Original Article

Nephroprotective Effect of *Citrus sinensis* L. on Mercury Exposed Wistar Rats

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Abstract

Context: Exposure to all forms of mercury has toxic effect on several biosystems. Disruption in structural, biochemical, and physiological renal integrity results in nephrotoxicity. *Citrus sinensis L* (sweet orange) has medicinal benefits for diverse ailments. **Aim:** This study evaluated the nephroprotective effect of ethanol fruit peel extract of *C. sinensis* (EPCS) against mercuric chloride ($HgCl_2$)-triggered nephrotoxicity in Wistar rats. **Materials and Methods:** Twenty-five Wistar rats (150–180 g) were separated into five Groups (I-V, $n = 5$): Group I received normal saline (1 ml/kg); Group II received $HgCl_2$ (5 mg/kg); Group III received reference drug, silymarin (100 mg/kg) +$HgCl_2$ (5 mg/kg); Group IV received EPCS (750 mg/kg) +$HgCl_2$ (5 mg/kg); and Group V received EPCS (1250 mg/kg) +$HgCl_2$ (5 mg/kg). Treatments were for 14 days. Nephroprotective effect was evaluated using the biochemical assay for renal function, histological, and histochemical assessments using H and E and periodic acid Schiff (PAS) stains and quantification of PAS staining intensity using Imagej® NIH, US. **Statistical Analysis Used:** One-way analysis of variance with Tukey *post hoc* test. **Results:** The results revealed remarkable ($P < 0.05$) alterations in renal functional biomarkers, especially urea, creatinine, and Na + in $HgCl_2$-treated group compared to the controls. However, EPCS treatment ameliorated alterations comparable to the silymarin and control groups. Histological and histochemical examinations revealed severe distortions in renal histoarchitecture such as shrunken glomerulus and Bowman-space dilatation for the rats exposed to $HgCl_2$ compared to controls. However, EPCS treatment ameliorated distortions by the preservation of renal histoarchitecture comparable to the silymarin and control groups. No remarkable difference for PAS staining intensity relative to controls. **Conclusion:** EPCS possesses potential nephroprotective effect against mercuric chloride-induced toxicity in Wistar rats.

Keywords: Histochemistry, histology, nephrotoxicity, silymarin, staining intensity

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Introduction

The earth's core is made up of some substances that exhibit metallic properties called heavy metals, such as lead (*Pb*), cadmium (*Cd*), and mercury (*Hg*).[1] These heavy metals are deleterious to body tissues which have generated great concerns in human and animal health.[2] Exposure to all forms of mercury through the consumption of contaminated water and food have toxic effect on several biosystems such as neurological, immune, and reproductive.[3-5] Nephropathy has been associated with exposure to conjugates of mercury. Mercury triggered-toxicity results in oxidative stress which causes cellular damage and disruption of homeostasis[1] attributed to the generation of reactive oxygen species (ROS).[6]

The kidney carries out several vital biochemical and physiological roles such as the regulation of homeostasis, extracellular environment regulation such as detoxification and excretion of xenobiotics substances, it can be said to mediate in the toxicity of numerous harmful substances.[7-9] Kidney structure is made up of numerous cells which form a unit called the nephron. Deleterious stimulations of the nephron could trigger cell death and tissue damage resulting in renal

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failure.[10] The nephrons are the primary sites for mercuric chloride toxicity.[11] Disruption in kidney functions results in nephrotoxicity.[7]

Mankind, from the dawn of time, has used natural agents as the source of remedy for ailments,[12] and majority of the populations in tropical countries depend on traditional medical remedies such as herbs.[13] In Nigeria, several traditional cultures use herbs as remedies for ailments.[12] *Citrus sinensis L* (sweet orange) fruit has been consumed for many centuries, and the peel has been found to have medicinal benefits for ailments such as gastrointestinal colic and gastric ulcers.[14,15] *C. sinensis* fruit tree is from the *Rutaceae* family.[16] Sweet orange according to the "encyclopaedia of life" is the most commonly grown tree fruit in the world. The flesh or pulp of the fruit is typically juicy and sweet and ranges in color from yellow to orange to red. The fruit's skin (rind or peel) contains numerous small oil glands.[17] The sweet orange peel is rich sources of bio-functional material, which is the importance of these by-products.[18] *C. sinensis* peel has a number of uses.[19] The peel contains beneficial phytochemicals such as flavonoid[20] and has been reported to have several pharmacological benefits such as antioxidant,[21] anti-inflammatory,[22] anticancer,[23] and anti-lipidemic[19] properties.[24]

This study evaluated the nephroprotective effect of ethanol extract of *C. sinensis L* peel (EPCS) against mercuric-triggered nephrotoxicity in Wistar rats.

