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CHRONIC CONSUMPTION OF *ABELMOSCHUS ESCULENTUS* AND *PIPER GUINEENSE* INDUCED HISTOPATHOLOGICAL CHANGES IN KIDNEY OF ADULT WISTAR RATS

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ABSTRACT

Histopathology of the kidney is one of the parameters used in assessing its microstructural integrity. In this study, the effect of the oral chronic exposure of 500mg/kg of *Abelmoschus esculentus* and 20mg/kg of *Piper guineense* on the kidney of wistar rats was assessed. Forty adult wistar rats, comprising of 20 females and 20 males (123-207g), divided into one control and three test groups of five rats each, were used in this study. The rats in the control group were administered with distilled water, while rats in group 2 and 3 were administered with 500mg/kg of *Abelmoschus esculentus* and 20mg/kg of *Piper guineense* respectively. Group 4 Animals received a combination of the two extracts. The results showed that exposure to *Abelmoschus esculentus* and *Piper guineense* caused tubular necrosis, atrophy, cellular degeneration, vascular congestion, interstitial odema, epithelial lining degeneration and glomerular inflammation in the experimental groups. Statistical value in the weight of the body and kidney showed significant value ($p < 0.05$) compared to control. The observations made from the tissue microscopic analysis, indicated an alteration in the filtration function of the kidneys in rats exposed to *Abelmoschus esculentus* and *Piper guineense*. Hence, the results obtained suggested that *Abelmoschus esculentus* and *Piper guineense* possess the potentials of inducing nephrotoxicity in rats.

Keywords: *Abelmoschus Esculentus, Piper Guineense, Histopathology, Kidney and Wistar Rat*

INTRODUCTION

The consumption of leafy vegetables is part of Africa's cultural heritage. The nutrient content of different types of vegetables varies considerably and they are the major sources of vitamins, essential amino acid, minerals and antioxidants (Fasuyi, 2006). Vegetables are included in meals mainly for their nutritional value. *Abelmoschus esculentus* is a popular health food due to its high fiber, calcium, potassium, vitamin C and foliate content. It also contains cytopropanoid fatty acids. It is often eaten for weight loss since it is fat and cholesterol free (Duvauchelle, 2011). It also possesses ethnomedical potentials and it is used as an antioxidant and in the treatment of urinary tract infection. It is also used for prevention and treatment of gastric acidity and duodenal ulcer.

Piper guineense contributes to the iodine content in the diet of the rural and urban dwellers. It has a high content of iodine among all vegetables (Ujoroundu *et al.*, 2011). *Piper guineense* is a spice used in seasoning food. Its high protein content and the presence of alkaloid makes it a supplement for daily protein requirement of the body and as an antimalarial remedy (Ekanem *et al.*, 2000). The seeds are consumed by women after childbirth to enhance uterine contraction for the extrusion of placenta and other remains from the womb (Udoh *et al.*, 1999). It is also used for the control of weight and as an adjuvant in the treatment of rheumatic pains.

The kidney is responsible for the maintenance of the constant extracellular environment through its involvement in the excretion of catabolites and regulation of water and electrolyte balance (Udoh *et al.*, 2011). Impairment of the renal functions may be caused by several diseased conditions and exposure to certain reactive or toxic metabolic (nephrotoxic substances) (Chatterjea and Shinde, 2002), (Ok, 2007). Renal function impairment manifests in a variety of different clinical presentations, (Patil *et al.*, 20097) some may be asymptomatic. The renal function impairment with asymptomatic presentation can only be detected by routine laboratory examinations. The kidney functions can be assessed from the histological

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analysis of the ultra structural status of the renal tissues (Nwankwo *et al.*, 2006, Uboh *et al.*, 2011) Nephrotoxic response, resulting in renal tissue dysfunction, has been reported to be characterized by this distortion in the architectural integrity of the glomerular tubules. In this study, the histopathological changes of the renal tissue (nephrotoxicity) associated with oral exposure of *Abelmoschus esculentus* and *Piper guineense* were assessed in albino wistar rats.

