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# EUGENOL RESTORES COGNITIVE FUNCTION AND NEUROTRANSMITTER BALANCE IN WISTAR RATS WITH LEAD-INDUCED NEUROTOXICITY

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## ABSTRACT

***Exposure to lead continues to pose a neurological risk due to its capacity to disturb neuronal signalling and behaviour. Alterations in monoamine neurotransmitters are believed to contribute to these functional deficits. Botanical antioxidants such as Phyllanthus emblica (amla) and eugenol demonstrate biological properties that may alleviate oxidative and neurochemical disturbances. This study examined the effects on behavioural performance and brain monoamine concentrations in rats subjected to prolonged lead exposure. Twenty-five adult male Wistar rats were randomly assigned into five groups (n=5): control, lead-exposed (30 mg/kg), and lead-treated groups receiving amla extract (500 mg/kg), eugenol (6 mg/kg), or vitamin C (2 g/kg) for 90 days. Behavioural assessments were conducted using standard cognitive and motor tests. Brain monoamines (dopamine, serotonin, and norepinephrine) were quantified by ELISA, and blood lead levels were measured using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). Lead exposure resulted in significant impairments in locomotion, spatial memory, motor coordination, and grip strength, along with reduced monoamine levels (dopamine, serotonin, and norepinephrine). Eugenol-treated animals showed significant improvement in behavioural performance and restoration of monoamine neurotransmitter levels, compared with the untreated lead-exposed group ($p < 0.05$ to $p < 0.001$). The results suggest a neuroprotective effect of eugenol against lead-induced neurotoxicity by restoration of neurotransmitter balance and behavioural function.***

**KEYWORDS:** Lead Neurotoxicity; Eugenol; Phyllanthus Emblica; Monoamine Imbalance; Cognitive Dysfunction; Wistar Rats.

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## 1. INTRODUCTION

Lead (Pb) is a pervasive environmental toxicant with no known physiological role in the human body and is capable of inducing multisystem toxicity even at low exposure levels [1,2]. Chronic exposure to lead continues to pose a substantial toxicological burden despite progressive regulatory control measures. Environmental persistence of this metal sustains human and animal exposure through industrial residues, informal battery recycling, contaminated water systems, deteriorating surface coatings, and dietary accumulation [3–6]. Following systemic absorption, lead distributes widely and gains access to neural tissue by breaching the blood–brain barrier. The brain is particularly vulnerable to toxic injury because of its high metabolic activity, abundant lipid content, and limited ability to regenerate damaged neurons. Prolonged exposure to toxic agents has been associated with impairment in memory, psychomotor speed, and behavioural control [3–6].

At the cellular level, lead exerts complex and overlapping harmful effects. A key mechanism involves disrupting calcium-dependent physiological pathways necessary for normal neuronal signalling, neurotransmitter release, and synaptic function. Lead competes with calcium at neuronal binding sites, disrupting intracellular signalling pathways crucial for synaptic transmission [7–10]. This disruption impairs the release of neurotransmitters, weakens synaptic ability, and alters communication across the neural network. At the same time, excessive generation of reactive oxygen species (ROS) leads to oxidative damage to lipids, proteins, and nucleic acids by overwhelming the body's own antioxidant defences [11–13]. Mitochondrial impairment, altered gene expression, and activation of apoptotic pathways contribute to progressive neuronal loss [14–16].

An imbalance of neurochemicals is another important part of toxicity. Experiments show that long-term exposure to lead affects the cholinergic and monoaminergic systems, including the pathways that use norepinephrine, dopamine, and serotonin. Such neurochemical alterations are strongly linked to deficits in memory, learning ability, emotional regulation, and coordinated motor function. The cumulative effects of oxidative damage, altered intracellular signalling, and disturbances in neurotransmitter systems contribute to the complex pathophysiology associated with lead-induced neurobehavioural deficits. Chelation therapy reduces systemic lead burden; however, it does not consistently restore neuronal function and may cause negative effects. Consequently, there is increasing interest in natural agents that can attenuate oxidative stress and restore neurochemical homeostasis in the brain [17–20].

