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(RESEARCH ARTICLE)

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# Therapeutic indication of caffeine and vitamin c on haematological profile in heparin, phenylhydrazine and aspirin induced haematological derangements in male Wistar rats

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## Abstract

**Background:** Caffeine and *Vitamin C*, key component in foods and beverages, have been investigated for potential haematological disease prevention.

**Aim:** To lend credence to the above studies, the current study in Wistar rats evaluated the effects of caffeine and vitamin C in the management of haematological disorders in rats.

**Methods:** Experimental animals were divided into three experimental phases (anaemic, leukocytopenic, and thrombocytopenic phases). Interventions with caffeine and vitamin C during anaemic, leukocytopenic, and thrombocytopenic phases were initiated for two weeks. Haematological parameters were all tested. All test, statistics were run using the Graph Pad Prism 8.1 at a confidence level of 0.05 or less (p-value).

**Results:** After analysis, caffeine and vitamin C shows a significant increase in RBC, PCV, Hb count in the anaemic treated rats when compared to control. Similarly, there was a significant increase in platelet count in the thrombocytopenic treated rats when compared to untreated thrombocytopenic group. There was also a significant increase in monocyte, lymphocyte and neutrophil counts in leukocytopenic animals compared to luukocytopenic group untreated.

**Conclusion:** In conclusion, this study suggests that caffeine and vitamin C have haemoprotective effects against anaemic, leukocytopenic and thrombocytopenic conditions.

**Keywords:** Anaemic; Leukocytopenic; Thrombocytopenic; Haemological indices; Caffeine

## 1. Introduction

Caffeine (chemically; 1, 2, 3-trimethylxanthine), a widely used pharmacological agent, has been studied for its potential benefits on cardiovascular and mental health, with debates surrounding its effects on animal models (1). Caffeine is a stimulant that temporarily reduces alertness and drowsiness in the human central nervous system and increases urine volume in intolerant subjects (2-4). Its common sources include coffee, tea, soft drinks, processed food and cocoa (5). Although some manufacturers claim that caffeine can be absorbed through the skin, which may unlikely due to its stimulatory effect. (6). Caffeine acts by antagonizing adenosine receptors in the brain, making it a competitive inhibitor (7-8).

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Plant bioactive compounds like quercetin and caffeine have significant applications in animal health and chemical industries (9-10). Despite caffeine toxicity, its wide use and increasing consumption have led to little attention to controversies surrounding its potential physiological effects (11-12). Recent scientific and public opinions raise concerns about caffeine's potential to reduce adverse health effects (13). Zhao's research on physiological changes in caffeine-fed rats, supports the beneficial effects of caffeine on living organisms (14).

On the other hand, vitamin C, commonly known as ascorbic acid, is essential for the growth, development, and repair of bodily tissues. It protects against free radical damage and harmful compounds, which contribute to diseases such as cancer, heart disease, and arthritis. Scurvy, fatigue, anemia, and loose teeth can all result from deficiency (15). Caffeine in coffee has a slight diuretic effect, increasing urine, which may deplete water-soluble vitamins like vitamin C. Regular ingestion is required to maintain healthy levels. Therefore, this study investigates the changes in haematological parameters after 14 days of caffeine and vitamin C administration to Wistar rats.

## 2. Material and methods

### 2.1. Chemicals

Pre-extracted caffeine product (1, 2, 3-trimethylxanthine) and vitamin C were purchased from Phil Hallmark Pharmacy, Benin City and Kunimed pharmachem ltd respectively. Phenylhydrazine hydrochloride, Aspirin, Heparin, and all chemicals utilized were bought from Sigma-Aldrich, St. Louis, MO, USA. All used chemicals were of top quality and analytical grade.

### 2.2. Animals

A total of ninety (90) male Wistar rats between 150 – 200 g were acquired and acclimatized for two weeks in the animal house of the aforementioned study area. Acclimatization of the animals was conducted within normal photoperiodic conditions while keeping them in standard laboratory settings. Water and standard rat chow were delivered *ad libitum* within the period of experimentation.

