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Embryofetal Toxicity of Artificially Ripened Fruits: Effects of Calcium Carbide Ripened Mango Fruits on Fetal Development in Wistar Rats

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Abstract

Background: Embryonic development is a critical process that relies on proper maternal nutrition, including the intake of fruits rich in essential vitamins and minerals. Mango (*Mangifera indica*) is widely consumed, but its perishability necessitates artificial ripening methods such as calcium carbide (CaC_2) treatment. The effects of calcium carbide-ripened fruits on fetal development remain largely unexplored, necessitating this study. ***Aim:*** This study aims to investigate the embryofetal toxicity of calcium carbide-ripened mangoes by assessing their effects on fetal development in Wistar rats. ***Methodology:*** Estrus cycling in female Wistar rats was determined via vaginal smear analysis. Forty-five pregnant rats were randomly assigned to nine groups, receiving 5mL or 10mL of blended mango paste from naturally ripened, market calcium carbide-ripened, laboratory calcium carbide-ripened (10g or 30g CaC_2), or unripe fruits. Treatment was administered for 20 days. Elemental analysis of mango samples was conducted to determine mercury, arsenic, and metallic content. Gestational weight gain, fetal growth indices, and placental weight were assessed. ***Results:*** Spectrometric analysis revealed elevated levels of mercury and arsenic in market and laboratory-ripened mangoes compared to naturally ripened samples. Elemental analysis showed increased concentrations of iron, manganese, and cadmium in artificially ripened mangoes. Pregnant rats consuming calcium carbide-ripened mangoes exhibited altered gestational weight gain patterns, with some groups showing significant weight loss. Fetal resorption was observed in groups administered 10mL of market or 30g laboratory carbide-ripened mangoes, indicating severe embryotoxic effects. Placental weights were significantly reduced in these groups. ***Conclusion:*** Calcium carbide-ripened mangoes contain elevated levels of toxic metals, which may contribute to embryofetal toxicity. Exposure to these fruits during pregnancy resulted in fetal growth restriction, increased fetal resorption, and altered maternal weight gain patterns.

Keywords: embryofetal toxicity, calcium carbide, artificially ripened mango, Wistar rats, fetal development, heavy metal contamination

1. Introduction

Embryonic development is a highly intricate process that involves a series of tightly regulated events, beginning from fertilization to the formation of a fully developed fetus. This process is vital for the proper formation of tissues, organs, and systems necessary for survival post-birth (Molnar & Gair, 2015). Early stages of development are particularly crucial, as they set the foundation for the overall health and viability of the offspring (Dahlen *et al.*, 2021). Proper nutrition during pregnancy plays an essential role in the optimal growth and development of the fetus, particularly through the intake of fruits and vegetables that provide vital nutrients such as vitamins, minerals, and antioxidants (Hossain *et al*., 2015). These nutrients contribute to various physiological processes, including vision, cellular differentiation, reproduction, and overall growth (Dibley, 2001).

Among the myriad of fruits consumed worldwide, the mango (*Mangifera indica*) holds a prominent place due to its nutritional value and widespread consumption. However, mangoes are highly perishable and are often harvested prematurely to avoid significant post-harvest losses (Ajayi & Mbah, 2018). To address the issue of premature ripening, artificial ripening agents such as calcium carbide (CaC_2) are commonly used in many countries, including Nigeria, where these agents are employed to accelerate the ripening process and reduce the economic losses associated with premature harvests (Al-Gubory, 2019; Zewter *et al*., 2019). Calcium carbide, although widely used, is not approved for food ripening in many regions due to concerns about its safety and potential toxicological effects. Despite its widespread application, there is a limited understanding of the potential risks associated with its use, particularly regarding its effects on fetal development (Gordian, 2021).

Calcium carbide releases acetylene gas upon contact with moisture, which serves as a ripening agent for fruits. However, the chemical composition of calcium carbide has raised concerns regarding its potential health risks. The substance contains impurities such as arsenic and phosphorus, both of which have been shown to possess toxic properties (Gordian, 2021). While previous studies have highlighted the negative impacts of calcium carbide on various organs, particularly reproductive organs (Ajayi & Mbah, 2018; Zewter *et al*., 2019), the effects of calcium carbide ripened fruits on fetal development remain underexplored. This gap in knowledge is concerning, as exposure to toxic substances during pregnancy can lead to adverse outcomes, including developmental malformations, fetal toxicity, and long-term health issues in offspring (Al-Gubory, 2019).

This study aims to investigate the potential embryo-fetal developmental toxicity of calcium carbide ripened mangoes by assessing their effects on fetal development in Wistar rats. Understanding the potential risks associated with consuming artificially ripened fruits is crucial for public health, as it can guide regulatory policies and inform consumers about the safety of such products. The importance of this study lies in its potential to provide valuable data that may influence future regulations surrounding the use of artificial ripening agents, particularly in developing countries where such practices are common. By exploring the relationship between artificial fruit ripening and fetal health, this research seeks to fill an important gap in the existing literature and contribute to the growing body of knowledge on the safety of food chemicals and their potential impacts on human health, particularly during critical stages of development.

2. Materials and Methods

2.1 Experimental Animals

In this study, a total of 45 female and 20 male Wistar rats, with body weights ranging from 140g to 200g, were utilized. The animals were sourced from the animal house at the University of Ibadan and subsequently housed in the animal facility at the Faculty of Basic Medical Sciences, Baze University, Abuja. The rats were acclimatized to their new environment for a period of two weeks prior to the initiation of the experiment. During this period, the animals were maintained on a 12-hour light/dark cycle and housed in clean, plastic cages. They were provided with commercial rat pellets and tap water *ad libitum*. The rat pellets were procured from the Vital Feeds shop of the National Institute for Pharmaceutical Research and Development (NIPRD) in Abuja.

2.2 Collection of Calcium Carbide

Calcium carbide, used for the artificial ripening of mangoes, was obtained from a welding shop located in Utako market, Federal Capital Territory, Abuja.

