

Original Research Article

Assessment of renoprotective effect of *Pentaclethra macrophylla* seed (Ugba) against Mercury induced acute kidney injury in male Albino Rats

UNDER PEER REVIEW

ABSTRACT

Aim: The aim of this study was to assess the renoprotective effect of *Pentaclethra macrophylla* seed (Ugba) against Mercury induced acute kidney injury in male Albino Rats

Study design: This is an interventional study.

Place and Duration of Study: Department of Animal and Environmental Biology Animal House, Rivers State University, Port Harcourt, Nigeria, between January, 2019 and January, 2021.

Methodology: Thirty six (36) adult male albino rats weighing approximately $135 \pm 1.5g$ were purchased from University of Port Harcourt, Rivers State, Nigeria. They were housed in plastic suspended cages, placed in well ventilated conditions and provided with rat diet and water and acclimatized for two weeks. Fresh matured seeds of *Pentaclethra macrophylla* (African oil bean seed) were sourced locally from markets in Imo state, Nigeria. The washed and sliced seeds were stored at room temperature and allowed to ferment. The fermented seeds were dried, ground and preserved in airtight container in the refrigerator at $4^{\circ}C$. The total weight of the powdered seed of *Pentaclethra macrophylla* produced was 250g. Seeds were thoroughly washed with distilled water, cooked for more than 2 hours at $100^{\circ}C$. Maceration technique was used to carry out an ethanolic extract of the plant seed. Mercury chloride salt was purchased in Port Harcourt. A standard dose of $3.0mg/kg$ body weight of mercury chloride obtained from acute toxicity study dose determination was administered to the rats for 30 days after they were divided into six groups of six rats per group. After 30 days, all the animals were weighed, anaesthetized using chloroform. Blood was collected by cardiac puncture into plain tubes. Samples were obtained by centrifugation of the clotted blood at 3500rpm for 10 minutes. The serum specimen were stored at $2^{\circ}C$ prior to the biochemical analysis (estimation of blood urea nitrogen, creatinine, cystatin C and Kidney injury molecule -1 (KIM-1)). Blood urea nitrogen was estimated using the Berthelot's enzymatic method creatinine by Jaffe Colorimetric - Kinetic method, Cystatin C and kidney injury molecule -1 by ELISA Method using specific rat kits. Histological examination of the kidneys were carried out and tissues were stained with H & E stain and examined under the light microscope. Data were expressed as mean \pm SD, and the statistical analysis was performed with the SPSS statistics 23.0 and p values less than .05 were considered statistically significant.

Results: The results showed that urea, creatinine, cystatin C and KIM-1 levels significantly increased ($p < .05$) after mercury intoxication. However, there was a significant decrease ($p < .05$) in the levels of all the parameters in PM seed treated groups 3, 4, 5 and 6 rats. There was also a corresponding histology results.

Conclusion: Mercury toxicity caused an acute kidney injury, however, the administration of PM ameliorated the toxic effect of mercury toxicity in the kidneys of albino rats.

Keywords: Renoprotective, *Pentaclethra macrophylla* seed (Ugba), Mercury toxicity acute kidney injury, male Albino Rats

1. INTRODUCTION

Toxicity of mercury is connected with severe harmful impacts exerted via pathological and biochemical effects [1]. The useful action and anatomic position of the liver and kidneys, render them susceptible to unpleasant effects of metals like, mercury [2][1][3]. Exposure to elevated concentration of mercury results in onset of oxidative stress and high production of free radicals, a situation involved in pathophysiology of hepatic and renal disorders [1][4]. The kidneys are really vulnerable to inorganic mercury accumulation and intoxication [2][4]. It is reported that mercuric chloride ($HgCl_2$) induces biological toxicity in different tissue via numerous mechanisms, like, lipid oxidation which have been known to take place in the liver, kidneys, Testis, and other tissues of investigational animals. Indeed, mercury accumulates in the epithelial cells of proximal tubules where it binds to intracellular sulfhydryl, phosphoryl and carboxyl groups, a situation that leads to inhibition of cell increase, enzyme inactivation, DNA disintegration, suppression of protein production and cell death [2]. Additionally, mercury intoxication disorganizes intracellular thiol content in a way that provokes oxidative stress, perturbation in heme metabolism, mitochondrial malfunction and lipid oxidation [5]. Oxidative stress driven histological alteration in kidney tissues exposed to mercury has been documented [6]. It has been reported in several literatures that the levels of blood urea nitrogen, creatinine, cystatin C and KIM-1 are all elevated in kidney diseases, especially due to acute or chronic toxicity [7][8].

