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The Negative Effects of Calcium Carbide Ripened Orange on the Haematological Indices of the Wistar Rats

OGOUN, TIMIPA RICHARD.
Department of Human Anatomy, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria.
Corresponding Author: Dr. Ogoun, Timipa Richard. Senior Lecturer, Head, Department of Human Anatomy, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria.

ABSTRACT
Background: *Fruits contains nutritional elements that aids digestion, cell, tissue, organ and systems proliferation and development. This study is aimed at investigating the hazardous consequences of the intake of Calcium Carbide forced ripened orange juice on the haematological properties of the Wistar rats.*
Materials and Methods*: Mature unripe oranges were plucked off from the parent plant in LEM-NAT farm in Yenagoa, Bayelsa State . The fruits were divide into two groups, one group was kept and allowed to ripe at normal room temperature and the other group was induced with Calcium Carbide to ripe. 600g of both the naturally ripened and calcium carbide ripened oranges were peeled separately and blended in an electric blender with 350ml/L of distilled water. The juice was filtered with a clean fine sieve and was poured into clean bottles labeled [CaC2 induced ripened juice and Naturally ripened orange juice] which was then stored in a refrigerator for subsequent use. 21 adult Wister rats weighing between 126.9- 213.3g were used. 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. LD_{50} was done using [15] method for administration of sample with nine Wistar rats. Twelve Wistar rats were divided into three groups [control, Treatment 1(natural juice) and treatment II(CaC2 forced ripened juice)] based on their weight and then 5ml/kg of the naturally ripened and calcium carbide induced ripened orange juice were administered orally. The rats were weighed, then one Wistar rat was sacrificed in the groups each week and blood samples were collected for hematological analysis.* ***Results:****The results showed significant increase in mean PCV, hemoglobin, RBC, Lymphocytes, Monocytes and Eosinophil while there was significant reduction in Total White Blood Cell count, Platelet, Neutrophil level ($p<0.05$).*
Conclusion:*Calcium Carbide is unsafe for fruit ripening and it is dangerous to human health. .*
Key Words*: Haematological indices, Calcium Carbide, Wistar Rats.*

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I. Introduction

Fruits and vegetables are highly nutritious and form a key food commodity for the human consumption. They are highly perishable due to their low shelf life. These food commodities are reported to be contaminated with toxic and health hazardous chemicals [1]. Ripening is a genetically programmed highly coordinated irreversible phenomenon which includes many biochemical changes including tissue softening, pigment changes, aroma and flavour volatile production, reduction in astringency, and many others [2]. The life of a fruit can be divided into three phases: fruit set, fruit development, and fruit ripening. Fruit ripening is the initiation of fruit senescence which is a genetically programmed highly coordinated process of organ transformation from unripe to ripe stage to yield an attractive edible fruit[3]. It is an irreversible phenomenon involving a series of biochemical, physiological, and organoleptic changes [4]. These changes include changes in carbohydrate content, increment of sugar content, changes in colour, texture, aroma volatiles, flavour compounds, phenolic compounds, and organic acids.A study by [5] compared physicochemical changes occurring during natural ripening and acetylene induced ripening in "Saba" bananas. Here it was shown that acetylene affects the rate of chemical changes during ripening; nevertheless it did not significantly affect the final levels of sugar and starch content in the ripe pulp. A study by[6], compared ripening techniques such as ethephon, smoking, and keeping in low density polyethylene plastic, in Teff straw, and in banana leaves. The results supported that smoking enhance faster ripening but led to least marketability, 28.67% at 10th day of storage when other treatments reported more than 83% of marketability. The reduction of marketability of smoked bananas was due to blackening and over softening.Study conducted by [7] on the Effect of induced ripening agents including calcium carbide, potash, and leaves of Irvingiagabonensisand Jatrophacurcas leaves on nutritional and mineral composition of bananas was studied. They reported that the chemical ripening agents

