EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 1
391-396
Year: 2021

CALCIUM CARBIDE RIPENED BANANA IMPAIR WHITE BLOOD CELL PROFILE BY ABATING LEUKOCYTE PROMOTING FACTORS IN RAT MODEL

Onwuka Osah Martins* **and Chamberlain Victoria Chiamaka**

Department of Human Physiology, College of Medical and Health Sciences, Gregory University Uturu, Abia State, Nigeria.

***Corresponding Author: Onwuka Osah Martins**
Department of Human Physiology, College of Medical and Health Sciences, Gregory University Uturu, Abia State, Nigeria.
DOI: https://doi.org/10.17605/OSF.IO/G3QFR

Article Received on 19/11/2020 **Article Revised on 09/12/2020** **Article Accepted on 29/12/2020**

ABSTRACT
The study was aimed at investigating mechanism of impairment of white blood cell profile in male Wistar rats orally administered Calcium Carbide (CaC_2) ripened banana (*Musa spp*). Twenty five (25) male Wistar rats weighing 150g – 210g were randomly divided into five (5) groups: Group 1 (control) received 2ml of distilled H_2O; Group 2 received 2ml of naturally ripened banana, Group 3, 4. & 5 received 2ml of 5g/kg of CaC_2, 15g/kg of CaC_2, and 25g/kg of CaC_2 ripened banana respectively. All administration was done orally for 28days. Thereafter blood samples were collected from the animals for laboratory assays. The samples were assayed for white blood cell profile and leukocyte promoting factors (leukopoietin, interleukin -3(IL-3), and prostaglandins). Results showed significant dose dependent decrease in leukocyte promoting factors: leukopoietin (Figure 1), interleukin -3 (Figure 2) and prostaglandin (Figure 3) when compared to Group 1 and Group 2, $P<0.05$. It also showed dose dependent significant reduction in white blood cell profile (Total WBC count, and WBC differential count) (Table1) when compared to Group 1 and Group 2, $P<0.05$. Conclusively, this study suggests that Calcium Carbide (CaC_2) ripened banana causes impairment of white blood cell profile by decreasing leukocyte promoting factors; leukopoietin, interleukin -3(IL-3), and prostaglandins- E_2 (PGE_2).

KEYWORDS: Calcium Carbide (CaC_2), leukocytes, leukopoietin, interleukin -3 (IL-3), prostaglandins- E_2 (PGE_2), white blood cells.

INTRODUCTION
Banana *(Musa spp)* is a fruit; it is a staple food for most developing regions of Africa and South East Asia. [1] In addition to the nutritional value of banana; it has several uses which includes; uses in medicines, beverages, flavorings, various religious and ceremonial practices.[2]

Banana is sweeter when it is ripened and its color ranges from green to yellow. Large amount of tropical fruits including bananas are produced in Nigeria.[1, 3] In order to reduce post-harvest spoilage, techniques for fruit preservation and post-harvest management which includes chemical ripening of fruits are employed.[4, 5]

In recent times consumption of fruits becomes extremely hazardous due to artificial ripening of fruits by different toxic chemical agents. [6] Calcium carbide (CaC_2) is one of the common chemicals used to ripen fruits (banana).[7] This chemical mimics a ripening hormone ethylene that induces the natural process of maturation.[6] Calcium carbide in contact with moisture produces acetylene which is an analogue of natural ripening hormone.[7, 8] Calcium carbide also contains trace amounts of toxic arsenic and phosphorous that makes the healthy fruits poisonous.[8] Study has shown that calcium carbide affects the nutritional values of banana. Acetylene gas produced by calcium carbide may affect the neurological system by inducing prolonged hypoxia gradually culminating to headache, dizziness, mood disturbances, sleepiness, mental confusion, memory loss, cerebral edema and seizures.[9, 10]

Studies have showed that calcium carbide (CaC_2) decreases blood cells, including white blood cell profile.[11, 12] But the mechanism of how calcium carbide causes reduction in white blood cell profile is not fully explicated. Hence this study is designed to investigate mechanism of impairment of white blood cell profile in male Wistar rats orally administered calcium carbide (CaC_2) ripened banana (*Musa spp*). In this study activity of calcium carbide ripened banana on leukocyte promoting factors; leukopoietin, interleukin -3(IL-3), and prostaglandins- E_2 (PGE_2), including leukocyte count were evaluated.