Though there is extensive consumption of African oil bean seeds in some parts of Nigeria, there is paucity of information on its effects on mercury-induced kidney toxicity, taking into consideration that the search for effective therapy in the management of mercury toxicity is a concern for researchers and health practitioners. Therefore, the aim of this study was to assess the renoprotective effect of *Pentaclethra macrophylla* seed (Ugba) against Mercury induced acute kidney injury in male Albino Rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

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Thirty - six (36) adult male albino rats weighing approximately $135\pm 1.5g$ were purchased from University of Port Harcourt, Rivers State, Nigeria and were used for this research work. The animals were housed in plastic suspended cages, placed in well ventilated conditions and provided with rat diet and water. The ethical regulations on animal care and handling of the National Academy of Science were observed and the rats were made to acclimatize for 14 days prior to experiment.

2.2 Collection and identification of Plant Materials

Fresh matured seeds of *Pentaclethra macrophylla* (African oil bean seed) were sourced locally from markets in Imo state, Nigeria. The seeds were identified by Dr. Mbagwu V. of the department of plant science and Biotechnology, Imo state University, Owerri.

2.3 Preparation of *Pentaclethra macrophylla* powder from Seed

Seeds were thoroughly washed with distilled water, cooked for 2hours at $100^{\circ}C$ to remove the seed coats, washed three times, sliced and cooked for another 2hours at $100^{\circ}C$, rinsed three times with distilled water [9]. The washed and sliced seeds were stored at room temperature and allowed to ferment. The fermented seeds were dried, ground and preserved in airtight container in the refrigerator at $4^{\circ}C$. The total weight of the powdered seed of *Pentaclethra macrophylla* produced was 250g.

2.4 Ethanol Extraction (Maceration method) [10]

Maceration technique was used to extract the phytochemical content of the plant seed used in this research work. 52g of the ground seed powder was soaked in 100mls of ethanol and allowed to stand at room temperature of $25^{\circ}C$ for 72 hours with agitation at interval of one hour. At the end of the third day, the content was filtered using Whatman no 1 filter paper and filtrate was subjected to rotary evaporator to remove the ethanol content. The recovered solute (powder) was then weighed again to enable the calculation of percentage yield.

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2.5 Calculation for Percentage yield of Ethanol Seed Extract

To calculate the percentage yield of ethanol seed extract (g) = $W1 \times 100 / W2$

Where,

W1 = Weight of dried powder of the extract obtained after solvent removal = 52grams.

W2 = Weight of the dried powdered seed sample before adding to solvent = 100grams.

To calculate the percentage yield of ethanolic seed extract = $\frac{52 \times 100}{100} = 52\%$

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2.6 Preparation of Seed extract for Treatment

Following the ethanolic extraction and evaporation of ethanol, a total of 52grams of the seed in powdered form were recovered. 52grams of the post – ethanolic extract powder was dissolved in 100mls of corn oil. Therefore, 1.0ml of the solution contain 0.5g/ml of the extract. Therefore, in $135\pm 1.5g$ rats, 0.5g/ml will be equivalent to 3.7g/kg body weight of rats.

2.7 Procurement and Preparation of Mercury Chloride Salt

Mercury chloride salt was purchased in Port Harcourt from Joe Kings Chemicals and made in China in a granular form. The salt is of industrial grade of 99.5% purity. Since the salt was purchased in a granular form, there was a need to dissolve the salt in Corn oil to facilitate oral treatment in the rats. 3.0mg of mercury chloride were weighed and dissolved in sterile container containing 8.0ml of corn oil. The contents of the container were mixed to ensure complete dissolution of the salt. This implies that 1.0ml of this solution contains 0.375mg of mercury chloride.

2.8 Administration of Mercury Chloride and *Pentaclethra macrophylla* seed extract

The method of treatment in the acute studies involved oral technique. In the oral treatment, mercury chloride salt and *Pentaclethra macrophylla* seed extract were administered using gavage tube inserted directly into the oesophagus of the rats through the mouth to ensure complete delivery of the salt and seed extract respectively.

