

Toxic effects of calcium carbide forced ripened pawpaw on the kidney of the Wistar rats

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World Journal of Advanced Research and Reviews, 2022, 16(01), 187-197

Publication history: Received on 24 August 2022; revised on 01 October 2022; accepted on 04 October 2022

Article DOI: <https://doi.org/10.30574/wjarr.2022.16.1.0992>

Abstract

There is a global upsurge in terminal diseases due to unnoticed intake of toxic chemicals. One of such chemical is Calcium carbide, which has been used to ripen fruits by vendors. The aim of this study is to showcase the toxic effect of Calcium carbide coerced ripened Pawpaw on the kidney. Mature unripe Pawpaw's were plucked off from the parent plant at Yenagoa, Bayelsa State. The fruits were dividing into two groups; one group was kept and allowed to rip at normal room temperature while the other group was induced with Calcium Carbide to ripe at the Histology Laboratory, Bayelsa Medical University, Yenagoa, Bayelsa State. 10gram of Calcium carbide was placed in a bowl containing 5ml of water for dissolution in a closed metal bucket containing 1kg of the fruit [pawpaw] rapped with black nylon and was allowed for two days[48 hours] for ripening. After ripening, sampled fruits were washed and juiced. 600g of both the naturally ripened and calcium carbide ripened pawpaw were peeled separately and blended in an electric blender with 350ml/1L of deionized water. The juice was filtered and poured into clean bottles labeled [CaC₂ induced ripened juice and naturally ripened pawpaw juice]; then stored in a refrigerator for subsequent use. 21 adult Wistar rats of both sexes weighing between 126.9- 214.3g were used. They were divided into three groups for each sex, based on the body weight and then different concentrations of naturally ripened and calcium carbide induced ripened Pawpaw were administered orally. LD50 was carried out using Lorke, 1983 [13] method for administration of samples using nine [9] Wistar rats. 12 Wistar rats were used for the main experiment. Group 1: Normal control group of 4 rats [2 males and 2 females] receive normal water and feeds only as placebo. Group 2 : Treatment Group [1] of 4 rats [2 males and 2 females] received 5ml/kg naturally ripened pawpaw juice. Group 3: Treatment Group [2] of 4 Wistar rats [2 males and 2 females] received Calcium Carbide ripened pawpaw for 4 weeks. 5ml/kg for both the natural fruit and the CaC₂ ripened fruits were administered against each body weight of the adult Wistar rats. The Wistar rats were weighed, one was sacrificed in the groups, each week and blood and organ samples were collected from the three groups for hematological and histopathological analysis. Results showed increase in Creatinine, Urea, and Albumin of the treated groups in contrast to the control ($p < 0.05$). Also there is moderate to severe renal tubular degeneration and tubular necrosis of the Calcium Carbide treated group as against the control. In conclusion Calcium carbide causes complete renal failure and subsequently death may arise.

Keywords: Calcium Carbide; Pawpaw; Hematology; Histopathology

1. Introduction

The kidney is one of the major vital organs of the body with various compartments like the major and minor calyces, renal tubules, glomeruli, and amongst others. It functions are enormous ranging from filtration to excretion. Any alteration in the structural and functional mass of the Kidneys will affect the general efficiency of the this organ. An assessment of the possible effect of feeding rats with CaC₂ ripened banana on the kidney and antioxidant status was done. Forty rats were divided into groups of 10 (A, B, C and D.), with A, serving as control. B, C and D were daily fed 2ml

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banana pulp (ripened with 2g, 4g and 6g CaC₂ respectively) for 6 weeks. Blood samples (5ml) were collected from each rat by cardiac puncture (after sacrifice) for the estimation of levels of Urea, Creatinine, GSH, activities of catalase and SOD. Two rats from each group were selected for histology. Significant differences exist among groups A, B, C & D respectively in levels of creatinine ($1.07 \pm 0.17, 1.87 \pm 0.39, 2.33 \pm 0.70$ & 2.70 ± 0.70 ; $P < 0.001$), urea ($46.88 \pm 5.16, 50.99 \pm 10.42, 58.42 \pm 10.00$ & 66.34 ± 16.49 ; $P < 0.05$) and catalase ($2.70 \pm 0.70, 0.49 \pm 0.34, 0.56 \pm 0.34$ & 0.30 ± 0.21 ; $P < 0.001$). Values were raised in Urea and creatinine and lower in Catalase in experimental groups than control. The degree of effects was highest in the group having the greatest dose of CaC₂, group D. Histological abnormalities were seen in both the glomerulus and the tubules. Findings from this study probably suggest hazardous effects of CaC₂ on the kidney via oxidative stress mechanism [1].

The global increase in the demand for ripe fruits has induced unhealthy use of toxic chemicals in fruit ripening. One of such chemicals in common use is calcium carbide (CaC₂). Due to its nature, commercial CaC₂ is consistently found to contain impurities such as Arsenic and other toxic and carcinogenic chemicals [2].

