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Research Article

Hematological effect of *Mucuna pruriens, Justicia carnea* and their combination on phenylhydrazine-induced anemia in rat

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Abstract

Objectives: Anemia, a widespread public health concern, affects millions globally, particularly in developing countries. Traditional medicinal plants, including *Mucuna pruriens* and *Justicia carnea*, have been used to manage anemia due to their potential hematopoietic and antioxidant properties. This study investigated the hematological effects of *Mucuna pruriens*, *Justicia carnea*, and their combination on phenylhydrazine-induced anemia in rats, aiming to provide insight into their therapeutic potential.

Methods: The acute oral toxicity test (LD50) was conducted using the Up-and-Down procedure. Anemia was induced in all rats, excluding the normal control group, via a single-dose intraperitoneal injection of 80 mg/kg phenylhydrazine. Thirty (30) adult male albino rats were assigned into six (6) groups of five (5) rats, consisting of the normal control, anemic control, and treated groups (standard drug—Astyfer 1.5 mg/kg, 200 mg/kg of ethanol leaf extracts of *Mucuna pruriens, Justicia carnea*, and their combined leaf extracts). Treatment was given orally once per day and lasted for 21 days. Blood samples were collected two weeks into treatment and three weeks after treatment. Hematological parameters were determined using standard biochemical methods. The parameters analyzed were hemoglobin (HB), packed cell volume (PCV), and red blood cell count (RBC).

Results: The LD50 results revealed no mortality or signs of toxicity at doses up to 5000 mg/kg body weight. The findings of this study revealed that, two weeks into treatment, all treatment groups showed a significant increase ($p<0.05$) in their HB, PCV, and RBC levels compared with the anemic control. At the end of the treatment (three weeks), the HB of groups treated with 200 mg/kg *Justicia carnea* and the combined extract were significantly higher ($p<0.05$) than in the other groups.

Conclusion: The combination of *Mucuna pruriens* and *Justicia carnea* offered a modest additional benefit, although the improvement was not substantially greater than the individual effects of each extract.

Keywords: Anemia, combination, *justicia carnea, mucuna pruriens*, phenylhyrazine-induced, rat

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Anemia, a prevalent blood disorder affecting individuals across all age groups, disproportionately impacts the elderly, women of childbearing age, and infants. Although not a disease itself, anemia often arises from underlying conditions [1]. With over 400 types, mostly rare, anemia is characterized by a reduced number of circulating red blood cells [2]. The most common form, iron-deficiency anemia, results from insufficient iron, a crucial component of hemoglobin. Heavy or persistent bleeding, particularly menstruation in women of childbearing age, is the primary cause of iron deficiency. Iron-deficiency anemia can also result from gastrointestinal bleeding caused by disorders like erosive gastritis, peptic ulcers, and inflammatory bowel disease [3].

The use of plants in treating anemia has garnered significant attention in recent research, highlighting various species with potential therapeutic effects. Anemia is reportedly managed

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with plant-derived treatments in traditional medicine practices. Several studies have demonstrated the efficacy of specific plant extracts in ameliorating anemia symptoms. For instance, *Mangifera indica* and *Telfairia occidentalis* showed significant anti-anemic effects in rabbits, with increased hemoglobin levels observed after treatment with their extracts [2]. A study on *Falcaria vulgaris* indicated that its aqueous extract significantly improved hematological parameters in rats, suggesting its potential as an anti-anemic agent [4]. Ethnobotanical research in Nigeria identified ten plants, including *Sorghum bicolor* and *Terminalia catappa*, traditionally used for anemia treatment, confirming their nutritional and phytochemical value [5]. Over the last decade, Nigeria has seen a surge in the cultivation and utilization of *Mucuna pruriens* and *Justicia carnea* for various medicinal applications, notably the treatment of anemia. These plants are increasingly becoming integral to traditional medicine practices in the region.

Mucuna pruriens (velvet bean/cowage), native to Africa and Asia, has been used in traditional medicine for its therapeutic properties, including nervous system regulation, reproductive health, and disease management [6,7]. Its seeds and extracts have been studied for their potential to treat infertility, nervous disorders, and male sexual dysfunction, as well as their aphrodisiac, neuroprotective, and anti-parkinsonian effects [8]. Research has also demonstrated its ability to increase hemoglobin levels and red blood cell count in anemic animal models [9].

