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Neuroprotective Efficacy of Eugenol Against Lead Acetate and Monosodium Glutamate Induced Neurotoxicity by Modulating Brain-Derived Neurotrophic Factor (BDNF) Gene Expression in Wistar Rats

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Abstract

The human nervous system is highly susceptible to various environmental toxins, which can lead to neurodegenerative conditions characterized by cognitive deficits, motor dysfunction, and even cell death. Among these toxins, lead (Pb) and Monosodium Glutamate (MSG) have been evaluated in this study for their neurotoxic effects. Lead exposure has been associated with detrimental effects on the central nervous system, similarly, MSG, a common food additive, has been reported to induce neurotoxicity through oxidative stress and excitotoxicity mechanisms. Eugenol, found in essential oils, have demonstrated promising antioxidant, anti-inflammatory and neuroprotective properties. Hence Eugenol was used as a therapeutic agent against lead acetate and MSG induced neurotoxicity by modulating Brain-derived Neurotrophic Factor. This in vivo study involved 48 Wistar albino rats, divided into eight groups consisting of Control, Lead acetate induction (100 mg/kg b.wt for 30 days), MSG induction (2 g/kg b.wt for 21 days) and subsequent treatment with Eugenol (250 mg/kg b.wt for 30 days) in comparison with positive control, memantine (20mg/kg b.wt for 15 days). Histopathological and BDNF gene expression were evaluated after the experimental period. Histopathological analysis confirmed that eugenol preserved neuronal integrity, reducing neuronal damage caused by lead acetate and MSG exposure by modulating free radical generation upon oxidative stress. Eugenol treatment in rats exposed to lead and MSG resulted in a significant upregulation of BDNF expression ($p<0.01$) compared to the untreated toxin-exposed groups. These outcomes suggest that Eugenol could be a possible therapeutic agent for protecting the neuronal tissues from Lead acetate and MSG-induced neurotoxicity.

Keywords: *BDNF, Eugenol, Lead, MSG, Neuroprotective, Neurotoxicity.*

Introduction

The human nervous system is highly susceptible to various environmental toxins, which can lead to neurodegenerative conditions characterized by cognitive deficits, motor dysfunction, and even cell death. Among these toxins, lead acetate (PbAc) and monosodium glutamate (MSG) have been extensively studied for their neurotoxic effects. Lead exposure has been associated with detrimental effects on the central nervous system, particularly during developmental stages, leading to deficits in learning, memory, and behaviour [1]. Similarly, MSG, a common food additive, has been reported to induce neurotoxicity through oxidative stress and excitotoxicity mechanisms [2].

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Lead acetate, a heavy toxic substance encountered in industrial settings, has been extensively studied for its detrimental impact on the central nervous system. Research has shown that lead exposure, especially during critical periods of development, can result in profound neurobehavioral deficits [3]. The toxic effects of lead are primarily mediated through oxidative stress, disruption of neurotransmitter function, and interference with synaptic plasticity, all of which contribute to the long-term damage observed in exposed individuals.

Monosodium glutamate, widely used as a flavour enhancer in the food industry, is another substance linked to neurotoxicity. Although generally recognized as safe at low levels of consumption, excessive intake of MSG has been associated with excitotoxicity, a process where exorbitant initiation of glutamate receptors prompts neuronal injury. This excitotoxicity is often accompanied by oxidative stress, which further exacerbates neuronal damage [4]. The potential risks posed by chronic MSG consumption, especially in populations with high dietary exposure, underscore the need for further investigation into protective strategies against its neurotoxic effects.