MATERIALS AND METHODS

Animals and grouping

Twenty five (25) male Wistar rats weighing 150g – 210g were housed in standard laboratory cages and maintained at room temperature with alternating 12-hour day and night cycles. They were fed standard rat feed and drinking water ad libitum. The animals were randomly divided into 5 groups of 5 rats each.

Administration protocol

Group 1 (control) received 2ml of distilled H_2O; Group 2 received 2ml of naturally ripened banana, Group 3, 4. & 5 received 2ml of 5g/kg of CaC_2, 15g/kg of CaC_2, and 25g/kg of CaC_2 ripened banana respectively.[13, 14] All administration was done orally for 28days. This study was approved by Department of Physiology, Gregory University Uturu. Animals received 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).

Procurement and preparation of banana

Banana fruit were freshly harvested from the Universities plantation. The banana fruits were divided into four (4) sets. The first set was allowed to ripen naturally while the remaining three (3) sets were ripened artificially using varying quantity (5g/kg, 15g/kg, 25g/kg) of calcium carbide (CaC_2). CaC_2 was gotten from a local market (Ukwunwangwu market) in Uturu, isiukwuato LGA of Abia State.

About 500g of banana from the different sets were blended separately and diluted with 500ml deionized water, then filtered to obtain the banana juice. This method was used to obtain banana juices both for the naturally and artificially ripened banana fruits. The juice obtained was properly labeled and stored in the refrigerator for further use.

Assay for Leukocyte promoting factors

After 28days of administration, blood sample was collected from animal in each group via cardiac puncture and put in sample bottles for assays.

Leukocyte promoting factors such as Leukopoietin, Interleukin-3 (IL3) and Prostaglandins PGE_2 were determined using their specific Enzyme-Linked Immunosorbent Assay (ELISA) kit (Bioassay Technology, China). The protocol of the kit was strictly adhered to as was stated in the manufacturer's manual.

Assay for Leukocyte Count

White blood cell count (WBC Count) and White blood cell differential count (WBC differential Count) were determined using blood samples of experimental animal on hematological analyzer (T6000 alpha swelab). The blood samples were aspirated into the hematological analyzer which automatically measured the parameters.

Statistical Analysis

The data obtained from the laboratory assays were statistically analyzed using GraphPad Prism (version 8). The results were analyzed using one-way analysis of variance (ANOVA) to determine statistical significance at $P \leq 0.05$. Multiple comparisons were done between all groups. Results were expressed as mean ± SEM.

RESULT

Levels of leukocyte promoting factors in male Wistar rats orally administered Calcium Carbide (CaC_2) ripened banana (*Musa spp*)

Leukopoietin (iu/ml) in Group 2 (Naturally ripened banana) (2.43±0.04) was significantly increased compared to Group 1(control) (1.86 ± 0.04) $P < 0.001$.Group 4 (15g/kg of CaC_2) (1.56 ±0.02), and Group 5 (25g/kg of CaC_2) (1.23 ±0.01)were significantly decreased compared to Group 1 (control) (1.86 ± 0.04) $P < 0.001$.Group 3 (5g/kg of CaC_2) (1.75 ±0.02), Group 4 (15g/kg of CaC_2) and Group 5 (25g/kg of CaC_2) were significantly decreased when compared to group 2 $P < 0.001$. There was significant dose dependent decrease between group 3, 4 and 5 $P < 0.05$. Leukopoietin (iu/ml) was not significant between group 3 and Group 1 (control) $P \leq 0.05$ (Figure 1).

