

# Calcium Carbide-Induced Ripening Alters Vitamin C Levels and Organ Histomorphology in Wistar Rats: Implications for Food Safety

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## Abstract

Background: The use of calcium carbide (CaC<sub>2</sub>) in fruit ripening is widespread in many developing countries, raising concerns about food safety and the potential health risks posed by chemical residues. This study investigates the effects of calcium carbide-induced ripening on vitamin C levels and organ histomorphology in Wistar rats. Aim: The aim of the study is to evaluate the impact of calcium carbide-induced ripening on vitamin C content in fruits and histological changes in the liver, kidneys, and ovaries of Wistar rats. *Methodology:* Wistar rats were divided into nine groups, with each group receiving different ripening treatments (naturally ripened, market ripened, and laboratory ripened with calcium carbide). The treatment duration was 20 days. Vitamin C levels were measured, and histopathological analyses were performed on liver, kidney, and ovary tissues. *Results:* The results showed that naturally ripened fruits had the highest vitamin C content  $(2.23\pm1.20 \text{ mg/ml})$ , while calcium carbide-treated fruits exhibited lower levels, with the 30g CC laboratory ripened fruits showing 1.28±0.50 mg/ml. Histological examination revealed vascular congestion and tissue degeneration in the liver and kidneys of rats fed calcium carbide-treated fruits. Liver and kidney weights were significantly altered in the calcium carbide-treated groups, with liver weights in Group D (4.40±0.40g) and Group G (5.00±0.90g) being notably lower. Conclusion: Calcium carbide-induced ripening adversely affects vitamin C content and induces histopathological changes in vital organs, indicating potential toxicity. These findings emphasize the need for stricter regulation of artificial ripening agents to protect public health.

Keywords: calcium carbide, ripening, vitamin C, histopathology, Wistar rats, food safety

#### 1. Introduction

In recent years, there has been increasing attention on the use of various chemicals in the ripening of fruits, particularly calcium carbide (CaC<sub>2</sub>), which is widely employed in many developing countries due to its low cost and accessibility (Ruchitha, 2008). The application of chemicals like calcium carbide accelerates the ripening process of fruits, leading to noticeable changes in the rate of softening, respiration, starch hydrolysis, and color and flavor development (Anwar *et al.*, 2008). This process, while beneficial for the timely ripening of fruits, raises significant concerns regarding food safety due to the potential health hazards associated with residual chemical components, such as arsenic and phosphorus, which may remain on the fruit after treatment (Mariappan, 2004). These concerns are compounded by the chemical's potential to cause toxicity when ingested, particularly in sensitive organs such as the liver and kidneys.

The health risks associated with calcium carbide-induced ripening extend beyond the fruits themselves. Research suggests that the ingestion of such treated fruits can lead to systemic toxicity, affecting vital organs such as the liver and kidneys (Kumar *et al.*, 2020; Singh *et al.*, 2021). Specifically, studies have shown that calcium carbide and its contaminants may exert oxidative stress, disrupt cellular integrity, and induce histopathological changes

in vital organs (Bose *et al.*, 2022). Given the widespread use of calcium carbide in the fruit industry, it is crucial to investigate its effects on the nutritional content of fruits and the potential consequences for consumer health.

Vitamin C, a key antioxidant in fruits, is known to be sensitive to various environmental and chemical factors, including those related to artificial ripening. As an essential nutrient, it plays a critical role in maintaining immune function and protecting cells from oxidative damage. However, the use of calcium carbide in fruit ripening has been reported to negatively impact vitamin C levels (Rai *et al.*, 2021). The degradation of this vital nutrient may compromise the health benefits of ripened fruits and exacerbate the risks posed by toxic chemical exposure.

The widespread use of calcium carbide as a ripening agent has led to public health alarms globally, especially in regions where its unregulated use is common (Ruchitha, 2008). Despite being banned or restricted in many countries, the chemical remains popular due to its affordability and effectiveness in ripening fruits, particularly in markets where alternative methods like ethylene gas or controlled temperature ripening are not readily available (Sajid *et al.*, 2020). The health risks posed by calcium carbide exposure have spurred a growing body of research into the physiological effects of its residues on the human body, especially regarding its impact on the liver, kidneys, and vitamin content in fruits (Sharma *et al.*, 2021).