2.3 Procurement, Identification, and Preparation of Mango Samples

Both unripe and ripe mango fruits used in this research were sourced locally from a farmer in the Federal Capital Territory, Abuja, Nigeria. The market-ripened mangoes were purchased from Utako market, also located in Abuja. The identification and authentication of the mango samples were conducted by a botanist from the Department of Biology, Faculty of Computing and Applied Sciences, Baze University, Abuja. A voucher specimen was issued with the reference number BU/1000/BUH. The preparation of the mango samples followed a modified version of the methodology outlined by Iroka *et al*. (2016). Mature unripe mangoes were weighed, and 1000g of these fruits were placed in a dark polybag. The first bag was then sealed in an airtight plastic container along with 10g of calcium carbide, while the second bag was treated with 30g of calcium carbide, in separate airtight containers. Both sets of containers were allowed to remain undisturbed for a period of four days, after which the fruits were fully ripened. Subsequently, 500g of each mango group (i.e., calcium carbide ripened with 10g and 30g) were washed, blended with 500mL of water, and stored in plastic bottles under refrigeration. The remaining 500g portions of each group were used for analysis of vitamins and elemental content. Similarly, both naturally ripened and market-ripened mangoes were separated into 500g portions, washed, blended, bottled, labeled, and refrigerated, with additional portions reserved for further analysis.

2.4 Mating, Pregnancy, and Treatment Protocol

Estrus cycling in female rats was determined by vaginal smearing with normal saline, distilled water, and a microscope. A small volume of distilled water was collected using a dropping pipette to rinse the vagina, followed by the collection of normal saline. The vaginal fluid was then transferred to a slide and examined under a microscope to determine the estrus cycle phase, which includes the following stages: di-estrus, pro-estrus, estrus, and met-estrus. Forty-five female Wistar rats were randomly selected and housed in pairs (two females and one male per cage) for a period of 24 hours. Conception was confirmed by the presence of spermatozoa in the vaginal smear and, in some cases, a vaginal plug, which marked the beginning of day zero of pregnancy (Olu *et al*., 2017). The pregnant rats were randomly assigned to nine treatment groups, with five rats per group. Each group received either 5mL or 10mL of blended mango paste from one of the four categories: naturally ripened, laboratory calcium carbide ripened, market calcium carbide ripened, or unripe. All treatments were administered for duration of 20 days, as detailed in Table 1.

Table 1. Animal Treatment Protocol

GROUPS	**TREATMENT**	**DURATION**
A	Feed and Water *ad libitum* (negative control)	20 days
B	Naturally Ripened Fruits (Positive Control) 5mL	20days
C	Naturally Ripened Fruits (Positive Control) 10mL	20 days
D	Market Ripened Fruits (MCR) 5mL	20days
E	Market Ripened Fruits (MCR) 10mL	20days
F	10 g CC. Laboratory Ripened Fruit (LCR) 5mL	20days
G	10 g CC. Laboratory Ripened Fruit (LCR) 10mL	20days
H	30g CC. Laboratory Ripened Fruit (LCR) 5mL	20days
I	30g CC. Laboratory Ripened Fruit (LCR) 10mL	20days

Note: CC = Calcium Carbide; MCR = Market Calcium Carbide Ripened; LCR = Laboratory Calcium Carbide Ripened.

2.5 Measurement of Gestational Weight

Upon confirmation of pregnancy, the initial body weights of the Wistar rats were recorded. Subsequently, their body weights were measured weekly throughout the gestational period to monitor gestational weight changes until euthanasia.

2.6 Euthanasia and Tissue Sample Collection

The pregnant rats were anesthetized using the chloroform inhalation method before being dissected. The uterus, along with the fetuses, was carefully harvested. The placentas and fetuses were blotted dry and weighed. Additional anthropometric parameters were measured before the fetuses were fixed in 10% formal saline for subsequent histological and morphological analysis.

2.7 Fetal Anthropometric Measurements

Fetal morphometric parameters, including fetal weight, placental weight, crown-rump length (CRL), crown-heel length (CHL), foot length, head circumference, abdominal circumference, and mid-arm circumference (MAC), were measured following the methodology described by Olu *et al*. (2017).

2.8 Statistical Analysis

All data were presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was employed to assess statistical differences among groups, followed by Duncan's Multiple Range Test (DMRT) for post hoc comparisons. Statistical significance was set at $P<0.05$ (Mathur *et al*., 2008). Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 20.0 (SPSS Inc., Cary, NC, USA).

2.9 Ethical Approval

This study was conducted in accordance with ethical standards and was approved by the Research Ethics Committee of the Department of Anatomy, Baze University, Abuja (Reference No. BU/URES/ANA/1001). The experimental procedures adhered to the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) under Section 15(1) of the Prevention of Cruelty to Animals

Act (1960) and were in compliance with the ethical principles outlined in the World Medical Association's Declaration of Helsinki for research involving experimental animals.

3. Result

3.1 Spectrometric Analysis of Mango Fruit Samples for Mercury and Arsenic

The spectrometric analysis of mango fruit samples for mercury and arsenic levels presented in Table 2 shows varying concentrations of these heavy metals across different sample types. The results indicate that all mango samples contain detectable levels of both mercury and arsenic, though in different quantities.

For mercury, the concentration ranges from 0.003 µg/g in the Naturally Ripened (NR) sample to a peak of 3.718 µg/g in the Market Calcium Carbide Ripened (MCR) sample. This suggests a considerable variation in mercury content, with the MCR sample showing the highest mercury concentration. On the other hand, the Laboratory Calcium Carbide Ripened (LCR) samples show mercury concentrations of 1.537 µg/g (10g) and 3.365 µg/g (30g), indicating a moderate presence of mercury in these variants, although still lower than the MCR sample.

For arsenic, all samples show arsenic concentrations above 0.02 µg/g, with the NR sample having the lowest concentration of 0.020 µg/g. The MCR sample again shows the highest concentration at 0.1213 µg/g, which is substantially higher than the levels observed in the LCR samples. The LCR samples (10g and 30g) show arsenic concentrations of 0.0674 µg/g and 0.0404 µg/g, respectively, indicating a moderate presence of arsenic compared to the NR sample.

These results suggest that mango fruits, especially the MCR sample, contain varying levels of mercury and arsenic, which may have implications for food safety and public health, particularly if these levels exceed regulatory thresholds for heavy metal contamination.

