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Research Article

Lead-induced Toxicities in Wistar rats: Mitigating Effects of Ethanol Leaf Extract of *Cymbopogon citratus* Stapf.

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Abstract
Herbal products have gained important applications in the management and treatment of several diseases. *Cymbopogon citratus* Stapf. (Lemon grass) is an all-season plant found throughout the entire world, particularly in the tropics with several bioactive components. Lead is a wide spread environmental pollutant reported to induce pathological conditions in humans. One possible mechanism of lead toxicity is oxidative stress. We evaluated the effects of ethanol leaf extract of *C. citratus* (EECC) on lead-induced toxicity in rats. Five groups of six rats each (control, EECC, lead acetate (PbAc), EECC+PbAc and PbAc recovery) were used in this study. Lead acetate (60 mg/kg) and EECC (500 mg/kg) were administered by gavage respectively. Lead reduced the antioxidants biomarkers, significantly increased the frequency of micronuclei formation in rat bone marrow cells and the level of DNA fragmentation in the liver tissue. The serum activities of the renal injury biomarkers were increased, the sperm count and motility were decreased while the percentage sperm abnormality was increased. However, administration of EECC significantly restored the antioxidant defense system, reduced the frequency of micronuclei formation and DNA fragmentation, offered protection against hepatorenal toxicities but did not protect against lead-induced damages on testicular tissues. Findings from this study, point out that the protective activity of EECC may be through its free radical scavenging properties.

Key Words: *Cymbopogon citratus*, genotoxicity, Lead acetate, oxidative stress, reactive oxygen species

INTRODUCTION

Continuous exposure of living organisms to environmental pollutants such as lead, a toxic heavy metal, remains a great burden and a serious challenge in many developing countries (Sunday et al., 2012). Cells of living organisms undergo division to replace worn out cells, differentiate to become specialized, and undergo programmed cell death (apoptosis) upon damages. These processes are tightly regulated to ensure continuity of life and a healthy system. However, dysregulation in any of these may result in different pathologies and/or diseases. Over the years, environmental pollution has continued to impose several health challenges globally thereby increasing economic burden on countries around the world (Tong et al 2000; Landrigan and Fuller, 2015).

Lead is a widespread environmental contaminant, which play an etiological role in many human pathological conditions via the depletion of antioxidant defense mechanisms and genotoxic effects (Gagan et al., 2012). The toxicity of lead has been reported decades before now (Needleman, 1999). According to a medical humanitarian organization, a toxic concentration of lead was associated with illegal mining of gold ore in Northern part of Nigeria (Zamfara State) which led to contamination of soil and household dust. This caused an estimated mortality rate as high as 40% among children and claimed over 400 lives (Medecins San Frontieres, 2012).

Oxidative stress, hepatic, renal, testicular and genetic toxicities have been reported in animals exposed to lead (Odunola et al., 2007; Flora et al., 2011; Debosree et al., 2012; Osula et al., 2014; Olugbami et al., 2015). Exposure to lead at high concentration (>60 µg/dL) or low concentration (~10 µg/dL) induces pathological conditions in both male and female reproductive system (Grant, 2008). According to Flora et al., (2011), abnormal spermatogenesis (reduced sperm count and motility), infertility, reduced libido, damage to chromosomes, irregular prostatic function and serum testosterone changes are the common effects observed in males exposed to lead while females are more susceptible to miscarriage, infertility, pre-eclampsia, pregnancy-induced hypertension and premature delivery.

Oxidative stress induction on exposure to lead in living organisms are enhanced by two different pathways occurring

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simultaneously: the first pathway is the reactive oxygen species (ROS) generation, like singlet oxygen, hydroperoxides and hydrogen peroxide, while the second is the depletion of the antioxidant reserves by the generated ROS (Patra et al., 2011; Lopes et al., 2016). Failure to rapidly mop up the generated reactive intermediates or the repair of the resulting damages can be said to be the hallmark of oxidative stress (Flora, 2002; Flora, 2009; Elias et al., 2014). The adverse effects emanating from the use of synthetic drugs necessitated the search for ethnomedicinal plants with potentials for therapeutic efficacy in the treatment of lead toxicity.

