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Assessment of Calcium Carbide and Natural Ripened Pawpaw (*Carica Papaya*) Fruits on Biochemical Parameters of Wistar Rats

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ABSTRACT

This research was aimed at assessing calcium carbide and natural ripened pawpaw (*Carica papaya*) fruit on the biochemical parameters of the Wistar rats. Twenty four (24) adult Wistar rats weighing between 126.9- 213.3 g was used for this study. The experimental Wistar rats were grouped into three and were allowed to acclimatize for two weeks at libitum. Five (5) ml/kg of natural and calcium carbide ripened pawpaw fruit juice were administered orally. At the end of the four weeks feeding period, the rats were sacrificed through cervical dislocation. Blood was collected by cardiac puncture, using 5ml syringes and 23G needles into blood sample containers for biochemical analysis using the standard biochemical methods. The renal, hepatic, cardiac, heart and lipid profile parameters analyzed were albumin, total protein, urea, creatinine, Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), total bilirubin, Aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and total cholesterol and were compared with the naturally ripened pawpaw fruit group. All mean values of total protein, total cholesterol, lactate dehydrogenase and creatinine levels of calcium carbide ripened pawpaw fruit juice fed group were significantly higher when compared with the natural ripened pawpaw fruit juice. Meanwhile, albumin, total bilirubin, urea, ALT, AST and ALP levels of calcium carbide ripened fruit juice fed group were lower when compared with the natural ripened pawpaw fruit juice. Statistically, there were no significant differences of albumin and total protein parameters at 95% confidence level ($P < 0.05$). In conclusion, the elevated levels of creatinine, total cholesterol and lactate dehydrogenase may result to kidney injury, cardiovascular and heart diseases. There is therefore need for institutional and legislative strengthening as well as enforcement to prevent the use of calcium carbide in the ripening of pawpaw and other fruits.

KEYWORDS: Calcium carbide, pawpaw (*Carica papaya*), Biochemical parameters, wistar rats, toxicity.

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INTRODUCTION

Fruit ripening process involves the production and decomposition of chlorophyll which reduces starch to glucose by amylase enzyme. Pectin is converted by pectinase enzyme and decomposition of pectin, in this case, unglues the fruit cells. The cells being able to slip past one another makes the fruit further soft. 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 [1].

Generally, 80% of fruits are ripened artificially through chemical agents [2, 3]. They are different commonly used artificial ripeners in the world including ethylene glycol, ethephon, acetylene gas and calcium carbide. Ethylene is a natural plant growth regulator, it is a gaseous hormone naturally produced in fruit. Ethylene being a natural hormone does not pose any health hazard for consumers of the fruits. It is a de-greening agent, which can turn the peel from green to perfect yellow and maintain the sweetness and aroma of the fruit, thus value addition in the fruit is possible as it looks more appealing. 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 [4].

Out of all the ripening agents, calcium carbide is commonly used, because it is inexpensive and easily purchased. The use

of this chemical for this purpose is illegal in most countries [4], but it is freely used in Nigeria and other Asian countries. The need for artificial fruit ripening is often encountered when fruit sellers offer fruits to the customers before the due season to meet high demand and make a high profit. Thus humans are at risk of short term and long term health effects simply by eating fruits that are induced to ripen. The use of calcium carbide ripening agent is of great concern as the activities of human beings have been said to contribute to exposure of food materials to heavy metal contamination [5]. Papaya (*Carica papaya* Linn.) is one of the highly metabolically active fruit that undergo significant changes during ripening. Papaya is also known as pawpaw that belongs to *Caricaceae* family [6]. The production of papaya (broadly cultivated fruit) is ranked the third after mango and pineapple around the world. India is placed at the first place in papaya production followed by Brazil [7]. *Carica papaya* contains the enzyme papain, which is present in the fruits, stem and leaves [8]. Meat can be tenderized by wrapping it in a papaya leaf before cooking. It contains biologically active compounds such as chymopain and papain which aids in digestion [9,10]. Apart from nutritive benefits, papaya has a wide range of applications in therapeutics to improve the human health by reducing the risk of occurrence of various diseases [11]. The alarming usage of various ripening practices/agents and their harmful effects on the fruit quality necessity an urgent need to understand the metabolic perturbations in papaya and their consequences on nutritional quality of the fruit.

Emerging literatures has shown that the use of artificial ripening agents in fruit ripening causes abdominal pain, diarrhea, vomiting, headache, dizziness and insomnia which may progress to memory loss and cerebral edema [3]. The upsurge of demand for food safety has motivated researchers into investigating possible risk common with the use of artificial methods in fruit ripening [12]. Owing to the importance of fruits to the health of man, this study was aimed at assessing calcium carbide ripened pawpaw (*carica papaya*) fruits on the biochemical parameters of the wistar rats.

MATERIALS AND METHODS

Experimental Design

This is an experimental study designed to investigate the Biochemical parameters of the First Filial Generation from the Wistar rats fed with naturally ripened and Calcium Carbide induced ripened pawpaw (*carica papaya*) fruits.

