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# Associated Risks and Pathogenic Effects of Consumption of Calcium Carbide-Ripened Banana Before and/or During Pregnancy on Maternal and Neonatal Livers

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## ABSTRACT

Calcium carbide ($CaC_2$) is a commonly used ripening agent, although its primary use is in welding. Use of $CaC_2$ in ripening fruits is considered harmful because it contains traces of phosphorous and arsenic. Aim of this study was to investigate associated risks and pathogenic (biochemical and histological) effects of consuming $CaC_2$-ripened banana as a meal, before and/or during pregnancy on maternal and neonatal livers of adult female wistar rats. 36 healthy-looking, nulliparous, adult female wistar rats weighing between 150-200g were utilized. Rats were divided into 2 groups namely Group A and Group B. Each group had 4 subgroups (A1, A2, A3 and A4) for Group A as well as (B1, B2, B3 and B4) for Group B. Rats in group A received $CaC_2$-ripened banana blended into their meal before and during pregnancy while group B rats received the same meal during pregnancy only. Subgroups A1 and B1 (positive control) received a naturally ripened banana with 0% $CaC_2$, subgroups A2 and B2 received 1% $CaC_2$-ripened bananas whereas subgroups A3 and B3 received 2% $CaC_2$-ripened bananas. Subgroups A4 and B4 received $CaC_2$-ripened bananas (bought from the market) randomly. The two groups A and B had one common negative control group which did not receive any banana ($CaC_2$-ripened or non-$CaC_2$-ripened) blended meal. Liver enzyme assay for group A showed that the positive control subgroup (A1) had mean AST (26.00±2.00 iu/L), ALT (33.00±3.00 iu/L), albumin (3.80±0.20 g/dL), direct (conjugated) bilirubin (1.10±0.10 mg/dL), and total bilirubin (1.95±0.15 mg/dL). These values were not significantly different ($p \geq 0.05$) compared with their respective values in the negative control subgroup except for ALT ($p < 0.05$). Group B showed that subgroups B2, B3, and B4 had mean AST, ALT, albumin, direct bilirubin and total bilirubin values which were not significantly different ($p \geq 0.05$) compared with their respective values in the negative control subgroup. Histological findings confirm a damaging effect on the liver's histoarchitecture of both mother and neonate. Consumption of $CaC_2$-ripened banana before and/or during pregnancy has associated risks and pathogenic effects on maternal and neonatal livers.

## INTRODUCTION

The sperm cell must fertilize an ovum in vivo or in vitro in order for conception (pregnancy) to occur. Gametes are ova and sperm cells which are haploid and have one copy of each type of chromosome i.e. 1–22 X or 1–22 Y (Ikwuka, 2023a). Pregnancy can be complicated by anemia due to sickle cell disease (SCD) and despite the significant need for effective treatment options for SCD patients, current treatments both traditional and newly developed, only ameliorate acute and chronic SCD manifestations without addressing the underlying cause (Musa, 2023).

During pregnancy, eating of varied healthy diet is important because the right nutrients contained in the food aid in the development and growth of the fetus, and eating fresh fruits during this period also ensures that both the fetus and mother remain healthy (Fan, 2021). Fresh fruits supply the essential growth-regulating factors which help maintain adequate and normal health for pregnant women (Hayes, 2005; Rossato, 2009). Fruits are nutritionally essential food commodities which are widely distributed in nature and also form part of a balanced diet. They are one of the best foods consumed in their raw form. Apart from the fruits' edible parts, the by-products, like the peels, could serve as materials for cosmetic, food or medicinal use.

## LITERATURE REVIEW

In particular, edible fruits have been propagated with the movements of animals and humans in a symbiotic relationship, as a way for nutrition and seed dispersal (Lewis, 2002). Many animals and humans have become dependent on fruits as their major food source. Hence, fruits account for a substantial fraction of the world's agricultural output (Per, 2007). To meet the markets' demand, farmers and retailers in most developing countries such as India, Bangladesh, Pakistan, Ghana, Cameroon and Nigeria hasten the process of fruit ripening by using ripening agents and chemicals (Dudley, 2004). Common examples of these ripening agents are calcium carbide ($CaC_2$), ethylene, acetylene, ethephon, ethrel (2-chloroethylphosphonic acid), propylene, putrescine, aminoethoxyvinylglycine, glycol, carbon monoxide, potassium dihydrogen sulfate, and ethanol



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(Per, 2007). Fruit vendors have been reported to use these agents to ripen multiple fruits such as pineapple, banana, mango, apple, tomato, date, citrus, avocado, pear, melon, pawpaw, coffee, groundnut, rubber and cucumber, making their nutritional value and natural taste a casualty in the process, and may cause serious health hazards (Kader, 2002).

Banana is one of the most popular fruits in the world and a member of the genus *Musa* (part of the family *Musaceae*). It is considered to be derived from the wild species *Musa balbisiana* and *Musa acuminata* (Rungnapa, 2007). Bananas are rich sources of potassium and carbohydrates while they tend to be low in protein (Happi, 2007). In recent years, there has been considerable research concerning the action of different types of ripening agents (Kader, 2002) and how these agents may affect pregnant women. Calcium carbide is commonly used to induce the ripening of fruits artificially in many countries because of its low cost and availability (Block, 1992). Once calcium carbide reacts with water, it liberates acetylene gas. The commercially graded $CaC_2$ is said to be impure as calcium arsenide ($Ca_3As_2$) and calcium phosphide ($Ca_3P_2$) present as impurities when $CaC_2$ reacts with water to form arsine ($AsH_3$) and phosphine ($PH_3$) respectively (Downie, 1997). Calcium carbide also contains traces of phosphorous and the heavy metal - arsenic (Fattah, 2010; Siddiqui, 2010). In inducing oxidative stress, major free radicals that are of physiological significance are superoxide anion, hydroxyl radical, and hydroperoxyl radical, while non-radical is hydrogen peroxide (Ikwuka, 2023b).