Cymbopogon citratus also referred to as "lemon grass" is an all season plant found throughout the entire world, particularly in the tropical and sub-tropical regions of the world (Francisco et al., 2011). It has been reported that *C. citratus* leaves contains many phytochemicals with biological properties such as anti-oxidant, anti-microbial, anti-fungal, antinociceptive, anti-bacteria, anti-obesity, anti-hypertensive, anti-fungi, anti-mutagenic and anti-inflammatory properties. (Oloyede, 2009; Pereira et al., 2009; Matasyoh et al., 2011; Olorunnisola et al., 2014). Synergistic interactions of bioactive components in medicinal plants might result in detoxification mechanisms to curtail the biochemical processes leading to pathological conditions, such as oxidative damage and genotoxicity. We therefore investigated the effects of ethanol leaf extract of *Cymbopogon citratus* (EECC) against lead-induced toxicity and oxidative stress in rats.

MATERIALS AND METHODS

Reagents and chemicals: Lead acetate $(CH_3COO)_2$ $Pb.3H_2O$, Mol. Wt 379.33, 99.999%, CAS No. 6080-56-4; (Aldrich Chemical Co. Inc. St. Paul Avenue, Wisconsin. USA) was dissolved in distilled water. All other chemicals/reagents used were of analytical grade.

Collection and extraction of plant material: Fresh leaves of *Cymbopogon citratus* were harvested at the Department of Chemistry, University of Ibadan, Ibadan, Oyo State, Nigeria. They were taken to the herbarium of the Department of Botany, University of Ibadan for identification and authentication, with authentication ID UIH-22637. Extraction was done according to the method prescribed by Agbafor and Akubugwo (2007) with slight modifications. The air-dried sample (*C. citratus*) was milled into powder. The powdered sample (200 g) was soaked in 500 mL of 80% ethanol for 72 hours. It was filtered and concentrated with rotary evaporator (Stuart, Barloworld and Model RE 300) at a temperature of 40 ± 2 ^oC to obtain crude extract which was thereafter lyophilized at the Multidisciplinary Central Research Laboratory, University of Ibadan, Nigeria.

Experimental animals and care: Thirty (30) male Wistar rats (90 ± 10 g) were procured from the Department of Physiology, University of Ibadan, Ibadan, Nigeria. They were acclimatized for one week under normal conditions in the Animal House, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. The animals were placed on standard commercial rat pellets and water *ad libitum* under standard environmental conditions of 50 ± 10% relative humidity, temperature of 27 ± 2^oC and 12-hour light/dark cycle. All the animals were handled in adherence to the guide for the care and use of experimental animals, as specified by the National Institute of Health (NIH publications number 85–93 revised in 1985).

Administration of test substances: Ethanol extract of *Cymbopogon citratus* (EECC) and lead acetate were administered by gavage to the rats at doses of 500 and 60 mg/kg body weights (i.e. 1/10th of the LD_{50} of lead acetate), respectively, according to Sujatha et al. (2011). EECC and the lead acetate were dissolved in distilled water as vehicle for administration.

Experimental protocol: The rats were randomly divided into five groups, with six animals per group. The rats in group I was designated as control and received distilled water only; those in group II received 500 mg/kg body weight of EECC. The rats in group III were given 60 mg/kg body weight of lead acetate (PbAc). Group IV received 500 mg/kg body weight of EECC and 60 mg/kg body weight of PbAc simultaneously. Group V received 60 mg/kg body weight of PbAc only and were left to recover for thirty days. All the treatments were administered by gavage for thirty days. Twenty-four hours after the last administration, rats were sacrificed; blood was collected into specimen bottles and allowed to clot for serum separation by centrifugation (4000 g, for 15 minutes). Liver, testes, femur and kidney were excised and rinsed in ice-cold solution of 1.15% potassium chloride, blotted dry, weighed, and processed for biochemical and histological analysis.