In the quest for potential neuroprotective agents, natural compounds have gained significant attention due to their ability to mitigate oxidative stress and inflammation. Eugenol, a phenolic compound found in essential oils such as clove oil, has demonstrated promising antioxidant, anti-inflammatory, and neuroprotective properties [5]. Eugenol's multifaceted biological activities include scavenging free radicals, reducing inflammation, and modulating signalling pathways involved in cell survival [6]. These properties make eugenol a compelling candidate for counteracting the neurotoxic effects of substances like lead acetate and MSG. Previous studies have suggested that eugenol may confer protection against various neurotoxic agents, but its specific role in counteracting the effects of lead acetate and MSG-induced neurotoxicity remains underexplored. Despite its potential, the particular mechanisms by which eugenol might protect against lead and MSG-induced neurotoxicity have not been fully elucidated. This study aims to fill this gap by employing histopathological analysis to assess the neuroprotective effects of eugenol. By examining the structural changes in neuronal tissues following exposure to lead acetate and MSG, and subsequent treatment with eugenol, this research seeks to provide a deeper understanding of how this natural compound may mitigate or prevent neurodegeneration caused by these toxic agents.

This study aims to investigate the neuroprotective efficacy of eugenol on lead acetate and monosodium glutamate-induced neurotoxicity using histopathological and immunohistochemistry of BDNF protein and BDNF gene expression analysis. By examining the structural integrity of neurons and the extent of neurodegeneration, this research seeks to elucidate the potential mechanisms by which eugenol may protect against these common neurotoxic agents.

Materials and Methods

Study Approval

The experiment was approved by the Institutional Animal Ethics Committee of Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (BRULAC/SDCH/SIMATS/IAEC/12-2019/042). The study adhered to the rigorous guidelines outlined by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), ensuring the humane treatment and welfare of animals used in research.

Chemicals Used

Lead acetate and eugenol were bought from Sigma-Aldrich Chemicals Private Limited (Merck Products) and the RT kit was procured from Eurogentec (Seraing, Belgium). All other chemicals and reagents are purchased from Sisco Research Laboratories Pvt. Ltd. (SRL), India.

Animal Model Used, Maintenance and Grouping

A healthy mature male Wistar albino rat (*Rattus norvegicus*) was used in this study, weighing about 150-200g obtained from a CPCSEA registered commercial grade agency. All animals were housed in proper cages in exact humidity (65 ± 5%) and warmth (25 ± 1˚C) with a regular 12-h daylight/dark cycle. They were fed with a regular rat diet and hygienic water ad libitum. The rats were acclimatized to the lab environment for a week before initiating experiments on quarantinization. 48 male rats were divided into 8 groups, Group-I: Control (Normal saline), Group-II: Drug control (Eugenol treatment), Group-III: Lead acetate induction, Group-IV: Lead acetate induction + Eugenol treatment, Group-V: Monosodium glutamate (MSG) induction, Group-VI: Monosodium glutamate + Eugenol treatment, Group-VII: Lead acetate induction + Memantine treatment (Positive control) and Group-VIII: Monosodium glutamate + Memantine treatment.

Experimental Toxicity Induction Procedure

Lead toxicity was induced in healthy adult rats that received lead acetate at a dose of 100 mg/kg body weight by oral route daily for 30 days [7]. Experimental neurotoxicity was induced by Monosodium glutamate at a dose of 2 g/kg body weight daily by oral route for 30 days [8]. Eugenol treatment was given as a daily dose of 250 mg/kg body weight and was administered orally via gastric intubation to rats after the 30 days of lead acetate and MSG induction period for consecutive 15 days [9]. Memantine treatment as positive control, was given to rats after lead acetate and MSG induction period of 30 days, orally at a dose of 20mg/kg body weight once a day for 15 days [10].

Histological Processing

Haematoxylin & Eosin

After 45 days of total experimental period, animals were sacrificed by CO_2 in a closed chamber. Brain tissues were dissected out and fixed in 4% paraformaldehyde and processed for histopathological analysis. For analysis of the hippocampus of brain tissue, paraffin sections of 5 µm thickness were stained with routine Haematoxylin & Eosin staining and observed under a light microscope [11].

