



(RESEARCH ARTICLE)



Nutritional programming assessment on the second filial generation pups of the Wistar rats fed with calcium carbide coerced ripened orange: The Haematological Perspective.

Ogoun Timipa Richard *

Department of Human Anatomy, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria.

Magna Scientia Advanced Biology and Pharmacy, 2022, 06(02), 017-022

Publication history: Received on 09 June 2022; revised on 18 July 2022; accepted on 20 July 2022

Article DOI: <https://doi.org/10.30574/msabp.2022.6.2.0077>

Abstract

The explicit use of chemicals for fruit ripening is increasing daily and with eminent consequences. The aim of this study is to check nutritional programming on the second filial generation pups of the Calcium Carbide coerced orange juice fed Wistar rats. Mature unripe oranges were gotten from the orange plant in Ogu, Yenagoa, Bayelsa state. The oranges were coerced to ripe with 10gram of Calcium Carbide which was placed in a bowl with 5ml of water for dissolution in a closed metal bucket containing 1kg of the matured orange rapped with black nylon and kept two days to ripe. After ripening, sampled fruits were washed and juiced. 600g of calcium carbide coerced ripened orange was peeled, blended in an electric blender with 350ml/L of distilled water to form the orange juice and was filtered with a clean fine sieve then poured into clean bottles which were labeled and stored in a refrigerator for future use. 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 Calcium carbide coerced ripened orange juice was administered orally based on their body weight. 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. The 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 was allowed free access to feed on the growers mash, clean water and copulation at libitum. In the second birthing 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 haematological analysis. The data was analyzed using Statistical Analysis Program for Social Sciences [SPSS 22.0 Version]. The results showed significant increase in PCV, hemoglobin, Total RBC, lymphocytes and reduction in Total WBC, Platelet and Monocytes in the second filial generation pups from the Wistar rats fed with Calcium carbide coerced orange juice. Haematological Indices are biomarkers that indicates functionality of the blood cells with regards to low, normal or high range. There is evidence of nutritional programming in the second filial generation pups as seen in this results.

Keywords: Nutritional Programming, Calcium Carbide. Haematological, Wistar rats

1. Introduction

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 [1]. Other

* Corresponding author: Ogoun Timipa Richard

Department of Human Anatomy, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria.

serious health hazards include failure of the neurological system resulting in dizziness, sleeplessness, confusion and seizures on a short term basis. In the long term, it can cause memory loss and cerebral edema [2].

Naturally, fruits ripen by the action of a ripening hormone, during which process a wide spectrum of biochemical changes such as chlorophyll degradation and biosynthesis of carotenoids (which are antioxidants, immune system boosters and anti-cancer agents), anthocyanins (powerful antioxidants), essential oils, as well as flavour and aroma components takes place [3]. A similar action is induced by CaC₂ in artificial conditions. Fruit vendors especially in our cities have thus used carbide gas from CaC₂ to ripen fruits, a practice CAP [3]. stated could portend hazard to the human body, especially as the chemical could also contain traces of arsenic and phosphorus. [4] have observed that direct consumption of acetylene could be detrimental as it reduces oxygen supply to the brain and can further lead to prolonged hypoxia. Further, impurities like arsenic and phosphorus found in industrial grade CaC₂ cause serious health hazards among workers who are indirect contact with these chemicals while applying the ripening agent. Effects may include dizziness, frequent thirst, irritation in mouth and nose, weakness, permanent skin damage, difficulty in swallowing, vomiting, and skin ulcer, among others. [5] states that higher exposures may even cause pulmonary edema [6] observed that chemicals including CaC₂ and acetylene gas also how other adverse effects including memory loss, neurological system failure, and cerebral edema. Other effects especially from contaminated foods include colonic lung cancer, quick-buck syndrome, DNA, RNA and hematological changes [7,8] as well as proliferation of bacteria, fungi and viruses which can cause diarrhea, peptic ulcer and other human diseases. The rising demand of fruit safety has inspired researchers about the risk related to the use of chemically ripened fruits and foods contaminated by pesticides [9].

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 [10]. 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 [11]. 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 [12,13]. Hence the need to evaluate the nutritional programming of calcium carbide coerced ripened fruits became imperative.

2. Material and methods

2.1. Design

This research is experimentally designed to assess the toxicity of Calcium carbide on the haematological indices of the second filial [F₂] generation of the Wistar rats fed with Calcium Carbide coerced ripened orange juice.