### 2.3. Experimental protocol

After acclimatization, the experimental animals were divided into 3 experimental Phases:

#### *2.3.1. Phase 1- (Anaemic phase)*

A total of 30 Wistar rats were divided randomly into five groups with six animals allocated in each group;

- **Group 1**: Received normal saline (10 ml/kg) and served as normal control
- **Group 2**: Anaemic group
- **Group 3:** Anaemic + caffeine (30 mg/kg)
- **Group 4:** Anaemic + caffeine (60 mg/kg)
- **Group 5:** Anaemic + Vitamin C (50 mg/kg)

#### *2.3.2. Phase 2- (Leukocytopenia Phase)*

A total of 30 Wistar rats were divided randomly into five groups with six animals allocated in each group;

- Group 1: Received normal saline (10 ml/kg) served as normal control
- **Group 2**: Leukocytopenia
- **Group 3**: Leukocytopenia + caffeine (30 mg/kg)
- **Group 4**: Leukocytopenia + caffeine (60 mg/kg)
- **Group 5**: Leukocytopenia + Vitamin C (50 mg/kg)

#### *2.3.3. Phase 3- (Thrombocytopenia Phase)*

A total of 30 Wistar rats were divided randomly into five groups with six animals allocated in each group;

- **Group 1**: Received normal saline (10 ml/kg) served as normal control
- **Group 2**: Thrombocytopenia
- **Group 3:** Thrombocytopenia + caffeine (30 mg/kg)

- **Group 4:** Thrombocytopenia + caffeine (60 mg/kg)
- **Group 5:** Thrombocytopenia + Vitamin C (50 mg/kg)

All treatments were made for two weeks after induction.

### 2.4. Ethical Consideration

All experimental procedures were performed strictly according to Delta State University's (DELSU's) ethical committee guidelines and protocol, ensuring animal care and safety. This is in accordance to the animal care regulations and standards that is approved by the Institute for laboratory animal research (ILAR, 1996). Ethical approval number: REC/FBMS/DELSU/21/125.

### 2.5. Preparation of Caffeine Stock Solution and administration

One hundred milligram (100 mg) commercial grade vitamin C tablet (Kunimed pharmachem ltd) was crushed and 50 mg dissolved in 0.5ml of distilled water and administered to rats weighing 150 g (75 mg/kg body weight of rat).Also, caffeine Stock solutions of 3 mg/mL and 6 mg/mL (30 mg/kg and 60 mg/kg respectively) were prepared using distilled water and administered. Thus, each rat thereafter received individual volume according to body weight. Caffeine was administered according to animals' body weight, such that 200 g, 170 g and 150 g rat received 2mL, 1.7mL and 1.5mL respectively. Administration was done orally using orogastric cannula.

### 2.6. Induction and confirmation of Anaemia, Leukocytopenia and Thrombocytopenia

Experimentally, rats were administered 30 mg/kg of phenylhydrazine hydrochloride for 8 days to induce haemolytic anaemia (16). Blood samples were collected to examine fragility profile and osmotic resistance. Aspirin was administered orally for leukocytopenia induction, with 150 mg/kg administered daily for 6 days a week for 5 weeks (17). Heparin was subcutaneously administered for thrombocytopenia induction, with platelet count confirmation after ten days (18).

### 2.7. Blood collection and preparation

About 0.5ml of blood was collected via retro bulbar puncture with a capillary tube for baseline tests and for confirmation of induced disorders.

### 2.8. Assessment of haematological indices

Freshly collected blood samples in ethylene diamine tetra-acetate (EDTA) bottles were analysed for haematological assay using an automatic haematological assay analyser (ERMA PCE 210, ERMA, Japan). Different tested haematological parameters were as follows: Packed cell volume (PCV) (19), White Blood Cell (WBC) and differential count (20), Red Blood Cells (RBC) (21), Haemoglobin (Hb) (22) and Platelet (PLT) (19). More so, blood was placed on a slide, stained with Leishman, and viewed under a microscope. Identified cells were counted per field using the differential WBC counter.

