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Comparative histomorphological assessment of Vitamin E and green tea (*Camellia sinensis*) extract-mediated amelioration of Lead-induced hepatopathy in experimental Wistar rats

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ABSTRACT

Objective: Hepatotoxins such as lead has potency of distorting hepatic histomorphology. Conversely, antitoxins exhibit anti-hepatotoxic effects by ameliorating their damaging effects. This study was carried out to comparatively assess ameliorative effects of Vitamin E and green tea extract on hepatic histomorphology of rat model of lead-induced hepatopathy.

Methods: Forty two (n = 42) animals were equally grouped into six: Group A received 5 ml/kg distilled water; Group B received 2 mg/ml lead acetate; Groups C and D received 2 mg/ml lead acetate + 100 mg/kg and 200 mg/kg Vitamin E respectively; Groups E and F received 2 mg/ml lead acetate + 5 mg/kg and 10 mg/kg green tea extract respectively. All treatment was via oral route and lasted for 35 days wherein animal body weight was regularly recorded. Serum ALT and AST levels were determined; hepatic tissues were harvested, weighed and processed for histomorphological study. Data were analyzed using IBM-SPSS (version 20) and compared using t-test and analysis of variance.

Results: There was significant ($p < 0.05$) body and organ weight decrease in Group B relative to Group A while Groups C-F only showed marginal reduction. Also, the serum ALT and AST levels were significantly ($p < 0.05$) increased in Group B while Groups C-F animals only showed marginal increase. The hepatic histomorphological features Groups C-F showed mild variations from normal histomorphology of Group A but intense variations were observed in Group B.

Conclusion: Vitamin E and green tea extract comparatively exhibited histomorphological reparation against damaging effects of lead on hepatic histomorphology. The most potent dosages for their hepato-reparative activity were 200 mg/kg and 10 mg/kg, respectively.

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Introduction

The liver is the largest visceral organ in vertebrates which performs diverse functions and largest gland, especially in humans that produces various physiological secretions [1]. It plays a major role in the accumulation and metabolism of macromolecules (such as carbohydrates and lipids) ingested into the body including chemicals that may cause hepatic structural damage or functional impairment [2]. These chemicals commonly referred to as hepatotoxins may be introduced into the human body during various domestic, environmental, or occupational activities [3]. These hepatotoxins include heavy metals, such as Lead, which has the potency of disrupting hepatic tissue morphology and physiological functions even at low levels of exposure. Its hepatotoxicity has been described to be due to the resultant lipid peroxidation, disruption of prooxidant-antioxidant balance, and production of reactive oxygen species following its exposure and may leads to hepatic tissue degeneration or necrosis. [4]. The initial hepatic tissue response to hepatotoxicity is stimulation of regeneration and

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activation of highly proliferating hepatocytes which constitute bulk (more than 70%) of the hepatic parenchyma except during chronic liver diseases whereby the hepatocyte proliferative response may be impaired [5–7]. Therefore, chemical agents that promote hepatocyte proliferation and/or exhibit antioxidant activity within hepatic parenchyma may be applied to potently counterbalance or neutralize deleterious effects of hepatotoxins, such as heavy metal (like lead) exposure. These chemical agents which exhibit anti-hepatotoxic or hepatoprotective effects through their antioxidant activity include Vitamin E and phytochemicals derived from green tea (*Camellia sinensis*) extract (GTE). Vitamin E (VE) is an antioxidant which functions as cytoprotective or detoxifying agent in tissues including hepatic tissues by inhibition or suppression of cytotoxic effects of free radicals generated by exposure of tissue to toxins, including hepatotoxins [8–10]. Similarly, the GTE through its constituent catechin polyphenols exhibit antioxidant effect against free radicals generated in hepatic and other tissues following exposure to cytotoxic agents and thereby ameliorating resultant tissue damage [11–13]. The relatively comparable chemical properties of VE and GTE may indicate comparable therapeutic activities and possibility of application of either for similar clinical conditions. Therefore, in this study, the objective was to comparatively assess hepatoprotective activity of VE and GTE (*Camellia sinensis*) against Lead acetate-induced hepatic tissue damage in adult male Wistar rats based on histological and histomorphometric profiling of the hepatic parenchyma of experimental Wistar rats.

