



Calcium Carbide Ripened *Musa sapientum*-Induced Reduction in White Blood Cell Count in Challenged Albino Wistar Rats

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ABSTRACT

The benefits of fruits in healthy living and disease prevention seem to be threatened due to the artificial ripening of fruits. This study investigated the effect of calcium carbide ripened *Musa sapientum* (banana) on some haematological variables of challenged Wistar rats. A total of 51 rats grouped into two main groups of 14 and 21 days treatment. Each group subdivided into rats fed with I. Naturally Ripened Banana with Immunization (NRB+IM), II. Naturally Ripened Banana without Immunization (NRB-IM), III. Chemically Ripened Banana with Immunization (CRB+IM), IV. Chemically Ripened Banana without Immunization (CRB-IM) and V. (control). The controls (11) were only fed with normal rat chow. NRB groups (20) were fed with normal rat chow and administered naturally ripened banana juice orally. The CRB groups (20) were fed with normal rat chow and administered chemically ripened banana juice. All rats were allowed free access to food and water during the study. Whole blood samples were collected on the 14th day and 21st day for complete blood count (CBC). The result showed a significant decrease in WBC in III (6460 ± 972.94) for 14th day when compared to other groups and control (10683 ± 218). Also, the 21st day WBC in III (5800 ± 1228.01) showed a significant decrease compared to other groups and control (12720 ± 326.19). Besides, there were decreases in PCV and Hb values in 21st day samples of III. We therefore, conclude that carbide ripened banana induces leucopenia probably due to suppressed haemopoiesis in SRBC immunized rats.

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Keywords: *Musa sapientum*, leucopenia, erythropoiesis, lymphocytes, haemopoiesis.

Introduction

Fruit ripening is a natural process in which the fruit goes through various chemical changes and gradually become sweet, flavoured, coloured, gets soft and become palatable.¹ In recent time, this process has been facilitated to meet the growing demand for fruits due to increasing awareness of its nutritional and health benefits.² Many marketers and farmers in most developing climes including Nigeria ripen their fruits with chemicals such as calcium carbide (CaC₂). One of the fruits commonly affected by this practice is banana.³ The natural ripening agent is ethylene gas which is produced by most fruits at maturity.⁴ In most developed countries, ethylene gas is employed in quickening ripening⁵ while CaC₂ ripening is prohibited.⁶ However, in many developing countries, this gas is not readily available, so the traders and farmers result to the use of calcium carbide as an alternative source. It can produce acetylene gas which seems to have the same ripening effect as ethylene gas.⁷ Many have reported various dangers of carbide ripened fruits.⁸⁻¹⁰ They reported that CaC₂ may contain traces of arsenic and phosphorus which is of great concern to human health. These hazardous and carcinogenic chemicals create short-term and long-term health problems.¹¹ Banana is a familiar tropical fruit. From its native Southwestern Pacific home, the banana plant spread to India by about 600 BC and later on it

spread all over the tropical world.¹² It is possibly the world's oldest cultivated crop. It even spread into the Islands of the Pacific and to the West Coast of Africa as early as 200-300 BC.^{12,13} Serotonin, nor-epinephrine, tryptophan, indole compounds, tannin, starch, iron, crystallisable and non-crystallisable sugars, vitamin C, B-vitamins, albuminoids, fats, mineral salts have been found in the fruit pulp.¹⁴⁻¹⁶ The usefulness of this fruit include antihypertensive,¹⁷ antimicrobial,¹⁸ antidiarrhoea,^{19,20} antiulcerative,²¹ hypoglycemic,²² hypocholesterolaemic,²³ antioxidant,²⁴ diuretic,²⁰ wound healing,²⁵ and anti-allergic,²⁶ where they all functions as effective immune response. Impure forms of calcium carbide have been found to contain arsenic and phosphorus.³ Early symptoms of arsenic or phosphorus exposure include diarrhoea, thirst and irritation in the eyes, mouth, nose and throat. Chronic exposure to the chemical could lead to peptic ulcers.²⁷ Calcium carbide has carcinogenic and neurological disorders properties. It can result in tingling sensation, numbness and peripheral neuropathy. If pregnant women consume fruit ripened with carbide, the children born could develop abnormalities.⁷ The use of calcium carbide is not only toxic to consumers, but it is also harmful to those who handle it. It affects the neurological system, resulting in headache, dizziness, mood disturbances, sleepiness, mental confusion and seizures on a short-term basis, while in the long-term it can cause memory loss and cerebral oedema.⁷ This work therefore, sets out to examine the effect of calcium carbide on some haematological variables of rats challenged with sheep red blood cells (SRBCs).

