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Sub-acute pathological effects of calcium carbide as an artificial fruit ripening agent on various organs of albino mice

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ABSTRACT

Introduction. Calcium carbide is the most commercially used artificial fruit ripener because it is inexpensive to produce though, involving the use of hazardous elements and can easily be purchased in local markets. This study aimed at investigating the architectural changes of organs extracted from albino mice fed with fruits that were ripened with calcium carbide.

Material and methods. About 40 mice of both males and females, weighing between 18 g–25 g were randomly used for this study. They were divided into five (5) groups, made up of six mice namely Groups 1, 2, 3, 4 & 5 respectively. A set of unripe mature pawpaw and banana were ripened with calcium carbide (CCRP & CCRB) and were fed orally to groups 3 and 5 respectively for four weeks with water. Another set of the unripe mature pawpaw and banana were ripened naturally (NRP & NRB) without subjecting them to any artificial ripening processes and were fed orally to groups 2 and 4 mice respectively for four weeks with water. Rat feed and water were also given to the control group 1.

Results. Increased body weights were observed in the calcium carbide ripened banana (CCRB) treated group when compared to the other groups (control and calcium carbide ripened pawpaw). Histological sections revealed increased numbers of inflammatory cells, presence of collapsed epithelial layer, ruptured muscle, disorganized clara cells, aggregation of fibroblasts in the lungs; mild interstitial edema in the brain between the cardiocytes: mononuclear cell infiltration with cloudy swelling of the renal epithelium; dendritic cells in the brain.

Conclusions. According to this study, eating fruits that are ripened with calcium carbide has adverse related health effects thus negatively altering the histological architecture of the organs such as the lungs, liver, kidney, heart as well as the brain. Fruit vendors must therefore use caution when applying calcium carbide and adhere to international regulations that strictly limit its use.

Introduction

The process of ripening causes the mature fruits to progressively change in color, texture, sweetness, and flavor as well as scent and taste. Fruits ripening artificially can be induced by using chemicals like calcium carbide [1]. Perotti et al. [2] state that ripening is the genetically planned, highly coordinated, irreversible process that turns an unripe fruit into a ripe fruit that is edible and visually appealing. Numerous physiological, biochemical, and organoleptic alterations are involved [3]. These alterations include the weakening of tissues, changes in color, and the creation of volatile flavors and aromas.

Ripe fruit changes both structurally and compositionally, making it more appetizing and edible. Among these variations are textural ones that vary depending on the species; for example, apples typically show less tissue softening than bananas, mangoes, and pawpaw, which all experience significant softening [4]. Depolymerization, loss of cell structure, and solubility of components of the cell wall causes tissue fruit to soften. Certain fruits like bananas, have a high concentration of starch in their epicarp, which is broken down by enzymes and contributes significantly to tissue softening. In citrus fruits, on the other hand, tissue softening is primarily brought about by changes in turgor pressure, which is a result of postharvest dehydration and/or dry matter loss [5].

For many fruits, changing color is a crucial indicator of maturity. Fruits that are orange, red, pink, blue, and purple are the consequence of pre-existing colors being unmasked by the breakdown of chlorophylls and the synthesis of anthocyanins and carotenoids during the ripening process. The volatile chemicals that give fruits their flavor and scent are mostly esters, alcohols, aldehydes, ketones, and terpenes [6]. Fruits that have naturally matured are a vital component of a balanced diet and are essential for sustaining human health and nutrition.

The banana (Musa spp. L), belonging to the Musaccae family, is a highly traded tropical fruit globally and ranks as the fourth largest food crop [7]. It is a widely grown commercial crop in Africa, providing a significant portion of the continent's income for over 70 million people. Nigeria is one of the world's top producers of bananas and plantains [8].The main reason bananas are grown is for their tasty, and nutritious fruits. They are also readily digested, providing the necessary calories and vitamins [9]. Given that bananas are climacteric fruits, they are typically picked when they are mature but still unripe, then allowed to ripen [9]. Although this method lessens post-harvest losses of bananas, particularly during transportation, it should be noted that artificial ripening with chemicals like calcium carbide and others, such ethylene glycol and ether, is frequently used in conjunction with bananas [10]. In Bangladesh, over 74% of banana wholesalers employed various ripening chemicals to expedite the ripening process of their bananas [11].