Eugenol is a naturally occurring phenolic constituent extracted from the clove (*Syzygium aromaticum*), exhibits potential antioxidant and anti-inflammatory properties through hydrogen donation and free radical scavenging [21–24]. These properties highlight its potential as a neuroprotective agent, warranting further investigation in experimental models of neurotoxicity.

## 2. MATERIALS AND PROCEDURES

### *1.1 Ethical Approval and Study Design*

The study aimed to determine whether eugenol could ameliorate chronic lead-induced alterations in the behaviour and brain chemistry of Wistar rats. All procedures were approved by the Institutional Animal Ethics Committee (136/IAEC/2020) and conducted according to CPCSEA guidelines and AVMA euthanasia standards, and followed OECD Test Guideline 408 for repeated 90-day oral toxicity studies in rodents [25-27].

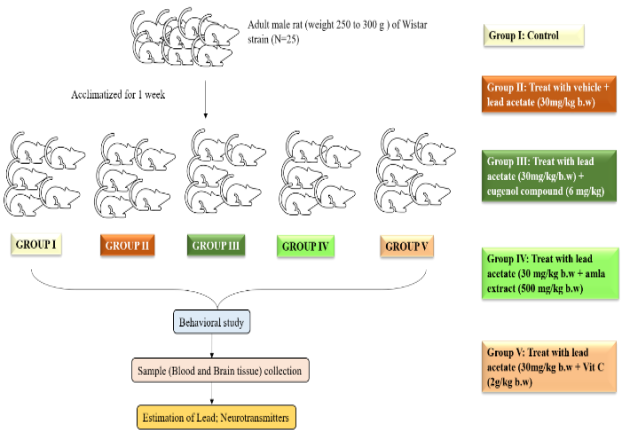


***Figure 1. Study Design***

### *1.2 Animals and Housing Conditions*

This study used 25 adult male Wistar rats (180 - 200 g). The animals were housed in polypropylene cages. Every day, there were 12 hours of light and 12 hours of dark. The temperature stayed between 22 ± 2°C. The humidity stayed between 50% and 60%. Rats were fed a pellet diet and clean drinking water and acclimatised for one week before the experiments.

After the animals had adjusted to their new environment, they were randomly assigned to five experimental groups, each comprising five rats. All treatments were delivered orally once daily for a duration of 90 days using an appropriately sized gavage needle to maintain precise dosing [28]. Lead

acetate administration and dosing procedures were performed according to standardised toxicological protocols [1, 19].

**Group I (Control):** Administered distilled water only.

**Group II (Lead-treated):** Received lead acetate at a dose of 30 mg/kg body weight per day.

**Group III (Lead + Ascorbic Acid):** Treated with lead acetate (30 mg/kg/day) in combination with ascorbic acid (2 g/kg/day).

**Group IV (Lead + Amla Extract):** Treated with lead acetate (30 mg/kg/day) and *Emblica officinalis* extract at 500 mg/kg/day.

**Group V (Lead + Eugenol):** Received lead acetate (30 mg/kg/day) along with eugenol at 6 mg/kg/day.

### *1.3 Assessment of Behavior*

All of the behavioural tests were done in a controlled and calm setting during the light phase. To reduce overall stress, there was at least 24 hours between each test. To avoid olfactory bias, the surfaces of the equipment were cleaned with 70% to 90% ethanol between animals. Three readings were taken for each parameter for each animal and then averaged.

### *1.4 Spatial Learning and Memory*

The Morris water maze paradigm was utilised to evaluate spatial cognition [28, 29]. A circular opaque water pool was used, and the escape platform was 1 cm below the water's surface. For four days in a row, rats were trained to locate the hidden platform. Escape latency, swim speed and the swim path were recorded. A probe trial was conducted 24 hours after the last training session to assess memory performance by measuring how long the subject stayed in the target quadrant after the platform was removed.