Immunohistochemical Analysis

For Immunohistochemistry, 4% paraformaldehyde-fixed paraffin-embedded brain tissue sections of 5 µm, mounted on a glass slide underwent heat-induced epitope retrieval in citrate buffer (pH 6.0) at 95°C for 20 minutes to unmask BDNF antigens, enhancing antibody binding [12]. After cooling, sections are incubated in 5% normal bovine serum albumin for 1 hour to block non-specific binding sites. Tissue sections are incubated overnight at 4°C with anti-BDNF primary antibody (dilution 1:200) for specific binding [13]. Sections are treated with a biotinylated secondary antibody conjugated with HRP (Horse Radish Per-oxidase) for 30 minutes for signal amplification [14]. DAB is applied as the chromogen, producing a brown precipitate on BDNF-positive cells, followed by haematoxylin nuclear counterstaining for contrast. Slides are dehydrated, cleared and covered with a permanent mountant. Slides are analysed using bright-field microscopy and photographed.

mRNA Expression Analysis

Gene expression analysis of BDNF was quantified via RT-PCR (Real-time polymerase chain reaction). We used the Total RNA Isolation Reagent Kit (TRIR, Invitrogen) to isolate total RNA for this investigation. In a nutshell, 1 millilitre of TRIR was mixed with 100 milligrams of fresh tissue from all the groups. Following homogenization, 0.2 ml of chloroform was added, and the mixture was vortexed for one minute before being stored at 4°C for five minutes. It was then centrifuged for five minutes at 12,000 × g for fifteen minutes at 4°C. After vortexing for 15 seconds, the upper aqueous phase was carefully transferred to a new microcentrifuge tube, and an equal volume of isopropanol was added. The tube was then placed on ice for 10 minutes. After centrifuging the contents at 12000 ×g for 10 minutes at 4°C, the supernatant was disposed of. The RNA pellet was then rinsed with 1 millilitre of 75% ethanol. Using spectrophotometry, the concentration of total RNA was determined and reported in micrograms. Following the manufacturer's instructions, a reverse transcriptase kit was used to convert 2 µg of total RNA into complementary DNA (cDNA) (Eurogentec, Seraing, Belgium). The primer sequence is described in Table 1. The PCR reaction mix containing the target gene-specific and internal control gene, water, cDNA and two reaction buffers (Takara SyBr Green Master Mix) was spun for the real-time PCR analysis. For 40 cycles, the reaction was set up at 95°C for 5 minutes, 95°C for 5 seconds, 60°C for 20 seconds, and 72°C for 40 seconds. The PCR equipment (CFX96 Touch Real-Time PCR Detection System) collected the findings and plotted them on a graph. For quantification, amplification and melting were employed.

Table 1. Primer Sequences for Real-Time Polymerase Chain Reaction Assay

Gene	**Forward Primer (5′ – 3′)**	**Reverse Primer (5′ – 3′)**
BDNF	CAGGGCAGTTGGACAGTCAT	TACGCAAACGCCCTCATTCT

Statistical Analysis

The data obtained from this study were analyzed using one-way analysis of variance (ANOVA) with multiple comparisons through the Student-Newman-keels test. Statistical analysis and plotting of graphs were carried out using GraphPad Prism software version 7, a p-value of less than 0.05 was deemed statistically significant.

Results

Efficacy of Eugenol on Histopathology of Hippocampus

In Group I (Control), the normal histological architecture of the hippocampus, with well-organized pyramidal cells was observed in the CA1, CA2 and CA3 regions. Neuronal cells appear healthy with intact nuclei and no evidence of neurodegeneration or gliosis (Figure 1: Group-I). In Group II (Drug Control Eugenol Group), hippocampal structure closely resembles the Control group with normal pyramidal cells and nil signs of gliosis. No significant histopathological alterations are observed indicating that the drug control eugenol alone group does not produce any toxic effects on hippocampal neurons (Figure 1: Group-II). In Group-III and Group-V, Lead and MSG-induced neurotoxicity groups, remarkable neuronal damage was observed in the hippocampal regions such as degeneration of pyramidal neurons, and many cells show darkly stained, shrunken nuclei (pyknosis), indicating cell death. Increased gliosis is indicative of a reactive astrocytic response due to neuronal injury. Edema and loss of cellular organization, reflecting severe neurotoxic

