

SOME HEMATOLOGICAL PARAMETERS OF WISTAR RATS ADMINISTERED ETHANOLIC LEAF EXTRACT OF *SPONDIAS MOMBIN* DURING PREGNANCY

Njoku-Oji N. N.^{1*}, Ifegwu N. O.², Umahi G. O.³, Maduka S. O.¹, Uchefuna R. C.¹, Enemuo I. C.⁴, Okonudo P. O.¹ and Dibia C. L.¹

¹Human Physiology Department, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

²Anatomy Department, Faculty of Basic Medical Sciences, College of Health Sciences, Abia State University Uturu, Nigeria.

³Physiology Department, Faculty of Medicine, Ebonyi State University, Abakaliki, Nigeria.

⁴Anatomy Department, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

*Corresponding Author: Njoku-Oji N. N.

Human Physiology Department, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

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ABSTRACT

Background: The World Health Organization (WHO) estimates that anemia affects over half of the pregnant women in developing countries. In Eastern Nigeria, fresh leaves of *S. mombin* are used during and after parturition.

Aim: To determine the effect of administration of ethanolic leaf extract of *Spondias mombin* on some hematological parameters of wistar rats during pregnancy. **Materials and Methods:** Twenty-eight (28) non-pregnant adult female rats weighing between 140-200g were used for this study. On confirmation of pregnancy, the animals were grouped into four groups of 7 rats each as follows: Groups I, II, III and IV. Administration of extract began on the first day of the second trimester (day 4) and terminated on the sixth day of third trimester (day 20). The ethanolic leaf extract was administered daily via oral gavage at 100, 200 and 400mg/kg body weight to groups II, III and IV respectively while group I received distilled water and served as the control group. At the end of extract administration, hematological analysis was carried out using the Automated Hematologic Analyzer. **Result:** Non-significant ($p > 0.05$) increase in PCV, RBC, WBC and Hemoglobin was recorded in groups II and III when compared with control group. There was significant increase ($p < 0.05$) in PCV, RBC, WBC and Hemoglobin in group IV when compared with the control group.

Conclusion: *Spondias mombin* may be useful in ameliorating the burden of dilutional anaemia in pregnancy.

KEYWORDS: *Spondias Mombin*, Hematological Parameters, Pregnancy, Wistar Rats.

INTRODUCTION

Anemia affects people of all ages especially the elderly, women of child bearing age and children.^[1] It is one of the major medical problems experienced during pregnancy. In developing countries, it is a cause of serious concern as it contributes significantly to high maternal mortality.^[2] It is characterized by reduction in circulating red blood cell, hemoglobin and hematocrit per unit of peripheral blood.^[3, 4, 5] A study has revealed that the most important risk factor for anemia in pregnancy is socioeconomic status of the women.^[6] Women are found to be anemic during pregnancy (dilutional anemia), this majorly is as a result of reduced concentration of hemoglobin as compared to the level of circulating red blood cells.^[7] The WHO estimates that anemia affects over half of the pregnant women in developing countries.^[8] Recent estimates in the developing countries including Nigeria put the

prevalence at 60.0% in pregnancy and about 7.0% of the women are said to be severely anemic.^[8,9]

Anemia in pregnancy is associated with increased risk of preterm birth and low birth weight babies.^[10,11] Preterm and LBW are still the leading causes of neonatal deaths in developing countries.^[12] It has also been associated with increased risk of intrauterine deaths (IUID) and intrauterine growth restriction (IUGR) which is a risk for stunting among children of less than two years.^[11, 13]

Spondias mombin (*S. mombin*) is a flowering plant with reported hematinic properties. It belongs to the family *Anacardiaceae*. It grows mostly in the rain forest and coastal areas of the world to a height of 15-22m.^[14] *S.mombin* is a native of tropical America and has been naturalized in parts of Africa, India and Indonesia and it is one of the medicinal herbs in Southern Nigeria.^[15] It is called hog plum in English, akika/iyeye in Yoruba,