Materials and Methods

Collection of Fruits

Unripe banana fruits were purchased in January 2017 from an open market in Benin City and their identity authenticated by Prof. (Mrs) Okungbowa of the Department of Plant Biology and Biotechnology, University of Benin.

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Experimental Animals

In this study, 51 Wistar albino rats were used. The rats weighing 150 -200 g were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. All experimental animals were handled in accordance with the US National Institutes of Health Guidelines for the care and use of laboratory animals.

Chemical Ripening of Fruits

The matured unripe banana was purchased in January 2017 from the open market in Benin City area of Edo State. The fruit was exposed to calcium carbide. The exposure was done by applying the calcium carbide powder (2 g/kg weight of fruit) on the surface of the fruits.¹²

The fruits were then placed in a sack, tied up, covered in a container, and stored for 24 hours

After a period of twenty-four hours, the fruits (now ripe fruits) was exposed by opening the sack.

Preparation of Fruit Juice

The fresh banana fruit, free of blemishes or obvious defects was used. The fruits were washed and manually peeled. Juice was obtained by blending the banana pulp using an electric blender and immediately diluted with distilled water to a volume of 1:5 for use. This was also done for the naturally ripened banana (NRB) except that it was not exposed to calcium carbide.

Preparation of Sheep Red Blood Cells (SRBCs)

Fresh sheep blood was collected at the point of slaughtering from central abattoir at Aduwawa Livestock Market in Benin City. This was collected for use in sterile Alsever's solution in 1:2 proportion of Alsever's solution (freshly prepared). Blood was kept in the refrigerator and processed, for the preparation of SRBC batch, by centrifuging at 2000 rpm for 10 minutes, washed with physiological saline 4-5 times and then suspended into buffered saline for further use.

Immunization of Rats with SRBCs

Rats were immunized with a single intravenous injection of 2×10^8 SRBC in 1 mL saline using Improved Neubauer Counting slide as described by Koganei *et al.*,²⁸

Grouping of Animals and Treatment Schedule

Healthy Wistar albino rats, of either sex, were used for the study. Ten groups of 5 rats (except the 21st day control with 6 rats) each, grouped into 14th and 21st day sample collection was used for the study. The animals were administered the juice with the aid of orogastric tube (gavage) daily before feeding. The cages were cleaned, and wood shavings, feed and water were replaced daily. The treatment schedule was as follows:

Group I (NRB+IM): naturally ripened banana juice treated for 14th and 21st day immunization with SRBC (3 mL/kg p.o)

Group II (NRB-IM): naturally ripened banana juice treated without immunization on the 14th and 21st day (3 mL/kg p.o)

Group III (CRB+IM): Carbide ripened banana juice treated 14th and 21st day immunization with SRBC (3 mL/kg p.o)

Group IV (CRB-IM): Carbide ripened banana juice treated without immunization on the 14th and 21st day (3 mL/kg/p.o)

Group V (Control): Control with no fruit and no immunization

The group V received vehicle (saline), while that of the other groups received treatment, as per the schedule for 14 days and 21 days respectively.