Another name for pawpaw (Carica papaya L.) includes papaya. It is a fruit of the Caricaceae family that is succulent and herbaceous. In the world's tropical climates, it is extensively planted and produced. Additionally, because it is a climacteric fruit, artificial ripening may be necessary to meet the growing demand. However, when fruits like banana or pawpaw are combined with other common chemicals, like ethylene (C_2H_4), calcium carbide (CaC₂), ethephone (2-chloroeth-ylphosphoric acid), ethylene glycol, acetylene gas, and other pesticides that are not advised for ripening immature fruits quickly and attractively, these can become harmful [12].

Although calcium carbide (CaC₂) is a colorless chemical molecule, most samples exhibit a color shift that ranges from black to greyish-white. Industrial production of CaC₂ involves heating a mixture of lime and coke to around 2000°C, which yields about 80% calcium carbide by weight. Since calcium carbide speeds up the ripening of immature fruits, it is frequently employed as an artificial fruit ripening agent in Africa [12]. The most widely used artificial fruit ripener in commerce is calcium carbide, which is readily available in local markets and affordable to make [13]. However, it does involve the usage of hazardous components.

Calcium carbide reacts with moisture (water) to produce acetylene and calcium cyanamide, which are the two main industrial uses for it. The majority of the time, calcium carbide is used to ripen fruits like pawpaw, mangoes, bananas, jackfruits, etc., however because of the health risks involved, its usage is discouraged globally [14]. Worldwide, the use of this chemical in the fruit industry is discouraged due to its industrial grade, in particular, as it is frequently found to be contaminated with trace amounts of arsenic and phosphorus hydride. These substances are known to cause a number of acute and chronic illnesses, including vomiting, diarrhea, burning sensations, skin ulcerations, coughing, shortness of breath, low blood pressure, and hormonal imbalances that can result in infertility [14]. In addition, stomach discomfort, mouth ulcers, headaches, mental disorientation, dizziness, mood swings, insomnia, memory loss, cerebral edema, and seizures have all been linked to calcium carbide. It has been observed that extended and direct exposure to ethylene gas and CaC₂ can cause hypoxia and neurological issues [15].

Following consumption of calcium carbide, humans produce free radicals, also known as reactive oxygen species (ROS) or oxidants, which have a negative impact on a number of organs. Antioxidants are abundant in fruits; yet, the harmful effects of artificial ripening render even healthful fruits toxic [16].

Acetylene gas is utilized as a fruit ripening agent, in the production of polyvinyl chloride plastics, and in acetylene torches for welding. Nevertheless, some research indicates that acetylene does nothing more than intensify the fruit skin's yellow color, leaving the flesh unripe [14]. Acetylene gas is carcinogenic, meaning it can change healthy human cells into cancerous ones [17].

These days, farmers and fruit vendors frequently use artificial fruit ripening to speed up the ripening process, improve consumer acceptance, make fruits available off-season, reduce transportation losses, and release fruit products on schedule at the desired ripening stage [18]. Although there are several safe ripening techniques that are advised, farmers and retailers typically choose low-cost, risky techniques that make use of readily available chemicals like calcium carbide (CaC₂) [19].

Furthermore, it has been shown that artificially ripened fruits have fewer mineral components and antioxidant potency than naturally ripened fruits [12]. However, during the actual ripening season, it is challenging to detect morphological distinctions between naturally ripened fruits and artificially ripened fruits [12, 20].

Most countries forbid using this chemical for this purpose [12], although others like Nigeria, India, Pakistan, Bangladesh, and Nepal allow it to be used openly.

Farmers are often forced to utilize artificial food ripening methods to meet the growing demand for fruits as a dietary source of vitamins, minerals, and dietary fiber. These methods facilitate the immediate ripening of fruits before the appropriate season, making them aesthetically pleasing and appealing to humans without taking into account the potential negative health effects of these chemicals [1]. Farmers also benefit financially from these methods. Because calcium carbide has such negative effects on humans, conducting clinical trials on humans is not only unethical but also impossible. This study is aimed at investigating the histopathological effects of calcium carbide as an artificial fruit ripening agent on various organs of albino mice.

Materials and methods

Study Area

This study was carried out in the Department of Biomedical Laboratory Science (BMLS) and Pathology, College of Medicine, University of Ibadan, Ibadan, Oyo State.

Study Design

This is an experimental study.

Ethical Clearance

The protocol for this study was applied and approved by the Ethics and Research Committee of Accurec (With number 24/046), University of Ibadan, Ibadan, Nigeria.