Materials and Methods

Experimental animals

Twenty-five Wistar rats weighing 150–180 g were procured from the Animal House, Department of Pharmacy, University of Abuja, Gwagwalada, Federal Capital Territory. Thereafter, they were kept at the Animal House, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University (ABU), Zaria where they were fed and clean water was provided *ad-libitum*. The rats were allowed to acclimatize for two weeks before the commencement of the study. Rats were housed under standard laboratory conditions, light and dark cycles of 12 h was provided.

Plant material

C. sinensis L osbeck fruit was obtained from a local market, Samaru market, Zaria, then taken to the Herbarium Unit of the Department of Biological Sciences, Faculty of Life Sciences, ABU, Zaria for authentication.

Drugs and chemicals

Mercuric chloride was used as a nephrotoxic agent in this study. The product is manufactured by May and Bakers Limited, Dagenham, England.

Silymarin (Silybon-140®), a well-known nephroprotective agent, manufactured by Micro Labs Limited 92, India, Silymarin was used as the reference drug to evaluate the properties of *C. sinensis* fruit peel extract.

Ketamine (Ketamine Hydrochloride injection USP, 50 mg/ml) was used as anaesthetic agent in this study. The product is manufactured by Swiss Parenterals PVT Ltd, Gujarat, India.

All drugs and chemicals were obtained from reputable suppliers in Samaru, Zaria.

Plant extraction

Preparation of ethanol fruit peel extract of *C. sinensis* (EPCS) was conducted as follows:

The fruits of *C. sinensis* were peeled off of their epicarp (fruit skin). The epicarps were shade-dried under laboratory temperature and pressure and grounded to powder with the aid of a pestle and mortar. The powder of 175 g was extracted using a Soxhlet extraction method (75% ethanol) in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria.

Acute toxicity study

The oral LD_{50} for EPCS was determined using the method described by Lorke.[25] No signs of toxicity or mortality were observed in the rats upon administration of the extract up to dose 5000 mg/kg. From this result, two different doses (750 mg/kg and 1250 mg/kg) were selected for the subsequent studies.

Experimental design

Twenty-five Wistar rats were randomly assigned into five Groups (I-V) consisting of five rats each. Group I served as the control group and was administered normal saline (1 ml/kg). Nephrotoxicity was induced in rats by the administration of mercuric chloride as reported by Sheikh *et al*. Group II was administered with 5 mg/kg $HgCl_2$ (12.5% LD_{50}; 40 mg/kg as reported by Sheikh *et al*.[26] Group III was administered silymarin at (100 mg/kg) as reported by Ahmed *et al*.[27] and $HgCl_2$ (5 mg/kg). Group IV was administered EPCS (750 mg/kg) and $HgCl_2$ (5 mg/kg). Group V was administered EPCS (1250 mg/kg) and $HgCl_2$ (5 mg/kg).

All administrations were through oral routes and lasted for 14 days [Table 1].

Physical observation and animal sacrifice

During the experimental period, physical behavioral patterns such as agility and feeding were observed. The rats were weighed before and after the study, and the body weight change

Table 1: Experimental protocol

Groups	Treatment
I	Normal saline (1 ml/kg)
II	$HgCl_2$ (5 mg/kg)
III	Silymarin (100 mg/kg) + $HgCl_2$ (5 mg/kg)
IV	EPCS (750 mg/kg) + $HgCl_2$ (5 mg/kg)
V	EPCS (1250 mg/kg) + $HgCl_2$ (5 mg/kg)

All administrations were via oral route for a duration of 14 days. *n*=5; EPCS: Ethanol fruit peel extract of *Citrus sinensis*, $HgCl_2$: Mercuric chloride

was calculated as the difference between final weight (FW) and initial weight. At the end of the experiment, rats were humanely sacrificed following ketamine (75 ml/kg) anesthesia.[28]

A mid-line abdominal incision was made to expose the abdominal cavity, and kidneys were collected and weighed using a digital weighing scale. Organosomatic index (OSI) was computed (kidney weight/body weight) ×100[29] and analyzed.

Harvested kidneys were fixed using neutral-buffered formalin for histological processing. Blood samples were collected through jugular vein method and transferred into plain sample bottles for the biochemical analysis.

Biochemical studies

Biochemical study involved kidney function tests. Serum kidney proteins and electrolytes analyzed include: Urea, creatinine, potassium (K+), hydrogen bicarbonate (HCO_3^-), sodium (Na+), and chloride (Cl^-). Biochemical analysis was conducted at the Department of Chemical Pathology, Faculty of Basic Clinical Sciences, ABU Teaching Hospital, Shika.

Histological and histochemical studies

The fixed kidneys were processed for light microscopy using the histological tissue processing techniques in the Histology Unit of the Department of Human Anatomy, ABU Zaria and photomicrography conducted in the Microscopy and Stereology Laboratory of the same facility. Histological sections were stained with hematoxylin and eosin (H and E) stains, as well as histochemical stain (periodic acid Schiff [PAS]).