Justicia carnea, native to Brazil, has been used in traditional South American medicine for its anti-inflammatory, analgesic, and antioxidant properties. It has also been employed to manage inflammation and gastrointestinal disorders [10–12]. Research has shown that *Justicia carnea* leaf extract may have hematinic effects, increasing hemoglobin levels and red blood cell count in experimental animals [13].

For centuries, traditional cultures have employed combined plant extract therapy, or polyherbal therapy, to enhance healing, balance bodily systems, and minimize side effects [14,15]. This approach leverages synergies between active compounds in multiple plants to achieve greater benefits than individual extracts. Rooted in ancient practices like Ayurveda, Traditional Chinese Medicine, and indigenous healing, combined therapy harnesses the power of plant interactions [16]. When applied correctly by knowledgeable practitioners, combined plant extract therapy offers numerous benefits. However, improper use can pose risks. Success relies on informed formulation, blending traditional wisdom with modern scientific insight [17]. In treating anemia, particularly iron-deficiency anemia, traditional medicine systems have explored combined plant extract therapies. Plant-based treatments incorporating iron-rich herbs, absorption enhancers, and vitality boosters can effectively manage anemia [18].

This study explored the potential therapeutic benefits of ethanol leaf extracts of *Mucuna pruriens* and *Justicia carnea* and their combined extract on hematological parameters in phenylhydrazine-induced anemia in rats. Anemia's significant impact on human health necessitates innovative treatments. *Mucuna pruriens* and *Justicia carnea* have been traditionally used to treat various health conditions, including anemia [13,19]. Investigating their combined effects may reveal synergistic or additive actions in alleviating anemia-related symptoms. Hematological parameters serve as crucial indicators of overall health [20]. By examining the changes in hematological parameters post-treatment with combined plant extracts, researchers can uncover valuable insights into the biological mechanisms that contribute to their therapeutic efficacy in combating anemia. While individual studies have investigated the effects of *Mucuna pruriens* and *Justicia carnea* extracts on hematological parameters [13,19], there is a notable gap in the literature regarding their combined effects. Few studies have explored the synergistic or additive effects of combining plant extracts, particularly in the context of treating anemia or related conditions [21]. This study aims to contribute to the existing body of knowledge by exploring the combined effects of *Mucuna pruriens* and *Justicia carnea* ethanol leaf extracts on hematological parameters in phenylhydrazine-induced anemia in rats. It also aims to contribute to the development of novel therapeutic strategies for managing anemia and related disorders.

Materials and Methods

Helsinki declaration – ethical approval

This study adhered to international guidelines for laboratory animal care and use, with approval from the Institutional Research and Ethical Clearance Committee, Faculty of Basic Medical Sciences, with the approval number: ESUCOM/FBMS/ETR/2024/04.

Sample collection and identification

Fresh leaves of *Mucuna pruriens* and *Justicia carnea* were harvested from a garden located at Ebeano Tunnel, Fidelity Estate, Enugu. They were identified by a botanist in the Department of Botany and deposited in the herbarium with voucher numbers NAUH-15 and NAUH-203B for *Mucuna pruriens* and *Justicia carnea*, respectively.

Sample preparation/extraction

The leaves of *Mucuna pruriens* and *Justicia carnea* were properly rinsed separately with distilled water and air-dried at room temperature (25 ^{o}C). The dried leaves were ground into a powdery form using a grinding mill. One hundred grams (100 g) of each of the powdered leaves was subjected to Soxhlet extraction as follows: the sample was wrapped in filter paper and placed in the thimble of the Soxhlet apparatus. The condenser and heating mantle were connected securely. An initial 500 ml volume of ethanol was added to the round-bottom flask via a funnel, passing through the sample-containing thimble. The condenser's inlet and outlet were connected to a hose for continuous cold water circulation during extraction. Finally, the heat source was activated, positioned 5 cm from the flask. The resulting filtrate was concentrated in a water

bath at 50 ºC. For the combined dose, the extracts of *Mucuna pruriens* and *Justicia carnea* were combined in equal proportions (1:1 ratio), reconstituted in distilled water to achieve the designated treatment group doses, and then administered orally once daily via gavage.

Experimental animal

A total of 30 male albino rats, weighing 150–200 g, were acquired from the animal house of Nnamdi Azikiwe University, Awka, Anambra State, and acclimatized for one week in the animal facility. The animals were housed in well-ventilated cages under controlled environmental conditions (27°C±3°C, 12-hour light/dark cycle). They were provided with commercial rodent chow and ad libitum access to water.

