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AMELIORATIVE EFFICACY OF ZINGERONE ON LEAD ACETATE INDUCED NEPHROTOXICITY IN WISTAR RATS

Akitha. S [1], Raj Kumar. D [2] and Karthik Ganesh Mohanraj [3]*

[1] Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India. Email: 152101085.sdc@saveetha.com
[2] Research Scholar, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India. Email: rajkumar080786@gmail.com
[3] Associate Professor, Department of Anatomy, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India. *Corresponding Author Email: karthikm.sdc@saveetha.com

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Abstract

Background: Lead is one of the heavy metals and considered as a dangerous environmental pollutant. It may be toxic to living beings when ingested directly or indirectly affecting the system as a whole and or organs individually such as liver and kidney. Ginger scientifically referred as *Zingiber officinale* of the family "zingiberaceae" and has important active ingredients such as zingerone, gingerol, paradol, etc. Zingerone has anti-inflammatory, antioxidant, anti-diabetic and anti-cancer activity. Aim of the work is to elucidate the protective effectiveness of zingerone against lead acetate induced nephrotoxicity in wistar rats. **Aim:** The aim of work is to research and analyse the antioxidant property of zingerone upon lead acetate induced renal toxicity in rats. **Materials and Methods:** Male adult rats of Wistar strain were separated into three groupings. Group I: Control, Group II: Lead acetate induction, given at a dose of 0.15% aqueous solution of lead acetate for 60 days and Group III: zingerone treatment (200 mg/kg body weight) daily for 30 days after lead induction period. At the termination of the study period, rats were euthanized, kidney tissues were taken out and processed for biochemical assay to decipher SOD, MDA, urea and creatinine levels and histopathological analysis. **Results:** Mean SOD level in Group I was 86.00 ± 2.09 U/ml, in Group II was 52.38 ± 3.21 U/ml and in Group III was 67.98 ± 2.78 U/ml. Mean MDA level in Group I was 6.48 ± 0.48 nmol/ml, in Group II was 19.11 ± 3.23 nmol/ml and in Group III was 12.95 ± 2.78 nmol/ml. Mean serum urea level in Group I was 32.71 ± 0.67 mg/dL, in Group II was 48.26 ± 1.87 mg/dL and in Group III was 41.28 ± 1.32 mg/dL. The mean serum creatinine level in Group I was 0.83 ± 0.01 mg/dL, in Group II was 1.92 ± 0.23 mg/dL and in Group III was 1.05 ± 0.03 mg/dL. Histopathological analysis showed severe renal steatosis in Group II compared to Group I control and reduced pathological features in Group III. After zingerone treatment, Group III revealed remarkable recovery from renal damage structurally. **Conclusion:** It was deciphered that zingerone revealed a tremendous antioxidant efficacy neutralising free radicals against lead acetate induced nephrotoxicity in rat models. Moreover the compound is a natural plant product and thus found to be a remedial agent for kidney metal toxicity at an *in vivo* scenario without any contra effects.

Keywords: Lead Acetate, Zingerone, Nephrotoxicity, Antioxidant, Anti-Inflammatory Activity, Environment, Pollution, Disease.

INTRODUCTION

The environment we are living in has been compromised and deteriorated by many factors at present such as pollution, nature exploitation, etc. The environment is contaminated severely by heavy metals like cadmium, mercury, lead, chromium, etc. All these environmental pollutants cause severe health related problems even at a low concentration when exposed. Among these, lead is considered one of the most poisonous heavy metal pollutants for which humans are exposed at a larger rate. Lead and other heavy metals produce reactive oxygen species (ROS) when intoxicated chronically causing deleterious effects on tissues (1). Lead is used widely in coating

poly vinyl chloride pipes, paint industries, colouring agents in toys, batteries, and so on. This gets into human exposure to people working in lead mines and industries and to children exposed to toys and materials made from lead (2). The compound, when accumulated in organs like liver and kidney, becomes toxic to cells and tissues leading to hepatotoxicity and nephrotoxicity (3). Particularly nephrotoxicity leads to oxidative stress induced apoptotic damage orchestrating an organ damage and subsequent pathological effects.

