

Assessment of the Biochemical Parameters of the Second Filial Generation Pups From the Wistar Rats Fed with Calcium Carbide Ripened Orange

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Abstract

Fruits are useful food substances that provide unique and essential nutrients for the body. This present study is aimed at evaluation the toxic effect on the Biochemical indices of the second filial generation pup from the Wistar rats fed with Calcium Carbide forced ripened orange fruits. Mature unripe oranges were plucked off from the orange plant in Ogu, Yenagoa, Bayelsa state. They were forced to ripe with 10g calcium carbide which was placed in a bowl containing 5ml of water apparently used to dissolved it in a closed metal bucket containing 1kg of the matured orange rapped with black nylon and was kept two days [48 hours] to ripe. After ripening, sampled fruits were washed and juiced. 600g of calcium carbide forced ripened orange was peeled and blended in an electric blender with 350ml/L of distilled water to form the orange juice. The orange juice was filtered with a clean fine sieve and was poured into clean bottles labeled [CaC₂ forced ripened orange juice] which was then stored in a refrigerator for further usage. 21 adult Wistar rats [10 males and 11 females] weighing between 126.9- 213.3g were used for this study. The experimental Wistar rats were cohort into two and was allowed to acclimatize for two weeks (fed with grower mash with clean water) at libitum then, different dosage of the fruit juice were administered orally based on their body weight. Group 1: Normal control group of 6 rats [3 males and 3 females] receive normal water and feeds only as placebo. Group 2: Treatment Group [2] of 6 rats [3 males and 3 females] received Calcium Carbide ripened orange juice. The treatment lasted for four weeks. The adult Wistar rats were allowed to copulate freely during and after the acclimatization and treatment period. Wistar rats birthed and the Pups of the different groups were collected according to the treatment protocols. The parent Wistar rats was still kept in their distinctive cages without treatment but was allowed free access to feed on the growers mash, clean water and copulation at libitum. They birthed again for the second time [Second Filial Generation] and the Pups were weighed at birth, at one week and two weeks, then they were sacrificed at the end of the second week and blood samples were collected from the two distinctive cohorts for Biochemical assay. Biochemical assay was done and results indicates reduction all the tested indices; AST, ALT, ALP, Creatinine, Urea, Albumin, Tottal protein, Total Cholesterol, Bilirubin, Lactate Dehydrogenase[LDH] in the Second Filial Generation Pups of the Calcium Carbide treated Wistar rat. Nutrients from the fruit induced with Calcium Carbide consumed during pregnancy permanently impact on the developing fetus of the Wistar rats which is expressed later in life.

Keywords: Calcium Carbide, Wistar rats, Second Filial Generation.

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INTRODUCTION

Artificial ripening agents are used to hasten up the process of ripening of fruits after they are picked before full ripening. This is done for faster and more uniform ripening. Generally, 80% of fruits are ripened artificially through these agents (Dhembare, 2013a; 2013b). Study seeks to investigate the possible effect of the consumption of fruits ripened with calcium carbide on some haematological and serum biochemical parameters of wistar rats. Three fruits (banana, apple

and oranges) were ripened using calcium carbide. These were fed to the animals orally and by mixing with their feed for six weeks. Haematological and serum biochemical analyses were carried out on whole blood and plasma respectively. The haematology result shows that there was a very high statistically significant difference ($p < 0.001$) in the total white blood cell count (WBC) of the rats mostly in group C (orange peel), H (orange juice) and F (banana juice); when compared with the control. Significant difference ($p < 0.05$) was