Calcium carbide releases acetylene gas upon contact with moisture, which mimics the natural ripening process triggered by ethylene, a plant hormone. However, the toxicological implications of acetylene's interaction with biological tissues remain underexplored, particularly concerning its effect on the liver and kidneys, which are primary detoxification organs in the body (Abd El-Ghany *et al.*, 2022). One of the critical concerns is its influence on the nutritional value of fruits, especially on vital nutrients like vitamin C, which plays a significant role in human health due to its antioxidant properties and its involvement in immune function, collagen synthesis, and the prevention of chronic diseases (Wang *et al.*, 2023).

This study aims to investigate the effects of calcium carbide-induced ripening on the levels of vitamin C and the histomorphology of vital organs, namely the liver and kidneys, in Wistar rats. By evaluating the biochemical and histological alterations associated with exposure to calcium carbide, this research seeks to enhance our understanding of the health risks posed by this ripening agent, providing insights into the broader implications for food safety and public health.

## 2. Materials and Methodology

## 2.1 Experimental Animals

Forty-five (45) female Wistar rats (150–180 g) were procured from the National Institute of Pharmaceutical Research and Development (NIPRID), Abuja, Nigeria. The animals were housed in the Animal Research Facility of the Department of Anatomy, Faculty of Basic Medical Sciences, Baze University, Abuja under controlled environmental conditions, including a 12-hour light/dark cycle, ambient temperature, and adequate ventilation. Prior to the commencement of the experiment, the rats were acclimatized for two weeks in standard plastic cages. Throughout the study, they were provided with a commercially available rodent pellet diet (obtained from NIPRID) and water *ad libitum*.

## 2.2 Collection of Calcium Carbide

Industrial-grade calcium carbide was sourced from a welding supply store located at Jabi Garage Shopping Complex, Abuja, Nigeria.

## 2.3 Plant Source and Identification

Both ripe and unripe mango (*Mangifera indica*) fruits were collected from a cultivated farmland within the Federal Capital Territory (FCT), Abuja. Additionally, mangoes that had undergone artificial ripening with calcium carbide were obtained from Utako Market, Abuja. The identity of the mango fruits was confirmed by a botanist in the Department of Biology, Faculty of Computing and Applied Sciences, Baze University, Abuja. A voucher specimen was deposited, and the reference number BU/1000/BUH was assigned.

#### 2.4 Ripening of Mangoes

Unripe mangoes were weighed and categorized into two experimental groups. The first group (500 g) was ripened under laboratory conditions using 10 g of calcium carbide, while the second group (500 g) was exposed to 30 g of calcium carbide. The mangoes were enclosed in plastic bags along with the calcium carbide and placed in an airtight container for four days to facilitate ripening. The ripening status was monitored and confirmed after the designated period. Naturally ripened mangoes were collected directly from the farmland, while market-ripened mangoes were obtained from Wuse Market, Abuja.

## 2.5 Elemental Analysis

Samples from all mango categories; naturally ripened, 10 g laboratory calcium carbide-ripened, 30 g laboratory

calcium carbide-ripened, and market calcium carbide-ripened, were subjected to elemental analysis. This analysis was conducted at the Sheda Science and Technology Complex (SHESTCO), Gwagwalada, Abuja, to determine the elemental composition of the mango samples.

## 2.6 Experimental Design

The rats were randomly grouped into nine (9) with five animals in each group and treated as shown in Table 1 below.

GROUPS	TREATMENT	DURATION
А	Feed and Water <i>ad libitum</i> (negative control)	20 days
В	Naturally Ripened Fruits (Positive Control) 5mL	20days
С	Naturally Ripened Fruits (Positive Control) 10mL	20 days
D	Market Ripened Fruits (MRF) 5mL	20days
E	Market Ripened Fruits (MRF) 10mL	20days
F	10 g CC. Laboratory Ripened Fruit 5mL	20days
G	10 g CC. Laboratory Ripened Fruit 10mL	20days
Н	30g CC. Laboratory Ripened Fruit 5mL	20days
Ι	30g CC. Laboratory Ripened Fruit 10mL	20days

Table 1. Animal Treatment Protocol

Note: CC = Calcium Carbide.

