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Administration of chemically ripened banana (*Musa acuminate*) juice to male Wistar rats depletes blood cells via impaired hematopoiesis

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Abstract

The use of chemicals such as calcium carbide to ripen fruits instigates toxicity; inclusion of the chemically ripened fruits in the preparation of juices may induce blood cells depletion by altering hematopoiesis via declined functionality of erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins, hematopoietic stem cells; resulting to reduction in cellular components of blood. The blood cell depletion potentials of chemically ripened banana juice were investigated in 20 male Wistar rats categorized into four; Group A (control), Group B (administered naturally ripened banana juice), Group C & D (administered juices composed of 15mg/kg and 25mg/kg of calcium carbide ripened banana) (n=5). Following the administration protocol of the banana juice for 28days, data was obtained and statistically analyzed using GraphPad prism (version 8). Chemically ripened banana juices significantly decreased erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins (PGE2), hematopoietic stem cells and cellular components of blood in a dose-dependent manner. Reduction in packed cell volume (PCV), hemoglobin concentration and increase in erythrocyte sedimentation rate (ESR); which suggests indication of anemia and red blood cell inflammation were also observed. The study suggests that chronic consumption of calcium carbide ripened banana juices induces alteration of hematopoiesis which results to depletion of blood cells.

Keywords: Banana, Blood cells depletion, Calcium carbide, Chemically ripened fruits, Hematopoiesis

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INTRODUCTION

Chemical ripening of fruits in Africa and Asia is a post-harvest practice that aid to manage spoilage of fruits as well as increase ripening in order to satisfy commercial consumption and demands (Sogo-Temi *et al*., 2014; Faqeerzada *et al*., 2018; Zhao *et al*., 2020). Fruits constitute the major content of most juices such as banana, mango, orange juices etc. (Salehi, 2020). Chemicals such as calcium carbide used to ripen fruits have been reported to have toxic effects (Asif, 2012; Okeke *et al*., 2022). Studies have also shown its toxic effect on the nutritional value of fruits (Adeyemi *et al*., 2018; Nura *et al*., 2018).

Pure fruit juices may be processed with fruits ripened with chemicals, hence contributing to the toxins reported on juices (Anastácio *et al*., 2018; Khazaei *et al* 2021); since fruits ripened with calcium carbide contains toxic arsenic and phosphorous which makes it extremely poisonous for consumption (Fattah and Ali, 2010). In this study, our hypothesis was whether pure fruit juice processed with calcium carbide ripened fruit (banana) would potentiate an adverse effect on blood as reports has showed that calcium carbide ripened fruits cause decrease in cellular components of blood (Andrew *et al.,* 2018; Onwuka and Chamberlain, 2021). Study also showed deterioration of blood components such as leukocytes and thrombocytes caused by calcium carbide ripened banana (Onwuka *et al.,* 2021); but the underlined mechanism is not fully explained and this study tend to elucidate an aspect.

Depletion of cellular components of blood can be due to impairment of physiological blood production (hematopoiesis) (Hormaechea-Agulla and King, 2020). Alteration of hematopoietic regulators such as erythropoietin, leukopoietin, thrombopoietin, interleukin-3 and prostaglandins can cause impaired production of hematopoietic stem cells such as Colony Forming Unit - erythrocyte, monocyte/macrophage, megakaryocyte (CFU-GEMM), Colony Forming Unit - granulocyte-macrophage (CFU-GM), Colony Forming Unit - granulocyte (CFU-G), Colony Forming Unit - macrophage (CFU-M), Burst-forming units - erythrocyte (BFU-E) hence resulting to depletion of cellular components of blood; because the hematopoietic stem cells give rise to the cellular component of blood (Weiss, 1986; Sotnezova *et al*., 2017; Martynenko *et al*., 2021). Thus, we investigated the activity of juice composed of calcium carbide ripened fruit (banana) on hematopoiesis in male Wistar rats. The study gave an insight on the pathophysiology of blood toxicity induced by chronic consumption of juice processed with calcium carbide ripened fruits.

MATERIALS AND METHODS

Animals

Twenty (20) male Wistar rats weighing averagely 175g were purchased from the animal farm of Gregory University, Uturu as approved by the Ethics Review Committee of the institution for the purpose of this study. Humane care and procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). The experimental rats were kept in standard laboratory cages under a dark/ light cycle and were acclimatized for 14 days before the experiment. They were fed standard rat chow and drinking water *ad libitum* for the duration of the study.