Procurement of Calcium Carbide

The calcium carbide was obtained from Gate market, Ibadan Oyo state.

Fruits Procurement and Preparation

Freshly harvested bunches of unripe matured green banana and pawpaw were purchased from Oje local market, Ibadan Oyo State. The fruits were divided into two experimental groups namely the naturally ripened fruits (non-treated with calcium carbide) and the artificially ripened fruits (treated with calcium carbide) of approximately same weight which were placed under same atmospheric condition. The matured banana and pawpaw not treated with calcium carbide, got naturally ripened in 4 to 5 days while the banana and pawpaw artificially ripened with calcium carbide got ripened in 2 days

After ripening, some sample fruits were washed, peeled and some of the fruits were unwashed, peeled, and were administered orally to the albino mice together with water for four weeks.

Experimental Animals

Forty albino mice (20 males and 20 females) with the weight ranging from 18–25 g were purchased from the animal house IAMRAT, University of Ibadan. The experimental animals were acclimatized for three (3) weeks under a conducive condition.

Experimental design

The experimental animals were separated into five (5) groups in clean local metal cages having both males and females (each group composed of four (4) males and four (4) females' albino mice).

Mice marked Group 1 were fed on the standard rodent feed (IAMRAT Animal House, formulated) and tap water which was considered the negative control group; the mice that fed on naturally ripened fruits marked groups 2 & 4 were considered to be the positive control group and the mice that fed on calcium carbide ripened fruits were marked groups 3 & 5 which were considered as sample groups. All the animals had free access to food specifically meant for each group.

Every procedure involving animals was carried out in compliance with the National Institutes of Health's handbook for the care and use of laboratory animals, as well as a protocol authorized by the IAMRAT Animal House Care.

Behavioural changes

Throughout the administration period, no behavioral changes were observed in the animals.

The above treatment was administered orally to each group respectively after acclimatization for a period of one month (4 weeks). After administration, various organs (brain, liver, lungs, kidney and heart) were obtained from the mice to investigate the histopathological effects of calcium carbide and in relations to any histopathological condition.

Sample collection and preparation for histology

After a month, (four weeks), all the mice from each group were sacrificed via a physical meanscervical dislocation. The mice were dissected in order to access and harvest the organs of interest- brain, liver, lungs, heart and kidney. Excised organs were fixed in 10% formal saline and then transferred to the Histology laboratory of the Department of Pathology, UCH, Ibadan, Oyo State.

These organs were grossed, each measuring few millimetres in thickness for tissue processing then the tissues was placed in the tissue cassettes appropriately labelled for tissue processing, then embedded in molten paraffin. The tissue blocks were sectioned with the aid of a rotating microtome at 3–5 micron in thickness. The sections were stained with Haematoxylin and Eosin stain then, mounted with distyrene plasticine xylene (DPX). The stained slides were viewed under the microscope for results interpretation.

Staining procedure

The tissue sections were taken to water through descending grades of alcohol $(99\% \rightarrow 90\% \rightarrow 80\% \rightarrow 70\%)$. The tissue sections were rinsed in water, thereafter stained in Harris heamatoxylin for 5 mins. Tissue sections were rinsed in water, then differentiated briefly in 1% acid alcohol and rinsed immediately in water. Tissue sections were blued in running tap water for 15 minutes, then counterstained with 1% aqueous eosin for 2 minutes. Tissue sections were rinsed in water, dehydrated in ascending grades of alcohol, cleared in xylene and latter mounted with Distyrene plasticine xylene (DPX).

Table 1. Distribution of mice into various groups (the experimental design).

Groups	Treatment
Group 1(4M & 4F)	Standard rodent feed and normal water
Group 2 (4M & 4F)	Naturally ripened pawpaw and normal water
Group 3 (4M & 4F)	Artificially ripened pawpaw and normal water
Group 4 (4M & 4F)	Naturally ripened banana and normal water
Group 5 (4M & 4F)	Artificially ripened banana and normal water

M = Male albino mice; F = Female albino mice

Data Analysis

The collected data are shown as mean and standard deviation (±SD). Statistical analysis for Social Sciences (SPSS) version 25 was the statistical package used to evaluate and compare the results for each group. Bonferroni post hoc test and Pearson Chi-Square were used to compare the means of the different analytes at p < 0.05statistical.