Materials and Methods

Reagents and extract used

Green tea was manufactured in China and purchased locally in the form of 50 g packet from Steven Chuks Global Associate Ltd. (Lagos, Nigeria). Other chemical reagents used in this study were analytical grade and purchased from Bristol Scientific Co. Ltd. (Lagos, Nigeria) and Sigma Chemical Co. (St Louis, MO). The GTE was prepared following the method by Khan et al. [14].

Experimental animals

Forty-two (42) male Wistar rats weighing between 150 and 170 g were sourced from the Central Animal Facility of Igbinedion University Okada, Nigeria. The animals were kept in cages under hygienic environmental conditions (room temperature of 25 ± 2ºC and relative humidity of 45%–50%), fed on standard animal feed and granted free access to drinking water *ad libitum.*

Experimental design

The experimental animals were equally grouped into six groups (A–F) comprising seven animals per group (n = 7). Group A animals were given distilled water (5 ml/kg body weight) representing the normal control group; Group B animals were given 2 mg/ml lead acetate in drinking water representing the test control group; Group C animals were given 2 mg/ml lead acetate in drinking water + VE (100 mg/kg b.w.); Group D animals were given 2 mg/ml lead acetate in drinking water + VE (200 mg/kg b.w.); Group E animals were given 2 mg/ml lead acetate in drinking water + GTE (5 mg/kg b.w.); Group F animals were given 2 mg/ml lead acetate in drinking water + GTE (10 mg/kg b.w.). The selected dosages of VE and GTE were considered safe with no associated toxic effects [13,15]. The route of all administrations was oral and orogastric gavage coupled to calibrated hypodermic syringe was employed in the daily administration which lasted for 35 days. The total body weight of experimental animals were taken and recorded on 0, 7, 14, 21, 28, and 35 days of study. This study was duly approved by Research and Ethics Committee of Igbinedion University, Okada, Edo State, Nigeria and all experimental protocols were in compliance with International guidelines for care and handling of laboratory animals.

Biochemical assay

The blood sample of experimental animals was collected from retro-orbital sinus, centrifuged at 4,000 rpm for 15 minutes to obtain clear serum. The serum was used to evaluate levels of enzyme antioxidants: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) according to method by Reitman and Frankel [16].

Tissue processing

After the study period, the experimental animals were sacrificed; their hepatic tissues were harvested, weighed and processed for tissue sectioning followed by histological staining. The processing of hepatic tissues of experimental animals were as follows: fixation in 10% neutral buffered formalin, dehydration using alcohol (including two changes each of 70%, 90% and absolute alcohol for 30 minutes each), clearing using xylene for 30minutes and embedding using molten paraffin to form tissue blocks.

Sectioning and staining of hepatic tissues

Sectioning of hepatic tissue blocks were done at 5 µ thickness by using rotary microtome (Leica RM2235; Leica Biosystems Inc, IL) and histological staining was done using the Haematoxylin and Eosin (H & E) technique. During the H & E staining technique, hepatic tissue sections were dewaxed in xylene, hydrated with alcohol, and distilled water, stained in Haematoxylin, rinsed in water, differentiated in 1% acid alcohol (1% HCl in 70% alcohol), blued in Scoh's tap water, rinsed in water, stained in Eosin, rinsed in water, dehydrated with alcohol, cleared in xylene and mounted with DPX [17].

Histomorphological study of hepatic tissues of experimental animals

The stained histological sections were examined under microscope to compare hepatic histomorphology of all experimental animals in treated Groups C–F with control Groups A and B animals. Photomicrographs of all stained sections were generated using 10MP digital camera for microscope and all observable histomorphological variations in the hepatic parenchyma of treated animals in comparison with the control animals were documented.