Preparation of pure banana fruit juice

Freshly harvested banana fruits from the university plantation were divided into three (3) categories; category 1 ripened naturally, while categories 2 and 3 were ripened with 15 mg/kg & 25 mg/kg of calcium carbide (CaC_2) obtained from Ukwunwangwu market in Uturu, Isiukwuato Local Government Area of Abia State, Nigeria.

Pure banana fruit juices were prepared using ripened banana fruits; 500g each from the various categories were blended separately and diluted with 500 ml of deionized water, filtered to obtain the banana juice. The juices obtained from the different category was properly labeled and stored in the refrigerator (20^oc) for further use. Category 1 = fruit juice composed of naturally ripened banana, categories 2 and 3 = fruit juice composed of banana ripened with 15 mg/kg and 25 mg/kg of calcium carbide (CaC_2) respectively.

Administration of banana juices

Administration of the banana juices was done orally on daily basis for 28 days on four (4) groups (A, B, C and D) of the male Wistar rats (n=5). Group A (control) received 2ml of deionized water, Group B received 2 ml of juice composed of naturally ripened banana, while Group C and D

received 2 ml of juice composed of banana ripened with 15 mg/kg and 25 mg/kg respectively.

Determination of erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins

Blood samples were collected 24 hrs. after last administration of banana juice via cardiac puncture from the animals on anesthesia (40 mg/kg Sodium pentobarbital i.p.); put into a sample bottle and centrifuged at 3000rpm for 15 minutes. The serum obtained was used for the assays using Enzyme-Linked Immunosorbent Assay (ELISA) kit obtained from Bioassay Technology, (China). The protocol of the kit was strictly adhered to as was stated in the manufacturer's manual.

About 50 µl of sample and 50 µl of sample diluents were added to the sample well. One hundred microliter of sample diluent was added to the blank well while 100µl of standard was added to the standard well. Fifty microliter of biotin-conjugate was added to all the wells covered with an adhesive and incubated at room temperature for 3 hours. After the incubation the adhesive was removed, micro wells emptied and washed 6 times with the wash buffer. The micro wells were tapped several times on an absorbent paper to remove water. The 100 µl of diluted streptavidin-HRP to all the wells was added. It was covered with an adhesive and incubated at room temperature for 20 minutes. After which the adhesive was removed and wells washed 6 times. Then 100 µl of TMBS substrate solution was added to all the wells and incubated for 10 minutes, after which 100 µl of stop, solution was added to all the wells and absorbance read using a spectrophotometer.

Determination of hematopoietic stem cell (Colony Forming Unit, CFU) counts

Hematopoietic stem cell (CFU) count was determined using STEMCELL Technologies (MethoCult GF M3434 semisolid medium) as was described by (Sotnezova *et al*., 2017): The cells were cultured in 35-mm Petri dishes (2×10^4 cell/dish), colonies were identified and counted on day 10 of culturing using dark field microscopy under a microscope X40, (Nikon Eclipse TiU).

Determination of cellular components of blood, hemoglobin concentration, PCV and ESR

Cellular components of blood (red blood cell RBC, White blood cell WBC, platelets), haemoglobin concentration, packed cell volume (PCV) and Erythrocyte sedimentation rate ESR were determined on blood samples aspirated into hematological analyzer (Swelab Alfa, Boule, USA) which automatically measured the parameters. Evaluation of the various juices on hemoglobin concentration (Hgb), PCV and ESR which are measures of blood cell inflammation and anemia were also ascertained; since they can be linked to hematopoiesis. Hence, we investigated the impact of the various juices on hemoglobin concentration, PCV and ESR to check whether the various juices have potentials of causing anemia and inflammation to the cellular component of blood (erythrocytes).

Statistical analysis

Statistical analysis was done using GraphPad Prism (version 8). Multiple comparisons were done between all groups using Boneferroni post hoc comparison test. One-way analysis of variance (ANOVA) was used to determine statistical significance at $P<0.05$. Results were expressed as mean ± SEM (standard error of mean).