As a result of this ripening technique, farmers harvest their fruits early before maturation to prevent attacks on a ripened fruit either from birds or other pests. However, the toxic effects of these agents on the nutritional values of fruits are yet to be completely understood. Calcium carbide is known to cause food poisoning, gastric irritation, mouth ulcers, cancer, seizures and cerebral edema (Per, 2007). Other health-related symptoms such as mood disturbances, sleepiness, dizziness, mental confusion, headache, and memory loss are some of the health challenges associated with exposure to calcium carbide (Anwar, 2008). It has been reported that consuming fruits ripened with $CaC_2$ causes alterations in the biochemical and hematological parameters (Igbinaduwa, 2016).

The rising demand for food safety (including fruits and vegetables) has however inspired researchers to study the associated risks and pathogenic effects of the consumption of fruits contaminated with heavy metals, toxins or pesticides (Dudley, 2004). It is for this reason that this study was undertaken to investigate the associated risks and pathogenic effects of consumption of $CaC_2$-ripened banana before and/or during pregnancy on maternal and fetal livers.

## MATERIALS AND METHODS

### Materials

### Chemicals

500g of calcium carbide ($CaC_2$) produced by S.K. Calcium Carbide Gasfield was purchased at the Industrial Site Mechanic Village, Abakaliki, Ebonyi State, Nigeria.

### Banana Collection

Green-colored, unripe and yellow-colored, ripe mature bananas (*Musa spp*) were purchased at Margaret Umahi International Market, Abakaliki. The bananas were authenticated at Ebonyi State University (EBSU)'s Herbarium, Abakaliki, in the Department of Applied Biology, EBSU. It is important to note that the seller of the ripe bananas confirmed ripening the fruit with $CaC_2$ using the market's method.

### Banana Ripening

The unripe bananas were grouped and labeled S1, S2, and S3.

Sample S1 - 5kg of the unripe banana soaked in sterile, clean water.

Sample S2 - 5kg of the unripe banana dipped in 1% $CaC_2$ solution (50g of $CaC_2$ in 5L of $H_2O$).

Sample S3 - 5kg of the unripe banana dipped in 2% $CaC_2$ solution (100g of $CaC_2$ in 5L of $H_2O$).

S1 was left in the sterile, clean water. S2 and S3 were left in the $CaC_2$ solution for 30 minutes before removing them, and then allowed to dry to remove adhering moisture. These samples were then placed in a 5kg capacity carton each and then allowed to ripen at ambient temperature. The standard quality for banana ripening was noted by the appearance of a yellow-green color on the banana skin.

### Experimental Design

36 healthy looking, nulliparous, adult female wistar rats weighing between 150-200g were acquired from the animal house of the Anatomy Department, EBSU. The rats were exposed to alternating 12 hours of light and darkness and were allowed free access to standard rat feed pellets and drinking water following the United States National Institute of Health Guidelines (NIHG) for the safe keeping and use of laboratory animals (National Research Council - US, 2011). 32 rats out of all the rats were divided into 2 groups – A and B, and each group had 4 subgroups (A1, A2, A3, A4) and (B1, B2, B3 and B4) containing 4 nulliparous rats each. The remaining 4 rats served as the negative control subgroup for both groups - A and B. Substance administration was carried out as follows:

Group A rats were fed before and during pregnancy. Subgroup A1 (positive control subgroup for Group A) rats were fed with naturally ripened bananas with 0% $CaC_2$. Subgroup A2 rats were fed with 1% $CaC_2$-ripened bananas. Subgroup A3 rats were fed with 2% $CaC_2$-ripened bananas. Subgroup A4 rats were fed with $CaC_2$-ripened bananas (bought from the market) randomly. Administration of feed lasted for 21 days, after which Udeh, *et al*'s method was deployed to impregnate and confirm pregnancy in the rats (Udeh, 2023a; Udeh, 2023b). The impregnated rats in the subgroups continued to receive their feed till the day of delivery.

Group B rats were fed only during pregnancy. Subgroups B1 (positive control subgroup for Group B) rats were fed with naturally ripened bananas with 0% $CaC_2$. Subgroup

B2 rats were fed with 1% $CaC_2$-ripened bananas. Subgroup B3 rats were fed with 2% $CaC_2$-ripened bananas. Subgroup B4 rats were fed with $CaC_2$-ripened bananas (bought from the market) randomly. Udeh, *et al*'s method was deployed to impregnate and confirm pregnancy in the rats (Udeh, 2023a; Udeh, 2023b). The impregnated rats in the subgroups started to receive their feed from confirmation of pregnancy till the day of delivery.

The mixed banana blends were mixed with the rat feed according to Igbinaduwa's method (Igbinaduwa, 2016). Approximately 20g of each of samples – S1, S2 and S3 were mixed thoroughly with 80g of rat feed which also served as a carrier. This blend was prepared for the subgroups separately and preserved in the refrigerator for subsequent consumption.

On the delivery day, blood samples were collected from the rats through ocular puncture into plain bottles. The animals were sacrificed by cervical dislocation. The maternal and fetal livers were harvested, weighed and processed as described by Ekechi, *et al* for histological analysis (Ekechi, 2023b).