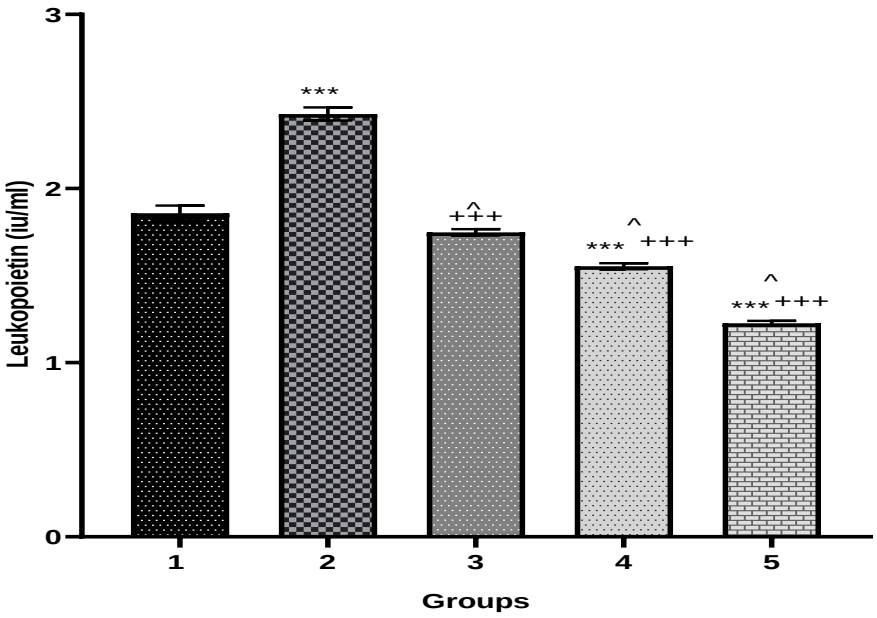

Figure 1: **Leukopoietin (iu/ml) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05) (**P < 0.01) (*** P < 0.001).+ indicates values that are significantly different from animals in Group 2(+P < 0.05) (++ P < 0.01) (+++P < 0. 001).^ indicates values that are significant between Group 3, 4 and 5.Group 1= control (Standard diet and water); Group 2 = Naturally ripened banana; Group 3 = 5g/kg of CaC_2; Group 4 = 15g/kg of CaC_2; Group 5 = 25g/kg of CaC_2.**

Interleukin-3 (IL-3) (pg/ml) was significantly increased in Group 2 (Naturally ripened banana) (837.3±5.52) compared to Group1 control (662.2±13.34) P < 0.001. Group 4 (15g/kg of CaC_2) (571.4±8.84), and Group 5 (25g/kg of CaC_2) (482.9±24.96) were significantly decreased compared to Group 1 (control) (662.2 ± 13.34) P < 0.001. Group 3 (5g/kg of CaC_2) (657.0±24.13), Group 4 (15g/kg of CaC2) and Group 5 (25g/kg of CaC_2) were significantly decreased when compared to group 2 P < 0.001. There was significant dose dependent decrease between group 3, 4 and 5, P < 0.05. There was significant dose dependent decrease between group 3, 4 and 5. Interleukin-3 (IL-3) (pg/ml) was not significant between group 3 and Group 1 (control) P ≤ 0.05 (Figure 2).