## 2.7 Animal Sacrifice and Sample Collection

On the twentieth day of the experiment, the animals were euthanized using chloroform anesthesia in a closed chamber. Following euthanasia, the liver, kidneys, and ovaries were carefully harvested, blotted dry, and weighed. The excised organs were subsequently fixed in 4% formal saline for histological analysis.

## 2.8 Histological Tissue Processing

Kidney and Liver tissues were embedded in molten paraffin wax and allowed to solidify in metallic tissue molds. The blocks were then cooled at 5°C for 15 minutes, removed from the molds, and trimmed. Serial sections (3  $\mu$ m thick) were obtained using a rotary microtome and floated in a water bath at 55°C. The sections were mounted onto clean frosted-end slides, placed on a hot plate for 40 minutes for proper adhesion, and then deparaffinized, hydrated, air-dried, and stored for staining.

## 2.9 Haematoxylin and Eosin (H&E) Staining

- 1) Sections were dewaxed in xylene (3 changes, 5 min each).
- 2) Rehydration was performed through descending ethanol concentrations (absolute, 95%, 80%, and 70%).
- 3) Staining was carried out using Harris hematoxylin (5 min).
- 4) Sections were rinsed in running tap water to remove excess stain.
- 5) Differentiation was performed in 1% acid alcohol (3 sec).
- 6) Sections were blued in running tap water (10 min).
- 7) Counterstaining with 1% eosin was done (1 min).
- 8) Dehydration was achieved through ascending ethanol concentrations (70%, 80%, 95%, and absolute).
- 9) Sections were cleared in xylene, air-dried, and mounted with dibutyl phthalate polystyrene xylene (DPX).

Slides were examined under a light microscope, and photomicrographs were captured.

#### 2.10 Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 23. Mean values were compared using one-way analysis of variance (ANOVA), and intergroup comparisons were performed using the least significant difference (LSD) post-hoc test. A p-value <0.05 was considered statistically significant.

2.11 Ethical Approval

This study was approved by the Ethical Committee of the Department of Anatomy, Faculty of Basic Medical Science Baze University, Abuja, and the reference number; BU/URES/ANA/1004 was issued. The animal experimental models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002).

# 3. Results

#### 3.1 Vitamin Contents in Samples of Mango Fruits Ripened in Various Methods

The results presented in Figure 1 show the mean vitamin content levels in mango fruits subjected to different ripening methods. Naturally ripened fruits (NRF) had the highest levels of both Vitamin C ( $2.23\pm1.20$  mg/ml) and Vitamin A ( $1.99\pm0.20$  mg/ml). In contrast, market ripened fruits (MRF) showed significantly lower concentrations of both vitamins, with Vitamin C ( $1.16\pm0.80$  mg/ml) and Vitamin A ( $1.56\pm0.40$  mg/ml) compared to NRF. Laboratory ripened fruits (LRF), using calcium carbide (CC) for ripening, also showed reduced vitamin contents. The LRF with 10g CC (Vitamin C:  $1.39\pm0.90$  mg/ml, Vitamin A:  $1.52\pm0.40$  mg/ml) and 30g CC (Vitamin C:  $1.28\pm0.50$  mg/ml, Vitamin A:  $1.44\pm1.20$  mg/ml) had significantly lower Vitamin C and Vitamin A levels compared to NRF. These results suggest that artificially ripened mango fruits, whether market or laboratory ripened, contain lower levels of essential vitamins compared to naturally ripened fruits.



Figure 1. Simple Bar Chart Showing the Mean Vitamin Levels in Mango Fruits under Different Ripening Methods

Values are expressed as MEAN $\pm$ SD; NRF = Naturally Ripened Fruit; MRF = Market Ripened Fruit; LRF = Laboratory Ripened Fruit; CC = Calcium Carbide; \**P*<0.05 Compared to the NRF.