also observed in the lymphocyte (LY) and granulocyte (GR) counts. There was a general transient elevation in the levels of creatinine, urea, total bilirubin, direct bilirubin, AST, ALT, Na⁺, K⁺, HCO₃⁻ and Cl⁻ and summarized that, the Consumption of fruits or skin of fruit ripened with carbide may cause some inflammatory effect, trigger some allergic reactions and have an adverse effect on the kidneys. (Igbinaduwa *et al*, 2016). A study examined the possible effect of selected fruits ripened with calcium carbide on some hormonal parameters, oxidative stress enzymes, haematology indices, histopathology of male and female Wistar rats and semen analysis of male rats. Three fruits (pawpaw, mango and plantain) were ripened using calcium carbide. Haematology results showed that there was a significant increase in total white blood cell count of female rats fed with artificially ripened mango while platelet count was significantly increased in rats fed with artificially ripened plantain. Degeneration of germ cells was observed in the testes of male rats fed with artificially ripened mango. This study suggests that consumption of fruits ripened with calcium carbide could lower the body's potential to resist infection by weakening the immune system, affect hormonal balance which could lead to infertility (Essien *et al*, 2018). It has been known for a long time that treatment of unripe fruits with ethylene would merely stimulate natural ripening until the fruit itself starts producing ethylene in large quantity in the fruit, (Siddiqui *et al*, 2010). It has been reported that though the cosmetic quality of artificially ripened fruits like the external color and the texture increases, organoleptic qualities, nutritional value and shelf life is reduced (Siddiqui *et al*, 2010); (Hakim *et al*, 2012). The results of (Gbakon *et al*, 2018) showed that calcium carbide ripened mangoes caused significant decrease in Red Blood Cell (RBC) count, Haemoglobin, Packed Cell Volume (PCV) and increased the White Blood Cells (WBC) and Lymphocytes. The data obtained showed the depletion in creatinine, protein, cholesterol, potassium and bicarbonate while albumin and bilirubin significantly increased. There was no remarkable change in the levels of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) during the period of the study. Findings from this study suggest that consumption of calcium carbide ripened mangoes is causing changes in the haematological and plasma biochemical profiles of consumers; hence that may increase erythrocytes destruction, have suppressive effect on the three major cell lines and interfere with some minerals and vitamins as well as lipid metabolism. This may consequently manifest in series of health hazards, lowering the body's ability to resist infection and weakening the whole immunity system when consumed. The key factors that influence the practice of artificial ripening include high demand of seasonal fruit and possible economic loss during fruit storage and distribution. The common practice currently is to harvest mangoes in bulk in a single picking, which

includes fruits of different stages of maturity thus contributing to 40-60% postharvest losses (Dhembare, 2013a; 2013b). Calcium carbide which is most commonly used because of its cheapness and availability liberates acetylene gas when it reacts with water. This gas initiates ripening in the mango fruits (Dhembare, 2013a; 2013b). There are increasing reports of food poisoning due to the use of chemical ripening agents such as calcium carbide. This ripening agent is alkaline in nature and irritates the mucosal tissue in the abdominal region. Cases of diarrhea, vomiting, abdominal pain and headache, after eating carbide ripened mangoes have been reported recently (Siddiqui *et al*, 2010). To evaluate the effects of Calcium carbide (CaC₂) in biological system, an *in vivo* study was carried out on Long Evans rats. CaC₂ was administered orally once daily for one month with specific. Histopathological analysis of liver, heart, spleen, kidney and lungs were performed to observe any change due to the administration of CaC₂. Remarkable changes were observed during the histopathological study of lungs and kidney only. The histopathological analysis of kidney showed the thickening of the lining of collecting tubules with changes in cell structure while lungs were found to be increased moderately in weight, with focal areas of consolidation that was found red-brown to red (Younus *et al*, 2007). (Ogoun *et al*, 2022) study revealed nephrotoxic and hepatobiliary dysfunction Cholestatic alterations effect of poly-herbal formulations thereby hindering renal and hepatic functions. CaC₂ and phosphine exposure were related to pulmonary symptoms (Noor *et al*, 2018). Thus, the need to evaluate the toxic effect of this ripening agent (Calcium carbide) on the offspring became necessary.

MATERIALS /METHODS

Experimental Design

This experimental study was designed to evaluate the toxic effect on Biochemical indices of the second filial [F₂] generation from the Wistar rats fed with Calcium Carbide induced ripened orange juice.

Fruit and Calcium Carbide Collection

Mature unripe oranges were plucked off from the orange plant in Ogu, Yenagoa, Bayelsa state. The mature oranges were forced to ripe with calcium carbide. 10gram of Calcium carbide which was bought at Swali market, Yenagoa, Bayelsa state, was placed in a bowl containing 5ml of water which apparently was used to dissolve it in a closed metal bucket containing 1kg of the matured orange rapped with black nylon and was kept two days (48 hours) to ripe. After ripening, sampled fruits were washed and juiced.