Fruit and Calcium Carbide Collection

Mature unripe pawpaw (*carica papaya*) fruits were plucked off from the pawpaw (*carica papaya*) plant in Yenagoa, Bayelsa State. The fruits were divide into two groups, one group was kept and allowed to rip at normal room temperature and the second category was induced to ripe with calcium carbide at the Histology Laboratory, Bayelsa Medical University, Yenagoa, Bayelsa State. Calcium carbide was bought at Swali Market, Yenagoa, Bayelsa State. 10gram of Calcium carbide was placed in a bowl and 5ml of water was used to dissolved it in a closed metal bucket containing 1kg of the fruit rapped with black nylon and was allowed for two days (48 hours) for ripening. After ripening, sampled fruits were washed and juiced.

Preparation of sample

In this study, 600g of both the naturally ripened and calcium carbide ripened pawpaw (*carica papaya*) fruits were peeled separately and blended in an electric blender with 350 ml/1L of distilled water. The juice was filtered with a clean fine sieve and was poured into clean bottles and labeled (CaC_2 ripened pawpaw fruits juice and naturally ripened pawpaw juice) then, stored in a refrigerator for further usage.

Experimental Wistar Rats

Twenty four (24) adult Wistar rats (12 male and female of each sex) weighing between 126.9- 213.3 g was used for this study. The experimental Wistar rats were grouped into three 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 Research Council, Guide for the Care and Use of Laboratory Animals, 2011 [13].

Administration of samples

LD_{50} was done using Lorke (1983) [14] Method for administration of samples.

Group 1: Normal control group of 8 rats (4 males and 4 females) received normal water and feeds only as placebo.

Group 2: Treatment Group [1] of 8 rats (4 males and 4 females) received 5 ml/kg naturally ripped pawpaw juice

Group 3: Treatment Group [2] of 8 rats (4 males and 4 females) received 5 ml/kg Calcium Carbide ripened pawpaw juice. The treatment lasted for four weeks.

Blood sample collection

The animals were observed in their cages for clinical symptoms daily and at the end of the four weeks treatment, the rats were sacrificed under chloroform anesthesia and blood was collected by cardiac puncture, using 5 ml syringes and 23G needles into blood sample containers. The blood was allowed to stand for 2 hours to coagulate and was centrifuged for 10 minutes at 2000 rpm and the supernatant (Serum) carefully collected for biochemical analysis.

Biochemical analysis

Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined following the principle described by [15] while the alkaline phosphates (ALP) were carried out according to the method described by [16] to assess liver function. Renal function was assessed by

measuring plasma creatinine (CREA) levels and blood urea was assayed following the method of [17, 18].

In order to assess the synthetic function of the liver, total protein (TP), Total bilirubin and albumin (ALB) concentrations were determined according to the principles based on the Biuret reaction [19, 20] and bromocresol green reaction [21] respectively. Total cholesterol (TC) and Lactate dehydrogenase concentrations were estimated following the method described by [22].

Analysis of Data

Data collected from this study was analyzed as Mean ± Standard Error of Mean (SEM). Significant difference among the groups were determined as $P<0.05$ by two-way ANOVA, using Statistical Analysis Program for Social Sciences (SPSS 22.0 Version).

RESULTS

Biochemical parameters

The Body Weight of wistar rats fed with natural and calcium carbide ripened pawpaw fruit juice is shown in Table 1. Table 2 shows the effects of natural and calcium carbide ripened pawpaw fruit juice on biochemical parameters of wistar rats at 5 ml/kg doses. The renal, hepatic, cardiac, heart and lipid profile indices analyzed were albumin, total protein, urea, creatinine, Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), total bilirubin, Aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and total cholesterol and were compared with the naturally ripened pawpaw fruit group. All mean values of total protein, total cholesterol, lactate dehydrogenase and creatinine levels of calcium carbide ripened pawpaw fruit juice fed group were significantly higher when compared with the natural ripened pawpaw fruit juice. Meanwhile, albumin, total bilirubin, urea, ALT, AST and ALP levels of calcium carbide ripened fruit juice fed group were lower when compared with the natural ripened pawpaw fruit juice. Statistically, there were no significant differences of albumin and total protein parameters at 95% confidence level ($P < 0.05$). However, the remaining biochemical parameters were statistically significant at 95% confidence level.