Table 2. Showing the UV/Visible Spectrometric Analysis of Mango for Arsenic and Mercury

SAMPLE ID	MERCURY (ug/g)	ARSENIC (ug/g)
NR	0.003	0.020
MCR	3.718	0.1213
LCR (10g)	1.537	0.0674
LCR (30g)	3.365	0.0404

Note: NR = Naturally Ripened; MCR = Market Calcium Carbide Ripened; LCR = Laboratory Calcium Carbide Ripened (10g/30g); ug/g: microgram per gram.

3.2 Elemental Analysis of Metallic Contents in Mango Fruit Samples

The elemental analysis of mango fruit samples revealed varying concentrations of metallic elements across different ripening methods. The results, presented in Table 3, show that the naturally ripened (NR) mango samples had the lowest concentration of metals, with notable values of 21.75 mg/kg for iron (Fe) and 20.54 mg/kg for copper (Cu). There was a detectable presence of chromium (Cr) at 0.96 mg/kg, cobalt (Co) at 0.19 mg/kg, and cadmium (Cd) at 0.29 mg/kg, but no lead (Pb) or manganese (Mn) was found.

In contrast, market calcium carbide ripened (MCR) mangoes showed a significant increase in iron (56.27 mg/kg), manganese (1.34 mg/kg), and cadmium (7.81 mg/kg), with copper levels remaining constant at 20.54 mg/kg. However, chromium, cobalt, lead, and manganese were absent in MCR samples. This suggests that the use of calcium carbide in ripening may elevate certain elemental concentrations, particularly iron, manganese, and cadmium, which could potentially pose health risks if consumed in excess.

The laboratory calcium carbide ripened mango samples, both at 10g (LCR 10g) and 30g (LCR 30g), had moderate levels of the metals analyzed. LCR 10g had iron at 50.76 mg/kg and manganese at 2.47 mg/kg, while LCR 30g contained similar concentrations of iron (47.59 mg/kg) and higher levels of manganese (4.84 mg/kg). Cadmium was present in LCR 30g samples at 0.77 mg/kg, but other metals like chromium, cobalt, lead, and copper were either minimal or absent.

The results suggest that the ripening method has a notable impact on the elemental composition of mango fruit. While natural ripening produced the least variation in metal concentrations, calcium carbide ripening, especially market and laboratory methods resulted in increased levels of potentially harmful metals such as iron, manganese, and cadmium.

Table 3. Showing Mean Values of Elemental Concentrations in Mango Samples

SAMPLE ID	Cr (mg/kg)	Fe (mg/kg)	Pb (mg/kg)	Co (mg/kg)	Mn (mg/kg)	Cd (mg/kg)	Cu (mg/kg)
NR	0.96±0.70	21.75±1.30	0.00±0.00	0.19±0.01	0.00±0.00	0.29±0.20	20.54±1.20
MCR	0.00±0.00	56.27±2.30	0.00±0.00	0.00±0.00	1.34±0.20	7.81±0.70	20.54±1.30
LCR (10g)	0.79±0.50	50.76±2.70	0.00±0.00	0.00±0.00	2.47±0.90	0.00±0.00	18.73±0.80
LCR (30g)	0.55±0.02	47.59±2.20	0.00±0.00	0.00±0.00	4.84±1.10	0.77±0.40	19.73±0.90

Note: NR = Naturally Ripened; MCR = Market Calcium Carbide Ripened; LCR = Laboratory Calcium Carbide Ripened (10g/30g); Cr = Chromium; Fe = Iron; Pb = Lead; Co = Cobalt; Mn = Manganese; Cd = Cadmium; Cu = Copper; mg/kg = milligram per kilogram.

3.3 Gestational Weight Gain in Pregnant Wistar Rats

Figure 1 shows the gestational weight changes in Wistar rats across different treatment groups. The positive control group had the highest weight gain by the second week of gestation, followed by a decline in the third week.

Among the groups administered naturally ripened mango fruit, those receiving 5mL showed the highest weight gain in the second week, whereas the 10mL group demonstrated the peak weight gain in the first week, followed by a gradual decrease.

For rats exposed to 5mL of market calcium carbide-ripened mango fruit, the highest weight gain was recorded in the third week, with the lowest in the first week. In contrast, those administered 10mL of the same fruit had peak weight gain in the second week and a significant decline by the third week.

In the group receiving 5mL of 10g laboratory-ripened mango fruit, maximum weight gain was observed in the third week, with no weight increase recorded in the first week. When the dose was increased to 10mL, weight gain remained highest in the third week but lowest in the first week.

For rats administered 5mL of 30g laboratory-ripened mango fruit, initial weight remained unchanged in the first week, followed by a decline in the second week and a slight increase in the third week. Administration of 10mL of 30g laboratory-ripened mango fruit did not result in significant weight gain in the first week, but the highest gain was observed in the third week.

