Protective role of eugenol on lead acetate induced neurotoxicity in wistar albino rats

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Type of Manuscript: Original Research

Running title: Protective role of eugenol on lead acetate induced neurotoxicity

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ABSTRACT

Lead acetate (PbAc) is a widespread form of global toxicity in addition to low level exposure of lead acetate induce neurotoxic and neurobehavioral changes. The current study was carried out to investigate the valuable properties of Eugenol in protecting the lead induced neurotoxicity in rats. Toxicity created by lead acetate in groups II, III, and IV rats. The toxic effect of lead is confirmed by using antioxidant assay, liver and renal functional test. Antioxidants enzymes are capable of improving free radicals before going to scavenge the cells. Additionally, PbAc administration significantly decreased the antioxidant enzymes like, GSH, GR, GPX, SOD& CAT and increased level of LPO. Moreover, PbAc induced neurotoxic which was evident that due to significant increase in the serum markers level of AST, ALT, ALP, total protein, albumin and bilirubin in liver. Urea and Creatinine in renal markers. Treatment with Eugenol at a dose of 250mg/kg b.wt, mixed with olive oil considerably shows good activity for all the enzymatic antioxidants. These outcomes indicate the neuroprotective activity and antioxidant of eugenol and it attests that it has a defensive role against the oxidative stress caused by lead acetate in rats.

Keywords: *Lead acetate, Eugenol, neurotoxic, antioxidant, serum markers, neuroprotective.*

INTRODUCTION

Lead Acetate (PbAc) is a severe environmental hazard that's still naturally available across the planet. Most people have been exposed to this metal in one way or another because of its pervasive usage in ecological contamination. In many nations today, it has emerged as a public health concern. It is present in food, water, and even in the air. It is mixed with a person for their daily activities. Lead is combined with paint and is also contained in cookware, furniture, and other household objects. It is also present in children's play materials and in a variety of other things. (1) It acts as a neurotoxic, the brain is one of the primary organs affected by lead poisoning, and the signs and indications range from severe neurological deficits to mild behavioral or psychological alterations. It interrupts normal neurological activity and causing damage to neural tissue, it can destroy or damage neurons, lead exposure can impair neurocognitive ability, and low-level lead exposure can cause "quiet" brain injury. High concentrations induce neuronal death (necrosis), which is the primary cause of neurodegenerative disorders. (2,3) Cells antioxidant responses may be compromised as a result of PbAc toxic activity on many enzymes, which leads the cells to be more exposed to oxidative stress. (4) Exposure to PbAc may have unwanted health issues like, neurological, respiratory, hematological, immunological, renal, kidney and reproductive illness (5)

Eugenol (4-allyl-2-methoxyphenol) is a fragrant chemical found in a variety of plants, including spices and medicinal herbs. Because eugenol is a component of the "major three spices," namely *Syzygium aromaticum* (clove), *Cinnamomum verum* (cinnamon), and *Myristica Fragrans* (nutmeg). Clove is a dried reddish brown flower bud of *Syzygium aromaticum* (Myrtaceae family). Eugenol is a bioactive chemical found in the traditional herbs like *Ocimum sanctum* Linn (Tulsi) and *Ocimum basilicum*, also known as holy basil in English. (6-8)

Eugenol, with the chemical formula $C_{10}H_{12}O_2$ and the molecular weight 164.21, is found mostly in clove oil, camphorated oil, cinnamon leaf oil, and nutmeg oil. In recent years, the anticancer activity of eugenol has gained prominence. Anticancer medicines made synthetically have various harmful adverse reactions and may eventually cause major damage to healthy neuronal cells in the nervous system (9) According to ancient Chinese pharmacopoeias, most herbal cures for Alzheimer's disease contain the plant *Rhizoma acori graminei* (RAG), which is high in eugenol. Clove buds have been utilized as an antiepileptic treatment in Iranian traditional medicine (10)

