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Antioxidant Activity Of L - Theanine On Cadmium Induced Oxidative Stress Mediated Neurodegeneration - An In Vivo Analysis

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ABSTRACT

Introduction: L-theanine, an amino acid found in green tea, has antioxidant, anti-inflammatory, and neuromodulatory properties. It improves dopamine, gamma-aminobutyric acid (GABA), and serotonin levels in the central nervous system and decreases neuronal death and mitochondrial failure (CNS). A complicated chain of circumstances, such as excitotoxicity, calcium excess, and mitochondrial malfunction contributes to oxidative stress-mediated neurodegeneration. By focusing on neurotrophins, polyphenols have neuroprotective effects and support neuronal survival.

Materials and Methods: Three groups of Wistar rats were assigned at random. Control group, Cadmium induction group, and L-theanine therapy group. Group I: Rats were given a dosage of 150 mg/kg body weight to induce cadmium. L-theanine was administered orally for 30 days at a dose of 100 mg/kg body weight after 60 days of lead induction. Rats were euthanized at the conclusion of the experiment, and the brain tissues were removed, fixed in 4% paraformaldehyde, and processed for histological examination. Using SPSS software, bar graphs for SOD and LPO levels were plotted.

Results: Group-II (Cadmium induced) rats showed neurodegeneration with severe neuronal damage, apoptotic and pyknotic neurons and gliosis. SOD levels were significantly decreased and MDA levels were significantly increased in this group. Group-III (L-theanine treated) rats showed relatively better improvement in neuronal recovery and significant increase in SOD level and a decrease in MDA level after treatment.

Conclusion: L-theanine can provide marked protective effects against cadmium induced brain dysfunction in rats through its neuroprotective and antioxidant properties on cadmium toxicity.

Keywords: *Neurodegeneration, Hippocampus, L-Theanine, Cadmium toxicity, neuroprotection*

INTRODUCTION

Cadmium has been long known to induce neurological degenerative disorders. It is found in the earth's crust combined with chlorine (CdCl2), oxygen (CdO) and Sulphur (CdS). (1)It is also a by-product of melting of Zinc, Lead, Copper Ores used mainly in metal plating, producing pigments, batteries, plastics and as a neuron absorbent in nuclear reactors. Cadmium poisoning is caused by excessive exposure to cadmium. It has no constructive purpose in human body. It is very toxic even in low concentrations. Cadmium toxicity is treated with potential antioxidants like Vitamin C, E Glutathione, Methionine, Glycine, Cysteine etc.(2,3)

L-theanine is a non-protein amino acid found in tea plants. It has a unique taste with caramel flavour which can elevate to bitter taste. It can be used as one of the significant indices to estimate the freshness of tea.(4) It is known to have many health benefits such as anti-cancer, anti-inflammatory, antioxidant, anti-anxiety, neuro-protective, metabolic regulatory, cardiovascular properties. (5)L-theanine is widely distributed in the roots of tea initially later it is transported to shoot off the plant. It plays a major role in improving the sleep quality, reduces spinal cord injury and reduces the risk of brain oxidation.(6)

Cadmium (Cd), a common hazardous contaminant with a poor rate of elimination from the body and a biological half-life of 20 to 30 years in humans, accumulates in human tissues.(7) Metalworking, the plastics sector, mining, pigments, chemical stabilizers, metal coatings, and battery manufacturing are some of the industries that expose workers to cd in the workplace and environment. A significant source of Cd exposure is contaminated food and agricultural soil(8). Additionally adding to human exposure is the fact that cd can be detected in tobacco smoke.

The kidney, pancreas, liver, lungs, bones, reproductive organs, neurological and cardiovascular systems, as well as other organs, are all adversely affected by the long-term deposition of Cd salts (8,9). Cd has also been related to the development of cancer, and the International Agency for Research on Cancer has classed it as a category I human carcinogen (10). Although Cd mostly occurs in the +2 oxidation state and does not produce free radicals directly through oxidation-reduction reactions, it has been observed that ROS such as superoxide, hydroxyl radical, hydrogen peroxide, and nitric oxide can develop indirectly from Cd (10,11).