A study to evaluate the effect of Calcium Carbide ripened fruit (Pawpaw) on the Haematological parameters of the Wistar rats was done and the results showed reduction in mean Pack Cell Volume, Total White Blood Count, Hemoglobin, Red Blood Cells, Platelets, Neutrophil, Monocytes for both the Wistar rats fed with Naturally ripened and CaC₂ ripened groups in comparison with the control group. There is evidence of increase in the mean Lymphocytes level for both the Wistar rats fed with naturally ripened and CaC₂ ripened groups in comparison with the control group, but the CaC₂ treated group tend to be higher. Eosinophil levels are higher in the CaC₂ treated group. In a nutshell, The consumption of fruits ripened with Calcium Carbide pose devastating effect on the bone marrow, deleterious effect on the circulating blood, heart and brain, that will evenly lead to myocardial infarction, Eosinophilia, Anemia, thrombocytopenia, paralysis, stroke, seizure and mortality may eventually arise [3].

Study to reveal the effect of Calcium Carbide forced ripened pineapple on the haematological indices of the Wistar rats was carried out, The results showed significant reduction in mean PCV, Total white blood count [TWBC], Hemoglobin, Total red blood cells [TRBC], Platelets, Neutrophils. But there was increase in lymphocytes, Monocyte and Eosinophil. In summary, Calcium carbide causes various health hazards to human even when consumed indirectly [4].

The explicit use of chemicals for fruit ripening is increasing daily and with eminent consequences. The aim of this study is to check nutritional programming on the second filial generation pups of the Calcium Carbide coerced orange juice fed Wistar rats. The results showed significant increase in PCV, hemoglobin, Total RBC, lymphocytes and reduction in Total WBC, Platelet and Monocytes in the second filial generation pups from the Wistar rats fed with Calcium carbide coerced orange juice. Haematological Indices are biomarkers that indicates functionality of the blood cells with regards to low, normal or high range. There is evidence of nutritional programming in the second filial generation pups as seen in this results [5]. Study aimed to evaluate the toxic effect on the Biochemical indices of the second filial generation pup from the Wistar rats fed with Calcium Carbide forced ripened orange fruits was conducted. Biochemical assay was done and results indicates reduction all the tested indices; AST, ALT, ALP, Creatinine, Urea, Albumin, Total protein, Total Cholesterol, Bilirubin, Lactate Dehydrogenase [LDH] in the Second Filial Generation Pups of the Calcium Carbide treated Wistar rat. Nutrients from the fruit induced with Calcium Carbide consumed during pregnancy permanently impact on the developing fetus of the Wistar rats which is expressed later in life [6]. Assessment of calcium carbide and natural ripened pawpaw (*Carica papaya*) fruit on the biochemical parameters of the Wistar rats was studied. The renal, hepatic, cardiac, heart and lipid profile parameters analyzed were albumin, total protein, urea, creatinine, Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), total bilirubin, Aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and total cholesterol and were compared with the naturally ripened pawpaw fruit group. All mean values of total protein, total cholesterol, lactate dehydrogenase and creatinine levels of calcium carbide ripened pawpaw fruit juice fed group were significantly higher when compared with the natural ripened pawpaw fruit juice. Meanwhile, albumin, total bilirubin, urea, ALT, AST and ALP levels of calcium carbide ripened fruit juice fed group were lower when compared with the natural ripened pawpaw fruit juice. Statistically, there were no significant differences of albumin and total protein parameters at 95% confidence level ($P < 0.05$). In conclusion, the elevated levels of creatinine, total cholesterol and lactate dehydrogenase may result to kidney injury, cardiovascular and heart diseases. There is therefore need for institutional and legislative strengthening as well as enforcement to prevent the use of calcium carbide in the ripening of pawpaw and other fruits [7]. Lipid profile and haematological indices of Wistar albino rats fed naturally ripened, unripe and artificially ripened mango pulp formulated diets were investigated. Results obtained indicated white blood cell count increase while Red blood cell count and hemoglobin concentration decreased in the artificially ripened groups compared to the control. Generally, the values of lipid parameters and hematological indices suggest that artificial ripening especially by the use of carbide may not be a good candidate in the ripening of mango fruits [8]. Calcium salts delayed the fruits ripening about 3 days as compared to the control fruits. however, it induced skin shriveling. CaSO₄ also delayed fruits ripening but improved the pulp color. The gradual increase in concentration of calcium salts delayed