## 3.2 Liver Weight Measurements

The liver weight measurements across the different experimental groups as shown in Figure 2 indicate significant variations due to different treatments. Group A (Positive Control) recorded the highest mean liver weight (7.50 $\pm$ 2.20 g). The Negative Control groups (B and C), which received 5 mL and 10 mL of naturally ripened mango fruit (NRF), showed slightly lower liver weights (6.40 $\pm$ 0.60 g and 6.90 $\pm$ 0.70 g, respectively), suggesting minimal impact of NRF on liver weight. A notable reduction in liver weight was observed in Group D (4.40 $\pm$ 0.40 g) and Group G (5.00 $\pm$ 0.90 g), both of which received calcium carbide (CC)-ripened mango fruit, with statistically significant differences (\**P*<0.05) compared to both the Positive Control and Negative Control groups. This suggests a potential hepatotoxic effect of market- and laboratory-ripened fruit treated with CC, especially at lower doses.

Conversely, Group E (10 mL CC MRF) recorded a higher liver weight ( $7.80\pm1.61$  g), which is comparable to the Positive Control, indicating a possible dose-dependent or adaptive response at higher CC-MRF exposure. Other CC-treated groups (F, H, and I) showed moderate liver weights (ranging from  $5.90\pm0.30$  g to  $6.00\pm2.00$  g), with no significant deviation from the Negative Control groups.

The result suggests that calcium carbide ripening, particularly at lower concentrations, negatively impact liver

weight, potentially reflecting hepatic stress or toxicity.



Figure 2. Simple Bar Chart Showing the Mean Liver Weight across Groups

Values are expressed as MEAN $\pm$ SD; N = 5; NC = Negative Control; NRF = Naturally Ripened Fruit; CC = Calcium Carbide; MRF = Market Ripened Fruit; LRF = Laboratory Ripened Fruit; \**P*<0.05 Compared to the Positive Control Group; +*P*<0.05 Compared to the Negative Control Groups.

#### 3.3 Kidney Weight Measurements

The kidney weight measurements presented in Figures 3 and 4 show significant differences between the experimental groups when compared to the positive and negative control groups. Group A (positive control) showed the highest kidney weight for both right and left kidneys, with values of  $1.50\pm0.06$ g and  $1.40\pm0.08$ g, respectively, which were significantly higher (P<0.05) than the negative control group (Group B). Group B (negative control: 5mL NRF) showed reduced kidney weights for both the right and left kidneys ( $0.40\pm0.20$ g and  $0.40\pm0.05$ g, respectively), with these values being significantly lower than those of the positive control.

Groups receiving various doses of market- or laboratory-ripened fruit (Groups C, D, F, G, H, and I) showed a range of kidney weights, lower than the positive control but higher than the negative control. Specifically, Group C (10mL NRF) had moderate kidney weight values (1.10 $\pm$ 0.30g for the right kidney and 0.70 $\pm$ 0.06g for the left kidney), while Groups D (5mL CC MRF) and F (5mL 10kg CC LRF) had significantly lower kidney weights (*P*<0.05) than the positive control.

Groups E (10mL CC MRF) and I (10mL 30kg CC LRF) had kidney weights that were closer to those of the positive control, with values of  $1.10\pm0.40g$  and  $0.60\pm0.04g$  for the right kidney, and  $1.05\pm0.50g$  and  $0.50\pm0.05g$  for the left kidney, respectively.

These data suggest that the different ripening methods and doses of the fruit extract have variable effects on kidney weight, with market-ripened and laboratory-ripened fruit formulations showing some kidney weight restoration, but generally lower compared to the positive control group.

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#### RIGHT KIDNEY WEIGHT ACROSS GROUPS (g)



Values are expressed as MEAN $\pm$ SD; N = 5; NC = Negative Control; NRF = Naturally Ripened Fruit; CC = Calcium Carbide; MRF = Market Ripened Fruit; LRF = Laboratory Ripened Fruit; \**P*<0.05 Compared to the Positive Control Group; +*P*<0.05 Compared to the Negative Control Groups.





Figure 4. Simple Bar Chart Showing the Mean Left Kidney Weights across Groups

Values are expressed as MEAN $\pm$ SD; N = 5; NC = Negative Control; NRF = Naturally Ripened Fruit; CC = Calcium Carbide; MRF = Market Ripened Fruit; LRF = Laboratory Ripened Fruit; \**P*<0.05 Compared to the Positive Control Group; +*P*<0.05 Compared to the Negative Control Groups.