MATERIALS AND METHODS

Drugs and Chemicals

Sodium chloride, formaldehyde, sodium trioxocarbonate V, sodium bicarbonate, xylene, 70% alcohol, 90% alcohol, absolute alcohol, haematoxylin, eosin, egg albumin, distilled water, paraffin wax were all procured from BDH Chemicals, England. All other chemicals were of analytical grade.

Animals

40 wistar rats (123-207g) were obtained from the University of Uyo Animal House. They were maintained on standard pellets (guinea feed) and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

Sourcing of Plant Material

Freshly fruit of *Abelmoschus esculentus* and *Piper guineense* were obtained in July, 2012 from Itam Market, Uyo, Akwa Ibom State, Nigeria. The plant was identified and authenticated by the Department of Botany, University of Uyo, Uyo, Nigeria.

Preparation of Extract

Abelmoschus esculentus was chopped and air dried. *Piper guineense* (seeds) was also air dried and after being dried they weighed 600g for *Abelmoschus esculentus* and 800g for *Piper guineense*. They were then macerated in 97% ethanol (SIGMA CO., UK) in a flat bottom flask and were kept for 72hrs at room temperature. At the end of 72hrs it was filtered. The filtrates were concentrated in water bath at 45 degree Celsius. The concentrated extract was preserved in refrigerator till commencement of research.

The weight of the extracts was 40.25g for *Abelmoschus esculentus* and 24g for *Piper guineense*.

Acute Toxicity Testing

The acute toxicity of *Abelmoschus esculentus* and *Piper guineense* on Wistar Albino rats were determined in two (2) stages for the two extracts.

For *Abelmoschus esculentus*, in stage one animals received 1000, 2000, 3000, 4000 and 5000mg/kg body weight while in stage two, animals received 2300, 2400, 2500, 2600, 2700mg/kg body weight.

And in acute toxicity of *Piper guineense* the same two stages was observed, in stage one; animals received 10, 50, 100, 200, 300 mg/kg body weight. Stage 2; received 85, 90, 95, 100, 105 mg/kg body weight.

All experimental animals were observed for physical signs of toxicity such as writhing, gasping, palpitation, decreased respiratory rate, body limb and death within 24hours. The extract was administered intraperitoneally (i.p). The LD50 was found to be 2500mg/kg for *Abelmoschus esculentus* and 100mg/kg for *Piper guineense*.

According to the modified lorke's method 500mg/kg and 20mg/kg per body weight were calculated respectively as middle doses for the *Abelmoschus esculentus* and *Piper guineense*. Doses were considered as stock solution, they were calculated further using 20 mls of distilled water for *Abelmoschus esculentus* and 10 mls of distilled water for *Piper guineense* to obtain working solution.

Experimental Design/Study Design

Matured 40 albino wistar rats (Age of 40 rats must be same) containing male and female, weighing between 123-207g were obtained from the faculty of Basic Medical Sciences Experimental Research Animal House of the University of Uyo, Uyo Nigeria. They were fed with standard laboratory diet and water *ad libitum*. Illumination was 12h light /dark cycle and room temperature was 25±2°C. The animals were divided into four groups, one control (I) and three experimental groups (II, III and IV), which consisted of 10 normal albino wistar rats per group. The control group was given distilled water while

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the experimental group II, III and IV were exposed daily to 500mg/kg body weight of *Abelmoschus esculentus* alone, 20 mg/kg of body weight *piper guineense* alone and 500 mg/kg of *Abelmoschus esculentus* combined with 20 mg/kg of *piper guineense* respectively by oral administration for 28 days. In this study, all the animals' experimentations were carried out following the guidelines for the care and use of laboratory animals obtained from the institutional animal ethics committee.

Sample Collection and Histopathological Analysis

Twenty four hours after last exposure, the animal were anesthetized with chloroform vapour and dissected. The harvested kidneys were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight.

On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the kidney, liver and pancreas. The sections were designated "vertical sections".

Serial sections of 5 µm thick were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

Gross Morphometrical Analysis

The initial and final weight of the rats and the weight of the kidney in each group were taken using the weighing balance. The values of all the morphometric analysis were compared statistically using SPSS 17 Software.

Photomicrography

Records of the Histological and histochemical results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health sciences, University of Uyo, Uyo, Akwa- Ibom, Nigeria as illustrated in Plate.1 to 5.

