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**Original Research Article**


# Investigation of the Haematological Indices of the First Generation of the Wistar Rats Fed with Calcium Carbide Ripened Orange


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**Abstract:** Various chemicals have been consumed unnoticed by human in the advent of solving problems. The study is aimed at investigating the haematological indices of the first generation of the Wistar rat fed with Calcium Carbide induced ripened orange. Mature unripe oranges were plucked off from the Orange 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 other group was induced with Calcium Carbide to ripe. 10gram of calcium carbide was dissolved in 5ml of water in a closed metal bucket containing 1kg of the orange 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 ripened orange 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 [CaC2 ripened orange juice and naturally ripened orange juice]; and was stored in a refrigerator for subsequent use. A total of 24 adult Wister rats [12 male and female of each sex] weighing between 126.9- 213.3g were used. They were kept in standard environmental condition, acclimatize for two [2] weeks and was fed with standard grower mash with clean water *ad libitum*. The rats were divided into three groups based on the body weight and then different concentrations of naturally ripened and calcium carbide induced ripened orange were administered orally. Group 1: Normal control group of 8 rats [4 males and 4 females] receive normal water and feeds only as placebo. Group 2: Treatment Group [1] of 8 rats [4 males and 4 females] received 5ml/kg of the naturally orange juice. Group 3: Treatment Group [2] of 8 rats [4 males and 4 females] received 5ml/kg of the Calcium Carbide ripped orange juice for 4 weeks. They were allowed to mate freely during the acclimatization and treatment period [One Month and two weeks]. The Wistar rats birthed and the pups of the three different groups were collected according to the treatment protocols. The Pups were weighed at birth, one week and two weeks and then sacrificed and blood samples were collected for haematological analysis. Results showed reduce Packed Cell Volume, Total White Blood Count, Hemoglobin, Red Blood Cell Count, Platelets, Lymphocytes, in the pups from the Calcium carbide treated Wistar rats ($p>0.05$). But there was an increase in Neutrophil, Monocytes and Eosinophil level of the pups from the Calcium carbide treated group. Calcium carbide has shown devastating effect on the haemtological parameters from the Pups of the Wistar rats fed with Calcium carbide. Blood cells production is impeded due its negative effect on hemapoietic stem cells, protection against foreign bodies is compromised due to low TWBC, resulting to tissue hypoxia, hemorrhage, and stunted growth and amongst other negative consequences; which are passed from parents to offspring as clearly exposed in this study.



**Keywords:** Calciun Carbide, Natural fruit, Haematological Indices.




## INTRODUCTION

Fruits provide vital nutrients in human diet by supplying the necessary growth regulating factors such as vitamins, minerals, complex carbohydrates, proteins, lipids and antioxidants essential for maintaining normal health of the human (Hayes, 2005; Rossato *et al*, 2009). They are widely distributed in nature, commercially important and nutritionally essential food commodity and can be consumed raw. Apart from the consumable part of the fruits, the by-products, such as the fruit peels