#### 3.4 Ovary Weight Measurements

The ovary weight measurements across different groups are shown in Figure 5. Group A (Positive Control) showed the lowest mean ovary weight  $(0.10\pm0.01g)$ , while Group E (10mL CC MRF) had the highest mean ovary weight (0.54±0.02g). Notably, Group E and Group F (5mL 10kg CC LRF) exhibited significantly higher ovary weights compared to the Positive Control group (*P*<0.05). Groups B, C, D, G, H, and I showed similar ovary weights to the Negative Control group (0.20±0.10g) and did not differ significantly.

These findings suggest that the use of calcium carbide (CC) in fruit ripening, especially in the 10mL CC MRF and 5mL 10kg CC LRF doses, has a significant impact on ovary weight, likely indicating hormonal or physiological alterations caused by the ripening agents.



OVARY WEIGHT ACROSS GROUPS (g)

Figure 5. Simple Bar Chart Showing the Mean Ovary Weights across Groups

Values are expressed as MEAN $\pm$ SD; N = 5; NC = Negative Control; NRF = Naturally Ripened Fruit; CC = Calcium Carbide; MRF = Market Ripened Fruit; LRF = Laboratory Ripened Fruit; \**P*<0.05 Compared to the Positive Control Group; +*P*<0.05 Compared to the Negative Control Groups.

#### 3.5 Histological Examination

Hematoxylin & Eosin (H&E) examination of the liver and kidney from the experimental groups reveal interesting histological appearances. The positive control group (Group A) showed normal hepatic and renal histology, with abundant hepatocytes and nephrons, intact space of Disse, central veins, normal renal capsules and tubules. The negative control groups (Groups B & C) treated with naturally ripened mango fruits also showed essentially normal liver and kidney histology. In groups D and E (treated with 5mL & 10mL market ripened mango fruit respectively), vascular congestions (degeneration of the central vein) were observed with depleted hepatocytes. The kidney sections also showed depletion of the nephrons, and vascular congestion. In the groups treated with 5mL and 10mL Laboratory ripened mango fruits (groups F – I), the liver section showed mild tissue degenerations, congested portal space, and less abundant hepatocytes, indicative of periportal hepatitis. The kidney sections revealed vascular congestions in the renal tissue, with mild interstitial nephritis.



**Plate 1A:** Liver Section from Group A Showing Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Normal Histoarchitecture (H&E x10)



**Plate 2A:** Liver Section from Group B Showing Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Normal Histoarchitecture (H&E x10)



**Plate 1B:** Kidney Section from Group A Showing Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Normal Kidney Histomorphology (H&E x10)



**Plate 2B:** Kidney Section from Group B Showing Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Normal Kidney Histomorphology (H&E x10)





Plate 3A: Liver Section from Group C Showing Plate 3B: Kidney Section from Group C Showing

Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Normal Histoarchitecture (H&E x10)



**Plate 4A:** Liver Section from Group D Showing Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Vascular Congestions & Depleted Hepatocytes (H&E x10)

Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Normal Kidney Histomorphology (H&E x10)



**Plate 4B:** Kidney Section from Group D Showing Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Depleted Neurons & Vascular Congestions (H&E x10)



**Plate 5A:** Liver Section from Group E Showing Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Vascular Congestions & Depleted Hepatocytes (H&E x10)



**Plate 5B:** Kidney Section from Group E Showing Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Depleted Neurons & Vascular Congestions (H&E x10)



**Plate 6A:** Liver Section from Group F Showing Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Mild Tissue Degeneration, Congested Portal Space & Depleted Hepatocytes (H&E x10)



**Plate 6B:** Kidney Section from Group F Showing Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Mild Tissue Degenerations & Vascular Congestions (H&E x10)



**Plate 7A:** Liver Section from Group G Showing Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Mild Tissue Degeneration, Congested Portal Space & Depleted Hepatocytes (H&E x10)





**Plate 7B:** Kidney Section from Group G Showing Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Mild Tissue Degenerations & Vascular Congestions (H&E x10)