RESULTS AND DISCUSSION

Results

Statistical Analysis Result

Table 1: Showing the effect of extracts on initial and final body weights

GROUPS	DRUG ADMINISTERED	INITIAL BODY WEIGHT(g)	FINAL BODY WEIGHT (g)
1	Control (no treatment)	137.90±6.16	169.50±6.53*
2	<i>Abelmochus esculentus</i> -500mg/kg-28 days	167.80±7.81	200.10±11.40**
3	<i>Piper guineense</i> -20mg/kg -28 days	181.10±8.56	198.10±12.22**
4	Combined <i>A.esculentus</i> + <i>P.guineense</i> -28days	151.78±7.86	205.44±12.73***

Result shown as=Mean ± SEM, *= $p < 0.05$, **= $p < 0.01$ ***= $P < 0.001$ compare to control

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Table 2: Showing the effect of *extracts* on percentage terminal kidney weights

GROUPS	EXTRACTS ADMINISTERED	KIDNEY WEIGHT(g)	% KIDNEY WEIGHT (%)
1	Control(no treatment)	0.96±0.03	0.7
2	<i>Abelmoschus esculentus</i> -500mg/kg-28 days	1.35±0.10 ^a	0.8
3	<i>Piper guineense</i> -20mg/kg -28 days	1.14±0.07 ^b	0.6
4	Combined <i>A.esculentus</i> + <i>P.guineense</i> -28days	1.17±0.08 ^a	0.8

Result shown as=Mean ± SEM, a= $p < 0.05$, b= $p < 0.01$ compared to control

Note All result were analyzed using one way ANOVA and using SPSS version 17.P-values of less than 0.05 were considered statistically significant.