**Biochemical Assay**

The blood samples were allowed to clot, then the sera were drained and centrifuged at 3,000 rpm for 15 minutes to get 100% serum with no blood clot. A liver function test was done on the sera to determine aspartate transaminase (AST), alanine transaminase (ALT), albumin, direct (conjugated) bilirubin and total bilirubin. AST, ALT, direct (conjugated) bilirubin, and total bilirubin were estimated according to the method of Reitman and Frankel using Randox test kits (Reitman, 1957). Albumin was estimated based on bromocresol green method using Agappe Diagnostics Albumin Kit (Doumasa, 1971).

**Statistical Analysis**

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 23. Data collected from each of the blood samples in the experiment were expressed as mean+standard error. For data comparison, one-way analysis of variance (ANOVA) was used. The level of significance was set at $p<0.05$.

**Ethical Approval**

This study was conducted with approval from the Ethical Committee of the Faculty of Basic Medical Sciences, College of Medicine, Ebonyi State University, Nigeria. The Ethical Approval ID Number is MPC/1702/04/0001.

## RESULTS

Table 1 shows the results of the liver enzyme assay of rats in group A and comparison with negative and positive controls. The mean values in the negative control subgroup were - AST (49.00±4.00 iu/L), ALT (71.00±5.00 iu/L), albumin (4.25±0.15 g/dL), direct bilirubin (1.05±0.25 mg/dL), and total bilirubin (2.20±0.10 mg/dL). The mean values in the positive control subgroup (A1) were - AST (26.00±2.00 iu/L), ALT (33.00±3.00 iu/L), albumin (3.80±0.20 g/dL), direct bilirubin (1.10±0.10 mg/dL), and total bilirubin (1.95±0.15 mg/dL). The mean values in the subgroup (A2) were - AST (42.00±4.00 iu/L), ALT (33.00±6.00 iu/L), albumin (4.00±0.10 g/dL), direct bilirubin (0.65±0.05 mg/dL), and total bilirubin (2.00±0.20 mg/dL). The mean values in the subgroup (A3) were - AST (46.50±12.50 iu/L), ALT (29.50±3.50 iu/L), albumin (3.80±0.20 g/dL), direct bilirubin (0.85±0.15 mg/dL), and total bilirubin (2.45±0.15 mg/dL). The mean values in the subgroup (A4) were - AST (58.50±9.50 iu/L), ALT (48.50±7.50 iu/L), albumin (3.90±0.30 g/dL), direct bilirubin (0.85±0.15 mg/dL), and total bilirubin (1.85±0.15 mg/dL).

Comparing the negative control subgroup with the other subgroups – A1, A2, A3, and A4, the mean AST, ALT, albumin, direct bilirubin, and total bilirubin for the negative control subgroup was higher than their respective values in A1, A2, A3, and A4, except AST for A4 and total bilirubin for A3 that were higher than their respective values in the negative control subgroup. However, these differences were not statistically significant for AST, ALT, direct bilirubin and total bilirubin ($p\geq0.05$), except ALT which was statistically significant in A1, A2, and A3 ($p<0.05$), but not in A4 ($p\geq0.05$).

Comparing subgroups A2, A3, and A4 with A1 (positive control subgroup), subgroup A1 had lower value for AST compared with other subgroups. There was lower ALT value in A1 compared with A3. Lower albumin value was noted in A1 compared with A2 and A4. Higher direct bilirubin value was noted in A1 compared with the other subgroups. Lower total bilirubin value was also noted in A1 compared with A2 and A3, but was higher compared with A4. All these differences in values were

**Table 1:** Liver Enzyme Assay Results for Female Wistar Rats in Group A, expressed in Mean±SEM and Comparison with Negative and Positive Control Subgroups

| Subgroups | AST (iu/L) | ALT (iu/L) | Albu (g/dL) | DBil (mg/dL) | TBil (mg/dL) |
|---|---|---|---|---|---|
| Negative Control | 49.00±4.00 | 71.00±5.00 | 4.25±0.15 | 1.05±0.25 | 2.20±0.10 |
| A1 - Positive Control | 26.00±2.00$^{**}$ | 33.00±3.00$^{*}$ | 3.80±0.20$^{**}$ | 1.10±0.10$^{**}$ | 1.95±0.15$^{**}$ |
| A2 | 42.00±4.00$^{**b}$ | 33.00±6.00$^{*b}$ | 4.00±0.10$^{**b}$ | 0.65±0.05$^{**b}$ | 2.00±0.20$^{**b}$ |
| A3 | 46.50±12.50$^{**b}$ | 29.50±3.50$^{*b}$ | 3.80±0.20$^{**b}$ | 0.85±0.15$^{**b}$ | 2.45±0.15$^{**b}$ |
| A4 | 58.50±9.50$^{**b}$ | 48.50±7.50$^{**b}$ | 3.90±0.30$^{**b}$ | 0.85±0.15$^{**b}$ | 1.85±0.15$^{**b}$ |

*Source: Fieldwork, 2023. * denotes significant difference when compared with negative control, $p<0.05$; ** denotes no significant difference when compared with negative control, $p\geq0.05$; b denotes no significant difference when compared with positive control, $p\geq0.05$*

not statistically significant (p≥0.05).