Lead is found in many rocks, as well as those that are qualified as over lead. This means that it can be absorbed through the soil and into water, where it can then end up in food, animal tissues, and even human tissue. The toxicity of lead metal is due to its chemical nature, when ingested orally or injected intravenously (through the bloodstream). Lead is more dangerous to humans than if absorbed through skin contact or inhaling vapours from contaminated air (4). Lead also accumulates throughout an organism's lifetime-the higher levels seen in adults occur due to cumulative exposure rather than a single large dose. It has been linked with serious health problems such as, liver damage, renal dysfunction, brain damage and infertility in both children and adults (5) (1). Lead can have serious consequences for the health of those who inhale it, including a decrease in intelligence quotient (IQ) and other cognitive impairments (6). Research has shown that excess lead can reduce mental development and intellectual performance in children and increase blood pressure among adults (7).

Several allopathic medicines and plant based herbal products are in use to treat the damage caused by lead poisoning but most of the available remedies are having side effects and fueling the kidney damage on long term medication. Thus the need of the hour relies on a plant based compound with no side effects and should improve tissue survival (8). Many researchers have been searching to alleviate these lacunae for several decades.

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 (9). Several authors have attempted to study the medicinal properties of plants and their antioxidant effect against tissue damage, free radical suppressing abilities (10) (11) (12). Many active components and isolated natural compounds from herbs are expensive and may not be affordable by all the people (13).

Ginger (*Zingiber officinale*) belongs to the zingiberaceae family containing the active ingredients zingerone, gingerol, parasol, etc. Among these active compounds zingerone has a wide variety of therapeutic properties like antioxidant, anti-ulcerative, anti-inflammatory, anti-cancer and anti-diabetic (14). Zingerone has a chemical structure of (4 - (4 - hydroxy - 3 - methoxy phenyl) - 2 - butanone). On pharmacological basis it is a very active constituent from zingerole converting into ‘zingerone’ when boiled and dried. Also, the content of gingerol and yet another compound of ginger includes 6-gingerol, 8-gingerol and 10-gingerol (15). Thus we made an attempt to investigate zingerone, because of its beneficial role in the kidney through its antioxidant defence mechanism. Previous studies have reported that zingerone is important in inhibiting radiation exposure from endogenous sources as well as scavenging free radicals. It also protects brain mitochondria by acting as an antioxidant (16). It is well established that antioxidants have several beneficial roles in

maintaining cellular homeostasis (17). Several plant based products possess anti-inflammatory properties which have targeted action (18,19). Phytochemical constituents are used against lead toxicity in animals and disorders affecting central or peripheral nervous systems (CNS), cardiovascular system (CV), kidney, liver, reproductive system (RS), among others (20).

We chose zingerone as our study drug for animal models, because its protective effects against lead acetate-induced nephrotoxicity have been well documented in experimental animals (21) (22). By understanding how zingerone protects the kidneys from cellular damage, we may be able to develop more effective treatments for kidney disease caused by exposure to this harmful metal. The aim of our research is to understand the antioxidant mediated nephroprotective effect of zingerone on lead acetate exposure in rats model.

MATERIALS AND METHODS

Study Design:

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 was authorised by Institutional Animal Ethics Committee (IAEC) with approval number BRULAC/SIMATS/IAEC/12-2019/044.

Chemicals:

Lead acetate and zingerone were procured from Sigma-Aldrich Chemicals Private Limited, India. Biochemical analysis was evaluated using the standard ELISA Kit method. Other basic chemicals were obtained from Sisco Research Laboratories Private Limited, India.

