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Original Article

Comparative amelioration of renal histomorphology by ascorbic acid and *Camellia sinensis* extract in Wistar rats exposed to Lead-induced nephropathy

Dayo Rotimi Omotoso[1], Wasiu Mathew Owonikoko[2], Winners Prince Ehiemere[1]

[1]Department of Anatomy, College of Health Sciences, Igbinedion University, Okada, Edo State, Nigeria
[2]Department of Physiology, College of Health Sciences, Igbinedion University, Okada, Edo State, Nigeria

Abstract

Aim: To compare the ameliorative effects of ascorbic acid and *Camellia sinensis* extract on renal histomorphology of lead-induced nephropathic rats.

Material and methods: Twenty four rats were randomly grouped into four groups (I–IV) as follows: Normal control group I administered distilled water (5 ml/kg), Test control group II administered lead acetate (2 mg/ml), Treated group III administered lead acetate (2 mg/ml) + ascorbic acid (100 mg/kg), Treated group IV administered lead acetate (2 mg/ml) + *Camellia sinensis* extract (10 mg/kg). All administrations were done through oral route and lasted for 30 days.

Results: Body and renal tissue weight of study animals were significantly reduced ($p < 0.05$) in test control relative to normal control while treated groups showed non-significant reduction. Histological examination of renal tissue showed significant prominence of histopathological features such as inflammation, necrosis and glomerular congestion in test control group while treated groups showed marked reduction.

Conclusion: According to the findings of this study, treatment with ascorbic acid or *Camellia sinensis* extract comparatively exert ameliorative effects on lead-induced nephropathy in Wistar rats which in turn culminates into reparative influence on their renal histomorphology.

Keywords: Ascorbic acid; *Camellia sinensis*; Lead-induced nephropathy; Wistar rats

INTRODUCTION

Lead is a heavy metal widely applied in the production of various domestic and industrial wares such as ceramics, plastics, batteries, and wire cables as well as many industrial activities like soldering, welding and furniture re-finishing (1). However, it is a common environmental pollutant that is abundantly released through car exhaust, automobile emissions and industrial wastes as well as during aforementioned industrial activities (1,2). The widespread cytotoxic effects of lead have been reported to affect numerous tissues in the body including liver, spleen, testis, brain and kidney (3-6). Such lead-induced cytotoxicity and/or tissue pathologies usually results from increased generation of reactive oxygen species (ROS) and accelerated peroxidation of tissue membrane lipids (7,8). In essence, naturally occurring substances including ascorbic acid and Camellia sinensis extract (CSE) with antioxidant properties may be applied to ameliorate the cytotoxic effects of lead exposure on body tissues. The ascorbic acid (AA) is a dietary antioxidant that has been widely used to ameliorate or prevent oxidative damage resulting from cytotoxins exposure (9-11). Similarly, Camellia sinensis (or green tea) is a common beverage with constituent phytochemicals that exhibit antioxidant properties which invariably confer therapeutic potentials on the beverage (12-14). In this study, the reparative potency of these two natural antioxidants against damaging effects of lead exposure on renal histomorphology of experimental Wistar rats was assessed and compared.

MATERIAL and METHODS

Chemical reagents and CSE

Reagents used in this study were of analytical grade and procured from Bristol Scientific Co. Ltd. (Lagos, Nigeria). Green tea beverage manufactured in China was purchased

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Corresponding Author: Dayo Rotimi Omotoso, Department of Anatomy, College of Health Sciences, Igbinedion University, Okada, Edo State, Nigeria, **E-mail:** dayohmts@gmail.com

locally from Steven Chuks Global Associate Ltd. (Lagos, Nigeria) and used to prepare the CSE following the method by Khan et al (15).

Experimental animals

This study involved twenty four adult Wistar rats having body weight ranging between 145-165 g. The animals were housed at the Central Animal Facility of Igbinedion University Okada, Nigeria. They were kept in cages under hygienic conditions and exposed to 12/12 h light/dark cycle, with room temperature of 25 ± 2ºC and relative humidity of 50-55%. The animals were fed on standard feed and allowed unrestricted access to drinking water.