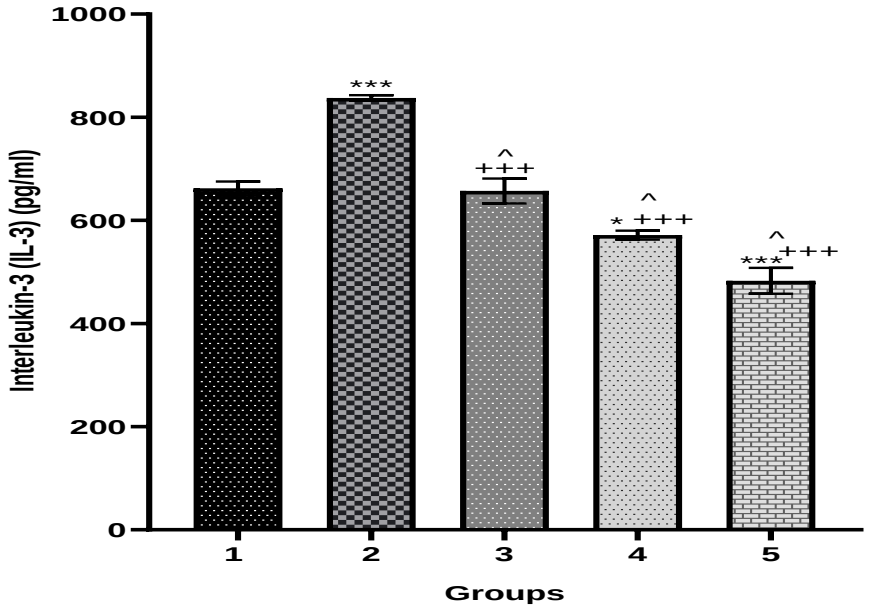

Figure 2: **Interleukin-3 (IL-3) (pg/ml) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05) (**P < 0.01) (*** P < 0.001). + indicates values that are significantly different from animals in Group 2 (+P < 0.05) (++ P < 0.01) (+++P < 0. 001). ^ indicates values that**

are significant between Group 3, 4 and 5. Group 1= control (Standard diet and water); Group 2 = naturally ripened banana; Group 3 = (5g/kg of CaC_2); Group 4 = 15g/kg of CaC_2; Group 5 (25g/kg of CaC_2).

Prostaglandins (PGE_2) (ng/ml) in Group 2 (Naturally ripened banana) (3.83 ±0.25) was significantly increased compared to Group 1(control) (3.08 ±0.19) P< 0.05. Group 4 (15g/kg of CaC_2) (2.36 ±0.02), and Group 5 (25g/kg of CaC_2) (1.84 ±0.15) were significantly decreased compared to Group 1 (control) (3.08 ±0.19) P < 0.05 and P < 0.001 respectively. Group 3 (5g/kg of CaC_2) (2.70 ±0.03), Group 4 (15g/kg of CaC_2) and Group 5 (25g/kg of CaC_2) were significantly decreased when compared to group 2 P < 0.001. There was significant dose dependent decrease between group 3 and 5 P < 0.05. Prostaglandins (PGE_2) (ng/ml) was not significant between group 3 and Group 1 (control), P ≤ 0.05 (Figure 3).