Animal Grouping:

Male adult Wistar albino rats of 200 - 220 grams weight were allocated into 3 separate groups (6 rats each). Group I: Control, Group II: Lead acetate induction, given at a dose of 0.15% aqueous solution of lead acetate in regular drinking water for 60 days and Group III: Lead acetate induction followed by zingerone treatment, given orally for 30 days at a dose of 200 mg/kg body weight.

Euthanasia and Sample Collection:

At the termination of the study period, the rats were anaesthetised in a carbon dioxide chamber and the blood was taken, then centrifuged and the separated serum was used for biochemical analysis to estimate superoxide dismutase (SOD) level and malondialdehyde (MDA) level in lipid peroxidation process. Renal function tests are done to assess urea and serum creatinine levels. The animals were euthanized, kidney tissues were obtained, fixed in neutral buffered formalin (10%) for two to three days and carried out for histopathological procedures.

Statistical Analysis and Interpretation:

All the biochemical antioxidant assay data were statistically explored by SPSS (version 23.0). ANOVA test was carried out for variance analysis and Tukey's HSD post-hoc test was done for inter group differences. Statistical significance was defined with the value $p<0.05$.

RESULTS

The biochemical antioxidant assay revealed the mean SOD level in Group I to be 86.00 ± 2.09 U/ml, in Group II was 52.38 ± 3.21 U/ml and in Group III was 67.98 ± 2.78 U/ml (Figure 1). Thus there was a significant decline in superoxide dismutase levels in Group II ($p<0.05$) when correlated with Group I (Control) and Group III (Lead induced + Zingerone treated) showed elevated levels significantly ($p<0.05$) than Group II (Lead induced).

The mean MDA level in Group I was 6.48 ± 0.48 nmol/ml, in Group II was 19.11 ± 3.23 nmol/ml and in Group III was 12.95 ± 2.78 nmol/ml (Figure 2). There is a significant increase in malondialdehyde level in Group II ($p<0.05$) in correlation with Group I, due to more lipid peroxidation in tissues upon oxidative stress. But, there was an improvement in cellular survival and decline in lipid peroxidation in Group III, lead induced rats treated with zingerone.

The mean serum urea level in Group I was 32.71 ± 0.67 mg/dL, in Group II was 48.26 ± 1.87 mg/dL and in Group III was 41.28 ± 1.32 mg/dL (Figure 3). The mean serum creatinine level in Group I was 0.83 ± 0.01 mg/dL, in Group II was 1.92 ± 0.23 mg/dL and in Group III was 1.05 ± 0.03 mg/dL (Figure 3). The renal function test indicated urea and serum creatinine levels in Group II were high significantly when correlated with Group I ($p<0.05$), surprisingly reduced in Group III after zingerone treatment.

Histopathological analysis (Figure 5) showed severe renal steatosis, apoptotic and distorted glomerulus in the basal peripheral zone of the renal cortex. Also there are numerous damaged distal convoluted tubules mentioned as 'DCT' and proximal convoluted tubules mentioned as 'PCT', in the renal cortex throughout. The tubules with steatotic damage showed micro vacuolization in the cytoplasm and ruptured cell wall in Group II, lead induced rats when compared to Group I, control rats. These pathological features were diminished in Group III, upon zingerone treatment. Group III revealed remarkable recovery from renal damage, cell death and structural restoration.

