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Calcium Carbide as an Artificial Fruit-Ripening Agent and its Physiological Effects on Wistar Rats

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Abstract

This study examined the possible effect of selected fruits ripened with calcium carbide on some hormonal parameters, oxidative stress enzymes, haematology indices, histopathology of male and female Wistar rats and semen analysis of male rats. Three fruits (pawpaw, mango and plantain) were ripened using calcium carbide. Fifty-six Wistar rats (28 males, 28 females) were used for this experiment and were in groups of four with male and female separated. The animals were fed 2ml per body weight with both natural and artificially ripened fruit juice orally for 30 days. Hormonal assay results for the female animals showed a significant increase ($p<0.05$) in estrogen levels of rats fed with artificially ripened mango, while that of the male rats showed a significant increase ($p<0.05$) in testosterone concentration of rats fed with artificially ripened mango. Oxidative stress enzymes for both male and female rats showed a significant increase in reduced glutathione concentration of animals fed with artificially ripened mango. Superoxide dismutase enzyme was significantly decreased in rats fed with artificially ripened mango. Haematology results 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.

Keywords: Artificial ripening, calcium carbide, fruits, hormonal assay, ovaries, testes, photomicrographs

Introduction

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. After certain chemical changes, these acids are transformed into sugars and the fruits turn sweet. In fruit ripening process, chlorophyll is produced and at the same time decomposed. Starch is reduced by amylase to produce sugar. Pectin is converted by pectinase and decomposition of pectin, in this case, unglues the fruit cells. The cells being able to slip past one another makes the fruit further soft.

Artificial ripening agents are used to hasten up the process of ripening of fruits after they are picked before full ripening. This is done for faster and more uniform ripening. Later on, researches showed that treating of fruits with high temperature also triggers fruit ripening, (Bhattarai *et al.*, 2005). Generally, 80% of fruits are ripened artificially through these agents (Dhembare, 2013a; 2013b). They are different commonly used artificial ripeners in the world including ethylene glycol, ethylene, ethephon, acetylene gas and calcium carbide. Ethylene is a natural plant growth regulator, it is a gaseous hormone naturally produced in fruit. Ethylene being a natural hormone does not pose any health hazard for consumers of the fruits. It is a de-greening agent, which can turn the peel from green to perfect yellow (in the case of bananas) and maintain the sweetness and aroma of the fruit, thus value addition in the fruit is possible as it looks more appealing. It has been known for a long time that treatment of unripe fruits with ethylene would merely stimulate natural ripening until the fruit itself starts producing ethylene in large quantity in the fruit, (Siddiqui & Dhua, 2010). Commercially, it is expensive to produce, involving the use of hazardous materials (Singal *et al*., 2012). Out of all the ripening agents, calcium carbide is commonly used, because it is inexpensive and easily purchased. The use of this chemical for this purpose is illegal in most countries (Siddiqui and Dhua, 2010), but it is freely used in India, Pakistan, Bangladesh, Nepal and other countries like Nigeria. The need for artificial fruit ripening is often encountered when fruit sellers offer fruits to the customers before the due season to meet high demand and make a high profit. However, it is harder to find physical differences between artificially ripened fruits and naturally ripened fruits during the actual season of ripening. It has been reported that though the cosmetic quality of artificially ripened fruits like the external color and the texture increases, organoleptic qualities, nutritional value and shelf life is reduced (Siddiqui and Dhua, 2010; Hakim *et al*., 2012). Moreover, most of the ripening agents are toxic and their consumption can cause serious health problems such as heart disease, skin disease, lung failure and kidney failure. Researchers have also reported that regular consumption of artificially ripened fruits may cause dizziness, weakness and heart-related disease (Asif, 2012). These ripening agents may also be composed of different chemicals as impurities which are toxic for human health.