Experimental design

The animals used for this study were randomly grouped into four groups (I–IV) comprising of six animals per group (n=6). The treatment regimen was as follows: Group I (normal control) comprised animals that were given distilled water (5 ml/kg body weight (b.w.)). Group II (test control) animals at were given lead acetate in drinking water (2 mg/ml). Group III comprised animals that were given lead acetate (as in group II above) + AA (100 mg/kg b.w.). Group IV animals were given lead acetate (as in group II above) + CSE (10 mg/kg b.w.). The route of treatment was oral using a flexible orogastric gavage and the period of treatment lasted for 30 days. During the treatment period, total body weight of animals were measured and recorded on days 0, 10, 20 and 30.

Ethical Approval

This study was carried out following approval by the Research and Ethics Committee of Igbinedion University, Okada, Edo State, Nigeria. All experimental procedures adhere to International guidelines for the use and handling of experimental animals.

Processing of study tissue

After the study period, study animals were sacrificed, the right and left kidney tissues collected, weighed and average weight value determined for each animal in control and treated groups. The tissues were subsequently processed as follows: fixation in 10% neutral buffered formalin, dehydration using ascending concentrations grades of alcohol (including 70%, 90% and absolute alcohol), clearing in xylene followed by paraffin embedding to form solid tissue blocks.

Sectioning of tissue blocks and staining of tissue sections

The tissue blocks were sectioned at thickness of 5 µ using rotary microtome and tissue sections were stained using Haematoxylin and Eosin (H & E) technique. The H & E staining technique is given as follows: Tissue sections were dewaxed in xylene and hydrated with descending grades of alcohol (including 100%, 90% and 70% alcohol). Tissue sections were stained in Haematoxylin and rinsed in water. Tissue sections were differentiated in 1% acid alcohol, blued in Scott's tap water and rinsed in water. Tissue sections were stained in Eosin and rinsed in water. Tissue sections were dehydrated with ascending grades of alcohol (including 70%, 90% and 100% alcohol), cleared in xylene and mounted with DPX (16).

Histopathological study

Stained tissue sections were viewed under microscope to assess the renal histomorphology of the study animals in control and treated groups. Photo-micrographs were generated for all stained tissue sections and further examination involved quantification using image-J software and comparative analysis of renal histopathological features of control and treated animals.

Statistical analysis

Data recorded during this study were analyzed using IBM-SPSS (version 20) and results presented as Mean ± standard error of mean (SEM). Comparison of statistical results was done using t-test and one way analysis of variance (ANOVA) with the probability level of $p < 0.05$ taken as statistically significant.

RESULTS

Evaluation of total body and study tissue weight

The mean values of total body weight of study animals in control and treated groups (I-IV) measured on days 0, 10, 20 and 30 were given in Table 1. In comparison to normal control group I, the mean body weight of test control Group II animals showed significant ($p < 0.05$) reduction during the period of study while treated groups III and IV showed non-significant reduction. In addition, the mean values of kidney tissue weight in study animals were given in Table 2. In comparison to normal control group I, the mean kidney tissue weight of test control group II animals showed significant ($p < 0.05$) reduction while treated groups III and IV only showed non-significant reduction.

Table 1. Mean values of body weight of study animals in control and treated groups I-IV measured at intervals between days 0 and 30 of study

	Day 0 (g)	Day 10 (g)	Day 20 (g)	Day 30 (g)
Group I	160.5 ± 2.13	162.5 ± 1.85	164.5 ± 2.46	165.5 ± 3.87
Group II	161.5 ± 1.55	156.0 ± 2.23*	153.5 ± 3.12*	151.5 ± 4.77*
Group III	160.5 ± 2.25	160.0 ± 3.44	159.5 ± 2.78*	161.5 ± 3.79
Group IV	161.5 ± 3.11	162.0 ± 1.98	161.5 ± 2.86	161.5 ± 4.13