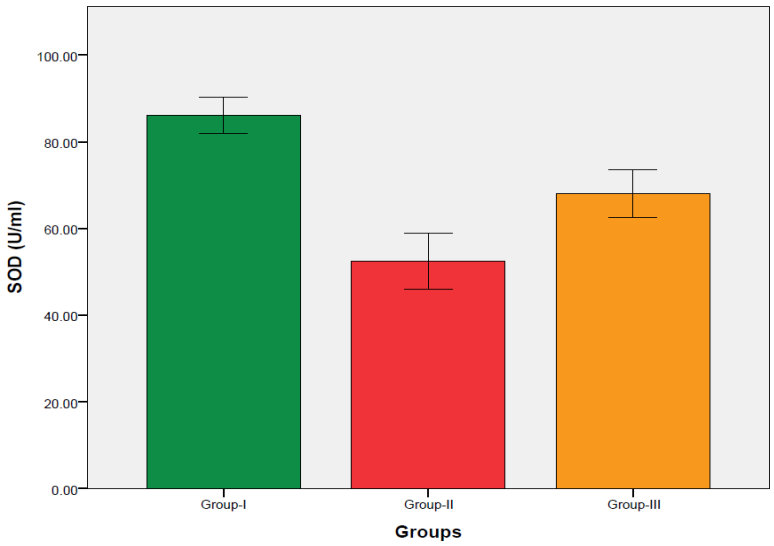

Figure 1: Graph Images Superoxide Dismutase (SOD) Level in all Groups. Comparing the 3 groups, Group-III has p = 0.00 (p<0.05) which is Indicative of Statistical Significant Increase from Group-II.

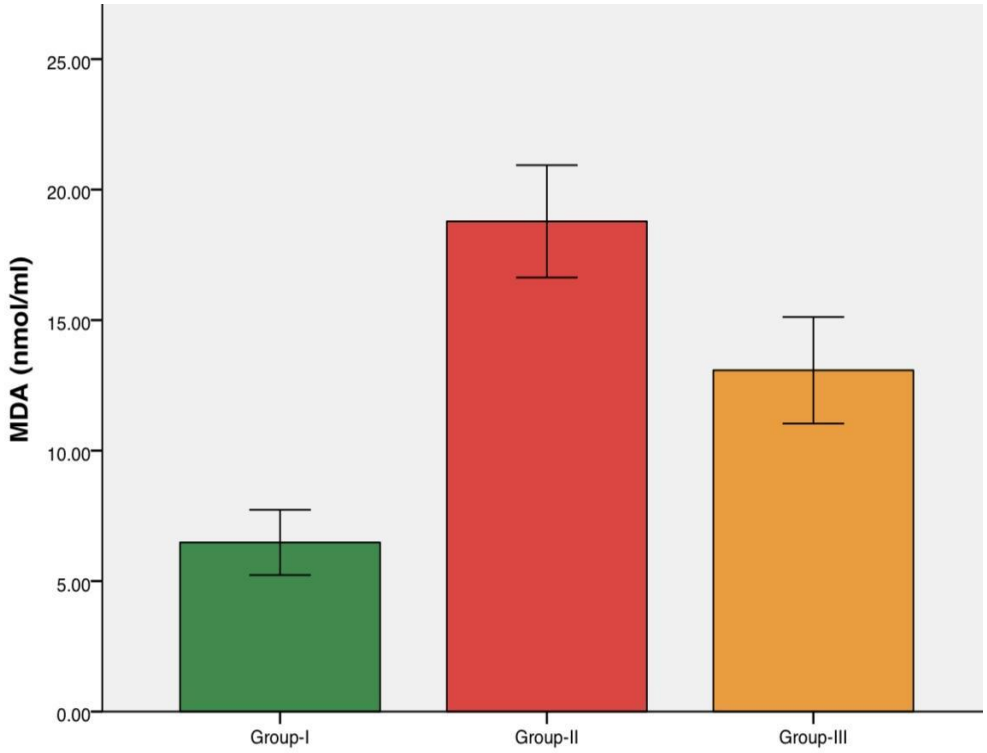

Figure 2: Graph Images Malondialdehyde (MDA) Level in all Groups. Comparison between the 3 Groups, Deciphered Group-III has p = 0.00 (p<0.05) Proving Statistically Significant Decrease from Group-II