L-theanine protected the brain against cadmium induced neurotoxicity through reducing brain Cadmium levels and relieved oxidative damage. It significantly reduced the levels of MDS and ROS and increased levels of SOD, CAT, GSW-Px in brain (12)

Our institution has produced several promising results in the field of research. Our team has extensive knowledge and research experience that has translated into high quality publications (13–22). The aim of this study is to analyze the antioxidant activity of L-theanine on Cadmium induced oxidative stress mediated neurodegeneration in Wistar rats. The main objective of this study is to examine the therapeutic efficacy of L-theanine on Cadmium induced neuronal toxicity through biochemical analysis. The antioxidant activity of L-theanine on cadmium induced neurodegeneration by histopathological analysis.

MATERIALS AND METHODS

An in-vivo evaluation of the experiment was done using an animal model. In comparison to the control group, the experimental and treatment groups were evaluated. Rats that were Wistar Albino were chosen at random for this investigation. Statistics indicated that the sample size was substantial. This research is an experimental examination using animal models that will be followed by pre-clinical trials to validate the findings. Institutional Animal Ethics Committee (IAEC) approval was obtained for this investigation with the following code: SU/CLAR/RD/O35/2017. The study also has some limitations, which are single-blinded study, only histopathological analysis, and then need to do molecular analysis.

This study made use of Wistar Albino rats. 18 people make up the entire sample (6 per group). It was confirmed that the animals on the case sheet had come from a breeder that had received CPCSEA approval. Verified information for age, gender, weight, and health. As part of the internal validation, the weight of the rats was associated with their sex in relation to the study, and their overall health was thoroughly confirmed.

The rats were randomly assigned to Group I, Control, Group II, Infected, and Group III,which was the group receiving the treatment. After 60 days of cadmium induction, rats received L- theanine orally at a dose of 100 mg/kg body weight for 30 days. Cadmium was induced in rats at a dosage of 150 mg/kg body weight and with normal drinking water.

The animal was euthanized in a CO2 chamber after receiving treatment for 30 days. The rat's tissues were removed, cleaned in saline, and then preserved in neutral buffered formalin. The preparation of all samples included H&E staining for histological analysis. After taking pictures and doing histopathology analysis on the stained samples, For the biochemical examination, unfixed tissues were used.

RESULTS

The accumulation of reactive oxygen species (ROS) enhances the susceptibility of brain tissue to harm, as has been well shown by earlier investigations. The amount of evidence showing Cadmium (Cd) is to blame for increased oxidative stress, ROS production, and neurodegeneration is growing(23).As a result, our research showed that the Cd-injected mice group had more ROS than the mice in the control group. However, the group of mice treated with Cd and l-theanine saw a significant decrease in the elevated level of ROS (Figure 1).

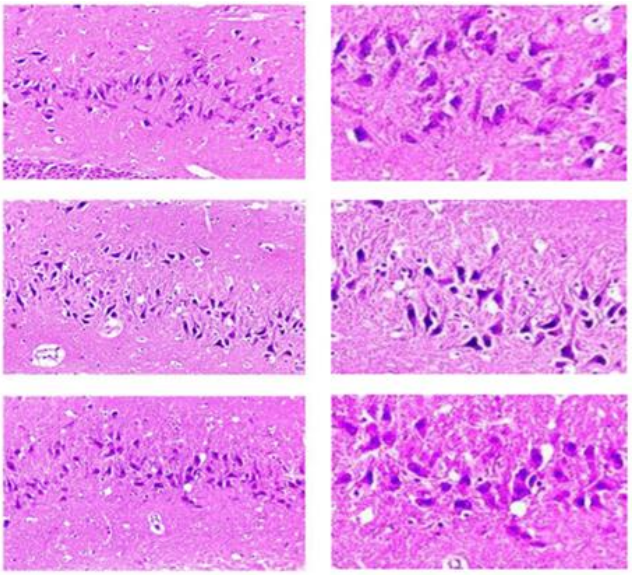

FIGURE 1: H&E stained image of hippocampus of Control group, Cadmium induction group, and L-theanine treatment group at 20X (First column) and 40X (Second column) magnification.