2.1.1. Fruit and Calcium Carbide Collection

Mature unripe oranges were gotten from the orange plant in Ogu, Yenagoa, Bayelsa state. The oranges were coerced to ripe with 10gram of Calcium carbide purchased from the Swali market, Yenagoa, Bayelsa state. The Calcium carbide was placed in a bowl containing 5ml of water for dissolution in a closed metal bucket containing 1kg of the matured orange rapped with black nylon and kept two days to ripe. After ripening, sampled fruits were washed and juiced.

2.2. Preparation of sample

In this study, 600g of calcium carbide coerced 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 which were labeled and then stored in a refrigerator for future use.

2.3. Experimental Wistar Rats

In line with 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 Calcium carbide coerced ripened orange juice was administered orally based on their body weight. The Wistar rats 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 [14].

2.4. Administration of samples

A sub-acute test [LD₅₀] was done [with 9 Wistar rats] to assess the lethal dose, using [15] 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.

2.5. Birthing Process

With the standard and design of this study, the 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 was 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 haematological analysis.

2.6. Analysis of Data

The data collected from the Haematological assessment 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].



Figure 1 Calcium Carbide

3. Results

The assayed Haematological Indices include, Packed Cell Volume [PCV] , Total White Blood Count [TWBC]. Hemoglobin [HB], Total Red Blood Cell Count [RBC], Platelet, Neutrophil, Lymphocytes, Monocytes, Eosinophil, Basophils. The data gotten from the Haematological assessment were statistically analyzed and results are presented on the tables below.

Table 1 mean weight of parent wistar rat[grams]

Group	Control	Natural fruits	Cac2 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±0.18	11.40±0.50	15.15±0.45
2	Treatment with calcium carbide [cac2]	2.08±0.31	12.81±0.93	22.83±4.93

Mean ±SEM

Table 3 haematological indices of the second filial generation

S/n	Haematological indices	Control	Cac ₂ ripened fruit
1	Packed Cell Volume [PCV]	15.03±0.09 ^C	45.20±0.20 ^A
2	Total White Blood Count [TWBC].	2.90±0.01 ^D	2.61±0.01 ^G
3	Hemoglobin [HB]	5.00±0.01 ^M	15.02±0.02 ^N
4	Total Red Blood Cell Count [TRBC]	1.50±0.01 ^Z	4.25±0.25 ^X
5	Platelet	740.00±1.16 ^Q	350.00±0.50 ^R
6	Neutrophil	30.00±0.01 ^E	30.04±0.04 ^E
7	Lymphocytes	65.02±0.01 ^B	67.40±0.40 ^T
8	Monocytes	4.01±0.01 ^X	2.05±0.05 ^H
9	Eosinophil	1.00±0.01 ^A	1.01±0.01 ^A
10	Basophils	0	0

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

4. Discussion

It is clear from this results [table 3] that there is vivid significant rise in the PCV of the second filial generation when compared with the control group (p<0.05).

There is reduction of Total White Blood Count of the second filial generation pups from the Wistar rats fed with Calcium carbide coerced orange juice. This is indicative of the fact that, there is metabolic programming. There will be compromised resistance of the body to combat infectious diseases by the due weakened immunity result is in consonance with the findings of This result is supported with the findings of [16] where the result showed a significant decrease in the WBC Count and lymphocytes of the female rats administered fruits treated with Calcium Carbide.

The result also showed a significant increase in hemoglobin level of the second filial generation pups of the Calcium carbide coerced orange juice fed Wistar rats (p<0.05). High hemoglobin level is an indication of lung disease like pulmonary fibrosis, congenital heart disease and heart failure and also in dehydration where your hemoglobin concentration will be very high artificially when fluid is low.

Findings from this results shows an elevation in the Total Red Blood Cell Count of the second filial generation pups of the Calcium carbide coerced orange juice fed Wistar rats (p<0.05). But there is conspicuous reduction of platelet of the second filial generation pups of the Calcium carbide coerced orange juice fed Wistar rats when compared with the pups of the control group. (p<0.05). Platelets which are also called thrombocytes are the smallest cells in the blood assist in blood clotting during bleeding are produced in the bone marrow. This reduction in the second filial generation pups could lead to thrombocytopenia. This platelet reduction can arise from autoimmune diseases, inherited conditions, likely from drugs or the bone marrow cannot produce enough platelets.

The results also showcased elevated lymphocytes in the second filial generation pups of the Calcium carbide coerced orange juice fed Wistar rats (p<0.05). It is obvious indication of foreigners in the body. In response to the invaders the body produce both the B and T- cells to perform specific functions.