Biochemical Assay: Pieces of liver sections were homogenized in phosphate buffer (pH 7.4). Oxidative stress biomarkers were investigated using liver homogenates following standard protocols: malondialdehyde (MDA), the activity of catalase (CAT), the activity of superoxide dismutase (SOD), the activity of glutathione-S-transferase (GST) and the activity of glutathione peroxidase (GPx) were determined according to the methods earlier described by Varshney and Kale, (1990); Claiborne, (1985); Misra and Fridovich, (1972); Habig *et al.* (1974); and Rotruck *et al.*, (1973); respectively. Furthermore, serum levels of renal injury biomarkers (creatinine, urea, albumin and total protein) were evaluated using Cypress® diagnostic kits. Percentage DNA fragmentation was investigated according to Wu *et al.* (2005) and the induction of micronuclei in the polychromatic erythrocytes (PCEs) were also assessed using rat bone marrow cells according to Schmid, (1975).

Reproductive study: Testicular functionality (sperm count, abnormality and motility) were assessed as previously described in Badkoobeh *et al.*, (2013).

Histological examination: Histological assessment of the liver, kidneys and testicular sections of the rats were done at the Veterinary Pathology Department, University of Ibadan, Ibadan, Nigeria. Liver and kidney tissues were fixed for 24 hours in 10% phosphate buffered formalin, and subsequently embedded in paraffin following dehydration serially in an ethanol gradient followed by xylene. Sections of 5µm thickness were cut, fixed on glass slides, deparaffinized, rehydrated and were stained with haematoxylin and eosin. Testes were fixed in Bouin's solution, processed, sectioned, mounted on slides and were stained with haematoxylin and eosin and examined by Carl Zeiss light microscope (Zeiss Axioscope 5).

Statistical analysis:
The biochemical data were subjected to analysis by one-way analysis of variance (ANOVA) using Predictive Analytical Software (PASW) LSD test was used to compare differences between means and $p < 0.05$ were considered to be statistically significant. Graphical data was subjected to analysis using Graph Pad Prism 6. Data are presented as Mean ± SEM.

RESULTS

Findings from this study show the effect of the treatments on oxidative stress biomarkers in the liver (Figure 1). Lead acetate significantly decreased the levels of the oxidative stress biomarkers in the liver tissue except for MDA which was significantly increased. Upon treatment with EECC, the antioxidant activities were restored.

