MSI Journal of
Multidisciplinary Research (MSIJMR)

EFFECT OF *Carica papya* SEED EXTRACTS ON RED BLOOD CELLS AND PLATELETS INDICES IN PHENYLHYDRAZINE INDUCED-ANAEMIA WISTAR RATS

Adesina Abraham Ayomide[1], Yakubu Abdulrahaman[2], Yusuf Yakubu[3], Paul Theophilus[4], Ayuba Obed lyocks[5], Khadijah Baaba Adamu[6], Maryam Mustapha Emigi[7], Mustapha Ibrahim Sihha[8], Joshua Yahaya B[9], Aminu Hafsat[10], Jafaru Muhammad Bunza[11*]

[1] Department of Hematology, Federal Medical Centre Bida.

[2] Department of Hematology, School of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto.

[3] Department of Chemical Pathology Modibbo Adama University Teaching Hospital Yola.

[4] Department of Hematology Federal Teaching Hospital Lokoja, Kogi State.

[5] Department of Hematology Ahmadu Bello University Teaching Hospital Zaria, Kaduna State.

[6] Department of Hematology Ministry of Defense, Abuja.

[7] Maryam, M. M., Department of Hematology, University of Abuja Teaching Hospital, Gwagwalada, FCT.

[8] Department of Hematology Aminu Kano Teaching Hospital, Tarauni Kano state.

[9] Yariman Bakura Specialist Hospital Gusau Zamfara State.

[10] Department of Hematology and Blood Transfusion Usmanu Danfodiyo university teaching hospital Sokoto.

[11*] Department of Chemical Pathology, School of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto.

*** Correspondence:** Jafaru Muhammad Bunza

The authors declare that no funding was received for this work.

OPEN ACCESS

Received: 10-October-2025

Accepted: 10-November-2025

Published: 13-November-2025

This article is published in the **MSI Journal of Multidisciplinary Research (MSIJMR) ISSN 3049-0669 (Online)**

The journal is managed and published by MSI Publishers.

Volume: 2, Issue: 11 (November-2025)

ABSTRACT: The haematological condition known as anemia is typified by a decrease in red blood cells or an inadequate oxygen-binding capacity of haemoglobin molecule to transport oxygen to different tissues and satisfy physiological needs. Anaemia remains a major health burden. The healthcare

system benefits greatly from the use of medicinal plants, particularly in rural areas. Carica papaya is one such plant that has a broad range of patronage. The goal of the current study was to find out how Carica papaya seeds affected the platelet and red blood cell indices in anaemic wistar rats models. In this study, thirty rats weighing 150 – 190g were split up into six groups, each with five rats. Anemia was not induced in the first group (group I) and served as normal control. Group II received phenylhydrazine40mg/kg only group III received phenylhydrazine40mg/kg and folic acid and B12treatment, group IV, V and VI were given papaya seed extracts for a14 day period at doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg, respectively. Result of red blood cell count in folic acid+ B12 treatment group (7.14±0.22) and papaya seed extract in anaemia + 200mg/kg(7.18±0.26), anaemia + 400mg/kg (6.82±0.37) and anaemia + 800mg/kg (7.43±0.19) showed a statistically significant increase compared to untreated control group (5.66±0.26).The haemoglobin concentration as well showed a statistically significant increase from untreated anaemic group(8.70±0.40) to anaemia folic +B12 treated (13.24±0.39) anaemia + 200mg/kg(13.66±0.50), anaemia+ 400mg/kg(3.34±0.39) and anaemia + 800mg/kg (13.37±0.12). There was also an observed improvement in the haematocrit from the anaemic untreated group(24.2±1.35) compared to the anaemic +folic + B12(35.6±0.82) and anaemia+200mg/kg(37.8±1.14), anaemia+ 400mg/kg(36.6±0.82) and anaemia +800mg/kg (36.9±0.57) The MCV showed only a statistically significant decrease in anaemic + folic acid+ B12 group(51.7±0.88) compared to the negative control group (54.6±1.06) for MCH ,MCHC RDW-CV and RDW-SD there was no statistical significance across the groups. The platelet count of anaemia + folic acid + B12 (636±27), anaemia + 200 mg/kg (699±49), anaemia + 400 mg (658±50), and anaemia + 800 mg/kg (620±37) were all statistically insignificantly higher than the anaemic and untreated group (557±64) group. The MPV as well as PCT showed similar trend in the study. In this research it was concluded that papaya seed extract has an effect on red cell and platelet but little effect on their indices and shows good potential as an anti-anaemic treatment.