and the seeds could represent excellent components for food, medicinal or cosmetic purposes. Calcium carbide is a chemical compound with a formula CaC2. Its main use now is as a source of acetylene. It is a toxic substance banned under the Prevention of Food Adulteration Act. Calcium carbide contains traces of hazardous arsenic and phosphorous compounds. Once dissolved in water, the carbide produces acetylene gas that quickens the ripening process, hence is used in ripening fruits (Halliwell *et al*, 1985). In human after ingestion, acetylene produces free radicals which initiate detrimental effects on various organs [Kaczmarski, 1999].Unripe fruits often contain various types of organic acids, namely citric acid, malic acid, ascorbic acid, formic acid, tartaric acid, etc. (Kendrick, 2009). These acids are responsible for the sour taste of fruits. The chemical [acetylene gas] emitted by calcium carbide on exposure to water, which quickens the ripening process of fruits, could affect the nutritional values when it is used in ripen fruits consumed by humans and inadvertently could affect man. The 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 (Essien *et al*, 2018). Consumption of fruits or skin of fruit ripened with carbide may cause some inflammatory effect, trigger some allergic reactions and have an adverse effect on the kidneys [Patrick and Rosemary 2016]. Fetal nutrition may permanently affect physiological properties of the new individual and hence the risk of future disease (Henriksen *et al*, 2005). Epidemiological studies indicate that fetal nutrition may significantly influence the risk of diabetes, cardiovascular disease, and cancer. Controlled animal studies show that even properties traditionally considered as exclusively genetic, like fur colour, may be modified by altered maternal nutrition (Henriksen *et al*, 2005] . Fetal and neonatal programming is the phenomenon describing deviations from normal developmental patterns. These deviations can increase risks for diseases later in life and are an example of phenotypic plasticity seen throughout nature (Nesterenko *et al*, 2009). A broad range of epidemiological evidence supports the hypothesis that risk of essential hypertension, coronary heart disease and non-insulin dependent diabetes is, in part, determined before birth (Marchand *et al*, 2001). This phenomenon, termed programming, is now the subject of intensive investigation in order to determine possible underlying mechanisms. It is widely accepted that maternal nutritional status in pregnancy is a major programming influence upon the fetus (Marchand *et al*, 2001). This review considers the hypothesis that nephron number in humans is determined by prenatal nutrition. An increasing number of human studies indicate that the developing kidney is particularly vulnerable to the adverse effects of fetal growth retarding influences. In animals, growth retarding diets or other insults which have an impact upon the development of cardiovascular functions, also appear to impact upon nephron number (Marchand *et al*, 2001]. Calcium Carbide releases acetylene which almost works like ethylene in terms of speeding up the ripening process. Direct consumption of acetylene has been found to be detrimental as it reduces oxygen supply to the brain and can further because prolonged hypoxia (Fattah *et al*, 2010). Calcium carbide is a corrosive and dangerous chemical containing traces of arsenic and phosphorus hydride as impurities.

## MATERIALS AND METHODS

### Design of the Experiment

This is an experimental study of Adult Wistar rats fed with naturally ripened and Calcium Carbide induced ripened fruits [oranges] in other to compare and investigate the haematological indices of their first generation pups.

### Fruits and Calcium Carbide Acquisition

Mature unripe oranges were plucked off from the Orange plant at Alu, University of Port Harcourt, Port Harcourt. The fruits were divide 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 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 [orange] 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 orange 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 [$CaC_2$ ripened orange juice and naturally ripened orange juice]; and was stored in a refrigerator for subsequent use.

### Experimental Animals

A total of 24 adult Wister rats [12 male and female of each sex] weighing between 126.9- 213.3g was 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 the body weight and then different concentrations of naturally ripened orange and calcium carbide induced ripened fruits[orange] 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 was administered. 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 Lorke (1983) Method for administration of samples.

**Group 1**

Normal control group of 8 rats [4 males and 4 females] receive normal water and feeds only as placebo.

**Group 2**

Treatment Group [1] of 8 rats [4 males and 4 females] received 5ml/kg naturally ripped fruits [orange juice] for 4 weeks [1month].

**Group 3**

Treatment Group [2] of 8 rats [4 males and 4 females] received Calcium Carbide ripped fruits [orange] for 4 weeks [1month].

5ml/kg each of both the natural fruit [Orange] and the Cac2 ripened fruits were administered against each body weight of the adult Wistar rats.

**Birthing**

The adult Wistar rats were allowed to mate freely during the acclimatization and treatment period [One Month and two weeks]. This period under review, the Wistar rats birthed and the Pups of the three different groups were collected and separated according to the treatment protocols. The pups were allowed to breast feed from the mother for 2 weeks and the pulps sacrificed.

**Blood Sample Collection**

The Pups were weighed at birth, one week and two weeks afterward they were then sacrificed and blood samples were collected from the three groups of pups for haematological analysis.

**Haematological Parameters**

The haematological parameters include PCV, T WBC. HB, RBC, Platelet. Neutrophil, Lymphocytes, Monocytes, Eosinophils and Basophils.