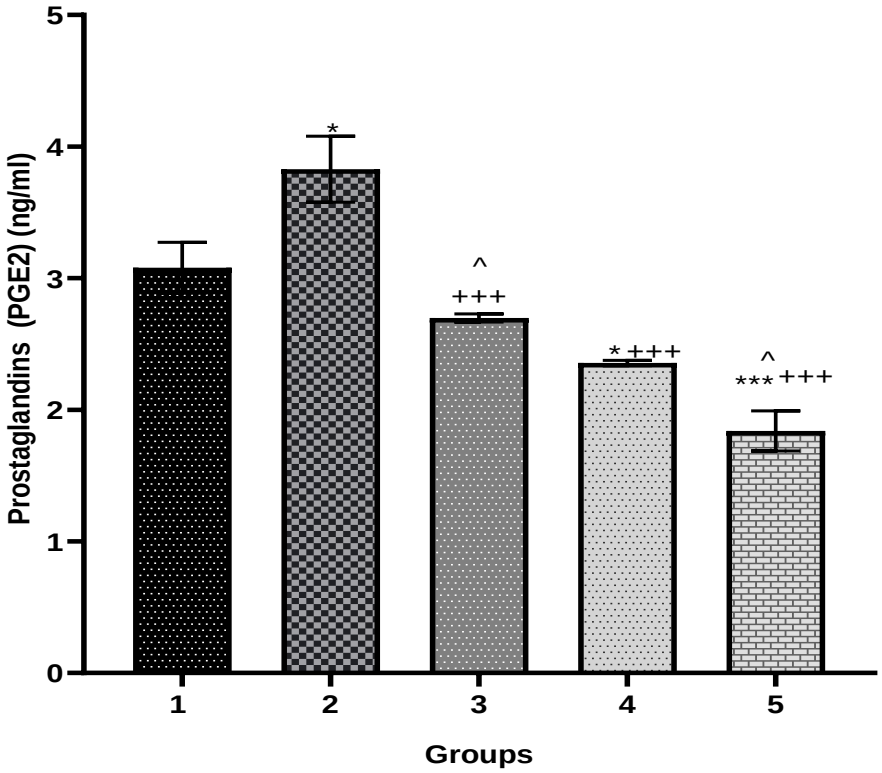

Figure 3: Prostaglandins (PGE_2) (ng/ml) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05) (P < 0.01) (*** P < 0.001). + indicates values that are significantly different from animals in Group 2 (+P < 0.05) (++ P < 0.01) (+++P < 0. 001). ^ indicates values that are significant between Group 3, 4 and 5. Group 1= control (Standard diet and water); Group 2 = naturally ripened banana; Group 3 = (5g/kg of CaC_2); Group 4 = 15g/kg of CaC_2; Group 5 (25g/kg of CaC_2).**

Leukocyte and leukocyte differential count of male Wistar rats orally administered CaC_2 ripened banana
There is significant decrease of WBC count, Lymphocytes, Monocytes, Neutrophils & Eosinophil in Group 3, 4, 5 when compared with Group 1, 2, P< 0.05. There is significant increase in of WBC count, Lymphocyte and Eosinophil in Group 2 when compared with group 1 P< 0.05.WBC count, Lymphocyte and Monocyte showed significant dose dependent decrease in Group 3, 4, 5 when compared with each other P <0.05. Eosinophil showed significant decrease between group 3 and 5 when compared with each other P <0.05. (Table 1)

Table 1: Leukocyte and leukocyte differential count in all groups of orally administered CaC_2 ripened banana.

Groups	WBC count x10^5 (µl)	Lymphocytes x10 (%)	Monocytes (%)	Neutrophils x10 (%)	Eosinophil (%)
1. Control	177.4 ± 1.71	60.18 ± 2.14	163.2 ±5.08	127.1 ±7.00	19.2 ±1.72
2.Natural	265.4 ± 15.19*	82.5 ± 4.11*	166.2 ±22.5	172.2 ±4.85*	29.8 ±3.23
3. 5g/kg CaC_2	144.4±6.71*+^	43.76±2.70*+^	148.3±5.05*+^	89.5 ± 3.76*+^	10.4 ±0.93*+^
4. 15g/kgCaC_2	118.4±3.15*+^	37.88±3.13*+^	122.4 ±1.95*+^	59.6 ± 2.98*+^	5.2 ± 0.80*+
5. 25g/kgCaC_2	84.72±8.52*+^	21.26± 4.03*+^	43.88 ±4.07*+^	48.96± 3.21*+	1.2 ± 0.37*+^

*Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05). + indicates values that are significantly different from animals in Group 2 (+P < 0.05). ^ indicates values that are significant between Group 3, 4 and 5 (^P < 0.05).*

DISCUSSION

Leukocytes promoting factors such as Leukopoietin, Interleukin-3 (IL3) and Prostaglandins PGE_2 are among factors that promote leukocytes (white blood cell) production in the bone marrow, spleen and lymph.[15] They stimulate bone cells or leukopoietic cells to initiate and carry out the activities of leukocytes production.[16]