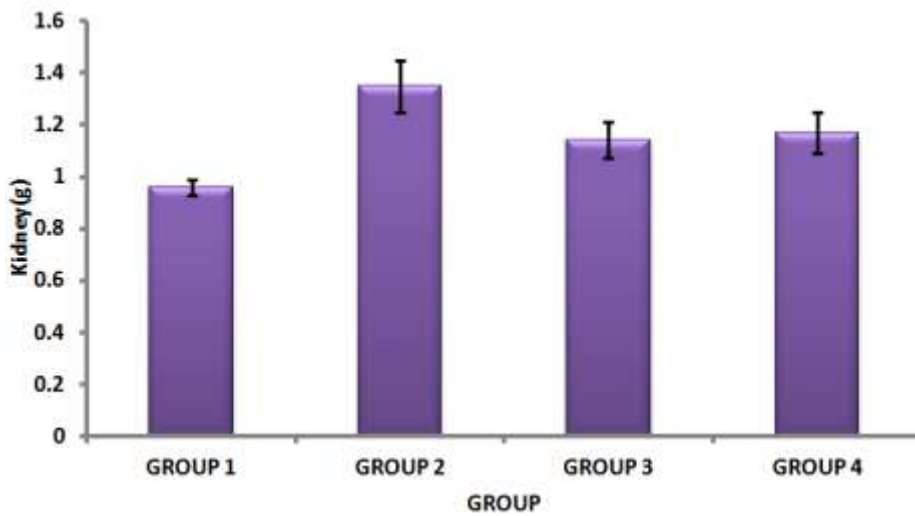


Figure 1: Showing mean kidney weight

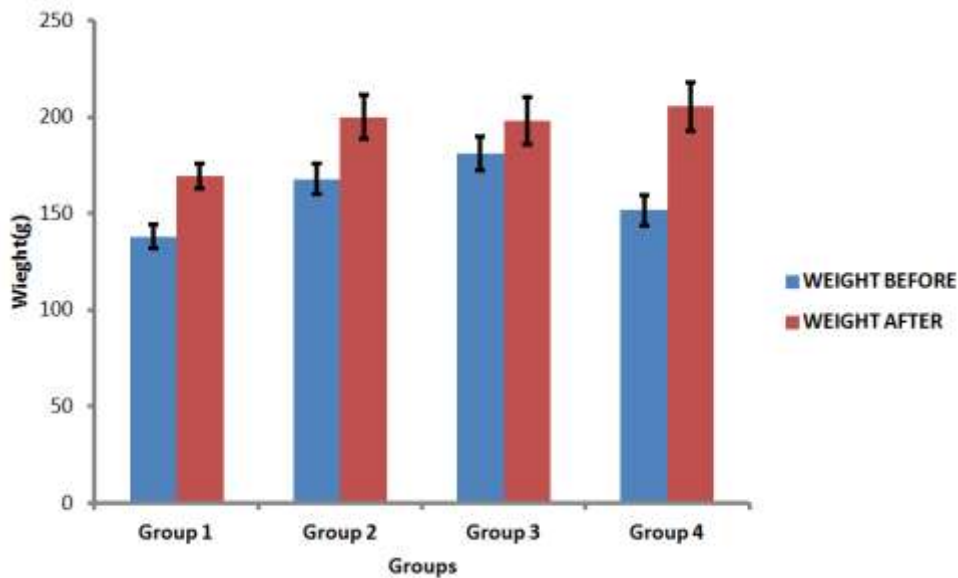


Figure 2: Showing comparison between initial and final body weight

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Histopathological Findings

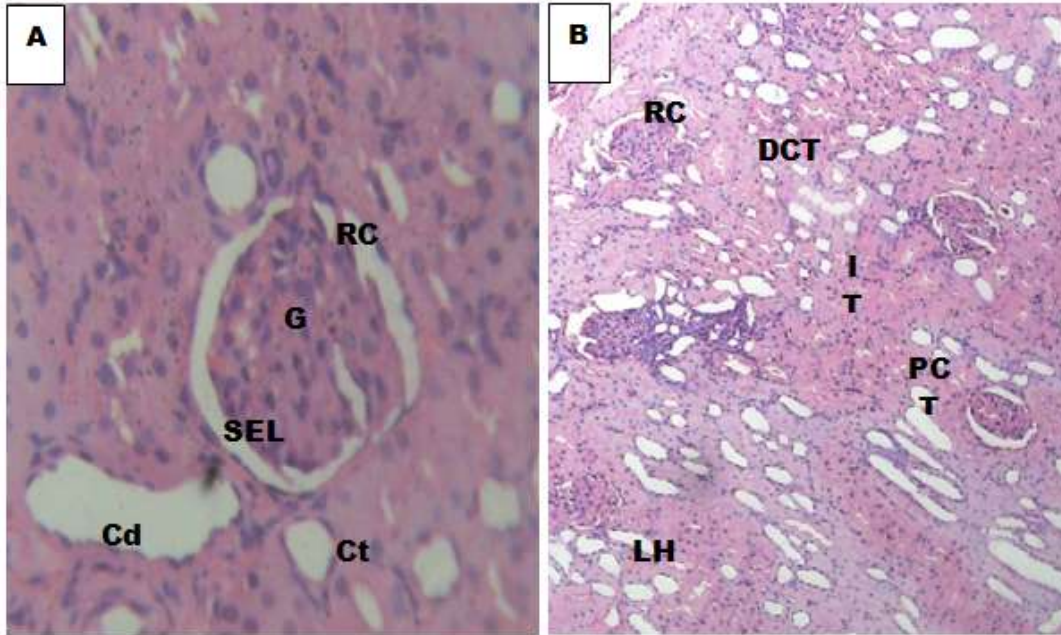


Plate 1: Normal control kidney at magnification A(x100) & B(x400) stained with H & E technique
 Note: *Cd*-collecting duct, *Ct*-collecting tubule, *G*-glomerulus, *RC*-renal corpuscle, *SEL*-Squamous epithelial lining, *DCT*-distal convoluted tubule, *IT*-interstitial tissue, *LH*- loop of henle, *PCT*-proximal convoluted tubule.