### *1.5 Behaviours Related to Anxiety*

The elevated plus maze was used to measure anxiety-like responses [30]. Each rat was given five minutes to adapt to the maze. Time spent in open arms and transfer latency were recorded as behavioural indices.

### *1.6 Spontaneous Movement*

Spontaneous movement was quantified using an actophotometer. After each animal was put in the device for 5 minutes, the total number of beam interruptions was counted as locomotor counts.

### *1.7 Balance and Coordination*

A rotating rod apparatus was employed to assess the effectiveness of the nervous and muscular systems in unison. The amount of time the animal stayed on the spinning rod (latency to fall) was recorded. There were three trials for each rat, with enough time in between for them to rest and not get tired.

### *1.8 Grip Strength of the muscles in the forelimbs*

Forelimb grip strength was assessed with the aid of a digital grip strength meter. Three consecutive measurements were taken with brief rest periods, and the average value was used for analysis.

### *1.9 Collecting Samples and Processing Tissues*

After 90-days of treatment period, animals were starved overnight (12 hours). Anaesthesia was induced via intraperitoneal administration of ketamine (75–90 mg/kg) in combination with xylazine (5–10 mg/kg). Depth of anaesthesia was confirmed by the lack of both pedal withdrawal and corneal reflex responses.

Blood samples were collected via cardiac puncture into EDTA vials. Guidelines of the American Veterinary Medical Association (AVMA) were followed during procedure. The brain was isolated, washed with cold phosphate-buffered saline (PBS), and dissected into cerebrum and cerebellum. Tissues were preserved at −80°C in acid-cleaned polypropylene containers for further biochemical analysis.

### *1.10 Quantification of Lead*

Lead concentrations in blood and brain tissues were quantified by inductively coupled plasma–mass spectrometry (ICP-MS). The samples were fully mineralised before being measured by being digested in acid with analytical-grade nitric acid and hydrogen peroxide. Calibration solutions and reagent blanks were prepared and analyzed in parallel to maintain accuracy and reliability of the measurements.

### *1.11 Analysis of Neurochemicals*

Brain concentrations of dopamine, serotonin, and norepinephrine were measured using ELISA kits according to manufacturer protocols. ELISA-based quantification was selected based on kit sensitivity and validated protocols.

### *1.12 Statistical Analysis*

All experimental data were compiled and analysed after completion of the study. Quantitative results are presented as mean ± standard deviation (SD). Statistical comparisons among multiple experimental groups were performed using one-way analysis of variance (ANOVA) to evaluate overall group differences. When a statistically significant F-

value was obtained, Tukey's post hoc multiple comparison test was applied to determine pairwise intergroup differences. The F-statistic and corresponding p-values were calculated and reported where appropriate. A two-tailed p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 5.1 (GraphPad Software Inc., San Diego, CA, USA).

All behavioural recordings and biochemical assays were conducted in triplicate to enhance reliability and reduce experimental variability.

## 3. RESULTS

### *Lead Estimation*

Table 1 demonstrates a significant elevation of lead levels in both blood and brain tissues following chronic exposure.

Blood lead concentration increased from 4.03 ± 0.64 µg/dL in controls to 22.48 ± 0.26 µg/dL in the lead-exposed group ($F(4,20) = 7.65$, $p < 0.001$). Post hoc analysis confirmed a significant difference between control and lead groups ($p = 0.0006$). Treatment with eugenol significantly reduced blood lead levels to 10.47 ± 0.60 µg/dL ($p = 0.0295$ vs lead). In contrast, amla (19.00 ± 0.13 µg/dL; $p = 0.8777$) and vitamin C (13.16 ± 0.09 µg/dL; $p = 0.1260$) did not show statistically significant reductions compared to the lead group.

