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ANTI-INFLAMMATORY ACTIVITY OF EUGENOL ON LEAD ACETATE INDUCED NEPHROTOXICITY IN ANIMAL MODEL

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Abstract

INTRODUCTION: The term "nephrotoxicity" describes a drug or agent's capacity to harm or poison the kidneys. The toxicants can damage the kidney tissue and leads to its dysfunction, causing the body system to have impaired function. Among them, heavy metals are considered more detrimental to humans. Lead is one among several available toxic heavy metals occurring naturally where human exposure is more. Eugenol is considered as a therapeutic agent in this study against lead induced nephrotoxicity. **AIM & OBJECTIVE:** The aim of this study is to assess the anti-inflammatory activity of eugenol on lead induced renal damage by histopathological analysis and deciphering its therapeutic efficacy through renal function tests. **MATERIALS & METHODS:** 18 adult male Wistar albino rats weighing 220 ± 20g are divided into 3 groups of 6 animals each. Group-I : Control, Group-II: Lead induction, Group-III: Lead induced + Eugenol treatment. Lead acetate was induced in rats at a dose of 0.15% aqueous solution of lead acetate in regular drinking water for 60 days. After this period of lead induction, eugenol was administered orally at a dose of 250 mg/kg body weight (Dissolved in olive oil) for 30 days. At the end of the experimental period the rats were anesthetized and blood was collected for biochemical analysis. Animals were then euthanized, kidney tissues were collected and fixed in 10% Neutral Buffered Formalin and processed for histopathological analysis. **RESULTS:** Eugenol demonstrated significant antioxidant activity leading to normalized renal function Histopathological analysis depicted significant glomerulus, proximal and distal convoluted tubule damage with renal steatosis. The anti-inflammatory and antioxidant potential of eugenol regulated lead induced renal toxicity. **CONCLUSIONS:** We conclude that eugenol could be used as a therapeutic drug on metal induced renal toxicity and even more scientific validation can be made to confirm its full potential and targeted action at cellular level.

Keywords: Metal Toxicity, Nephrotoxicity, Lead Acetate, Eugenol, Anti-Inflammatory.

INTRODUCTION

Nephrotoxicity is the rapid decline in kidney function brought on by the toxic effects of drugs and substances (1). Nephrotoxicity can cause rhabdomyolysis, thrombotic microangiopathy, inflammation, tubular cell toxicity, changes in glomerular hemodynamics, and nephropathy (2). Renal toxicity can be caused by several factors and the most common one is by heavy metals. Several heavy metals such as cadmium, lead, chromium, etc., were found to be the most prevalently occurring metals in the environment which are directly and indirectly associated to humans. Among these metals, lead acetate is a probable toxic pollutant and carcinogen to humans. There is some proof that inorganic lead compounds induce kidney cancer in animals and lung, brain, stomach, and kidney cancer in people. Because of its extreme toxicity, lead is one of the polluting metals that is harmful when exposed to the public

posing alarming health issues. These metallic elements are considered systemic toxicants because they have been shown to induce multiple organ damage even at low exposure levels (3). An earlier study demonstrated the detrimental effects of lead acetate on albino adult rats by lowering the hemoglobin and RBC counts of intoxicated animals' blood as well as the plasma levels of T3, T4, and blood WBCs (4).

Drug overdose, drug-drug interactions, and unfavorable drug effects are risk factors for drug-induced nephrotoxicity. Understanding the pathogenic processes of these medications' neurotoxicities is essential to reducing the prevalence of kidney damage because the use of some nephrotoxic pharmaceuticals is still unavoidable in the therapeutic situation..

Realistic strategies to prevent the last stage of renal failure include the early diagnosis of drug-induced nephrotoxicity and the decrease of therapeutic side effects (5). To explore renal cell damage, animal models may be used as early detection methods for food and drug-induced nephrotoxicity (6).

Medicine or other chemicals that lessens bodily inflammation, including pain, swelling, and redness are called anti-inflammatory drugs. Anti-inflammatory medications prevent some bodily chemicals from causing inflammation. The main active ingredient in *S. aromaticum*, is eugenol, has promising qualities that include anti-inflammatory, antioxidant, and anticancer effects. The primary source of eugenol (4-allyl-2-methoxyphenol), a musky oil, is cloves.