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2.9 Reagents and Chemicals

Mercury chloride (Kermel, China), tetraoxosulphate vi acid, sulphanilamide, hydrochloric acid, thiobarbituric acid, trichloroacetic acid, sodium hydroxide, ethylene - diaminetetraacetic acid, sodium pyrophosphate, 5, 5 – dithiobis – 2 – nitrobenzoate, sodium nitroprusside, urease, phenol, picric acid, 2, 4 – dinitrophenyl hydrazine (UK), were obtained in Port Harcourt.

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2.10 Toxicity Induction with Mercury Chloride ($HgCl_2$)

After two weeks of acclimatization, toxicity was induced in the male albino rats with mercury chloride ($HgCl_2$). A standard dose of 3.0mg/kg body weight of mercury chloride obtained from acute toxicity study dose determination was used to induce organ toxicity in the experimental rats. Organ and Tissue damage were determined by evaluating liver, kidney and testicular parameters under review.

Comment [S14]: $HgCl_2$

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2.11 Experimental Design

Thirty six (36) male adult albino rats weighing approximately 135±1.5g/kg were used for this research study. The animals were placed into six groups, each containing six rats and the duration of the experiment was 30 days. Mercury (II) chloride (HgCl₂) was dissolved in corn oil and administered three times a week by oral gavage. The administered dose of 3.0mg/kg body weight was done orally using gavage tube. *Pentaclethra macrophylla* seed extract was dissolved in corn oil and administered to the rats daily for 30 days.

Group 1: Control (rats received corn oil at 2ml/kg)

Group 2: HgCl₂ (rats were administered 3.0 mg/kg HgCl₂)

Group 3: 100mg/kg P.M + HgCl₂ (rats were treated with 100mg/kg P. M. and 3.0mg/kg HgCl₂)

Group 4: 200mg/kg P.M + HgCl₂ (rats were treated with 200mg/kg P. M. and 3.0mg/kg HgCl₂)

Group 5: 100mg/kg P.M (rats were treated with 100mg/kg *Pentaclethra macrophylla* alone)

Group 6: 200mg/kg P.M (rats were treated with 200mg/kg *Pentaclethra macrophylla* alone)

2.12 Blood samples Collection and Preparation.

After 30 days, all the animals were weighed, anaesthetized using chloroform. Blood was collected by cardiac puncture into plain tubes. Samples were obtained by centrifugation of the clotted blood at 3500rpm for 10minutes. The serum specimen were stored at 2°C prior to the biochemical analysis (estimation of blood urea nitrogen, creatinine, cystatin C and Kidney injury molecule – 1 (KIM – 1).

2.13 Laboratory Procedures

2.13.1 Determination of serum Urea by Berthelot's enzymatic method.

Principle.

Urea in serum is hydrolysed to ammonia in the presence of Urease. The quantity of ammonia produced is directly proportional to the concentration of Urea in the specimen. The ammonia (in form of coloured solution) is measured spectrophotometrically against reagent blank to obtain the concentration of Urea in the specimen.

$\text{Urea} + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2$.

$\text{NH}_3 + \text{hypochlorite} + \text{phenol} \rightarrow \text{Indophenol. (Blue compound)}$.

Comment [S18]: CH₄N₂O

2.13.2 Determination of serum Creatinine by Jaffe Colorimetric – Kinetic method

Principle.

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The intensity of colour complex formed is directly proportional to the concentration of creatinine in the specimen.

2.13.3 Determination of Cystatin C by ELISA Method

Principle.

This is based on the interaction between antibody, enzyme – antigen conjugate and native antigen. Mixture of biotinylated antibody, enzyme – antigen conjugate and a serum containing the native antigen which results in a competitive interaction between the native antigen and the enzyme – antigen conjugate for a limited number of antibody binding sites. The yellow color formed is measured spectrophotometrically at a wavelength of 450nm. The concentration of Cystatin C in the samples was then determined by comparing the O.D. of the samples to the standard curve.

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2.13.4 Determination of Kidney injury molecule – 1 by Elisa Method

Principle.

This is based on the interaction between antibody, enzyme – antigen conjugate and native antigen. Mixture of biotinylated antibody, enzyme – antigen conjugate and a serum containing the native antigen which results in a competitive interaction between the native antigen and the enzyme – antigen conjugate for a limited number of antibody binding sites. The yellow color formed is measured spectrophotometrically at a wavelength of 450°c. The concentration of Kidney injury molecule – 1 (KIM – 1) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

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2.13.5 Histological and morphometric analyses of the Kidneys

Histological examination of the kidneys were carried out according to the procedure described by Drury and Wallington (1980). Bouin's fixed kidney tissue of the experimental rats were dehydrated in graded series of ethanol and embedded in ethanol. Serial sections (5 µm) for histological evaluation with light microscope by an experienced pathologist.