Table 2 shows the results of the liver enzyme assay of rats in group B and comparison with negative and positive control subgroups. The mean values in the negative control subgroup were - AST (49.00±4.00 iu/L), ALT (71.00±5.00 iu/L), albumin (4.25±0.15 g/dL), direct bilirubin (1.05±0.25 mg/dL), and total bilirubin (2.20±0.10 mg/dL). The mean values in the positive control subgroup (B1) were - AST (39.50±7.50 iu/L), ALT (77.00±8.00 iu/L), albumin (4.80±0.20 g/dL), direct bilirubin (1.10±0.10 mg/dL), and total bilirubin (1.70±0.50 mg/dL). The mean values in the subgroup (B2) were - AST (35.00±7.00 iu/L), ALT (72.50±5.50 iu/L), albumin (4.55±0.55 g/dL), direct bilirubin (0.90±0.10 mg/dL), and total bilirubin (2.10±0.10 mg/dL). The mean values in the subgroup (B3) were - AST (45.00±6.00 iu/L), ALT (45.50±10.50 iu/L), albumin (4.50±0.50 g/dL), direct bilirubin (1.00±0.00 mg/dL), and total bilirubin (2.15±0.15 mg/dL). The mean values in the subgroup (B4) were - AST (59.50±4.50 iu/L), ALT (57.50±7.50 iu/L), albumin (3.70±0.30 g/dL), direct bilirubin (0.90±0.10 mg/dL), and total bilirubin (1.90±0.40 mg/dL).

Comparing the negative control subgroup with the other subgroups, the mean AST in the negative control subgroup was higher than the values in B1, B2, and B3,

**Table 2:** Liver Enzyme Assay Results for Female Wistar Rats in Group B, expressed in Mean±SEM and Comparison with Negative and Positive Control Subgroups

| Subgroups | AST (iu/L) | ALT (iu/L) | Albu (g/dL) | DBil (mg/dL) | TBil (mg/dL) |
|---|---|---|---|---|---|
| Negative Control | 49.00±4.00 | 71.00±5.00 | 4.25±0.15 | 1.05±0.25 | 2.20±0.10 |
| B1 – Positive Control | 39.50±7.50$^{**}$ | 77.00±8.00$^{**}$ | 4.80±0.20$^{**}$ | 1.10±0.10$^{**}$ | 1.70±0.50$^{**}$ |
| B2 | 35.00±7.00$^{**b}$ | 72.50±6.00$^{**b}$ | 4.55±0.55$^{**b}$ | 0.90±0.10$^{**b}$ | 2.10±0.10$^{**b}$ |
| B3 | 45.00±6.00$^{**b}$ | 45.50±10.50$^{**b}$ | 4.50±0.50$^{**b}$ | 1.00±0.00$^{**b}$ | 2.15±0.15$^{**b}$ |
| B4 | 59.50±4.50$^{**b}$ | 57.50±7.50$^{**b}$ | 3.70±0.30$^{**b}$ | 0.90±0.10$^{**b}$ | 1.90±0.40$^{**b}$ |

*Source: Fieldwork, 2023. $^{**}$ denotes no significant difference when compared with the negative control subgroup, p≥0.05; $^{b}$ denotes no significant difference when compared with the positive control subgroup, p≥0.05*

but was lower compared with B4. ALT in the negative control subgroup was lower when compared with ALT in B1 and B2, but was higher compared with B3 and B4. Albumin in the negative control subgroup was lower compared with B1, B2, and B3, but was higher compared with B4. Direct bilirubin in the negative control subgroup was lower compared with B1, but was higher compared with B2, B3, and B4. Total bilirubin in the negative control subgroup was higher compared with other subgroups in group B. The differences in these values were not statistically significant (p≥0.05).

Comparing B1 (positive control subgroup) with B2, B3, and B4, B1 has a lower value for AST compared with B3 and B4, but was higher compared with B2. Higher ALT, albumin, and direct bilirubin values were noted in B1 compared with B2, B3, and B4. Lower total bilirubin value was noted in B1 compared with B2, B3, and B4. The differences in these values were not statistically significant (p≥0.05).

Table 3 shows the comparison between the results of the positive control subgroups A1 and B1 in groups A and B respectively. The mean AST, ALT, and albumin values in subgroup B1 were higher. Direct bilirubin values were the same in both subgroups, whereas total bilirubin value was lower in subgroup B1. The differences in these values were not significant (p≥0.05) except for ALT (p<0.05).

Table 4 shows the comparison between the results of subgroups A2 and B2. The mean ALT, albumin, direct

**Table 3:** Comparison of Results of Positive Control Subgroups - A1 and B1

| Subgroups | AST (iu/L) | ALT (iu/L) | Albu (g/dL) | DBil (mg/dL) | TBil (mg/dL) |
|---|---|---|---|---|---|
| A1 | 26.00±2.00 | 33.00±3.00 | 3.80±0.20 | 1.10±0.10 | 1.95±0.15 |
| B1 | 39.50±7.50$^{**}$ | 77.00±8.00$^{*}$ | 4.80±0.20$^{**}$ | 1.10±0.10$^{**}$ | 1.70±0.50$^{**}$ |

*Source: Fieldwork, 2023. $^{*}$ denotes significant difference between the subgroups, p<0.05; $^{**}$ denotes no significant difference between the groups, p≥0.05*

bilirubin, and total bilirubin values in subgroup B2 were higher, except for AST value which was lower. The differences in these values were not statistically significant (p≥0.05) except for ALT (p<0.05).

Table 5 shows the comparison between the results of subgroups A3 and B3. The mean ALT, albumin, and direct bilirubin values in subgroup B3 were higher, except for AST and total bilirubin values which were lower. The differences in these values were not statistically significant (p≥0.05).

Table 6 shows the comparison between the results of subgroups A4 and B4. The mean AST, ALT, direct bilirubin, and total bilirubin values in subgroup B4 were higher, except for albumin which was lower. The differences in these values were not statistically significant (p≥0.05).