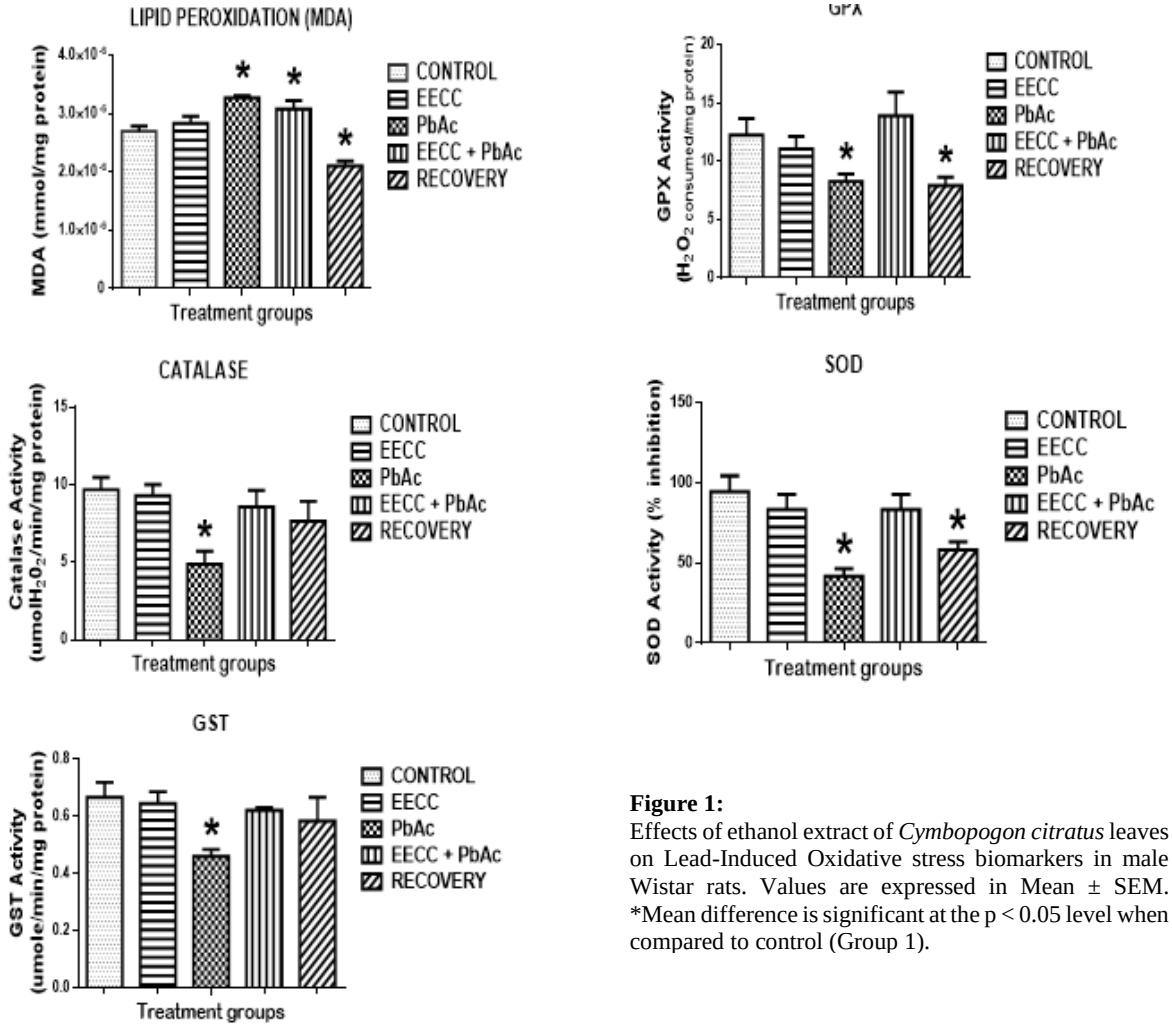


Figure 1:
Effects of ethanol extract of *Cymbopogon citratus* leaves on Lead-Induced Oxidative stress biomarkers in male Wistar rats. Values are expressed in Mean ± SEM. *Mean difference is significant at the $p < 0.05$ level when compared to control (Group 1).

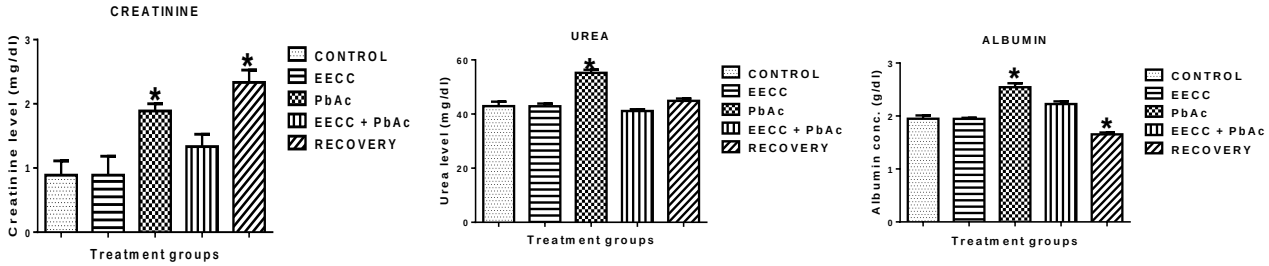


Figure 2:
Effect of ethanol extract of *Cymbopogon citratus* leaves on biomarkers of Lead-Induced nephrotoxicity in rats. Values are expressed in Mean ± SEM. *Mean difference is significant at the $p < 0.05$ level when compared to control.