Because of its numerous benefits, including antibacterial, anticancer, anti-inflammatory, and antioxidant qualities, it has been used for a very long time all over the world (7). Additionally, eugenol is a very intriguing bioactive molecule with broad-spectrum antibacterial activity against planktonic and sessile cells of human diseases as well as food-decaying microorganisms (8). The antioxidant properties of many medicinal plants have been studied extensively by many research scientists and have proved their therapeutic roles in the development of a disease prognosis (9) (10).

Antioxidants are important for protecting against free radicals and have been shown, useful in the therapy of enormous diseases. They can also help reduce heavy metal toxicity, kidney damage, and more (11). Several authors have attempted to study the medicinal properties of plants and their antioxidant effect against tissue damage, free radical suppressing abilities (12)(13). Many active components and isolated natural compounds from herbs are expensive and may not be affordable by all the people (14). The aim of our research is to understand the antioxidant mediated nephroprotective effect of eugenol on lead acetate exposure in rat model.

MATERIALS AND METHODS

The animal experiment protocol was in compliance with national ethical guidelines of “The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). This study and the experimental protocol were authorized by the Institutional Animal Ethics Committee (IAEC) with approval number BRULAC/SDCH/SIMATS/IAEC/12-2019/042. In this study of animal model, 18 adult male Wistar albino rats weighing 220 ± 20g are divided into 3 groups of 6 animals each. Group-I : Control, Group-II: Lead induction, Group-III: Lead induced+Eugenol treatment. Lead acetate was induced in rats at a dose of 0.15% aqueous solution of lead acetate in regular drinking water for 60 days.

After 60 days of lead induction, Eugenol was administered orally at a dose of 250 mg/kg body weight (Dissolved in olive oil) for 30 days. At the end of the experimental period the rats were anesthetized using isoflurane and blood was collected, centrifuged and the serum was separated, which was used for biochemical analysis.

The animals were then euthanized using CO_2 then kidney tissues were collected and fixed in 10% Neutral Buffered Formalin and processed for histopathological analysis. Biochemical analyses were statistically interpreted using SPSS software version 23.0 considering ANOVA test for various analyses was performed followed by least significant difference test for multiple comparison to examine differences among the group.Statistical significance was defined with the value p values less than 0.05. Tukey's HSD post-hoc test was done for inter group differences.

RESULTS

The mean serum creatinine level in Group I was 0.842 ± 0.13 mg/dL, in Group II was 1.451 ± 0.24 mg/dL and in Group III was 1.176 ± 0.19 mg/dL (Figure 1). The mean serum urea level in Group I was 31.33 ± 2.94 mg/dL, in Group II was 48.62 ± 3.54 mg/dL and in Group III was 40.17 ± 4.52 mg/dL (Figure 2). The renal function test indicated serum creatinine and serum urea levels in Group II were high significantly when correlated with Group I ($p<0.05$), surprisingly reduced in Group III after eugenol treatment of 30 days.

The histopathological findings (Figure 3) in Group I depicted typical healthy glomeruli. These images showcase the normal functioning and structure of these vital kidney components, devoid of any evident abnormalities or irregularities. This is a valuable reference point for comparison when assessing other samples or groups with potential renal issues, as it establishes a clear baseline of what constitutes a healthy glomerulus.

The Group 2 image provides a compelling representation of glomerular damage caused by lead acetate-induced nephrotoxicity. These visuals illustrate the adverse effects of lead acetate on the delicate structures of the glomeruli, revealing a notable deterioration in their integrity and function. The presence of histological alterations and cellular damage in this group serves as a significant indication of the harmful impact of lead acetate exposure on renal health.

The Group 3 image depicts a remarkable improvement in glomerular health attributed to the administration of eugenol. These visuals highlight a notable restoration of glomerular structure and function, with a substantial reduction in histological abnormalities and cellular damage when compared to the previous group. The application of eugenol appears to have a beneficial effect on mitigating the damage caused by nephrotoxicity, leading to a visibly healthier state of the glomeruli.