Eugenol exhibit its anti-depressive activity in a different manner from that of imipramine, which may provide an alternative treatment to patients who are resistant to typical antidepressant. pharmacological activities of eugenol like central and neuroprotective, anthelminthic & anti leishmanial activities with possible mechanism of action. (11) Eugenol is taken orally seems to penetrate the blood-brain-barrier to enter brain and acts *in situ*. It has been shown to protect neuronal cells from the cytotoxic effect (12) Mostly studies focused on the effectiveness of the eugenol as an analgesic, anticonvulsant, anti-inflammatory, antiseptic, antimicrobial and antifungal activity but neuroprotective and antioxidant role were not been examined against the lead induced toxicity. Objective of our research study was to evaluate the toxic level and damages in the organs of lead acetate, to assess the effect of eugenol on oxidative stress, Evaluate the protective efficacy of eugenol against lead acetate induced toxicity in wistar rats by measuring the status of lipid profile, renal markers, and antioxidants enzymes against lead acetate group.

MATERIALS & METHODS

Animal Model and Maintenance

An in-vivo evaluation of the experiment was done using an animal model in BRULAC, Saveetha Dental College and Hospitals. A Healthy mature male Wistar albino rat (*Rattus norvegicus*) was used in this study. A total of 24 rats, weighing about 150-200 gm was randomly divided in to 4 groups containing 6 animals per group. All animals were housed in proper cages in exact humidity (65 ± 5%) and warmth (25 ± 1°C) with regular 12-h daylight/dark cycle. They fed with regular rat diet and hygienic water was given *ad libitum*. The rats were acclimatized to the lab environment for a week prior to the initiation of experiments. Institutional Animal Ethics Committee (IAEC) approval was obtained for this investigation with the number: BRULAC/SDCH/SIMATS/IAEC/12-2019/042.

Chemicals

Eugenol, lead acetate and memantine were obtained from Sigma Chemical Company.

Grouping the animals:

Animals were grouped into 4, Group-1- Control (normal saline), Group-2- Lead acetate (toxic induction), Group-3- Lead acetate + eugenol (treatment group), Group-4- Lead acetate+ memantine treated (positive control)

Drugs and treatments:

LEAD ACETATE:

Lead toxic induction was induced to healthy adult wistar albino rats that received lead acetate at a dose of 100 mg/kg b.w by orally on a regular basis for 30 days. (13).

EUGENOL:

Eugenol mixed with olive oil treatment was given as a daily dose of 250 mg/kg b.w was administered orally via gastric intubation to rats for after 30 days of induction period for consecutive 15 days (14)

MEMANTINE:

Memantine treated as positive control, it was given to rats after induction period, orally at a dose of 20mg/kg in once in a day for 15 days (15)

Biochemical Studies:

At the end of the experimental period, blood samples were collected from the rats in the medial canthus of the eye through retro-orbital venous sinus puncture. Blood was subjected to centrifugation at 3000 rpm for10 min in order to enable serum separation. Renal function test like, urea, creatinine, uric acid and Liver function test like, total protein, albumin, total bilirubin, aspartate amino transferase (AST), alanine amino transferase (ALT), Alkaline phosphatase (ALP), were analyzed by using commercial kit method. After collecting the blood, the animals were euthanized by CO_2 inhalation in a CO_2 chamber. The brain tissue was dissected out from skull and the cerebrum alone was removed and processed for antioxidant analysis. Antioxidant level was estimated by Lipid peroxidation (LPO/MDA level), Superoxide dismutase (SOD), Catalase activity, Glutathione peroxidase, Glutathione reductase activity was analyzed by the ELISA kit method.

Statistical Analysis

The results were expressed as the Mean ± SE. The data obtained from this study were analyzed by using one-way analysis of variance (ANOVA) with multiple comparison through Student-Newman-keuls test. Statistical analysis and plotting of graphs were carried out using GraphPad Prism software version 7, the p value <0.05 was considered as statistically significant.

Results

As a result of our research work showed that lead acetate induced rat group had more elevation in the liver markers like., AST, ALT, ALP, Albumin, Protein and bilirubin level and renal markers like., Urea and Creatinine than compared with the control group. Though, the rats treated with lead and eugenol treated group showed a significant decrease in the liver markers and in renal markers (Table 1).