Results

Table 2 Shows the mean and standard deviation of the body weight of the mice in their respective groups taken per week for a period of four (4) weeks, with p < 0.001 being significant when compared to the control group. **Table 3** compared the difference in mean body weights measured among the group 1 with the other groups

Table	2.	Body	weight	analysis.
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Groups	Mean (µ)	Standard Deviation(s)	p-value
Group 1	28.1111	4.48359	0 .0001*
Group 2	18.8571	5.33631	
Group 3	17.5714	2.14920	
Group 4	15.9091	3.01511	
Group 5	18.8182	2.27236	
Total	22.1587	6.47129	

Note: asterick (*) means significant p < 0.001. Group 1: Control; Group 2: Naturally ripened pawpaw (NRP); Group 3: Calcium carbide (Artificially) ripened pawpaw (CCRP); Group 4: Naturally ripened banana (NRB); Group 5: Calcium carbide (Artificially) ripened banana (CCRB)

	Table 3.	Multiple com	parison (Bon	ferroni post	hoc test).
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(I) Weight Group	(J) Weight Group	Mean Difference (I-J)	p-values (SIG)
Group 1	Group 2	9.25397*	.0001*
	Group 3	10.53968*	.0001*
	Group 4	12.20202*	.0001*
	Group 5	9.29293*	.0001*
Group 2	Group 1	-9.25397*	.0001*
	Group 3	1.28571	1.000
	Group 4	2.94805	1.000
	Group 5	.03896	1.000
Group 3	Group 1	-10.53968*	.0001*
	Group 2	-1.28571	1.000
	Group 4	1.66234	1.000
	Group 5	-1.24675	1.000
Group 4	Group 1	-12.20202*	.0001*
	Group 2	-2.94805	1.000
	Group 3	-1.66234	1.000
	Group 5	-2.90909	.823
Group 5	Group 1	-9.29293	.0001*
	Group 2	03896	1.000
	Group 3	1.24675	1.000
	Group 4	2.90909	.823

Note: asterick (*) means significant p < 0.001. Group 1: Control; Group 2: Naturally ripened pawpaw (NRP); Group 3: Calcium carbide (Artificially) ripened pawpaw (CCRP); Group 4: Naturally ripened banana (NRB); Group 5: Calcium carbide (Artificially) ripened banana (CCRB)

Table 4. Food consumption analysis.

Groups	Mean(µ)	Standard Deviation(s)	p-value
Group 2	104.3571	19.24181	0.357
Group 3	106.1429	19.45748	
Group 4	100.7500	18.13468	
Group 5	110.3793	15.73761	
Total	106.3014	17.72920	

P > 0.05. Note: Group 2: Naturally ripened pawpaw (NRP); Group 3: Calcium carbide (Artificially) ripened pawpaw (CCRP); Group 4: Naturally ripened banana (NRB); Group 5: Calcium carbide (Artificially) ripened banana (CCRB).

106

(2, 3, 4, & 5); group 2 with the other groups (1, 3, 4, & 5); group 3 with the other groups (1, 2, 4, & 5); group 4 with the other groups (1, 2, 3, & 5) and group 5 with the other groups (1, 2, 3, & 4). There was a significant mean difference found between Group 1 and every other group. **Table 4** shows the mean and standard deviation of the food consumption of the mice in their respective

Histological description of photomicrographs

groups taken per day, with p = 0.357 being insignificant. The table compared the mean food consumption among the groups of mice fed with the fruits (banana and pawpaw); groups (2, 3, 4, & 5). It revealed the group fed with the artificially ripened fruits (banana and pawpaw) had the highest fruits consumption which is group 3, then group 2 and group 4 with the least food consumption.

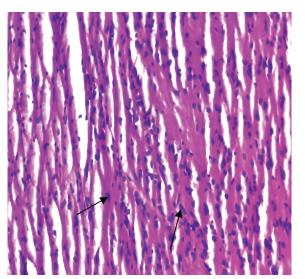


Plate A. Histological findings for the control brain samples showed normal brain histological features of neuronal cells with no inflammatory cells observed (H&E-X100)

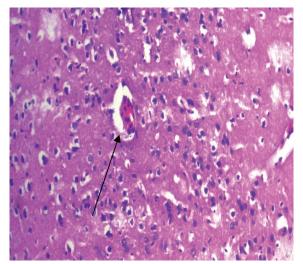


Plate C. Revealed brain section from the group fed with calcium carbide ripened banana (CCRB) which shows dendritic cell infiltration with mixed inflammation including histiocytes, lymphocytes, plasma cells and rare eosinophils (H&E-X100).