Effect on kidney function biomarkers: The nephroprotective effects of EECC against lead induced toxicity were evaluated by accessing the kidney biomarkers activities (Figure 2). Findings showed an increased albumin level in lead treated group when compared to control, serum creatinine level increased in lead treated group and the recovery group when compared to control; urea level increased significantly in lead treated group. However, the

administration of EECC reduced the kidney functional parameters close to that of the negative control.

Genotoxic effect: The genotoxic effect of lead acetate and the ameliorative effect of EECC were evaluated in the rat bone marrow to detect chromosomal damage and percentage DNA fragmentation level (Figure 3). Induction of micronucleated polychromatic erythrocytes (mPCEs) and percentage DNA fragmentation were increased significantly in lead acetate treated group; however, co-administration with EECC remarkably reduced these parameters.

Reproductive effects: Reproductive studies were assessed by analysis of sperm indices (Figure 4). The sperm count and sperm motility reduced significantly, while percentage sperm abnormality was significantly increased in lead acetate treated group. However, co-administration of EECC and lead showed non-significant increase in sperm motility and sperm count and non-significant decrease in percentage sperm abnormality when compared with the lead acetate treated group.

Histological analysis

Histological analysis of liver, kidney and testes sections is represented in Figures 5A, 5B and 5C respectively. The liver section showed mild sinusoidal congestion, severe periportal cellular infiltration, and moderate to severe sinusoidal congestion in EECC treated group, lead treated group and recovery group respectively as depicted in Figure 5A. However, co-administration of EECC and PbAc showed no visible lesion on the liver tissue, this portrays the hepatoprotective activity of EECC. Photomicrograph of kidney section revealed mild renal cortical congestion, mild congestion around the glomeruli, protein cast in the lumen of few tubules and degeneration on the glomeruli in EECC group, PbAc group, co-administered group of EECC with PbAc, and recovery group respectively as depicted in Figure 5B. Photomicrograph of testes showed immature cellular clumps in the lumen of seminiferous tubules in PbAc treated group. However, no visible lesion was observed in EECC group and the co-administered (EECC and PbAc) group respectively, as depicted in Figure 5C.

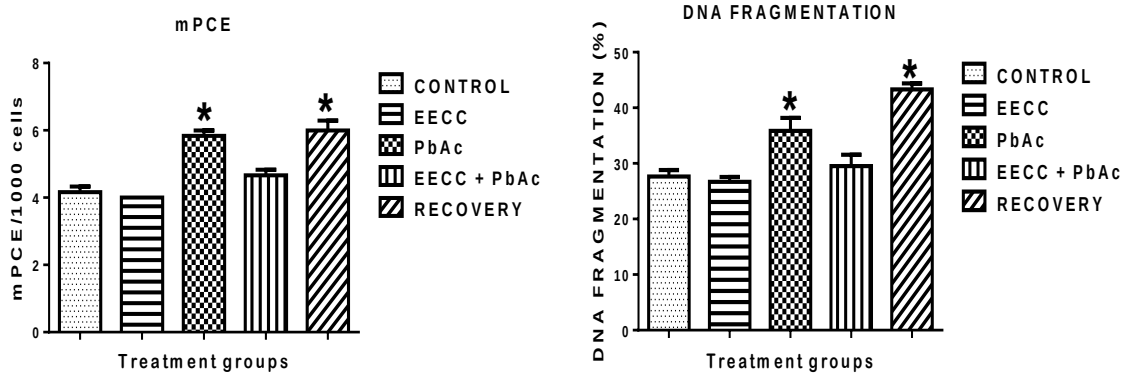


Figure 3:
Induction of micronucleated polychromatic erythrocytes (mPCEs) in rat bone marrow and evaluation of % DNA fragmentation in male wistar rats following lead acetate and ethanol extract of *Cymbopogon citratus* leaves administration. Values are presented as Mean ± SEM. * Mean difference is significant at the $p < 0.05$ level when compared with control.