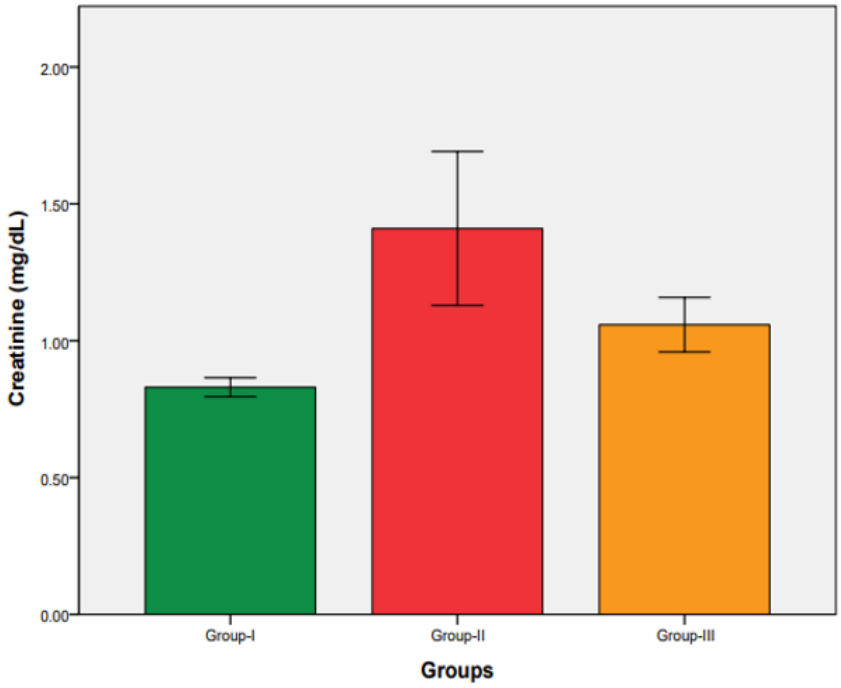

Figure 1: Bar Chart Shows the Level of Serum Creatinine in Control and Experimental Groups

The x-axis represents the animal grouping and Y-axis represents the creatinine level in mg/dL. All the values are expressed as Mean ± Standard Deviation. Between the groups, Group-III showed p= 0.013 ($p<0.05$) indicating statistically significant decrease in creatinine when compared to Group-II.

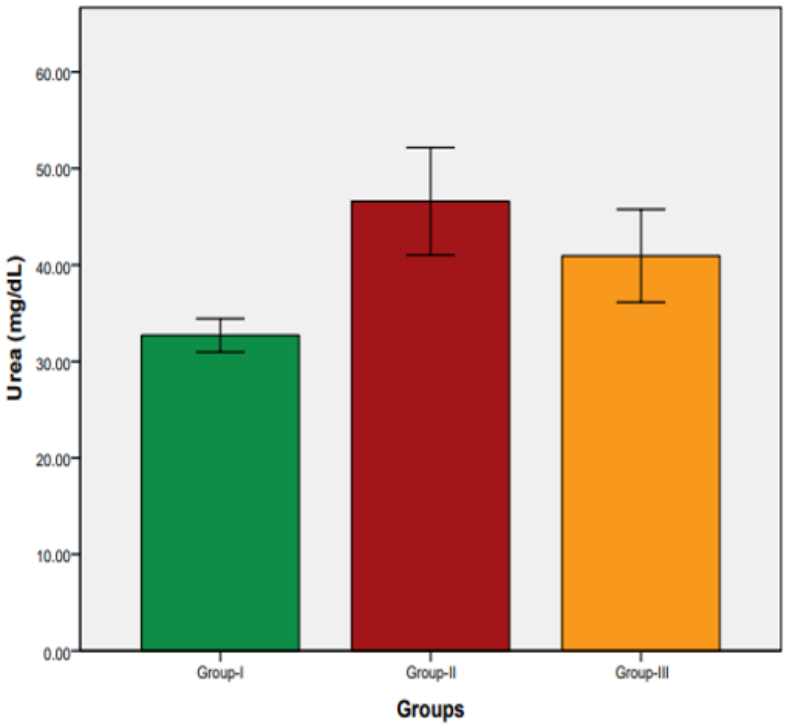

Figure 2: Bar Chart Shows the Level of Serum Urea in Control and Experimental Groups

The x-axis represents the animal grouping and Y-axis represents the urea level in mg/dL. All the values are expressed as Mean ± Standard Deviation. Between the groups, Group-III showed p= 0.041 ($p<0.05$) indicating statistically significant decrease in urea level when compared to Group-II.