This study showed significant dose dependent decrease in leukocytes promoting factors of Wistar rats orally administered Calcium carbide (CaC_2) as seen in figure 1, 2 and 3 when compared with group1 (control) and group 2 (natural). This suggests that CaC_2 ripened banana affects white blood cell profile adversely since it decreases the promoting factors. leukopoietin is produced by neutrophils when they encounter a foreign antigen.[15] Leukopoietin replaces neutrophils that have inevitably been phagocytized by foreign antigens; by stimulating the bone marrow microenvironment to increase the rate of production of white blood cells.[15, 16] Hence the decrease in the levels of leukopoietin (figure 1) suggests an increase in foreign antigens as a result of consumption of Calcium carbide ripened banana which caused decrease in neutrophil.

Interleukin-3 is a multipotent hematopoietic growth factor produce by activated T-cells monocytes/macrophages and stromal cells.[17] Interleukin-3 promotes the differentiation and proliferation of hematopoietic cells of various lineage including neutrophils, eosinophil, basophils, megakaryocyte and erythroid lineages.[18] The increase in interleukin-3 seen in group 2 when compared with the group 1 is attributed to the immune stimulating function associated with banana intake. While the significant dose dependent decrease in Interleukin-3 levels (figure 2) suggests that calcium carbide ripened banana decreases hematopoietic growth factor which in return reduces white blood cell production.

Prostaglandins are synthesized by many cell types in the marrow.[19] PGE2 and other metabolites in the prostaglandins pathway expand the hematopoietic stem and progenitor cells (HSPCs) and thereby improve their repopulating ability.[20] Hence dose dependent decrease in prostaglandins PGE2 (figure 3) suggests that calcium carbide ripened banana affects white blood cell profile by decreasing repopulating ability of progenitors as a result of reduction in prostaglandins which reduces its activity.

The reduction in leukocyte count and leukocyte differential count (table 1) suggests that calcium carbide ripened banana decreases white blood cell profile. The observed decrease in leukocyte promoting factors suggest that the mechanism through which calcium carbide ripened banana impair white blood cell profile includes; decreasing leukopoietin, interleukin-3 and prostaglandin which plays vital role in white blood cell production.

CONCLUSION

This study suggests that Calcium Carbide (CaC_2) ripened banana causes impairment of white blood cell profile by decreasing leukocyte promoting factors; leukopoietin, interleukin -3(IL-3), and prostaglandins- E_2 (PGE_2) in a dose dependent manner.

ACKNOWLEDGEMENT

I primarily acknowledge Almighty God for giving me the inspiration grace and strength to carry out this work and supernatural provisions. I sincerely appreciate Department of Human Physiology, Gregory University, Uturu, Abia State, Nigeria for giving me the privilege of using their facilities for this study. My gratitude also goes to Chief Chamberlain Isiuwa and Mrs. Blessing Chamberlain for their financial and moral support towards the success of this research work. To my wonderful research team and co-author; Chamberlain Victoria Chiamaka, I sincerely appreciate all your efforts towards the success of this work. To my family, friends, colleagues and other staff of Gregory University, Uturu, Abia State, Nigeria who one way or the other contributed to this study I appreciate all of you. Best regards!

REFERENCES

1. Heslop-Harrison JS, Schwarzacher T. Domestication, genomics and the future for banana. Annals of botany. 2007; 100(5): 1073-84.
2. Ashokkumar K, Elayabalan S, Shobana VG, Sivakumar P, Pandiyan M. Nutritional value of cultivars of Banana (Musa spp.) and its future prospects. Journal of Pharmacognosy and Phytochemistry. 2018; 7(3): 2972-7.
3. Singh R, Kaushik R, Gosewade S. Bananas as underutilized fruit, having huge potential as raw materials for food and non-food processing industries: a brief review. The Pharma Innovation Journal. 2018; 7(6): 574-80.
4. Singh V, Hedayetullah M, Zaman P, Meher J. Postharvest technology of fruits and vegetables: An overview. Journal of Postharvest Technology. 2014; 2(2): 124-35.
5. James A, Zikankuba V. Postharvest management of fruits and vegetable: A potential for reducing poverty, hidden hunger and malnutrition in sub-Sahara Africa. Cogent Food & Agriculture. 2017; 3(1): 1312052.
6. Siddiqui MW, Dhua RS. Eating artificially ripened fruits is harmful. Current science. 2010: 1664-8.