the fruits ripening, but had negative effects on fruit quality by increasing skin shriveling and lowering flavor and taste of the mango fruits. On the other hand, the calcium ammonium nitrate had a poor influence in the mango fruits ripening. Although calcium salts delayed the ripening for some days, but it as well as decreased the fruits quality, thus, responsible for poor eating quality. CaC₂ is a fast ripening agent and it was seen that, after spraying the CaC₂ solution on the mango, they were ripped within 1/2days as compare to the controls were ripped within at least 5 days [9]. A study was conducted to investigate the first time the outcome of ingestion of calcium carbide-ripened fruit on some female reproductive parameters. An increased serum oestrogen level and uterus weight were detected in the CCRB and oestradiol treated groups. Histopathology showed increased numbers of myometrial cells, presence of secondary follicles and regressing corpus luteum as well as thickened cervix epithelia which were evidence of oestrogenic disruptions. This study has shown that consumption of fruits ripened with calcium carbide negatively alters the female reproductive physiology, accelerates puberty onset and increases serum oestrogen levels [10]. The use of calcium carbide is being discouraged worldwide, due to associated health hazards. Calcium carbide treatment of food is extremely hazardous because it contains traces of arsenic and phosphorous, and once dissolved in water, it produces acetylene gas. Arsenic, phosphorous and acetylene gas may affect the different body organs and causes various health problems like headache, dizziness, mood disturbances, sleepiness, mental confusion, memory loss, cerebral edema, seizures and prolonged hypoxia [11]. The indecent use of calcium carbide for fruit ripening is on the increase thus, the need for this study.

2. Material and methods

2.1. Materials

Materials include Wistar rats, Calcium carbide, Water, Pawpaw, Syringes and Needles, Hand Gloves, Incubator, stop watch, Oven, centrifuge Model 800, cotton wool, Chloroform, 40% formaldehyde, Desiccator, Methylated spirit, EDTA bottles, normal sample bottles, Animal weighing balance, Water bath, and amongst others.

2.2. Design of the Experiment

This is an experimental study of adult Wistar rats were fed with naturally ripened and Calcium Carbide induced ripened fruits [pawpaw] in other to compare and investigate the effect of Calcium Carbide on the kidney of the Wistar rats.

2.3. Fruit Collection

Mature unripe pawpaws were plucked off from the parent plant at Yenagoa, Bayelsa State. The fruits were dividing into two groups; one group was kept and allowed to rip at normal room temperature at the Histology Laboratory, Bayelsa Medical University, Yenagoa, Bayelsa State. The other group was induced with Calcium Carbide to ripe.

2.4. Weight and application of Calcium Carbide

Calcium carbide was bought at Swali Market, Yenagoa, Bayelsa State. 10gram of Calcium carbide was placed in a bowl containing 5ml of water for dissolution in a closed metal bucket containing 1kg of the fruit [pawpaw] rapped with black nylon and was allowed for two days[48 hours] for ripening. After ripening, sampled fruits were washed and juiced.

2.5. Homogenized sample preparation

600g of both the naturally ripened and calcium carbide ripened fruits [pawpaw] were peeled separately and blended in an electric blender with 350ml/1L of deionized water. The juice was filtered with a clean fine sieve and was poured into clean bottles labeled [CaC₂ induced ripened juice and naturally ripened pawpaw juice]; and was stored in a refrigerator for subsequent use.

2.6. Experimental Animals

21 adult Wister rats of both sexes weighing between 126.9- 214.3g were used for this study. The animals were purchased and kept in standard environmental condition, given standard rodent food (formulated) and water ad libitum in the animal house of the Bayelsa Medical University. The rats were divided into three groups for each sex, based on the body weight and then different concentrations of naturally ripened and calcium carbide induced ripened Pawpaw were administered orally. Animals were allowed to acclimatize for two [2] weeks and was fed with standard grower mash with clean water before the commencement of treatment following the protocols of [12].

2.7. Sample Administration

LD50 was carried out using [13] method for administration of samples. A total of nine [9] Wistar rats were used for this section grouped into three [3] each group containing three [3] rats. 12 Wistar rats were used for the main experiment.

- Group 1: Normal control group of 4 rats [2 males and 2 females] receive normal water and feeds only as placebo.
- Group 2: Treatment Group [1] of 4 rats [2 males and 2 females] received 5ml/kg naturally ripened ripened fruits [pawpaw juice] for 4 weeks [A month].
- Group 3: Treatment Group [2] of 4 Wistar rats [2 males and 2 females] received Calcium Carbide ripened fruits [pawpaw] for 4 weeks [A month].

5ml/kg for both the natural fruit and the CaC2 ripened fruits were administered against each body weight of the adult Wistar rats.

2.8. Organ Collection

The Wistar rats were weighed, then one Wistar rat was sacrificed in the groups, each week and samples were collected from the three groups for histological tissue preparation.

3. Results

The results from both the hematological and histopathological analysis are displayed in the Tables and Photo plates below.

Table 1 Body Weight of Adult Wistar Rats Before Treatment [Grams]

| Group | Control | Natural fruits | Cac2 ripened fruits |
|------------|--------------|----------------|---------------------|
| Mean value | 214.30±10.53 | 184.53±19.53 | 174.28±17.35 |

Mean ±SEM

Table 2 Toxic effects of Natural and CaC₂ ripened fruit on the Kidney Biomarkers

| Kidney Biomarkers | Control | Natural Ripened Pawpaw | CaC2 Forced Ripened Pawpaw |
|-------------------|-------------|------------------------|----------------------------|
| CREATININE [M/D] | 0.615 ±0.02 | 0.75 ±0.01 | 0.77 ±0.04 |
| UREA [MG/DL] | 15.5 ±0.3 | 24 ±0.2 | 23.85 ±0.95 |
| ALBUMIN [G/DL] | 4.5 ±0.1 | 5.05 ±0.25 | 5.05 ±0.25 |

Mean ±SEM

The results of the histological analysis of the kidney from the three different groups [Control, Natural and CaC₂ treated groups] for two [2] and four [4] weeks are shown on the photo Figure below.