2.14 Statistical Analyses

Data were expressed as mean \pm SD, and the statistical analysis was performed with the SPSS statistics 23.0 (SPSS Inc. Chicago, IL). All the statistical analysis were analysed by ANOVA followed by Tukey's multiple test. $P < .05$ were considered to indicate statistical significance.

3. RESULTS AND DISCUSSION

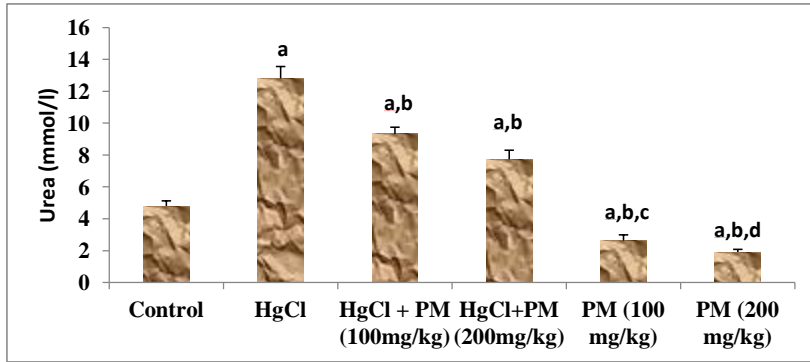


Figure 1: Serum Urea level of rats co – treated with mercury chloride and *Pentaclethra macrophylla* PM seed extract for thirty (30) days.

Each bar represents mean \pm S.D.

^aSignificant as compared with control; $p < 0.05$

^bSignificant as compared with HgCl₂ group; $p < 0.05$

^cSignificant as compared with HgCl₂ +PM (100mg/kg) group; $p < 0.05$

^dSignificant as compared with HgCl₂ +PM (200mg/kg) group; $p < 0.05$

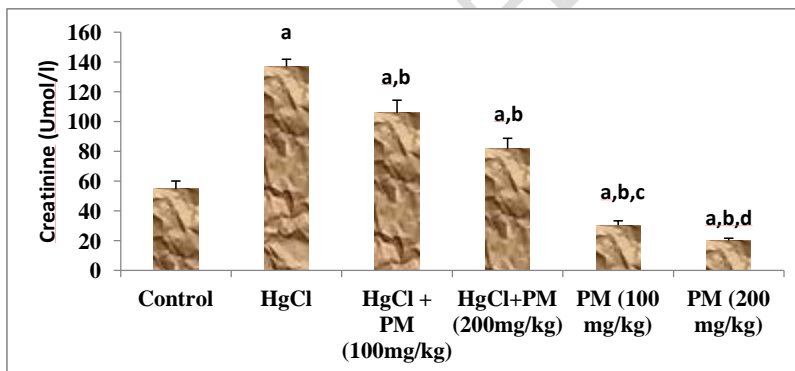


Figure 2: Serum Creatinine level of rats co-treated with mercury chloride and *Pentaclethra macrophylla* PM seed extract for thirty (30) days.

Each bar represents mean \pm S.D.

^aSignificant as compared with control; $p < 0.05$

^bSignificant as compared with HgCl₂ group; $p < 0.05$

^cSignificant as compared with HgCl₂ +PM (100mg/kg) group; $p < 0.05$

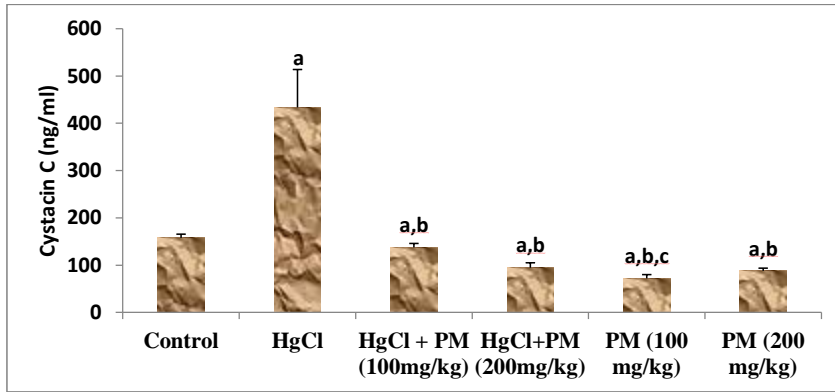
^dSignificant as compared with HgCl₂ +PM (200mg/kg) group; $p < 0.05$