A marked accumulation of lead was also observed in the cerebrum. Lead exposure increased cerebral levels from 2.21 ± 0.12 µg/g in controls to 138.80 ± 3.24 µg/g ($F(4,20) = 1042.0$, $p < 0.0001$). Eugenol treatment significantly reduced cerebral lead concentration to 28.12 ± 0.68 µg/g ($p < 0.0001$ vs lead). This reduction was significantly greater than that observed with amla (75.73 ± 3.88 µg/g) and vitamin C (70.02 ± 6.25 µg/g) ($p < 0.0001$ for both comparisons vs eugenol).

Similarly, cerebellar lead levels increased significantly following exposure, rising from 1.83 ± 0.04 µg/g in controls to 75.91 ± 1.55 µg/g ($F(4,20) = 536.9$, $p < 0.0001$). Eugenol reduced cerebellar lead concentration to 22.08 ± 2.88 µg/g ($p < 0.0001$ vs lead), demonstrating a stronger effect compared with amla (52.90 ± 1.40 µg/g) and vitamin C (46.17 ± 5.05 µg/g) ($p < 0.0001$ vs lead; $p < 0.0001$ vs eugenol). Amla showed a modest but statistically greater reduction than vitamin C ($p = 0.0078$). Collectively, eugenol produced the most pronounced reduction in systemic and neural lead accumulation among the tested interventions.

***Table 1. Lead Concentration in Blood and Brain Tissues of Wistar Rats (n = 5 per group)***

| Sample | Control | Lead | Lead + Eugenol | Lead + Amla | Lead + Vit C | ANOVA (F, p-value) |
|---|---|---|---|---|---|---|
| Blood (µg/dL) | 4.03 ± 0.64 | 22.48 ± 0.26 | 10.47 ± 0.60 | 19.0 ± 0.13 | 13.16 ± 0.09 | $F(4,20) = 7.65$, $p < 0.001$ |
| Cerebrum (µg/g) | 2.21 ± 0.12 | 138.8 ± 3.24 | 28.12 ± 0.68 | 75.73 ± 3.88 | 70.02 ± 6.25 | $F(4,20) = 1042.0$, $p < 0.0001$ |
| Cerebellum (µg/g) | 1.83 ± 0.04 | 75.91 ± 1.55 | 22.08 ± 2.88 | 52.9 ± 1.40 | 46.17 ± 5.05 | $F(4,20) = 536.9$, $p < 0.0001$ |

Values are expressed as Mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test.

### *Assessment of Behavior*

### *Locomotor Activity*

Lead exposure significantly reduced locomotor activity **(101.4 ± 7.43 movements/min)** compared with controls **(126.9 ± 9.65 movements/min; p < 0.0001)**. Amla treatment restored activity to levels comparable to controls *(p = 0.0016 vs lead)*. Eugenol showed partial improvement **(116.9 ± 9.65 movements/min)**, although this did not reach statistical significance *(p = 0.0775)*. Vitamin C did not produce a significant effect *(p = 0.8955)*, Figure 2(A).

### *Elevated Plus Maze*

Exploratory behaviour was significantly reduced in the lead group (71.4 ± 7.43 s) compared to controls (105.3 ± 8.27 s; $p < 0.0001$), Amla (98.93 ± 6.06 sec; $p = 0.0002$), and Vitamin C (99.07 ± 8.41 sec; $p = 0.0002$) significantly improved performance, Figure 2(B).

### *Strength of the Grip*

Lead significantly diminished grip strength (1785 ± 185.8 lb) in comparison to controls (3056 ± 289.7 lb; $p < 0.0001$). Eugenol brought grip strength back up to 3346 ± 204.8 lb ($p < 0.0001$ vs lead), which is higher than the control values. Amla (2718 ± 182.1 lb; $p < 0.0001$) and Vitamin C (2930 ± 289.7 lb; $p < 0.0001$) exhibited moderate enhancement. Eugenol was found to be more effective than Amla ($p = 0.0035$), Figure 2(C).