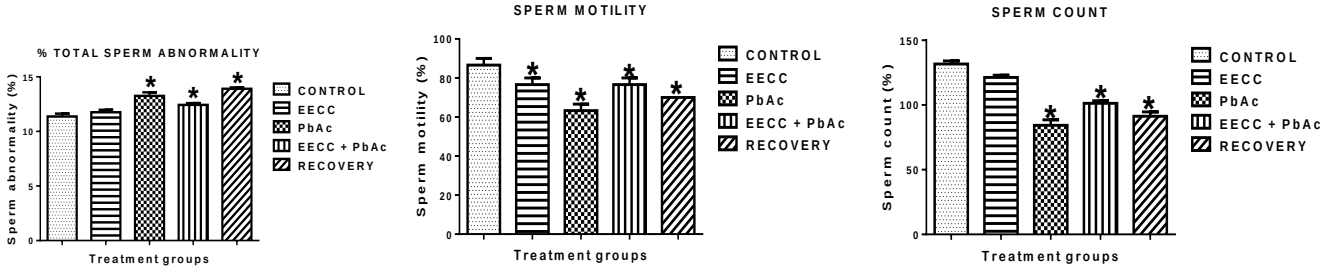
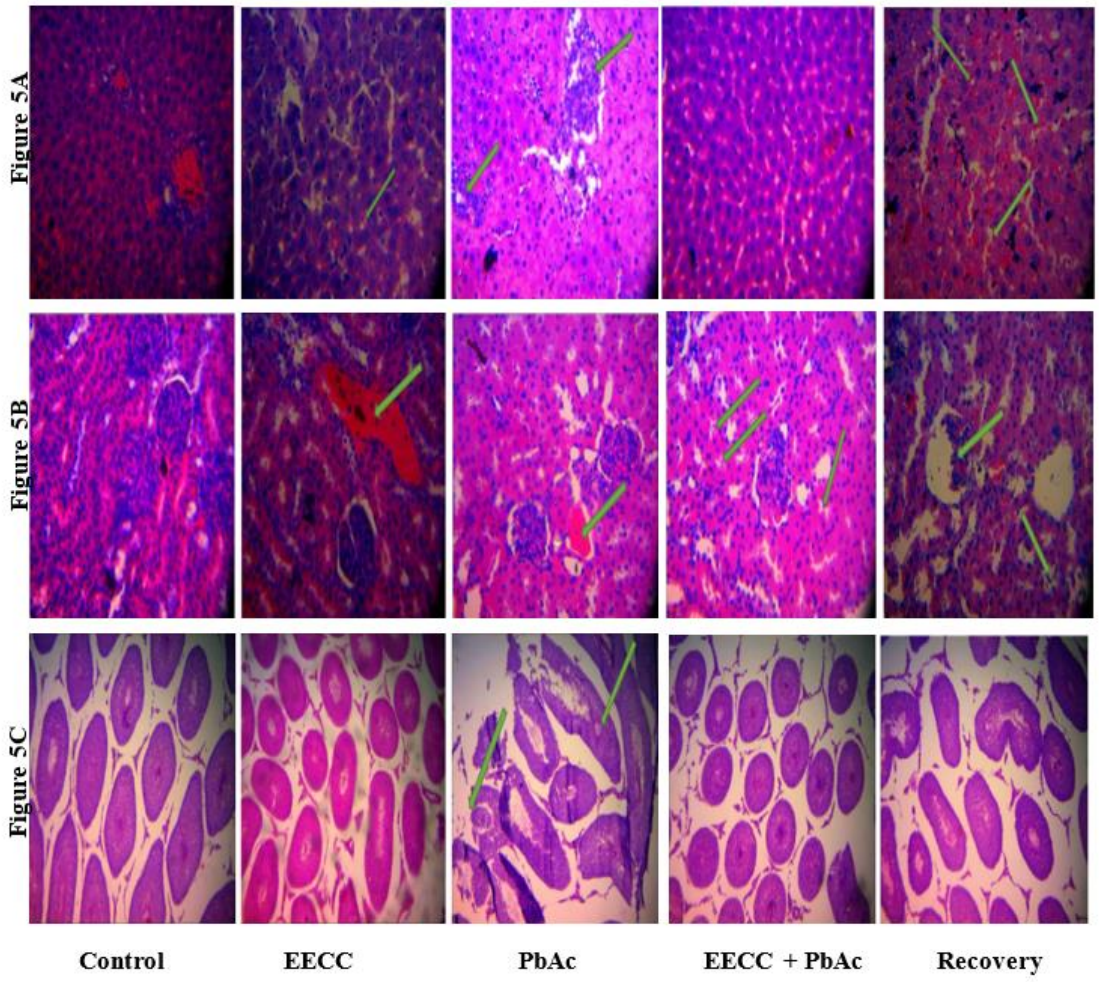