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Figure 3: Serum Cystatin C level of rats exposed to mercury chloride and *Pentaclethra macrophylla* PM seed extract for thirty (30) days

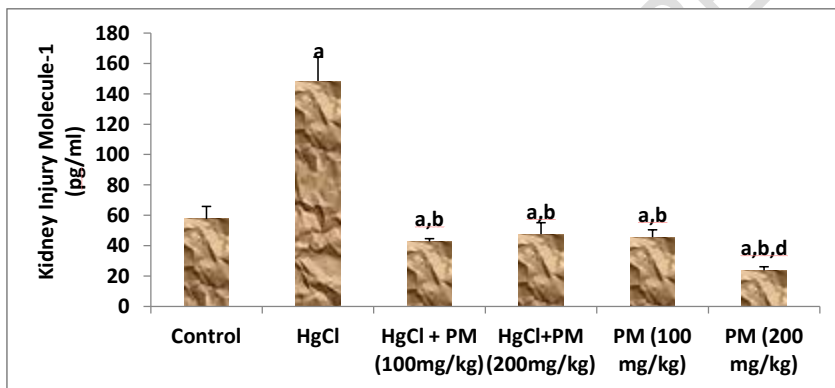
Each bar represents mean \pm S.D.

^aSignificant as compared with control; $p < 0.05$

^bSignificant as compared with HgCl₂ group; $p < 0.05$

^cSignificant as compared with HgCl₂ +PM (100mg/kg) group; $p < 0.05$

^dSignificant as compared with HgCl₂ +PM (200mg/kg) group; $p < 0.05$



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Figure 4 : Serum Kidney injury molecule – 1 level of rats exposed to mercury chloride and *pentaclethra macrophylla* PM seed extract for thirty (30) days.

Each bar represents mean \pm S.D.

^aSignificant as compared with control; $p < 0.05$

^bSignificant as compared with HgCl₂ group; $p < 0.05$

^cSignificant as compared with HgCl₂+PM (100mg/kg) group; $p < 0.05$

^dSignificant as compared with HgCl₂ +PM (200mg/kg) group; $p < 0.05$

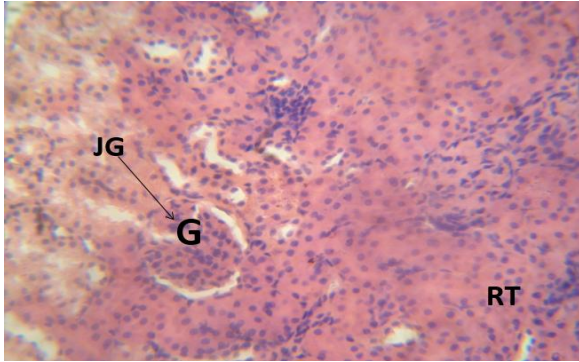


Plate 1: Photomicrograph of renal cortex from control group 1. H&E, MAG: 400X, DOSE: Normal control group. Treatment substance. NIL. The section showed a renal cortex with moderate glomeruli (G) with its tuft of endothelia ensuring ultrafiltration. Juxtaglomerular (JG) apparatus and renal tubules (RT) were intact.

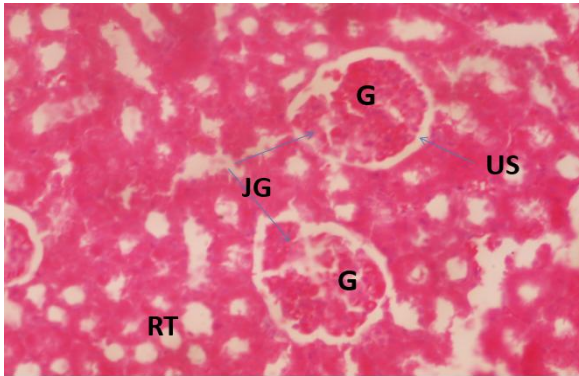


Plate 2: Photomicrograph of section of renal cortex from group 2. H&E, MAG: 400X, DOSE: 3.0mg/kg Hgcl alone for 30 days. Treatment substance. Mercury chloride. The section showed a renal cortex with moderate glomeruli (G) with intact tuft of endothelia. The urinary space (US) appeared constricted and Juxtaglomerular (JG) apparatus appeared distorted. Renal tubules (RT) showed clear lumen.