Thus humans are at risk of short term and long term health effects simply by eating fruits that are induced to ripen. 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 (Orisakwe *et al*., 2012). With the ever-increasing demand in consumption of fruits as dietary source of minerals, vitamins and dietary fiber, farmers who are not able to meet up with these demands and some because of greed now tend to use artificial fruit ripening agents to facilitate immediate ripening fruits to make them presentable and appealing to man without considering the side effects of these chemicals to human health.
It is therefore important to perform qualitative and quantitative analysis of ripening agents within the fruit skin and flesh to understand the relevant health hazard, (Mursalat *et al*., 2013). It is also important to quantify the presence of these chemicals within fruit flesh and to analyze their impact on the food value of artificially ripened fruits. The present study, therefore, is aimed at ascertaining the effect of an artificial ripening agent (Calcium carbide) on some biological parameters of Wistar rats.

Materials and Methods

Reagent and chemicals

Accu-Bind Elisa Kit used include: Testosterone kit (Monobind Inc. Lake Forest CA 92630, USA), Follicle stimulating hormone kit (Monobind Inc. Lake Forest CA 92630, USA), Luteinizing hormone kit (Monobind Inc. Lake Forest CA 92630, USA), Estradiol kit (Monobind Inc. Lake Forest CA 92630, USA) and Thyrotropin (TSH) kit (Monobind Inc. Lake Forest CA 92630, USA). All other reagents used were of analytical grades.

Procurement and Preparation of Samples

Matured unripe pawpaw, mangoes and plantains were bought from Choba market, Obio/Akpor Local Government Area of Rivers State. The sampled fruits were separated into two equal parts. One part was left to ripen naturally at room temperature. The other part was ripened artificially with 5g of CaC_2 kg^{-1} weight of fruits in a sack and left in a properly covered bucket. After ripening, sampled fruits were washed and juiced. Five hundred grams (500g) of pawpaw, mango and plantain were separately blended in an electric blender together with 500ml/1L deionised water and the juice filtered with a fine sieve. The juices were poured into clean rubber bottles, well labelled and stored in the refrigerator immediately for further use.

Experimental design

Fifty-six Wistar rats (28 males, 28 females) with weight ranging from 150-275 g, were obtained from the animal house of Department of Biochemistry, University of Port Harcourt. They were separated in groups of 4 in clean plastic cages with male and female separated. All animal procedures were performed under a protocol approved by the Laboratory Animal house care of the Department of Biochemistry,

University of Port Harcourt and in accordance with National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The experimental animals were left to acclimatize for two (2) weeks in a conducive condition and fed with standard growers mash with clean water before administration of the juices.

Groups	**Treatment**
Group 1 (A & B)	Normal feed and water
Group 2 (A & B)	2ml of naturally ripened pawpaw
Group 3 (A & B)	2ml of artificially ripened pawpaw
Group 4 (A & B)	2ml of naturally ripened mango
Group 5 (A & B)	2ml of artificially ripened mango
Group 6(A & B)	2ml of naturally ripened plantain
Group 7 (A & B)	2ml of artificially ripened plantain

A = Male Wistar Rats

B = Female Wistar Rats

The juices were administered orally to the animals for 4 weeks (30 days). After 30 days of administration, the animals were sacrificed after being anaesthetized with chloroform. Five ml of blood was obtained from animals through cardiac puncture. The testes and epididymis were collected from male rats while ovaries were collected from the female rats for analysis.

Statistical Analysis

Statistical analysis was done using SPSS. Descriptive statistic was done using mean and standard deviation and results displayed using Graphs. Reference statistics was done using ANOVA and the p-value was set at 0.05 significant levels.

Results

Effect of Ingestion of Calcium Carbide Ripened Fruit on Body Weight of Male Wistar Rats

Table 1 shows results of the effect of ingestion of calcium carbide ripened fruit on bodyweight of experimental rats. Values obtained showed no significant difference ($p>0.05$) between the treated groups when compared with the control.