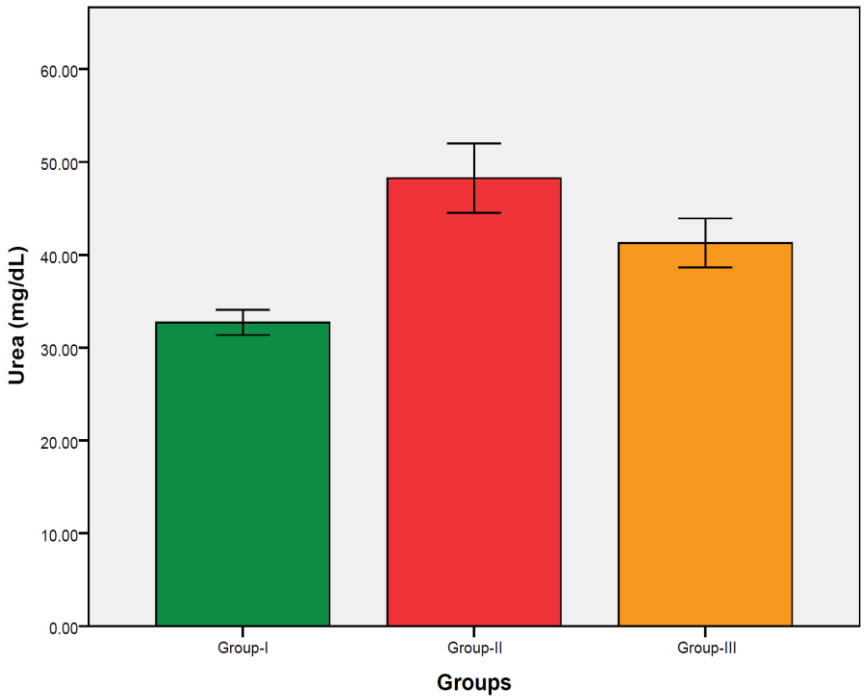

Figure 3: Graph Images Serum Urea Level in all Groups. When Comparing, Group-III exhibited p = 0.00 (p<0.05) Proving Statistically Significant Decrease from Group-II

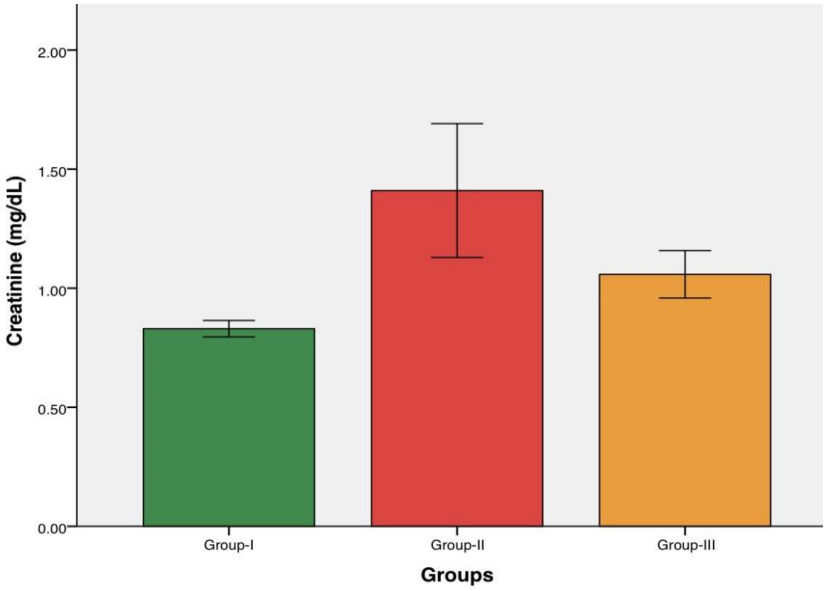

Figure 4: Graph Images Serum Creatinine Level in all groups. Comparing the 3 Groups, Group-III has p = 0.00 (p<0.05) Proving Statistically Significant Decrease from Group-II