Preparation of Sample

600g of calcium carbide forced ripened orange was peeled, blended in an electric blender with 350ml/1L of distilled water to form the orange juice. The orange juice was filtered with a clean fine sieve and

was poured into clean bottles labeled (CaC₂ forced ripened orange juice) which was then stored in a refrigerator for further usage.

Experimental Wistar Rats

Due to the experimental design of this study, 21 adult Wistar rats [10 males and 11 females] weighing between 126.9- 213.3g were used for this study. The experimental Wistar rats were cohort into two and was allowed to acclimatize for two weeks (fed with grower mash with clean water) at libitum then, different dosage of the fruit juice were administered orally based on their body weight. They were kept in standard environmental condition in the animal house of the Bayelsa Medical University; following the guidance of National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Administration of Samples

A sub-acute test [LD₅₀] was done [with 9 Wistar rats] to assess the lethal dose, using Lorke (1983) formula for administration of samples.

Group 1: Normal control group of 6 rats [3 males and 3 females] receive normal water and feeds only as placebo.

Group 2: Treatment Group [2] of 6 rats [3 males and 3 females] received Calcium Carbide ripened orange juice. The treatment lasted for four weeks.

Wistar Rats Birthing

The adult Wistar rats were allowed to copulate freely during and after the acclimatization and treatment period. Wistar rats birthed and the Pups of the different groups were collected according to the treatment protocols. The parent Wistar rats were still kept in their distinctive cages without treatment but were allowed free access to feed on the growers mash, clean water and copulation at libitum. They birthed again for the second time (2nd Filial Generation).

The Pups were weighed at birth, at one week and two weeks, then they were sacrificed at the end of the second week and blood samples were collected from the two distinctive cohorts for Biochemical assay.

Analysis of Data

The data collected from the Biochemical assay was analyzed as Mean \pm Standard Error of Mean [SEM]. Significant difference among the groups was determined as P<0.05; by One-way ANOVA; using Statistical Analysis Program for Social Sciences [SPSS 22.0 Version].

RESULTS

Biochemical assay was done on the following Liver parameters

1. Aspartate Aminotransferase [AST]
2. Alanine Transaminase [ALT]
3. Alkaline Phosphatase [ALP]
4. Total Bilirubin
5. Albumin

The kidney parameters are

1. Creatinine
2. Urea

Lipid profile

1. Total Protein
2. Total Cholesterol

Heart assessment parameter was

1. Lactate Dehydrogenase

The results gotten from the data analysis are presented on the tables below with different mean body weight of the parent Wistar rats and the Second filial generation pups. Also the different Biochemical Indices [markers] showing the various mean values are shown.

Table 1: Mean Weight of Parent Wistar Rat [Grams]

GROUP	CONTROL	NATURAL FRUITS	CaC ₂ RIPENED FRUITS
MEAN VALUE	214.30 \pm 10.53	184.53 \pm 19.53	174.28 \pm 17.35

Mean \pm SEM

Table 2: Mean Body Weight of the Second [2nd] Filial Generation [Grams]

S/N	GROUP	BIRTH	WEEK1	WEEK 2
1	CONTROL	2.10 \pm 0.18	11.40 \pm 0.50	15.15 \pm 0.45
2	TREATMEN WITH CALCIUM CARBIDE [CaC ₂]	2.08 \pm 0.31	12.81 \pm 0.93	22.83 \pm 4.93

Mean \pm SEM

Table 3: Biochemical Parameters of the V second Filial Generation

BIOCHEMICAL PARAMETERS	CONTROL	CaC ₂ RIPENED FRUIT
AST[u/l]	52.43 \pm 0.30 ^A	46.10 \pm 0.10 ^B
ALT[u/l]	25.53 \pm 0.29 ^C	20.20 \pm 0.20 ^D
ALP[u/l]	70.40 \pm 0.31 ^D	51.70 \pm 0.10 ^Z
CREATININE[mg/dl]	0.49 \pm 0.01 ^R	0.45 \pm 0.01 ^F
UREA[mg/dl]	15.59 \pm 0.30 ^M	12.10 \pm 0.10 ^Q