**Plate 8A:** Liver Section from Group H Showing Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Mild Tissue Degeneration, Congested Portal Space & Depleted Hepatocytes (H&E x10) **Plate 8B:** Kidney Section from Group H Showing Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Mild Tissue Degenerations & Vascular Congestions (H&E x10)



**Plate 9A:** Liver Section from Group I Showing Hepatocytes (H), Central Vein (CV) & Space of Disse (SOD), with Mild Tissue Degeneration, Congested Portal Space & Depleted Hepatocytes (H&E x10)



**Plate 9B:** Kidney Section from Group I Showing Renal Capsule (RC), Bowman's Space (BS) & Renal Tubule (RT), with Mild Tissue Degenerations & Vascular Congestions (H&E x10)

## 4. Discussion

The results presented in this study highlight the significant impact of calcium carbide (CC)-induced ripening on the histomorphology of the liver, kidneys and ovary in Wistar rats. The findings underscore potential hormonal and physiological alterations associated with the use of ripening agents, particularly at higher concentrations of calcium carbide.

The observed reduction in vitamin C and vitamin A levels in artificially ripened mango fruits compared to naturally ripened ones suggests that the use of calcium carbide (CC) and other artificial ripening agents compromises the nutritional quality of the fruit. Naturally ripened mangoes retained the highest levels of vitamin C and vitamin A, while fruits ripened using CC at different concentrations exhibited significantly lower vitamin contents. These findings align with previous studies indicating that artificial ripening accelerates metabolic processes, leading to rapid degradation of essential nutrients (Kumar *et al.*, 2022). Studies by Sharma *et al.* (2021) reported a significant reduction in vitamin C content in CC-ripened bananas and papayas, corroborating the findings that CC exposure negatively impacts antioxidant vitamin levels.

Market-ripened fruits (MRF) also displayed lower vitamin C and vitamin A concentrations, which could be attributed to improper post-harvest handling, storage conditions, or the presence of multiple ripening chemicals. Similar trends were observed by Rahman *et al.* (2023), where mangoes subjected to artificial ripening in commercial settings exhibited a 40% reduction in vitamin C compared to naturally ripened counterparts. These findings reinforce concerns regarding the impact of artificial ripening on fruit quality and human health.

The significant reduction in liver weight in groups receiving CC-ripened fruit suggests hepatotoxic effects associated with artificial ripening agents. Groups D and G exhibited markedly lower liver weights compared to both the positive control and naturally ripened fruit groups, implying that prolonged consumption of CC-treated fruits may induce hepatic stress. This aligns with the findings of Adekunle *et al.* (2022), who reported histopathological alterations in rat liver following CC exposure, including hepatocyte degeneration and lipid accumulation.

Conversely, the increased liver weight observed in Group E suggests a possible compensatory or adaptive response at higher doses. Similar results were documented by Singh *et al.* (2021), where a biphasic response in liver weight was noted in rats exposed to CC, with lower doses inducing hepatotoxicity and higher doses triggering hepatomegaly. The slight variations in liver weights across different CC-treated groups further indicate that the extent of hepatic effects may be dose-dependent and influenced by the duration of exposure.

Kidney weight variations among experimental groups suggest potential nephrotoxic effects of CC-ripened fruit consumption. The highest kidney weights were observed in the positive control group, while significantly lower kidney weights were recorded in groups consuming artificially ripened mangoes. This suggests that CC exposure adversely affects renal health, possibly due to oxidative stress and nephrotoxicity. A similar study by Ibrahim *et al.* (2023) demonstrated that chronic exposure to CC-ripened fruits in animal models resulted in reduced renal mass, histopathological damage, and compromised renal function.

Interestingly, some CC-treated groups (E and I) displayed partial restoration of kidney weight, suggesting potential adaptive mechanisms or metabolic adjustments. However, the overall trend remains consistent with studies linking artificial ripening agents to renal toxicity (Chaudhary & Gupta, 2024). Given that the kidney plays a crucial role in detoxification, exposure to harmful ripening agents may result in renal dysfunction and metabolic imbalances.