RESULTS

Impact of the various category of juice on erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins

We investigated the impact of the various category of juices prepared in this study on variables that promote hematopoiesis (erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins). Erythropoietin, leukopoietin, thrombopoietin promotes erythrocytes (RBC), leukocytes (WBC), thrombocytes (platelets) respectively, while interleukin-3 and prostaglandins facilitate hematopoiesis processes (Weiss, 1986). Fruit juice composed of naturally ripened banana enhanced the hematopoiesis promoting variables whereas juices composed of banana ripened with 15 mg/kg and 25 mg/kg of calcium carbide decreased them in a dose dependent manner (Table 1).

Impact of the various category of juice on hematopoietic stem cells (CFU counts)

Hematopoietic stem cells mature into cellular components of blood (Anthony and Link, 2014). This study showed that fruit juice composed of naturally ripened banana increased hematopoietic stem cells (CFU counts), while the category of juice composed of banana ripened with 15 mg/kg and 25 mg/kg of calcium carbide reduced the CFU counts (Table 2). This could be as result of the effects of the various categories of juices on hematopoiesis promoting factors (Table 1) observed in this study.

Impact of the various category of juice on the cellular components of blood

The cellular components of blood (erythrocytes, thrombocytes, leukocytes and leukocytes differential counts) were increased on administration of juice composed of naturally ripened banana. Fruit juice composed of calcium carbide (15 mg/kg and 25 mg/kg) ripened banana showed dose dependent decrease in the cellular components of blood (Table 3). These effects correspond to that of CFU counts (Table 2) which was a result of the effects of the various category of juice on the hematopoiesis promoting factors (Table 1).

Impact of the various juices on hemoglobin concentration, PCV and ESR

Following the outcome of this study that showed alterations of hematopoiesis variables (Table 1-3); The study showed that fruit juice composed of naturally ripened banana can ameliorate anemia or inflammation to erythrocyte by enhancing hemoglobin concentration, PCV and decreasing ESR, whereas fruit juice composed of banana ripened with calcium carbide (15 mg/kg and 25 mg/kg) decreased hemoglobin concentration, PCV and increase in ESR (Table 4) suggesting a potential anemic condition and inflammation to the red blood cells.

Table 1: Values of erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins on administration of the various category of banana juice

Groups	A	B	C	D
Erythropoietin (pg/mL)	172.2±5.88	199.8±3.96*	141.8±3.06**++	113.6±7.026**++^
Leukopoietin (iu/ml)	1.744±0.05	2.666±0.17**	1.286±0.04*++	1.002±0.08**++
Thrombopoietin (pg/mL)	498.4±12.8	660.4± 47.3**	356.0±20.9*++	181.8± 24.3**++^
Interleukin-3 (pg/mL)	610.4±22.2	819.4±24.3**	472.4±19.9**++	354.2±14.93**++^
Prostaglandins-E2 (ng/ml)	2.924±0.14	4.696±0.39**	1.790±0.11*++	1.012±0.17**++

Values are expressed as mean ± standard error of mean (SEM), Group A: Control, Group B: juice composed of naturally ripened banana, Group C and D: juice composed of banana ripened with 15mg/kg and 25mg/kg of calcium carbide respectively. *P<0.05, **P<0.01 indicates significant difference on comparison with Group A, +P<0.05, ++P<0.01 indicates significant difference on comparison with Group B while ^P<0.05 indicates significant difference on comparison between Group C and D.

Table 2: Hematopoietic stem cells (CFU counts) on administration of the various category of banana juice

Groups	A	B	C	D
CFU-GEMM	24.2±1.28	39.0±2.43**	16.2±1.28++	8.4±1.21**++
CFU-GM	38.8±1.66	48.0±1.30	25.6±2.21*++	13.8±1.36**++^
CFU-G	80.4±2.54	94.8±1.93**	59.8±3.11**++	43.8±3.61**++^
CFU-M	54.6±3.09	73.8±4.53**	57.6±2.50++	38.8±3.22**++^
BFU-E	9.6±1.21	18.2±1.28	4.0±0.45++	1.2±0.37++
Total CFU count	207.2±3.84	273.8±6.87**	163.2±4.74**++	106.6±3.70**++^

CFU/20×10^3 values are expressed as mean ± standard error of mean (SEM), Group A: Control, Group B: juice composed of naturally ripened banana, Group C and D: juice composed of banana ripened with 15mg/kg and 25mg/kg of calcium carbide respectively. *P<0.05, **P<0.01 indicates significant difference on comparison with Group A, +P<0.05, ++P<0.01 indicates significant difference on comparison with Group B while ^P<0.05 indicates significant difference on comparison between Group C and D.