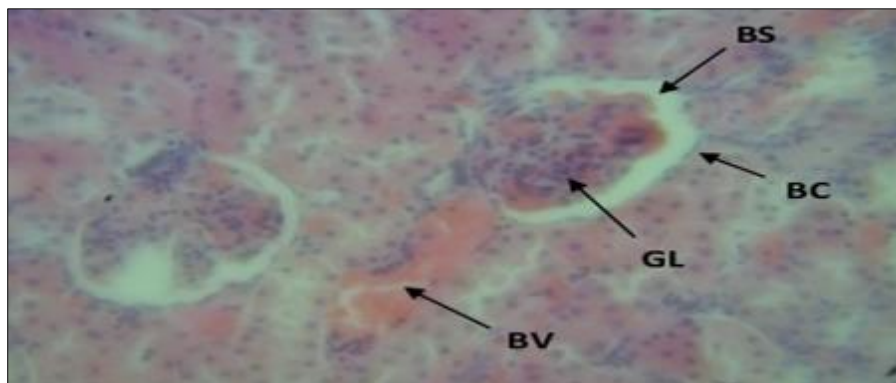


Figure 1 Photomicrograph (H&E x400) of the kidney of a Wistar rat fed with normal grower mesh [Control]. showing normal architecture: visible Malpighian corpuscle with Glomeruli (GL), Bowman's capsule (BC) and Bowman's Space (BS). Normal renal tubules with blood vessel (BV) in view

Lesion Diagnosis: Normal renal architecture.

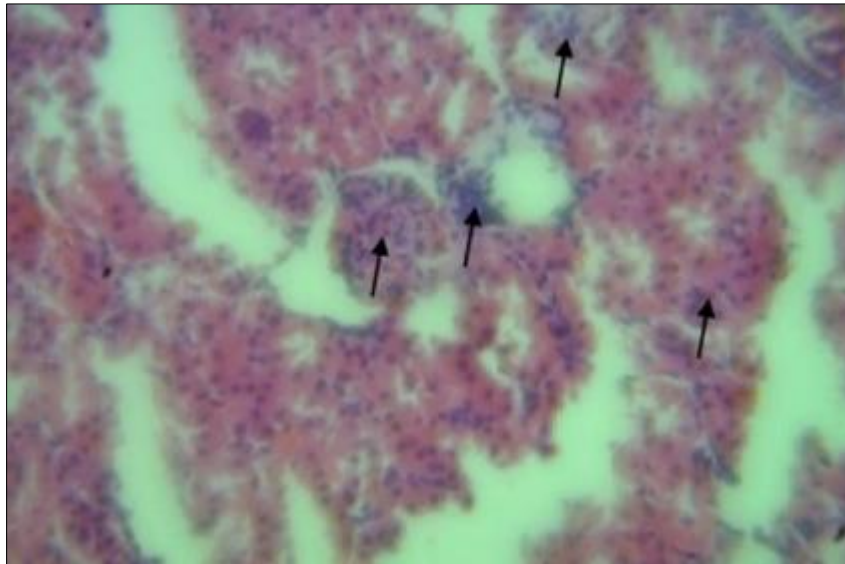


Figure 2 Photomicrograph (H&E Stain x400) of the kidney from male Wistar fed with CaC2 for [2 weeks] showing severe renal tubular degeneration with an associated inflammatory cell infiltration (arrows)