Acute toxicity study (LD50)

The acute oral toxicity was assessed using the Up-and-Down Procedure (UDP) as described by Bruce [22]. Six rats were randomly selected and subjected to a limit test with a dose of 5000 mg/kg of the extract. Each animal was then closely monitored for 48 hours for death or signs of toxicity.

Induction of anemia

Anemia was induced in the rats through a single-dose intraperitoneal administration of phenylhydrazine (80 mg/kg b.w.). Anemia was confirmed after 48 hours. Blood samples were collected via retro-orbital sinus puncture for hematological analysis. Blood samples were also collected before anemia induction to establish baseline hematological parameters.

Experimental design and treatment

Thirty (30) rats were assigned into six groups of five rats each.

- **Group 1:** Normal control with no induction or treatment.
- **Group 2:** Negative control with anemia and no treatment.
- **Group 3:** Anemic rats treated with the standard drug (Astyfer 1.5 mg/kg).
- **Group 4:** Anemic rats treated with 200 mg/kg of *Mucuna pruriens*.
- **Group 5:** Anemic rats treated with 200 mg/kg of *Justicia carnea*.
- **Group 6:** Anemic rats treated with 200 mg/kg of the combined *Mucuna pruriens* and *Justicia carnea*.

Treatments were administered orally once daily for a period of three weeks.

Blood sample collection and hematological analysis

Blood samples were collected at two weeks into treatment and again at the end of the three-week treatment period via orbital sinus puncture using a sterile syringe. Samples were transferred to heparinized tubes for hematological analysis. The following hematological parameters were analyzed: packed cell volume (PCV), hemoglobin (HB), and red blood cell (RBC) count.

Packed cell volume (PCV) estimation

Packed cell volume (PCV) was estimated using the microhematocrit technique described by Ochei and Kolhatkar [23]. Blood samples (2 ml) were collected in heparinized capillary tubes, sealed with plasticine to prevent leakage, and subjected to centrifugation at 12,000 g for five minutes. Following centrifugation, the tubes were placed on a microhematocrit reader scale, and the PCV was measured as a percentage.

Red blood cell (RBC) count

Total red blood cell counts were determined according to Ochei and Kolhatkar's [23] hematological protocol. A 0.02 ml aliquot of EDTA-anticoagulated whole blood was diluted (1:20) with 10% sodium carbonate (Na_2CO_3), loaded into a Neubauer hemocytometer, and examined under a light microscope. RBCs were counted in designated squares and summed to obtain the total RBC count.

Determination of hemoglobin (HB) concentration

Hemoglobin (HB) concentration was determined using the cyanmethemoglobin method, as described by Ochei and Kolhatkar [23].

Principle

Hemoglobin reacts with Drabkin's solution, containing potassium ferricyanide, potassium cyanide, and potassium dihydrogen phosphate, to form cyanmethemoglobin. This reaction produces a colored compound that is measured colorimetrically.

Procedure

A 0.02 ml whole blood sample was diluted 1:250 with 5 ml of Drabkin's solution in a test tube. After mixing and a 10-minute incubation, the absorbance was measured at 540 nm using a colorimeter, with Drabkin's solution as the blank. The absorbance reading was then multiplied by a standard hemoglobin factor (36.8 g/dl) to calculate the actual hemoglobin concentration.

Statistical analysis

Data analysis was performed using IBM SPSS version 29 statistical package (SPSS Inc., Chicago, Illinois, USA). Values were presented as mean ± standard deviation (SD). The statistical significance of the results between groups was determined using one-way analysis of variance (ANOVA), and multiple comparisons were carried out using Tukey's post hoc test. Differences between means were considered statistically significant at $p<0.05$.

Results

Acute toxicity test

The extract demonstrated a high margin of safety in rats, with no behavioral changes or mortality observed at doses as high as 5000 mg/kg body weight during the 48-hour toxicity study.