Statistical analysis

Data recorded were analyzed using IBM-SPSS (version 20, IBM Corp, NY) and results presented as Mean ± SEM. Multiple comparisons were done using one way analysis of variance and comparison of significance between groups was done using *t*-test with the probability level of $p < 0.05$ regarded as statistically significant.

Results

Evaluation of body weight of experimental animals

The mean body weight of experimental animals in normal control Group A, test control Group B, and treated Groups C–F measured at days 0, 7, 14, 21, 28, and 35 of the study were given in Figure 1. In comparison with the mean body weight of normal control Group A animals, there was non-significant mean body weight reduction in the treated Groups C–F but significant ($p < 0.05$) weight loss recorded in test Group B animals.

Evaluation of hepatic tissue weight of experimental animals

The mean hepatic tissue weight of experimental animals in normal control Group A, test control Group B, and treated Groups C–F measured after the study period was given in Figure 2. In comparison with the mean hepatic tissue weight of normal control Group A animals, there was non-significant reduction in mean tissue weight of treated Groups C–F but significant ($p < 0.05$) weight loss recorded in test Group B animals.

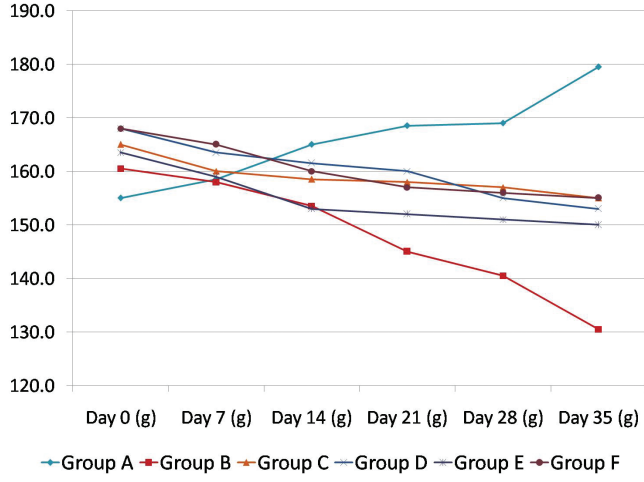

Figure 1. Mean values of body weight measured at regular intervals between days 0 and 35 of study for experimental animals in normal control Group A, test control Group B, and treated Groups C–F administered with 2 mg/ml lead acetate + 100 mg/kg VE, 2 mg/ml lead acetate + 200 mg/kg VE, 2 mg/ml lead acetate + 5 mg/kg GTE, and 2 mg/ml lead acetate + 10 mg/kg GTE, respectively.

Biochemical assay

The mean ALT and AST values in the serum of experimental animals in normal control Group A, test control Group B, and treated Groups C–F were given in Figure 3. The serum levels of ALT and AST in treated Groups C–F animals were non-significantly elevated but significantly ($p < 0.05$) elevated in test control group B in comparison to levels in the normal control Groups A.

Histomorphological study

The histological results of this study revealed hepatic histomorphology of experimental animals in normal control Group A, test control Group B, and treated Groups C–F (Figure 4). The hepatic histomorphology of treated Groups C–F compared relatively with those of normal control Group A, while the test control Group B showed prominent histomorphological distortions following exposure to lead toxicity. However, observable histomorphological variations in hepatic tissues of all experimental animals in normal control Group A, test control Group B, and treated Groups C–F including hepatocyte diameter, sinusoidal size, hepatic

necrosis, centrilobular hepatic congestion, and mononuclear leukocyte infiltration were compared and documented (Table 1).

