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Toxic Assessment of Calcium Carbide Ripened Pawpaw on the Haematological Parameters of the Wistar Rats

Dibagha I. J.[1], Ogoun T.R.[2*]

[1]Department of Human Anatomy, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria
[2]Senior Lecturer, Head, Department of Human Anatomy, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria

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***Corresponding author:** Ogoun, Timipa Richard
Senior Lecturer, Head, Department of Human Anatomy, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria

Abstract

Fruits are sweet fleshy products of a plants, that contain various nutrients and when consumed help the human in diverse ways. Some build up muscles, some burst immune system, some are anti-oxidant and amongst other functions. Because of the high commercial demand, naturally ripe fruits are scarce, thus; alternative (artificial) ways of making them ripe for consumption has become inevitable and uncontrollable. The aim of this study is to evaluate the effect of Calcium Carbide ripened fruit (Pawpaw) on the Haematological parameters of the Wistar rats. Mature unripe pawpaw's were plucked off from the parent plant and were divide into two groups, one group was kept and allowed to rip at normal room temperature and the other group was induced with Calcium Carbide to ripe for 48 hours.10g of the CaC_2 was placed in a bowl and 5ml of water was used to dissolved it in a closed metal bucket containing 1kg of the fruit [Pawpaw] rapped with black nylon and was allowed for two days [48 hours] for ripening. After ripening, sampled fruits were washed and juiced. 600g of both the naturally ripened and calcium carbide induced ripened fruits [Pawpaw] were peeled separately and blended in an electric blender with 350ml/1L of deionized water. The two sets of juice was filtered with a clean fine sieve and was poured into clean bottles labeled [CaC2 ripened and naturally ripened pawpaw juice]. A total of 21 adult Wister rats of both sexes weighing between 126.9g- 213.3g were used for this study. 9 Wistar rats were used for Sub Acute Test (LD_{50}). The rest 12 Wistar rats were divided into three groups (Control, Natural and CaC2). 5ml/kg of the naturally ripened and calcium carbide induced ripened fruits [Pawpaw] were administered orally to the rats based on the body weight. The rats were weighed weekly, then one rat was sacrificed in each group and blood samples were collected from the three groups for hematological analysis. The result showed reduction in mean Pack Cell Volume, Total White Blood Count, Hemoglobin, Red Blood Cells, Platelets, Neutrophil, Monocytes for both the Wistar rats fed with Naturally ripened and CaC2 ripened groups in comparison with the control group. There is evidence of increase in the mean Lymphocytes level for both the Wistar rats fed with naturally ripened and CaC2 ripened groups in comparison with the control group, but the CaC2 treated group tend to be higher. Eosinophil levels are higher in the CaC2 treated group. In a nutshell, The consumption of fruits ripened with Calcium Carbide pose devastating effect on the bone marrow, deleterious effect on the circulating blood, heart and brain, that will evenly lead to myocardiac infarction, Eosinophilia, Anemia, thrombocythopenia, paralysis, stroke, seizure and mortality may eventually arise.

Keywords: Calcium Carbide, artificial ripening, Fruits, Haematological Parameters.

INTRODUCTION

Various chemical, drugs and other agents are constantly and unconsciously consumed in diverse ways. There is globally high demand to urgently ripen fruits for commercial purposes with these chemicals which inadvertently induced health risk to mankind. Amongst the agent is calcium carbide (CaC2). Due to its nature, commercial CaC2 is consistently found to contain impurities such as Arsenic and other toxic and carcinogenic chemicals [1]. Calcium carbide is a corrosive and dangerous chemical containing traces of arsenic and phosphorus hydride as impurities [2]. Natural fruit ripening is a combination of physiological, biochemical and molecular processes [3, 4]. It involves coordination of different metabolisms with activation and deactivation of various genes, which leads to changes in colour, sugar content, acidity, texture and aroma volatiles [3, 5]. The change in colour during the fruit ripening process is a result of the unmasking of pigments by degradation of chlorophyll, synthesis of different types of anthocyanins and their accumulation in vacuoles and accumulation of carotenoids. Fruits ripened with calcium carbide may develop a uniform and attractive surface colour, but are poor in flavour and the inside may remain unripe. Though the use of calcium carbide accelerates ripening, it may affect the physicochemical characteristics of the fruits [2].