Figure 1, showed a result of biochemical serum marker glutathione level, when compared with control group, serum marker GSH level decreased in lead induced toxic group, but in eugenol treated group showed a significant rise. In figure 2 bar graph showed a Superoxide dismutase an antioxidant enzyme level is decreased in lead toxic group, however, it tremendously increased in eugenol treated as almost near the level of memantine treated positive control group. Likewise in figure 3, 4, 5 bar graph showed a Glutathione reductase, Catalase and Glutathione peroxidase level, these three intracellular antioxidant enzymes which levels are reduced, showed a reduce in the hydrogen peroxide to its harmful effect in lead toxic group, but when we treated eugenol group it showed a double fold increase in these antioxidant serum markers, these results proves that the antioxidant effect of eugenol against with lead toxic group. In figure 6, lipid peroxidation level significantly increased in lead toxic group when compared with control and eugenol treatment group. Our research findings revealed that eugenol has a protective role and proves an antioxidant activity against the lead acetate toxic group. As well as when eugenol treatment group compared with memantine as a positive control group showed almost equal and it seems higher protective role than the positive control group.

Table-1: Role of eugenol on renal function test and liver function test against lead induced toxicity in rats

PARAMETERS	GROUPS			
	Control	PbAc	PbAc + Eug	Memantine
LIVER MARKERS				
AST	64.05±0.70	67.81±0.87[a]	66.75±1.01[b]	65.36±0.68
ALT	48.75±1.11	54.88±1.04 [a]	52.36±0.55 [b]	49.4±0.89
ALP	170.8±0.89	175.6±1.35 [a]	172.8±1.19 [b]	171.2±1.36
ALBUMIN	2.85±0.06	2.99±0.04 [a]	2.95±0.05 [b]	2.88±0.04
TOTAL PROTEIN	6.89±0.05	6.95±0.11 [a]	6.91±0.06 [b]	6.90±0.07
BILRUBIN	0.423±0.01	0.455±0.01 [a]	0.432±0.01 [b]	0.443±0.20
RENAL MARKERS				
UREA	32.63±0.59	36.48±0.69 [a]	34.25±0.96 [b]	33.29±0.65
CREATININE	0.86±0.01	0.99±0.67 [a]	0.92±0.02 [b]	0.82±0.01

a denotes significantly difference from control group, b denotes significantly difference from lead group at $p < 0.05$ mean ± SE, (n = 6).

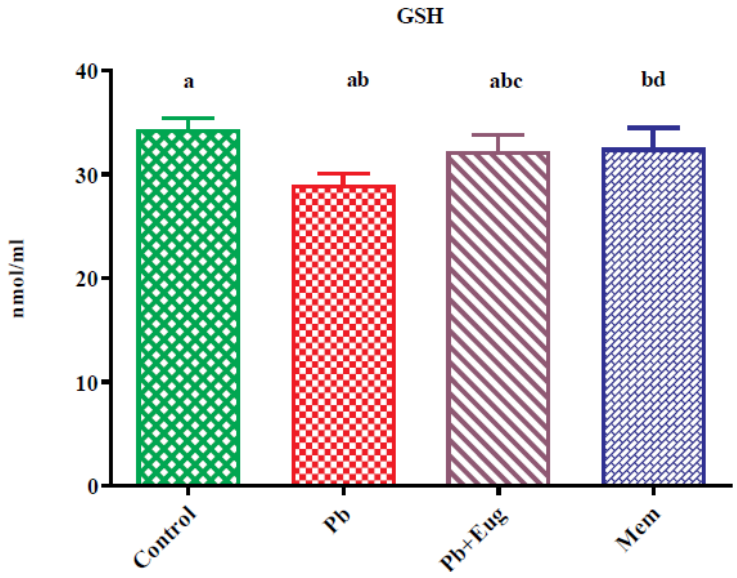

Figure 1: Bar graph shows the range of GSH in Control, Lead acetate, Lead acetate + eugenol treated, Lead + memantine group in rat serum. Bars carrying different letters (a, ab, abc, bd) are significantly different ($p < 0.05$) (mean ± SE, n = 6)