Discussion

The cytotoxic effect of lead exposure which is usually not limited to hepatic tissue of animals has been reported to affect other tissues such as spleen [18], bone marrow [19], testis [20,21], brain, and kidney [22,23]. Such widespread toxicity, affecting several body tissues, can result into significant reduction of individual organ weight as well as the total body weight. According to the results of this comparative study (Figure 1), the significant ($p < 0.05$) reduction of total body weight of test control Group B animals in comparison with the normal control Group A can be linked to the widespread damaging effect of lead exposure. Conversely, the non-significant reduction of the body weight of experimental animals in treated Groups C–F compared to the normal control Group A may be attributed to the attenuation of deleterious effects of lead exposure by the administered VE and GTE. In furtherance, the findings of this study (Fig. 2) showed non-significant weight reduction of the hepatic tissue of experimental animals in treated Groups C–F compared to those of normal control Group A. However, there was significant ($p < 0.05$) hepatic tissue weight loss in test Group B animals following exposure to lead toxicity and null treatment.

As a major organ of metabolism, the liver tissue is prominently exposed to various macromolecules ingested into the body including potential hepatotoxic agents [24]. These potential hepatotoxins may be environmentally derived substances such as heavy metallic pollutants like lead. The exposure to these hepatotoxins has earlier been linked with multiple organ morphological damages or physiological impairment. These morphological

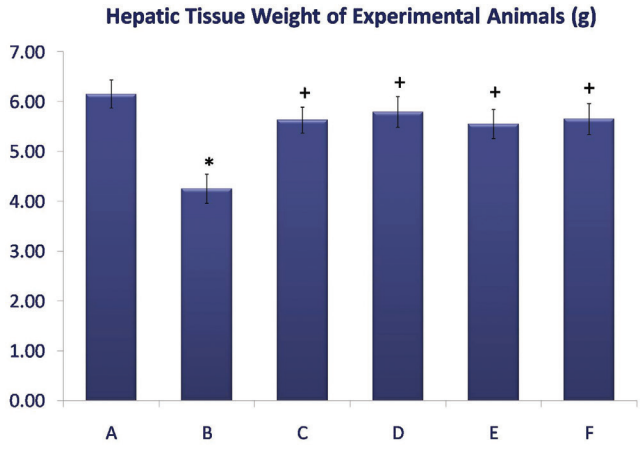

Figure 2. Mean values of hepatic tissue weight of experimental animals in normal control Group A, test control Group B, and treated Groups C–F administered with 2 mg/ml lead acetate + 100 mg/kg VE, 2 mg/ml lead acetate + 200 mg/kg VE, 2 mg/ml lead acetate + 5 mg/kg GTE, and 2 mg/ml lead acetate + 10 mg/kg GTE, respectively. (* = significant *p* value when compared with Group A; + = significant *p* value when compared with Group B).