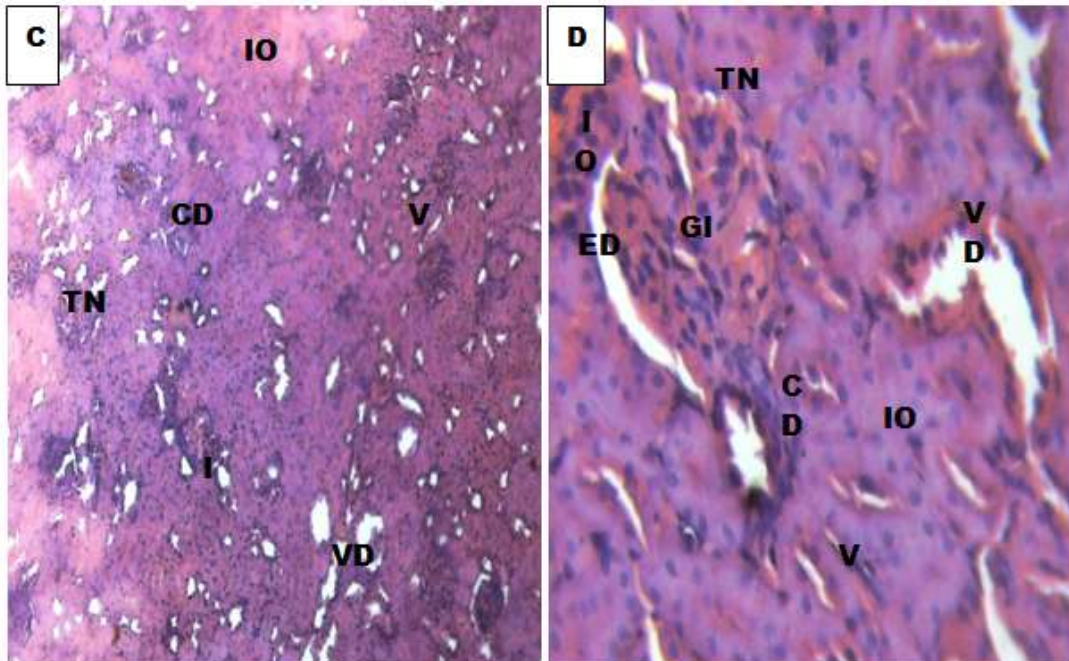


Plate 2: Kidney treated with ABELMOSCHUS ESCULENTUS (500mg/kg) at magnification C(x100) & D(x400) stained with H & E technique
 Note: *CD*-cellular degeneration, *ED*-epithelial degeneration, *GI*-glomerular infiltration, *IO*-interstitial odema, *V*-vacuolization, *VD*-vascular degeneration and *TN*-tubular necrosis.

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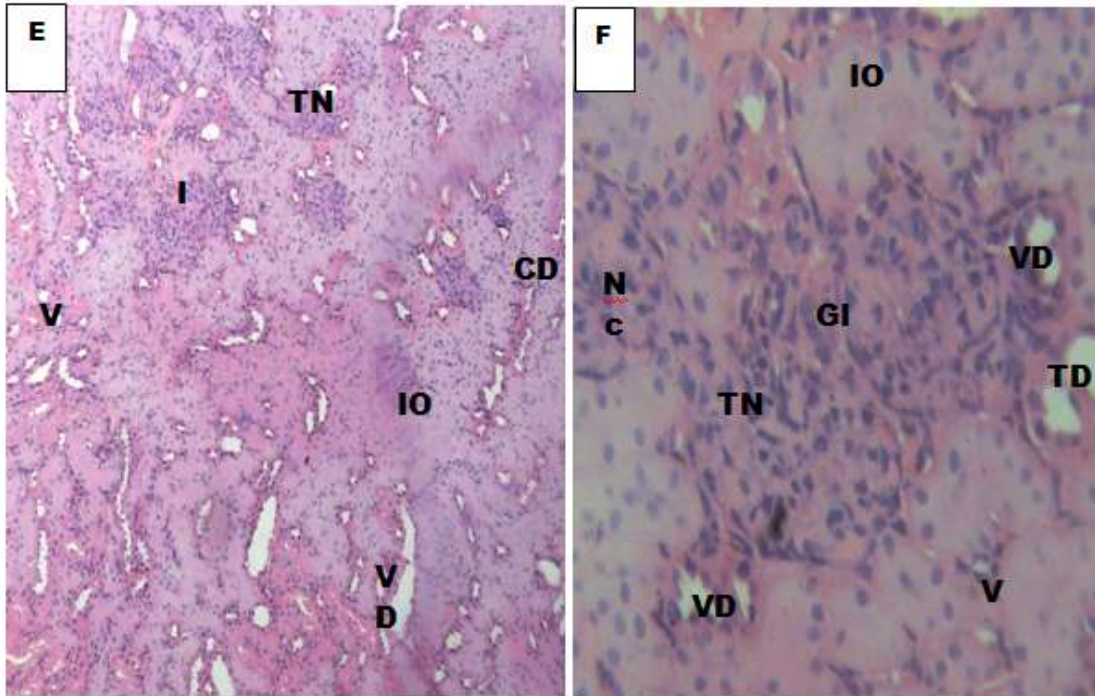