Image analysis

Quantification of PAS reactivity to glycogen moiety distribution involved measuring the staining intensity of PAS-stained micrographs using a computer running image analysis software (imageJ® NIH, US) according to the manufacturer's instruction. The ImageJ region of interest (ROI) manager tool for the analysis of specific areas of the micrographs was employed to limit bias values resulting from nonidentical image quality (image acquisition setting and exposure times). The mean gray values for three ROI were obtained, means computed and analyzed [Figure 1].

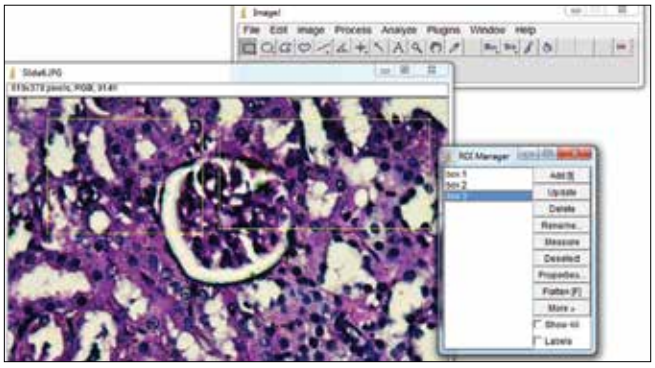

Figure 1: Measuring periodic acid schiff staining intensity using ImageJ software technique

Data analysis

Data collected were expressed as mean ± standard error of the mean. One-way analysis of variance was used to compare the mean difference between groups and Tukey's range test was applied as *post hoc* to compare the level of significance. $P < 0.05$ was considered statistically significant. Statistical analysis was carried out using the Statistical Package for the Social Sciences (IBM SPSS version 21, Armonk, NY: IBM Corp).

Results

Physical observation

In this study, rats in the control group exhibited normal agility and feeding habit. However, less agility and sluggish movement were observed in rats exposed to 5 mg/kg mercuric chloride. Rats exposed to EPCS showed improved agility and less sluggishness compared to rats exposed to 5 mg/kg mercuric chloride.

In this study, it was observed that there was no significant difference in FW when compared with the control. However, a significant difference in body weight change was observed in rats treated with 5 mg/kg mercuric chloride when compared to controls [Table 2].

OSI for all groups was not significantly different when compared to the control group [Figure 2].

Biochemical analysis

In this study, there was a significant increase in serum urea concentration levels of all groups when compared to control except rats exposed to silymarin + mercuric chloride. On the other hand, there was no significant difference in the urea concentration of rats exposed to EPCS compared to rats exposed to silymarin [Figure 3].

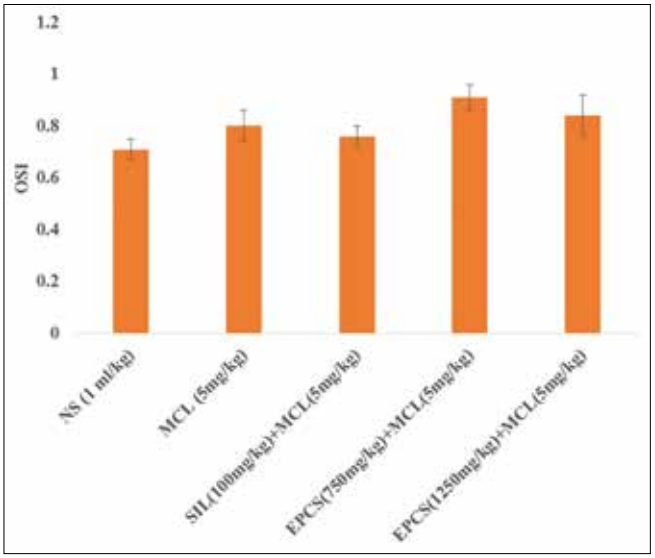

Figure 2: The effect of EPCS on organosomatic index (OSI) of mercuric chloride exposed Wistar rats. $n = 5$; mean ± standard error of mean, one-way analysis of variance; $P > 0.05$ when compared with the control; EPCS: Ethanol fruit peel extract of *Citrus sinensis*, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride

Table 2: Effect of ethanol fruit peel extract of *Citrus sinensis* on body weight in Wistar rats

Group/treatment	IW (g)	FW (g)	WC (g)
NS (1 ml/kg)	166.40±8.44	191.02±7.52	24.60±3.14
MCL (5 mg/kg)	169.60±3.69	178.20±3.25	8.60±1.75*
SIL (100 mg/kg) + MCL (5 mg/kg)	160.20±2.49	176.80±5.67	16.60±2.23
EPCS (750 mg/kg) + MCL (5 mg/kg)	171.20±7.49	185.40±5.41	14.20±5.03
EPCS (1250 mg/kg) + MCL (5 mg/kg)	168.21±7.74	192.60±6.42	21.40±2.77

n=5; mean±SEM, One-way ANOVA followed by Tukey *post hoc* test; *P<0.05 when compared with the control (1 ml/kg NS), EPCS: Ethanol fruit peel extract of *Citrus sinensis*, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride, IW: Initial Body Weight; FW: Final Body Weight, WC: Weight Change, SEM: Standard error mean