Ripening is done naturally or artificially and this leads to fruit maturity before consumption or processing [6]. 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 [7]. Investigation of the effects of consumption of calcium carbide ripened banana on the liver showed statistically significant increases in the levels of plasma total protein, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) ($p<0.001$) [8]. Histology of the liver showed varying degrees of hepatic injury such as fatty change, sinusoidal dilation, congestion and dilatation of the central vein. Findings suggest that consumption of calcium carbide ripened banana may have deleterious effects on the liver architecture as well as its functions. Ripening can then take place outside the parent plant [9]. During the process of natural ripening, ethylene gas, which serves as ripening agent, is released at the respiratory peak, to coordinate and increase the rate of the ripening process [10, 11 & 9]. This process is well coordinated, genetically programmed and non-reversible. It results in different physiological, biochemical and organoleptic changes which produce attractive edible fruits [12, 13]. These changes include increment in sugar content, aroma production, colour development, reduction in astringency, and textural changes among others [9]. With natural ripening, it is difficult to meet population demands; therefore, artificial ripening is embarked upon, for commercial purposes. It is practiced so as to prevent losses due to handling during transportation and storage [14]. The investigation of Lipid profile and haematological indices of Wistar albino rats fed naturally ripped, unripe and artificially ripped mango pulp formulated diets shows that at 10, 20 and 30% levels of incorporation of samples into the formulated diets, White blood cell count increased while Red blood cell count and haemoglobin concentration decreased in the artificially ripped groups compared to the control. Generally, the values of lipid parameters and haematological indices suggest that artificial ripening especially by the use of carbide may not be a good candidate in the ripening of mango fruits [15]. The significant alterations in hematological parameters of rats treated with the drugs may provide evidence of toxicity. The reduction in PCV (piroxicam, Ibuprofen and diclofenac), RBC (Ibuprofen) and Hb (piroxicam) values in the above drug-treated groups may suggest drug-induced toxicity, characterized by excessive destruction of red blood cells resulting in anaemia [16]. It may also be due to loss of erythrocytes as a result of gastrointestinal bleeding. When there is a substantial loss of blood from the body, the RBC picture may indicate microcytic hypochromic anaemia [17, 18]. The upsurge of demand for food safety has motivated researchers into investigating possible risk common with the use of artificial methods in fruit ripening [19]. Hence the need for this study to be carried out. When calcium carbide comes in contact with moisture, it produces acetylene gas, which is quite similar in reaction to the natural ripening agent ethylene. Acetylene acts like ethylene and accelerates the ripening process. Industrial-grade calcium carbide may also contain traces of arsenic and phosphorus which makes it a human health concern hence, the use of this chemical for this purpose is illegal in most countries because, it is extremely hazardous to the human body [20]. Packed cell volume (PCV) also referred to as haematocrit or erythrocyte volume fraction (EVF), is the proportion of the total blood volume composed of the red blood cells [21]. It is typically about 50-52% for men and 36-48% for women. Diseases condition such as chronic obstructive pulmonary diseases (COPD) can cause increased packed cell volume when it occurs. Reduced packed cell volume may suggest anemia. At both levels of packed cell volume, the disorders are life-threatening [22].

MATERIALS AND METHODS

Materials

Wistar rats, Calcium carbide, Water, Pawpaw , Syringes and Needles, Hand Gloves, Incubator, stop watch, Oven, centrifuge Model 800, cotton wool, Chloroform, 40% formaldehyde, Desiccator, Methylated spirit, EDTA bottles, normal sample bottles, Animal weighing balance, Water bath, and amongst others.

Design of the Experiment

This study is experimental in nature. Adult Wistar rats were fed with naturally ripened and Calcium Carbide induced ripened fruits [pawpaw] in other to compare and investigate the effect of Calcium Carbide on the hematological parameters of the Wistar rats.