Plate 3: Kidney treated with PIPER GUINEENSE (20mg/kg) at magnification G(x100) & H(x400) stained with H & E technique

Note: GI-glomerular infiltration, I-inflammation, IO-interstitial oedema, V-vacuolization, VD-vascular degeneration, TN-tubular necrosis.

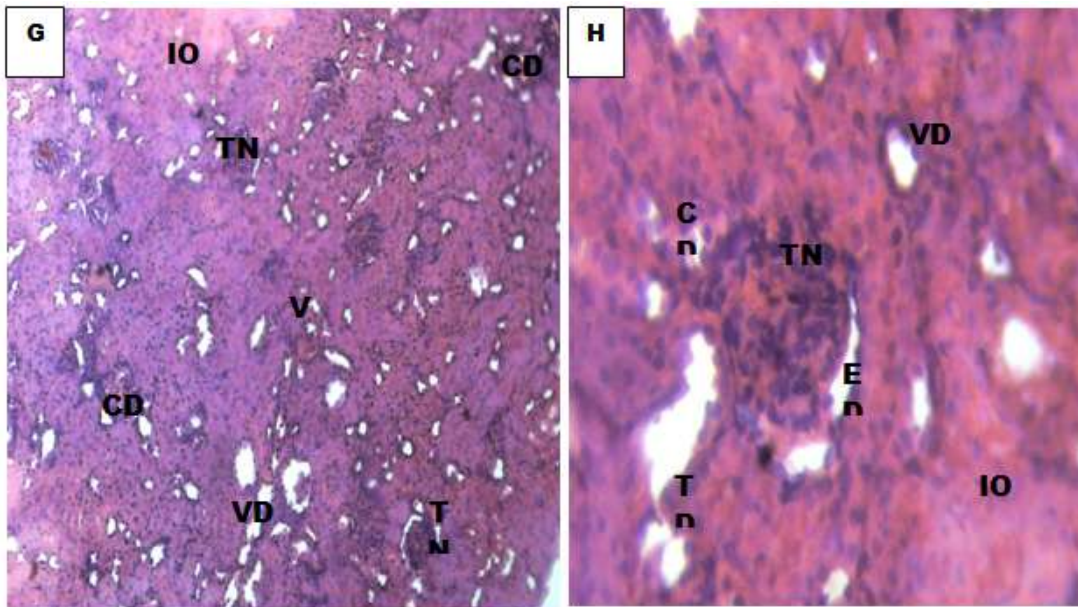


Plate 4: Kidney treated with combined extract of ABELMOSCHUS ESCULENTUS (500mg/kg) and PIPER GUINEENSE (20 mg/kg) at magnification A(x100) & B(x400) stained with H & E technique.

Note: CD-cellular degeneration, ED-epithelial degeneration, IO-interstitial odema, V-vacuolization, VD-vascular degeneration, TN-tubular necrosis.

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Plate 1: Control group of kidney showed normal cellular pattern of cortex and medulla, with numerous renal corpuscles lined with squamous epithelial lining, the glomerulus within the corpuscle and surrounding proximal and distal convoluted tubules, collecting duct and loop of henles all within normal limit.

Plate 2: Kidney treated with *Abelmoschus esculentus*-500mg/kg for 28 days showed severe (if possible the changes could have been written as no./unit microscopic area) cellular abnormalities of cortex and medulla, with numerous (no./unit microscopic area) tubular necrosis lined with epithelial degeneration, degenerated tubules, vacuolization, interstitial odema, inflammation, cellular and vascular degeneration as compared to the control group.