Diagnostic Lesion: Severe Renal Tubular Degeneration

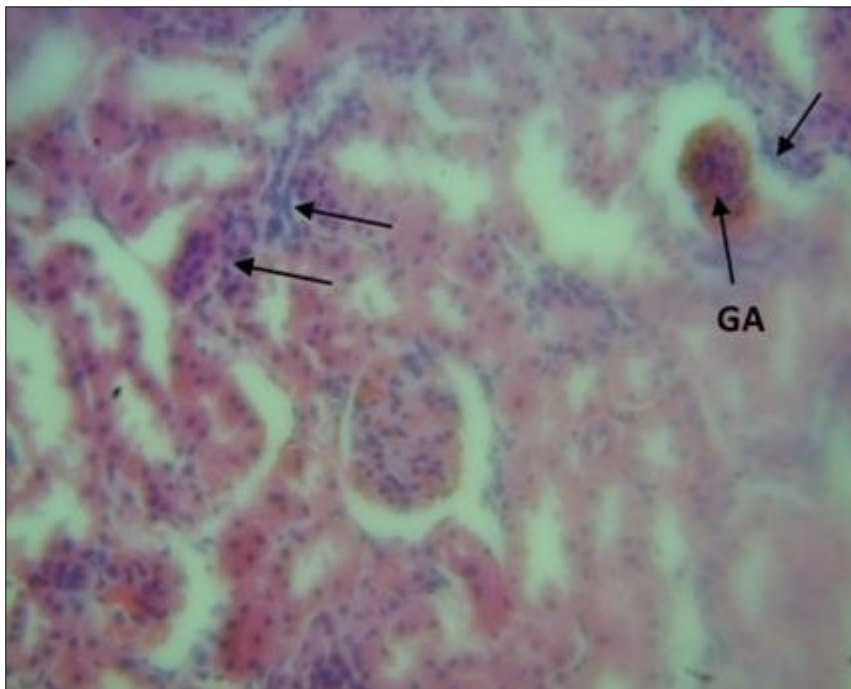


Figure 3 Kidney X400 (H&E Stain) Photomicrograph (H&E x400) of the kidney from CaC2 fed female Wistar rat for [2 weeks]: There is moderate renal tubular degeneration with associated glomerular atrophy (GA) and inflammatory cell infiltration (arrows)

Diagnostic Lesion: Moderate Renal Tubular Degeneration

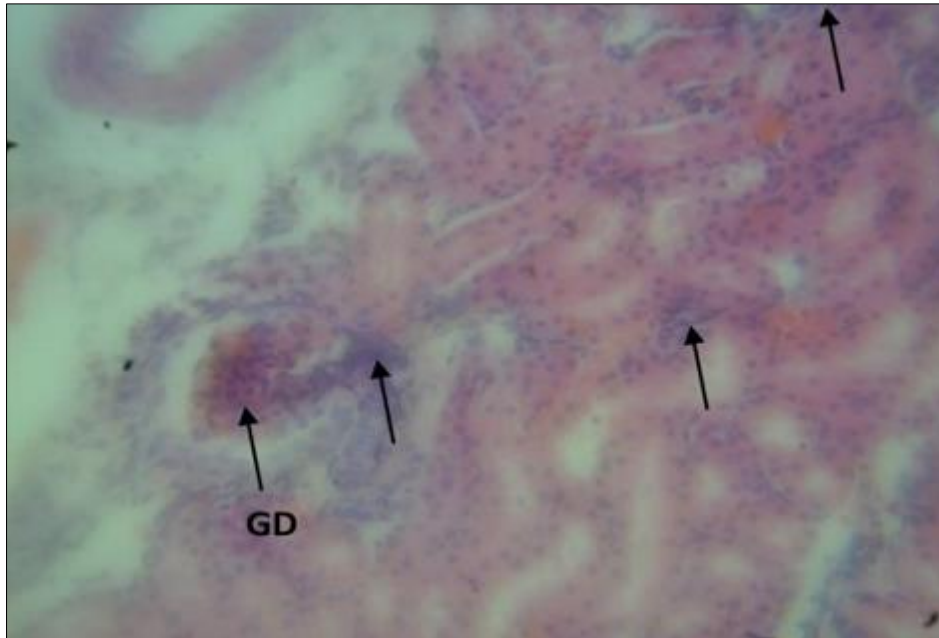


Figure 4 Photomicrograph (H&E x400) of the kidney from a male Wistar rat fed with naturally ripened pawpaw for [2 weeks]: There is a mild renal tubular degeneration with associated glomerular degeneration (GD) and minimal inflammatory cell infiltration (arrows)