Figure 4:
Effect of ethanol extract of *Cymbopogon citratus* leaves on biomarkers of Lead-Induced testicular toxicity in rats. Values are expressed in Mean ± SEM. *Mean difference is significant at the $p < 0.05$ level when compared to control.

DISCUSSION

The induction of oxidative stress by lead is mediated via the generation of ROS and the depletion of antioxidant reserves (Elias *et al.,* 2014; Patri *et al.,* 2017). Toxic heavy metals in the environment are primarily stored, biotransformed and detoxified in the liver, thus, liver is an important organ in the metabolism of heavy metals (Ohkawa *et al.,* 1979; Aliyu *et al.,* 2015). Lead as a toxic heavy metal, generates oxidative damage in the liver by promoting lipid peroxidation (Can *et al.,* 2008; Lopes *et al.,* 2016).

Findings from this study clearly demonstrated that lead acetate induced oxidative stress in liver tissue which is in tandem with the previous reports of Abdel-Moneim (2016) who reported that lead acetate induces oxidative stress, hepatotoxicity and apoptosis in rats. In this very study, treatment with EECC significantly restored the antioxidant activities against lead-induced toxicity in rats.



Figures 5A-C:
Photomicrographs of liver (5A), kidney (5B) and testes (5C) sections (Magnification x100). 5A: Control: No visible lesions, **EECC:** Mild Sinusoidal congestion, **PbAc:** Severe periportal cellular infiltration, **EECC + PbAc:** No visible lesion, **Recovery:** Moderate to severe sinusoidal congestion. **5B: Control:** No visible lesion, **EECC:** Mild renal cortical congestion, **PbAc:** Mild congestion around the glomeruli**. EECC + PbAc:** Few tubules have protein cast in their lumen, **Recovery:** There is a focus of glomeruli degeneration/necrosis. **5C: Control:** No visible lesion, **EECC:** No visible lesion**. PbAc:** Seminiferous tubules have immature cellular clumps in their lumen, **EECC + PbAc:** No visible lesion**. Recovery:** No visible lesion.

The reduced level of the oxidative biomarkers (Catalase, GPx, SOD and GST) observed in the liver tissue is in tandem with a biochemical insight upon tissue damage. Biochemically, the liver cells lose integrity upon damage which explains the reduced levels of oxidative biomarkers in the liver tissue observed in this study. The free radical scavenging properties of EECC may be due to its constituent bioactive secondary metabolites capable of eliminating unstable molecules/electrons by donating or sharing of its molecules/electrons. The antioxidant activities of EECC observed in this study is in tandem with the report of Koh *et al.,* (2012) who reported the antioxidant potential of *Cymbopogon citratus* extract against oxidative stress and toxicity.

