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LEUKOCYTE AND THROMBOCYTE DETERIORATING EFFECT OF CALCIUM CARBIDE EXPOSED FRUIT ON RATS

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ABSTRACT

This study is aimed at investigating the effect of calcium carbide (CaC_2) exposed fruit on Leukocyte and thrombocyte in rats. In this study banana fruits were exposed to different concentration (5g/kg, 15g/kg & 25g/kg) of calcium carbide in order to induce ripening. 25 Wistar rats weighing approximately 200g were divided into five (5) groups: Group 1 was given 2ml of distilled H_2O; Group 2 was given 2ml of banana not exposed to calcium carbide, Group 3, 4. and 5 were given 2ml of banana fruit exposed to (5g/kg, 15g/kg and 25g/kg) of CaC_2 respectively. The fruit administrations were done by oral for 28days. Blood samples were collected from the animals for laboratory assays of leukocyte count, leukocyte differential count and thrombocyte count. Results showed significant dose dependent decrease in leukocyte count (Figure 1); it also showed significant decrease in leukocyte differential count (Table 1) and thrombocyte count (Figure 2) when compared to Group 1 and Group 2, $P<0.05$. Conclusively, this study suggests that Calcium Carbide (CaC_2) exposed fruit (banana) has deteriorating effect on leukocyte and thrombocyte in rats, which may in turn compromise the immune system.

KEYWORDS: Leukocyte, thrombocyte, calcium carbide (CaC_2), banana, ripening

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INTRODUCTION

Leukocyte also known as white blood cells is the cells of the immune system that are involved in protecting the body against foreign invaders and infectious diseases. [1] It is a type of blood cell that is made in the bone marrow and found in the blood and lymph tissue: Its types include neutrophils, eosinophils, basophils, monocytes, and lymphocytes (T cells and B cells). [2, 3]

Thrombocytes (Platelets) are derived from megakaryocytes in the bone marrow and they are inactive and execute their actions only when activated. [4, 5] Platelets are responsible for the formation of intrinsic prothrombin activator which is responsible for the onset of blood clotting. Hence leukocyte and thrombocytes are associated with immune and inflammatory response. [3, 6]

Studies have shown that fruits such as banana has vital nutrient that helps to nourish and improve the blood cells such as leukocyte, erythrocyte and thrombocyte. Thus humans are exposed to fruits for its dietary need. [7, 8]

Banana is mainly consumed when ripened and its color ranges from green to yellow. Due to post-harvest spoilage and need to meet up demands of increase in fruit consumption chemical ripening of fruits are used as techniques for fruit preservation and management. [8, 9]

Calcium carbide (CaC_2) is among chemicals used to ripen fruits including banana. Calcium carbide in contact with moisture produces acetylene which is an analogue of natural ripening hormone (ethylene). Hence it mimics ethylene gas. [10]

Calcium carbide contains trace amounts of toxic arsenic and phosphorous that makes fruits poisonous when ripened with it. Study has also shown that calcium carbide affects the nutritional values of banana; and acetylene gas produced by calcium carbide may cause health issues such as headache, dizziness, mood disturbances, mental confusion, memory loss, cerebral edema etc. [11, 12]

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Since leukocytes and thrombocytes play vital role in immune and inflammatory response; this study is designed to investigate the role of calcium carbide exposed fruit (banana) on leukocyte and thrombocyte in Wistar rats.

MATERIALS AND METHODS

Animals and grouping

Twenty five (25) Wistar rats weighing approximately 200g were housed in laboratory cages and maintained at room temperature. The animals were randomly divided into 5 groups of 5 rats each and were fed standard rat feed and drinking water ad libitum.

Administrations

Group 1 (control) - 2ml of distilled H_2O;

Group 2 - 2ml of banana not exposed to CaC_2;

Group 3, 4 & 5 - 2ml of 5g/kg of CaC_2, 15g/kg of CaC_2, and 25g/kg of CaC_2 exposed fruit (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).