**Table 4:** Comparison of Results of Subgroups A2 and B2

| Subgroups | AST (iu/L) | ALT (iu/L) | Albu (g/dL) | DBil (mg/dL) | TBil (mg/dL) |
|---|---|---|---|---|---|
| A2 | 42.00±4.00 | 33.00±6.00 | 4.00±0.10 | 0.65±0.05 | 2.00±0.20 |
| B2 | 35.00±7.00** | 72.50±5.50* | 4.55±0.55** | 0.90±0.10** | 2.10±0.10** |

*Source: Fieldwork, 2023. * denotes significant difference between the subgroups, p<0.05; ** denotes no significant difference between the subgroups, p≥0.05*

**Table 5:** CComparison of Results of Subgroups A3 and B3

| Subgroups | AST (iu/L) | ALT (iu/L) | Albu (g/dL) | DBil (mg/dL) | TBil (mg/dL) |
|---|---|---|---|---|---|
| A3 | 46.50±12.50 | 29.50±3.50 | 3.80±0.20 | 0.85±0.15 | 2.45±0.15 |
| B3 | 45.00±6.00** | 45.50±10.50** | 4.50±0.50** | 1.00±0.00** | 2.15±0.15** |

*Source: Fieldwork, 2023. ** denotes no significant difference between the subgroups, p≥0.05*

**Table 6:** Comparison of Results of Subgroups A4 and B4

| Subgroups | AST (iu/L) | ALT (iu/L) | Albu (g/dL) | DBil (mg/dL) | TBil (mg/dL) |
|---|---|---|---|---|---|
| A4 | 58.50±9.50 | 48.50±7.50 | 3.90±0.30 | 0.85±0.15 | 1.85±0.15 |
| B4 | 59.50±4.50** | 57.50±7.50** | 3.70±0.30** | 0.90±0.10** | 1.90±0.40** |

*Source: Fieldwork, 2023. ** denotes no significant difference between the subgroups, p≥0.05*

**Histological Findings and Interpretation of Hepatic Histoarchitecture for Maternal Rats and their Neonates (Pups) Using Hematoxylin & Eosin Stains**

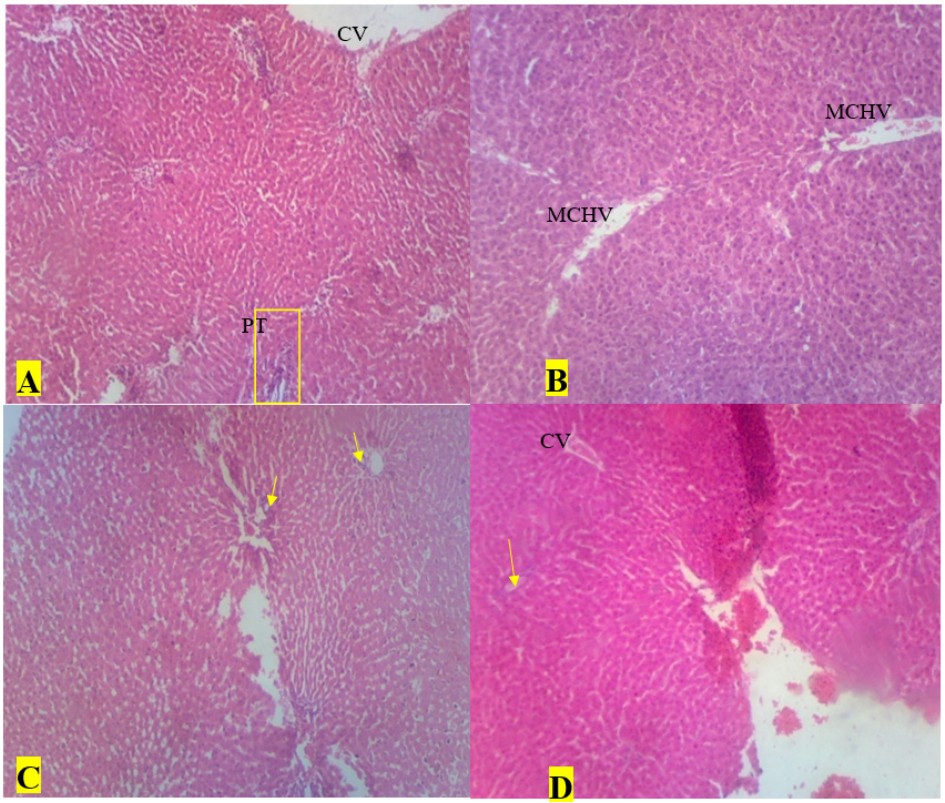


**Figure 1:** Photomicrographs of the livers of maternal rats in group A.
*A: Negative control subgroup shows normal histological structure; normal hepatocytes, central vein (CV), sinusoids, and portal triad (PT). B: Subgroup A2 shows proper perfusion of hepatocytes but with a few degenerating hepatocytes, and mild congested hepatic vessel (MCHV). C: Subgroup A3 shows dilatation of sinusoids, steatosis, widespread nuclear degeneration in the hepatocytes, cholangitis (yellow arrows), and distortion of hepatic tissues. D: Subgroup A4 shows cholangitis (yellow arrow), nuclear degeneration in the hepatocytes around the congested central vein, and extensive distortion of hepatic tissue architecture. [Stain used: Hematoxylin and eosin (H&E), magnification at X60].*