Blood Collection from Experimental Rats

On the 14th day (i.e. 7th day Post-immunization with SRBCs) and 21st (i.e. 14th day Post-immunization) of the treatment, 5 mL blood samples were collected from all relevant groups by cardiac puncture into plain tubes containing potassium EDTA. The samples were analyzed using Sysmex autohaematology analyzer (XP, Japan) at the Haematology laboratory of the University of Benin Teaching Hospital (UBTH), Benin City.

Statistical Analysis

Statistical analysis including descriptive statistics were carried out using the Statistical Package for Social Scientists (SPSS) version 21.0 (IBM, USA). All values were expressed as Mean \pm Standard Error of Mean. The One-way Analysis of Variance (ANOVA) was used to compare means in the five study groups of participants. Sources of the differences in means

were indicated by the Post hoc Analysis. Different superscripts letters indicate statistical difference in mean values. Comparisons of different samples of the same group data were performed by using the Student t-test. In all cases, $p < 0.05$ was considered significant.

Results and Discussion

The haematological findings on the 14th day (7 days post-immunization) as shown in Table 2, reveal that the total white blood cell count (6460 ± 972.94) in the rats fed with CRB+IM was observed to be significantly lower than all other study groups. The outcome was however similar in NRB-IM (8420 ± 720.69), CRB-IM (8080 ± 86.02) and control (10683.33 ± 218.20) which were significantly lower than NRB+IM (11800 ± 230.21). The reduced WBC count in the challenged CRB rats could be due to impaired immune response as a result of the carbide used in ripening the banana which may have led to arsenic toxicity.³ Alimbe,²⁹ stated that one, among the validated methods for investigating the immune-toxic potentials of toxicants, primarily in rodents is changed in cellular components of blood. This finding agrees with those of previous reporters^{3,30} who observed comparatively lower white blood cell counts in mice exposed to toxicant (CaC₂) concentrations than the unexposed group. However, it seems not to agree with the finding of Igbinaduwa and Aikpitanyi¹⁰ who found higher leucocyte count and however increased percentage lymphocyte count with exposure to CaC₂ in rats without immunization compared to controls. The finding with NRB+IM as being significantly higher is noteworthy despite NRB-IM being similar to the control group. It has further confirmed reports,³¹⁻³³ that regular intake of fruits boosts immune capabilities. It is reported that increasing the consumption of fruits is a practical strategy for significantly reducing the incidence of chronic diseases.³⁷ The lymphocyte counts in CRB+IM (89.66 ± 0.27) and CRB-IM (86.28 ± 0.47) were similar but significantly higher compared with NRB-IM (80.26 ± 2.91), NRB+IM (78.42 ± 4.90) and control (70.78 ± 0.64) which differed significantly across the groups. The high lymphocyte in the two CRB groups could be due to the recognition of the fruit administered as being toxic or antigenic in nature and the effect of the SRBCs in the case of CRB+IM. However, the percentage lymphocyte is only a function of the available leucocyte count. The granulocyte count in NRB+IM (9.48 ± 4.96) and NRB-IM (8.72 ± 1.42), were similar but varied significantly across other groups: CRB+IM (4.00 ± 0.13); CRB-IM (5.56 ± 0.17); control (15.87 ± 0.30). The least count found again in the CRB groups could be indicative of weak response to invasion as granulocytes are normally first line defenders.³⁴ The platelet count showed that CRB+IM (408800 ± 1392.84) with NRB-IM (412800 ± 41230.33), NRB+IM (433600 ± 61881.01) and control (509666.67 ± 1763.54) were similar and are significantly higher than CRB-IM (331600 ± 3854.87). The high platelet count in the CRB+IM could be due to haemostatic response as a result of tissue injury during immunization since it is significantly lower in CRB-IM.³⁸ There was no change however in weight, PCV and Hb values. This finding appears different from that of Dhembare and colleagues^{30,35,36} who worked with rabbits.