Fruit Collection:

Mature unripe pawpaws were plucked off from the parent plant at Yenagoa, Bayelsa State. The fruits were dividing into two groups; one group was kept and allowed to rip at normal room temperature at the Histology Laboratory, Bayelsa Medical University, Yenagoa, Bayelsa State. The other group was induced with Calcium Carbide to ripe.

Calcium Carbide Acquisition, weighing and Application

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 [pawpaw] rapped with black nylon and was allowed for two days[48 hours] for ripening. After ripening, sampled fruits were washed and juiced.

Homogenized Sample preparation

600g of both the naturally ripened and calcium carbide ripened fruits [pawpaw] were peeled separately

and blended in an electric blender with 350ml/1L of deionized water. The juice was filtered with a clean fine sieve and was poured into clean bottles labeled [CaC_2 induced ripened juice and naturally ripened pawpaw juice]; and was stored in a refrigerator for subsequent use.

Experimental Animals

A total of 21 adult Wister rats of both sexes weighing between 126.9- 213.3g were used for this study. The animals were purchased and kept in standard environmental condition, given standard rodent food (formulated) and water *ad libitum* in the animal house of the Bayelsa Medical University. The rats were divided into three groups for each sex, based on the body weight and then different concentrations of naturally ripened and calcium carbide induced ripened fruits [pawpaw] were administered orally to the rats. Animals were allowed to acclimatize for two [2] weeks fed with standard grower mash with clean water before treatment. The process was in tandem with the guidance of National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Sample Administration

LD_{50} was done using [23] method for administration of samples. A total of nine [9] Wistar rats were used for this section grouped into three [3] each group containing three [3] rats.

In the second section [main] experiment, twelve [12] Wistar rats were used.

Group 1: Normal control group of 4 rats [2 males and 2 females] receive normal water and feeds only as placebo.

Group 2: Treatment Group [1] of 4 rats [2 males and 2 females] received 5ml/kg naturally ripped fruits [pawpaw juice] for 4 weeks [A month].

Group 3: Treatment Group [2] of 4 rats [2 males and 2 females] received Calcium Carbide ripped fruits [pawpaw] for 4 weeks [A month].

5ml/kg for both the natural fruit and the CaC_2 ripened fruits were administered against each body weight of the adult Wistar rats.

Blood Sample Collection

The rats were weighed, then one Wistar rat was sacrificed in the groups each week and blood samples were collected from the three groups for hematological analysis.

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HAEMATOLOGICAL PARAMETERS

The haematological parameters include Pack Cell Volume [PCV], Total White Blood Count [TWBC], Hemoglobin [Hb], Red Blood Cells [RBC], Platelet, Neutrophil, Lymphocytes, Monocytes, Eosinophil and Basophils.

Data analysis

The obtained data was analyzed with SPSS version 16.0. Descriptive statistics was done and ANOVA was used to compare mean value for statistical significance difference.

RESULTS

Table 1: Body Weight of Adult Wistar Rat [Grams]

GROUP	CONTROL	NATURAL FRUITS	CaC2 RIPENED FRUITS
MEAN VALUE	214.30±10.53	184.53±19.53	174.28±17.35

Mean ±SEM

Table 2: Effect of Calcium Carbide Ripened Pawpaw on the Haematological Parameters of the Wistar Rats

HAEMATOLOGICAL PARAMETERS	Group 1 [CONTROL]	Group 2 NATURAL PAWPAW	Group 3 CaC_2 PAWPAW
PCV	54.5 ± 4.50^{a}	43.0 ± 1.00^{b}	44.0 ± 1.00^{c}
TWC	5.75 ± 1.75^{b}	2.9 ± 0.90^{c}	2.25 ± 0.05^{a}
HB	17.5 ± 1.50^{c}	14.0 ± 0.00^{a}	14.5 ± 0.50^{b}
RBC	5.45 ± 0.45^{c}	4.30 ± 0.10^{b}	4.40 ± 0.10^{a}
PLATELET	569.0 ± 97.00^{a}	524.5 ± 36.50^{c}	561.5 ± 105.50^{b}
NEUTROPHIL	56.5 ± 1.50^{b}	30.0 ± 10.00^{a}	27.5 ± 2.50^{c}
LYMPHOCYTES	38.5 ± 1.50^{c}	65.0 ± 10.00^{b}	67.5 ± 2.50^{a}
MONOCYTE	3.0 ± 0.00^{a}	2.0 ± 0.00^{b}	2.5 ± 0.50^{c}
EOSINOPHIL	2.0 ± 0.00^{c}	2.0 ± 1.00^{a}	2.50 ± 0.50^{b}
BASOPHIL	0	0	0