Table 1: Effects of Ingestion of Calcium Carbide Ripened Fruit on Body Weight of Male and Female Wistar Rats

Groups	Male weight before administration (g)	Male weight after administration (g)	Female weight before administration (g)	Female weight after administration (g)
Control	193.75±12.5	218.75±23.93	156.25±12.50	175±20.41
N.R Pawpaw	206.25±12.5	225±20.41	168.75±12.50	212.5±14.43
A.R Pawpaw	206.25±23.93	250±50	175±0.01	200±0.01
N.R Mango	206.25±12.5	243.75±23.93	175±20.41	193.75±12.50
A.R Mango	225±20.41	250±50	150±0.01	200±0.01
N.R Plantain	225±20.41	256.25±12.5	181.25±23.94	200±0.01
A.R Plantain	225±20.41	256.25±51	181.25±23.94	243.75±37.50

Results are expressed as mean ±SEM

N.R =Naturally Ripened

A.R =Artificially Ripened

Effects of Ingestion of Calcium Carbide Ripened Fruit on Haematological indices of Female Wistar Rats

The results of the effect of ingestion of Calcium Carbide ripened Fruit on haematological indices of Female Wistar Rats is as shown in Table 2. The study revealed that these fruits significantly increased the PCV level both for the naturally ripened and artificially (Calcium Carbide) ripened fruits, though fruits that were artificially ripened show lower PCV as compared to the naturally ripened fruit. The study also showed that there was no statistically significant effect on the Hb and RBC levels of the Female Wistar Rats. The study revealed that these fruits significantly increased the WBC and Eosinophil levels of rats both for the naturally ripened and artificially (Calcium Carbide) ripened fruit, with rats administered artificially (Calcium Carbide) ripened Pawpaw showing a higher increase, while rats administered naturally ripened Mango showed a significant decrease in WBC level. This study revealed that these fruits significantly increased the platelet counts of experimental animals especially in those administered naturally ripened plantain. Result obtained showed that these fruits elicited slight reduction of lymphocytes with little or no significant increase in Monocytes and basophil when compared with the control at $p \leq 0.05$. However, a significant increase was observed in the number of Neutrophils count present in the artificially ripened fruits.

Table 2: Effects of Ingestion of Calcium Carbide Ripened Fruit on Haematological indices of Female Wistar Rats.

Parameters	Control (A)	N.R pawpaw (B)	A.R pawpaw (C)	N.R mango (D)	A.R mango (E)	N.R plantain (F)	A.R plantain (G)
PCV(%)	33.75±0.96	41.00±2.16a	39.25±5.80a	42.00±6.98a	38.00±2.00*	40.75±4.11	39.00±4.58a
Platelet count($\times10^3$/l)	52.50±81.77	25.50±4.04a	89.75±10.11ab	36.75±7.68a	40.33±4.48*α	307.50±51.50a	128.67±11.27ab
Hb(g/l)	10.35±0.29	12.58±0.65	12.12±1.91	12.96±2.28	11.66±0.61	12.49±1.26	11.95±1.41
WBC($\times10^9$/l)	1.79±0.36	2.59±1.09a	2.86±2.36a	1.38±0.17a	1.93±1.00ab	2.43±0.96a	2.58±1.75
RBC($\times10^6$/µl)	6.53±0.28	6.77±0.65	6.66±2.26	2.90±2.47a	6.31±0.41b	3.01±0.43a	5.53±0.83ab
Lymphocytes(%)	61.33±2.08	55.2±5±5.91a	53.50±5.97a	59.00±5.57a	56.25± 5.91a	50.50±3.70a	55.75±5.66a
Monocytes (%)	30.00±2.08	31.00±6.32	29.25±6.18	27.67±5.51	27.60±4.65	30.50±1.29	30.25±3.77
Basophils(%)	4.33±0.58	7.75±1.26a	8.00±2.16a	7.67±2.08a	9.75±1.71a	9.75±2.06a	8.25±2.06a
Neutrophils(%)	2.67±0.58	3.50±1.29a	6.25 ±1.26a	3.67±1.15a	4.50±0.58a	3.75±0.96a	6.25±1.71ab
Eosinophil(%)	1.67±0.58	2.50±0.58a	3.00±0.82a	2.33±1.15a	2.00±0.82a	3.00±1.41a	2.00±0.82ab