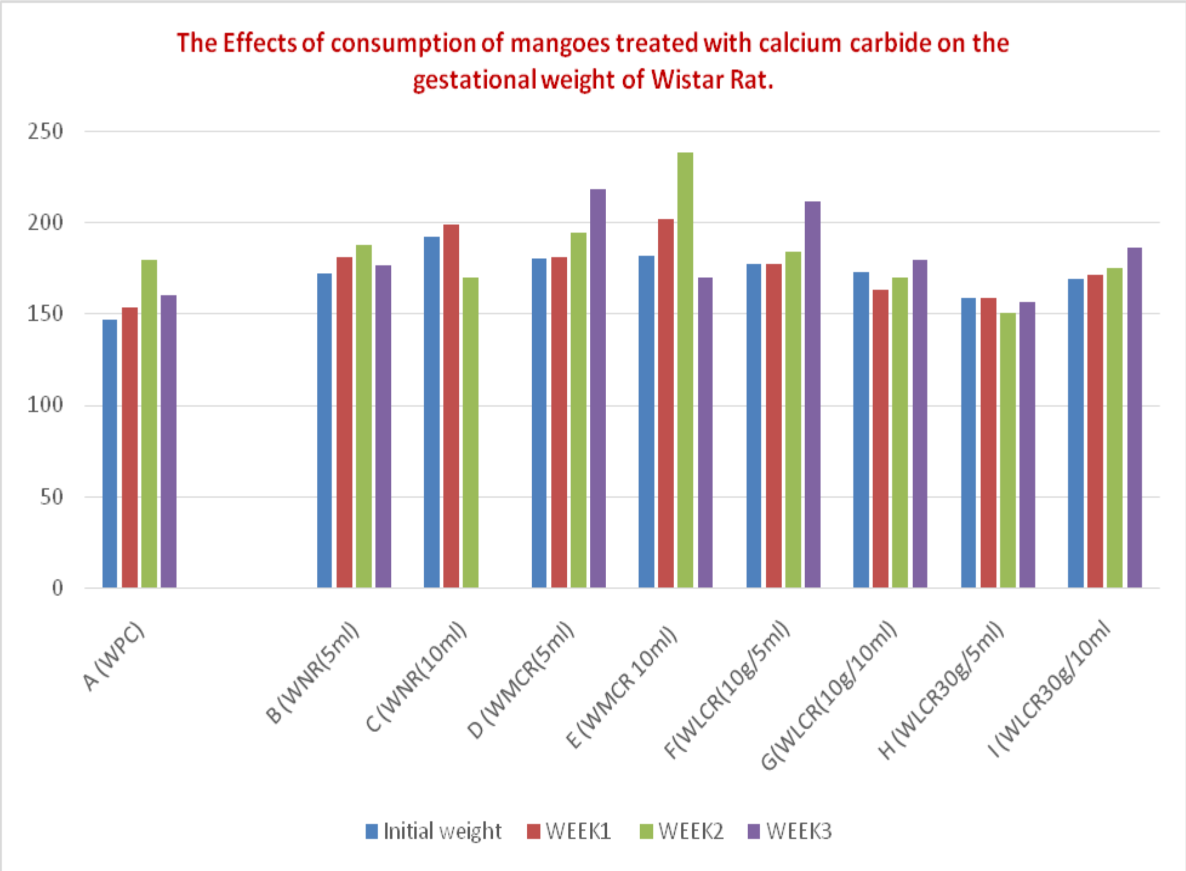

Figure 1. Simple Bar Chart Showing Gestational Weight of Pregnant Rats for each Trimester (represented in weeks)

Note: WNR: Weight of rats in Naturally Ripened; WMCR: Weight of rats in Market Calcium Carbide Ripened Mango; WLCR: Weight of rats in Laboratory Calcium Carbide Ripened Mango (10mg/30mg); PC: Positive Control.

3.4 Fetal Growth Indices and Placental Weight

Analysis of fetal growth indices, including fetal and placental weights as shown in Figure 2, revealed that the group treated with 10mL of naturally ripened mango fruit exhibited the highest fetal weight gain ($P<0.05$). Conversely, the lowest fetal weight was recorded in the group dosed with 5mL of 10g laboratory carbide-ripened mango fruit.

Notably, total fetal resorption was observed in the groups administered 10mL of market carbide-ripened mango fruit and 10mL of 30g laboratory carbide-ripened mango fruit, resulting in the absence of measurable fetal growth parameters in these groups.

Placental weight analysis indicated that the positive control group had the lowest mean placental weight. The highest placental weight was observed in both the 10mL naturally ripened mango fruit group and the 5mL market carbide-ripened mango fruit group.

Among fetal morphometric parameters, the highest crown-rump length was recorded in the 10mL naturally ripened mango fruit group, whereas the lowest value was found in the positive control group. Similarly, the highest foot length was observed in the 5mL market carbide-ripened mango fruit group, with the lowest value in the positive control group.

Crown-heel length was significantly greater in fetuses from the 10mL naturally ripened mango fruit group ($P<0.05$), while the lowest value was recorded in the 5mL market carbide-ripened mango fruit group.

Head circumference measurements revealed that the highest mean value was found in fetuses from the 5mL 10g laboratory carbide-ripened mango fruit group, whereas the lowest was in the 5mL 30g laboratory carbide-ripened mango fruit group.

Abdominal circumference was significantly highest in the 10mL naturally ripened mango fruit group, while the lowest value was recorded in the 5mL 10g laboratory carbide-ripened mango fruit group.

Mid-arm circumference analysis indicated that the highest mean value occurred in the 5mL market carbide-ripened mango fruit group, whereas the lowest was recorded in the positive control group.