effects on the hippocampus shown in (Figure 1: Group-III & V). Lead + Eugenol and MSG + Eugenol Group (Treatment Group) showed preservation of hippocampal architecture with reduced apoptotic neurons compared to neurotoxic groups. Upon reduction in neuronal degeneration, few neurons exhibit pyknosis, with improved nuclear integrity. Less vacuolation and reduced gliosis were also observed, suggesting a protective or neuro-restorative property of Eugenol on lead-induced neurotoxicity (Figure 1: Group-IV & VI). All these restorative features of histopathology on rats treated with Eugenol are much better than the rats treated with the standard drug Memantine upon lead and MSG-induced neurotoxicity (Figure 1: Group-VII & VIII).

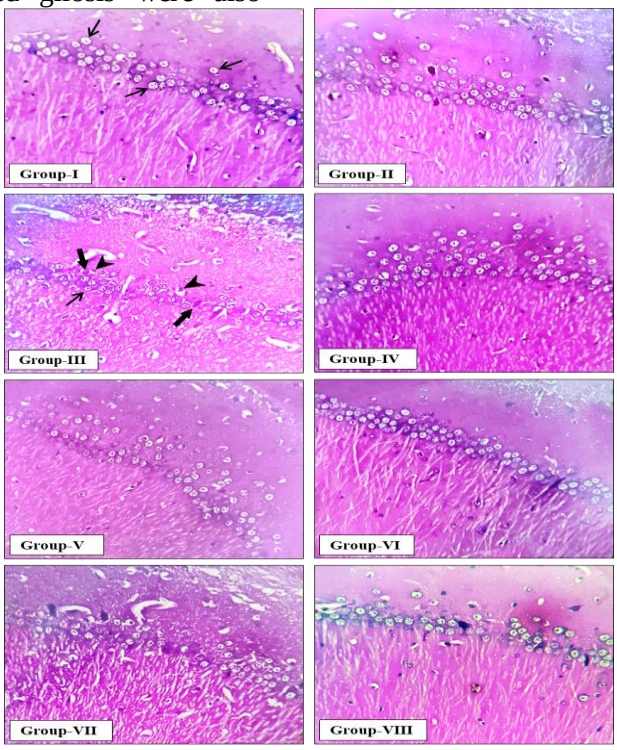

Figure 1. Photomicrograph Showing Histopathology of Hippocampus Stained with H&E of Group-I: Control (Normal Saline); Group-II: Drug Control (Eugenol); Group-III: Lead Acetate (Pb); Group-IV: Lead Acetate + (Eugenol); Group-V: Monosodium Glutamate (MSG); Group-VI: Monosodium Glutamate + (Eugenol); Group-VII: Lead Acetate + Memantine; Group-VIII: Monosodium Glutamate + Memantine at 10X Magnification. Thin Arrows – Normal Healthy Neurons; Thick Arrows – Immature Neurons; Arrowheads – Apoptotic Pyknotic Neurons.

Efficacy of Eugenol on BDNF Immunohistochemistry of Hippocampus

In the Control group strong and widespread expression of BDNF in the hippocampus, particularly in the CA1, CA3, and dentate gyrus (DG) regions was seen. Intense BDNF immunoreactivity in pyramidal neurons and glial cells, with clearly defined positive brown staining indicating healthy neurotrophic activity, was observed (Figure 2: Group-I). In the Eugenol group (Drug Control), BDNF expression is similar to the Control group, with strong immunoreactivity in the hippocampal regions. No significant reduction in BDNF staining, indicating that eugenol alone does not negatively affect BDNF levels (Figure 2: Group-II). The lead and MSG (Neurotoxicity) group showed that substantial reduction in BDNF expression compared to the Control group. Weak immunoreactivity in the CA1, CA3, and DG regions, suggests a decrease in neurotrophic support due to lead and MSG-induced neurotoxicity. Fewer BDNF-positive