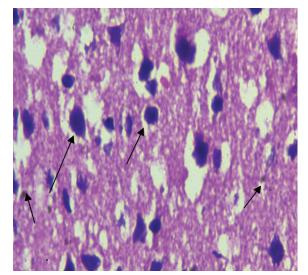


Plate B. Revealed brain section of the control group fed with naturally ripened pawpaw shows normal histological feature of subcortical white matter largely composed of axons and oligoden-droglia (H&E –X400).

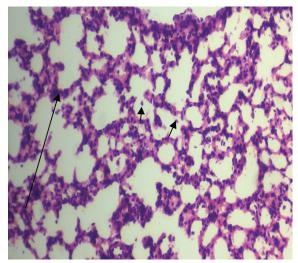


Plate D. Revealed normal histology of the lungs obtained from the control group fed with rat feed and water which shows the bronchiole and blood vessel with few inflammatory cells (H&E-X100).

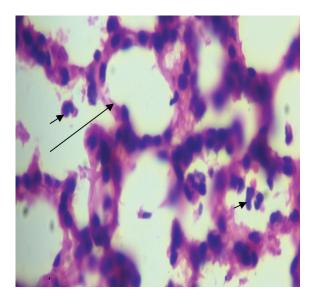


Plate E. Lung tissue section from the control group fed with naturally ripened pawpaw (NRP), shows normal lung histology with bronchiole and blood vessel with few inflammatory cells (H&E X400).

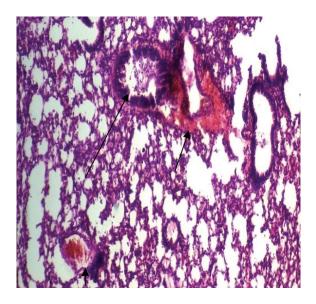


Plate F. Histological lung findings of the group fed with CCRB shows collapsed inner epithelial layer, ruptured muscular layer of lung tissues with disorganized clara cells and aggregation of fibroblasts (H&E-X100).

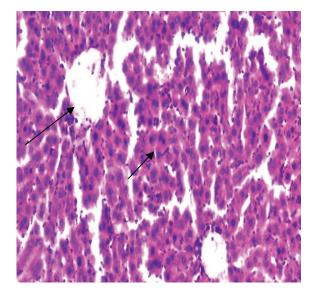


Plate G. Photomicrograph of liver section obtained from the control group fed with rat feed and normal water. Shows normal histological appearance of hepatic cords with hepatocytes, normal central vein and normal bile ducts in the hepatic area (H&E-X100).

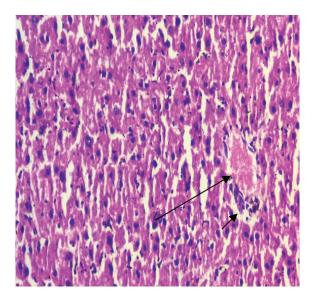


Plate H. Liver section harvested from the control group fed with naturally ripened banana (NRB). Shows the normal liver histological appearance of hepatic cords and normal bile ducts in the hepatic area (H&E-X100).

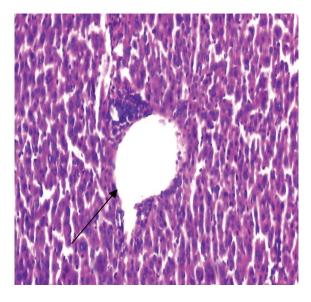


Plate I. Photomicrograph of liver section from the group fed with CCRB and normal water shows mild infiltration of inflammatory cells within the hepatic area within focal aggregation of lymphocytes and macrophages in the hepatic area (H&E-X100).

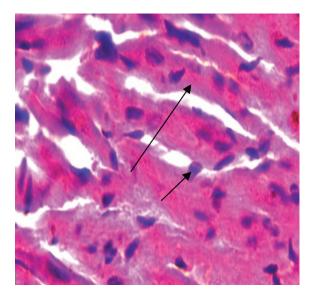


Plate J. Heart tissue section from the control group fed with rat feed. Heart section shows normal heart histology with cardiac muscles and blood vessels (H&E-X400).