7. Asif M. Physico-chemical properties and toxic effect of fruit-ripening agent calcium carbide. Annals of tropical medicine and public health. 2012; 5(3): 150.
8. Fattah SA, Ali MY. Carbide ripened fruits-a recent health hazard. Faridpur Medical College Journal. 2010; 5(2): 37-37.
9. Sogo-Temi CM, Idowu OA, Idowu E. Effect of biological and chemical ripening agents on the nutritional and metal composition of banana (Musa spp). Journal of Applied Sciences and Environmental Management. 2014; 18(2): 243-6.
10. Per H, Kurtoğlu S, Yağmur F, Gümüş H, Kumandaş S, Poyrazoğlu MH. Calcium carbide poisoning via food in childhood. The Journal of emergency medicine. 2007; 32(2): 179-80.
11. Ogbuagu DH, Ujowundu CO, Izunobi LC. Calcium carbide-induced haematological alterations in the albino mice-Mus musculus. J. Environ. Sci. Toxicol. Food Technol. 2016; 10:100-4.
12. Osakue JO, Okungbowa MAO. Calcium Carbide Ripened Musa Sapientum Induced Leucopenia in Challenged Albino Wistar Rats. Trop J Nat Prod Res., 2018; 2(3): 114-7.
13. Essien EB, Onyegeme-Okerenta BM, Onyema JO. Calcium carbide as an artificial fruit-ripening agent and its physiological effects on Wistar rats. Clin Exp Med Sci. 2018; 6(1): 47-61.
14. Nura A, Dandago MA, Wali NR. Effects of artificial ripening of banana (Musa spp) using calcium carbide on acceptability and nutritional quality. Journal of Postharvest Technology. 2018; 6(2): 14-20.
15. Ghai CL. A textbook of practical physiology. JP Medical Ltd. 2013; 8(1): 65.
16. Ijeoma OL, Ifeoma UE, Emmanuel O, Ifeanyi AU, Andrew A. Comparative Study of Cd8+ T-Cell Count and Leukopoietin Levels in Human Immunodeficiency Virus Infection in Umuahia, Nigeria. Int.J.Curr.Res.Biosci.PlantBiol., 2015; 2(4): 83-85.
17. Dagar VK, Khasa YP. High-level expression and efficient refolding of therapeutically important recombinant human Interleukin-3 (hIL-3) in E. coli. Protein Expression and Purification. 2017; 131: 51-9.
18. Ackermann M, Haake K, Kempf H, Kaschutnig P, Weiss AC, Nguyen AH, Abeln M, Merkert S, Kühnel MP, Hartmann D, Jonigk D. A 3D iPSC-differentiation model identifies interleukin-3 as a regulator of early human hematopoietic specification. Haematologica. 2020.
19. Kern K, Pierre S, Schreiber Y, Angioni C, Thomas D, Ferreirós N, Geisslinger G, Scholich K. CD200 selectively upregulates prostaglandin E2 and D2 synthesis in LPS-treated bone marrow-derived macrophages. Prostaglandins & other lipid mediators. 2017; 133: 53-9.
20. Heffner GC, Bonner M, Christiansen L, Pierciey FJ, Campbell D, Smurnyy Y, Zhang W, Hamel A, Shaw S, Lewis G, Goss KA. Prostaglandin E2 increases lentiviral vector transduction efficiency of adult human hematopoietic stem and progenitor cells. Molecular Therapy. 2018; 26(1): 320-8.