neurons and those that are positive show diminished staining intensity. Widespread neuronal damage and reduced BDNF levels are indicative of impaired neuronal survival and synaptic plasticity, which correlates with the neurotoxic effects of lead and MSG in Groups III and V (Figure 2: Group III & V). However, In Eugenol + Lead and Eugenol + MSG (Treatment Group) showed that, moderate restoration of BDNF expression compared to the lead and MSG neurotoxic group. Increased BDNF immunoreactivity was observed in the CA1 and CA3 regions, as well as the dentate gyrus. More BDNF-positive neurons with improved staining intensity, suggest that eugenol enhances and promotes neuronal survival and plasticity. Although BDNF expression does not fully return to control levels, the partial recovery of BDNF-positive neurons indicates that eugenol has a neuroprotective effect, mitigating the neurotoxicity induced by lead and MSG (Figure 2: Group-IV & VI). Healthy, well-defined BDNF-positive neurons, propose that eugenol may support normal neurotrophic function without inducing neurotoxicity. The immunostaining intensity on rats treated with Eugenol was much better than the rats treated with the standard drug Memantine upon lead and MSG-induced neurotoxicity (Figure 2: Group-VII & VIII).

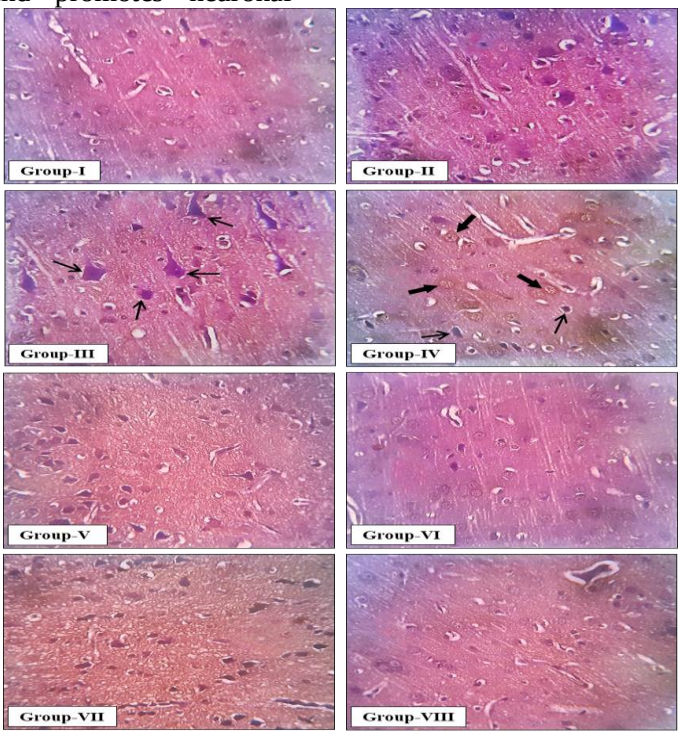

Figure 2. Photomicrograph Showing Immunohistochemistry Localization of BDNF in Hippocampus of Brain in Control and Experimental Groups at 20X Magnification. Group-I: Control (Normal Saline); Group-II: Drug Control (Eugenol); Group-III: Lead Acetate (Pb); Group-IV: Lead Acetate + (Eugenol); Group-V: Monosodium Glutamate (MSG); Group-VI: Monosodium Glutamate + (Eugenol); Group-VII: Lead Acetate + Memantine; Group-VIII: Monosodium Glutamate + Memantine. Thick Arrows indicating BDNF Positive Neurons with Dark Brown Coloration. Thin Arrows Indicating BDNF Negative Neurons with Purple Coloration. BDNF Positive Neuronal Expression is up-Regulated in Group-IV: Lead Acetate + (Eugenol)

Efficacy of Eugenol on BDNF Gene Expression in Hippocampus

In the Control Group, normal expression of the BDNF gene, with stable mRNA levels was detected in the hippocampal region. Baseline BDNF expression was consistent with healthy hippocampal function and proper neuronal survival, synaptic plasticity, and neurogenesis.