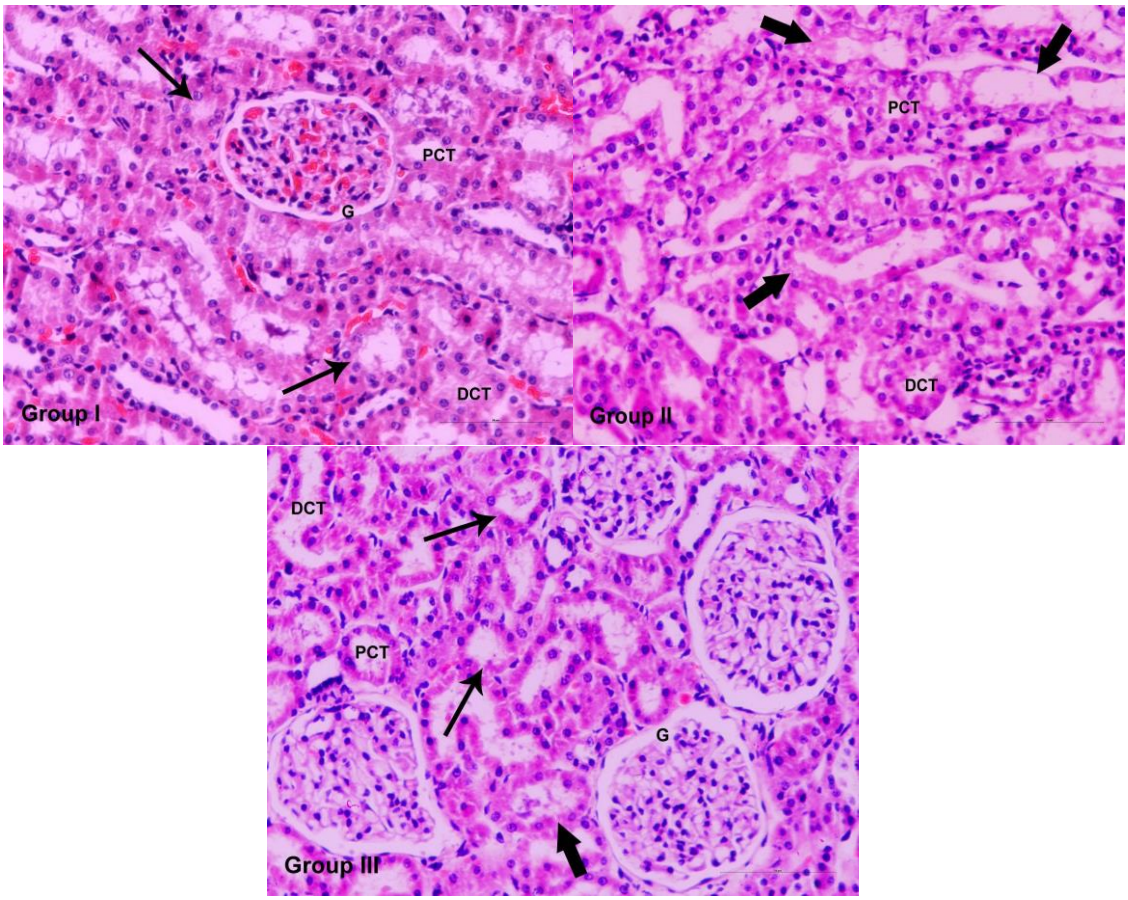

Figure 5: Histopathological Images of Kidneys Stained with Haematoxylin & Eosin in Group I (Control), Group II (Lead Acetate Induction) and Group III (Lead acetate induced + Zingerone Treated) at 10X Magnification. DCT - distal Convoluted Tubule; Thin Arrow - Normal Tubules; Thick Arrow - Damaged Tubules