BIOCHEMICAL PARAMETERS	CONTROL	CaC ₂ RIPENED FRUIT
TOTAL BILURIBIN[mg/dl]	0.32±0.01 ^Q	0.31±0.01 ^Q
ALBUMIN[g/dl]	5.50±0.25 ^X	4.24±0.04 ^S
TOTAL PROTEIN[g/dl]	6.61±0.01 ^G	5.61±0.01 ^H
TOTAL CHOLESTEROL[mg/dl]	58.50±0.12 ^K	53.50±0.30 ^M
LACTATE DEHYDROGENASE[u/l]	146.33±0.24 ^C	132.30±0.10 ^A

Keys:

1. All values are in Mean ±SEM
2. The Means with Different superscript alphabets in the same row indicates significant difference at 95% confidence level (p<0.05).

DISCUSSION

The use of toxic chemicals such as Calcium carbide for fruit ripening has raise serious concerns in the world today. The evidence of fetal programming and genetic transfer is still obscure. The findings from these present results have shown that there is statistically significant reduction in the AST, ALT and ALP of the second filial generation pups of the CaC₂ treated Wistar rats. According to Staff Writer, 2020, a low AST, ALT result is not an indicator of poor health. A low level means that your liver is functioning well and normally. It is only high results (above 25 IU/L in females and 33 IU/L in males) that would concern a physician. ALP is found in the liver, bile duct, bones, kidneys, intestines, placenta in pregnant people.

The present results showed reduction in the level of Creatinine and urea in the second filial generation pups from the CaC₂ treated Wistar rats (p<0.05). This reduction could be as a result of muscular dystrophy, liver disease and excess loss of water which is in line with written by Corey, 2020. Reduced Urea and Creatinine level could arise from low protein intake as seen in malnutrition and starvation, advanced liver disease, when the liver can't produce enough urea and in Sickle cell anemia were the kidneys reabsorb less urea and more of it is lost in the urine (Puya, 2021).

There is also significant reduction in the in the mean serum level of Albumin in the second filial generation pups from the CaC₂ treated Wistar rats (p<0.05). This point towards liver cirrhosis since Albumin is manufactured by the hepatocytys of the liver and that reduction depicts liver anomaly. In the same vein, there is reduction of the Total Protein in the second filial generation pups from the CaC₂ treated Wistar rats (p<0.05). This is also an indication of liver mass interference by the CaC₂ which may likely lead to hypertrophy of the liver. One of the major organs of the human body is the liver which performs basic functions such as protein and other biochemical synthesis, detoxification, glycogen storage, production of hormones, hence all these function are likely to be impaired which is an index of problem. This is in line with the narrative by Mayo Clinic, 2022; that Albumin

is one of several proteins made in the liver. Your body needs these proteins to fight infections and to perform other functions. Lower-than-normal levels of albumin and total protein may indicate liver damage or disease.

The result of the present study has shown significantly low level of Cholesterol in the second filial generation pups from the CaC₂ treated Wistar rats (p<0.05) as compared to the normal control group. Low cholesterol is tantamount to cause cancer, depression, anxiety, preterm birth stroke. Low cholesterol is associated with coronary heart disease [Behar *et al*, 1997].

In this result, there is significant reduction of Lactate dehydrogenase in the second filial generation pups from the CaC₂ treated Wistar rats (p<0.05) as compared to the normal control. Lactate dehydrogenase, an enzyme that aids in the conversion of sugar to energy, in the bloodstream. Low levels can be caused by genetic mutations were people experience fatigue and muscle pain during exercise or a large intake of vitamin C.

CONCLUSION

From this study, it is evident that, the nutrients from the fruit induced with Calcium Carbide consumed during pregnancy permanently impact on the developing fetus of the Wistar rats which is expressed later in life. The quality and quantity of the nutrients consumed during pregnancy permanently impact the developing fetus [Nutritional or Metabolic Programming]. According to the "Developmental Origins of Health and Disease" paradigm, the conditions during peri- conception (2–3 months before getting pregnant), pregnancy, and lactation strongly influence the developing child. There is substantial evidence showing that an unfavorable environment during these periods creates a predisposition to certain diseases, mostly inflammatory, that manifest later in adult life (Tarrade, 2015).

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