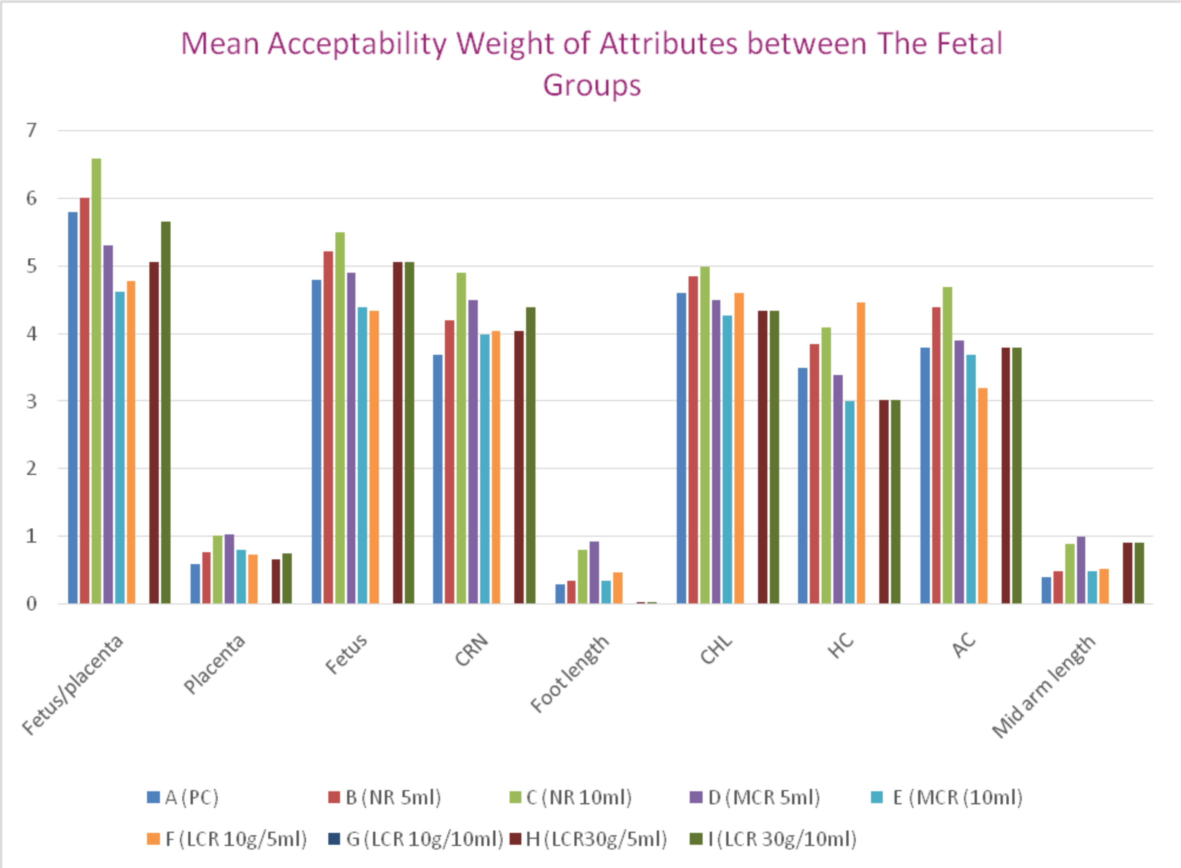

Figure 2. Simple Bar Chart Showing Measurements of Fetal Growth Indices in the Pups of Wistar Rats

Note: CRL = Crown Rump Length; CHL = Crown Heel Length; HC = Head Circumference; AC = Abdominal Circumference; PC = Positive Control; NR = Naturally Ripened (5mls/10mls); MCR = Market Calcium Carbide Ripened (5mls/10mls); LCR = Laboratory Calcium Carbide Ripened (10g: 5mls/10mls; 30g: 5mls/10mls).

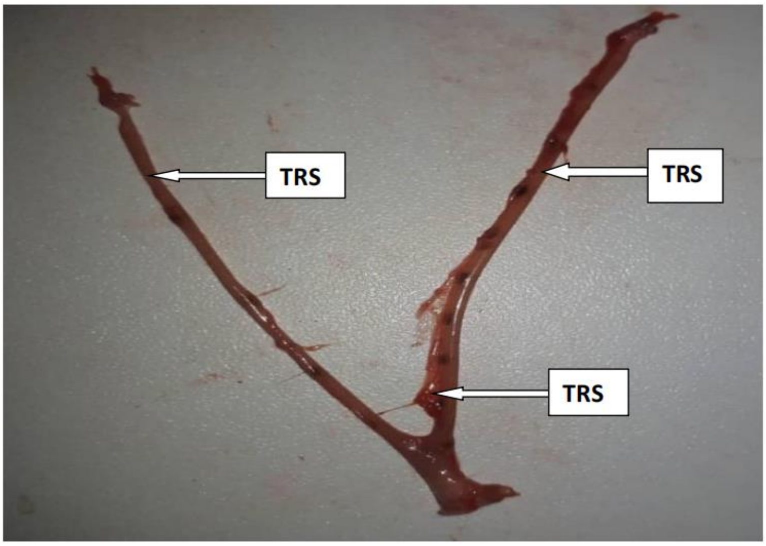

Figure 3. Showing Total Resorption sites (TRS) in the group (group I) treated with 10ml of 30g of Calcium Carbide Ripened Mangoes (30gLCR 10ml)

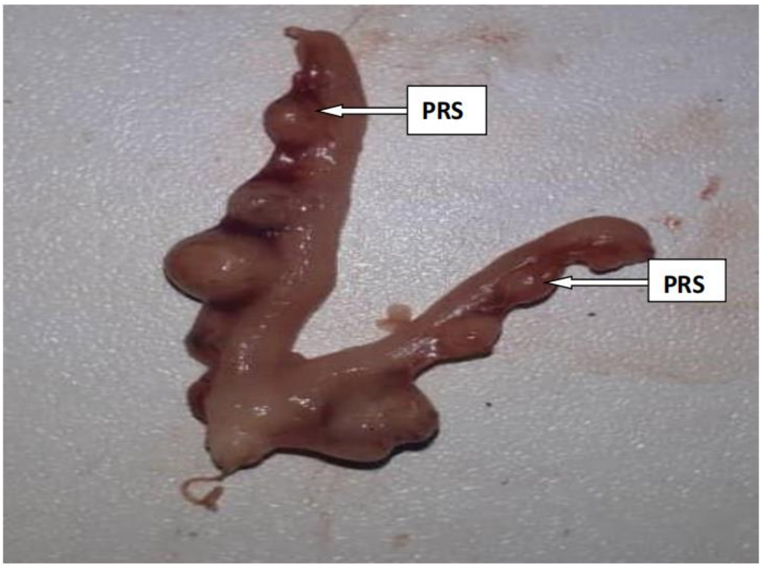

Figure 4. Showing Partial Resorption Sites (PRS) in the group (group E) treated with 10ml of Market Calcium Carbide Ripened Mangoes (MCR 10ml)

3.5 Histological Profile

Hematoxylin and Eosin (H&E) examination of the fetal brain tissue (cerebrum) across the experimental groups showed normal fetal cerebral histology across groups. All the examined groups A – D and F – H showed essentially normal brain tissue with intact cell layer, abundant pyramidal cells and neurocytes.

This result suggests that treatment with mango fruits subjected to different ripening methods had no observable effect on the brain (cerebral) histology of the fetal Wistar rats in this study.