Ijikara/Ichikara in Igbo and tsardarmaser in Hausa.^[16] The plant has been known traditionally for its medicinal and food values.^[17, 18] The plant leaves are common animal forage. The tender leaves and ripe fruits are also edible. The bark, leaf and fruit juice have been widely used for varied folk medical purposes. In Eastern Nigeria, fresh leaves of *S. mombin* are used during and after parturition. It is a common midwife's remedy to help induce labor, reduce bleeding and pain during and after childbirth, to bring on the flow of breast milk, and as a vaginal wash to prevent or treat uterine or vaginal infections after childbirth.^[16] In Senegal, Mandika women with a history of miscarriage take young leaves of the plant for maintenance of pregnancy.^[19]

S. mombin has been reported to have a significant hematinic^[16, 20] and anti-anaemic activities^[21] thus supporting its traditional use by women shortly after child birth to increase their blood content as claimed by traditional birth attendants. Kramer et al^[22] recommended its use for pregnant women but only after five months of pregnancy. Phytochemical screening and quantification carried out by Njoku and Akumefula^[23], revealed the presence of bioactive compounds tannins (3.82%), saponins (7.6%), Flavonoids (3.0%), alkaloids (6.0%) and phenol (1.0%). Vitamins results showed the plant leaves contained ascorbic acid (19.35mg100⁻¹g) Niacin (3.75mg100⁻¹g), Riboflavin (0.25mg100⁻¹g) and Thamine (0.05mg100⁻¹g). Mineral analysis revealed in the plant leaves K (22.55%), Mg (0.30%), Na (0.10%), Ca (1.31%) and P (0.20%).

The increased prevalence rate of anemia in pregnancy and the reported hematinic activities of the leaf extract of *S. mombin* necessitated this study. Meanwhile, no activity of this extract has been reported in this regard.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Spondias mombin* were hand-picked from local farms in Okofia, Otolu-Nnewi, Anambra state, Nigeria. They were certified and authenticated by Mr. Tochukwu Egboka a Taxonomist in Botany Department, Nnamdi Azikiwe University, Awka, Anambra state. A voucher specimen was deposited and stored as a reference specimen in the herbarium with the voucher number (N.A.U.H #56). It was classified under the family *Anacardiaceae*.

Preparation of Extract

The fresh leaves were washed under running water to remove debris and then air-dried under ambient temperature in a shade. The dried leaves were pulverized using a mechanical blender (model MS-223; Blender/Miller III, Taiwan, China), weighed (1.5kg of powder) and kept air-tight prior to extraction.

About 1.4 kg of the powdered leaf was soaked in 3.5L of 75 % ethanol in a 5 L beaker and covered with aluminum foil. The mixture was stirred every 3 hours for proper

mixing and allowed to stand for 24 hours. The resulting decoction was filtered and the filtrate was subjected to a slow but complete solvent evaporation using a regulatory hot plate (Techmel, USA) at a temperature of 40-60°C yielding 27.71g (1.98%) of crude ethanol extract. The extract was packaged in an air-tight container, labeled and stored below 4°C in a freezer until required.

Experimental Animals

In this experimental study, twenty-eight (28) non-pregnant adult female albino rats weighing between 140-220g were housed under standard conditions (24 hour light/dark cycle) in the Animal House of Nnamdi Azikiwe University, Nnewi campus, using standard animal cages. They were then acclimatized for two weeks, with water and feed provided *ad libitum*.

Experimental Research Design and Laboratory Testing

At the end of acclimatization, the oestrus cycle of the sexually matured female rats were monitored by examining the daily Vaginal Smears under Light Microscopy. The female rats with two consecutive regular four-day cycles were paired with sexually matured male rats at a ratio of 2:1 on the pro-oestrus phase of the oestrus cycle. Successful mating was ascertained by the presence of sperm cells in fresh vaginal smear made on clean microscope slide and observed under the x10 magnification of wide angle eyepiece of the light microscope and this day was designated Day 1 of pregnancy.^[24]

Thereafter, the animals were randomly assigned to four groups of 7 rats each as follows: Groups A, B, C and D. Administration of extract began on the first day of the second trimester and terminated on the sixth day of third trimester (day 8-20). The ethanolic leaves extract of *S. Mombin* was administered daily via oral gavage at 100mg/kg, 200mg/kg and 400mg/kg to groups B, C and D respectively while group A received distilled water and served as the control group.