Keys: [Mean ±SEM], *Means of different superscript alphabets in the same row shows no significant difference at 95% confidence levels (p>0.05).*

DISCUSSION

The results of this present study have shown that the mean Packed Cell Volume [PCV] of the Wistar rats fed with both the naturally and the CaC_2 ripened fruits tend to be lower when compared with the PCV of the control group. This finding is in tandem with the result of [24] were the Packed Cell Volume is lower with the Wistar rats fed with CaC_2 ripened fruits. This depletion in PCV is suggestive of anemia [22].

The mean Total White Blood Count of the Wistar rats fed with both the naturally ripened and the CaC2 ripened fruits tend to be lower when compared with the control group. The Total White Blood Count of the CaC_2 treated group tend to be lower, this is a pointer that consumption of fruits ripened with calcium carbide could lower the body's resistance to infection by way of weakening the immune system. This result is supported with the findings of [24] were the result showed a significant decrease in the WBC Count and lymphocytes of the female rats administered these fruits. This result went contrary with findings of [25] were the Wistar rats fed with calcium carbide ripened fruit possesses significantly higher value of total white blood count. From the result presented in [table 2], the Total Red Blood Cell count [RBC] and Platelets of the Wistar rats fed with both the naturally ripened and artificially [Calcium Carbide] ripened fruit [pawpaw] is lower in contrast to the mean value of the control group. The reduction in the mean values of the treated groups may suggest chemical-induced toxicity, depicted by excessive extermination of red blood cells leading to anaemia. It may be due to erythrocytes loss as a result of gastrointestinal bleeding which was also posited by [16]. As a result of substantial loss of RBC, microcytic hypochromic anaemia may have resulted which was a vivid from the analyzed film. This finding is in tandem with the result of [17, 18].

The result has shown mean reduction of Neutrophils of the Wistar rats fed with both the naturally ripened and artificially [Calcium Carbide] ripened fruit [pawpaw] in contrast to the mean value of the control group. Neutrophils constitutes 55-73 percent of the Total White Blood Cell Count, the first cells to combat invaders, and also signal senders to other cells of the immune system; their reduction is an indication of massive death due to combat with external invaders. Because of the deleterious and reduction effects, the body is prone to various illnesses and infections. The mean value of the Lymphocytes of the Wistar rats fed with artificially [Calcium Carbide] ripened fruit is higher than the control group. This is suggestive of the fact that there are foreign invaders.

There is reduction in the Monocyte value of the Wistar rats fed with both the naturally ripened and artificially [Calcium Carbide] ripened pawpaw in comparison with the control. But the Wistar rat fed with Calcium Carbide ripened fruit possess higher value than the Natural ripened group. The Eosinophil level of the Wistar rat fed with artificially [Calcium Carbide] ripened fruit tend to be elevated as compared to the Wistar rat fed with natural ripened fruit and the control group. This is possibly, caused by response to the presence of allergies or parasites in Wistar rats since these leukocytes are active in a wide range of inflammatory problems resulting to Eosinophilia.

CONCLUSION

It is clear, that consumption of fruits ripened with Calcium Carbide pose devastating effect on the bone marrow, deleterious effect on the circulating blood that will evenly lead to myocardiac infarction, Eosinophilia, Anemia, thrombocythopenia, paralysis, stroke, seizure and mortality may eventually arise. The use of Calcium Carbide as a ripening agent should be abolished.

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