Keywords: *Anaemia, carica papaya seeds, phenylhydrazine, red blood cells, platelets.*

INTRODUCTION

Anaemia is commonly described the decrease in the quantity of red blood cells or hemoglobin in circulation, which lowers the amount of oxygen delivered to tissues (Freeman *et al*., 2023). The World Health Organization (WHO, 2008) defines anemia as having hemoglobin (Hb) levels of less than 12.0 g/dl in women and less than 13.0 g/dl in men.

Anaemia is major global health issue affecting both developed and developing nations. It manifests through symptoms such as fatigue, weakness, dizziness, shortness of breath, reducing overall quality of life, impairing daily functioning and, in severe cases, death. Anaemia is rarely a standalone condition; its aetiology is multifactorial, influenced by diet, physiological state, infections, genetics. In developing nations, its prevalence is heightened by malnutrition, parasitic infections (*Plasmodium*, trypanosomes, helminths), and widespread use of medications such as and exposure to certain drugs and chemicals such as phenylhydrazine, dapsone, hydroxylamine, ammonium chloride. (Cappellini & Motta, 2015; Pingali, 2015; Phillips and Henderson, 2018; Aloke et al., 2021).

phenylhydrazine induces haemolytic anaemia due to its interaction with hemoglobin, which results in the production of hydrogen peroxide and the oxidized derivatives and free radicals of hydrazine that destroy the pigment. Increased lipid peroxidation brought on by these free radicals promotes membrane damage. Furthermore, phenylhydrazine stimulates the immune system, which encourages phagocytosis and prevents erythropoietin from binding. (Aloke *et al*., 2021).

Humans have had a close relationship with their surroundings since the beginning of time and have used elements found there to make food and medicine (Jamshidi-Kia *et al.*, 2017). Human knowledge on plants has long been progressively communicated through generations and over the years through trial by error has led to man discovering uses of plants for food and medicine. Today modern societies work to Ensuring the efficacy, safety, and purity of medicinal plants and herbal remedies (Jamshidi-Kia *et al.*, 2017). Approximately 70–95% of people in developing nations use plants as their primary source of medication. These plants

have a lot of biofunctional substances which exhibit antibacterial, antioxidant, anti-inflammatory and anthelmintic properties. (Van Wyk & Wink, 2018; Khan & Ahmad, 2019). *Carica papaya* seeds which are small, black structures which have a flavour similar to black pepper usually discarded as waste have been used in herbal medicine for a very long time (Omobolanle *et al*., 2017; Saba and Pattan, 2022).

Studies have reported the antithrombocytopenic, antiulcerogenic, larvicidal, and antidiabetic effects of carica papaya seeds (Amin *et al*., 2019; Ugbogu *et al*., 2023; Agada *et al*., 2020). Papaya seed widespread availability, low cost, and diverse pharmacological activities make them a promising candidate for developing plant-based therapies.

Platelet indices (platelet count, mean platelet volume, platelet distribution width) and corpuscular indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width)are critical diagnostic parameters for classifying anaemia types, monitoring platelet function, and evaluating treatment responses (Kliegman *et al.,* 2022.

The purpose of this study is to assess how papaya seed extracts affect platelet and red blood cell indices in a phenylhydrazine-induced anaemia Wistar rat.