tested contributed to lower levels of protein compared to biological ripening agents. Also levels of Pb, Cu, Zn, and Mn were higher in calcium carbide treated fruits than other ripening agents.[8] treated bananas with different concentrations of ethephon. Here it was found out that different concentrations of ethephon significantly influence chemical changes during ripening and 1000 ppm was found as the best concentration of ethephon for early ripening. [9] showed that prestorage treatments of mangoes (Mangiferaindica L., cv. Kensington Pride) with ethephon (500 mg/L) for five minutes increased TSS, TSS/acid ratio, and sugars and reduces chilling injury. [10] compared ethephon treatment and traditional kerosene smoke treatment and their effect on ripening of "Cavendish" bananas, where it was shown that ethephon treated fruits demonstrated higher sensory quality. Apart from being used to initiate ripening, ethephon has been recorded as plant growth regulator which can be used to increase fruit size, induce flowering, enhance colour, and induce flower abscission [11]. According to gene expression analysis it has been revealed that ethylene directly regulates the transcription of both a softening-related PpPG gene that encodes an α-L-arabinofuranosidase/β-xylosidase (PpARF/XYL) and an expansin (PpExp3) [12]. In some fruits such as bananas which contain high level of starch in the fruit flesh, enzymatic hydrolysis of starch is a major factor in fruit softening. In citrus fruit, softening is mainly associated with change in turgor pressure, a process associated with the postharvest dehydration and/or loss of dry matter [13].

II. 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

This experimental study is aimed at investigating the effect of Calcium Carbide forced ripened orange juice on the hematological parameters of the Wistar rats.

Fruit Collection:

Mature unripe oranges were plucked off from the parent plant in LEM-NAT farm in Yenagoa, Bayelsa State . The fruits were divide into two groups, one group was kept and allowed to ripe at normal room temperature and 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 oranges 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

600g of both the naturally ripened and calcium carbide ripened oranges 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_2induced ripened juice and Naturally ripened orange juice] which was then stored in a refrigerator for subsequent use.

Experimental Animals

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 based on their weight and then different concentrations of naturally ripened and calcium carbide induced ripened orange juice were administered orally. Animals were allowed to acclimatize for two weeks and was fed with standard grower mash with clean water before treatment.The process was in tandem with the guidance of [14].

Sample Administration

LD_{50} was done using [15] method for administration of samples. A total of nine [9] Wistar rats were used for this section grouped into three each group containing three rats.

In the main experiment, twelve 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 [orange juice] for 4 weeks [A month].

Group 3: Treatment Group [2] of 4 rats [2 males and 2 females] received Calcium Carbide ripped fruits [orange] 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.

III. Results

The haematological indices 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 22.0. Descriptive statistics was done and ANOVA was used to compare mean value for statistical significance difference. The results gotten from the analyzed data are presented in the tables below.

TABLE 1: BODY WEIGHT OF ADULT WISTAR RATS [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 PINEAPPLE ON THE HAEMATOLOGICAL PARAMETERS OF THE WISTAR RATS

HAEMATOLOGICAL PARAMETERS	Group 1 [CONTROL]	Group 2 NATURAL PAWPAW	Group 3 CaC_2 PAWPAW
PCV	54.50 ± 4.50^{A}	51.50 ± 16.50^{B}	61.50 ± 6.50^{C}
TWC	5.75 ± 1.75^{D}	2.25 ± 0.25^{W}	3.10 ± 1.20^{S}
HB	17.50 ± 1.50^{Q}	13.50 ± 1.50^{A}	20.00 ± 2.00^{Z}
RBC	5.45 ± 0.45^{K}	5.30 ± 1.50^{K}	6.10 ± 0.65^{V}
PLATELET	569.00 ± 96.00^{B}	$235.00\pm64.50_{V}$	478.00 ± 1.28^{C}
NEUTROPHIL	56.50 ± 1.50^{A}	56.00 ± 14.00^{A}	37.00 ± 9.00^{W}
LYMPHOCYTES	38.50 ± 1.50^{D}	40.00 ± 14.00^{S}	53.50 ± 8.50^{C}
MONOCYTE	3.00 ± 0.01^{G}	2.00 ± 0.01^{F}	6.50 ± 1.50^{N}
EOSINOPHIL	2.00 ± 0.01^{P}	2.00 ± 1.00^{P}	3.00 ± 1.00^{I}
BASOPHIL	0	0	0