**Fig 1: Pup from Wistar rat fed with naturally ripened orange juice**

**Fig 2: Pup from Wistar rat fed with Calcium Carbide ripened orange juice**

## RESULTS

**Table 1: Mean adult Rat Weight Before Treatment[Grams]**

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

**Table 2: Mean Values of the Pulps Weight at Two [2] Weeks [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 NATURAL FRUIT | 2.13±0.58 | 13.65±1.25 | 28.75±6.25 |
| 3 | TREATMEN WITH CALCIUM CARBIDE [CaC2] | 2.03±0.03 | 11.96±0.60 | 16.90±3.60 |

**Table 3: Haematological Values of the Pups**

| PARAMETER | CONTROL | NATURAL FRUITS | $CaC_2$ RIPENED FRUIT |
|---|---|---|---|
| PCV | $15.03\pm0.09^{c}$ | $27.83\pm0.22^{a}$ | $10.01\pm0.04^{b}$ |
| T WBC | $2.90\pm0.06^{b}$ | $1.73\pm0.04^{c}$ | $1.90\pm0.01^{a}$ |
| HB | $5.00\pm0.01^{a}$ | $9.02\pm0.02^{c}$ | $3.0\pm0.01^{b}$ |
| RBC | $1.5\pm0.01^{m}$ | $2.7\pm0.02^{n}$ | $1.00\pm0.01^{o}$ |
| PLATELET | $740.00\pm1.16^{f}$ | $450.33\pm0.88^{e}$ | $521.04\pm0.03^{d}$ |
| NEUTROPHIL | $30.00\pm0.01^{x}$ | $25.00\pm0.01^{y}$ | $47.03\pm0.03^{z}$ |
| LYMPHOCYTES | $65.02\pm0.01^{J}$ | $70.02\pm0.01^{K}$ | $43.2\pm0.02^{L}$ |
| MONOCYTES | $4.00\pm0.05^{C}$ | $3.00\pm0.00^{D}$ | $6.07\pm0.01^{E}$ |
| EOSINOPHILS | $1.00\pm0.03^{R}$ | $2.01\pm0.6^{S}$ | $4.01\pm0.01^{T}$ |
| BASOPHILS | 0 | 0 | 0 |
| FILM REPORT | ANISOLYTOSIS [+] | ANISOLYTOSIS [+] | ANISOLYTOSIS [+] |
| | POITILICYTOSIS [+] | POITILICYTOSIS [+] | POITILICYTOSIS [+] |
| | NUCLEATED RED CELLS | NUCLEATED RED CELLS | NUCLEATED RED CELLS |

Keys: [Mean ±SEM], Means of different superscript alphabets in the same row shows significant difference at 95% confidence levels ($p>0.05$).

## DISCUSSION

The results of the haematological analysis showed that the Packed Cell Volume of the pup of the CaC2 carbide treated Wistar rats is significantly lower when compared with the control [Table 4]. But the Wistar rats treated with naturally ripened fruits tends to be higher than the control which shows that fruits build up blood cells ($p<0.05$). This difference reveal that calcium carbide effect haemapoietic stem cells [bone marrow] of the Wistar rats. The results also revealed reduction in Total White Blood Count of the pup from the CaC2 carbide treated Wistar rats in contrast to the control group, which is an evidence of Leukopenia. It is clear in [Table 4] that the hemoglobin level of the pups of the CaC2 carbide treated Wistar rats is reduced in comparison to the control group. This reduction in hemoglobin concentration could have resulted in tissue damage, generalized weakness or malaise, easy fatigability, body aches, or myalgias, Orthostatic symptoms which was evident on the pups. This is in contrast with pups of the Wistar rats fed with naturally ripened fruits, which had high level of hemoglobin; suggestive of good nourishment from the fruits. There is also clear indication from the results that the Total Red Blood Count of the pup from the Wistar rats fed with CaC2 is lower as compared with the control group ($p<0.05$), which specify anemia and other organ damage like the bone marraow. This result is in variance with the pups of the Wistar rats fed with naturally ripened fruits, which had higher level of RBC predicting the nutritional value of natural fruits. There is evidence of low platelet for both the pups of the Wistar rats fed with CaC2 and naturally ripened fruits with the pups of the control group. This result suggests that there is clothing thrombocytopenia in the calcium carbide fed Wistar rats. White blood cells like Neutrophils tend to increase in the body system when there are bacteria to combat with. This is the results from this present study; where the pups of the Wistar rats fed with CaC2 had higher neutrophil concentration and the pups from the Wistar rats fed with natural fruits had lower neutrophils. Lymphocytes which are blood cells that produce immune proteins called antibodies that target and fight specific disease-causing organisms tend to very low in the pups of the Wistar rats fed with CaC2 as against the pups of the control group. This is a pointer to the fact that calcium carbide is dangerous to the body, causing depletion in both the B- Lymphocytes and the T- lymphocytes. But there is high level of lymphocytes in the pups from the Wistar rats fed with natural fruits; which is a clear indication of the health benefit the natural fruits possess. Monocytes; the frontline defenders that attack any foreign agent the immune system considers abnormal in the body will tend to be high if there are invaders. This premise correlates with the present findings where monocytes in the pups from the Wistar rats fed with CaC2 are higher as against the pups of the control group. There is clear evidence of the rise in the level of the Eosinophils in the pups of the Wistar rats fed with CaC2 indicating the presence of parasitic agents in the pulps of the Wistar rats fed with CaC2.

