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Nephrotoxic Effects of Calcium Carbide on Wistar Albino Rats – A Histological Study

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ABSTRACT

Background: Calcium carbide (CaC2) is commonly used as an artificial fruit ripening agent. Once CaC2 comes in contact with moisture, it releases acetylene gas (Carbide gas), similar to the fruit ripening agent 'ethylene'. The main objective of the study was to investigate the effect of calcium carbide on the kidney via the evaluation of gross and histological changes.

Data & Methods: Twenty-four male healthy Wistar-albino rats weighing 130-200gm were randomly divided into 4 groups i.e. control group (received normal diet) and group I (normal diet mixed with 0.5gm CaC2), group II (received normal diet mixed with 0.25gm CaC2), group III (normal diet mixed with 0.125gm CaC2). Each group included 6 rats. All groups were treated over 21 consecutive days. On the 22nd day, all rats were anesthetized by inhalation of ether and scarified by cervical dislocation. Organs were removed and preserved in 10% formalin solution for histological examination. The diameters of renal components were measured using oculo-micrometers.

Results: CaC2 did not affect the body weight of albino rats. Compared to the control group, the weight of kidney of all experimental groups decreased significantly (p-value 0.001). Histological study of the kidney showed a statistically significant decrease in the diameter of renal corpuscle and capsular space of group I compared to the control group and a statically significant increase in diameter of that of group II and III (p-value <0.01). The diameter of the glomerulus of group I and group III decreased but that of group II diameter was increased compared to the control group (p-value <0.01). Similarly, the diameter of distal convoluted tubules decreased in all experimental groups (p-value <0.001). However, there was no effect in the diameter of proximal convoluted tubules of all experimental groups (p-value 0.052).

Conclusion: CaC2 caused both morphological and histological changes in rat kidney. The biochemical markers and pathological changes should be included in future studies.

Keywords: Nephrotoxicity, Kidney Histopathology, Oxidative Stress, Calcium Carbide Toxicity

INTRODUCTION

Fruits play a vital role in human nutrition by supplying the necessary growth-regulating factors essential for maintaining normal health.[1] So, traders pick green fruits before maturation and ripen artificially to serve in the market earlier than the season for higher profit. Moreover, green fruits are transported easily with minimum damage and ripened at the place of retail sale.[2] The rising demand for fruit safety has inspired researchers to think about the risks related to the use of fruit contaminated by pesticides, heavy metals, or toxins.[3] The different ripening agents used by the farmers and/or traders are calcium carbide, acetylene, ethylene, propylene, glycol, and smoke.[4]

The CaC2 is used as the common ripening agent for mangoes, bananas, jackfruits, litchis, and other fruits. Calcium carbide powder is kept in the container of fruits wherein contact with moisture produces acetylene gas and acts as a ripening agent. During the ripening process, a wide spectrum of biochemical changes takes place such as chlorophyll degradation, and biosynthesis of carotenoids, anthocyanins, essential oil, and flavor and aroma components. A similar condition induced by CaC2 with the help of acetylene gas has good peel color but is poor in flavor5 which is due to the production of a complex mixture of volatile compounds ocimene and myrcene and the degradation of alkaloids sesquiterpene, which are responsible for flavor and aroma.[6]

A study done by Ugbeni OC et al showed glomeruli atrophy and tubular necrosis in the kidneys of rats that were fed with CaC2-ripened plantain.[7,8]

Therefore, we did this study to evaluate the gross effects of CaC2 on the body of rats and the weight of their kidneys as well as histological changes in different components of the nephron viz., renal corpuscle, glomerulus, capsular space, proximal convoluted tubule, and distal convoluted tubule. Due to the paucity of such types of literature in our setting, this would be useful in policymaking and public awareness of the use of calcium carbide.