Findings from this study revealed significant differences in the renal injury biomarkers of lead treated groups when juxtaposed with the control. Lead induced renal damage observed in this study corroborates the previous report of Offor *et al.,* (2017) who reported lead induced hepato-renal damage in male rats. As observed from the results of this study, lead exposure affected the renal tissues resulting in a significantly increased renal injury biomarkers activities in lead treated rats when juxtaposed with the control. However, upon treatment with EECC, there was nephroprotective effect which agrees with the previous report of Ademuyiwa *et al.,* (2017) who reported studies on the nephroprotective and nephrotoxic effects of extract of *Cymbopogon citratus* in Wistar rats. Summarily, EECC possess nephroprotective properties against lead-induced toxicity. The protective properties of EECC could be attributed to the presence of some bioactive compounds, this is similar to the report of Olorunnisola *et al.,* (2014) who reported that *Cymbopogon citratus* possess myriads of biologically active compounds with health promoting properties.

DNA fragmentation level and mPCEs induction were investigated in this study as biomarkers of genotoxicity. Lead induction of DNA fragmentation observed in this study is in line with the report of Akram *et al.,* (2019) who reported lead induced DNA damage, alteration of aminolevulinic acid (ALAD) and antioxidant genes mRNA expression in construction site workers. The induction of mPCEs observed in this study also agrees with the previous report of Odunola *et al.,* (2007) who reported lead induction of mPCEs in bone marrow cells of rats. From our findings, EECC showed a protective potential against lead induced genotoxicity in

Wistar rats, this could be due to the bioactive constituents of the extract and its antioxidant activity which may act by reducing the generation of ROS thereby enhancing balance between pro-oxidant and the antioxidant defense system. The geno-protective effect of EECC against lead induced toxicity observed in this study corroborate the previous report of Olorunnisola *et al*., (2014) who reported the antioxidative and antimutagenic properties of *Cymbopogon citratus*.

Spermatic indices (sperm count, motility and morphology) were assessed in this study for testicular functionality. The quality and functionality of spermatic indices are vital for male fecundity (Zinaman *et al.,* 2000; Eliason, 2003). Lead exposure triggers free radicals production and also inhibits the antioxidant scavenging potentials in the reproductive tissue resulting in testicular oxidative stress induction (Owolabi *et al.,* 2012; Sudjarwo *et al.,* 2017). In this study, there were significant reduction in sperm count and sperm motility while percentage sperm abnormality was significantly increased in lead acetate treated group. Lead-induced testicular toxicity observed in this study is mediated via the induction of oxidative stress which is in tandem with the previous report of Sudjarwo *et al.,* (2017) who reported that lead acetate induced testicular toxicity in Wistar rats. Interestingly, our study showed that co-administration of EECC and lead showed no significant differences when compared with the lead alone treated group, suggesting no significant protective effect on the testicular parameters. However, our findings at the present dose contrasted the report of Rahim *et al.,* (2013) who reported a protective effect of *Cymbopogon citratus* against testicular oxidative stress induction in male rats.

EECC offer protective properties against oxidative stress, hepatotoxicity, nephrotoxicity and genotoxicity induced by lead exposure. These effects might be linked to its ability to modulate antioxidant enzymes necessary for maintaining balance between the pro-oxidants and antioxidant defense system and thereby protect liver and kidney tissues, thus restoring the normal metabolic processes. This result may offer an insight for future therapeutic development especially for areas where human beings are occupationally or environmentally exposed to lead. The EECC may also serve as a possible nutritional supplement to circumvent tissue damages induced by oxidative stress among humans exposed to lead.

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