Table 3: Impact of the various categories of juices on the cellular components of blood

Groups	A	B	C	D
Erythrocytes (x 10^6/µl)	5.32±0.15	6.58±0.17*	4.06±0.16+	2.48±0.19*++^
Leukocytes (x 10^5/µl)	145.2±2.82	168.2±3.17*	139.8±7.61*++	119.0±2.9**++^
Lymphocytes (%)	56.40±2.73	72.8±2.22*	39.80±3.59**++	20.2±2.91**++^
Monocytes (%)	156.40±2.73	192.2±15.7**	132.2±2.35*++	101.4±12.4**++^
Neutrophils (%)	146.0±2.17	168.8±2.67	125.8±1.88*++	57.0±11.76**++^
Eosinophil (%)	18.80±2.71	33.2±2.48**	8.40±1.44**++	2.80±0.58**++^
Thrombocytes (10^3/µl)	364.0±27.3	704.0±35.6**	256.6±17.7**++	156.6±5.04**++^

Values are expressed as mean ± standard error of mean (SEM), Group A: Control, Group B: juice composed of naturally ripened banana, Group C and D: juice composed of banana ripened with 15mg/kg and 25mg/kg of calcium carbide respectively. *P<0.05, **P<0.01 indicates significant difference on comparison with Group A, +P<0.05, ++P<0.01 indicates significant difference on comparison with Group B while ^P<0.05 indicates significant difference on comparison between Group C and D.

Table 4: Impact of the various categories of juices on hemoglobin (Hgb), PCV and ESR

Groups	A	B	C	D
Hgb (g/l)	11.12±0.63	14.28±0.26	7.56±0.34*++	3.54±0.53**++
PCV (%)	36.8±1.49	50.2±2.63**	24.4±1.99**++	12.4±1.43**++^
ESR (mm/hr.)	0.582±0.05	0.28±0.03	1.08±0.11*++	2.04±0.10**++

Values are expressed as mean ± standard error of mean (SEM), Group A: Control, Group B: juice composed of naturally ripened banana, Group C and D: juice composed of banana ripened with 15mg/kg and 25mg/kg of calcium carbide respectively. *P<0.05, **P<0.01 indicates significant difference on comparison with Group A, +P<0.05, ++P<0.01 indicates significant difference on comparison with Group B while ^P<0.05 indicates significant difference on comparison between Group C and D.

DISCUSSION

The health benefits of pure fruit juice cannot be overemphasized (Ogundele *et al*., 2016). Several fruits (banana, orange, mango etc.) used to process these juices has been reported to have beneficial effects on the body (Kardum *et al*., 2015; Zhang *et al.*, 2021). Banana fruit is a known immune booster, antioxidants, hematopoiesis enhancer (Imataki *et al.,* 2006; Singh *et al*., 2016; Yang *et al.,* 2019). This study has also showed that the fruit juice composed of naturally ripened banana actually enhanced hematopoiesis by increasing the hematopoiesis promoting variables (Table 1), hematopoietic stem cells (Table 2), and cellular components of blood (Table 3) as well as enhancing hemoglobin concentration, PCV and reducing ESR (Table 4). This is suggested to be as a result of the bioactive component of banana (*Musa acuminate)* such as pantothenic acid, pyridoxine, cholin, vitamin C, zinc, which becomes part of the fruit juice and have been reported to cause increase in blood cell profile (Akhtar and Ciji, 2020; Khan and Jameel, 2020; Lopes *et al.,* 2020).

Calcium carbide ripened fruits has been reported to cause health hazard (Essien *et al.,* 2018; Okeke *et al.,* 2022). In this study we elucidated the pathophysiological impact of fruit juice made of calcium carbide ripened banana on blood cells production; we established that consumption of juice processed with banana ripened with calcium carbide can impair hematopoiesis by reducing hematopoiesis promoting variables (Table 1) which in turn reduced hematopoietic stem cells (Table 2) and cellular components of blood (Table 3) as well as reducing hemoglobin concentration, PCV and enhancing ESR (Table 4). This physiological alteration observed could be as a result of calcium carbide used to ripen the banana fruit. This suggests that the hematopoiesis was decreased in the experimental animals since there is reduction in its promoting factors. In order to elucidate the impact of the decrease in the hematopoiesis promoting variable, there is need to evaluate the impact of the various categories of juices on hematopoietic stem cells and cellular component of blood. The findings of our study agree with scientific reports of various authors who concluded that hematopoiesis is influenced by erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins which results to production of hematopoietic stem cells (Weiss 1986; Anthony and Link, 2014; Sotnezova *et al.,* 2017).