Results are expressed as mean ±SEM

"a" shows significant difference when compared to the control

"b" shows significant difference when compared to the naturally ripened fruits

Effects of Ingestion of Calcium Carbide Ripened Fruit on Haematological indices of Male Wistar Rats

The results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on haematological indices of Male Wistar Rats is as shown in Table 3. The study revealed no significant difference in RBC except for a significant increase in rats administered artificially ripened mango and plantain and little or no change in PCV level both for the animals administered the naturally and artificially ripened (Calcium Carbide) fruits. The result showed a significant decrease in the WBC Count and lymphocytes of the female rats administered these fruits while no significant difference in the level of monocytes was observed. Basophil and Neutrophil levels increased while Eosinophils were elevated significantly in the female rats given artificially ripened mango and Pawpaw. The result revealed that the Hb levels increased significantly in rats administered artificially ripened Pawpaw and plantain with no notable differences in other groups when compared to the control. The study revealed an elevated level of platelets especially in rats fed with artificially ripened plantain and naturally ripened Pawpaw and plantain.

Table 3: Effects of Ingestion of Calcium Carbide Ripened Fruit on Haematological indices of Male Wistar Rats

Parameters	Control (A)	N.R pawpaw (B)	A.R pawpaw (C)	N.R mango (D)	A.R mango (E)	N.R plantain (F)	A.R plantain (G)
PCV(%)	35.67±3.51	41.50±6.19*	42.00±7.15*	46.00±3.46*	45.00±6.98*	40.00±1.00	42.00±5.10*
Platelet count($\times10^3$/l)	33.33±6.51	61.50±48.18*	28.75±9.95	41.00±6.63*	41.50±35.91*	61.33±14.01*	331.00±40.97
Hb(g/l)	10.94±1.08	12.81±2.03	15.03±1.99	14.18±1.21	13.95±2.30	12.26±0.31	12.95±1.71
WBC($\times10^9$/l)	2.45±0.91	2.88±0.73	1.80±0.29*α	2.16±0.85*	2.00±0.71	2.27±0.55*	2.18±0.64*
RBC($\times10^6$/ml)	5.20±0.27	5.55±3.39	5.38±2.97	5.19±2.77	6.77±0.73*	7.59±0.64*α	5.41±0.42
Lymphocytes (%)	65.67±1.15	62.00±5.35	54.75 ±4.72	58.33± 2.52	56.00 ±6.32	53.25 ±6.85	60.50 ±1.91
Morocytes (%)	28.00 ±2.00	3.00 ±3.74	33.75 ±3.77	28.33 ±2.62	30.00 ±4.69	31.25± 4.99	25.50± 3.00
Basophils(%)	3.33 ±0.58	2.75± 1.71	8.00 ±1.83	7.00 ±1.00	6.75± 0.96	9.50± 1.29	8.25± 1.71
Neutrophils(%)	1.67 ±0.58	2.75 ±0.96	3.50± 1.29	4.00± 1.29	4.50± 1.29	3.50± 1.29	3.75± 1.26
Eosinophil(%)	1.33 ±0.58	1.75± 0.96	3.00 ±0.82	2.75 ±1.00	2.75± 0.96	2.00± 0.82	2.00± 0.82

Results are expressed as mean ±SEM

"a" shows significant difference when compared to the control

"b" shows significant difference when compared to the naturally ripened fruits

Effects of Ingestion of Calcium Carbide Ripened Fruit on Sex Hormones in Female Wistar Rats

The results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on FSH, LH, TSH, level of Female Wistar Rats is as shown in Table 4. This result showed significant increased ($p<0.05$) level of all tested hormones when compared to the control Group both for the Groups administered the naturally ripened and artificially (Calcium Carbide) ripened fruits.