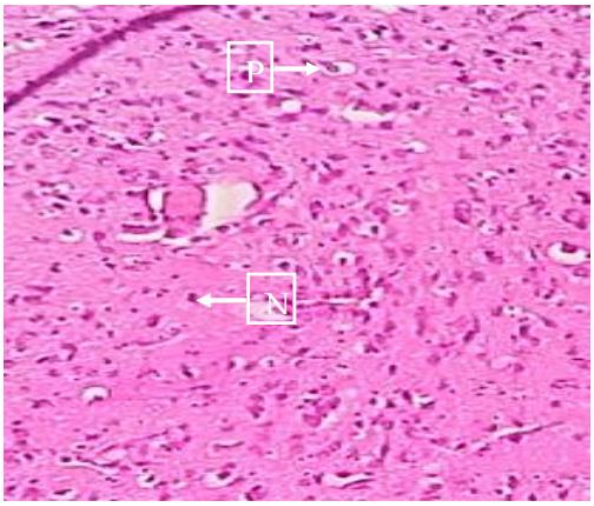

Plate 1: Photomicrograph of the brain tissue (cerebrum) from Group A showing normal cerebral histology with intact cell layers, abundant Pyramidal Cells (P), & Neurocytes (N) (H&E x40)

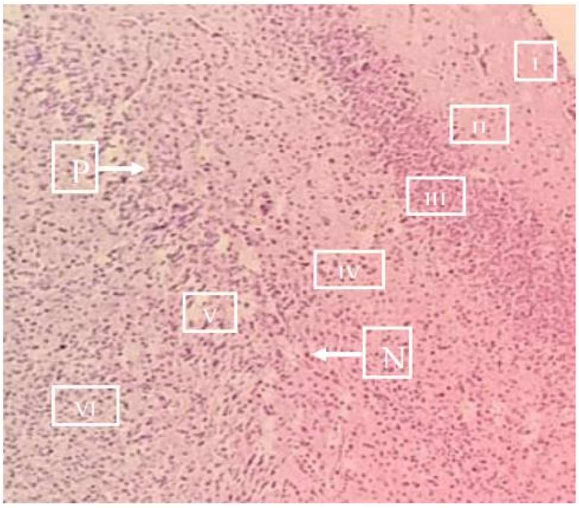

Plate 2: Photomicrograph of the brain tissue (cerebrum) from Group B showing normal cerebral histology with intact cell layers (I – VI), abundant Pyramidal Cells (P), & Neurocytes (N) (H&E x40)

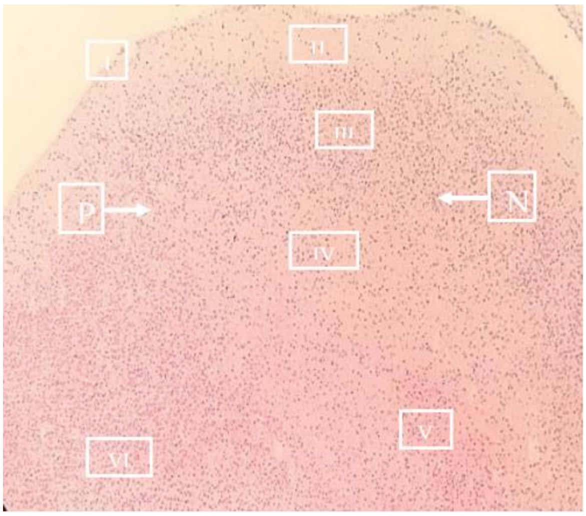

Plate 3: Photomicrograph of the brain tissue (cerebrum) from Group C showing normal cerebral histology with intact cell layers (I – VI), abundant Pyramidal Cells (P), & Neurocytes (N) (H&E x40)

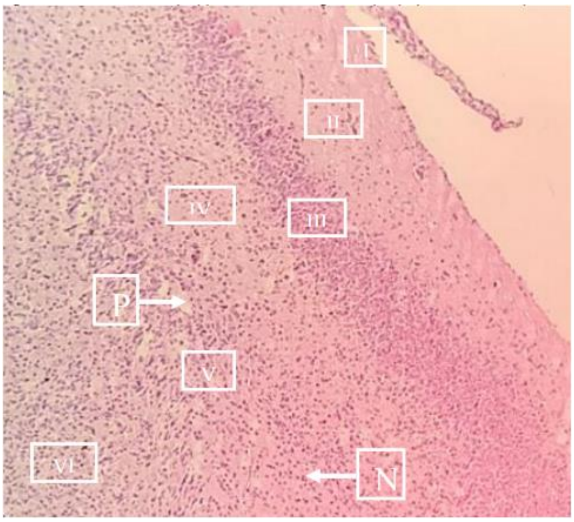

Plate 4: Photomicrograph of the brain tissue (cerebrum) from Group D showing normal cerebral histology with intact cell layers (I – VI), abundant Pyramidal Cells (P), & Neurocytes (N) (H&E x40)

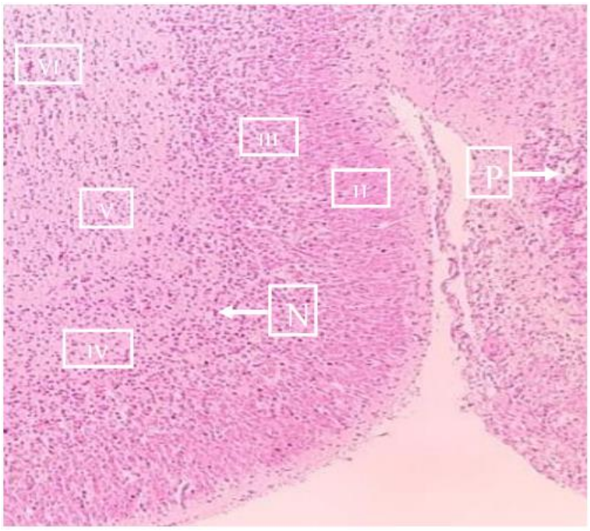

Plate 5: Photomicrograph of the brain tissue (cerebrum) from Group F showing normal cerebral histology with intact cell layers (I – VI), abundant Pyramidal Cells (P), & Neurocytes (N) (H&E x40)

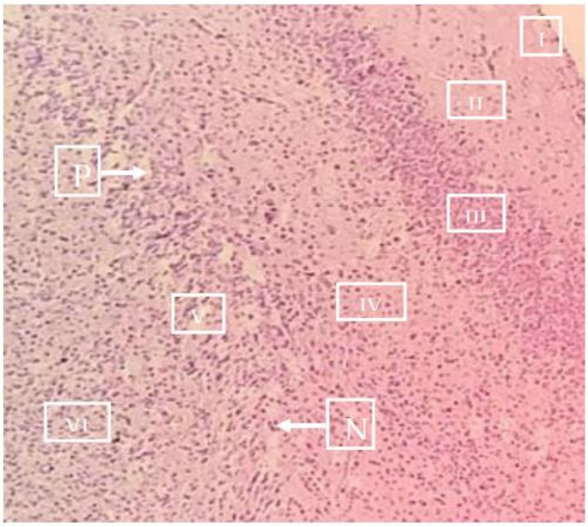

Plate 6: Photomicrograph of the brain tissue (cerebrum) from Group G showing normal cerebral histology with intact cell layers (I – VI), abundant Pyramidal Cells (P), & Neurocytes (N) (H&E x40)