#### *2.8.1. Determination of Erythrocyte Sedimentation Rate (ESR)*

About 1:5 dilution of collected blood with 3.8 % sodium citrate anticoagulant in an ESR container was made. Next, the Westergren ESR tube was inserted into the container till blood gets to the "O" mark of the tube (to avoid air bubbles). The tube was then allowed to stand vertically and left for an hour. The exact meeting level between plasma and RBC (in mm) was read and recorded (19).

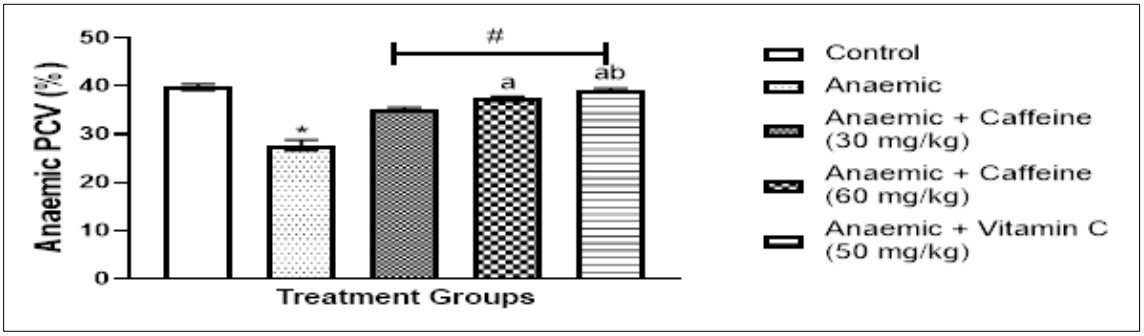
### 2.9. Statistical Analyses of Data

For each group, obtained results were presented as mean ± Standard error of mean (SEM) of sample size (n = 5). Average values were statistically compared by one-way analysis of variance (ANOVA) followed by post hoc Turkey's test for multiple comparison (where necessary) using Graph pad version 8.0. P-value < 0.05 was regarded as significant, statistically.

## 3. Results

### *3.1.* Effect of caffeine and vitamin C on packed cell volume level in phenylhydrazine induced anaemia in male rats

Figure 1 results show a marked decrease in PCV levels in anaemic rats. Caffeine and vitamin C intervention restored these changes to near normal, with vitamin C administration significantly increasing PCV levels.

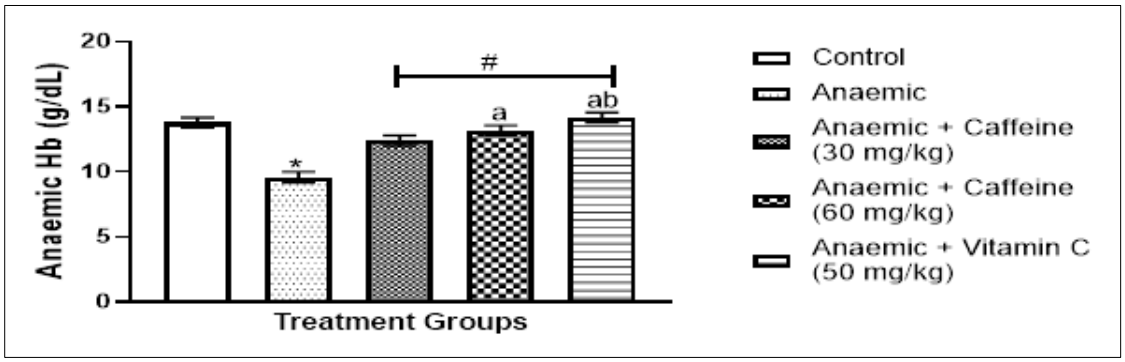



Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}P < 0.05$ relative to controls. $^{\#}p < 0.05$ relative to anaemic group; $^{a}p < 0.05$ relative to 30 mg/kg of caffeine treated group. $^{b}p < 0.05$, relative to 60 mg/kg of caffeine treated group.