Diagnostic Lesion: Mild Renal Tubular Degeneration

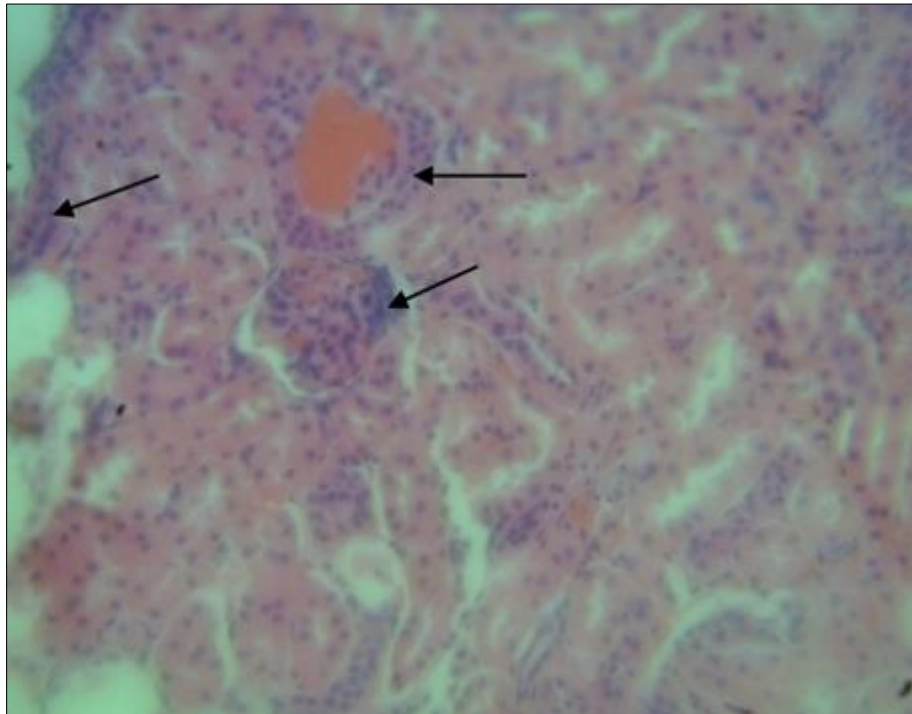


Figure 5 Photomicrograph (H&E x400) of the kidney from a female Wistar rat fed with naturally ripened pawpaw: with multi-focal areas of mild cellular infiltration (arrows)

Diagnostic Lesion: Mild Cellular Infiltration

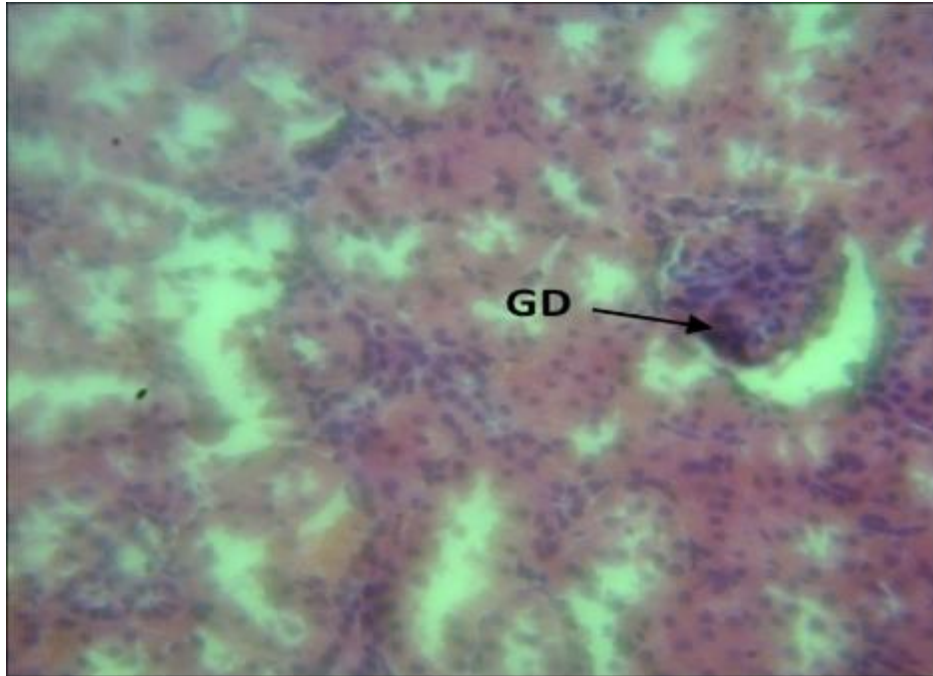


Figure 6 Kidney X400 (H&E Stain) Photomicrograph (H&E x400) of the kidney from a male Wistar rat fed with CaC2 ripened pawpaw for [4 weeks]: There is moderate renal tubular degeneration with an associated glomerular inflammation (GA) and severe diffuse inflammatory cell infiltration

