because of this, this study has demonstrated the antioxidant effectiveness of *Celastrus paniculatus* in scavenging free radicals and preventing oxidative stress.

Only the rats treated with 200 and 300 mg/kg body weight of the aqueous extract, the doses that are used to detect lipid peroxidation and the production of free radicals, exhibited a substantial reduction in the levels of malondialdehyde in the brain. Levels of glutathione, a tripeptide found in all cells that reacts with free radicals to shield cells against superoxide radical, hydroxyl radical, and singlet oxygen, increased concurrently and significantly. These findings imply that the natural antioxidant enzymes are increased by the hexane extract of CP, which lowers oxidative stress. Our results showed that stress significantly inhibited SOD. This enzyme's decline brought on by increased lipid peroxidation under the stress state may be the cause of this discovery(45).

Conclusion

The effects of repeated restraint stress which increases oxidative damage and behavior in rat. Furthermore, *Celastrus paniculatus* is a prime antioxidant potential for preventing stress-induced lipid peroxidation and that chronic stress significantly alters antioxidant enzymes. CP has the beneficial therapeutic agent in clinical disorders linked to free radical damage because of its capacity to function as an antioxidant in fat- and water-soluble tissues in both its oxidized and reduced forms. There is also a need to investigate studies including the isolation of a particular chemical with the greatest therapeutic potential, including antibacterial and antioxidant capabilities.

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Conflict of Interest

The authors declare that they no conflicts of interest concerning this research article

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