

Examination Of The Haematological Indices Of The Wistar Rats Fed With Calcium Carbide Ripened Pineapple

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Abstract—The use of chemicals to ripen fruits has raised exponential health issues in recent times. This study is to reveal the effect of Calcium Carbide forced ripened pineapple on the haematological indices of the Wistar rats. Mature unripe Pineapples were plucked off from the parent plant at Lem- Nat farm in Yenagoa, Bayelsa State . The fruits were divide into two groups, one group was kept and allowed to rip at normal room temperature while the other group was forcefully ripened with 10g of Calcium Carbide which was placed in a bowl and 5ml of water to dissolved and both were enclosed in a metal bucket containing [1kg of the fruit] rapped with black nylon for 48hours. 600g of both the naturally ripened and calcium carbide forced ripened pineapple were peeled separately and blended in an electric blender with 350ml/L of water. The juice was filtered with a clean fine sieve and was poured into two separate clean bottles and stored in the refrigerator of further use. 21 adult Wister rats of both sexes weighing between 126.9- 213.3g were used for this study. The rats were divided into three groups based on the body weight and then different concentrations of naturally ripened and calcium carbide induced ripened pineapple were administered orally. The animals were allowed to acclimatize for two [2] weeks fed with standard grower mash with clean water before treatment. LD50 was done using [14] method for administration of samples. Nine [9] Wistar rats were used for this section and were grouped into three [3] each group containing three [3] rats. In the main experiment, twelve [12] Wistar rats were divided into three [3] groups; control, treatment 1[natural pineapple] and treatment II [Calcium Carbide forced ripened pineapple]. 5ml/kg for both the natural fruit and the CaC2 ripened fruits were administered against each body weight of the adult Wistar rats for 4 weeks. 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. The results showed significant reduction in mean PCV, Total white blood count [TWBC], Hemoglobin, Total red blood cells [TRBC], Platelets, Neutrophils. But there was increase in

lymphocytes, Monocyte and Eosinophil. In summary, Calcium carbide causes various health hazards to human even when consumed indirectly.

Keywords—*Haematological Indices, Calcium Carbide, Pineapple, Wistar rats.*

INTRODUCTION

The increased request and demand for fruits has caused the use toxic chemicals to induce fruits to ripe to meet the demand by vendors globally. [1] listed some of the most common chemicals used in fruit ripening and growth as, “calcium carbide, acetylene, ethylene, propylene, ethrel (2-chloroethyl phosphonic acid), glycol, ethanol and further stated that, “calcium carbide (CaC 2) is the commonly used chemical for ripening of fruits, due to its low price and availability in local market; however, use of this chemical in fruit industry is being discouraged worldwide due to dangers of explosion and carryover of toxic materials like arsenic and phosphorus to consumers, thus making the healthy fruit poisonous. [2] said “indiscriminate use on different types of fruits can lead to poisonous effects. Due to lack of awareness and education people consume chemically ripened fruits”. [3] in his paper, Bitter Truth about Fruit with Reference to Artificial Ripener also joins the chorus in highlighting the harmful effects of these chemicals and states that, “If pregnant women consume fruit ripened with carbide, the children born could develop abnormalities”. Calcium carbide is a corrosive and dangerous chemical containing traces of arsenic and phosphorus hydride as impurities [4]. Globally, banana (*Musa spp.*) is one of the most commonly consumed fruit crops and artificial ripening is commonly practiced for commercial purposes. Sadly, many had unknowingly suffered serious damage to their internal organs due to the intakes of banana ripened with artificial ripening agents such as calcium carbide [5]. 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 [5]. Calcium carbide in the presence of water releases

acetylene (an analogue of ethylene) which behaves in the same way with ethylene [6]. The internal ethylene concentration of climacteric fruits varies widely, but that of non-climacteric fruits changes little during development and ripening [7]. The rise in respiration in response to ethylene may occur more than once in non-climacteric fruits in contrast to the single respiration increase in climacterics[8]. [9] study on the haematological indices using during herbal drink concluded that, The Consumption Vino Gano Ginger and Herbal Liquor by man to sexually satisfy a woman is a banquet of plague and index of death. [10] results has shown that drugs can cause significant alterations in the hematological and biochemical indices of rats. It can therefore be concluded that Indomethazine, Celecoxib, Aspirin, Diclofenac, Piroxicam and Ibuprofen at the various respective doses and duration of study might cause toxic effects on vital animal tissues, resulting in hematological disorder, hepatic and renal impairments. Haematological findings by [11] 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 [11]. The use of toxic and suspicious ripening agents is of great concern as the activities of human beings have been said to contribute to exposure of food materials to heavy metal contamination [12].

MATERIALS AND METHODS

MATERIALS

Wistar rats, Calcium carbide, Water, Pineapple, 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 is an experimental study of Wistar rats that were fed with naturally ripened and Calcium Carbide induced ripened pineapple in other to examine the effect of Calcium Carbide on the hematological indices.

Collection of Pineapple and Calcium Carbide Application

Mature unripe Pineapples were plucked off from the parent plant at 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 while the other group was induced with Calcium Carbide to ripe.

Calcium carbide was bought at Swali Market, Yenagoa, Bayelsa State. 10g 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 Pineapple rapped with black nylon and was allowed for two days[48 hours] for ripening. After ripening, sampled fruits were washed and juiced.