DATA AND METHODS

Twenty-four healthy male Wistar-albino rats weighing 130-200 grams were randomly selected for the study. The rats were acclimatized for 4 weeks before enrolling in the study. The animals were housed in plastic cages and were placed in a room at atmospheric temperature and humidity with 12-hour light-dark cycles without any stressful stimuli. All the rats were provided free access to a standard mashed-rodent diet and water ad libitum. Calcium carbide was obtained from Chamunda Importer and Exporter (Manufacturer: TYWH, made in China)

After the quarantine period, rats were randomly divided into four groups, each consisting of six members. The control group received a normal diet that is 120gm Bengal

gram. Group I received 120gm Bengal gram mixed with 0.5gm/kg of CaC2. Group II received 120gm of Bengal gram mixed with 0.25gm/kg of CaC2. Group III received 120gm of Bengal gram mixed with 0.125gm/kg of CaC2. All groups were treated over a period of 21 consecutive days. Twenty-four hours after the administration of the last doses, on the 22nd day, rats were anesthetized by inhalation of ether and sacrificed by cervical dislocation. The kidney was taken out by abdominal dissection, weighed, and preserved in a 10% buffered formaldehyde solution. After tissue processing, slides were prepared by Hamatoxylin & Eoson staining, and were observed for histological changes.

A minimum of 8 fields for each kidney section was examined under light microscopy and oculo-micrometers were used for quantitative measurements of different parameters like diameter of renal corpuscles (RC), glomerulus (G), proximal convoluted tubule (PCT), distal convoluted tubule (DCT), and dimension of Bowman's space . The diameter of 100 renal corpuscles, glomerulus, PCT and DCT were measured and the mean value of each group was calculated. The Bowman's space observed between two layers of Bowman's capsule was also measured by using the ocular micrometer. Study done by Ugbeni OC rats fed with carbide-ripened plantain had a significantly high level of plasma bicarbonate, the levels of plasma potassium (K+) and chloride (Cl−) were significantly low, the levels of urea and creatinine were significantly high and glomeruli atrophy and tubular necrosis in kidneys.7 A study done by Dibagha and Ogoun in calcium carbide forced ripened pawpaw showed an increase in creatinine, urea, albumin and severe renal tubular degeneration and tubular necrosis.14 Since calcium carbide affected both biochemical markers and cytoarchitecture of the kidney we selected the above-mentioned parameters like PCT, DCT and Bowman's space.

Statistical analysis was performed using SPSS software (Statistical Package for the Social Sciences) v22. Results were presented as mean values ± standard deviation. The one-way analysis of variance (ANOVA) test was used for data analysis and probability values (P) less than 0.05 was considered statistically significant.

RESULTS

Body weight of rats: The details of body weight before and after the experiment are shown in Table 1. The mean body weight of rats before the experiment was lower than their body weight after three weeks of the experiment. In ANOVA testing, the mean body weight among control and three experimental groups I, II, and III were not significant before (p=0.128) and after the experiment (p=0.438).

Table 2. Weight of Kidney in grams (mean ± SD)

Groups' name	Kidney weight (g) [Mean ± SD]
Control	0.77 ± 0.046
Group I	0.69 ± 0.083
Group II	0.63 ± 0.048
Group III	0.66 ± 0.038
P-value	0.001

After the experimental period, the weight of kidneys was as shown in Table 2. Compared to the control group kidney weight, the weight of all experimental groups I, II, and III kidneys were less and were statistically significant at a p-value of less than 0.001.

As shown in Table 3, CaC2 caused a statistically significant decrease in the diameter of the renal corpuscle of group I compared to that of the control group but caused a statistically significant increase in the diameter of the renal corpuscle of group II and III (P-value <0.001). About the

Table 1. Body weight of rat before and after experiment in gram (mean ± SD)

Grouping of the experimental Rats	Body weight in grams (mean ± SD)		Test Statistics for Paired t-test	p-value
	Before experiment	After experiment		
Control	132 ± 7.583	176 ± 11.14	9.67	0.0002
Group I	134 ± 6.44	181 ± 11.94	9.64	0.0002
Group II	136 ± 0.81	179 ± 4.54	23.2	<0.00001
Group III	128 ± 5.78	173 ± 5.75	19.17	<0.00001
P-value of One-way ANOVA	0.128	0.438		

Table 3. Diameter of renal corpuscle (RC), glomerulus (G), capsular space (CS), proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) in micrometer [Mean ± SD]