The significant increase in ovary weight observed in Group E (10mL CC MRF) and Group F (5mL 10kg CC LRF), when compared to the Positive Control group, suggests a possible endocrine disruption or physiological alteration induced by calcium carbide. These higher ovary weights could indicate hormonal changes, such as the stimulation of ovarian growth or alteration in the regulation of reproductive hormones. The fact that Groups B, C, D, G, H, and I showed similar ovary weights to the Negative Control group further suggests that the observed increase is likely linked to the CC treatment.

The increase in ovary weight in response to higher concentrations of calcium carbide may be a result of the calcium carbide's effect on gonadotropins or ovarian function. Recent research has demonstrated that environmental or chemical agents, like calcium carbide, can interfere with endocrine function, leading to reproductive alterations. For instance, Soni *et al.* (2022) reported that exposure to certain chemical ripening agents altered ovarian function and caused increased ovarian weight in rodents, similar to what was observed in the present study. Similarly, in a study by Adefolalu *et al.* (2023), rats exposed to calcium carbide for prolonged periods exhibited altered ovarian morphology, which aligns with the findings of increased ovary weight in the present experiment.

The histological examination of liver and kidney tissues revealed significant changes in the experimental groups treated with calcium carbide — ripened fruit. Group A (Positive Control) and Groups B and C (treated with naturally ripened mangoes) showed normal liver and kidney histology, which is consistent with the expected baseline health of the organs. However, the groups treated with market-ripened mangoes (Groups D and E), which contained higher concentrations of calcium carbide, exhibited significant histological alterations, including vascular congestion, depletion of hepatocytes, and kidney damage. These pathological changes are indicative of the toxic effects of calcium carbide.

The findings of liver degeneration and vascular congestion in the market-ripened mango groups (D and E) are particularly concerning, as they suggest that prolonged exposure to calcium carbide could lead to liver dysfunction. In contrast, Groups F, G, H, and I, which were exposed to laboratory-ripened mangoes, showed milder histological changes, including mild tissue degeneration and congestion of the portal space, indicative of periportal hepatitis. These results suggest that ripening with calcium carbide, regardless of its source (market or laboratory), may have a detrimental impact on liver and kidney health, with varying degrees of severity.

The kidney damage observed in the treatment groups, characterized by vascular congestion and nephritis, aligns with previous studies that have shown that exposure to calcium carbide can induce nephrotoxicity. For instance, Bhatia *et al.* (2021) observed kidney damage in rats treated with chemicals commonly used in fruit ripening, including vascular congestion and nephron depletion. Similarly, Sharma *et al.* (2022) highlighted that toxic chemical like calcium carbide can lead to renal impairment, manifesting as vascular changes and nephritis, which were evident in the current findings.

The results of this study raise important concerns about the use of calcium carbide in fruit ripening, especially considering its widespread use in commercial settings. The alteration in ovary weight and the histological changes in liver and kidney tissues observed in the experimental rats suggest that the consumption of fruit ripened with calcium carbide could have potential health implications for humans, particularly in terms of reproductive health and organ function.

Several studies have reported similar findings regarding the toxicity of calcium carbide and its effects on various organs. For example, Adefolalu *et al.* (2023) examined the effects of calcium carbide on reproductive health in rats and found alterations in ovarian function, supporting the findings of increased ovary weight. Likewise, Bhatia *et al.* (2021) and Sharma *et al.* (2022) observed kidney and liver damage following exposure to chemicals used in fruit ripening, reinforcing the histological findings in the current study. These studies, in agreement with the current study, suggest that calcium carbide-induced ripening may pose significant risks to organ health, particularly the liver and kidneys, and highlights the need for stricter regulations regarding its use.

### 5. Conclusion

Calcium carbide-induced ripening significantly alters vitamin C levels and adversely affects organ histomorphology in Wistar rats. The study reveals reduced vitamins C and A content in artificially ripened fruits, with notable histopathological changes in the liver, kidneys, and ovaries, indicating potential toxicity. The observed hepatic, renal, and reproductive effects suggest that prolonged consumption of CC-ripened fruits may pose health risks. These findings underscore the need for stricter regulations on artificial ripening agents to safeguard food quality and public health.

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