Superoxide dismutases (SODs) are an essential antioxidant defense mechanism in the body against oxidative stress.(24) The enzyme is an effective treatment for illnesses brought on by reactive oxygen species. The enzyme, however, has some restrictions when used in medical settings. SOD conjugates and mimics have thus been created to boost its therapeutic effectiveness(2). Because they scavenge one of the reactive oxygen species, superoxide anion, superoxide dismutases (SOD), a group of metal-containing enzymes, play a crucial antioxidant function in maintaining human health.

SPSS software was used to acquire the results. The excel file was inserted once the software was opened. A different variable perspective was used, and an analysis was conducted. LSD and Tukey were chosen together with one-way ANOVA-Post hoc. The results were graphically plotted using a bar histogram.

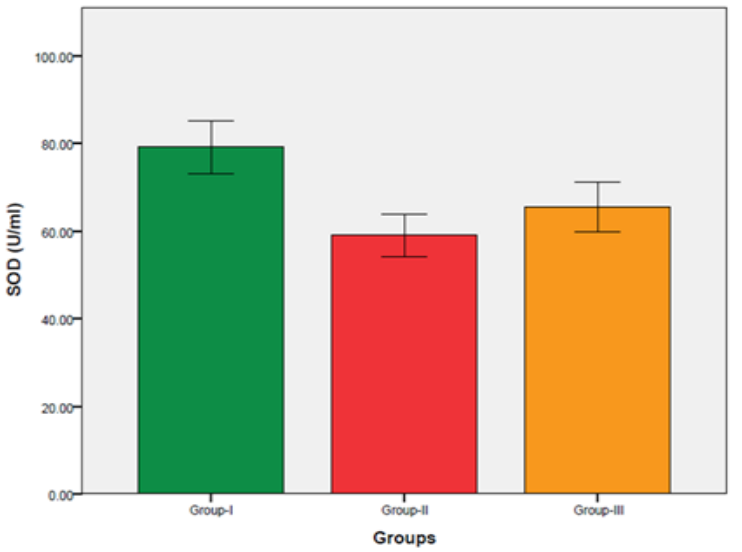

FIGURE 2: Bar graph shows the level of superoxide dismutase (SOD) in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the SOD level in U/ml. All the values are expressed as Mean ± Standard Deviation. On comparing between the 3 groups, Group-III showed p= 0.015 ($p<0.05$) indicating statistically significant increase from Group-II.

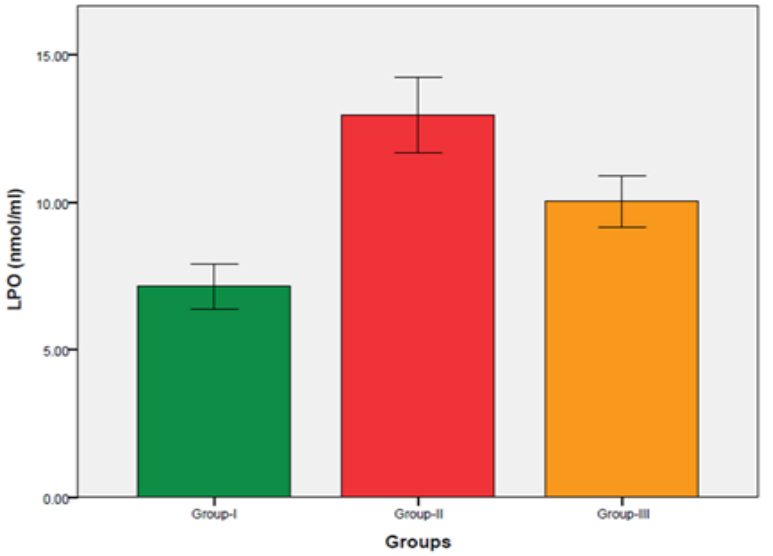

FIGURE 3: Bar graph shows the level of malondialdehyde (MDA) upon lipid peroxidation in Control and experimental groups. The x-axis represents the grouping and the Y-axis represents the MDA level in nanomole/ml. All the values are expressed as Mean ± Standard Deviation. On comparing between the 3 groups, Group-III showed p= 0.009 ($p<0.05$) indicating statistically significant decrease from Group-II.