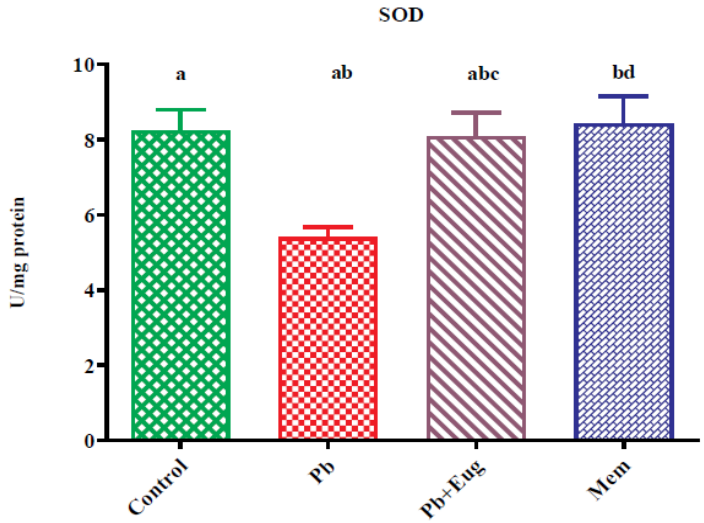

Figure 2: Bar graph shows the range of SOD in Control, Lead acetate, Lead acetate + eugenol treated, Lead + memantine group in rat serum. Bars carrying different letters (a, ab, abc, bd) are significantly different ($p < 0.05$) (mean ± SE, n = 6)

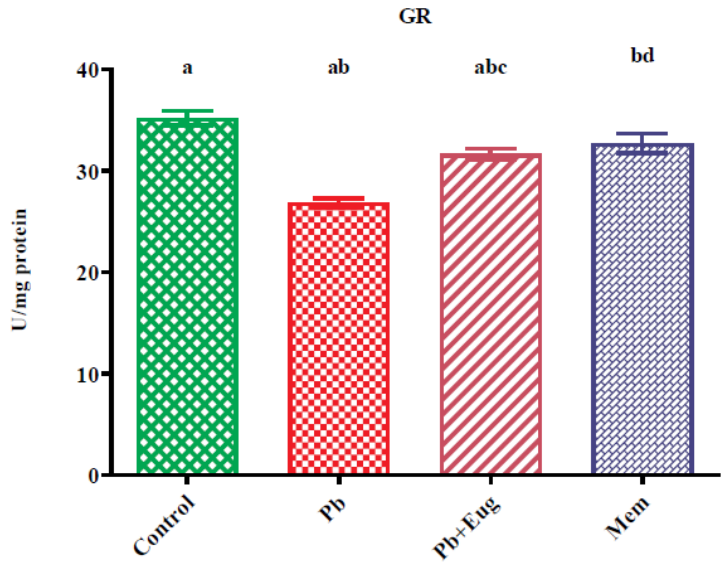

Figure 3: Bar graph shows the range of GR in Control, Lead acetate, Lead acetate + eugenol treated, Lead + memantine group in rat serum. Bars carrying different letters (a, ab, abc, bd) are significantly different ($p < 0.05$) (mean ± SE, n = 6)