Sample preparation:

600g of both the naturally ripened and calcium carbide ripened pineapple were peeled separately and blended in an electric blender with 350ml/1L of distilled water. The juice was filtered with a clean fine sieve and was poured into clean bottles labeled [CaC₂ forced ripened and Naturally ripened pineapple juice]; and was stored in a refrigerator for subsequent use.

Experimental Animals and Sample Administration

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 pineapple were administered orally. Animals were allowed to acclimatize for two [2] weeks and was fed with standard grower mash with clean water before treatment. The process was in tandem with the guidance of National Research Council, Guide for the Care and Use of Laboratory Animals, 2011.[13].

LD₅₀ was done using [14] method for administration of samples. Nine [9] Wistar rats were used for this section and they were grouped into three [3] each group containing three [3] rats.

For the 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 pineapple juice.

Group 3: Treatment Group [2] of 4 rats [2 males and 2 females] received Calcium Carbide ripped Pineapple juice for 4 weeks.

5ml/kg for both the natural fruit and the CaC₂ 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.

RESULTS

Haematological Parameters

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

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 | 47.50±2.50 ^D | 49.50±6.50 ^S |
| TWC | 5.75±1.75 ^D | 1.80±0.30 ^W | 3.55±0.55 ^S |
| HB | 17.50±1.50 ^Q | 16.00±1.00 ^E | 16.50±2.50 ^E |
| RBC | 5.45±0.45 ^K | 4.75±0.25 ^G | 4.95±0.65 ^G |
| PLATELET | 569.00±96.00 ^B | 405.00±50.00 ^P | 319.00±1.14 ^H |
| NEUTROPHIL | 56.50±1.50 ^A | 50.00±10.00 ^D | 52.50±17.50 ^C |
| LYMPHOCYTES | 38.50±1.50 ^D | 42.50±12.50 ^X | 40.00±15.00 ^E |
| MONOCYTE | 3.00±0.01 ^G | 4.00±1.00 ^G | 3.50±0.50 ^G |
| EOSINOPHIL | 2.00±0.01 ^P | 3.50±1.50 ^I | 4.00±2.00 ^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

Assaying the haematological indices of the Wistar rats fed Calcium Carbide forced ripened pineapple and the naturally ripened pineapple showed significant reduction in the Packed Cell Volume [PCV] of both the Wistar rats fed Calcium Carbide forced ripened pineapple and the naturally ripened pineapple in comparison with the control ($p<0.05$). This depletion of the packed cell volume is showing a compromised blood cell production and excessive internal hemorrhage which could cause anemia. This finding is supported by the results of [15]. There is also reduction in the Total White Blood Cell count of the Wistar rats fed Calcium Carbide forced ripened pineapple and the naturally ripened pineapple. This reduction is in lieu of the body's compromise to fight infections as a result of weakened immunity. This finding is in tandem with [16] result which showed reduction in mean WBC of the haematological indices of the first generation of the Wistar rats fed with calcium carbide ripened orange. The result of [11] also support this findings.

Consequently, there is depletion in the hemoglobin level of the Wistar rats fed Calcium Carbide forced ripened pineapple. If hemoglobin which transport oxygen is reduced, then there will be tissue hypoxia and hypoxemia, which will lead to ischemia.

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.

TABLE 1: BODY WEIGHT OF ADULT WISTAR RAT [Grams]

| GROUP | CONTROL | NATURAL FRUITS | CaC ₂ RIPENED FRUITS |
|------------|--------------|----------------|---------------------------------|
| MEAN VALUE | 214.30±10.53 | 184.53±19.53 | 174.28±17.35 |

Mean ±SEM

Significant reduction was also noticed in the Total Red Blood Cell count of the Wistar rats fed Calcium Carbide forced ripened pineapple ($p<0.05$). This reduction could lead to anemia, shock, brain damage and eventually death may arise. Because oxygen supply is impeded, cell multiplication and differentiation could be compromised. The red blood cells production by the bone marrow may have been compromised or there is acute internal hemorrhage resulting microcytic hypochromic anemia. This result supports the findings of [16, 17]. In this study, the platelet value also reduced drastically in the Wistar rats fed Calcium Carbide forced ripened pineapple. Platelets [Thrombocytes] are responsible for blood clotting during bleeding. The reduction in platelets could result to thrombocytopenia. The Neutrophil reduced in the Wistar rats fed Calcium Carbide forced ripened pineapple as shown in [table 2] depicts excessive death of these cells due to combat with foreign invaders.

Findings from this present study highlight increase in the mean value of Lymphocytes of both the Wistar rats fed Calcium Carbide forced ripened pineapple which is an indicator of external invaders in the body. The body respond to external attack by producing T-lymphocytes and B- lymphocytes. There is also evidence of mild increase in monocytes level of the Wistar rats fed Calcium Carbide forced ripened

pineapple. In addition, significant increase was recorded in the level of the eosinophil in the Wistar rats fed Calcium Carbide forced ripened pineapple.

CONCLUSION

The indirect consumption of various chemicals [Calcium Carbide] used in fruits ripening evidentially causes inimical and pernicious effect on human. The Government, Multinational Companies, Corporate bodies, Non-Governmental Organizations, Philanthropists, Radio and Television stations, should endeavor to disseminate this information to the populace on a regular basis to reduce this unsafe act and unsafe conditions in order to sustain the healthy nature of living.

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