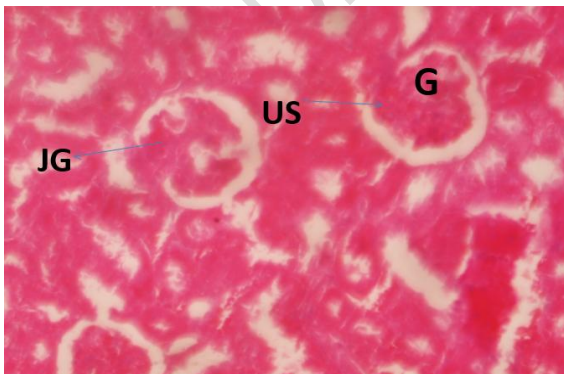


Plate 3: Photomicrograph of renal cortex from group 3. H&E, MAG: 400X, DOSE: 3.0mg/kg Hgcl + 100mg/kg PM Seed for 30 days. Treatment substance. Mercury chloride and PM Seed. Moderate glomeruli (G) with its tuft of endothelia intact. Juxtaglomerular (JG) apparatus were intact. The urinary spaces (US) were slightly wider. Renal tubules were with occluded lumen.

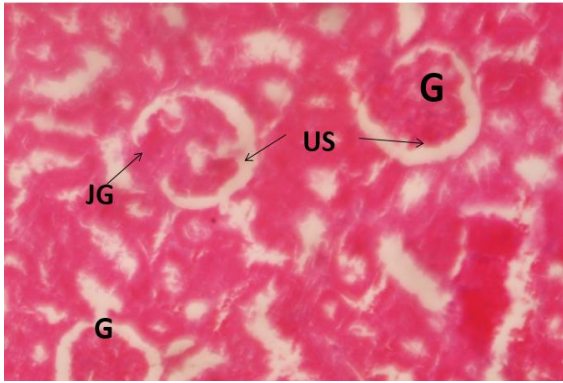


Plate 4: Photomicrograph of renal cortex from group 4. H&E, MAG: 400X, DOSE: 3.0mg/kg Hgcl + 200mg/kg PM Seed for 30 days. Treatment substance. Mercury chloride and PM Seed. Moderate glomeruli (G) with its tuft of endothelia intact. The nuclei were not distinct. Juxtaglomerular (JG) apparatus were slightly distorted. The urinary spaces (US) were slightly

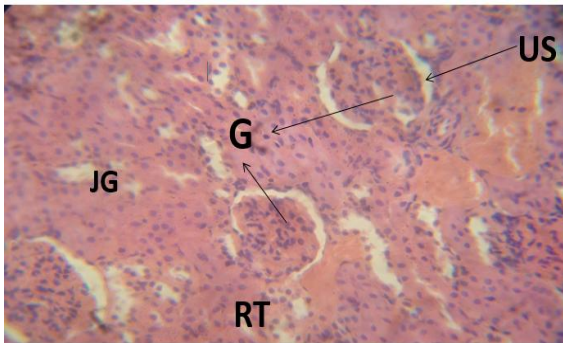


Plate 5: Photomicrograph of the section of renal cortex from group 5. H&E, MAG: 400X, DOSE: 100mg/kg PM Seed alone for 30 days. Treatment Substance. *Pentaclethra macrophylla* seed. The section showed the renal cortex with Moderate glomeruli (G) with intact tuft of endothelia. The urinary spaces (US) were normal. The renal tubules (RT) presented with normal lumen.

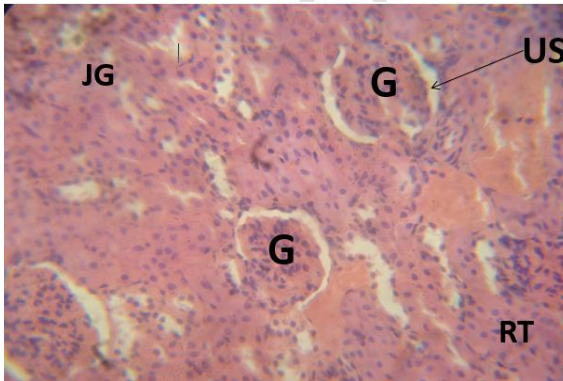


Plate 6: Photomicrograph of renal cortex from group 6. H&E, MAG: 400X, DOSE: 200mg/kg PM Seed for 30 days. Treatment substance. *Pentaclethra macrophylla* Seed. Moderate glomeruli (G) with intact tuft of endothelia and distinct nuclei ensuring ultrafiltration. Juxtaglomerular (JG) apparatus and renal tubules (RT) were intact and normal.