Table 4: Effects of Ingestion of Calcium Carbide Ripened Fruit on Sex Hormones in Female Wistar Rats

Parameters	Control (A)	N.R pawpaw (B)	A.R pawpaw (C)	N.R mango (D)	A.R mango (E)	N.R plantain (F)	A.R plantain (G)
FSH(ng/ml)	0.33±0.32	1.38±0.04a	1.60±0.43ab	1.08±0.21a	1.30±0.66a	1.30±0.32a	1.03±0.15ab
LH (ng/ml)	0.09±0.03	1.53±0.75a	2.53±0.81ab	0.83±0.25a	1.00±0.99ab	0.98±0.71a	0.37±0.06ab
EST(ng/l)	25.50±4.80	73.25±26.55a	72.50±6.24a	43.00±10.89a	101.67±26.50ab	69.25±12.37a	54.00±36.17ab
TSH(ng/ml)	1.85±0.13	1.68±0.61a	1.10±0.57ab	1.50±0.96a	1.17±0.59ab	1.70±0.63a	2.27±0.38abs

Results are expressed as mean ±SEM

"a" shows significant difference when compared to the control

"b" shows significant difference when compared to the naturally ripened fruits

Effects of Ingestion of Calcium Carbide Ripened Fruit on Sex Hormones in Male Wistar Rats

The results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on FSH, LH, Testosterone, and TSH levels of Male Wistar Rats is as shown in Table 5. The result shows that FSH, LH and testosterone levels increased significantly both for the Groups administered the naturally and artificially (Calcium Carbide) ripened fruits, except for the Group administered artificially ripened plantain which showed little or no significant effect. TSH showed no significant difference in treated rats except for those administered with artificially ripened pawpaw.

Table 5: Effects of Ingestion of Calcium Carbide Ripened Fruit on Sex Hormones in Male Wistar Rats

Parameters	Control (A)	N.R pawpaw (B)	A.R pawpaw (C)	N.R mango (D)	A.R mango (E)	N.R plantain (F)	A.R plantain (G)
FSH(ng/ml)	0.11±0.06	1.18±0.77a	2.20±1.52a	1.05±0.26a	1.00±0.22a	1.10±0.53	0.13±0.05a
LH(ng/ml)	0.09±0.03	1.40±0.94a	2.23±0.42ab	1.60±0.63a	0.43±0.29ab	1.00±0.70a	0.18±0.10ab
TEST9ng/ml)	0.06±0.03	0.95±0.48a	1.38±0.51ab	0.85±0.34a	2.15±0.31ab	0.93±0.31a	0.13±0.05ab
TSH(ng/ml)	1.33±0.25	1.10±0.53a	2.45±2.03ab	0.68±0.22a	1.28±0.46ab	1.63±1.01a	1.05±0.39ab

Results are expressed as mean ±SEM

"a" shows significant difference when compared to the control

"b" shows significant difference when compared to the naturally ripened fruits

Effects of Ingestion of Calcium Carbide Ripened Fruit on Oxidative Stress Markers in Female Wistar Rats

The results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on Oxidative Stress Markers in Female Wistar Rat are as shown in Table 6. The study revealed a significant decrease in SOD activity except in animals administered artificially ripened plantain where there is a significant increase in SOD activity. CAT activities was not affected as no significant difference in CAT activity were observed when compared to normal control. There was no significant difference in MDA and GSH activities in all Groups administered the fruit samples.

Table 6: Effects of Ingestion of Calcium Carbide Ripened Fruit on Oxidative Stress Markers in Female Wistar Rats

Parameters	**Control(A)**	**N.R pawpaw (B)**	**A.R pawpaw (C)**	**N.R mango (D)**	**A.R mango (E)**	**N.R plantain (F)**	**A.R plantain (G)**
SOD(µmol/ml)	4.42±1.48	3.87±0.38a	4.02±0.53ab	3.77±0.15a	3.88±0.67a	4.59±0.33a	4.51±0.33a
CAT(µmol/ml)	0.44±0.15	0.39±0.04	0.40±0.05	0.38±0.01	0.39±0.07	0.46±0.00	0.45±0.03
MDA(nmol/ml)	1.10±0.26	1.48±0.11a	1.12±0.10α	1.91±1.28a	1.10±0.09a	1.04±0.27a	1.08±0.09
GSH(µmol/ml)	4.25±0.17	3.55±0.21a	4.02±0.19ab	2.17±0.32a	4.11±0.23ab	3.92±0.16a	4.01±0.19