The 21st day sample results as shown in table 2 indicates that PCV and Hb in CRB+IM (37.88 ± 0.55 ; 13.04 ± 0.21) and CRB-IM (38.34 ± 0.21 ; 13.16 ± 0.07) are now significantly lower than that of NRB-IM (41.90 ± 1.02 ; 14.56 ± 0.40), NRB+IM (43.00 ± 0.56 ; 15.02 ± 0.18) and control (42.90 ± 0.47 ; 14.92 ± 0.16) groups. This could mean a gradual decline in the erythropoietic response of the CRB groups. Since the major stimulant of erythropoiesis is erythropoietin produced by the kidneys, there could be possible onset of kidney damage with prolong intake of CaC₂ ripened banana. This finding appears consistent with Igbinaduwa and Aikpitanyi,³² who had reported a higher level of Creatinine indicating a reduced glomerular filtration rate in unchallenged rats. Again, of significance, is the improved value with NRB+IM indicative of a more effective erythropoietic function in spite of the challenge. The total WBC of CRB+IM (5800 ± 1228.01) is again significantly lower than CRB-IM (8600 ± 18.32), NRB-IM (9220 ± 578.27), NRB+IM (12280 ± 208.33) and control (12720 ± 326.19) which also varied significantly. The consistent reduction in the CRB groups could further confirm sustained immunosuppression due to CaC₂.^{30,31} The change in the NRB groups could further confirm the immune-modulatory role of normal fruits.³⁷ The lymphocytes are significantly higher in CRB+IM (90.60 ± 9.68) than NRB+IM (86.82 ± 0.47), CRB-IM (87.68 ± 0.65), NRB-IM (84.48 ± 1.13) and control (81.90 ± 2.15) which again varied significantly across the groups. It is worthy of note that high percentage of lymphocyte do not necessarily connote higher lymphocyte count as it is only a percentage of

Table 1: Weight and Some Haematological Variables of Rats Administered NRB, CRB, ± Immunization with SRBCs and Controls on Day 14

Variables	Treatment Groups					F- Value	P value
	NRB+IM	NRB-IM	CRB+IM	CRB-IM	Controls		
	N=5	N=5	N=5	N=5	N=5		
Wt (g)	198.68 ± 12.92	202.54 ± 2.11	201.80 ± 3.72	187.00 ± 9.82	204.30 ± 8.87	0.65	0.63
PCV (%)	41.30 ± 1.46	43.10 ± 0.12	40.92 ± 0.31	41.78 ± 0.64	42.60 ± 0.77	1.24	0.33
Hb (g/dl)	14.24 ± 0.64	14.72 ± 0.19	13.56 ± 0.16	14.26 ± 0.34	14.88 ± 0.32	1.97	0.14
WBC(/ul)	11800 ^a ± 230.21	8420 ^b ± 720.69	6460 ^c ± 972.94	8080 ^b ± 86.02	10683.33 ^b ± 218.20	16.11	0.001
L%	78.42 ^c ± 4.90	80.26 ^b ± 2.91	89.66 ^a ± 0.27	86.28 ^a ± 0.47	70.78 ^d ± 0.64	10.45	0.001
G%	9.48 ^b ± 4.96	8.72 ^b ± 1.42	4.00 ^d ± 0.13	5.56 ^c ± 0.17	15.87 ^a ± 0.30	17.26	0.001
M%	12.10 ^a ± 2.28	11.66 ^a ± 1.60	6.34 ^b ± 0.29	9.36 ^a ± 1.10	13.47 ^a ± 0.72	4.38	0.10
PLT(/uL)	433600 ^a ± 61881.01	412800 ^a ± 41230.33	408800 ^a ± 1392.84	331600 ^b ± 3854.87	509666.67 ^a ± 1763.54	4.21	0.01

Note: NRB= Naturally Ripened banana; CRB= Chemically Ripened Banana; PCV= Packed Cell Volume; Hb= Haemoglobin Concentration; WBC= White Cell Count; %L= %Lymphocyte; %G= %Granulocytes; %M= %Monocytes; PLT= Platelet Count
Significance: P < 0.05.