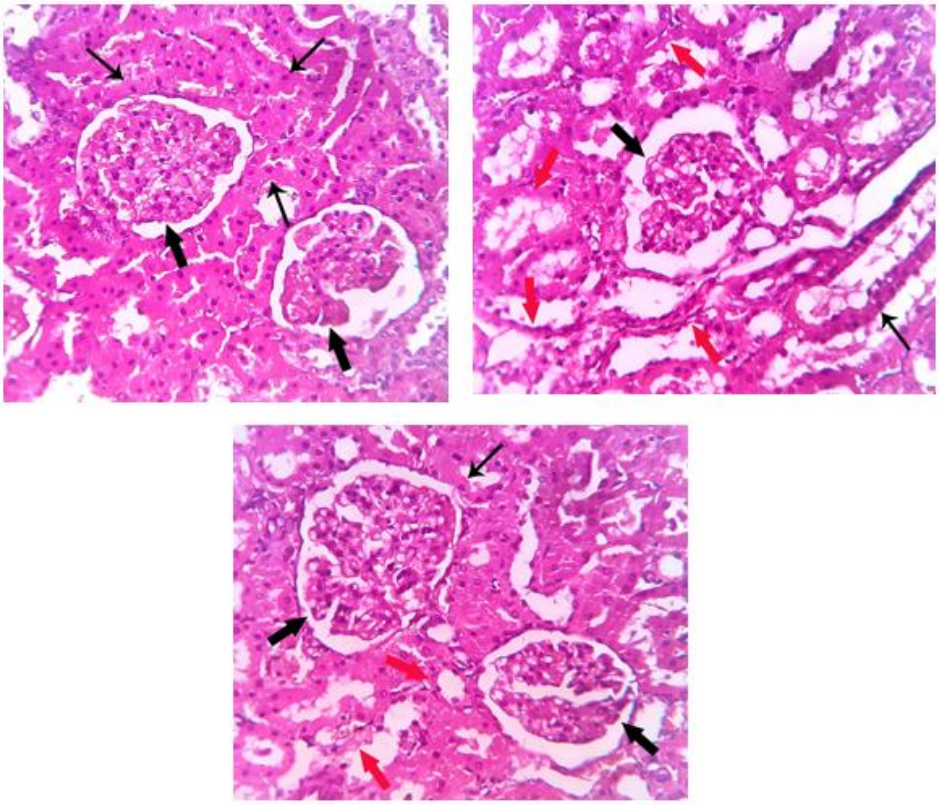

Figure 3: Histopathological Images of Kidneys Stained with Haematoxylin & Eosin in Group I (Control), Group II (Lead Acetate Induction) and Group III (Lead Acetate Induced + Eugenol Treatment) at 10X Magnification. Thin Arrow - Normal Tubules; Black Thick Arrow - Glomerulus; Red Thick Arrow - Damaged Tubules

DISCUSSION

According to the study, eugenol, a naturally occurring substance included in a variety of essential oils, has encouraging benefits in terms of reducing nephrotoxicity. because eugenol has anti-inflammatory and antioxidant qualities that can aid in lowering inflammation and oxidative stress in the kidneys, eventually protecting renal function. In a previous research study, Gentamicin-induced nephrotoxicity was effectively treated with Eugenol. Gentamicin, a commonly used antibiotic, is known to have nephrotoxic side effects, potentially causing kidney damage (15). The study demonstrated that Eugenol's antioxidant and anti-inflammatory properties played a crucial role in protecting renal tissue from damage caused by Gentamicin. This finding not only highlights the importance of investigating natural compounds for their therapeutic potential but also suggests that Eugenol could be a valuable candidate for alleviating drug-induced kidney injury in clinical settings.