Diagnostic Lesion: Moderate Renal Tubular Degeneration

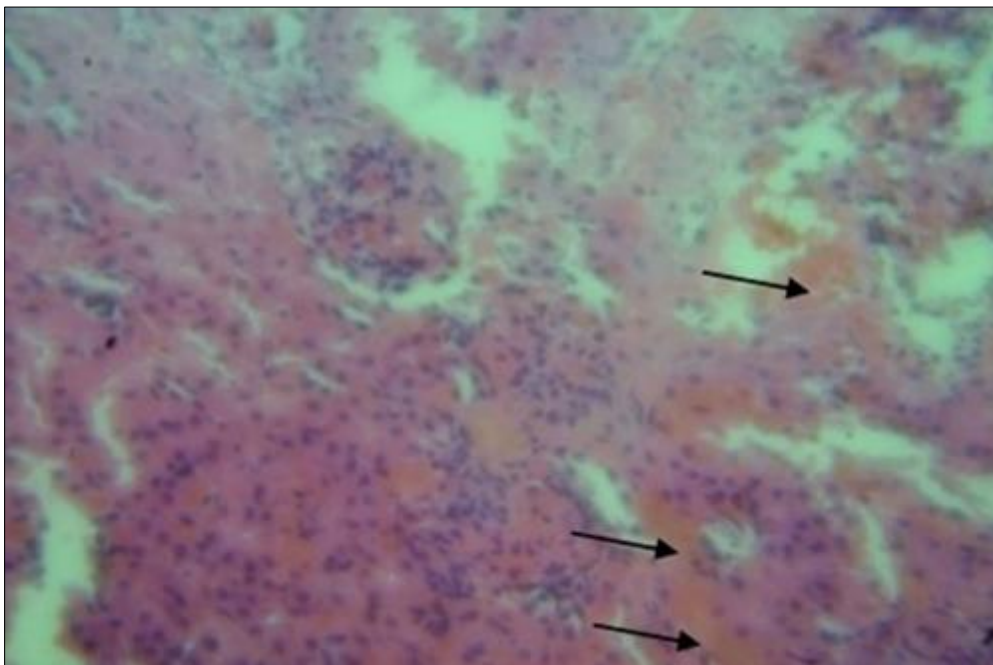


Figure 7 Photomicrograph (H&E x400) of the kidney from a female Wistar rat fed with CaC2 ripened pawpaw for [4 weeks]: There is tubular necrosis with associated pigmented accumulation (arrows)

Diagnostic Lesion: Tubular Necrosis

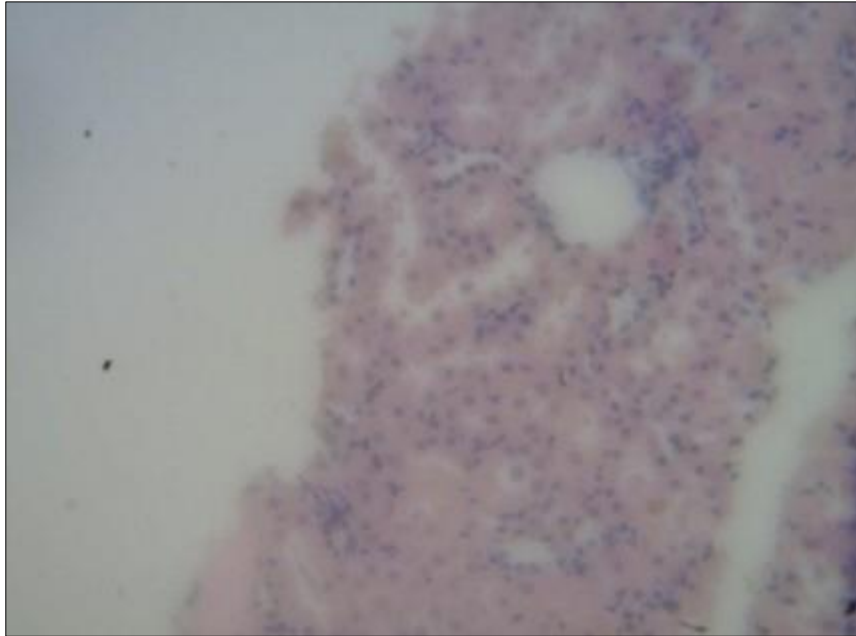


Figure 8 Photomicrograph (H&E x400) of the kidney from a male Wistar rat fed with naturally ripened pawpaw for [4 weeks]; : minimal cellular infiltration (arrows)

Diagnostic Lesion: Minimal Cellular Infiltration

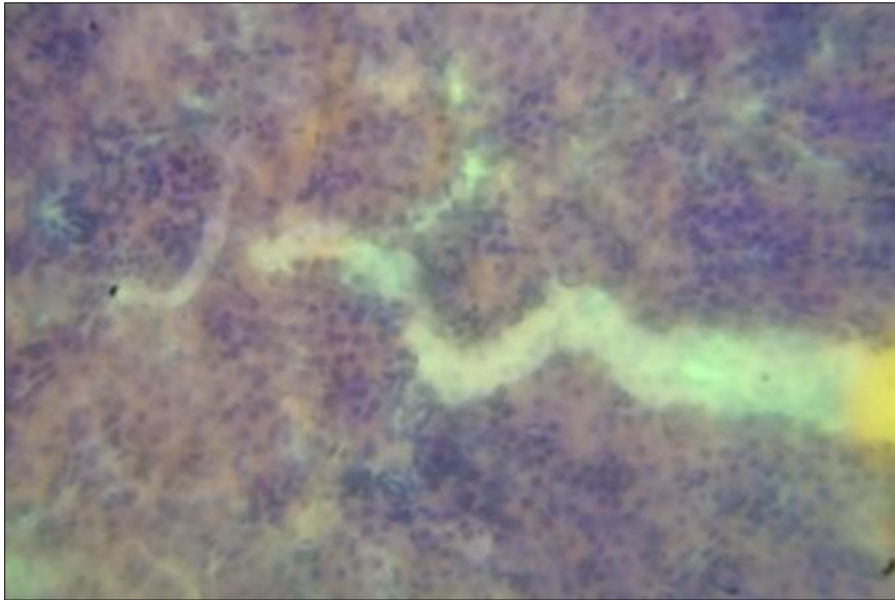


Figure 9 Photomicrograph (H&E x400) of the kidney from a female Wistar rat fed with naturally ripened pawpaw for [4 weeks]: Showing Severe inflammation – diffuse infiltration of inflammatory cells