**Figure 1** Effect of Caffeine and vitamin C on PCV in phenylhydrazine hydrochloride induced anaemic rats

### 3.2. Effect of caffeine and vitamin C on Haemoglobin in phenylhydrazine induced anaemia in male rats

Figure 1 results show a marked decrease in Hb levels in the anaemic group, but higher doses of caffeine and vitamin C restored them to near-normal levels. Vitamin C showed a more significant increase in Hb levels.

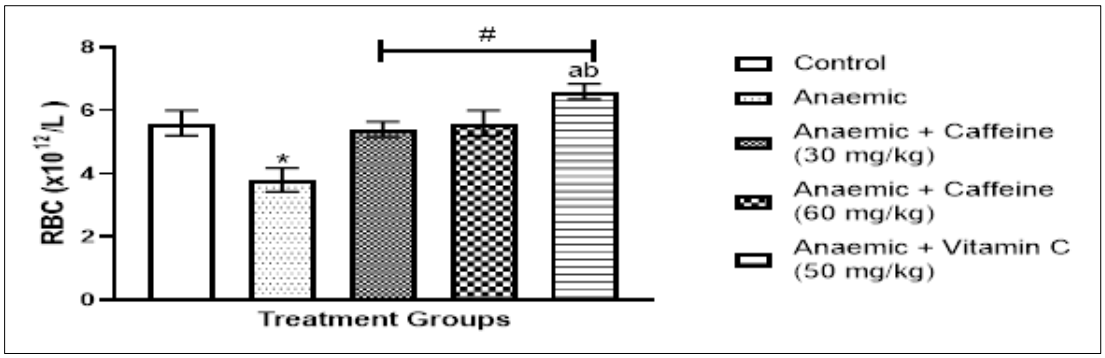



Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}P < 0.05$ relative to controls. $^{\#}p < 0.05$ relative to anaemic group; $^{a}p < 0.05$ relative to 30 mg/kg of caffeine treated group. $^{b}p < 0.05$, relative to 60 mg/kg of caffeine treated group.

**Figure 2** Effect of Caffeine and vitamin C on Hb in phenylhydrazine hydrochloride induced anaemic rats

### 3.3. Effect of caffeine and vitamin C on Red Blood Cell level in phenylhydrazine, induced anaemic in male rats

Figure 3 results show marked significant decrease in RBC levels in the anaemic group, but caffeine and vitamin C intervention restored them to near normal levels.

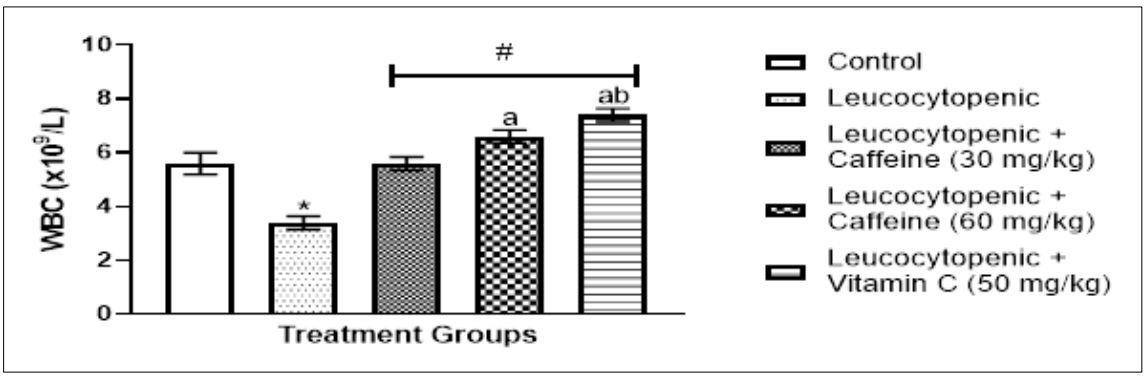



Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}P < 0.05$ relative to controls. $^{\#}p < 0.05$ relative to anaemic group; $^{a}p < 0.05$ relative to 30 mg/kg of caffeine treated group. $^{b}p < 0.05$, relative to 60 mg/kg of caffeine treated group.