Effect of the extracts on packed cell volume (PCV)

As presented in Table 1, a statistically significant reduction in packed cell volume (PCV) was observed ($p<0.05$) in all experimental groups (2–6) relative to the normal control group (1) following induction. After two weeks of treatment with the standard drug and extracts, all treatment groups (3–6) exhibited recovery, with no statistically significant difference ($p>0.05$) compared to the normal control group (1) but significantly increased ($p<0.05$) compared to group 2. At three weeks, a significant elevation in PCV was observed in groups 3–6 relative to group 2, surpassing initial baseline levels. Notably, groups 5 and 6 displayed the highest PCV values.

Effect of the extracts on hemoglobin (HB)

Table 2 indicates that HB levels decreased significantly ($p<0.05$) in all the experimental groups following induction. However, the HB levels of all treatment groups (2–6) demonstrated a significant increase ($p<0.05$) in weeks 2 and 3, with group 5 showing the most significant increase ($p<0.05$), followed closely by group 6 compared to the other groups.

Effect of the extracts on red blood cell (RBC) count

Table 3 shows that RBC counts decreased significantly ($p<0.05$) in all groups following induction. However, the RBC counts in all treatment groups (3–6) showed a significant increase ($p<0.05$) by the third week of treatment compared to group 2, with groups 4 and 5 showing the highest RBC counts at the final measurement, approaching normal control levels.

Discussion

The primary objective of this study was to assess the effect of *Mucuna pruriens* and *Justicia carnea* leaf extracts, alone and in combination, on hematological parameters in phenylhydrazine-induced anemia in albino rats, with a focus on potential synergistic effects. The results of the acute oral toxicity study demonstrated that the extracts did not induce any behavioral alterations or mortality in rats at doses up to 5000 mg/kg over a 48-hour observation period. This suggests a high safety profile for the extract, indicating potential safety for short-term use.

The outcomes of hematological investigations are crucial for the diagnosis, management, and monitoring of various diseases, as well as for evaluating the severity of blood-related damage. This study demonstrated a statistically significant ($p<0.05$) decline in packed cell volume (PCV), hemoglobin (HB) concentration, and red blood cell (RBC) count following phenylhydrazine administration in all experimental groups (2–6). Phenylhydrazine damages RBCs by generating reactive oxygen species, causing lipid peroxidation, hemoglobin oxidation, and premature destruction [24].

PCV measurement provides valuable diagnostic information on red blood cell proportion, aiding in the detection and monitoring of various conditions, including anemia, dehydration, and polycythemia. This parameter is essential for evaluating oxygen-carrying capacity and hematological well-being. Notably, from the results of this study, a marked increase in packed cell volume (PCV) levels was observed in all treatment groups following the induction-induced decrease. It was also observed that the untreated group exhibited a modest spontaneous recovery by the final week, suggesting the presence of intrinsic compensatory mechanisms that may mitigate the initial damage over time. Groups treated with *Mucuna pruriens* and *Justicia carnea*, alone or combined, showed notable PCV improvements. Combination treatment yielded the highest PCV levels, closely followed by the group treated with *Justicia carnea* alone, indicating effectiveness in restoring PCV levels and blood health. The results suggest that *Mucuna pruriens* and *Justicia carnea* possess therapeutic potential in managing hypochromic conditions, such as anemia, characterized by reduced PCV. The observed PCV increase implies stimulation of erythropoiesis or enhanced erythrocyte survival. Notably, synergism was observed with combined administration.

Hemoglobin (HB), a vital protein within erythrocytes, facilitates the transport of oxygen from the pulmonary system to peripheral tissues and concurrently returns carbon dioxide to the lungs [25]. Hemoglobin concentrations serve as critical biomarkers for assessing the blood's oxygen transport capacity, facilitating the diagnosis of anemia and monitoring the efficacy of therapeutic interventions aimed at reestablishing optimal hematological function. This study examined how different treatments affected hemoglobin levels in induced anemia across four treatment groups. As noted earlier, post-induction, a pronounced decline in hemoglobin concentrations was observed across all experimental groups, demonstrating that the induced condition effectively diminished the blood's capacity to transport oxygen—a typical consequence of anemia.

Over the course of treatment, a significant increase ($p<0.05$) in hemoglobin concentrations was observed across all treatment groups, with the exception of the untreated control group. The most pronounced improvement in hemoglobin levels was observed in the *Justicia carnea*-treated group, followed closely by the group treated with the combined extract. The *Mucuna pruriens*-treated group demonstrated a notable, albeit lesser, increase. Standard drug treatment resulted in moderate recovery, slightly lower than the extract treatments. The results indicate that *Mucuna pruriens* and *Justicia carnea* exhibited beneficial effects on hemoglobin concentrations, with *Justicia carnea* demonstrating the most significant effect, which was statistically similar ($p>0.05$) to that of the combined extract. The combination of *Mucuna pruriens* and *Justicia carnea* appears to offer a synergistic effect, leading to higher hemoglobin recovery than *Mucuna pruriens* treatment alone.