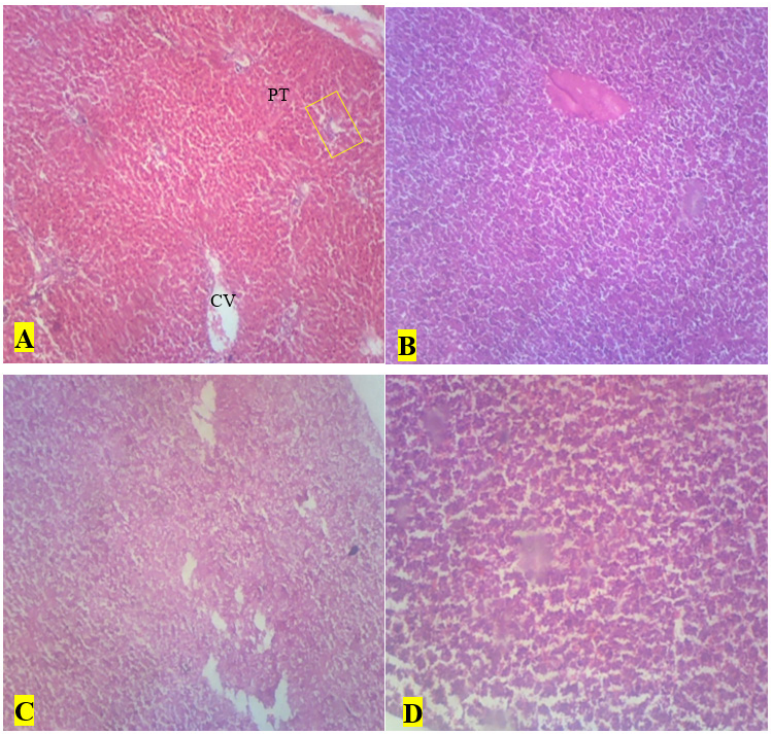


**Figure 2:** Photomicrographs of the liver of neonatal rats (pups) in group A.
*A: Negative control subgroup shows normal histological structure, normal hepatocytes, central vein (CV), sinusoids, and portal triad (PT). B: Subgroup A2 shows mild congestion of immature looking hepatocytes, large and clumpy nuclei in the hepatocytes, and inconspicuous plate-like arrangement of hepatocytes. C: Subgroup A3 shows distortion of hepatic architecture, steatosis, degenerating immature hepatocytes with clumpy nucleus. D: Subgroup A4 shows distortion of hepatic architecture with mild infiltration of inflammatory cells. [Stain used: H&E, magnification at X60].*

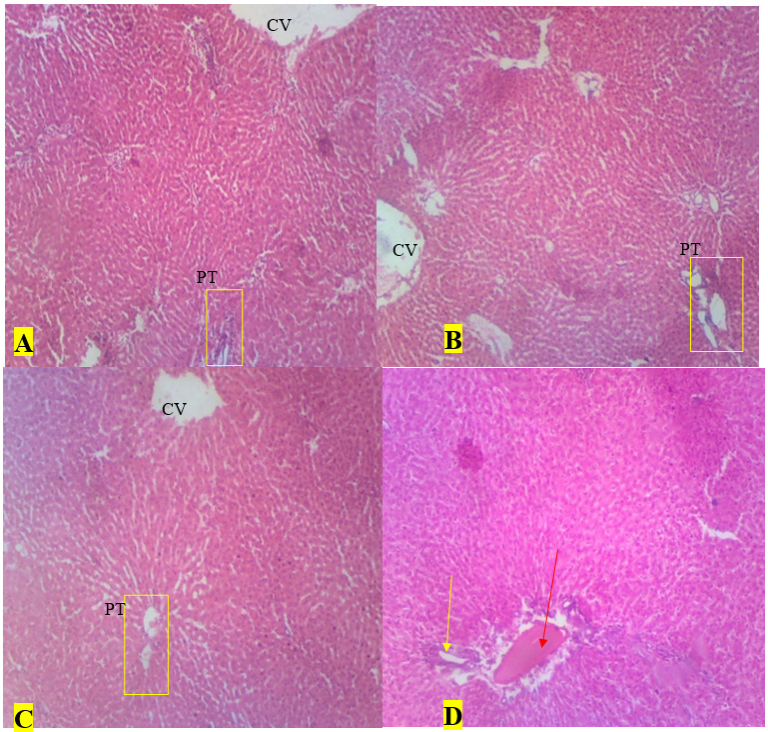


**Figure 3:** Photomicrographs of the livers of maternal rats in group B.
*A: Negative control subgroup shows normal histological structure, normal hepatocytes, central vein (CV), sinusoids, and portal triad (PT). B: Subgroup B2 shows mild dilatation of sinusoids and mild infiltration of hepatocytes. C: Subgroup B3 shows mild distension of sinusoids, and widespread increased perfusion of hepatocytes with indistinctive borders. D: Subgroup B4 shows cholangitis (yellow arrow) in portal area, congested portal vein (red arrow), clumpy nucleus in degenerating hepatocytes. [Stain used: H&E, magnification at X60].*