## CONCLUSION

The result of this study have shown that, calcium carbide tend to have devastating effect on the haemtological parameters of the Pups of the Wistar rats fed with Calcium carbide. Blood cells production is impeded due its negative effect on hemapoietic stem cells, protection against foreign bodies is compromised due to low TWBC resulting tissue hypoxia, hemorrhage, stunted growth and amongst other negative consequences; which are passed from parents to offspring as clearly exposed in this study. The public should be enlightened of the consequences of using chemicals like Calcium Carbide in fruits ripening.

## REFERENCES


- Essien, E. B., Onyegeme-Okerenta, B. M., & Onyema, J. O. (2018). Calcium carbide as an artificial fruit-ripening agent and its physiological

effects on Wistar rats. *Clin Exp Med Sci*, *6*(1), 47-61.

- Fattah, S. A., & Ali, M. Y. (2010). Carbide ripened fruits-a recent health hazard. *Faridpur Medical College Journal*, *5*(2), 37-37.
- Gbakun, S. A., Ubwa, T. S., Ahilem, U. J., Obochi, O. G., Nwannadi, I. A., & Yusufu, M. I. (2018). Calcium carbide treatment on some physicochemical characteristics of broken and mummy mango fruits. *American Journal of Food Technology*, *13*(1), 23-31.
- Halliwell, B., & Gutteridge, J. M. C. (1985). The chemistry ofoxygen radicals and other oxygen-derived species. *In: Free Radicals in Biology and Medicine. New York: Oxford University Press. 20-64.2*.
- Hayes, D. P. (2005). The protective role of fruits and vegetables against radiation-induced cancer. *Nutrition reviews*, *63*(9), 303-311.
- Henriksen, T., Haugen, G., Bollerslev, J., Kolset, S. O., Drevon, C. A., Iversen, P. O., & Clausen, T. (2005). Fetal nutrition and future health. *Tidsskrift for den Norske Laegeforening: Tidsskrift for Praktisk Medicin, ny Raekke*, *125*(4), 442-444.
- Igbinaduwa, P., & Aikpitanyi-Iduitua, R. (2016). Calcium carbide induced alterations of some haematological and serum biochemical parameters of wistar rats. *Asian J Pharm Health Sci*, *6*(1), 1396-1400.
- Kaczmarski, M., Wójcicki, J., Samochowiec, L., Dutkiewicz, T., & Sych, Z. (1999). The influence of exogenous antioxidants and physical exercise on some parameters associated with production and removal of free radicals. *Die Pharmazie*, *54*(4), 303-306.
- Kendrick, M. (2009). The origin of fruit ripening. *Scientific American™. New York: Nature America Inc*. www.scientificamerican.comRetrived:2 June 2012.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, *54*(4), 275-287.
- Marchand, M. C., & Langley-Evans, S. C. (2001). Intrauterine programming of nephron number: the fetal flaw revisited. *Journal of Nephrology*, *14*(5), 327-331.
- Nesterenko, T. H., & Aly, H. (2009). Fetal and neonatal programming: evidence and clinical implications. *American Journal of Perinatology*, *26*(03), 191-198.
- Rossato, S. B., Haas, C., Raseira, M. D. C. B., Moreira, J. C. F., & Zuanazzi, J. A. S. (2009). Antioxidant potential of peels and fleshes of peaches from different cultivars. *Journal of medicinal food*, *12*(5), 1119-1126.