This study findings is in correspondence with the report that hematopoiesis is influenced by erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins which causes production of hematopoietic stem cells that give rise to cellular component of blood. Erythropoietin, leukopoietin and thrombopoietin are hormones that stimulate the production of erythrocytes, leukocytes and thrombocytes respectively in the bone marrow by stimulating hematopoietic stem cells (CFU-GEMM, CFU-GM, CFU-G, CFU-M, BFU-E, and Total CFU count) (Weiss 1986; Anthony and Link, 2014; Sotnezova *et al.,* 2017). The various hematopoietic stem cells differentiate into cellular components of blood (Figure 1). Thus, the various categories of fruit juices were able to impact on the hematopoiesis by following the stated physiological mechanisms.

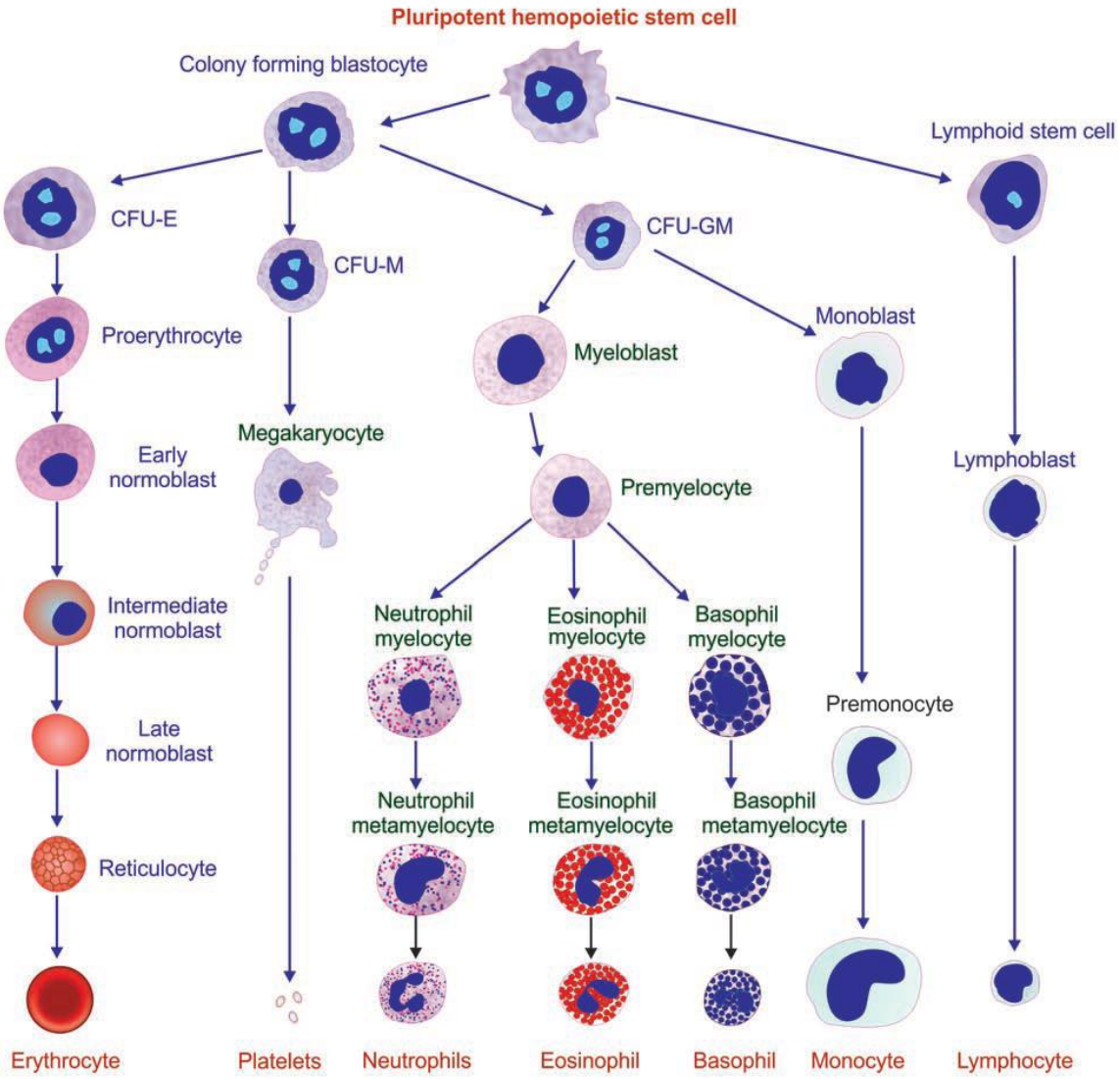

Figure 1: Differentiation of hematopoietic stem cells into cellular components of blood (Sembulingam and Sembulingam, 2012).

CONCLUSION

The various category of juice influenced hematopoiesis; juice composed of naturally ripened banana enhanced hematopoiesis whereas depletion of blood cells mediated via impaired hematopoiesis were observed on administration of juice composed of calcium carbide ripened banana. Thus, chronic consumption of fruit juice composed of chemically (calcium carbide) ripened banana impairs hematopoiesis leading to reduction in cellular

component of blood which can result to anemia and inflammation to erythrocytes.

Author contributions

OMO participated in conceptualization, methodology, validation, data analysis, Investigation, providing resources, writing of the original draft, review & editing, supervision, project administration and funding. ALO participated in methodology, validation, investigation, providing resources, writing of the original draft, review, editing and funding. NCN participated in the methodology, validation, providing resources, writing of the original draft, review, editing and funding.

Conflict of interest

Authors have no conflict of interest to declare.

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REFERENCES

Adeyemi, M. M., Bawa, M. H., & Muktar, B. (2018). Evaluation of the effect of calcium carbide on induce ripening of banana, pawpaw and mango cultivated within Kaduna Metropolis, Nigeria. *Journal of Chemical Society of Nigeria*, ***43***(2):108-118.