Group I = Normal control group (Distilled water 5 ml/kg)
Group II = Test control group (Lead acetate 2 mg/ml)
Group III = Treated group (Lead acetate 2 mg/ml + Ascorbic acid 100 mg/kg)
Group IV = Treated group (Lead acetate 2 mg/ml + Camellia sinensis extract 10 mg/kg)
Data are expressed as Mean ± SEM of six animals per group (n=6).
* represented significant difference relative to Group I (p value < 0.05)

Table 2. Mean values of right kidney, left kidney and average kidney tissue weight of study animals in control and treated groups I-IV

	Right Renal Tissue Weight	Left Renal Tissue Weight	Average Renal Tissue Weight (g)
Group I	6.25 ± 0.35	6.05 ± 0.38	6.15 ± 0.28
Group II	4.55 ± 0.24*	4.25 ± 0.25*	4.25 ± 0.29*
Group III	5.65 ± 0.33+	5.45 ± 0.36+	5.63 ± 0.26+
Group IV	5.85 ± 0.21+	5.65 ± 0.27+	5.79 ± 0.31+

Group I = Normal control group (Distilled water 5 ml/kg)
Group II = Test control group (Lead acetate 2 mg/ml)
Group III = Treated group (Lead acetate 2 mg/ml + Ascorbic acid 100 mg/kg)
Group IV = Treated group (Lead acetate 2 mg/ml + Camellia sinensis extract 10 mg/kg)
Data are expressed as Mean ± SEM of six animals per group (n=6).
* represented significant difference relative to Group I (p value < 0.05)
+ represented significant difference relative to Group II (p value < 0.05)

Histopathological result

Assessment of stained tissue sections under microscope revealed renal histomorphology of study animals in normal control group I, test control group II and treated groups III and IV showing histopathological features which include inflammation, necrosis and glomerular congestion in the renal parenchyma following the exposure to heavy metal - lead (Figures 1). Evaluation of renal histopathological features showed that, in comparison to normal control group I, the test control group II animals exhibited significant ($p < 0.05$) prominence while in treated groups III and IV, they were non-significantly increased (Table 3).

Table 3. Evaluation of kidney tissue inflammation, necrosis and glomerular congestion within the renal parenchyma of study animals in control and treated groups I-IV

	Renal Tissue Inflammation	Renal Tissue Necrosis	Glomerular congestion
Group I	0.00	0.00	0.00
Group II	4.83 ± 0.60*	4.34 ± 0.52*	3.48 ± 0.43*
Group III	1.67 ± 0.32+	1.80 ± 0.41+	1.49 ± 0.38+
Group IV	1.76 ± 0.42+	1.91 ± 0.31+	1.43 ± 0.31+

Group I = Normal control group (Distilled water 5 ml/kg)
Group II = Test control group (Lead acetate 2 mg/ml)
Group III = Treated group (Lead acetate 2 mg/ml + Ascorbic acid 100 mg/kg)
Group IV = Treated group (Lead acetate 2 mg/ml + Camellia sinensis extract 10 mg/kg)
Data are expressed as Mean ± SEM of six animals per group (n=6).
* represented significant difference relative to Group I (p value < 0.05)
+ represented significant difference relative to Group II (p value < 0.05)