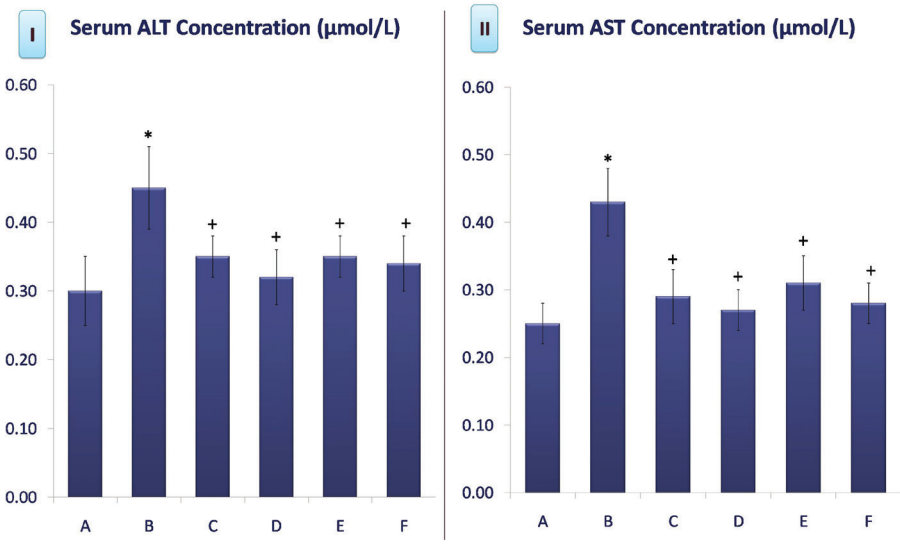

Figure 3. Mean values of serum ALT (I) and AST (II) concentrations of experimental animals in normal control Group A, test control Group B, and treated Groups C–F administered with 2 mg/ml lead acetate + 100 mg/kg VE, 2 mg/ml lead acetate + 200 mg/kg VE, 2 mg/ml lead acetate + 5 mg/kg GTE, and 2 mg/ml lead acetate + 10 mg/kg GTE, respectively. (* = significant *p* value when compared with Group A; + = significant *p* value when compared with Group B).

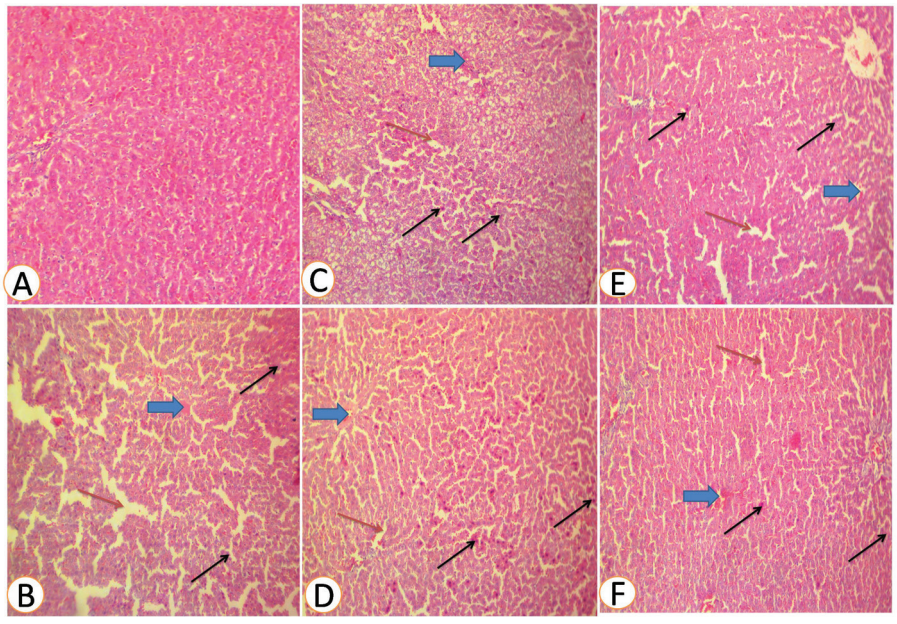

Figure 4. Representative hepatic histological photomicrographs of normal control Group A, test control Group B, and treated Groups C–F administered with 2 mg/ml lead acetate + 100 mg/kg VE, 2 mg/ml lead acetate + 200 mg/kg VE, 2 mg/ml lead acetate + 5 mg/kg GTE, and 2 mg/ml lead acetate + 10 mg/kg GTE, respectively (H&E ×100). The hepatic histological appearances of experimental animals showed various histomorphological features—blue arrows pointing at centrilobular congestion, red arrows pointing at enlarged sinusoids, black arrows pointing at neutrophil infiltration.

Table 1. Hepatic histomorphological features of experimental animals in test control Group B and treated Groups C-F compared to normal control Group A.