The elevation in Urea and Creatinine levels in group 2 animals reached significant levels at the end of the experiment. This could be attributed to chronic renal failure provoked by mercury intoxication to renal cells and tissues, resulting to

low glomerular filtration rate (GFR) and accumulation of Urea and Creatinine in biological system. This corresponds with Cheesbrough in [11], in his research work on renal function, that a marked and prolonged serum or plasma Urea and Creatinine levels were indicative of impaired renal failure. The significant decrease in Urea and Creatinine levels in groups 3 & 4 rats on application of PM seed could be due to great ameliorative potentials of PM seed extract which contains rich phytochemicals [11][12]. **The Urea and Creatinine levels in groups 5 & 6 rats where only PM seed extract was administered at different concentrations were further reduced by PM seed.**

The elevation in kidney **cystatin c** and kidney injury molecule – 1 levels in group 2 rats reached significant level. This could be attributed to acute renal failure precipitated by mercury intoxication to renal cells and tissues, resulting to low glomerular filtration rate (GFR) and accumulation of Cystatin C and kidney injury molecule – 1 in biological system. Kidney **cystatin c** and **Kim – 1** rise proportionately as glomerular filtration rate reduces and remain emerging biomarkers for early detection of kidney injury. These findings tallied with previous submissions by some research teams who reported that serum **cystatin c** and **Kim – 1** levels rise during renal toxicity, and are specific and reliable indicators for early detection of kidney injury [14]. The significant reduction in kidney **cystatin c** and **Kim – 1** levels in PM seed treated groups 3 & 4 rats, could be as a result of ameliorative potentials exhibited by PM seed to renal cells and tissues. This corresponds with the report of Shahidi and Nacz [15] and Prior and his team in 2006 on the usefulness of phenols in the treatment of wounds and **are** active in curing kidneys and stomach problems. The reduction in kidney **cystatin c** level in groups 5 & 6 rats where only PM seed extract was given at different concentrations is attributable to PM seed effect on renal tissues and cells. The **Kim – 1** level in groups 5 & 6 rats were reduced significantly, reflecting ameliorative influence from PM seed.

In group 2 rats, the renal urinary space appeared constricted with distorted juxtaglomerular apparatus, and could be as a result of mercury intoxication to renal tissues and cells. Oxidative stress driven histological distortion in kidney tissue administered mercury has been reported [6]. The liver and kidney histopathology showed severe abnormalities as a result of mercury exposure [16]. In PM seed treated groups 3 & 4 rats, the urinary spaces were slightly wider with some renal tubules occluded with lumen. However, juxtaglomerular apparatus, some renal tubules, and endothelia cells were all intact, attributing it to remarkable ameliorative potentials exhibited by PM seed extract to renal tissues and cells. In groups 5 & 6 rats, the renal histo-architectures were maintained, with renal tubules, juxtaglomerular apparatus, and urinary spaces intact.

Plate 1 is the photomicrograph of renal cortex from control group 1. The section showed renal cortex with moderate glomeruli with its tuft of endothelia ensuring ultrafiltration. Juxtaglomerular apparatus and renal tubules were intact. Plate 2 represents photomicrograph of section of renal cortex from group 2 where only 3.0mg/kg of Hgcl were administered to the rats. The section showed a renal cortex with moderate glomeruli and with intact tuft of endothelia. The urinary space appeared constricted while Juxtaglomerular apparatus appeared distorted. Renal tubules showed clear lumen. Plate 3 is the photomicrograph of renal cortex from group 3 (3.0mg/kg Hgcl + 100mg/kg PM seed). Moderate glomeruli with its intact tuft of endothelia observed. Juxtaglomerular apparatus were intact and normal. The urinary spaces were slightly wider while renal tubules were with occluded lumen. Plate 4 is the photomicrograph of renal cortex from group 4 (3.0mg/kg Hgcl + 200mg/kg PM seed). Moderate glomeruli with its tuft of endothelia were intact. The nuclei were not distinct. Juxtaglomerular apparatus were slightly distorted while the urinary spaces were slightly degenerated. Plate 5 represents the photomicrograph of the section of renal cortex from group 5 (100mg/kg PM seed alone). The section showed the renal cortex with moderate glomeruli and intact tuft of endothelia. The urinary spaces were normal. The renal tubules presented with normal lumen. Plate 6 is the photomicrograph of renal cortex from group 6 (200mg/kg PM seed alone). Moderate glomeruli with intact tuft of endothelia and distinct nuclei ensuring ultrafiltration were seen. Juxtaglomerular apparatus and renal tubules were intact and normal.