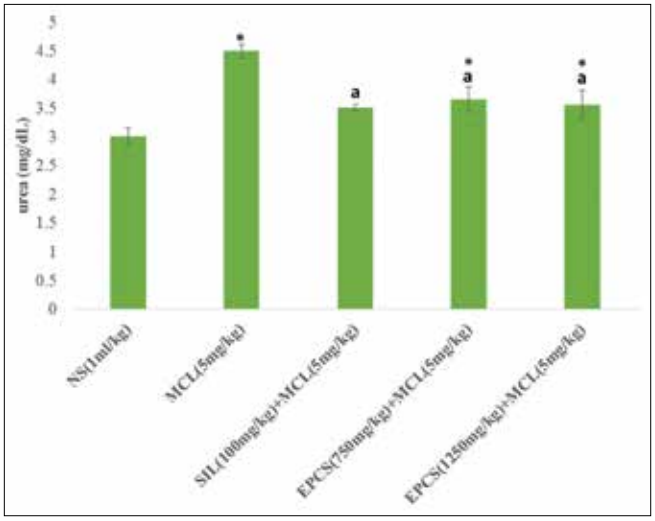

Figure 3: The effect of EPCS on serum urea concentration in mercuric chloride exposed Wistar rats. $n = 5$; mean ± standard error of mean, One-way analysis of variance followed by Tukey *post hoc* test; *$P < 0.05$ when compared with the control (1 ml/kg NS). [a]$P < 0.05$ when compared with the MCL. EPCS: Ethanol fruit peel extract of *Citrus sinensis*, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride

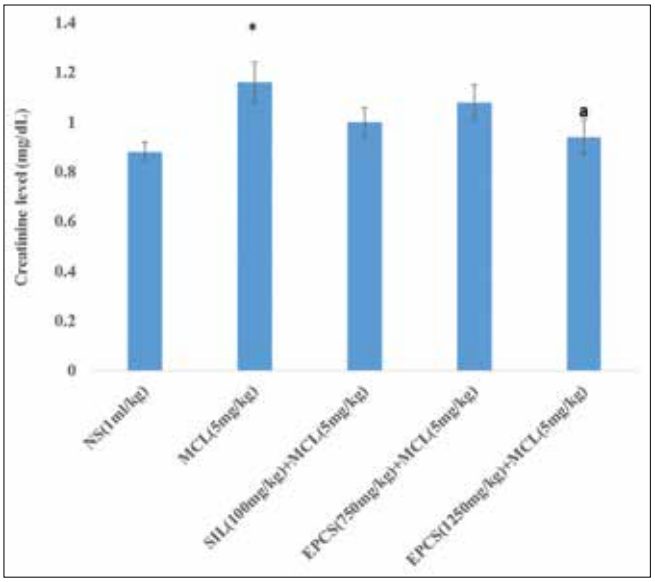

Figure 4: The effect of EPCS on serum creatinine concentration in mercuric chloride exposed Wistar rats. $n = 5$; mean ± standard error of mean, One-way analysis of variance followed by Tukey *post hoc* test; *$P < 0.05$ when compared with the control (1 ml/kg NS). [a]$P < 0.05$ when compared with the MCL. EPCS: Ethanol fruit peel extract of *Citrus sinensis* peel, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride

In this study, it was observed that there was no significant difference in the creatinine level of rats in all groups when compared with controls except rats administered with mercuric chloride. However, there was significant difference in the creatinine levels of groups exposed to EPCS and mercuric chloride except rats treated with 1250 mg/kg of EPCS [Figure 4].

In this study, there was significant difference in serum sodium ion (Na+) levels of all groups compared to controls, except for rats exposed to silymarin + mercuric chloride [Figure 5]. There was no significance difference in serum chloride ion (Cl^-) levels compared to controls, except for rats exposed to EPCS (1250 mg/kg) + mercuric chloride [Figure 6]. There was no significant difference in serum potassium (K+) and bicarbonate ion ($HC0_3^-$) levels of all groups compared to controls [Figures 7 and 8].

Histology and histochemical studies

In this study, histological examinations were carried out on sections of the kidney stained with H and E stains for general histoarchitecture and histochemical staining with PAS for glycogen moiety. Rats in the control group administered with normal saline (1 ml/kg) showed normal histoarchitechture, glomerulus, proximal convoluted tubules, distal convoluted tubules, and Bowman's space. Histochemically, sections demonstrated a well-defined basement membrane of the renal corpuscles and tubular epithelium with PAS staining [Figures 9a and 10a].