DISCUSSION

The study result revealed that the lead exposure caused kidney dysfunction which was evident from the impaired histopathological features and antioxidant enzymes. This deleterious effect was restored in cells and tissues of the kidney in rats treated with zingerone after lead acetate induction. Being a major metal toxicant in the environment, lead can also bring severe damaging effects to humans upon chronic accumulation in cells and tissues. Poisoning with lead is at high risk as it hampers cell survival affecting both kidney physiology and structure. Previous studies reported that adenine induced kidney damage has remarkable effect on organ function and orchestrating the cell level defects to system level impairment. They also depicted that lead toxicity affected the kidney adversely more than most metals but therapeutic targeted drug delivery systems should be engineered for rapid cellular recovery (23) (24).

Heavy metal poisoning, especially like lead, is a condition of serious health related issues making pavement for important organ damage such as blood, kidney, liver, lung and brain (25) (26). Humans might have direct or indirect contact with lead through materials made of lead, lead paints, cosmetics, etc. It can also get into humans via lead contaminated animals and fish consumption. Once it gets accumulated in human major organs such as the liver and kidneys, causing alarming effects leading to severe organ dysfunction (27). Under such circumstances, the primary treatment regimen could be the chelation therapy for a long period of time. This therapy instead of treating the disease, worsens the condition and the related organs with excess side effects. To tackle the scenario of lead toxicity induced stress, herbal remedy could be a better and wise choice. It is a well known fact that natural products and herbal medicines are a long standing choice for many human ailments though without much scientific validation. Among them, ginger is a potential plant rhizome with enormous medicinal properties from ancient times.

This finding was in correlation with our study that in the lead acetate induced group, the cells lining glomerulus, PCT and DCT were affected severely with renal steatosis which was evident from the histopathological analysis. In the same condition, zingerone treated lead acetate induced group, exhibited significant recovery at structural level with intact cellular architecture in glomerulus, PCT and DCT. This could be due to the neutralising action of zingerone on lipid peroxidation (28) (29).

Offor et al., showed in his experiment that lead induction in rats generated multiple organ deterioration such as liver, kidney and testis. The hepatic and renal parameters were considerably declined upon lead exposure and altered both structural and functional level. The pathophysiology behind the metal toxicity relies on overproduction of free radicals and reactive oxygen species (9).

Rehman et al., in his literature review established that there is a relation between limits of lead exposure with response rate of the tissues of kidney. They also investigated and portrayed the mechanism behind the poisoning at acute and chronic level. The metal poisoning and the cellular response in *in vitro* and *in vivo* studies have been elucidated with scientific points (30).

Amin et al., drafted that zingerone has the capability to nullify the damages caused by lead induction and has the potential to repair the toxic manifestations created by lead through its bioactive and bioavailable property. They also defended that zingerone has the ability to prevent hepatic and renal toxicity via its antioxidant and aminolevulinic

acid dehydratase modulatory properties (31). From our study we inferred that zingerone has capability to enhance and prevent lead from inhibiting aminolevulinic acid dehydratase (ALAD), thereby preventing aminolevulinic acid (ALA) accumulation in blood and inside tissues. It recovers cellular damages and restores tissue architecture for better structural and functional support (32). Thus this natural compound renders a significant ameliorative effect on lead toxicity in an *in vivo* animal model (33) (34).

CONCLUSION

The present experimental study revealed zingerone as a good therapy against kidney dysfunction in rats. Thus, our result defends that zingerone, a natural material, could be more useful to help kidney damage from lead acetate exposure than the persisting active antioxidants in society.

It could be a statement that zingerone via its enormous antioxidant capacity, scavenging the free radicals and protects the cells from lead acetate induced renal damage. It can be an all-time available natural product for hampering the lead acetate inflicted toxic effects.

Author Contributions

Author 1 - Akitha. S, carried out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis and the preparation of the manuscript.

Author 2 - Raj Kumar. D, assisted in carrying out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis.

Author 3 - 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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Conflict of Interest

There is no conflict of interest to declare by the authors.

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