MATERIALS AND METHODS

Experimental Animals

We bought thirty adult wistar rats, weighing between 140 and 190 grams, from the animal house of the pharmaceutical sciences faculty at Usmanu Danfodiyo University in Sokoto, Nigeria. For the duration of the study, the rats were kept in a stainless-steel cage in a ventilated room in the animal house of Usmanu Danfodiyo University Sokoto's faculty of pharmaceutical sciences. We fed the wistar rats with commercial livestock feed obtained and allowed unlimited access to clean drinking water throughout the study. Six groups of five rats each were randomly selected from among the animals.

Group I: these animals were un-induced group

Group II: The Animals were induced with 40mg/Kg of phenylhydrazine for 2 days to induce anaemia. Untreated group

Group III: The Animals were induced with 40mg/Kg of phenylhydrazine of appropriate dosage for 2 days to induce anaemia and were treated with appropriate dosage of oral Folic acid(50ug/kg) and intraperitoneal B12(0.3mg/kg)

Group IV: The Animals were induced with 40mg/Kg of phenylhydrazine for 2 days to induce anaemia and then treated with 200mg/kg papaya seeds extract

Group V: The Animals were induced with 40mg/Kg of phenylhydrazine of for 2 days to induce anaemia and were treated with 400mg/Kg papaya seeds extract

Group VI: The Animals were induced with 40mg/Kg of phenylhydrazine for 2 days to induce anaemia and were treated with 800mg/Kg papaya seeds extract

PLANT PREPARATION AND EXTRACTION

The Fresher *Carica papaya* seeds from which sample was identified was washed and allowed to dry for a duration of five weeks. The dried C. Papaya Seeds were roughly crushed. 375.98g of the roughly crushed powder material was soaked in 1000 mL of aqueous solvent (distilled water) for 24 hours inside a volumetric flask with continuous stirring every 2hours to ensure maximum extraction. The resulting crude extract was filtered with (Whitman® No. 1 filter paper) to get rid of the chaff (dregs). Filtrate was concentrated in an oven at 40ºC and the derived extract was weighed 74.67g and stored in a vial at refrigeration of 4ºC

INDUCTION OF EXPERIMENTAL ANAEMIA

Based on animal welfare rationality and simplicity of Anaemia model, phenyl hydrazine was used to induce anaemia. In this experiment a phenyl hydrazine suspension was loaded intraperitoneally at a dosage of 40mg/kg for two days (D_0 & D_1). Rats were observed for the formation of anaemia for 48hours by observing their eyes and ears and feet, alertness and agility.

After induction of Anaemia in experimental animals in all groups except the positive control group, all other group of animals were divided and subjected to treatment

with standard treatment receiving 50ug/kg of Folic acid and 0.3mg/kg of vitamin B12, papaya seeds extract 200mg/kg, papaya seed extract 400mg/kg and papaya seed extracts 800mg/kg.

The animals were anesthetized with chloroform for few seconds. 3mls of blood was collected via cardiac puncture into an EDTA sample container.

HAEMATOLOGICAL ANALYSIS

The Red blood cells and platelets indices were analysed using Mythic™ 60 5+ differential hematology analyzer. Which employs the principle of impedance to give the red cell and platelet parameters.

Results were expressed as mean ± standard error of the mean (SEM). Multiple comparisons between the control and experimental groups, as well as among the experimental groups, were performed using one-way ANOVA. Significant differences among treatments were determined using the LSD post hoc test, and values of $P < 0.05$ were considered statistically significant. All results were presented in tabular form.