In this study, rats treated with mercuric chloride (5 mg/kg) revealed severe histoarchitectural distortion such as shrunken glomerulus and Bowman space dilatation. Reduced staining intensity was observed with PAS staining relative to controls [Figures 9b and 10b].

In this study, rats treated with silymarin (100 mg/kg) and mercuric chloride (5 mg/kg) showed mildly distorted histoarchitecture. A normal staining intensity with PAS staining was observed compared to controls [Figures 9c and 10c].

In this study, rats exposed to 750 mg/kg EPCS and mercuric chloride (5 mg/kg) showed relatively normal histoarchitecture. Reduced staining intensity was observed with PAS staining [Figures 9d and 10d].

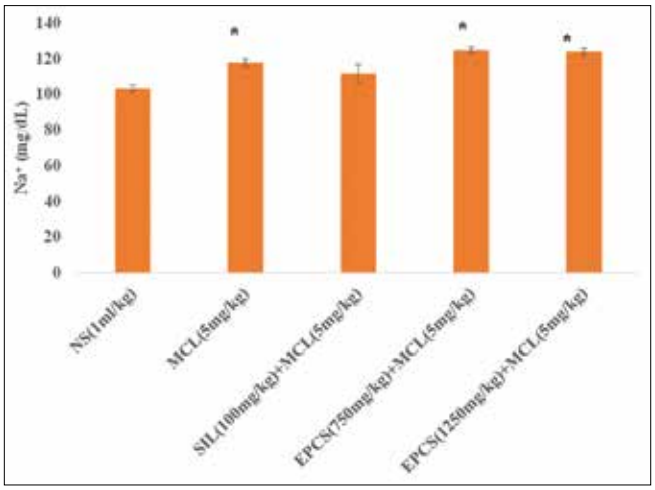

Figure 5: The effect of EPCS on serum Sodium ion (Na+) concentration in mercuric chloride exposed Wistar rats. $n = 5$; mean ± standard error of mean, One-way analysis of variance followed by Tukey *post hoc* test; *$P < 0.05$ when compared with the control (1 ml/kg NS). EPCS: Ethanol extract of *Citrus sinensis* peel, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride

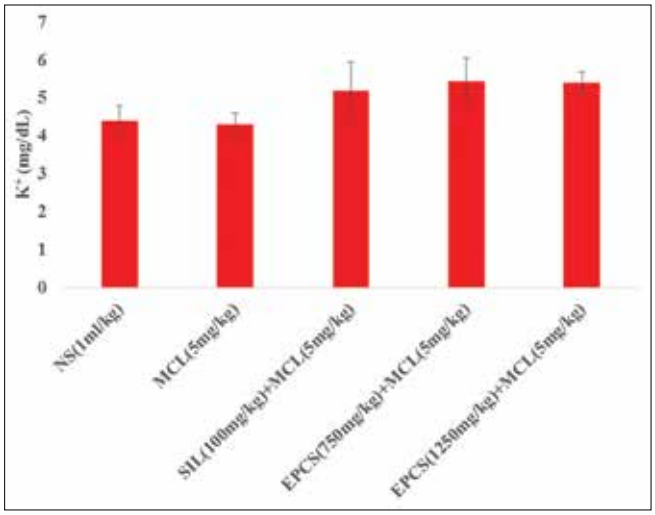

Figure 7: The effect of EPCS on serum Potassium ion (K+) concentration in mercuric chloride exposed Wistar rats. $n = 5$; mean ± standard error of mean, One-way analysis of variance followed by Tukey *post hoc* test; $P > 0.05$ when compared with the control (1 ml/kg NS) EPCS: Ethanol extract of *Citrus sinensis* peel, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride

In this study, rats treated with 1250 mg/kg EPCS and mercuric chloride (5 mg/kg) showed relatively normal histoarchitecture. Normal staining intensity with PAS staining when compared with controls [Figures 9e and 10e].

Image analysis

There was no significant difference in staining intensity for all groups when compared with controls [Figure 11].

Discussion

In this study, physical observations, biochemical, histological, and histochemical assessments were carried out to evaluate the nephroprotective effect of EPCS on Wistar rats exposed to mercuric chloride.