### *Rota Rod Performance*

Retention time was nearly halved in the lead group (206.9 ± 6.07 sec) compared to controls (423.3 ± 8.27 sec; $p < 0.0001$). All treatments improved performance. Eugenol (394.9 ± 6.07 sec; $p < 0.0001$ vs lead) showed the greatest recovery and significantly

outperformed Amla (299.9 ± 6.07 sec; $p < 0.0001$) and Vitamin C (344.9 ± 6.07 sec; $p < 0.0001$), Figure 2(D).

### *Morris Water Maze*

### *Speed of Swimming*

Lead exposure decreased swim speed (15.91 ± 0.36 cm/s) in contrast to controls (21.17 ± 0.37 cm/s; $p < 0.0001$). Eugenol brought values back to almost normal levels (20.14 ± 0.45 cm/s; $p < 0.0001$ vs lead), which was better than Amla (17.27 ± 0.31 cm/s; $p = 0.0002$) and Vitamin C (17.44 ± 0.45 cm/s; $p < 0.0001$), Figure 2(E).

### *Length of Path*

Rats treated with lead (526.7±41.38 cm) exhibited a markedly increased path length in contrast to controls (246.4±30.33 cm) ($p < 0.0001$). Eugenol (247.9±25.81cm) ($p < 0.0001$ vs lead), but Amla (473.6±67.87 cm), and Vitamin C (491.2±57.60 cm) continued to be ineffective compared to lead, Figure 2(F).

### *Time to Escape*

The lead group (78.61±6.176 s) had a significant increase in escape latency compared to the control group (37.00±3.852 s) ($p < 0.0001$). Eugenol (45.22±3.861 s) ($p < 0.0001$ vs lead) restored normal function, but the magnitude of improvement was less pronounced in Amla (74.56±3.564 s), and Vitamin C (76.46±6.055 s), Figure 2(G).

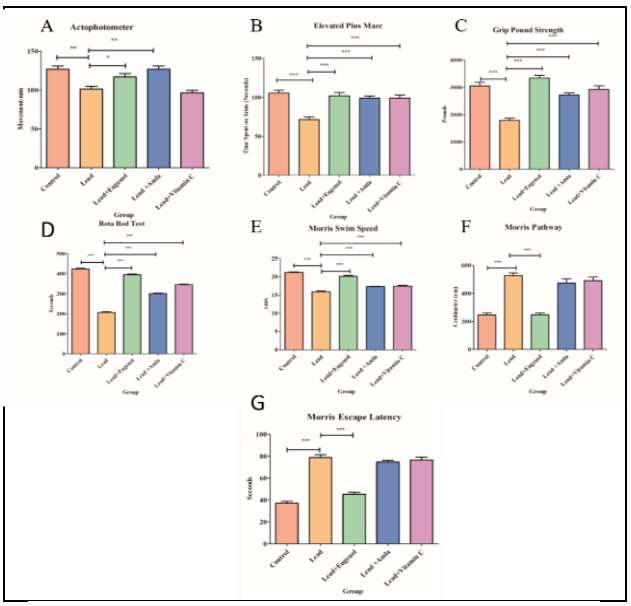


***Figure 2. Effect of lead exposure and antioxidant interventions on behavioural performance in Wistar rats (n = 5 per group). (A) Locomotor activity; (B) Elevated plus maze performance; (C) Grip strength; (D) Rotarod retention time; (E) Morris water maze swim speed; (F) Morris water maze path length; (G) Morris water maze escape latency***

Data are presented as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Symbols indicate statistical comparison versus the lead-treated group unless otherwise specified.