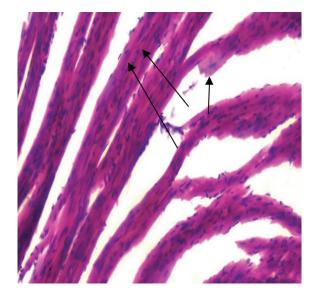


Plate K. Histological heart section of the NRP administered control group shows cardiac muscle of the normal heart tissue with few polymorphs (H&E-X100).

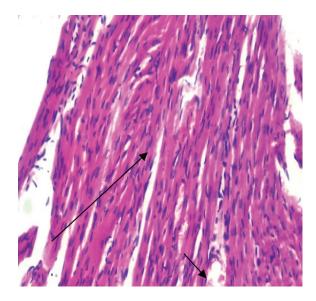


Plate L. Heart section from the CCRB and normal water fed group shows mild lymphocyte infiltration between cardiomyocyte, mild Interstitial edema between myocytes with lymphocyte infiltrations (H&E-X100).

109

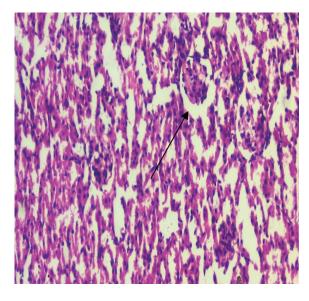


Plate M. Histological section of the kidney section from the rat feed and normal water fed group shows proximal tubules, normal architecture of glomerulus in normal in normal kidney with intact tubular cells and brush border (H&E-X100).

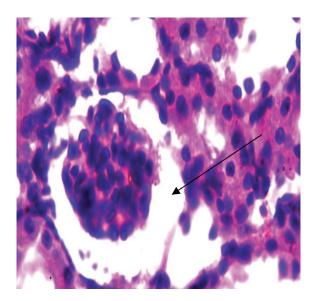


Plate N. Kidney section fed with the NRP shows normal kidney with intact tubular cells and increased Bowman capsule (H&E-X400).

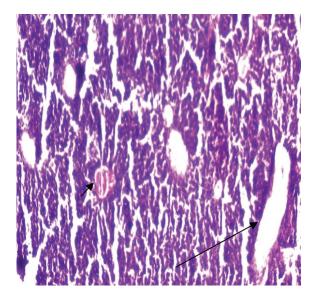


Plate O. Histology of the kidney, showed proximal tubules with cloudy swelling of renal tubular epithelium and mild mononuclear cell infiltration (H&E-X100).

Discussion

The majority of climacteric fruits are commonly ripened using industrial-grade calcium carbide (CaC₂). Commercial-grade calcium carbonate (CaC2) poses a risk to human health due to the presence of impurities such as phosphorus and arsenic, the potential toxic effects of which are directly correlated with their quantity. This leads to the negative effects of CaC₂ on the brain, lungs, heart, liver, and kidney of mice that are induced to ripen bananas (CCRBs) [21].

In this study, fruits that were naturally ripened took an average of four days to completely ripen, whereas those that were artificially ripened with calcium carbide took two days. This is consistent with a study by Bafor et al. [22] that found that under the same conditions, naturally ripened fruit containing calcium carbide took 2 days to ripen, whereas artificially ripened fruit with the same substance took 4 days.

In this study it was observed that the naturally ripened banana, the epicarp which is the outer part was hard while peeling it, not uniformly colored and with patches, but for the calcium carbide ripened banana, the epicarp had a softer texture when peeling, with a uniformly yellow colour but with a black stem. This result aligns with the findings of [9] which revealed that fruits that ripen naturally possess superior sensory qualities, including fresh color, flavor, and acceptability, when compared to fruits treated with calcium carbide. Depolymerization, the solubility of cell wall components, and the loss of cell structure are the causes of this [23].

This study revealed a significant increase in the mean body weight among the control group 1, followed by groups 5 and 3 then groups 2 and 4 with the least body weight. According to Chakraborty et al. [24], their research showed that the non-artificially ripened banana and calcium carbide ripened banana groups had significantly higher body weights than the other groups, which may have been caused by the additional banana meal given to those particular animals. Interestingly, the mice treated with calcium carbide showed the greatest increase in body weight when compared to the mice treated with natural ripening bananas. This suggests that the weight gain in the calcium carbide ripened banana groups was caused by something more than merely the fruit's high calorie content. The accelerated weight gain in this study for the calcium carbide ripened banana may be due to the presence of a component-phosphorus in calcium carbide employed in artificial ripening [24].