RESULTS

Table 1 Effects of *Carica papaya* seeds extract on haematological parameters in phenyl hydrazine anaemic wistar rats

Groups	N	RBC($x10^6$/µl)	HGB(g/dl)	HCT (%)	MCV (fL)
I	5	6.75 ± 0.29^a	13.20 ± 0.33^a	36.8 ± 1.15^a	54.6 ± 1.06^a
II	5	5.66 ± 0.26^b	8.70 ± 0.40^b	24.2 ± 1.35^b	51.7 ± 0.88^a
III	5	7.14 ± 0.22^a	13.24 ± 0.39^a	35.6 ± 0.82^a	50.0 ± 0.61^b
IV	5	7.18 ± 0.26^a	13.66 ± 0.50^a	37.8 ± 1.14^a	52.7 ± 1.01^a
V	5	6.82 ± 0.37^a	13.34 ± 0.39^a	36.6 ± 0.82^a	54.2 ± 2.28^a
VI	5	7.43 ± 0.19^a	13.37 ± 0.12^a	36.9 ± 0.57^a	52.3 ± 0.063^a

Legend: RBC = Red Blood Cell Count; HGB = Haemoglobin Concentration; HCT = Haematocrit; MCV = Mean Cell Volume. Values are expressed as mean ± SEM.

Values in the same column bearing different superscripts are significantly different at $P < 0.05$. N = 5 rats per group.

Table 2 Effects of *Carica papaya* seeds extract on red cell indices in phenyl hydrazine induced anaemic Wistar rats

Groups	N	MCH(pg)	MCHC(g/dL)	RDWCV(%)	RDWSD(fL)
I	5	19.6±0.38[a]	35.9±0.31[a]	12.7±0.53[a]	24.8±1.15[a]
II	5	18.7±0.34[a]	36.1±0.33[a]	11.8±0.63[a]	21.9±0.44[a]
III	5	18.6±0.12[a]	37.1±0.29[a]	11.1±0.23[b]	19.9±0.49[b]
IV	5	19.0±0.15[a]	36.1±0.49[a]	12.1±0.79[a]	23.2±1.84[a]
V	5	19.7±0.59[a]	36.4±0.47[a]	12.1±0.51[a]	24.6±2.63[a]
VI	5	18.4±0.35[a]	36.7±0.19[a]	11.4±0.20[a]	20.4±1.03[a]

Legend: MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; RDW-CV = Red Cell Distribution Width Coefficient of Variation; RDW-SD = Red Cell Distribution Width Standard Deviation. n = 5 rats per group. Values are expressed as mean ± SEM. Values in the same column bearing different superscripts are significantly different at $P < 0.05$

Table 3: Effects of papaya seed extract on platelet count, mean platelet volume, plateletcrit, platelet distribution width, and platelet–large cell ratio in phenylhydrazine-induced anaemic Wistar rats.

Groups	N	PLT(x10^3/µl)	MPV(fL)	PCT (%)	PDW (%)	PLCR(%)
1	5	890±71[b]	7.1±0.19[b]	0.626±0.045[b]	31.4±3.31[a]	7.0±0.46[a]
II	5	557±64[a]	6.8±0.05[a]	0.378±0.04[a]	36.7±5.00[a]	6.8±0.14[a]
III	5	636±27[a]	6.3±1.06[c]	0.400±0.023[a]	38.0±2.22[a]	4.5±0.44[b]
IV	5	699±49[a]	6.4±0.13[a]	0.446±0.03[a]	33.9±2.10[a]	4.7±0.38[b]
V	5	658±50[a]	6.6±0.16[a]	0.434±0.037[a]	31.8±4.26[a]	5.9±0.47[a]
VI	5	620±37[a]	6.4±0.15[a]	0.397±0.030[a]	40.6±2.72[a]	4.9±0.75[b]

Legend: Values are expressed as mean ± SEM. Values in the same column bearing different superscripts are significantly different at $P < 0.05$. PLT = Platelet Count;

MPV = Mean Platelet Volume; PCT = Plateletcrit; PDW = Platelet Distribution Width; PLCR = Platelet Large Cell Ratio.

DISCUSSION

The purpose of this study was to evaluate the effects of papaya seed extract on red blood cell and its indices as well as platelet and platelet indices in a phenyl hydrazine induced anaemia wistar rats.