Preparation of CaC_2 Exposed fruit (Banana)

Banana fruit were harvested from the Universities plantation. The banana fruits were divided into four (4) categories; the first category was not exposed to calcium carbide while the remaining three (3) were exposed to 5g/kg, 15g/kg, and 25g/kg of calcium carbide (CaC_2). CaC_2 was gotten from a local market (Ukwunwangwu market) in Uturu, isiukwuato LGA of Abia State.

After exposure 500g of banana from the different categories were blended separately and diluted with 500ml deionized water, and then filtered to obtain the banana fruit juice for both

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CaC_2 and non CaC_2 exposed banana fruits. The obtained juice was properly labeled and preserved in the refrigerator for further use.

Sample collection

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

Laboratory Assay for Leukocyte count, leukocyte differential count and thrombocyte count

Leukocyte count, leukocyte differential count and thrombocyte count were determined using blood samples of experimental animal on hematological analyzer (T6000 alpha swelab). The blood samples were aspirated into the hematological analyzer and the parameters were measured automatically.

Statistical Analysis

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

Leukocyte count of Wistar rats in all groups of experimental protocols

Leukocytes count x 10^5 (µl) was significantly increased in Group 2 compared to Group 1 (control) $P < 0.01$. Group 3, Group 4 and Group 5 were significantly decreased compared to Group 1 (control) and Group 2, $P < 0.05$. There was significant dose dependent decrease between group 3, 4 and 5 compared to each other $P < 0.05$ (Figure 1).

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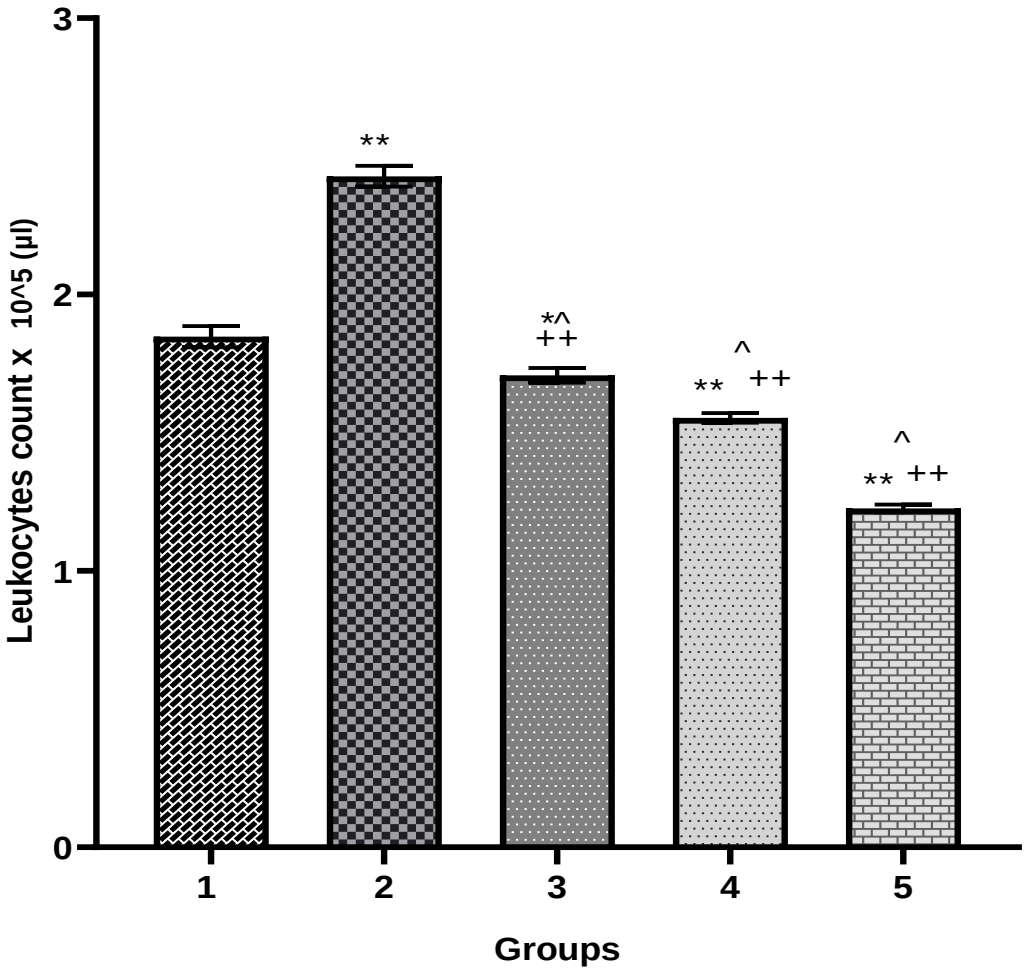