Groups	Hepatocyte diameter decrease	Sinusoidal size increase	Hepatic Necrosis	Centrilobular Congestion	Leukocyte Infiltration
A	–	–	–	–	–
B	++	+++	+++	+++	+++
C	+	++	+	++	++
D	+	+	+	++	++
E	+	++	+	++	++
F	+	+	+	++	++

–: comparative baseline; +: mild; ++: moderate; +++: intense.

distortions or functional deficit that followed exposure to hepatotoxins have been described as resultant damaging effects of generated free radicals (such as reactive oxygen species) leading to lipid peroxidation [25]. The consequent free radical-activated membrane lipid peroxidation has been associated with diverse tissue pathologies and disease conditions [26,27]. This has underscored the role of antioxidants in neutralizing toxic effects of cytotoxic agents, including hepatotoxins via inhibition of oxidative damage thereby counteracting against pathogenesis of various diseases [27,28].

The study by Sharma and Bhattacharya [20] had posited that oxidative damage constitutes the prominent basis for lead-induced tissue damage. Hence, antioxidants or chemical agents with antioxidant properties may potently exert protective or ameliorative effects against such damage. The antioxidant defense system includes endogenous enzymes, such as ALT and AST, which have been described as serum biomarkers of hepatic tissue damage [29]. According to the findings of this study (Fig. 3), the non-significant elevation of serum ALT and AST concentrations in experimental animals in treated Groups C–F (compared to the values in normal control Group A) may also be associated with VE and GTE treatments that helped to attenuate oxidative responses following lead exposure.

The absence of such treatment in test control group B can be related with significant elevated levels of these antioxidant enzymes, indicating prominent hepatic tissue damage.

Furthermore, the findings of this study (Fig. 4) showed that treatment with VE and GTE account for relative reparation of hepatic histomorphology in treated Groups C–F making them comparable with histomorphology of the normal control Group A but the test control Group B showed prominent hepatic histomorphological alterations. As a result, the hepatic histomorphological features such as sizes of hepatocytes, hepatic sinusoids, and hepatic necrosis showed marginal variation while centrilobular congestion and leukocyte infiltration was moderately increased in treated Groups C–E compared to normal control Group A (Table 1). However, prominent variation was observed in hepatic histomorphological features of test control Group B compared to normal control Group A. These can be attributed to the ameliorative activity of VE and GTE treatment against deleterious effect of lead exposure in treated Groups C–E animals and unavailability of such treatment in test control Group B.

From previous studies, the VE has been described as an important unit of the antioxidant defense system which actively binds oxidizing free radicals, potently scavenges reactive oxygen species, thereby inhibiting lipid peroxidation and helping to prevent or ameliorate various tissue pathological conditions that result from oxidative stress [20,30–32]. Similarly, GTE has been described to exhibit various medicinal properties, including antioxidant and anti-lipid peroxidation activities [33–35]. Its antioxidant effects, which is often due to its potent free radical scavenging activity, is supported by the chelation of metal ions by their constituent cathecins to further offer cytoprotection against damaging effects of hepatotoxins exposure, including lead-induced hepatotoxicity [36,37].

On the whole, the exposure to environmental toxic agents (such as heavy metals like lead) causes negative alterations of physical, biochemical, histomorphological status of many vertebrate tissues, including the hepatic tissue. Conversely, chemical agents (like Vitamin E) or phytochemicals of herbal mixtures (like green tea) with intrinsic anti-oxidative properties can each potently suppress or attenuate the deleterious effects of heavy metals (like lead) on tissues including hepatic tissue. Further studies are suggested on the possibility of therapeutic synergy of VE and GTE against the harmful effects of heavy metal (such as Lead) exposure.

Conclusion

The Vitamin E and green tea extract comparatively exhibited potent ameliorative effects against the damaging effects of lead exposure on hepatic histomorphology resulting in historeparation of the hepatic tissue of experimental animals. The most potent dosages for their hepato-reparative activity were 200 and 10 mg/kg, respectively. Hence, the therapeutic choice may be based on other considerations such as accessibility and affordability.

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Nil.

Conflicts of interest

There are no conflicts of interest

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