Graph I is plotted with SOD Vs Groups. SOD stands for superoxide dismutase, which is an antioxidant. We can infer from this graph that for group I and II SOD levels and disease are inversely related. The hippocampus along with increased levels of lipid peroxidation and concurrent decrease in the antioxidants enzyme level of SOD (Figure 2).

Graph II, is plotted with LPO Vs Groups. LPO stands for lipid peroxidation. The series of

oxidative lipid breakdown reactions are known as lipid peroxidation. Cell damage is the result of the process by which free radicals "steal" electrons from the lipids in cell membranes. For group I and II LPO levels and disease are directly correlated (Figure3).

On administration of L-theanine along with cadmium-induced rats were found a significant improvement in these levels which inevitably confirms that L-theanine has a prominent role in preventing brain damage upon toxicity.

DISCUSSION

Antioxidants are endogenous or exogenous substances that protect the biological system from the harmful effects of oxidative stress in any form. To prevent oxidative stress, they could neutralise ROS and other types of free radicals (25).A wide range of antioxidant supplements are presentin many of the foods we eat, including flavonoids, phenolic compounds, lipoic acid (thioctic acid),ubiquinone, idebenone, beta-carotene, and vitamin C. These natural antioxidants operate as an upstream therapeutic barrier to oxidative stress by preventing the oxidation of proteins, lipid peroxidation, and the formation of ROS (reactive oxygen species) (26). One effect of ROS production is the beginning of excitotoxicity, which is controlled by glutamate receptor overactivity.

The creation of ROS and the biological system's capacity to detoxify the reactive intermediates are out of balance, which leads to oxidative stress[q].

The progression of neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD), and others has been linked to oxidative stress(27). Oxidative stress, which causes free radicals to destroy brain cells, plays a disastrous role in neurodegeneration. Protein misfolding, glia cell activation, mitochondrial malfunction, and ultimately cellular apoptosis are all caused by the toxicity of ROS.(28)

The develops toxicity as a result of ongoing exposure to cadmium. Toxic cells have a proclivity to deteriorate. Apoptosis and necrosis are two of its processes. The process of necrosis cannot be reversed. Apoptosis can be reversed. The cells in the current investigation are going through Apoptic Pyknosis. Degenerated cells emit free radicals. These radicals adhere to the cell membrane and begin the peroxidation of lipids. As a result, the lipid layer being porous, the cell loses its stability, and homeostasis begins. On administering L-theanine to rats for 30 days at a dose of 100 mg/Kg of body weight. There was an improvement noted, which was observed in the slides.

Chronic and prolonged Cadmium exposure affects BBB (Blood Brain Barrier) permeability mainly due to the weakening of the cellular antioxidant defenses that in turn allow more cadmium entering the brain (29).

CONCLUSION

The present study indicated that L-theanine can provide marked protective effect against cadmium induced brain dysfunction in rats. Therefore our study suggests that L-theanine, a natural plant compound could be useful to prevent oxidative brain damage from cadmium exposure. Cadmium is widespread in our environment Among all the problems it effects in many organs and tissues, it plays a crucial role in CNS, reacting BBB and increasing its permeability, allowing for in turn growing entrance in the brain.

AUTHOR CONTRIBUTIONS

Author 1: Souparnika. V, carried out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis and in the preparation of the manuscript.

Author 2: Karthik Ganesh Mohanraj, aided in conception of the topic, designing the study and supervision of the study, correction and final approval of the manuscript.

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CONFLICTS OF INTEREST

There is no conflict of interest to declare.

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