The red blood cell count, haematocrit, and haemoglobin concentration of all treatment groupsof all the treatment groups (standard folic acid & B12 treatment, papaya seed extract 200mg/kg 400mg/kg and 800mg/kg) showed a statistically significant increase ($P < 0.05$) compared to the induced, untreated group. This indicates papaya seed extracts have protective effects on circulating red blood cells or promotes erythropoiesis or both, boosting their values significantly back to normal. These findings were consistent with studies by (Ikpeme *et al*., 2011; Tedwins *et al*., 2015; Amin *et al*., 2019) who reported on the haematological effect of papaya seeds. Phenylhydrazine administration inhibits various enzymes, immune mechanisms, generating free radicals that leads to the oxidative damage of red blood cells (Berger, 2007)**.** The phytochemical profile of papaya seed extract contains alkaloid and flavonoid and vitamins. which have antioxidant activity scavenging free radicals produced, preventing membrane damage, ultimately keeping heme iron in its ferrous state. Also, similarity of the extract's effect to that of folic acid and B12 in the present study supports the idea that the seed extract provides both protective and nutritive components that support haemoglobin synthesis and erythroid recovery.

Findings from this study also showed that the red cell indices (MCV, MCH, MCHC) of the treatment groups Papaya seed extracts 200mg/kg 400mg/kg and 800mg/kg showed no statistically significance when compared with both control groups. This suggests that treatment restored the number of normocytic, normochromic erythrocytes rather than producing a notable shift toward macrocytosis or microcytosis. In other words, the extract appears to recover blood oxygen-carrying capacity by increasing RBC mass while preserving normal cell morphology. Findings

from this study was in agreement with (Gheith and El-Mahmoudy, 2019) (Alhassan *et al.*, 2020) (Adewuyi *et al*., 2024)) and (Taiwo *et al.*, 2021).

Phenylhydrazine caused thrombocytopenia when compared to the uninduced control. Treatment with papaya seed extracts 200mg/kg 400mg/kg and 800mg/kg produced modest increases in platelet counts that was not statistically significant (P> 0.05), compared to the induced, untreated animals. This finding are consistent with research conducted by (Adewuyi *et al*., 2024) on pre-clinical protective potentials of Carica papaya constituents in experimentally induced anemia but in contrast to studies by (Pandita *et al.*, 2019) and (Okon *et al.*, 2023) who reported an increase in platelet count after treatment with papaya leaves extracts following lead nitrate administration as well as the treatment of thrombocytopenia in dengue fever patients within 24hours of administration of papaya leaf extract drugs. Suggesting longer duration required by papaya seeds in alleviating thrombocytopenia or a lack of active antithrombocytopenic ingredients in aqueous papaya seed extracts as opposed to alcoholic extracts.

In this study The MPV, PCT and PDW in the treatment groups (standard folic acid & B12, papaya seed extract 200mg/kg, 400mg/kg and 800mg/kg) revealed no statistically significant (P>0.05) increase following the course of treatment when compared to the induced untreated group. this is in corroboration with (Taiwo *et al.*, 2021) who in his study showed there was no statistical significance in platelet indices after administration of Carica papaya seeds extracts. In this study, comparison of the platelet–large cell ratio between the induced, untreated group and the treatment groups showed a statistically significant decrease (P < 0.05). P-LCR were reduced in treated animals, suggesting a shift toward smaller, less reactive platelets and a lowering of the proportion of large platelets. These observations are consistent with a partial recovery from a reactive thrombopoietic response and may reflect changes in marrow megakaryocyte activity or platelet survival and concur with (Ofem *et al.*, 2012) Smith *et al.* (2015) explaining the role of platelet indices in unexplained anaemia. furthermore studies carried out by (Baig, 2015) which associated its increase to the increase in MPV as a result of the increase destruction of platelets after phenyl hydrazine administration.

CONCLUSION

Findings from this study showed that Papaya seed extract has an observed effect on the haemoglobin concentration, haematocrit and red blood cell count, in experimental animals. It however showed no effect on red cell indices. Platelets and its indices as well were not significantly affected by papaya seed extracts.

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