Red blood cell count serves as a crucial diagnostic tool, measuring the number of red blood cells and aiding in the detection of various blood-related disorders [26]. This study examined how various treatments affected red blood

Table 1. Effect of the leaf extracts *Mucuna Pruriens, Justicia carnea* and their combination on the packed cell volume of anemic rats

	Normal control	Anemic control	Standard drug Astyfer -1.5mg/kg	200mg/kg *Mucuna pruriens*	200mg/kg *Justicia carnea*	200mg/kg *Mucuna pruriens* +*Justicia carnea*	p
Initial (%)	44.00±2.12	40.60±4.34	41.30±2.33	48.00±5.83	46.70±5.61	46.00±4.69	0.074
After induction (%)	48.00±2.10	26.00±3.37*	24.13±0.85*	24.75±3.77*	23.13±2.10*	23.13±0.85*	0.00*
2 Weeks into treatment (%)	51.80±1.20**	36.00±1.41	53.00±4.08**	53.25±3.10**	52.75±2.06**	52.50±2.08**	0.00**
3 weeks into treatment (final) %	55.14±2.17**	46.34±2.84	50.25±4.90**	55.67±2.24**	57.78±2.63**	57.10±0.56**	0.00**

Values are mean±SD; *p: Significantly different from the control (p<0.05); **p: Significantly different from anemic control (p<0.05). SD: Standard deviation.

Table 2. Effect of the leaf extracts *Mucuna pruriens, Justicia carnea* and their combination on the hemoglobin of anemic rats

	Normal control	Anemic control	Standard drug Astyfer-1.5mg/kg	200 mg/kg *Mucuna pruriens*	200 mg/kg *Justicia carnea*	200 mg/kg *Mucuna pruriens* +*Justicia carnea*	p
Initial (g/dl)	20.88±1.05	20.80±3.74	18.56±1.15	21.14±2.71	20.90±2.23	19.46±1.23	0.40
After induction (g/dl)	22.18±2.06	8.90±1.12*	8.00±0.14*	8.90±0.29*	8.32±0.87*	9.20±0.64*	0.00*
2 Weeks into treatment (g/dl)	25.10±3.20**	9.05±0.34	11.90±1.61**	12.60±1.10**	12.48±0.96**	11.10±0.26**	0.00**
3 weeks into treatment (g/dl)	27.58±4.31	26.10±1.55	25.08±2.96	22.4±3.84	30.10±1.19***	28.85±1.51***	0.016***

Values are mean±SD; *p: Significantly different from the control (p<0.05); **p: Significantly different from anemic control (p<0.05); ***p: Significantly different from normal control, anemic control, standard drug and 200 mg/kg *Mucuna pruriens* groups (p<0.05).

Table 3. Effect of the leaf extracts *Mucuna Pruriens, Justicia carnea* and their combination on the red blood cell count of anemic rats

	Normal control	Anemic control	Standard drug Astyfer-1.5mg/kg	200 mg/kg *Mucuna pruriens*	200 mg/kg *Justicia carnea*	200 mg/kg *Mucuna pruriens* +*Justicia carnea*	p
Initial ($\times10^{12}$/L)	4.90±0.40	5.02±0.31	4.80±0.21	4.82±0.34	4.92±0.41	4.38±1.06	0.51
After induction ($\times10^{12}$/L)	6.89±0.80	3.58±0.38*	3.35±0.34*	3.35±0.51*	3.33±0.39*	3.30±0.18*	0.00*
2 Weeks into treatment ($\times10^{12}$/L)	8.90±0.60**	3.53±0.21	3.45±0.20**	4.80±0.16**	4.40±0.16**	4.95±0.26**	0.00**
3 weeks into treatment ($\times10^{12}$/L)	10.78±0.59***	7.75±0.37	8.35±1.32	10.13±0.54***	9.98±1.38***	9.15±0.95***	0.002***

Values are mean±SD; *p: Significantly different from the normal control (p<0.05); **p: Significantly different from anemic control and standard drug (p<0.05); ***p: Significantly different from anemic control and standard drug (p<0.05).

cell (RBC) counts after inducing anemia in five groups. Initially, RBC levels were similar and not significantly different ($p>0.05$) across all groups. Following induction, RBC counts significantly ($p<0.05$) dropped in all treatment groups, demonstrating the successful induction of anemia. Notably, the untreated group also showed a substantial decrease in RBC count, which remained consistently low throughout the study, emphasizing the severity of the condition when left untreated.