Evidence in (table 3), show high reduction of Monocytes in the second filial generation pups of the Calcium carbide coerced orange juice fed Wistar rats ($p < 0.05$) there will be accumulation of dead cells in the body.

5. Conclusion

The Haematological Indices are biomarkers that indicates functionality of the blood cells with regards to low, normal or high range. There is evidence of nutritional programming in the second filial generation pups as seen in this results. Human development in-utero is characterized by cell multiplication and differentiation. Nutritional variations in a mother's diet can influence the multiplication or differentiation process. Adverse conditions during proliferation can cause organs to be smaller, even though they have normal cell characteristics. And adverse conditions during differentiation can result in normal-sized tissue having an altered profile and functionality.

Compliance with ethical standards

Acknowledgments

I sincerely thank Mr. Preye Sidney for his assistance during the laboratory process.

Statement of ethical approval

The Bayelsa Medical University Research and Ethics Committee approved this research.

References

- [1] Siddiqui MW, Dhua RS. Eating Artificial Ripened Fruits is Harmful. *Current Science*. 2010; 99(12): 1664-1668.
- [2] Dhembare AJ. Bitter truth about fruit with reference to artificial ripener. *Archives of Applied Science Research*. 2013; 5(5): 45-54.
- [3] Consumers Association of Penang (CAP). Ban the Use of Carbide to Ripen Fruits, Retrieved July 20, 2014. www.Cap.org.my
- [4] Fattah SA, Ali MY. Carbide Ripened Fruits -A Recent Health Hazard, *Faridpur Medical College Journal*. 2010; 5(2): 37.
- [5] USA Department of Health (USDH), Description of a Hazardous Substance, Fact Sheet, USA: Trenton, NJ. 2000; 1-4.
- [6] Kjuus, H., Andersen, A. Langård, S. Incidence of cancer among workers producing calcium carbide. *British Journal of Industrial Medicine*. 1986;43:237-242.
- [7] Rahman WS, Chowdhury. MB Artificial Ripening: What we are Eating. *Journal of Medicine*. 2008; 9: 42-44.
- [8] Dhembare AJ, Gholap AB, Vandana V. Bitter Truth About Artificial Ripener. *Journal of Experimental Zoology India*. 2011; 14(1): 187-189.
- [9] Ruchitha G. Effects of diluted ethylene glycol as a fruit-ripening agent. *Global J Biotechnology Biochem*. 2008; 3: 8-13.
- [10] Pauline N. Iheagwam, Eugene N. Onyeike, Benjamin A. Amadi. Lipid Profile and Haematological Indices of Wistar Albino Rats fed Ripened, Unripe and Artificially Ripened *Mangifera indica* (Mango) Pulp Formulated Diets. *European Scientific Journal*. 2019; 15(15): 30-45.
- [11] Enendu AC, Unekwe PC, Esimone CO, Obi E, Chilaka KC. Protective effect of phenylalanine and glycine on chloramphenicol-induced bone marrow toxicity in albino rats infected with *Klebsiella Pneumoniae*. *Int J Biol Chem Sci*. 2016;10(1): 369-383.
- [12] Okolo I, Owolabi OA, James BD, Sallau AB, Andongma BT, Moses CA. The haemoglobin regeneration potential of fermented and unfermented *Telfaira occidentalis* and *Gnetum africanus* leaves in iron deficient albino rats. *Int J Biol Chem Sci*. 2015; 9(4): 1742-1754.
- [13] Basavraj T, Fefar DT, Prajapati KS, Jivani BM, Thakor KB, Patel JH, Ghodasara DJ, Joshi BP, Undhad VV. Haematobiochemical alterations induced by diclofenac sodium toxicity in Swiss albino mice. *Vet World*. 2012; 5(7): 417-419.

- [14] National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK54050/> doi: 10.17226/12910.
- [15] Lorke D. A New Approach to Practical Acute Toxicity Testing. Archives of Toxicology. 1983; 54: 275-287.
- [16] Eka Basseyy Essien, Blessing MinaopunyeOnyegeme-Okerenta and Jennifer Obiajulu Onyema. Calcium Carbide as an Artificial Fruit-Ripening Agent and its Physiological Effects on Wistar Rat Clinical and Experimental Medical Sciences. 2018; 6(1): 47 – 61.
- [17] Langley-Evans SC. Nutritional programming of disease: unravelling the mechanism. Journal of Anatomy. 2009; 215(1): 36-51.