**Figure 3** Effect of Caffeine and vitamin C on RBC in phenylhydrazine hydrochloride induced anaemic rats

### 3.4. Effect of caffeine and vitamin C on White Blood Cell level in aspirin induced leukocytopenic in male rats

Figure 4 results show that caffeine and vitamin C significantly increased WBC levels in leukocytopenic rats, while vitamin C showed a more significant increase in WBC.

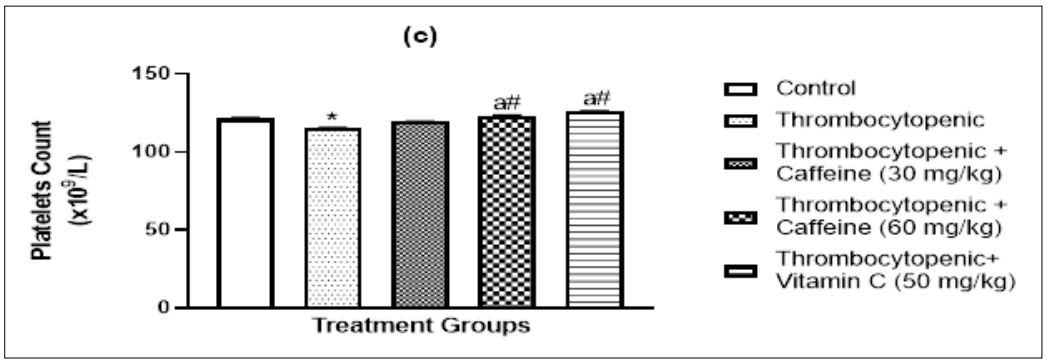



Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{\#}p < 0.05$ as compared to leukocytopenic group; $^{a}p < 0.05$ relative to 30 mg/kg of caffeine treated group. $^{b}p < 0.05$, relative to 60 mg/kg of caffeine treated group.

**Figure 4** Effect of Caffeine and vitamin C on WBC in aspirin induced leukocytopenic rats

### 3.5. Effect of caffeine and vitamin C on platelet level in heparin-induced thrombocytopenia in male rats

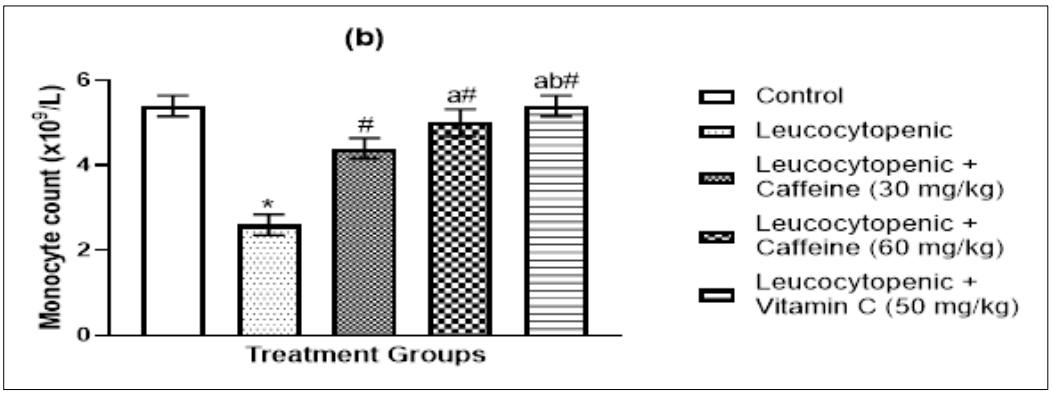
Caffeine and vitamin C treatment significantly increase the platelet count in male rats with heparin-induced thrombocytopenia, compared to the heparin groups alone (Figure 5).




Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{\#}p < 0.05$ as compared to thrombocytopenic group alone; $^{a}p < 0.05$ as compared to 30 mg/kg of caffeine treated group.

**Figure 5** Effect of Caffeine and vitamin C on platelet count in heparin induced thrombocytopenic rats

### 3.6. Effect of caffeine and vitamin C on Monocyte level in aspirin induced leukocytopenia in male rats



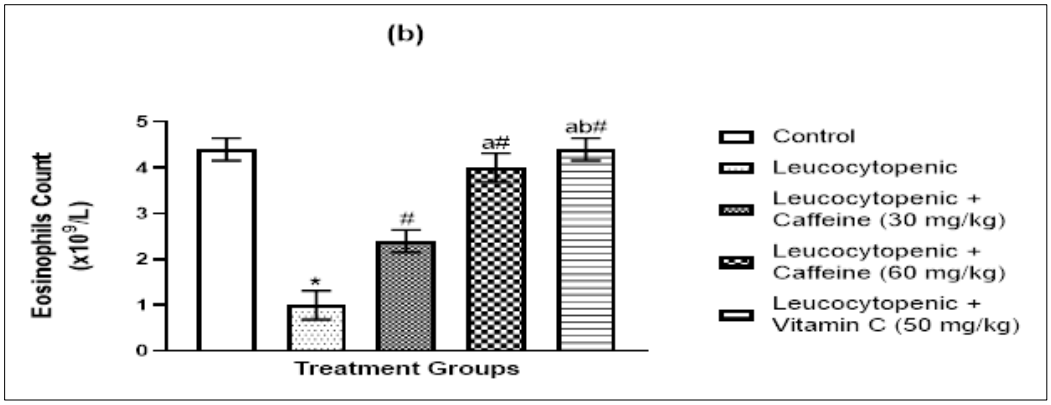

Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{\#}p < 0.05$ as compared to the leukocytopenic group; $^{a}p < 0.05$ as compared to the to 30 mg/kg of caffeine treated group. $^{b}p < 0.05$, as compared to the to 60 mg/kg of caffeine treated group.

**Figure 6** Effect of Caffeine and vitamin C on Monocyte in aspirin induced leukocytopenic rats

Figure 6 results show that caffeine and vitamin C significantly increased monocyte levels in the leukocytopenic group, with vitamin C showing a more significant increased as compared to caffeine treated groups.

### 3.7. Effect of caffeine and vitamin C on Eosinophil level in aspirin and induced leukocytopenia in male rats

Figure 7 results show that caffeine and vitamin C significantly increased eosinophil levels when compared to the leukocytopenia group, with vitamin C showing a more significant increase.

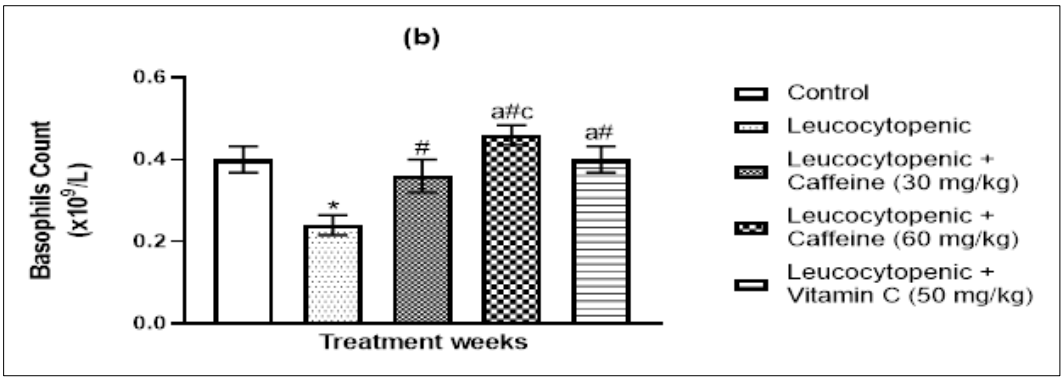



Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test). # p < 0.05 as compared to leucocytopenic group; ap < 0.05 relative to 30 mg/kg of caffeine treated group. bp < 0.05, as compared to 60 mg/kg of caffeine treated group.