Table 2: Weight and Some Haematological Variables of Rats Administered NRB, CRB, ± Immunization with SRBs and Controls on Day 21

Variables	Treatment					F- value	Pvalue
	NRB+IM	NRB-IM	CRB+IM	CRB-IM	Controls		
Wt (g)	218.40 ± 5.53	206.20 ± 7.70	212.08 ± 6.54	198.68 ± 2.86	199.20 ± 7.36	0.81	0.53
PCV (%)	43.00 ^a ± 0.56	41.90 ^a ± 1.02	37.88 ^b ± 0.55	38.34 ^b ± 0.21	42.90 ^a ± 0.47	16.20	0.001
Hb (g/dl)	15.02 ^a ± 0.18	14.56 ^a ± 0.40	13.04 ^b ± 0.21	13.16 ^b ± 0.07	14.92 ^a ± 0.16	17.23	0.001
WBC (/uL)	12280 ^a ± 208.33	9220 ^c ± 578.27	5800 ^d ± 1228.01	10600 ^c ± 18.32	12720 ^a ± 326.19	19.40	0.001
L (%)	86.82 ^b ± 0.47	84.48 ^c ± 1.13	90.60 ^a ± 9.68	87.68 ^b ± 0.65	81.90 ^c ± 2.15	7.70	0.001
G (%)	5.62 ^b ± 0.09	6.44 ^b ± 0.09	4.46 ^b ± 0.09	4.30 ^b ± 0.07	10.92 ^a ± 1.86	9.37	0.001
M (%)	7.76 ^a ± 0.64	9.08 ^a ± 0.58	5.12 ^c ± 0.65	7.42 ^b ± 0.28	7.16 ^b ± 0.45	7.12	0.001
PLT (/uL)	538800 ^a ± 3152.78	388400 ^c ± 22209.46	487400 ^b ± 35815.64	348800 ^c ± 3507.57	453600 ^b ± 3931.92	15.94	0.001

Note: NRB = Naturally Ripened banana; CRB = Chemically Ripened Banana; IM = Immunization; PCV = Packed Cell Volume; Hb = Haemoglobin Concentration; WBC = White Cell Count; %L = %Lymphocyte; %G = %Granulocytes; %M = %Monocytes
Significance: P < 0.05.

the leucocytes available. The granulocyte count shows that CRB+IM (4.46 ± 0.09) which appear similar to others, is significantly lower than the control group (10.92 ± 1.86). This probably is because control group seems to be in a state of rest immunologically.³⁴ The Monocytes (M) count is significantly lower in CRB+IM (5.12 ± 0.65) than NRB-IM (9.08 ± 0.58), NRB+IM (7.76 ± 0.64), control (7.42 ± 0.28) and CRB-IM (7.42 ± 0.28). This is probably due to the bypass of other tissue line of defence during immunization with SRBCs i.e not involving the tissue macrophages.³⁴ The platelet is significantly higher in CRB+IM (487400 ± 35815.64) and NRB+IM (538800 ± 3152.78) than control (453600 ± 3931.92), CRB-IM (348800 ± 3507.57) and NRB-IM (348800 ± 3507.57) respectively. This could also be due to good haemostatic mobilization.³⁸

Conclusion

Chemically ripened banana seems to be on the increase in our local markets. This is probably due to increased demand for fruits resulting from increased awareness of its value. Our finding suggests that regular consumption of chemically ripened banana will probably lead to reduced total white blood cell count, packed cell volume and haemoglobin. This reduced count is also more pronounced during exposure to antigenic stimulation by sheep red blood cells. We therefore, conclude that calcium

carbide ripened banana juice causes reduction in the white blood cell counts in immunized albino Wistar rats. Calcium carbide ripened banana could possess some immunotoxicological effects and advocate further studies on the mechanism involved. Moreover, this study has further confirmed the immunomodulatory role of banana juice. We therefore, advocate for increased sensitization of the public on the intake of good naturally ripened banana fruit.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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