Table 1. Body weight of Wistar Rat in grams

Group	Control	Natural ripened pawpaw fruit juice	CaC_2 ripened pawpaw fruit juice
Mean ±SEM	214.30±10.53	184.53±19.53	174.28±17.35

Table 2. Effects of Natural and CaC_2 ripened pawpaw fruits on Biochemical parameters of Wistar rats

Biochemical parameters	Control	Natural ripened pawpaw fruit juice	CaC_2 ripened pawpaw fruit juice
AST (u/l)	50.73 ± 2.08^{A}	67.50 ± 0.70^{B}	64.90 ± 0.30^{C}
ALT (u/l)	31.10 ± 0.10^{F}	54.70 ± 3.50^{G}	49.50 ± 1.10^{H}
ALP (u/l)	70.60 ± 2.00^{I}	88.70 ± 0.20^{J}	84.05 ± 1.55^{K}
Creatinine (mg/dl)	0.62 ± 0.02^{M}	0.75 ± 0.01^{N}	0.77 ± 0.04^{O}
Urea (mg/dl)	15.50 ± 0.30^{P}	24.00 ± 0.20^{Q}	23.85 ± 0.95^{R}
Total bilirubin (mg/dl)	0.34 ± 0.02^{S}	0.61 ± 0.03^{T}	0.55 ± 0.05^{U}
Albumin (g/dl)	4.50 ± 0.10^{B}	5.05 ± 0.25^{B}	4.40 ± 0.10^{B}
Total Protein (g/dl)	8.50 ± 0.10^{A}	8.40 ± 0.20^{A}	8.95 ± 0.15^{A}
Total Cholesterol (mg/dl)	73.00 ± 3.40^{W}	93.95 ± 2.95^{X}	94.20 ± 1.00^{Y}
Lactate Dehydrogenase (u/l)	151.00 ± 2.40^{X}	191.00 ± 1.60^{Y}	195.00 ± 1.25^{Z}

(MEAN±SEM), The Means with Different superscript alphabets in the same row indicates significant difference at 95% confidence level ($p<0.05$).

DISCUSSION

The health benefits of pure fruit juice cannot be overemphasized [23]. Several fruits used to process these juices have been reported to have beneficial effects on the body [24,25].Calcium carbide ripened fruits has been reported to cause health hazard [26,27]. The estimation of AST, ALT and ALP is suitable in the early diagnosis of viral or toxic hepatitis and thus people exposed to hepatotoxic chemicals [28]. Generally, perturbation of parenchymal cells of the liver by xenobiotics results in elevation of both transaminases (AST and ALT) in the blood [29, 30]. AST has both mitochondrial and cytoplasmic origin and any elevation could be taken as a first sign of cell damage that leads to the appearance of these enzymes in the serum [31].

Therefore, wistar rats were tested for ALP, ALT, AST, total bilirubin and Albumin levels to check for liver effects. Thus, the calcium carbide ripened pawpaw fruits were observed to decrease in concentration of the liver enzymes when

compared with the natural ripened pawpaw fruits but higher than the control. This implies that at the four weeks of feeding with calcium carbide ripened fruit juice does not pose any toxic effects to the liver of the wistar rats. The findings of this study were in agreement with the works of Gbakon et al. [32]. Creatinine and urea levels are used as markers of kidney function. Elevated serum levels of creatinine may indicate kidney injury, with resultant reduced glomerular filtration. While Urea is formed in the liver, representing the principal waste product of protein catabolism and is excreted by the kidney. In this study, Creatinine and urea levels of calcium carbide ripened pawpaw fruits show lower when compared with the natural ripened pawpaw fruits. The results are synonymous with the findings of Gbakon et al. [32].

In lipid profile and heart function test, the slight increase in total cholesterol and total protein levels observed in calcium carbide ripened pawpaw fruit juice in the rats may predispose the animals to arthrosclerosis and other related diseases [33]. Increase may be attributed to decrease in the metabolism of acetyl CoA arising probably from reduced beta - oxidation of fatty acid [34]. Changes in the lipid profile of animals such as cholesterol, and total protein may be employed in obtaining beneficial information on lipid metabolism and would give an insight to the cardiovascular health status of the animals [35]. Furthermore the significantly increased concentration of Lactate dehydrogenase in the calcium carbide ripened fruit juice in the rats observed in this study could be attributed to heart and other related disease. These findings were in agreements with the studies of Pauline et al. [36].

CONCLUSION

Calcium carbide hastens the ripening of mango fruits which also results in attractive surface colour. However, consumption of such mango fruits poses great health hazards. In this study, the renal, hepatic, cardiac, heart and lipid profile parameters assessed were albumin, total protein, urea, creatinine, Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), total bilirubin, Aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and total cholesterol and were compared with the naturally ripened pawpaw fruit group. All mean values of total protein, total cholesterol, lactate dehydrogenase and creatinine levels of calcium carbide ripened pawpaw fruit juice fed group were significantly higher when compared with the natural ripened pawpaw fruit juice. Meanwhile, albumin, total bilirubin, urea, ALT, AST and ALP levels of calcium carbide ripened fruit juice fed group were lower when compared with the natural ripened pawpaw fruit juice. In conclusion, the elevated levels of creatinine, total cholesterol and lactate dehydrogenase of the calcium carbide induced pawpaw fruit juice fed group may result to kidney injury, cardiovascular and heart diseases. There is therefore need for institutional and legislative strengthening as well as enforcement to prevent the use of calcium carbide in the ripening of pawpaw and other fruits.

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