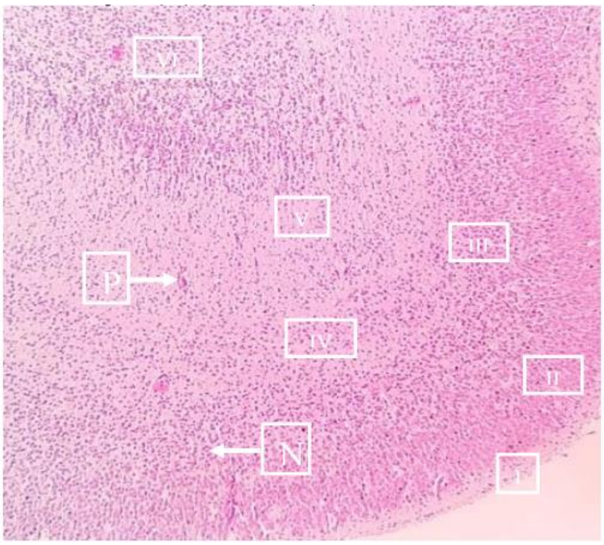

Plate 7: Photomicrograph of the brain tissue (cerebrum) from Group H showing normal cerebral histology with intact cell layers (I – VI), abundant Pyramidal Cells (P), & Neurocytes (N) (H&E x40)

4. Discussion

The spectrometric and elemental analyses of mango fruit samples have provided valuable insights into the presence of heavy metals and their concentrations across different ripening methods, with significant findings regarding mercury (Hg) and arsenic (As) levels, as well as various metallic elements such as iron (Fe), copper (Cu), chromium (Cr), and cadmium (Cd). These findings highlight the potential risks associated with artificially ripened fruits, particularly those treated with calcium carbide, and the possible implications for both consumer health and food safety.

The spectrometric analysis revealed a notable variation in mercury and arsenic concentrations, with the highest levels detected in the Market Calcium Carbide Ripened (MCR) mango samples. The MCR samples exhibited a mercury concentration significantly higher than the Naturally Ripened (NR) samples, which had a much lower concentration. Similarly, arsenic levels in the MCR samples were substantially elevated compared to the NR sample, which showed the lowest arsenic concentration. The Laboratory Calcium Carbide Ripened (LCR) samples also exhibited moderate levels of both mercury and arsenic, though not as elevated as the MCR samples.

The higher concentrations of mercury and arsenic in the MCR samples are consistent with previous studies that have raised concerns about the contamination of food products with heavy metals due to the use of calcium carbide in fruit ripening. Several studies have highlighted that calcium carbide, often used in the artificial ripening of fruits, may contain trace amounts of hazardous substances such as mercury and arsenic. For instance, research by Khan *et al*. (2022) found that the use of calcium carbide in the ripening of fruits, particularly mangoes, can lead to the accumulation of toxic heavy metals, thereby posing health risks to consumers. Similarly, studies by Sharma *et al*. (2023) confirmed the presence of significant levels of mercury and arsenic in artificially ripened fruits, emphasizing the potential for bioaccumulation and its harmful effects on human health.

The elemental analysis revealed a striking increase in certain metallic elements, such as iron, manganese, and cadmium, in the MCR mango samples. The iron concentration in the MCR samples was markedly higher when compared to the NR samples. Likewise, the manganese concentration in the MCR samples was notably elevated, suggesting that calcium carbide ripening may alter the nutritional composition of mangoes, potentially increasing the levels of metals that could be harmful if consumed in excess. This finding is corroborated by studies such as those by Gupta *et al*. (2021), which reported an increase in heavy metal concentrations, including cadmium and manganese, in fruits ripened with artificial methods.

The presence of cadmium in the MCR mangoes is particularly concerning, as cadmium is a well-known toxic metal that can accumulate in the human body, leading to adverse health effects such as kidney damage and bone demineralization. Cadmium contamination in food products has been the subject of multiple studies. According to a study by Bhatti *et al*. (2024), the use of artificial ripening methods such as calcium carbide significantly contributes to elevated cadmium levels in fruits, raising concerns about its potential long-term effects on human health.

Furthermore, the laboratory-calcium carbide-ripened mango samples (LCR 10g and LCR 30g) showed moderate levels of iron and manganese. These findings are in line with research by Singh *et al*. (2022), which found that while laboratory ripening methods may not produce metal concentrations as high as those observed in market-ripened fruits, they still pose a potential risk of heavy metal exposure.

The elevated concentrations of mercury, arsenic, and other metallic elements in artificially ripened mangoes highlight the potential risks to consumer health, particularly for vulnerable populations such as pregnant women and young children. Long-term exposure to mercury and arsenic can lead to serious health conditions, including developmental delays, neurological damage, and an increased risk of cancers.

The results of this study are consistent with several recent researches that have explored the toxicological effects of calcium carbide on food safety. Khan *et al*. (2022) documented similar findings, where mangoes artificially ripened with calcium carbide exhibited higher concentrations of mercury and arsenic, raising concerns about their safety for consumption. Similarly, a study by Sharma *et al*. (2023) confirmed that calcium carbide-treated fruits contained significantly elevated levels of toxic metals compared to naturally ripened fruits, underscoring the need for stricter regulations regarding its use in the food industry.

Bhatti *et al*. (2024) further reinforced these concerns by examining the cadmium and manganese levels in artificially ripened fruits and their potential health impacts. Their findings align with the present study's observation that calcium carbide ripening can lead to increased concentrations of cadmium, which is known to have detrimental effects on kidney function and bone health.