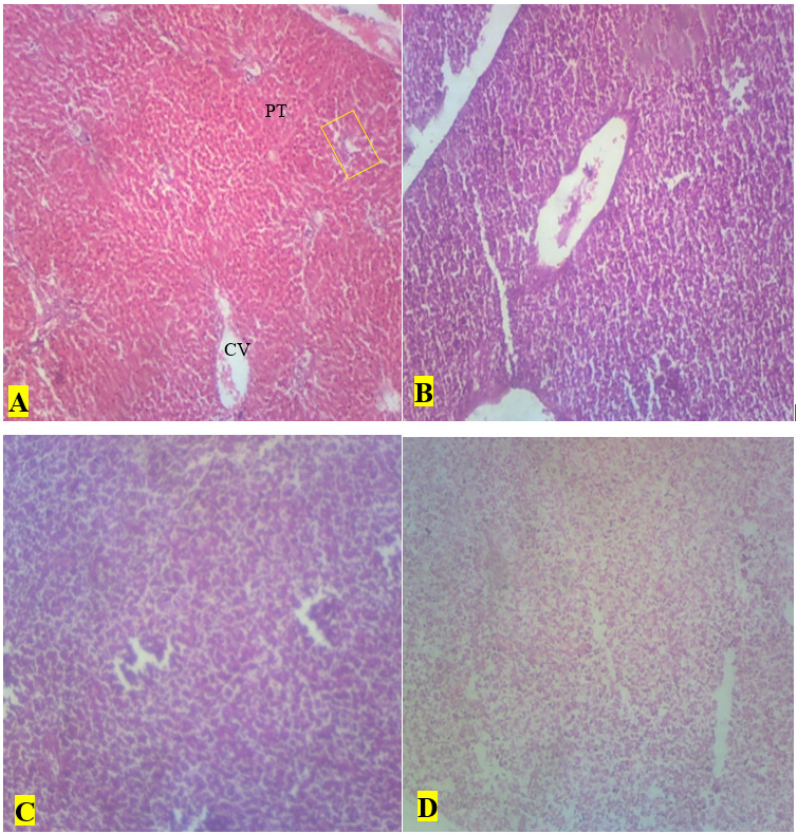


**Figure 4:** Photomicrographs of the liver of neonatal rats (pups) in groups B.
*A: Negative control subgroup shows normal histological structure, normal hepatocytes, central vein (CV), sinusoids, and portal triad (PT). B: Subgroup B2 shows mild congested central vein, and immature hepatocytes. C: Subgroup B3 shows distortion of hepatic architecture seen as distended sinuses with steatosis. D: Subgroup B4 shows hepatic pallor, severe steatosis, and overall distortion of hepatic histology. [Stain used: H&E, magnification at X60].*

## DISCUSSION

Fruits are essential in human nutrition particularly during pregnancy (Rahman, 2008). Fruit vendors in trying to meet up with demand, usually ripen their products in large quantities by using chemicals such as calcium carbide (Siddiqui, 2010). Artificial ripening of fruits undoubtedly hastens the ripening process. However, the health safety and nutritional qualities of these fruits are altered (Hossain, 2015). Banana is a popular fruit all over the world either locally grown or imported. This study investigated the alterations in some selected liver enzymes following the consumption of $CaC_2$-ripened banana before and/or during pregnancy, as well as its associated risks and pathogenic effects on maternal and neonatal livers.

AST and ALT are transaminase enzymes that catalyze amino transfer reactions and also play a role in amino acid catabolism and biosynthesis (Ayepola, 2013). They are majorly found in the hepatocytes and serve as biomarkers for hepatic damage (Ekechi, 2023b; Udeh, 2023b). Increased serum levels of AST and ALT indicate a hepatic injury due to exposure to toxic substances. The increase is directly proportional to the extent of the tissue damage, and of the two, ALT is more sensitive (Huang, 2006; Liu, 2014). These enzymes with albumin, direct (conjugated) bilirubin, and total bilirubin are biomarkers that give wholistic evaluation of hepatic injury (Ahmadvand, 2012; Udeh, 2023b).

The findings in this study as seen in Table 1 shows that eating naturally (A1)- or $CaC_2$-ripened (A2 and A3) bananas before and during pregnancy insignificantly lowers serum AST and ALT levels, but market-derived $CaC_2$-ripened (A4) bananas insignificantly lowered serum ALT and increased AST. This means that it might be worse not to include bananas in a woman's diet before and during pregnancy. Moreover, it was observed that it is healthier to eat naturally-ripened bananas before and during pregnancy because the positive control subgroup (A1) had the lowest mean AST value when compared with subgroups A2, A3, and A4 that ate $CaC_2$-ripened bananas, and an insignificant lower mean ALT value when compared with the mean ALT value of subgroup A4 that ate market-derived $CaC_2$-ripened bananas ($p \geq 0.05$).

Table 2 shows that eating naturally- or 1% $CaC_2$-ripened bananas only during pregnancy insignificantly lowered serum AST ($p \geq 0.05$) but increased serum ALT ($p \geq 0.05$). Market-derived $CaC_2$-ripened bananas lowered only ALT ($p \geq 0.05$), while 2% $CaC_2$-ripened bananas lowered both AST and ALT ($p \geq 0.05$). This implies that not eating bananas during pregnancy would not relieve the liver of metabolic stress, eating 2% $CaC_2$-ripened bananas could not be safe if eaten during pregnancy, while naturally-,

1% $CaC_2$-, and market-derived ripened bananas could not be said to be 100% safe or toxic to the liver. This can be restated that naturally ripened bananas might not be 100% safer than $CaC_2$- and market-derived $CaC_2$-ripened bananas.

The increased serum AST in subgroups A4 and B4 and the increased ALT in subgroup B2 could be related to the action of $CaC_2$ causing the enzymes to leak from the hepatocytes into blood circulation. This leakage could be related to the mild dilatation of sinusoids, mild infiltration of hepatocytes, congested portal vein, and clumpy nucleus in degenerating hepatocytes seen in Figures 1D, 3B, and 3D. The very high ALT level in subgroup B1 that ate naturally ripened bananas raises concern about the nutritional composition of the banana species. Disturbance of liver functions could contribute to dyslipidemia which is a metabolic syndrome disease. Metabolic syndrome diseases, MSDs (Hypertension, Adiposity, Diabetes mellitus and Dyslipidemia) are interrelated diseases with very high morbidity and mortality rates (Ikwuka, 2015; Ikwuka, 2017a; Ikwuka, 2017c; Ikwuka, 2023c; Virstyuk, 2016).