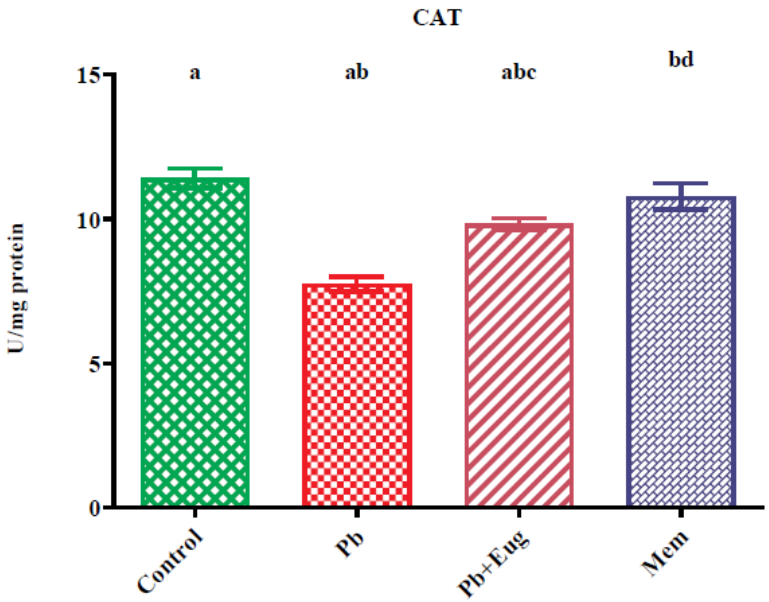

Figure 4: Bar graph shows the range of CAT in Control, Lead acetate, Lead acetate + eugenol treated, Lead + memantine group in rat serum. Bars carrying different letters (a, ab, abc, bd) are significantly different ($p < 0.05$) (mean ± SE, n = 6)

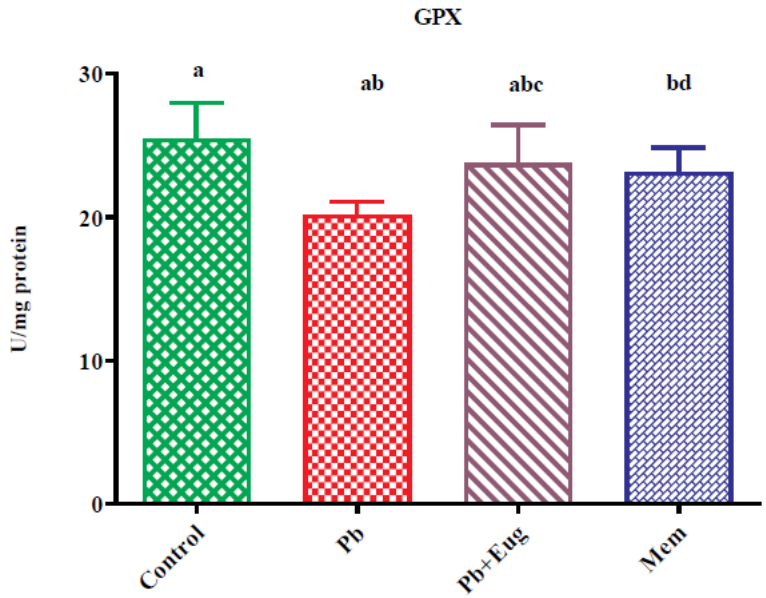

Figure 5: Bar graph shows the range of GPX in Control, Lead acetate, Lead acetate + eugenol treated, Lead + memantine group in rat serum. Bars carrying different letters (a, ab, abc, bd) are significantly different ($p < 0.05$) (mean ± SE, n = 6)

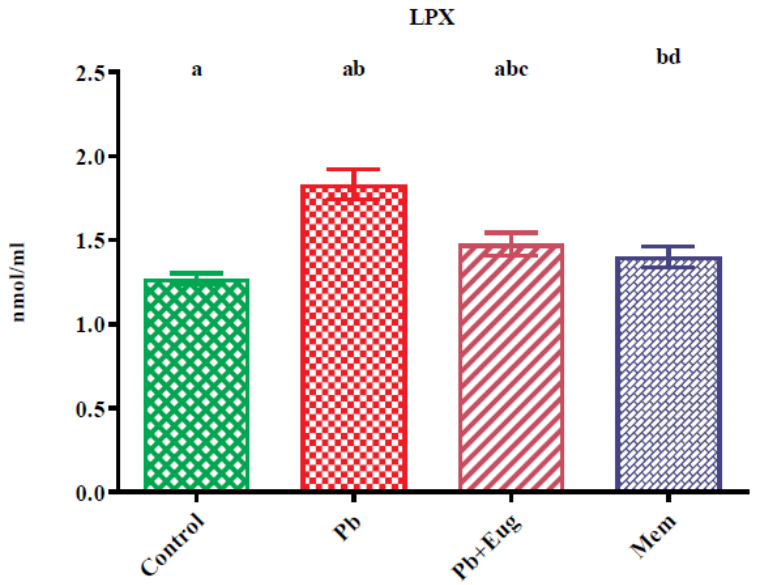

Figure 6: Bar graph shows the range of LPX in Control, Lead acetate, Lead acetate + eugenol treated, Lead + memantine group in rat serum. Bars carrying different letters (a, ab, abc, bd) are significantly different ($p < 0.05$) (mean ± SE, n = 6)