Results are expressed as mean ±SEM

"a" shows significant difference when compared to the control

"b" shows significant difference when compared to the naturally ripened fruits

Effects of Ingestion of Calcium Carbide Ripened Fruit on Oxidative Stress Markers in Male Wistar Rats

The results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on Oxidative Stress Markers in Male Wistar Rat are as shown in Table 7. The result showed a significantly decreased SOD activities though with elevated values when compare to the animals on naturally ripened fruits. CAT activities was not affected as no significant difference in CAT activity was observed when compared to normal control. The MDA levels increased significantly, however the values obtained from male rats administered artificially ripened fruits was significantly lower than that observed in the Groups administered the naturally ripened fruits. GSH was significantly increased in the Groups administered the artificially ripened pawpaw and mango juices.

Table 7: Effects of Ingestion of Calcium Carbide Ripened Fruit on Oxidative Stress Markers in Male Wistar Rats

Parameters	**Control (A)**	**N.R pawpaw (B)**	**A.R pawpaw (C)**	**N.R mango (D)**	**A.R mango (E)**	**N.R plantain (F)**	**A.R plantain (G)**
SOD(µmol/ml)	4.37±0.69	3.35±0.39a	3.97±0.29ab	2.80±1.94a	4.20±0.12ab	3.87±0.40a	4.41±0.74b
CAT(µmol/ml)	0.44±0.07	0.33±0.04a	0.40±0.03a	0.28±0.19a	0.42±0.01a	0.39±0.04a	0.44±0.07b
MDA(nmol/ml)	0.90±0.10	1.53±0.17a	1.00±0.18a	1.26±0.27a	0.99±0.10b	0.90±0.10	1.02±0.20
GSH(µmol/ml)	4.71±0.28	3.01±0.33a	4.05±0.15ab	3.57±0.28a	4.71±0.09b	4.52±0.11	3.77±0.23ab

Results are expressed as mean ±SEM

"a" shows significant difference when compared to the control

"b" shows significant difference when compared to the naturally ripened fruits

Effects of Ingestion of Calcium Carbide Ripened Fruit on Semen potency of Male Wistar Rats

The results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on Semen potency of Male Wistar Rats is as shown in Table 8. The result showed that particulate debris measured by debris quantitative and microscopic examination of semen (D1) and morphological structures (MS) increased significantly in rats administered artificially ripened Mango, Pawpaw and plantain. However, there was a reduction in sperm count (C) in rats administered with artificially ripened mango and plantain as well as a significant decrease in motility quantitation of both motile and non-motile spermatozoa (M1) of rats administered with artificially ripened pawpaw and plantain.

Table 8: Effects of Ingestion of Calcium Carbide Ripened Fruit on Semen potency of Male Wistar Rats

Parameters	Control (A)	N.R pawpaw (B)	A.R pawpaw (C)	N.R mango (D)	A.R mango (E)	N.R plantain (F)	A.R plaintain (G)
M1(%)	65.00±8.66	72.50±3.54a	61.25±14.36ab	70.00±0.001	65.00±15.81	77.50±3.54a	57.50±3.54ab
D1 (%)	5.00±0.00	5.00±0.00	8.75±2.50ab	5.00±0.001	8.75±2.50ab	10.00±0.001a	5.00±0.001
C (%)	61.67±2.89	68.00±4.24a	73.25±8.85ab	83.50±4.95a	74.50±5.26ab	79.50±2.12a	73.00±4.24ab
MS (%)	5.00±0.001	10.00±0.001	7.50±2.89a	5.00±0.001	7.50±2.89a	5.00±0.001	10.00±0.001a

Results are expressed as mean ±SEM

"a" shows significant difference when compared to the control

"b" shows significant difference when compared to the naturally ripened fruits

Histology Result

Photomicrographs of the testes of male rats from the group fed with artificially ripened mango showed that there were losses of germs cells of the seminiferous tubules and they also appeared degenerated, some germ cells were also detached from the epithelium and found in the lumen.