### *Neurotransmitter Analysis*

### *Norepinephrine*

In the cerebrum, norepinephrine levels were reduced in lead-treated rats (2649 ± 619.6 pg/mg) compared with controls (3646 ± 138.3 pg/mg); however, this difference did not reach statistical significance in post hoc comparisons despite overall ANOVA significance. In contrast, cerebellar norepinephrine levels were significantly decreased following lead exposure (1948 ± 589.0 vs 3399 ± 316.1 pg/mg; $F(4,20) = 10.14$, $p = 0.0001$). Treatment with eugenol significantly increased cerebellar norepinephrine levels (4340 ± 1156 pg/mg; $p < 0.0001$ vs lead). Amla (3114 ± 125.8 pg/mg; $p = 0.0433$) and vitamin C (3380 ± 115.9 pg/mg; $p = 0.0096$) also produced significant improvements, although to a lesser extent (Figure 3A).

### *Dopamine*

Cerebral dopamine levels were significantly reduced in the lead group (2010 ± 175.4 pg/mg) compared to controls (2787 ± 141.2 pg/mg; $p < 0.0001$). Eugenol significantly elevated dopamine levels (2965 ± 37.7 pg/mg; $p < 0.0001$ vs lead), with a markedly greater effect than amla (2314 ± 139.0 pg/mg; $p < 0.0001$) and vitamin C (2533 ± 157.4 pg/mg; $p = 0.0007$) (Figure 3B).

In the cerebellum, dopamine levels decreased from 2185 ± 98.7 pg/mg in controls to 1751 ± 203.5 pg/mg in lead-treated rats ($p < 0.0001$). Both eugenol (2405 ± 56.2 pg/mg; $p < 0.0001$ vs lead) and vitamin C (2355 ± 4.65 pg/mg; $p < 0.0001$) demonstrated substantial restoration, whereas amla showed a comparatively modest effect (1972 ± 117.0 pg/mg; $p = 0.0496$ vs lead) (Figure 3B).

### *Serotonin*

Lead exposure resulted in a marked reduction in cerebral serotonin levels (42.31 ± 1.76 ng/mg) compared to controls (73.49 ± 6.41 ng/mg; $p < 0.0001$). Eugenol significantly increased serotonin levels (85.35 ± 9.42 ng/mg; $p < 0.0001$ vs lead), exceeding control values. Vitamin C produced partial recovery (62.07 ± 3.72 ng/mg; $p = 0.0003$), whereas amla did not show a statistically significant effect (51.31 ± 4.87 ng/mg; $p = 0.1464$). Post hoc

analysis confirmed that eugenol was significantly more effective than both amla and vitamin C ($p < 0.0001$) (Figure 3C).

In the cerebellum, serotonin levels were significantly reduced following lead exposure (16.50 ± 0.60 vs 45.61 ± 0.70 ng/mg; $p < 0.0001$). Eugenol restored serotonin levels to 55.41 ± 3.02 ng/mg ($p < 0.0001$ vs lead), which was significantly higher than amla (25.23 ± 3.42 ng/mg) and vitamin C (34.60 ± 3.63 ng/mg) ($p < 0.0001$). Amla-treated animals exhibited significantly lower levels compared to vitamin C ($p = 0.0001$) (Figure 3C).