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

IV. Discussion

The results shown in [table 3] indicates increased Packed Cell Volume [PCV] of the Wistar rats fed with Calcium Carbide ripened orange ($p>0.05$). PCV is the percent of the circulating red blood cell. An increase, is generalized with over production of the red blood cells by the bone marrow, enlarged spleen, or dehydration. Which could lead to polycythemia vera.The total white blood cell count significantly decreased in the Wistar rats fed with Calcium Carbide ripened orange ($p>0.05$). White blood cells are produced in the bone marrow and their decrease depicts hematopoietic infringement or cell death during combat with invaders which in turn exposes the wistar rats to infection, aplastic anemia splenic sequestration, leucopenia and if not properly managed the resultant effect will be death . This finding is corroborated by the results of [16]on the effect of Vino Gano Ginger and Herbal Liqueur on the Heamatological Parameters of the Wistar Rats.There is an increase in the hemoglobin concentration in the Wistar rats fed with Calcium Carbide ripened orange ($p>0.05$). This increase could result from excessive production of red blood cells by the bone marrow or the excess production of the hemoglobin itself to compensate for oxygen deficit. Other likely causes of increased hemoglobin level are heart, kidney and liver diseases.

The result in (table 2) also shows an increase in the Total Red Blood Cells count of the Wistar rats fed with Calcium Carbide ripened orange in contrast with the control ($p>0.05$). Excessively increased in red blood cell count [Erythrocytosis]if occurred, can make your blood more thicker than normal and could inadvertently

cause blood clotting issues. Platelet otherwise called thrombocyte tend to decrease in the wistar rat fed with Calcium Carbide induced orange juice ($p>0.05$). This evidence of low platelet indicates clothing thrombocytopenia. Most of the clotting factors [proteins] are synthesized in the liver. Thus, severe damage to the liver mass will be associated with coagulopathy.In the present result, there is drastic reduction of Neutrophil in the wistar rat fed with Calcium Carbide induced orange juice ($p>0.05$). Neutrophil are the body's first line of defense and constitute about 55-73 percent of the total white blood cell count. Their decrease in the body of the Wistar rats fed Calcium Carbide induced orange explain massive cell death in response to combat with foreign bodies, thus the resultant effect will be weakness, infections, organ damage, and or death. The result published by [17] on the "Toxic Assessment of Calcium Carbide Ripened Pawpaw on the Haematological Parameters of the Wistar Rats" confirmed this present finding.

Lymphocytes of the wistar rat fed with Calcium Carbide induced orange juice is higher than the control group as presented in (table 2). Lymphocytes such as T- lymphocytes and B- lymphocytes produce antibodies [immune response] to deter and destroy foreign bodies within the body system.There increase is a pointer to the presence of foreign invaders, and that the body's immune system is responding to the invading agents. In addition to the increased lymphocytes, Monocytes are also higher in the wistar rat fed with Calcium Carbide induced orange juice when compared with the control group ($p>0.05$). Monocytes increase is also an indicator of an attack from foreign agents. This is finding in consonance with the result of [18] "Investigation of the Haematological Indices of the First Generation of the Wistar Rats Fed with Calcium Carbide Ripened Orange".The mean Eosinophil level is increased in thewistar rat fed with Calcium Carbide induced orange juice ($p>0.05$).This rise in eosinophil [Eosinophilia] is a response to cancer, allergy or parasitic infection in the body. This result is in tandem with the findings of [18].

V. Conclusion

The usefulness of fruits to the human body can be over emphasized, as they are a source of vitamins, minerals, fiber which helps in preventing heat stroke, high blood pressure, heart. Liver, kidneys diseases. Forcing these fruits to ripe other than the natural means [with chemicals such as Calcium Carbide] will alter the chemical composition and deplete the nutritional content and when consumed will engender various health complications some of which can't be forestalled back to normal even with medications.

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