4. CONCLUSION

Mercury toxicity caused an acute kidney injury, however, the administration of PM ameliorated the toxic effect of mercury toxicity in the kidneys of albino rats.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the **appropriate** ethics committee.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these

products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Othman, M. S., Safwat, G., Aboulkhair, M., & Moneim, A. E. A. The potential effect of berberine in mercury-induced hepatorenal toxicity in albino rats. *Food and chemical toxicology*, 2014; 69: 175-81.
2. Zalups, R.K. Molecular interactions with mercury in the kidney. *Pharmacological reviews*, 2000; 52(1): 113-44.
3. Carvalho, C.M., Chew, EH, Hashemy, SI. Lu, J., & Holmgren, A. Inhibition of the human thioredoxin system: A molecular mechanism of mercury toxicity. *Journal of Biological Chemistry*, 2008; 283(18): 11913–23.
4. Abarikwu, S. O., Njoku, R. C. C. & Onuah, C. L. Aged coconut oil with a high peroxide value induces oxidative stress and tissue damage in mercury-treated rats. *Journal of basic and clinical physiology and pharmacology*, 2018; 29(4): 365-76.
5. Aleo, MF., Morandini, F., & Bettoni, F. In vitro study of the nephrotoxic mechanism of mercury chloride. *La medicina. Del Lavoro*. 2002; 4(93): 267–78
6. Apaydin, F. G., Baş, H., Kalender, S., & Kalender, Y. Subacute effects of low dose lead nitrate and mercury chloride exposure on kidney of rats. *Environmental toxicology and pharmacology*, 2016; 42: 219-24.
7. Walter, F. Medical physiology: A cellular and Molecular Approach on Urea and Creatinine as metabolic wastes by the kidney. Elsevier/Saunders. 2004.
8. Randers, E., Erlandsen, EJ., Pedersen, OL., Hasling, C., & Danielsen, H. Serum cystatin c as an endogenous parameter of the renal function in patients with normal to moderately impaired kidney function. *Clin. Nephrol.* 2000; 54: 203–9.
9. Osabor, V. N., Okonkwo, P. C., & Ikeuba, A. I. Chemical profile of leaves and seeds of *Pentaclethra macrophylla* Benth. *Journal of Medicinal plant and Herbal Therapy Research*, 2017; 5: 11 – 17.
10. Handa, SS., Khanuja, SPS., Longo, G., & Rakesh, DD. Extraction Technologies for Medicinal and Aromatic plants, United Nations Industrial Development Organization and International Centre for science and High Technology, 2008 ; 66(8):1-6.
11. Cheesbrough, M. District Laboratory in Tropical Countries, part 1, 2nd Edition, Cambridge University Press, Cambridge, 2005; Pp. 358 – 361.
12. Dongmo, F. G., Enyong, O. J., Noumessing, M. C., & Enyegue, M. D. Phytochemical constituents and Antioxidant potential of some Camroonian Medicinal Plants. *Pharmacology on line*, 2007; 2: 436 – 452.
13. Oyeleke, G. O., Odedeji, J. O., Ishola, A. D., & Afolabi, O. Phytochemical Screening and Nutritional Evaluation of African Oil Bean Seed (*Pentaclethra macrophylla*) IOSR. *Journal of Environmental Science, Toxicology and Food Technology*, 2014; 8: 14–17.
14. Han, WK., Bailly, V., Abichandani, R., Thadhanin, R., & Bonventre, JV. Kidney Injury Molecule – 1 (KIM – 1), a novel biomarker for human renal proximal tubule injury. *Kidney Int*, 2002; (62): 237–244
15. Shahidi, F. & Naczki, M. Phenolics in food and Nutraceuticals, Sources, Applications and health effects, CRC Press, Boca Raton, FL.
16. Clarkson, T. W., Magos, L., & Myers, G. J. The toxicology of mercury current exposures and clinical manifestations. *N. Engl. J. Med*, 2003; 349: 1731–1737.

Comment [S29]: Year?