During the treatment phase, groups administered *Mucuna pruriens* and *Justicia carnea*, both as individual treatments and in combination, exhibited significant recovery in RBC counts. Notably, *Mucuna pruriens* and *Justicia carnea* individually yielded comparable results, indicating equivalent efficacy. The combined treatment of both herbs resulted in a modestly enhanced recovery, although the expected synergistic effect was not prominently observed. These findings suggest that *Mucuna pruriens* and *Justicia carnea* may have additive, rather than synergistic, effects on RBC count recovery. The standard drug treatment group also exhibited an increase in RBC counts, although the recovery was less pronounced compared to the leaf extract treatment groups. In contrast, the normal control group demonstrated a significant surge in RBC count by the final week, likely attributable to the natural recovery process or enhanced erythropoietic activity in the absence of any stressors or interventions. This finding suggests that the leaf extract treatments may have promoted a more robust recovery in RBC counts compared to the standard drug treatment. The results indicate that *Mucuna pruriens* and *Justicia carnea* are effective in restoring RBC levels following the induced reduction, indicating their potential as therapeutic agents in treating conditions like anemia. The combination of *Mucuna pruriens* and *Justicia carnea* offered a modest additional benefit, although the improvement was not substantially greater than the individual effects of each extract.

These results align with previous research, which has established that *Mucuna pruriens* and *Justicia carnea* possess erythropoietic properties. Previous research on *Mucuna pruriens*' hematological effects revealed its erythropoietic potential, attributed to its high L-DOPA content, which promotes red blood cell production [27]. Research conducted on *Justicia carnea* has substantiated its ethnopharmacological application in anemia treatment, attributed to its considerable iron content and potential erythropoietic effects [13]. Our study confirms these findings, demonstrating improved hematological parameters. The synergistic effect observed is consistent with other studies showing improved therapeutic outcomes from combining herbal remedies [21].

Based on these results, the combination of *Mucuna pruriens* and *Justicia carnea* offered a modest additional benefit in improving hematological parameters, indicating potential synergistic effects. Therefore, *Mucuna pruriens* and *Justicia carnea* may stimulate erythroid progenitor cells, promoting erythropoiesis and increasing red blood cell production. The extracts may enhance hemoglobin synthesis, leading to improved oxygen delivery to tissues and organs. The study provides and adds to preliminary evidence for the use of these traditional medicinal plants in the management of anemia. It also contributes to the development of novel treatment options for anemia, providing an alternative to conventional therapies. However, further studies are needed to elucidate optimal dosages and potential interactions with conventional medications.

Limitations of the study

The treatment duration of 21 days may not be sufficient to fully assess the long-term effects of the extracts and therefore, further study on long term effect is recommended. The study only tested a single dose of 200mg/kg, which may not be the optimal dose for therapeutic effects. There is need for graded dosage which may potentially boost the synergistic effect of the extracts.

Conclusion

The efficacy of *Mucuna pruriens* and *Justicia carnea* treatments was assessed in rats with phenylhydrazine-induced anemia through various hematological parameters. Notably, hemoglobin levels exhibited significant enhancements in treatment groups, with the combination of *Mucuna pruriens* and *Justicia carnea* yielding the most substantial improvements. Furthermore, red blood cell counts demonstrated marked recovery in treated groups, particularly with *Mucuna pruriens* and *Justicia carnea*, suggesting potential erythropoietic benefits. These results indicate that these treatments effectively replenish red blood cells and promote overall blood health. This study provides evidence supporting the traditional use of *Mucuna pruriens* and *Justicia carnea* for enhancing erythropoiesis, suggesting their potential as natural remedies for improving red blood cell production and potentially treating related health conditions.

Ethics Committee Approval: The study was approved by The ESUT Institutional Research Ethics Committee (No: ESUCOM/FBMS/ETR/2024/04, Date: 12/02/2024).

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