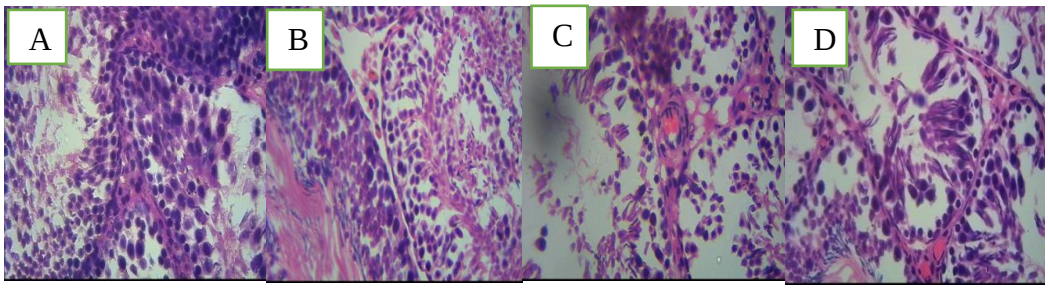

Plate 1: Photomicrographs of sections of the testes of male rats fed with: **A** (control) – Normal rat feed, **B** - naturally riped mango, C & D - artificially ripened mango. H & E X40.

Discussion

Results obtained, have elucidated the effects of ingestion of calcium carbide ripened fruit on male and female Wistar rats. The study showed that this fruit ripening agent has little or no effect on the body weight of the control and treated male and female Wistar rats. However, some of the haematology indices of the animals were compromised. PCV, Hb, and RBC Levels of Female Wistar Rats administered these fruits had significantly increased PCV level both for the Groups on the naturally and artificially (Calcium Carbide) ripened fruits, though Groups that were administered the artificially ripened fruits show lower PCV compared to those given the naturally ripened fruits, higher than the PCV of the control Group.

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 (Purves *et al.*, 2003). 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 (Zubieta *et al.*, 2007). The study also showed little or no significant effect on the Hb and RBC levels of the Female Wistar Rats. Haemoglobin failure may be as a result low haemoglobin concentrations (anaemia) or by the inability of each molecule to bind oxygen. Generally, haemoglobin failure is differentiated from hypoxemia (low pressure of oxygen in the blood) (McGaffigan, 1996). Other causes of decreased haemoglobin concentration are cancers that affect the bone marrow, kidney diseases, loss of blood, nutritional deficiencies and chemotherapy. Haemoglobin concentration that is increased is as a result of increased numbers or red blood cells size known as polycythemia. This elevation may be as a result of lung disease, smoking, dehydration, and some tumours (Padmanaban and Todra, 2011).

The results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on WBC, and Eosinophil Levels of Female Wistar Rats revealed that these fruits significantly increased the WBC and Eosinophil level at $p \leq 0.05$ both for the rats administered the naturally and artificially (Calcium Carbide) ripened fruit, though those administered artificially (Calcium Carbide) ripened Pawpaw showed higher levels, while those administered naturally ripened Mango showed marked reduction in WBC level. White blood cells (WBC(s) (leukocytes), are cells of the immune system associated with preserving the body from infectious disease and foreign bodies. Hematopoietic stem cells are multipotent cells in the bone marrow where white blood cells are produced. Leukocytes are found all over the body, including the blood and lymphatic systems. Eosinophils consists of about 2-4% of the total white blood cell. This count alternates throughout the day, and during menstruation. It increases in response to allergies, parasitic infections, diseases of collagen, spleen and central nervous system. They are not usually found in the blood, but abundance in the mucous membranes of the respiratory, digestive and urinary tracts (Saladin, 2012).