Albumin is the most abundant plasma protein, synthesized by hepatocytes, and it maintains normal oncotic pressure and acts as a transporter for endogenous ligands e.g. bilirubin, ions, fatty acids, and exogenous ligands e.g. drugs (Moman, 2022). The synthesis of albumin is favored by adequate nourishment. In a poor nutritional state, hepatotoxicity, and inflammation, its secretion is inhibited while dehydration has been linked to its increase in the blood (Moman, 2022). Results from different studies have shown that high levels of blood pressure, glucose and lipid metabolic disorders, asymptomatic hyperuricemia, activation of systemic immune inflammation and fibrogenesis, contribute to kidney damage (Ikwuka, 2017d; Ikwuka, 2017e; Ikwuka, 2018a; Ikwuka, 2018c; Ikwuka, 2018d; Ikwuka, 2019a; Ikwuka, 2019c; Ikwuka, 2022; Ikwuka, 2023d; Ikwuka, 2023f; Virstyuk, 2017a; Virstyuk, 2018a; Virstyuk, 2019; Virstyuk, 2021a; Virstyuk, 2021b).

In this study, the rats in negative control subgroup had the highest serum albumin level, while subgroups A1 and A3 had the lowest albumin level as seen in Table 1. However, the values were within or close to 3.0-4.0g/dL which is the normal albumin range for wistar rats. The serum albumin levels for rats in group B show that subgroup B1 had the highest albumin level, while subgroups B2 and B3 had albumin levels higher than the negative control subgroup and B4. The very high levels of albumin noted in group B (subgroups B1, B2, and B3) could be attributed to dehydration causing the release of more albumin by the hepatocytes to maintain oncotic pressure.

Metabolic Syndrome Diseases require new and effective treatment regimens. Dapagliflozin which is a Sodium-Glucose Linked Transporter 2 (SGLT-2) inhibitor and Liraglutide which is a Glucagon-like Peptide 1 Receptor Agonist (GLP-1 RA) have been found to increase the effectiveness of treatment and improve the clinical course of type 2 diabetes mellitus and hypertension in patients with such comorbidities (Ikwuka, 2017b; Ikwuka, 2018b; Ikwuka, 2019b; Ikwuka, 2021; Ikwuka, 2023f; Virstyuk, 2017b; Virstyuk, 2018b; Virstyuk, 2018c). In addition, Rauwolfia vomitoria has a neuroprotective ability at it elevates antioxidants and suppresses lipid peroxidation (Ekechi, 2023a).

Bilirubin is a bile pigment produced from the breakdown of heme from hemoglobin in the red blood cell (hemolysis) (Ikwuka, 2023e). An elevated total bilirubin therefore suggests an excessive hemolysis, poor processing of bilirubin volume by hepatocytes, hepatic obstruction and/or biliary obstruction during bilirubin excretion (Udeh, 2023b). In this study, rats in subgroup A1 that ate naturally ripened bananas before and during pregnancy had the highest direct (conjugated) bilirubin level but with a low total bilirubin level when compared with other subgroups as seen in Table 1. It can be deduced that eating naturally ripened bananas is safe at this period. The high level of total bilirubin in subgroups A2 and A3 relates to the mild congested hepatic vessels, dilatation of sinusoids, cholangitis, steatosis (fatty change), and hepatocyte degeneration seen in Figures 1B and 1C, an these show that 1% and 2% $CaC_2$-ripened bananas are toxic before and during pregnancy. Group B proves that it is best to eat naturally ripened bananas during pregnancy as the total bilirubin level is lowest in subgroup B1. Non-inclusion of bananas in the diet of a pregnant woman exposes her liver to metabolic stress even if the liver histology appears normal. Market-ripened bananas even with the low total bilirubin level as expressed in Table 3 and the severe hepatic injury (cholangitis in portal area, congested portal vein, clumpy nucleus in degenerating hepatocytes) are proofs that market-ripened bananas could be harmful.

Histopathological examination of the maternal and neonatal liver sections summarizes the findings of the effect of consuming $CaC_2$-ripened bananas before and/or during pregnancy. The results of AST, ALT, albumin, direct bilirubin, and total bilirubin did not totally confirm the toxicity or safety of $CaC_2$-ripened bananas as most of the parameters were not statistically significant ($p \geq 0.05$), although the histological analysis gave a better insight into the hepatic effects of the diet. In groups A and B, these varying calcium carbide exposures harmed the livers of the respective subgroups that received 1%, 2%, and market-derived $CaC_2$-ripened bananas in their diets, which supports the reports of a study where $CaC_2$-ripened fruits were reported to be toxic to the liver as they altered some hematological and serum biochemical parameters of wistar rats (Igbinaduwa, 2016).

Histological findings for both groups ranged from hepatic vessel congestion to degeneration of hepatocytes, steatosis, cholangitis, and dilatation of sinusoids as seen in Figures 1B, 1C, 1D, 3B, 3C, and 3D. These alterations were also visible in the neonatal (pup) liver. For pups in group A, the manifestations were mild congestion of immature hepatocytes, clumpy nucleus in hepatocytes,

distortion of hepatic architecture, steatosis, degenerating immature hepatocytes, mild infiltration of inflammatory cells as shown in figures 2B, 2C, and 2D. The pups in group B had mild congested central vein, immature hepatocytes, distended sinuses, steatosis, hepatic pallor, and overall distortion of hepatic histology as seen in figures 4B, 4C, and 4D.

## CONCLUSION

Consumption of calcium carbide-ripened fruits have associated risks and poses a great health threat to pregnant women and their neonates due to various degrees of alteration in some parameters of liver enzymes and thus can lead to impairment of liver functions. The activities of fruit sellers should be adequately and strictly monitored. Life should be considered first, before gain in order to avoid later pain.

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