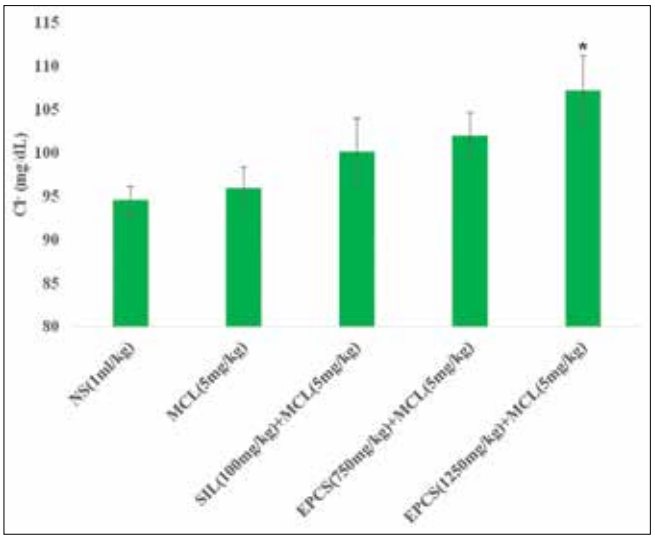

Figure 6: The effect of EPCS on serum Chloride ion (Cl−) concentration in mercuric chloride exposed Wistar rats. $n = 5$; mean ± standard error of mean, One-way analysis of variance followed by Tukey *post hoc* test; *$P < 0.05$ when compared with the control (1 ml/kg NS). EPCS: Ethanol extract of *Citrus sinensis* peel, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride

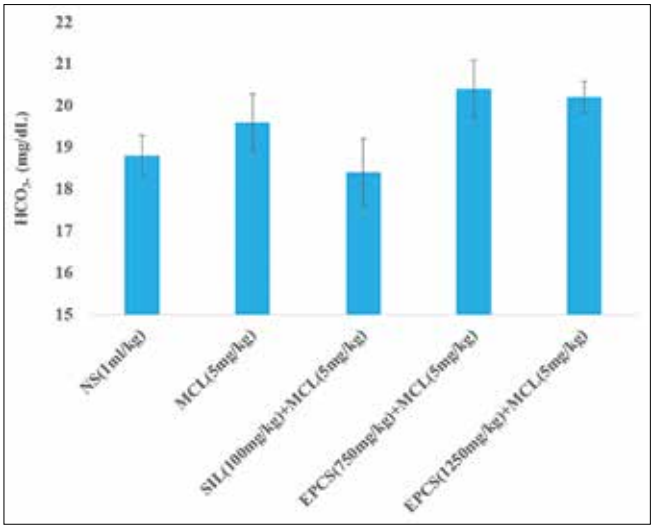

Figure 8: The effect of EPCS on serum Bicarbonate ion (HCO3−) concentration in mercuric chloride exposed Wistar rats. $n = 5$; mean ± standard error of mean, One-way analysis of variance followed by Tukey *post hoc* test; $P > 0.05$ when compared with the control (1 ml/kg NS). EPCS: Ethanol extract of *Citrus sinensis* peel, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride

Physical observation

The rats exposed to mercuric chloride showed less agility and reduced feeding activity when compared to the control group. This could be due to mercuric chloride toxicity which has been reported to trigger physiological changes.[30] Body weight changes have been reported as the indicator of the health status of animals.[18] A significant lower absolute body

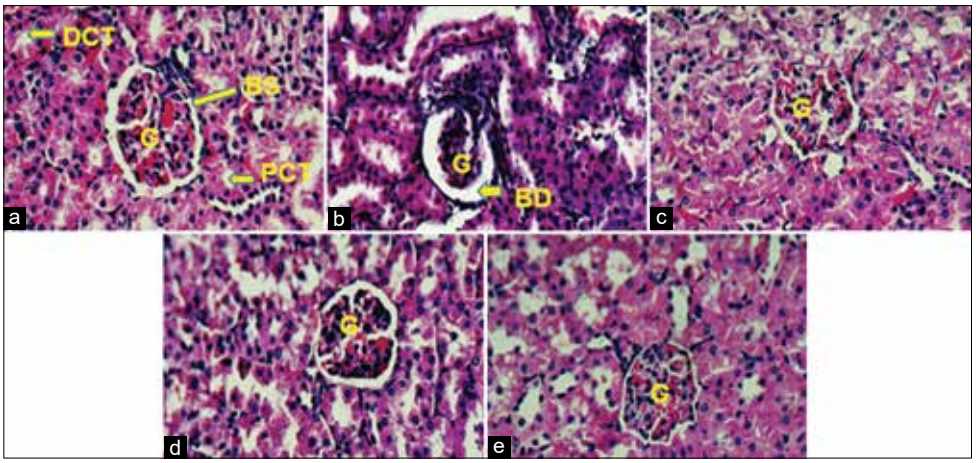

Figure 9: Micrograph of kidney section of Wistar rat (H and E ×250). (a) Control, treated with normal saline (1 ml/kg) with normal histoarchitecture. Glomerulus (G); Bowman's capsule space (BCS); proximal convoluted tubules (PCT); distal convoluted tubules (DCT). (b) Group treated with Mercuric Chloride (5 mg/kg) with histoarchitectural distortion. Shurken glomerulus (G); bowman space dilatation (BD). (c) Group treated with Silymarin (100 mg/kg) and mercuric chloride (5mg/kg) with relatively normal histoarchitecture. Glomerulus (G). (d) Group treated with 750 mg/kg of ethanol extract of *C. sinensis* peel and Mercuric Chloride (5mg/kg) with relatively normal histoarchitecture. Glomerulus (G). (e) Group treated with 1250 mg/kg ethanol extract of *C. sinensis* and Mercuric Chloride (5 mg/kg) with relatively normal histoarchitecture. Glomerulus (G)