Groups' name	Renal Corpuscle (RC)	Glomerulus (G)	Capsular Space (CS)	Proximal Convoluted Tubule (PCT)	Distal Convoluted Tubule (DCT)
Control	37.62 ± 4.76	31.19 ± 4.95	3.86 ± 1.25	15.42 ± 2.64	14.15 ± 2.36
Group I	33.10 ± 7.24	26.63 ± 6.87	3.53 ± 1.28	14.48 ± 2.37	12.02 ± 2.26
Group II	41.07 ± 5.83	32.39 ± 6.68	5.64 ± 1.19	14.07± 2.15	11.84 ± 2.10
Group III	38.10 ± 5.95	30.59 ± 6.06	4.07 ± 1.32	14.50 ± 2.82	13.21 ± 2.68
p-value for ANOVA	<0.001	<0.001	<0.001	0.052	<0.001

proximal convoluted tubule, calcium carbide had no effect on experimental groups (p-value 0.052). About the distal convoluted tubule, calcium carbide caused a significant decrease in the diameter of all experimental groups (p-value <0.001). However, about glomerulus, group I and group III diameters were decreased, and group II diameter was increased compared to the control group (p-value 0.052). In Bowman's space the dimension was decreased in group I however increased in group II & group III (p-value <0.001).

Photomicrograph of a rat kidney section from the control group, displaying normal histological features. The renal cortex and medulla exhibit intact cytoarchitecture with clearly visible glomeruli, tubules, and interstitial spaces (magnification 100X) (Figure 1). Photomicrograph of a rat kidney section from Group I (treated group), showing alterations in the cortical cytoarchitecture. Notable changes include distortion of glomerular structures and tubular irregularities, indicative of tissue damage (magnification 100X) (Figure 2). Photomicrograph of a rat kidney section from Group I, demonstrating the presence of hemorrhage in the medullary region. Disruption of normal tissue architecture and extravasation of red blood cells are evident (magnification 100X) (Figure 3).

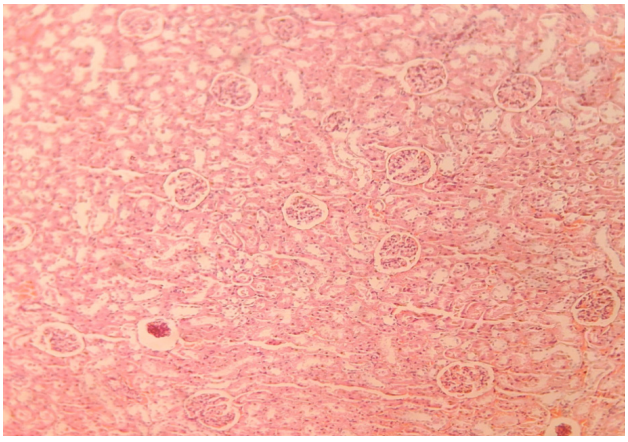

Figure 1. Photomicrograph of rat kidney section showing normal histology of control group (100X)

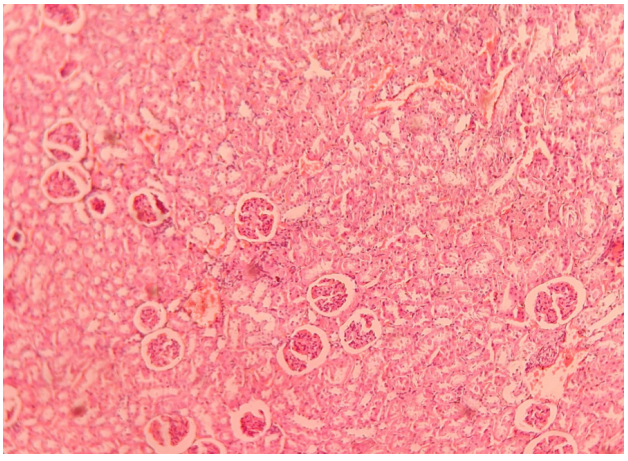

Figure 2. Photomicrograph of rat kidney showing changes in cytoarchitecture of group I (100X)

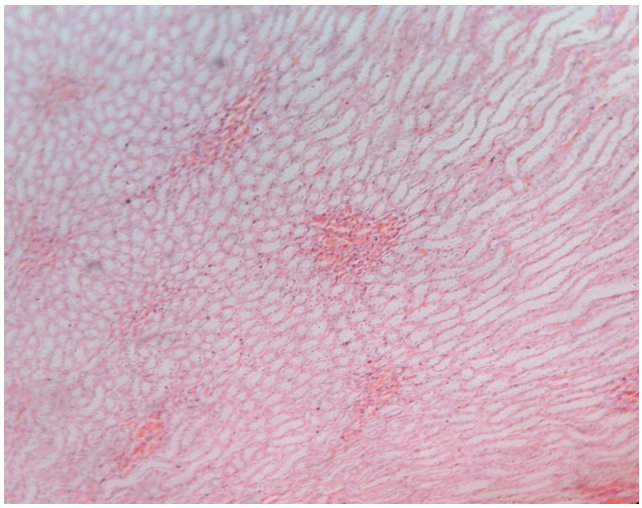

Figure 3. Photomicrograph of rat kidney showing hemorrhage in medullary region of group I (100X)