Diagnostic Lesion: Severe inflammation

4. Discussion

The kidney is on the most vital organ in the body . Alteration in the structural is an index of problem in the functionality of the general wellbeing of the body. Specific biomarkers have been used to explicitly analyze the kidney functions. Amongst these biomarkers is Creatinine. Arise in creatinine level is a pointer of kidney injury and could lead to low glomerular filtration rate as seen in [table 2] . Albumin is also a biomarker that test the efficiency of the kidney filtration process. When normal kidneys filter blood, albumin remains in the blood. When kidney damage occurs, this filter is

unable to properly filter out albumin, which is then excreted in the urine - a symptom known as albuminuria - and can be measured and is used as a marker of kidney damage. In this study, there is high albumin in the both the treated groups resulting to hyperalbuminemia. While Urea is formed in the liver, representing the principal waste product of protein catabolism and is excreted by the kidney. In this present study, Urea levels is extremely high in the treatment groups as against the control which could result to uremia [table 2]. This condition is a terminal clinical manifestation of kidney [renal] failure. The results corroborates the findings of [7, 14].

Results from the histological assay showed, normal kidney structure with clear Malpighian corpuscle with Glomeruli (GL), Bowman's capsule (BC) and Bowman's Space (BS). Normal renal tubules with blood vessel (BV) of the control group [Photo Figure 1]. The mammalian kidney is composed of roughly 1.3 million nephrons- the structural and functional unit of kidneys. Each of which is composed of four regions [15]. The glomerulus is a complex web of capillaries derived from the afferent arteriole. Glomeruli can be located in the cortex of the kidney or the cortico-medullary junction. The glomeruli contains Efferent / Afferent Arterioles: The blood enters the glomerular tuft via the afferent arteriole and exits via

Mesangium – provides structural integrity to the glomerular tuft and modulates glomerular perfusion through smooth muscle-like activity.

Bowman's space and Bowman's capsule: Bowman's space is the space between the capillary tuft and Bowman's capsule. Bowman's capsule is lined by parietal epithelium, which is simple squamous epithelium. The ultra-filtrate drains toward the urinary pole and enters the proximal convoluted tubule.

Proximal tubule (PT): There is a convoluted portion, which is tortuous and often seen as rings of epithelial cells lining a basement membrane, and a straight portion which is more linear running from the cortex to the medulla.

Loop of Henle: The thin descending limb of Henle travels from the PT and forms a hairpin loop to become the thin ascending limb and the thick ascending limb before terminating into the distal convoluted tubule.

Distal convoluted tubule (DCT): The DCT is composed of simple cuboidal epithelium. The DCT is located in the cortex and corticomedullary junction.

Collecting duct: The collecting ducts span the renal cortex and medulla. The collecting duct is composed of simple cuboidal epithelium. There are two distinct cell types present in the collecting tubules: intercalated cells and principal cells.

Papillary duct: These are continuous with the collecting ducts and their lumens widen slightly as they reach the renal papilla. The epithelium lining these tubules transitions smoothly to the urothelium that lines the renal papilla.

The Results indicate that the Wistar rat fed with CaC₂ for both male and female for two[2] weeks [photo Figure 2&3] showed severe and moderate renal tubular degeneration. Renal Tubular Degeneration is a nonspecific entity that can arise from any number of etiologies that perturb cell function and is often an early indicator of necrosis. Degeneration, in some cases, is preceded by vacuolation. In general, degeneration is characterized by several morphologic and variable cell features, such as cell swelling with or without cytoplasmic vacuolation and pale staining and fragmented cytoplasm [15, 16]. While [photo Figure 4&5] showing results of the kidney of Wistar rats fed with naturally ripened pawpaw with Mild Renal Tubular Degeneration and Mild Cellular Infiltration. The results also showcased the kidneys of male and female Wistar rats fed with CaC₂ forced ripened pawpaw [Photo Figure 6&7] with Moderate Renal Tubular Degeneration and Tubular Necrosis. The term tubular necrosis is a misnomer, as true cellular necrosis is usually minimal, and the alteration is not limited to the tubular structures. The pattern of injury that defines tubular necrosis includes renal tubular cell damage and death. Intrarenal vasoconstriction or a direct effect of drug toxicity is caused by an ischemic event, nephrotoxic mechanism, or a mixture of both [17]. While [photo Figure 4&5] showing results of the kidney of Wistar rats fed with naturally ripened pawpaw for four [4] weeks with Minimal Cellular Infiltration and Severe inflammation.

5. Conclusion

Calcium carbide consumed directly or indirectly pose a devastating effects of the kidney of the Wistar rats. Ranging from low filtration rate, moderate to severe renal tubular degeneration and tubular necrosis which could lead complete renal failure and subsequently death. We therefore, advocate complete stoppage of the use of calcium carbide as a fruit ripening agent.

Compliance with ethical standards

Acknowledgments

We sincerely, appreciate TETFund for sponsoring this research.

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

The Bayelsa Medical University Ethics Committee approved the conduct of this research.

Funding

Tertiary Education Trust Fund [TETFund]

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