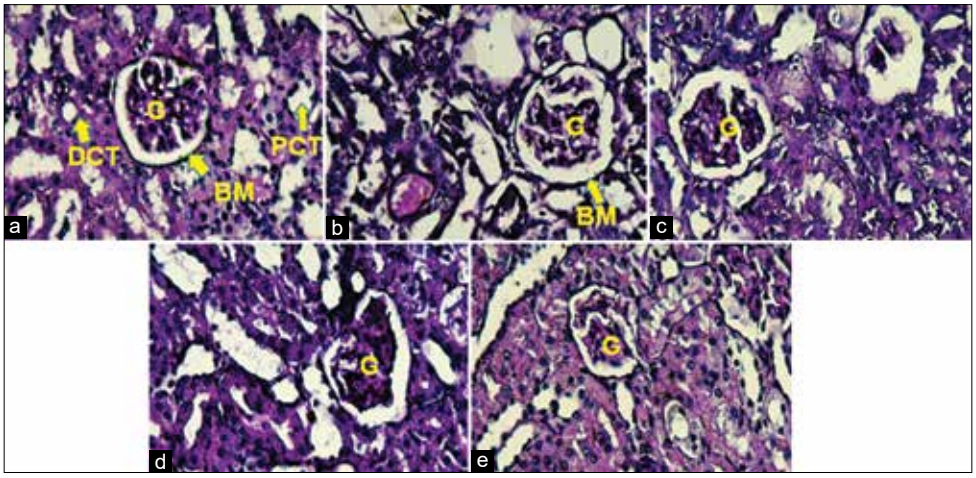

Figure 10: Micrograph of kidney section of Wistar rats (periodic acid schiff ×250). (a) Control, administered normal saline (1 ml/kg) with normal histoarchitecture and increased periodic acid schiff stain intensity. Basement membrane (BM); distal convoluted tubules (DCT); proximal convoluted tubules (PCT); Glomerulus (G). (b) Group treated with mercuric chloride (5 mg/kg) with relatively distorted histoarchitecture and reduced periodic acid schiff stain intensity; Glomerulus (G); Basement membrane (BM). (c) Group treated with Silymarin (100 mg/kg) and mercuric chloride (5 mg/kg) with relatively normal histoarchitecture and periodic acid schiff stain intensity. Glomerulus (G). (d) Group treated with 750 mg/kg ethanol extract of *Citrus sinensis* peel and mercuric chloride (5 mg/kg) with relatively normal histoarchitecture and reduced periodic acid schiff stain intensity. Glomerulus (G). (e) Group treated with 1250 mg/kg ethanol extract of *Citrus sinensis* peel and mercuric chloride (5 mg/kg) with relatively normal histoarchitecture and periodic acid schiff stain intensity. Glomerulus (G)

weight change was observed in rats treated with 5 mg/kg mercuric chloride when compared to controls. This could be due to mercuric chloride toxicity which affected appetite for feeding. This finding agrees with Thomas *et al*.[31] and Jadhav *et al*.[32] whom in their work observed that the body weight of rats treated with mercury chloride decreased significantly compared to the controls. The body weight change of rats exposed to 1250 mg/kg EPCS was significantly higher than rats exposed to mercuric chloride, and this is consistent with reports by Acar *et al*.[33] and Abbasi *et al*.,[34] which revealed that sweet orange peel essential oil can act as a growth promoter. Similarly, Salem and Abdel-Ghany[35] suggested that sweet orange peels have the ability to influence growth rate and improve feed intake in rats.

In this study, kidney SI showed no significant difference when compared to the control group. This is suggestive that the treatment had no effect on SI values. This is consistent with Abbasi *et al*.[34] whom reported that dried sweet orange pulp had no effect on organ weight of chickens after 35 days of exposure to *C. sinensis* dried peel. Similarly, Ajibade *et al*.[36] reported that there was no significant difference in organ-body weight ratio when exposed to mercuric chloride. In this study,