DISCUSSION

The misuse of CaC2 to induce fruit ripening is a global challenge with potential adverse effects to human health. This study illustrated both morphological and histological changes caused by calcium carbide in the kidneys of Wistar albino rats.

Normally, fruits produce a ripening hormone ethylene that induces the natural process of maturation. This process is artificially accelerated by using different chemicals of which calcium carbide is the commonest. Acetylene is a highly reactive substance used mainly in welding and allied industries. Industrial grade calcium carbide also contains trace amounts of more toxic arsenic and phosphorous that converts healthy fruits to poisonous ones.[10]

After experimental period the body weight of rat was increased in all groups. The accelerated weight gain in this study may be due to the presence of a component-phosphorus in calcium carbide. This study was similar to study done by Ajileye AB. Also in the study done by Ajileye AB, histopathological analysis of kidney revealed proximal tubules with cloudy swelling of renal tubular epithelium and mild mononuclear cell infiltration which is consistent with our finding.

In our study, after the experimental period, the weight of rats was increased equally in all groups which showed CaC2 caused no effect on body weight. In the study done by Ouma et al., there was no effect of CaC2 on the weight of rats, illustrating similar findings to our study.11Contrary to this in the study done by Ajileye AB calcium carbide caused increase in body weight. [14]

In the study done by Ajileye AB histopathological analysis of kidney revealed proximal tubules with cloudy swelling of renal tubular epithelium and mild mononuclear cell infiltration which is consistent with our finding showing changes in cytoarchitecture of kidney.

Histological study of the kidney under the light microscope showed a decrease in the size of the renal corpuscle, glomerulus, capsular space, and distal convoluted tubule of group I but no change or increase in the sizes among group II and group III. It showed that only a high dose affects the diameter but a low dose has no such

effect. These changes in size are caused by adverse effect of calcium carbide. This is consistent with the findings of Ugbeni OC study, which similarly showed high level of plasma bicarbonate, the levels of plasma potassium (K+) and chloride (Cl−) were significantly low, the levels of urea and creatinine were significantly high and glomeruli atrophy and tubular necrosis in kidneys.

This investigation bears similarities to those conducted Dibagha and Ogoun showing increase in creatinine, urea, albumin and severe renal tubular degeneration and tubular necrosis. Also supported by the findings from the study by Patoare et al., and Dibagha et al., which showed thickening of the lining of the collecting tubules, moderate to severe renal tubular degeneration, glomerular atrophy in the condition of the CaC2 treated group against the control group.[12,13] Atrophy of the glomerulus may be the cause of a decrease in the diameter of the glomerulus and capsular space.

Hemorrhagic areas were seen in medulla of group I kidney (Figure 3). This investigation bears similarities to those conducted by Bini et al., wherein they reported congestion resulting from hemorrhage and degradation of renal corpuscles upon exposure to calcium carbide.[15] This is as a result of cell death (apoptosis).

Compared to control group the weight of kidney of all experimental was decreased due to effect of calcium carbide.

Currently, the majority of fruit vendors utilize harmful chemicals like CaC2 to mature their fruits, raising serious worries about food safety and health security. Based on study by Cruzan et al.CaC2 is very dangerous for human health when consumed since it includes quantities of arsenic and phosphorus that can harm the kidney, heart, and central nervous system (brain).[16]

CONCLUSIONS

From this study, it was evident that calcium carbide causes adverse effects on the kidneys of Wistar albino rats. Morphological study showed a decrease in the weight of the kidney and histological study showed a decrease in the diameter of the renal corpuscle, glomerulus, capsular space, and distal convoluted tubule. It is recommended to include biochemical markers and pathological changes in future research. The result of this research will help in policymaking of food and agriculture ministry about harmful effect of calcium carbide

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