Stable BDNF mRNA levels indicate that eugenol does not alter the gene expression of BDNF under normal conditions. The lack of significant changes in BDNF expression suggests that eugenol alone maintains normal neurotrophic gene activity without causing neurotoxic or excessive stimulatory effects. Lead and MSG neurotoxicity Group results showed that, significant downregulation of BDNF gene expression compared to the control group as shown in Figure 3 ($P < 0.01$). Decreased BDNF mRNA levels in the hippocampus demonstrated a disabled neurotrophic flagging pathway. The suppression of BDNF expression correlates with lead and MSG-induced neurotoxicity, reflecting a disruption in neuronal survival, synaptic plasticity, and neurogenesis. In Eugenol + Lead and Eugenol + MSG-Treatment Group, partial restoration of BDNF gene expression compared to the lead and MSG neurotoxic group. Significant upregulation of BDNF mRNA levels in the hippocampus, though not fully reaching the levels of the Control group ($P < 0.01$). The increase in BDNF gene expression suggests that eugenol has a neuroprotective effect, promoting the recovery of neurotrophic signalling that was impaired by lead and MSG toxicity (Figure 3).

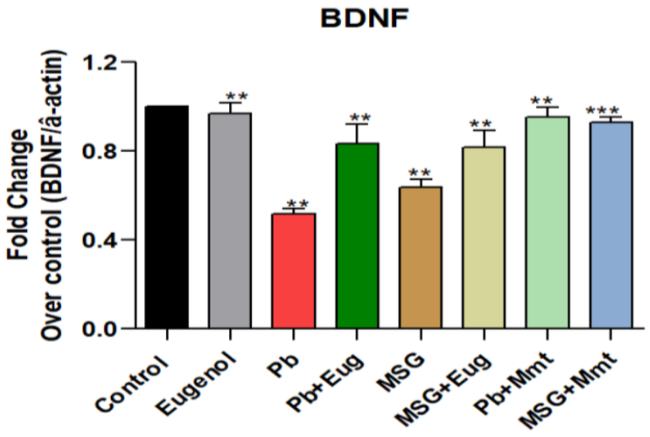

Figure 3. Efficacy of Eugenol on Lead and MSG induced Neurotoxicity on mRNA Expression of BDNF in Hippocampal Tissues of Rats. The X-axis represents the Animal Grouping and the Y-axis represents the BDNF mRNA Expression Levels in the Fold Change Over the Control Group. The Mean ± standard Deviations are used to Represent the Data. One-way ANOVA and Tukey's Post Hoc Test for Multiple Comparisons were used to Evaluate the Data. Significant Differences from the control Group are Denoted as * - $P < 0.05$, ** - $P < 0.01$ and *** - $P < 0.001$.

Discussion

The hippocampus is a critical brain region involved in literacy, memory, and cognitive function. It's particularly vulnerable to neurotoxic cuts, similar to exposure to lead and monosodium glutamate, both of which can lead to neuronal damage and cognitive poverties. Recent studies suggest that eugenol, a natural emulsion set up in clove oil, may have neuroprotective parcels due to its antioxidant, anti-inflammatory, and neuroprotective goods [15, 16]. Lead and MSG are well-proven neurotoxic agents. Lead exposure disrupts synaptic transmission, impairs neurogenesis, and induces oxidative stress, which results in neuronal apoptosis and brain atrophy, particularly in the hippocampus [17]. In H&E stained sections of the hippocampus from lead-exposed rats, the histopathological changes include loss of neurons, pyknotic nuclei, vacuolization, and overall neuronal loss. These differences are reflective of necrosis and degeneration of hippocampal neurons, especially in the CA1 and CA3 regions, which are responsible for memory conformation and spatial literacy.