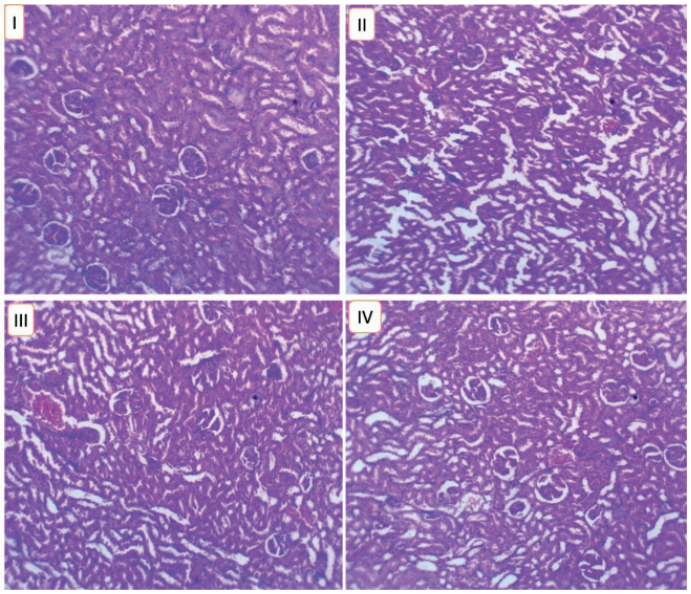

Figure 1. Histological micrograph showing renal histomorphology of study animals in control and treated groups I – IV. Group I = Normal control group (Distilled water 5 ml/kg); Group II = Test control group (Lead acetate 2 mg/ml); Group III = Treated group (Lead acetate 2 mg/ml + Ascorbic acid 100 mg/kg); Group IV = Treated group (Lead acetate 2 mg/ml + Camellia sinensis extract 10 mg/kg)

DISCUSSION

Lead has long been described as the most popular among toxic heavy metals whose exposure results into accumulation in body tissues including the kidney. The kidney tissue contains certain proteins which readily bind with lead and promotes its accumulation in the tissue (17). Such bioaccumulation results into lead poisoning characterized by diverse histopathological presentations including necrosis, fibrosis, tubular atrophy and so on (18,19). These damaging effects of lead poisoning following toxic exposure to bodily tissues including kidney can result in marked loss of organ and body weight (3). According to the results of this study, lead exposure caused significant ($p < 0.05$) decrease in body weight of animals exposed without treatment relative to non-exposed animals. However, the body weight of exposed and treated animals showed no significant decrease relative to the non-exposed animals (Table 1). Similar results were obtained for kidney tissue weight of study animals (Table 2). The import of these findings is that the treatment with either AA or CSE may ameliorate the damaging effect of lead poisoning thereby forestalling destruction of parenchyma of multiple body tissues (including kidney tissue) and consequent loss of body or kidney tissue weight.

Further, histopathological presentations associated with lead poisoning have been described to result from oxidative stress and damage of multiple bodily tissues including the kidney (20,21). However, oxidative damage due to generation of highly reactive free radicals can be actively prevented or ameliorated by substances with

potent antioxidant activity (21,22).

According to the histopathological results of this study (Figure 1), exposure to lead caused significant ($p < 0.05$) increase in renal histopathological features of animals exposed without treatment relative to the non-exposed animals. However, there was no significant prominence of histopathological features in animals exposed and treated when compared to non-exposed animals (Table 3). These findings can be related with earlier report that the AA exhibited antioxidant property by enhancing antioxidant enzymes activity and suppressing ROS production, free radicals activity and membrane lipid peroxidation (9-11). Also, CSE has been profiled to contain phytochemicals that exhibit anti-oxidant properties which in turn promote the therapeutic effects of the beverage (14,23). Hence, based on the findings of this study, the ascorbic acid and Camellia sinensis extract can distinctly ameliorate the renal histomorphology of lead-induced nephropathic Wistar rats and such therapeutic effect can be comparatively linked to their antioxidant activity.

CONCLUSION

Dietary supplements like ascorbic acid or herbal beverages like green tea (Camellia sinensis), often used in traditional medicines, contain chemical or phytochemical constituents with intrinsic antioxidant property. This in turn promotes their comparative therapeutic activity including the amelioration of renal histomorphology of lead-induced nephropathic rats.

Recommendation

Further studies are recommended to probe therapeutic potentials of distinct active ingredients of the herbal green tea and to relate possible mechanisms of such therapeutic activity with other natural antioxidants.

Conflict of interest: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: This study was carried out following approval by the Research and Ethics Committee of Igbinedion University, Okada, Edo State, Nigeria.

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