**Figure 7** Effect of Caffeine and vitamin C on Eosinophil in aspirin induced leukocytopenic rats

### 3.8. Effect of caffeine and vitamin C on Basophil level in aspirin induced leucocytopenia in male rats

Figure 8 results show that caffeine and vitamin C significantly increased Basophil levels when compared to the leucocytopenic group alone. Notably, treatment with caffeine at 60 mg/kg increased basophils levels significantly when compared to vitamin C treated group as well as the caffeine treated group.

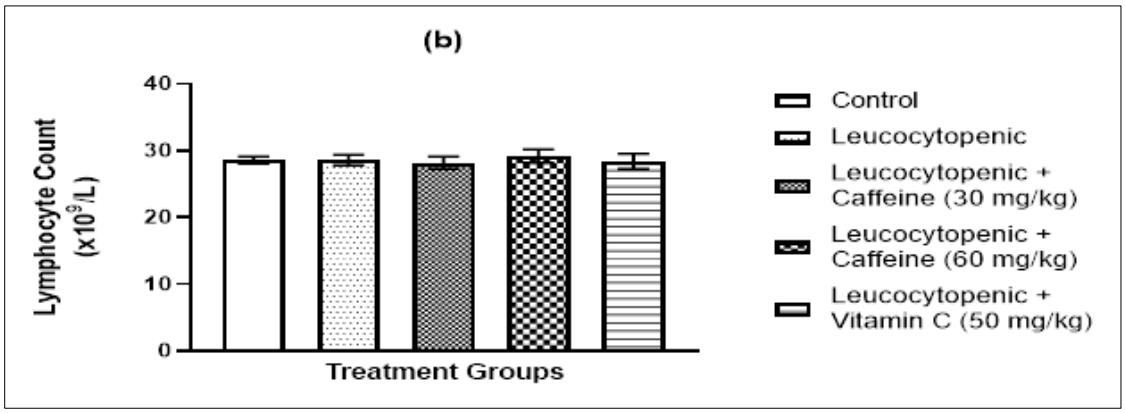



Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{\#}p < 0.05$ as compared to leucocytopenic group; $^{a}p < 0.05$ as compared to 30 mg/kg of caffeine treated group. $^{b}p < 0.05$, as compared to 60 mg/kg of caffeine treated group.

**Figure 8** Effect of Caffeine and vitamin C on Basophil in aspirin induced leukocytopenic rats

### 3.9. Effect of caffeine and vitamin C on Lymphocyte level in aspirin induced leukocytopeniain male rats

In figure 9, Caffeine and vitamin C shows no significant effects on lymphocyte levels in leukocytopenic male rats. No significant changes were also observed in the caffeine and Vitamin C treated groups as compared to the control group.




Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test).

**Figure 9** Effect of Caffeine and vitamin C on Lymphocyte in aspirin induced leukocytopenic rats

### 3.10. Effect of caffeine and vitamin C on neutrophil level in aspirin induced leukocytopenia in male rats

Figure 10 results show a significant decrease in neutrophil level in the leukocytopenic group, but caffeine and vitamin C intervention restored decreased neutrophil level to near normal levels.