The gestational weight gain pattern observed in the Wistar rat model across the different treatment groups highlights variations in weight dynamics related to the type of mango fruit administered. The positive control group, which did not receive mango fruit treatment, exhibited the highest weight gain by the second week of gestation. This could reflect the inherent physiological changes during gestation in a healthy, untreated population, where natural weight gain is expected during pregnancy. The subsequent decline in weight during the third week could be attributed to natural hormonal shifts or changes in food intake, as gestation nears its later stages.

For the groups administered naturally ripened mango fruits, the 5mL dose exhibited the highest weight gain in the second week, suggesting an initial favorable impact of the fruit on the metabolic and nutritional status of the pregnant rats. This aligns with findings from previous studies suggesting that fruit consumption can positively influence weight gain during pregnancy due to the presence of beneficial nutrients, such as vitamins and minerals (Sarker *et al*., 2021). However, the 10mL dose exhibited peak weight gain in the first week, followed by a gradual decline, which may be attributed to the metabolic adjustments required to process higher doses of the fruit. These findings suggest a potential dose-dependent effect of mango fruit on gestational weight, with lower doses favoring gradual weight accumulation.

The rats exposed to artificially ripened mango fruits, particularly those treated with 5mL of market calcium carbide-ripened mango fruit, demonstrated the highest weight gain in the third week, indicating a delayed but significant impact. The lower dose of 10mL in carbide-ripened mango fruit resulted in peak weight gain in the second week, followed by a marked decline. This suggests that artificial ripening may have a delayed but potent influence on weight gain, possibly due to the chemical changes induced by calcium carbide in the fruit, which might alter the nutritional bioavailability of the fruit or induce a stress response in the rats (Mukherjee *et al*., 2022).

Interestingly, laboratory-ripened mango fruit groups exhibited delayed or inconsistent weight gains. The 10g dose in particular did not result in a significant weight increase in the first week, with the highest weight gain observed in the third week. This suggests that the laboratory-ripened mango fruit may have different compositional qualities compared to naturally ripened mangoes, potentially influencing metabolic processes differently (Nair *et al*., 2023). The 30g dose of laboratory-ripened mango fruit did not result in significant weight gain in the first week, and weight gain remained highest in the third week, indicating a possibly slower metabolic response or reduced nutritional uptake.

These observations underline the significant variability in gestational weight gain influenced by the ripening method and dosage of mango fruit, with natural ripening demonstrating a more consistent and gradual weight increase compared to the artificial ripening methods.

Fetal growth and placental weight are essential markers of prenatal development, and in this study, the analysis of these parameters further highlights the differential effects of mango fruit ripening methods. The group administered 10mL of naturally ripened mango fruit exhibited the highest fetal weight gain, which is consistent with the known nutritional benefits of naturally ripened fruits, including higher levels of antioxidants, vitamins, and minerals that support fetal growth (Park *et al*., 2021).

Conversely, the group receiving 5mL of 10g laboratory carbide-ripened mango fruit demonstrated the lowest fetal weight, which may be attributed to the presence of harmful chemical residues such as calcium carbide, a known carcinogen and growth inhibitor in animal models (Ghosh *et al*., 2022). The absence of measurable fetal growth parameters in the groups administered with 10mL of market carbide-ripened mango fruit and 10mL of 30g laboratory carbide-ripened mango fruit further confirms the adverse effects of carbide-ripened mango fruits on fetal development. Calcium carbide, which is often used to artificially ripen mangoes, has been shown to disrupt normal metabolic processes and impair fetal development, possibly due to the presence of toxic residues (Rahman *et al*., 2020).

Placental weight analysis revealed the highest placental weight in the 10mL naturally ripened mango fruit group and the 5mL market carbide-ripened mango fruit group. This suggests that naturally ripened mangoes may offer better nutritional support for both fetal growth and placental development compared to artificially ripened fruits, which could hinder normal placental function. Similar studies have reported that the placenta serves as a crucial organ for nutrient transfer during pregnancy, and any disturbance in placental development may impair fetal growth (Yin *et al*., 2022).

Moreover, fetal morphometric parameters such as crown-rump length, foot length, crown-heel length, and head circumference revealed marked differences, with the highest values observed in the 10mL naturally ripened mango fruit group. These results emphasize the positive impact of natural mango ripening on fetal development. Conversely, the lowest values were recorded in groups receiving artificially ripened mango fruit, particularly the 5mL 10g laboratory carbide-ripened mango fruit group, which supports the hypothesis that chemical residues from carbide ripening may be detrimental to fetal growth (Sahu *et al*., 2023).

Total and Partial resorption sites were seen in 30gLCR 10mMCR 10ml respectively indicating disrupted pregnancies or abortion in the present study. Endocrine disrupting compounds (EDCs) have been described as key heavy metals that exhibit endocrine disrupting properties and they include arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) (Georgescu *et al*., 2011). There are adverse birth outcomes observed when there is exposure to these heavy metals during pregnancy both on the mother and fetus (Rahman *et al.*, 2016). Studies have shown association between these heavy metals and spontaneous abortion, still birth and neonatal death (Rahman et al 2016; Amadi *et al.*, 2017). Pregnancy outcomes of these groups treated with higher doses of calcium carbide ripened mango fruits therefore corroborate with previous studies that exposure to heavy metals could lead to abortions.

The histological examination of fetal brain tissue (cerebrum) did not reveal significant differences across the experimental groups, with all groups displaying essentially normal cerebral histology. This finding suggests that, despite the observed differences in growth indices and placental weight, the various ripening methods did not cause overt histopathological damage to the fetal brain tissue in this study. This could indicate that while mango fruit ripening methods might influence overall growth parameters, they may not necessarily cause severe structural damage to the brain tissue in the early stages of gestation. Similar findings have been reported in studies examining the effects of dietary exposures on fetal brain histology, where subtle biochemical changes may occur without clear histological alterations (Xu *et al*., 2023).

5. Conclusion

This study highlights the potential hazards of consuming artificially ripened mangoes treated with calcium carbide, particularly regarding the elevated concentrations of toxic heavy metals such as mercury, arsenic, and cadmium. The gestational and fetal outcomes in the Wistar rat model revealed that calcium carbide ripened mangoes adversely affect maternal weight gain, fetal growth, and placental development, with natural ripening methods showing more favorable results. The findings underscore the need for stricter regulation of artificial ripening methods to safeguard public health, especially for pregnant women and developing fetuses.

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