Akhtar, M. S., & Ciji, A. (2020). Pyridoxine and its biological functions in fish: Current knowledge and perspectives in aquaculture. *Reviews in Fisheries Science & Aquaculture*, **29** (2): 260-278.

Anastácio, M., Dos Santos, A. M., Aschner, M., & Mateus, L. (2018). Determination of trace metals in fruit juices in the Portuguese market. *Toxicology reports*, **5**:434-439.

Andrew, G. S., Simon, U. T., John, A. U., Godwin, O. O., Alexander, N. I., & Ikagu, Y. M. (2018). Studies on changes in some haematological and plasma biochemical parameters in wistar rats fed on diets containing calcium carbide ripened mango fruits. *International Journal of Food Science and Nutrition Engineering*, **8** (2): 27-36.

Anthony, B. A., & Link, D. C. (2014). Regulation of hematopoietic stem cells by bone marrow stromal cells. *Trends in Immunology*, **35** (1): 32-37.

Asif, M. (2012). Physico-chemical properties and toxic effect of fruit-ripening agent calcium carbide. *Annals of Tropical Medicine & Public Health*, 5(3): 150-156.

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. *Clinical and Experimental Medicine*, **6** (1):47-61.

Faqeerzada, M. A., Rahman, A., Joshi, R., Park, E., & Cho, B. K. (2018). Postharvest technologies for fruits and vegetables in South Asian countries: a review. *Korean Journal of Agricultural Science*, **45** (3): 325-353

Fattah, S. A., & Ali, M. Y. (2010). Carbide ripened fruits-a recent health hazard. *Faridpur Medical College Journal*, **5** (2): 37-37.

Hormaechea-Agulla, D., Le, D. T., & King, K. Y. (2020). Common sources of inflammation and their impact on hematopoietic stem cell biology. *Current Stem Cell Reports*, **6** (3), 96-107.

Imataki, O., Nakatani, S., Hasegawa, T., Kondo, M., Ichihashi, K., Araki, M., & Takaue, Y. (2006). Nutritional support for patients suffering from intestinal graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *American Journal of Hematology*, **81** (10), 747-752.

Kardum, N., Milovanović, B., Šavikin, K., Zdunić, G., Mutavdžin, S., Gligorijević, T., & Spasić, S. (2015). Beneficial effects of polyphenol-rich chokeberry juice consumption on blood pressure level and lipid status in hypertensive subjects. *Journal of medicinal food*, **18** (11):1231-1238.