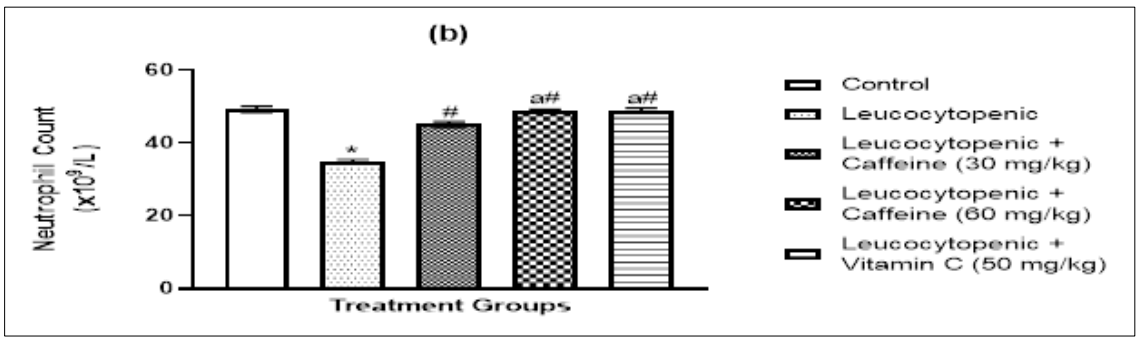



**Figure 10** Effect of Caffeine and vitamin C on neutrophil in aspirin induced leukocytopenic rats

Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{\#}p < 0.05$ relative to leukocytopenic group; $^{a}p < 0.05$ relative to 30 mg/kg of caffeine treated group. $^{b}p < 0.05$, relative to 60 mg/kg of caffeine treated group.

## 4. Discussion

This study has confirmed that vitamin C and caffeine have a direct relationship with hematological indices (24), and that they are both helpful in preventing anemia by raising RBC, PCV, and HB levels as well as restoring WBC, monocyte, eosinophil, basophil, and neutrophil in rats with induced leuokocytopenia. However, caffeine showed no lymphocytic benefits.

Caffeine and vitamin C may increase erythropoiesis and raise Hb and reticulocyte numbers in red blood cells, but they may not promote haemolysis (25). The decreased red blood cells in the anaemic untreated groups could be attributed to low flow rates, arteriolar oxygen ($O_2$) depletion, and decreased blood partial pressure of oxygen ($PO_2$). These changed RBCs have a higher surface area-to-volume ratio, which reduces $O_2$ uploading by stiff RBCs in the lung. These possibilities, however, remain theoretical, particularly in low RBC circumstances where decreased ATP release by changed RBCs has not been explored (26).

Monocyte levels in rats treated with coffee and vitamin C increased significantly, indicating increased monocyte phagocytosis activity. This could perhaps mitigate the harm caused by these illnesses. Caffeine consumption raises the number of basophils, eosinophils, and neutrophils. Caffeine at high doses may activate more active neutrophils, resulting in increased free radical generation and tissue damage, which could contribute to multiple organ failure and severe lung injury. More research is needed to determine the role of neutrophil activation in caffeine-related illnesses. Caffeine administration at high doses enhanced leukocytosis in rats, implying that it boosts the immune system and improves the body's resistance to infection.

Furthermore, whereas caffeine consumption increased platelet levels in thrombocytopenic rats, heparin-induced thrombocytopenia followed by vitamin C also increased platelet levels, demonstrating a thrombocytic action of vitamin C and caffeine. The rise in platelet count in the test animals could be attributed to the antioxidant properties of both caffeine and vitamin C (27).

## 5. Conclusion

Given the findings of this study, it is possible to draw the conclusion that both caffeine and vitamin C have haemoprotective effect against anaemic, leukocytopenic, and thrombocytopenic syndromes in animals

## Compliance with ethical standards

### *Acknowledgments*

The authors thank the technical staff of the Department of Physiology, Faculty of Basic Medical Sciences, Delta State University in Abraka, Nigeria

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of ethical approval*

The Delta State University Ethical Use of Animals Research Committee (RC) prepared protocols that were followed for using adult male Wistar rats in this work. The reference number for these protocols was given as REC/FBMS/DELSU/21/125.

### *Author Contributions*

The study was designed by OMO and OO, who also measured biochemical parameters, did statistical analysis, and produced and evaluated the report. OMO, JCI, helped organize the protocol and designed the animal grouping. OMO and OO contributed to the manuscript's review. All authors approved the final version after reviewing the manuscript

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