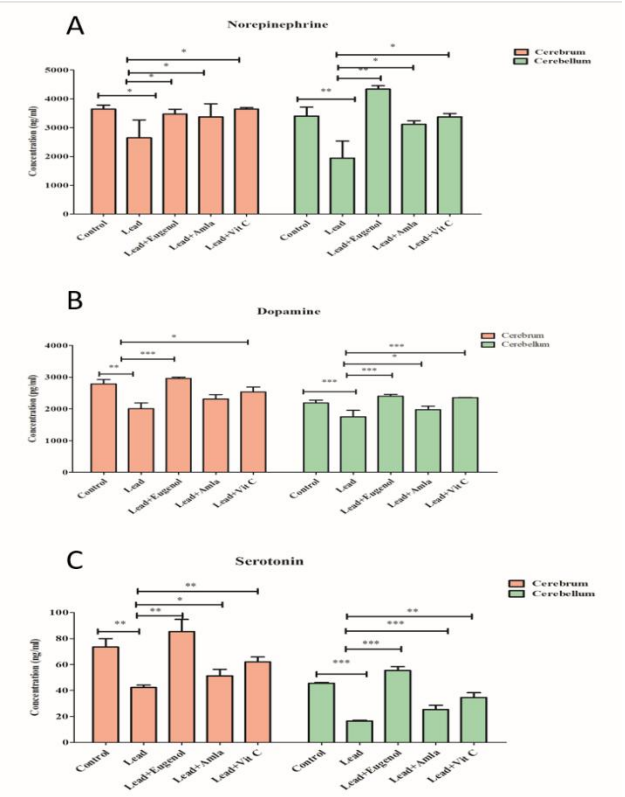


***Figure 3. Effect of lead exposure and antioxidant treatments on brain monoamine neurotransmitter levels in Wistar rats (n = 5 per group). (A) Norepinephrine (B) Dopamine (C) Serotonin***

Data are expressed as mean ± SD. Group differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Symbols represent statistically significant differences compared with the lead-exposed group unless otherwise specified.

## 4. DISCUSSION

These findings are consistent with previous reports demonstrating antioxidant-mediated restoration of monoaminergic systems under toxic stress. Lead exposure remains a major public health problem. Chronic exposure of lead causes impairments in cognitive functions, exploration, motor coordination and normal neurotransmitter release [3–6, 11–16]. Spatial learning deficits have been linked to alterations in N-methyl-D-aspartate (NMDA) receptor function and structural changes in hippocampal synapses [7–10]. Lead disrupts calcium-dependent signalling pathways, thereby impairing memory encoding and consolidation through oxidative damage to hippocampal circuits. Alterations in monoamine neurotransmitters, including dopamine, norepinephrine, and serotonin, are strongly associated with synaptic dysfunction and neuronal damage mediated by oxidative stress [11–13, 17–20]. The present study demonstrates that eugenol administration markedly attenuated the observed neurochemical and behavioural alterations. The enhancement of task performance, normalization of monoamine concentrations, indicates the restoration of synaptic integrity and functional neurotransmission. These findings are biologically possible, as phenolic compounds are widely documented to exhibit antioxidant and neuromodulatory effects [17–20]. The observed neuroprotective effects may be attributed to the antioxidant properties of eugenol, including reduction of oxidative stress, inhibition of lipid peroxidation, preservation of mitochondrial function, and the maintenance of redox homeostasis in cells [21–24].

This study employed a control and biologically uniform model of adult male Wistar rats to ensure unambiguous interpretation of behavioural and neurochemical results, thereby enhancing internal validity. The emphasis on validated behavioural evaluations and monoamine quantification establishes a robust functional framework for comprehending lead-induced neurotoxicity and therapeutic efficacy. The 90-day subchronic model provides a reproducible foundation for future investigations incorporating broader biomarker profiling and extended evaluation of sustained recovery.

## 5. CONCLUSION

Chronic lead exposure in Wistar rats produced substantial neurobehavioural deficits, evidenced by diminished locomotor activity, impairment in spatial learning and memory, and pronounced depletion of dopamine, serotonin, and norepinephrine in cerebrum and cerebellum tissues. These results indicate that monoaminergic systems are susceptible to neurotoxicity induced by lead, possibly through mechanisms involving oxidative stress and the

disruption of calcium dependent synaptic signaling. Eugenol exhibited the most consistent neuroprotective effect among the tested interventions, resulting in significant improvement in behavioural performance and restoration of monoamine levels toward control values. Vitamin C and *Phyllanthus emblica* (amla) showed partial benefit; their effects were comparatively modest. Overall, eugenol demonstrates potential as a neuroprotective adjunct in experimental models for mitigating lead-induced neurotoxicity. Further investigations are required to elucidate the molecular mechanisms that facilitate effects and enhance their translational relevance.

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