MSG, extensively used as a flavour enhancer, has been shown to induce excitotoxicity in the hippocampus by over-activating glutamate receptors, resulting in neuronal damage. Histopathological evaluation of hippocampal sections exposed to MSG reveals signs of neuronal lump, nuclear fragmentation, and cytoplasmic vacuolation [18]. This excitotoxic damage, analogous to lead exposure, leads to disruption in hippocampal function and is associated with cognitive impairment.

Eugenol has been considerably studied for its antioxidative and anti-inflammatory properties making it a seeker for guarding neurons from poison- convinced damage. In histopathological studies, eugenol treatment has been shown to alleviate lead and MSG convinced damage in the hippocampus by reducing oxidative stress and inhibiting inflammation [19]. In H&E-stained sections of eugenol-treated creatures, the hippocampal structure appears saved, with lower neuronal degeneration, reduced vacuolization, and smaller pyknotic nuclei, compared to the rats exposed to lead or MSG. In contrast, BDNF expression can be significantly altered in various pathological conditions, including neurodegenerative diseases like Alzheimer's disease. Several studies have demonstrated that in Alzheimer's disease, BDNF levels may be reduced in regions like the hippocampus and cortex, contributing to the progressive neurodegeneration observed in these patients [20]. The altered expression pattern in these conditions often correlates with the extent of neural damage and cognitive decline, reflecting BDNF's vital role in maintaining neural health and functionality. Reduced BDNF staining in Alzheimer's patients highlights the neurotrophic factor's potential as a therapeutic target for slowing disease progression [21]. Exposure to lead and MSG has been well-documented to cause neurotoxic effects. Lead disrupts neuronal function by increasing oxidative stress, inducing neuroinflammation, and impairing neurotransmission. Its impact on neurogenesis includes the downregulation of critical neurotrophic factors like BDNF. Similarly, MSG, when consumed in excessive amounts, has been shown to exert excitotoxic effects, leading to neuronal damage and oxidative stress, ultimately downregulating BDNF expression. Both toxins contribute to cognitive dysfunction, memory deficits, and behavioural abnormalities, often correlating with reduced levels of BDNF in brain regions like the hippocampus and cortex. BDNF plays a vital role in maintaining neuronal plasticity and survival, regulating synaptic function, and protecting against neurodegenerative processes [22]. Decreased BDNF articulation in the cerebrum is a sign of a few neurodegenerative and mental problems. In the context of lead and MSG toxicity a downregulation of BDNF impairs the brain's ability to recover from toxic insults, exacerbating neurodegeneration [23]. Eugenol has demonstrated potent neuroprotective effects, primarily attributed to its ability to modulate oxidative stress, reduce inflammation, and maintain synaptic integrity. In the present study, eugenol treatment in rats exposed to lead and MSG resulted in a significant upregulation of BDNF expression compared to the untreated, toxin-exposed groups.

Immunohistochemistry (IHC) is used to detect specific antigens (proteins) in tissue sections with antibodies. Brain-derived neurotrophic Factor (BDNF) is a key player in the development, maintenance, and plasticity of neurons. It plays a critical role in synaptic modulation, neurogenesis, and cell survival, making it a valuable marker in neurodegenerative, psychiatric, and neurological disorders [24]. Immunohistochemical analysis allows the precise visualization of BDNF expression in tissue samples, providing insights into the spatial distribution and localization of neurotrophin within different brain regions. The decreased staining intensity of Group-III

was due to the continuous toxic actions of the free radicals generated by lead and MSG increasing lipid peroxidation and subsequent damage to the cells via inflammation-mediated apoptosis. But the Lead and MSG rats upon treatment with Eugenol showed improved cellular survival and recovery from neuronal damage as it has the modulatory effect on the AMPK/PI3K/p-AKT/mTOR (Adenosine Monophosphate-activated Protein Kinase /Phosphoinositide 3-Kinase / Phosphor-Protein Kinase B / Mammalian Target of Rapamycin) signalling pathway for cell growth, cell proliferation and cell survival upon oxidative stress conditions which was in concurrence with the present study [25].