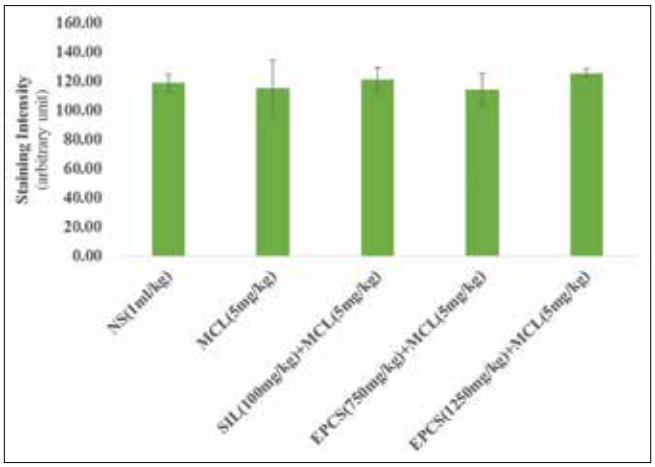

Figure 11: The effect of EPCS on periodic acid Schiff staining intensity in the kidney section of mercuric chloride exposed Wistar rats. n = 5; mean ± standard error of the mean, One-way analysis of variance followed by Tukey *post hoc* test; $P > 0.05$ when compared with the control (1 ml/kg NS). EPCS: Ethanol extract of *Citrus sinensis* peel, SIL: Silimarin, NS: Normal saline, MCL: Mercuric chloride

EPCS demonstrated more efficacy on body weight changes relative to the reference drug, silymarin following body weight assessment.

Biochemical assessments

Nephrotoxicity has been associated with alteration of renal-associated proteins (urea and creatinine) and electrolytes (K+, Na+ and Cl^-) concentrations in serum. In this study, elevated levels of urea and creatinine were observed in mercury-treated group. Renal-related diseases and functional disorders could be attributed to serum urea accumulation exceeding its clearance rate.[37] Ajami *et al*.[38] reported increased urea and creatinine levels to be strongly correlated with kidney injury and oxidative stress. Elevated ROS production may distort the filtration surface area and modify the filtration coefficient; this actually leads to decrease in glomerular filtration hence the accumulation of creatinine and urea in the blood serum. Mercuric chloride is an established nephrotoxic agent.[39,40] Regulation of serum urea and creatinine levels, a vital biomarker of renal functionality, is an important renal physiological process.[41] In this study, silymarin and EPCS-treated groups had lower urea and creatinine levels when compared to mercuric chloride-treated group. The finding is in agreement with Chen *et al*.[42] and Nasution *et al*.[43] on the ameliorative effect of EPCS on cellular damage. Urea and creatinine levels were comparable to that of the reference drug, silymarin, which implies similar efficacy in the regulation of serum renal protein levels following exposure to nephrotoxins such as mercury chloride.

In this study, observed remarkable increased concentration of Na^+ and Cl^- in the serum could be attributed to impaired renal tubular function. The kidney is responsible for fluid and electrolyte balance in the body. Chronic or acute toxicity can lead to electrolyte imbalance in distribution within the body.[44-46]

Histology and histochemical studies

Renal injury has been associated with alteration in the integrity of structural and functional unit of the kidney which impacts deleteriously on its functionality.[47] Mercuric chloride remains to be the most vital cause of nephrotoxicity in many parts of the world among all metals toxicities.[48,49]

In this study, rats treated with mercuric chloride revealed severe histoarchitectural distortion such as shrunken glomerulus and Bowman space dilatation. This was in agreement with Oda and El-Ashmawy[50] who observed necrotic tubules, glomerulus atrophy, and dilatation of Bowman's space.

In this study, rats exposed to EPCS showed relatively normal histoarchitecture similar to the reference drug silimarin and a relatively normal staining intensity with PAS staining when compared with controls. Observed histoarchitecture preservation with EPCS administration is in line with the report of Adil *et al*.[51] and Kosasih *et al*.[52] who stated that the nephroprotective effect of citrus by-products extracts may be due to presence of phytoconstituents such as polyphenolic compounds.

The ameliorative effects of EPCS could be associated with the antioxidant property of constituent phytochemicals such as flavonoids. These findings are in concurrence with those obtained by Mostafa *et al*.[53] and Kosasih *et al*.[52] that reported increasing dose of *C. sinensis* peel extract showed increased nephroprotective activity in experimentally-induced nephrotoxicity in Wistar rats exposed to gentamicin. Antioxidants have been established to mitigate oxidative stress-related pathologies directly, by scavenging of ROS.[54,55] The flavonoids, which are one of the main phytochemical constituent of orange peel, are is known to play a role as free radical scavengers or antioxidants in biological systems.[56] Flavonoids have many biological effects such as antioxidant, antibacterial, anticancer, antimutagenic, and anti-inflammatory properties.[57] In this study, the nephroprotective property of EPCS could be comparable to the reference drug, silymarin following histological assessments.

Conclusion

Ethanol extract of *C. sinensis L* peel possesses potential nephroprotective effect against mercuric chloride induced toxicity in Wistar rats. The nephroprotective property could be attributed to antioxidant properties of constituent phytochemicals such as flavonoids. Therefore, *C. sinensis L* peel could be useful in the management and treatment of mercury induced renal toxicity. Further studies are recommended to elucidate the mechanism involved in therapeutic potentials of this extract as a nephroprotective agent.

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Conflicts of interest

There are no conflicts of interest.

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