Khan, N.T., & Jameel, N. (2020) Pantothenic acid -a dietary abundant vitamin. *International Journal of Biotechnology and Allied Fields*, **8** (5): 283-286

Khazaei, S., Talebi Ghane, E., Bashirian, S., & Mehri, F. (2021). The concentration of potentially toxic elements (PTEs) in fruit juices: a global systematic review, meta-analysis and probabilistic health risk

assessment. *International Journal of Environmental Analytical Chemistry*, 1-13.

Lopes, S., Borges, C. V., de Sousa Cardoso, S. M., de Almeida Pereira da Rocha, M. F., & Maraschin, M. (2020). Banana (Musa spp.) as a source of bioactive compounds for health promotion. Handbook of Banana Production, Postharvest Science, Processing Technology, and Nutrition, Pp.227-244.

Martynenko, R. V., Shepitko, V. I., Stetsuk, Y. V., Boruta, N. V., Yakushko, O. S., & Martynenko, V. B. (2021). Complex histological and immunohistochemical study of monocytopoiesis in rats with determination of proliferative activity by expression Ki67 and CD68+ in the experiment. *World of Medicine and Biology*, **17**(78): 229-232.

Nura, A., Dandag, M. A., & Wali, N. R. (2018). Effects of artificial ripening of banana (Musa spp) using calcium carbide on acceptability and nutritional quality. *Journal of Postharvest Technology*, **6**(2):14-20.

Ogundele, O. M., Awolu, O. O., Badejo, A. A., Nwachukwu, I. D., & Fagbemi, T. N. (2016). Development of functional beverages from blends of *Hibiscus sabdariffa* extract and selected fruit juices for optimal antioxidant properties. *Food science & Nutrition*, ***4*** **(**5), 679-685.

Okeke, E. S., Okagu, I. U., Okoye, C. O., & Ezeorba, T. P. C. (2022). The use of calcium carbide in food and fruit ripening: potential mechanisms of toxicity to humans and future prospects. *Toxicology*, **468** (28) 153112.

Onwuka, O. M., & Chamberlain V. C. (2021). Calcium carbide ripened banana impair white blood cell profile by abating leukocyte promoting factors in rat model. *European Journal of Biomedical and Pharmaceutical Sciences*, **8** (1), 391-396.

Onwuka, O. M., Olorunfemi O. J., Nwafor, A. (2021), Leukocyte and thrombocyte deteriorating effect of calcium carbide exposed fruit on rats. *International Journal of Research (IJR),* **8** (10), 206-215

Salehi, F. (2020). Physicochemical characteristics and rheological behaviour of some fruit juices and their concentrates. *Journal of Food Measurement and Characterization*, **14** (5): 2472-2488.

Sembulingam, K., & Sembulingam, P. (2012). *Essentials of medical physiology*. JP Medical Ltd.

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2016). Bioactive compounds in banana and their associated health benefits–A review. *Food Chemistry*, **206**: 1-11.

Sogo-Temi, C. M., Idowu, O. A., & Idowu, E. (2014). Effect of biological and chemical ripening agents on the nutritional and metal composition of banana (Musa spp). *Journal of Applied Sciences and Environmental Management*, **18** (2), 243-246.

Sotnezova, E. V., Markina, E. A., Andreeva, E. R., & Buravkova, L. B. (2017). Myeloid precursors in the bone marrow of mice after a 30-day space mission on a Bion-M1 biosatellite. *Bulletin of Experimental Biology and Medicine*, **162** (4),:496-500.

Weiss, L. (1986). Cellular Regulation in Hematopoiesis. In The Reticuloendothelial System (pp. 1-22). Springer, Boston, MA.

Yang, J., Tu, J., Liu, H., Wen, L., Jiang, Y., & Yang, B. (2019). Identification of an immunostimulatory polysaccharide in banana. *Food Chemistry*, **277**: 46-53.

Zhang, S., Hu, C., Guo, Y., Wang, X., & Meng, Y. (2021). Polyphenols in fermented apple juice: Beneficial effects on human health. *Journal of Functional Foods*, **76**:104294.

Zhao, P., Ndayambaje, J. P., Liu, X., & Xia, X. (2020). Microbial spoilage of fruits: a review on causes and prevention methods. *Food Reviews International*, 1-22.