Gene expression analysis of BDNF is a powerful tool to understand the transcriptional regulation of this neurotrophin in both normal and disease states. The combined use of RNA extraction, qPCR and validation techniques provides a comprehensive approach to studying BDNF's role in neurobiology and its potential as a therapeutic target. Gene expression analysis of BDNF provides crucial insights into its regulation in response to environmental factors (e.g., stress, exercise), and its role in neurodegenerative diseases like Alzheimer's and Parkinson's [26]. Reduced BDNF expression is linked to impaired synaptic plasticity and cognitive decline in Alzheimer's disease and other neurodegenerative conditions [27]. In psychiatric disorders, altered BDNF levels have been observed in depression and schizophrenia, influencing therapeutic interventions [28]. The restorative property of mRNA expression levels in Lead treated with Eugenol group and MSG treated with Eugenol group is attributed to the anti-inflammatory property of Eugenol via inhibition of NF-κB, TNF-α and IL-1β inflammation gene expression levels [29], antioxidant activity of Eugenol through decrease in ROS generation with increased production of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase levels [30] and BDNF gene modulating property for cell proliferation, survival and repair. In the adult brain, BDNF's binding to its receptor promotes neuronal survival and controls excitatory and inhibitory synaptic transmission as well as activity-dependent plasticity [31]. The "neurotrophin hypothesis of depression" is mostly supported by data showing that antidepressant therapy increases BDNF expression and that stress-induced depressed behaviours are associated with lower hippocampus BDNF levels [32]. Eugenol's hydrophobic characteristic enables it to effectively cross the blood-brain barrier, enter the brain, and carry out its function in vivo. Eugenol protects neurons against oxidative and excitotoxic damage caused by N-methyl-D-aspartate (NDMA). Due to its ability to lower brain-derived neurotrophic factor (BDNF) and delay amyloid-β peptide (A-β)-induced cell death by abnormally blocking Ca2+ (a result of A-β), eugenol exhibits neuroprotective potential on hippocampus tissues [33, 34].

The neuroprotective effect of eugenol against lead acetate was discovered in the current investigation. Previous research has also shown that eugenol has a neuroprotective effect against a variety of other neurotoxic substances at varying dosages. It has been observed that eugenol lessens the hippocampal-dependent deficit in neuronal damage and gene expression brought on by eugenol usage. Because of its water-repellent, antioxidant, anti-apoptotic, and neurotrophic qualities, eugenol protects rats' brains from lead-induced neurotoxicity. In recent research, eugenol's ability to scavenge free radicals and act as an antioxidant has been linked to its therapeutic impact in alleviating neurodegeneration and promoting BDNF mRNA expression brought on by lead and MSG-induced brain damage in rats.

Conclusion

In conclusion, the histopathological analysis of the hippocampus, BDNF protein expression

in immunohistochemistry and gene expression analysis of the BDNF gene, provide a clear visualization of the cellular damage induced by lead and MSG neurotoxicity and subsequent ameliorated action Eugenol upon treatment. Both neurotoxic substances cause huge neuronal degeneration through the process of putrefaction, vacuolization and neuronal pyknosis. The antioxidative and anti-inflammatory properties of eugenol make it a promising therapeutic candidate for mitigating lead and MSG-induced neurotoxic damage in the hippocampus of rats. More detailed signalling pathways underlying the interaction of eugenol with cellular metabolism concerning the brain are still not fully understood and further future investigations are fully warranted.

Disclosures

Human Subjects

All authors have confirmed that this study did not involve human participants or tissue.

Animal Subjects

The Institutional Animal Ethics Committee of Saveetha Dental College and Hospitals Issued protocol number (BRULAC/SDCH/SIMATS/IAEC/12-2019/042).

Payment/Services Info

All authors have declared that no financial support was received from any organization for the submitted work.

Financial Relationships

All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.

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Conflicts of Interest

All the authors have no conflict of interest to declare.

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