In this study, an increase in food consumption was observed in group 5, followed by group 3, then group 2. This is in accordance with a study carried out by Chakraborty et al. [24], where they observed an increase in weight gain in the calcium carbide ripened banana group which may have resulted into higher body weight in this group. This study revealed dendritic infiltration with mixed inflammation including the histiocytes, lymphocytes, plasma cells and rare eosinophils in the histologically investigated brain of the group 5 mice. This is in line with a study done by Uzzal et al. [25], where they observed haemorrhage and increased intra myocardial spaces in the histological brain section of their mice group exposed to calcium carbide. This could be due to the fact that industrial grade of CaC2, when dissolved in water, produces the actual ripening agent called acetylene gas, which affects the central nervous

system by reducing oxygen supply to the brain, as a result of the presence of impurities such as phosphorus and arsenic with the appearance of dendritic cells affecting an area in the brain known as the pituitary gland. These impurities act as neurotoxic substances thus causing hypoxia [26].

In this study, the histological section of the liver of group 5 mice revealed a mild infiltrate of inflammatory cells with the hepatic area showing focal aggregation of lymphocytes and macrophages in the hepatic area. This is in agreement with the study of Kjuus *et al.* [27], who found CaC_2 exposure produced liver toxicity and nephrotoxicity along with colonic and prostatic cancer. Similar results were also found from the study of Vecchia *et al*, [28], where it was revealed that exposure to calcium carbide may be cancerous as a result of the free radicals released from calcium carbide that may interact with the gene to change the basic information coding in DNA, which subsequently leads to cancer [28].

The histological details of the lungs tissues gotten from the group 5 subjects revealed collapsed inner epithelial layer, ruptured muscular layer with disorganized Clara cells and aggregation of fibroblasts. These results are consistent with those of Patore et al. [29], who observed a reddish-brown to brown focal area of consolidation. Pulmonary edema and cardiac arrest are caused by phosphorus, which targets the respiratory and cardiovascular systems when it enters the lungs [30]. This lung toxicity could be the result of phosphine inhibiting mitochondrial cytochrome C oxidase, which disrupts mitochondrial morphology and causes oxidative respiration to be lowered by 70%, ultimately leading to the premature death of the cell.

In this study, histopathological analysis of kidney revealed proximal tubules with cloudy swelling of renal tubular epithelium and mild mononuclear cell infiltration in group 5 mice. This is consistent with the findings of Patore et al. [29] study, which similarly showed certain glomeruli in a ruptured state and thickening of the collecting tubule lining with a change in cell shape. This investigation bears similarities to those conducted by Bini et al. [31], wherein they reported congestion resulting from haemorrhage and degradation of renal corpuscles upon exposure to calcium carbide. This is as a result of cell death (apoptosis) [31]. The study revealed in the brain section of the group 5 mice, mild interstitial edema between the myocytes and lymphocytes resulting in infiltration.

Currently, the majority of fruit vendors utilize harmful chemicals like CaC_2 to mature their fruits, raising serious worries about food safety and health security. Based on study by Cruzan et al. [32] CaC_2 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) [32]. Therefore, the exhibited histopathological effects may be due to the arsenic and the phosphorus which are part of the constituents of calcium carbide.

Conclusion

This study showed that the artificial fruit ripening agent CaC_2 has toxic effects on a number of albino mice's organs, including the liver, kidney, and brain. It also revealed altered organ architecture, including microvesicular fatty changes and kidney corpuscle degeneration and lung, heart, and brain lymphocyte infiltration. In this study, exposure to calcium carbide is being unsafe and toxic to the body organs.

Acknowledgements

Ethical Clearance

The protocol for this study was applied and approved by the Ethics and Research Committee of Accurec, University of Ibadan, Ibadan, Nigeria.

Funding

The three Authors provided funding for this study.

Availability of data and materials

All data and materials that were collected during this study are available with the corresponding author upon reasonable request.

Authors' contributions

ABA, OOA and MNA conceived the idea. ABA, OOA and MNA designed the study methodology. ABA, OOA and MNA conducted the study. ABA, OOA and MNA analyzed the data. ABA, OOA and MNA interpreted the results. ABA, OOA and MNA wrote the draft manuscript. ABA, OOA and MNA revised and edited the final manuscript. ABA, OOA and MNA approved the manuscript.

Competing interests

The authors declare that they have no competing interests.

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