Plate 3: Kidney treated with *Piper guineense*-20mg/kg for 28 days showed severe cellular abnormalities of cortex and medulla, with numerous tubular necrosis lined with lost of epithelial lining, degenerated tubules, vacuolization, interstitial odema, inflammation, cellular and vascular degeneration as compared to the control group.

Plate 4: Kidney treated with a Combination of *Abesmoschus esculentus* and *Pipper guineense* for 28days showed severe cellular abnormalities of cortex and medulla, with numerous tubular necrosis lined with epithelial degeneration, degenerated tubules, vacuolization, interstitial odema, inflammation, glomerular inflammation, cellular and vascular degeneration as compared to the control group.

Finally, the result of the microscopic examination showed histopathological damage to the renal tissues of rats exposed to *Abelmoschus esculentus* and *piper guineense*, compared to the tissues from the control rats (plate 1). The kidney of rats in the test groups were observed to have developed interstitial odema, nuclear clumping, valcuolization, tubular necrosis, cellular degeneration, tubular atrophy epithelial degeneration and infiltration both in females and males albino rats of each group (plate 2-4). This gave an indication that exposure to *Abelmoschus esculentus* and *Piper guineense* may induce cellular alterations of normal kidneys. Cytoarchitecture, distorting the functional integrity of the renal tissues. The observations made from the tissue microscopic analysis, indicated the existence of disturbances in the filtration function of the kidneys in rats exposed to *Abelmoschus esculentus* and *piper guineense*.

Discussion

This study investigated the effect of *Abelmoschus esculentus* and *Piper guineense* on the kidney functions in rat model. In assessing the renal effects of these extracts, the histopathology of the renal tissues were examined. This study showed a significant increase in weight of animals and a significant decrease in organ weight in each group. The decrease in the weight of the kidneys of the experimental groups may have occurred as a result of tubular necrosis and loss of cytoplasmic constituent of the kidney. Significant distortions in the architectural integrity of the ultra structural status of the renal tissues were observed. Specifically, the cytostructure of the tubules, vacuolations with damaged external membrane were observed. Acute Inflammation of the kidney tubules were indicated by infiltration of polymorphonuclear cells into the tubules. The observations made from this study indicated a condition of nephrotoxicity and correlated the previous report on the nephrotoxicity effects of cayenne pepper, another specie of pepper which contains capsaicin a known active ingredient in piper guineense, (Mbongue *et al.*, 2005). The results of this study therefore provide a clear indication that *Abelmoschus esculentus* and *Piper guineense* contain some chemical substances with nephrotoxic potential. The specific chemical constituents and mechanisms responsible for the nephrotoxic effect reported in this study are not clear. It may be assumed that the reactive metabolites of *Abelmoschus esculentus* and *Piper guineense* constituents could have interacted with the renal tissues to cause derangements in glomerular functions.

The combination of the two extracts showed severe nephrotoxic effects on the kidneys, indicating that in combination their effect to the kidney potentiates each other. The interaction of these metabolites with the renal tissues may be responsible for cellular injury and subsequent damage to the tissues. The functionality of the kidneys may be compromised due to damage of the renal tissues.

Conclusion

Results obtained in this study show that exposure to *Abelmoschus esculentus* and *Piper guineense* induced adverse and detrimental effects on the renal function in rat model. These observations indicated

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that exposure to *Abelmoschus esculentus* at doses as high as 500mg/kg body weight and *Piper guineense* at doses of 20mg/kg body weight and above is a risk factor for renal function impairment and the associated disorders. This work showed high level of cellular distortion in all experimental groups. Further study is recommended with isolated components of these extracts and with lower concentration of extract of *Abelmoschus esculentus* and *Piper guineense* to confirm the underlying mechanism and active constituents responsible for the observed activity documented by the results of this study.

Conflict Interests

The authors declared that they have no competing interests.

Authors' Contributions

All the Authors contributed equally.

ACKNOWLEDGEMENT

This research was not supported by any organization or funding body, however the authors contributed collectively to carry out the experiment.

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