This result showed that these fruits significantly increased the platelet counts, especially in Groups administered the naturally ripened plantain while Groups admini-

stered both naturally and artificially (Calcium Carbide) ripened mangoes showed a marked reduction in platelet counts. However, rats administered with artificially (Calcium Carbide) ripened Plantain and pawpaw showing a significant rise in platelet counts. Basophil and Neutrophil levels increased in all Groups while Eosinophils were significantly increased in Groups administered artificially ripened mango and pawpaw. All the Groups, however, showed little or no significant difference in the level of monocytes. Hb levels were observed to have increased significantly in Groups administered artificially ripened pawpaw and plantain with no notable difference in other Groups when compared to the control.

The study further revealed a decrease in SOD activities except in artificially ripened plantain where there was a significant increase in SOD level. Superoxide dismutase is an enzyme that intermittently catalyses the dismutation of the superoxide (O_2^-) radical into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is generated as a by-product of oxygen metabolism; cells may be destroyed if not regulated (Hayyan *et al.*, 2016). Therefore, increase in the activity of SOD observed in male rats especially in the groups fed with artificially ripened fruits is an indication that there was an increased generation of superoxide (O_2^-) radical as result of consumption of calcium carbide ripened fruits.

CAT activities were not affected as no significant difference in CAT activity was observed when compared to normal control. The MDA levels increased significantly, however, the values obtained from male rats fed with artificially ripened fruits were lower than that recorded in the group fed with naturally ripened fruit. GSH activity increased in the Groups given artificially ripened pawpaw and mango, with a significant reduction in the groups given naturally ripened pawpaw and mango. Oxidative stress can be measured by the ratio of reduced glutathione to oxidized glutathione in the cell. (Pastore *et al.*, 2001). In normal cells and tissues, above 90% of the total glutathione is found in the reduced form (GSH) and lower than 10% are in the disulphide form (GSSG). An elevated level ratio of GSSG to GSH is an indication of oxidative stress (Halprin and Dhkawara, 1967). Moderately reduced level of GSH causes systematic deterioration of the cell while extremely low levels result in rapid cell death (Hali, 1999).

Results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on FSH, LH, TSH, level of Female Wistar Rats showed a significant increase in the levels of all tested Hormones in rats fed with naturally and artificially ripened fruit when compared to the control group.

There was a significant increase in oestrogen level in all rat groups administered various samples of ripened fruits. In the male rats, there was a significant increase in FSH, LH and testosterone levels at $p \leq 0.05$ both for the animals on the naturally and artificially ripened fruit, except for the animals administered the artificially ripened plantain which showed little or no significant effect on the male rats. TSH value was not significant in treated rats except for those administered artificially ripened pawpaw where a significant increase in TSH was observed.

The results of the Effects of Ingestion of Calcium Carbide Ripened Fruit on Semen potency of Male Wistar Rats showed the presence of particulate debris D1 and MS which was significantly increased in rats fed with artificially ripened Mango, pawpaw

and plantain. The study also revealed a significant decrease in M1 (morphology) and sperm count (C) of Semen in rats fed with artificially ripened Mango, pawpaw and plantain when compared with group feed with naturally ripened fruits.
Photomicrographs of the ovaries of female rats showed no abnormalities in the control as well as in all the treated groups. However, photomicrographs of the testes male rats from the group fed with artificially ripened mango showed that there were losses of germs cells of the seminiferous tubules and they also appeared degenerated, some germ cells were also detached from the epithelium and found in the lumen. Histopathology effect was observed more in the groups fed with mango than pawpaw and plantain. This may be attributed to the fact that the animals feed on the whole fruit including the outer coat which contains the ripening agent as compared to pawpaw and plantain whose outer coat has to be peeled off and discarded before feeding the animals the fleshy part.

Conclusion

Some countries use Calcium carbide for artificially ripening of fruits. 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 (Siddiqui and Dhua, 2010; Mohammad, 2012). This study, however, has demonstrated that consumption of fruits ripened with calcium carbide could be a potential human health risk, as it tends to lower the body's ability to resist infection, affect hormonal balance and could affect the overall reproductive function and health of the male species especially, causing infertility.

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