Figure 1: Leukocytes count x 10^5 (µl) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05) (**P < 0.01). + indicates values that are significantly different from animals in Group 2 (+P < 0.05) (++ P < 0.01). ^ indicates values that are significant between Group 3, 4 and 5 (P<0.05).Group 1= control; Group 2 = banana not exposed to CaC_2; Group 3 = 5g/kg of CaC_2; Group 4 = 15g/kg of CaC_2; Group 5 = 25g/kg of CaC_2.

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Leukocyte differential count of Wistar rats in all groups of experimental protocols

When compared to Group 1 there is significant increase in Group 2 and decrease in group 3, 4 and 5 of the leukocyte differential count, $P < 0.05$. There is significant dose dependent decrease in Group 3, 4 and 5 in the differential count compared to Group 2, $P < 0.05$. (Table 1)

Table 1: Leukocyte differential counts in all groups of experimental protocols

Groups	Lymphocytes x10 (%)	Monocytes (%)	Neutrophils x10 (%)	Eosinophil (%)
1. Control	64.44 ± 2.56	171.5 ± 7.78	137.3 ± 7.07	16.4 ± 1.03
2. No CaC_2	86.50 ± 3.59*	200.2 ± 35.9*	173.2 ± 4.98*	30.6 ± 2.99*
3. 5g/kg CaC_2	44.56 ± 3.53*+^	146.7 ± 3.20*+^	88.68 ± 3.76*+^	10.0 ± 0.73*^
4. 15g/kgCaC_2	28.72 ± 1.85*+^	123.2 ± 2.52*+^	58.52 ± 2.22*+^	5 ± 0.7*+^
5. 25g/kgCaC_2	18.66 ± 3.13*+^	41.7 ± 2.70*+^	47.98 ± 2.59*+	0.8 ± 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).*

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Thrombocyte count of Wistar rats in all groups of experimental protocols

There is dose dependent significant decrease in thrombocyte count in group 3, 4 and 5 when compared to group 1 and 2, $P < 0.05$. There are also significant dose dependent variations in group 3, 4 and 5 when compared to each other, $P < 0.05$. Comparison between group 1 and 2; showed significant increase in group 2, $P < 0.01$. (Figure 2)