Furthermore, it is worth noting that Eugenol has also demonstrated efficacy in treating nephrotoxicity induced by silver nanoparticles (AgNP). Silver nanoparticles have

gained attention in various industries and biomedical applications but are associated with potential renal toxicity. In recent research, Eugenol has shown promise in mitigating the nephrotoxic effects of AgNP. Eugenol's multifaceted pharmacological properties, including its ability to scavenge free radicals and reduce oxidative stress, make it a valuable candidate for protecting renal tissue from AgNP-induced damage (9). This underscores the versatility of Eugenol as a potential therapeutic agent in addressing various sources of nephrotoxicity, offering a promising avenue for future investigations into its broader applications in renal protection.

Occupational exposure to lead presents significant and well-documented hazards to workers across various industries. Lead is a toxic heavy metal that can adversely affect multiple organ systems in the human body, with the greatest concern centering on its impact on the nervous system and the kidneys. Four of the eight participants who were suspected of having an excessive amount of lead exposure at work had abnormal renal function found (16). In addition to the aforementioned concerns, chronic lead nephrotoxicity can manifest in a range of severe symptoms and health complications. Azotemia, characterized by elevated levels of nitrogenous waste products in the blood, is a common consequence, reflecting impaired kidney function. Renal failure, interstitial fibrosis, and progressive nephron loss are also potential outcomes, indicating the progressive damage inflicted upon the renal structures by chronic lead exposure. Moreover, gout, a painful disorder brought on by the buildup of uric acid crystals in joints, and hypertension, a persistently elevated blood pressure, can both be influenced by lead nephropathy. (17).

In a study conducted by Fathy M *et al.,* (2022), it was researched that, eugenol for dosage of 100 milligram/kilogram suppressed the upregulated oxidative stress, inflammation, and apoptosis in rats treated with CCl4. Significantly, eugenol co-administration decreased the protein expression levels of pAkt and TGF-β in rat kidney induction with CCl4 in comparison to the CCl4 group. In conclusion, eugenol's promising antioxidant, efficient anti-inflammatory and evident anti-fibrotic properties demonstrated a strong nephroprotective impact against CCl4-induced kidney injury (18).

Eugenol has also exhibited promising therapeutic potential in the treatment of acute pancreatitis. Eugenol's anti-inflammatory and antioxidant properties make it a compelling candidate for managing this condition. Research has shown that Eugenol can help alleviate the severity of pancreatitis by reducing inflammation, oxidative stress, and the associated tissue damage. By targeting these key mechanisms, Eugenol may offer relief to individuals suffering from acute pancreatitis and contribute to the development of novel therapeutic approaches for this challenging medical condition. However, further clinical studies are required to validate its effectiveness and establish appropriate treatment protocols (19) (20).

CONCLUSION

Treating lead-induced nephrotoxicity and could be a valuable asset in addressing occupational hazards associated with lead exposure. Lead-induced nephrotoxicity is a serious concern, given the detrimental effects of lead on kidney function. Research suggests that Eugenol's antioxidant and anti-inflammatory properties may help mitigate the damage caused by lead in renal tissues. By reducing oxidative stress and inflammation, Eugenol holds promise in protecting the kidneys from the toxic effects

of lead exposure. This not only highlights the therapeutic potential of Eugenol but also underscores its relevance in occupational health and safety measures. Incorporating Eugenol-based interventions could be a valuable strategy in safeguarding the well-being of workers in lead-exposed environments and mitigating the risk of lead-induced nephrotoxicity. Further research and clinical studies are necessary to establish its efficacy and optimal usage in this context. We conclude that eugenol could be used as a therapeutic drug on metal induced renal toxicity and even more scientific validation can be made to confirm its full potential and targeted action at cellular level.

Author Contributions

Author 1: Majumdar Protim, 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: Vidya. S, carried out the study by collecting data and assisted in carrying out the necessary statistical analysis.

Author 3: Karthik Ganesh Mohanraj, aided in conception of the topic, designing the study and supervision of the study, statistical analysis, 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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