Discussion:

Lead acetate is a highly toxic metal due to its deadly potential to plants, animals, and the human population, as well as its widespread occurrence and dispersion. The barrier between the blood and the brain is another probable target for lead acetate's neurotoxic action (16, 17). One of the key mechanisms of lead toxicity is oxidative stress (18). Furthermore, oxidative stress has been linked to lead-related tissue damage in the brain, kidney, liver, and other organs (19). By joining sulfhydryl and nucleophilic functional compounds and causing oxidative stress, lead interferes with a variety of biological processes (20). Previous research revealed that administering lead acetate caused oxidative stress in the brain and other organs by increasing free radical, as seen by enhanced lipid peroxidation (21). The treatment of lead acetate to rats resulted in a significant increase in the activities of ALP, AST, and ALT, Albumin, Total protein as well as bilirubin levels in the current investigation. The alteration in liver function under lead acetate insult was studied. These events set off a chain reaction of cellular dysfunction, including disruption of cellular signaling and impairment of cytoskeletal integrity owing to protein hyperphosphorylation, which results in the leaking of liver enzymes that mark liver cells into the blood stream (22). But there is a significant decrease in the eugenol treated group, also there is a significant difference was found in the lead combined with memantine which was a positive control group.

Antioxidant assay results showed that, LPO levels increased statistically significantly in the lead acetate group compared to the control group, but decreased significantly ($p< 0.05$) in the lead acetate combined with eugenol group compared to the lead group only. High lead levels cause oxidative damage by increasing free radical generation while also reducing the cellular antioxidant defense system by lowering GSH levels. Inhibiting the activity of antioxidant enzymes and promoting lipid peroxidation (23). Furthermore, the oxidative stress caused by lead exposure revealed that antioxidant enzymes such as GR, GPX, GSH, SOD, and CAT values intensely decreased. However, a significant ($p< 0.05$) increase in the activation of these enzymes was observed with eugenol treatment group, implying that the free radical scavenging activity of eugenol may facilitate thiol status maintenance and up regulation of anti-oxidant enzymes, and eugenol treated group showed a significant rise in the level of LPO. These findings suggest that lead acetate-induced cognitive impairment is caused by a formation of oxidative stress in brain regions, which is mitigated by eugenol treatment. The results show that eugenol has the strongest antioxidant and elimination of free rad icals activity. (24) results also revealed that almost equal values and shows more effective than the positive control group.

Because of its important role in metal accumulation and excretion from the body, the kidney is especially vulnerable to the damaging effects of long-term lead exposure (25). Lead exposure was found to cause renal injury associated with renal markers in serum such as., urea and creatinine levels were significantly elevated in lead acetate treated rats compared to the control group. This impact might be related to a decrease in glomerular filtration rate caused by an increase in ROS generation caused by the synthesis of renal vasoconstriction mediators (26, 27). The current investigation found that whereas eugenol treated rats had lower levels of serum renal indicators, eugenol intoxicated animals had significant repair of oxidative stress and kidney enzyme markers in serum. This demonstrated eugenol's nephroprotective effect.

Conclusion:

In the present study, we had postulated that eugenol inhibited the lead- induced toxicity by decreasing oxidative stress, and decline in the liver and renal markers level. It is therefore assumed through the evidence provided that might treat in lowering the toxic heavy lead acetate accumulation in rats. Currently available drugs are expensive with several adverse effects. Eugenol a phenylpropanoid it is safe and may be used as a dietary supplement to make clear its protective potential against lead acetate induced toxicity in rats.

Acknowledgement:

I thank Saveetha Dental College, SIMATS for providing all research facilities and permission in carrying out this research study.

Conflict of interest:

The authors declare that they have no conflict of interest.

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