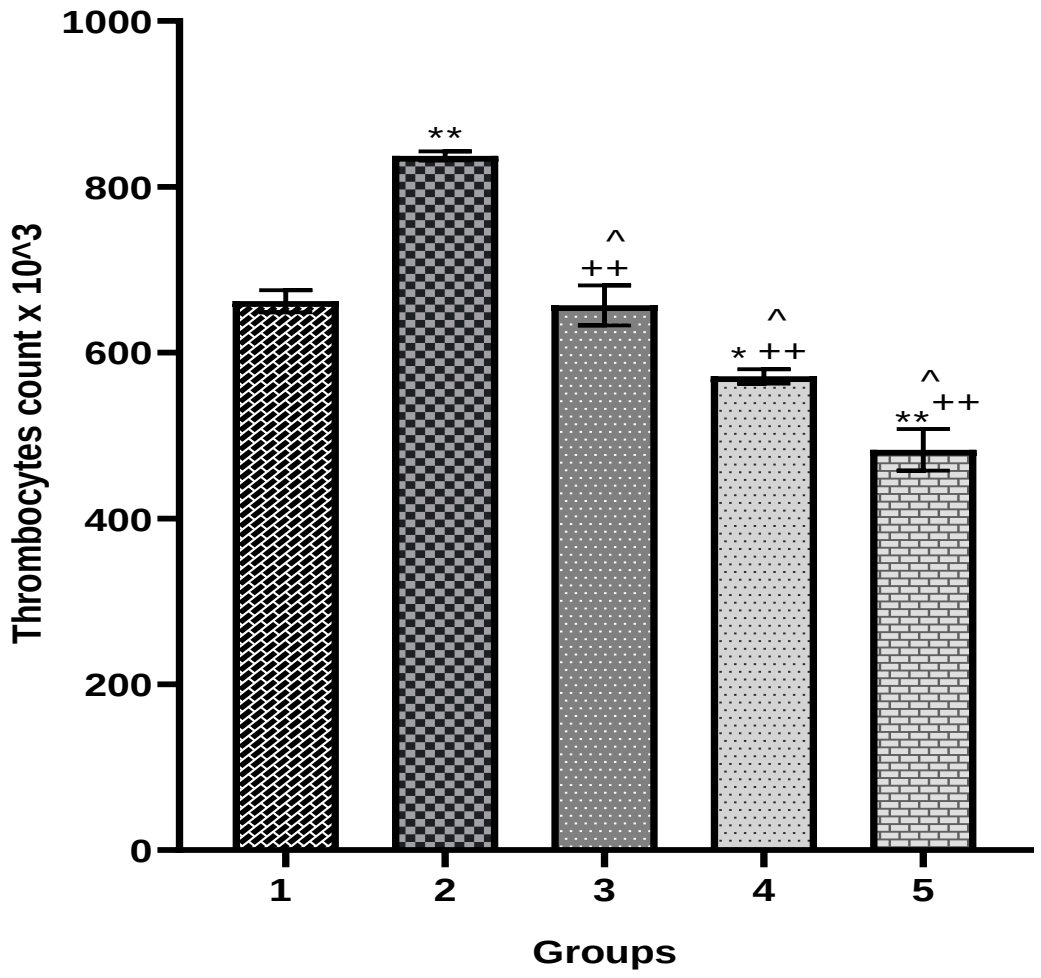

Figure 2: Thrombocytes count x 10^3 (µl) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05) (**P < 0.01). + indicates

values that are significantly different from animals in Group 2 (+P < 0.05) (++ P < 0.01). ^ indicates values that are significant between Group 3, 4 and 5 (P<0.05). Group 1= control; Group 2 = banana not exposed to CaC_2; Group 3 = 5g/kg of CaC_2; Group 4 = 15g/kg of CaC_2; Group 5 = 25g/kg of CaC_2.

DISCUSSION

The decrease in leukocyte count (Figure 1) and leukocyte differential count (Table 1) suggests that calcium carbide exposed fruit (banana) decreases Leukocytes profile. Leukocytes are immune cells that are involved in protecting the body against foreign invaders and infectious diseases; hence they function in immune responses. [3, 14] The results therefore suggest that calcium carbide exposed fruit is capable of compromising the immune system and reduce the body's ability to fight against foreign invaders.

Thrombocyte which is a vital cell for inflammatory response, healing process and blood clotting during injury [6, 15] was also significantly decreased (Figure 2) in the groups exposed to calcium carbide exposed fruits. This also suggests that calcium carbide exposed fruits such as banana compromises thrombocyte; hence the calcium carbide exposed fruit can affect the inflammatory and healing process of the body.

The observed increase in leukocyte, leukocyte differentials and thrombocytes as seen in Figure 1, Table 1 and Figure 2 of Wistar rats exposed to banana not treated with calcium carbide suggests that banana is a good fruit that enhances leukocytes and thrombocytes of the body, hence banana can be a good immune boaster and can play a role in wound healing which corresponds to previous reports [8, 16, 17]

CONCLUSION

Conclusively, Calcium Carbide (CaC_2**)** exposed fruit (banana) has deteriorating effect on leukocyte and thrombocyte in rats and this suggest that it can affect the immune system adversely and impede wound healing.

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