On day twenty of gestation, extract administration was terminated and the animals were put under light anaesthesia using diethylether, blood samples were collected via oculo-puncture and stored in EDTA containers. The hematological analysis (determination of the Red Blood Cell count, White Blood Cell count, Hemoglobin concentration and Packed Cell Volume) was carried out using the Automated Hematologic Analyzer (Sysmex, KX-21, Japan). All procedures used in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of animals.^[25]

Statistical Analysis

All Data were tabulated and statistically analyzed using the Statistical Package for Social Sciences Version 20.0 (SPSS V20.0). Results were expressed as Mean ±

Standard Deviation ($M \pm SD$). One-way Analysis of Variance (ANOVA) followed by Bonferroni's Post-hoc Test, were used for Data Comparison and the level of significance set at $p < 0.05$.

RESULTS

Figure 1 shows non-significant ($P > 0.05$) difference in PCV in groups II (32.50 ± 1.92) and III (35.50 ± 1.29) when compared with the control (32.33 ± 3.79) while group IV (38.25 ± 1.26) showed significant ($P < 0.05$) increase in PCV when compared with the control.

Figure 2 shows non-significant ($P > 0.05$) difference in RBC in groups II (6.45 ± 0.28) and III (6.58 ± 0.43) when compared with the control (6.15 ± 0.99) while group IV

(7.56 ± 0.48) showed significant ($P < 0.05$) increase in RBC when compared with the control.

Figure 3 shows non-significant ($P > 0.05$) difference in WBC in groups II (6.13 ± 0.54) and III (6.48 ± 0.50) when compared with the control (5.93 ± 0.42) while group IV (7.70 ± 0.29) showed significant ($P < 0.05$) increase in WBC when compared with the control.

Figure 4 shows non-significant ($P > 0.05$) difference in HB in groups II (11.75 ± 0.60) and III (12.48 ± 0.81) when compared with the control (11.53 ± 1.51) while group IV (14.33 ± 0.62) showed significant ($P < 0.05$) increase in HB when compared with the control.

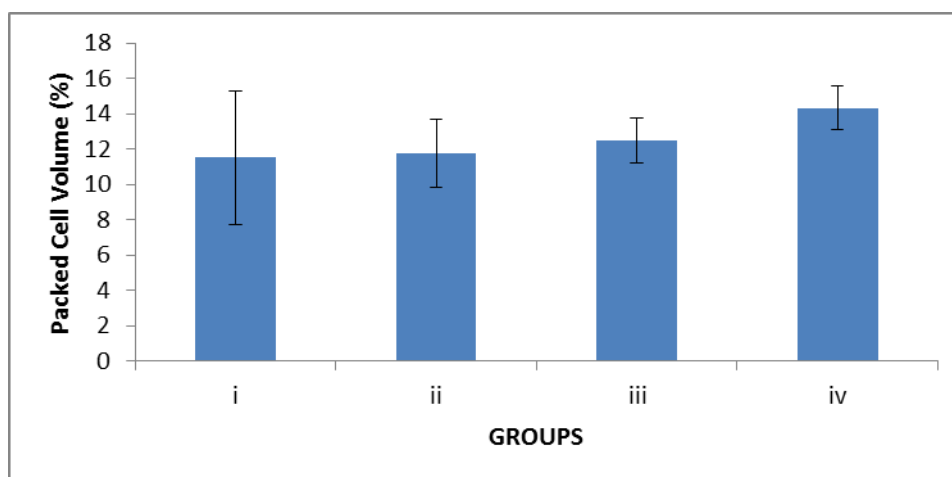


Figure 1: Effect of ethanolic leaf extract of *S.mombin* administration during pregnancy on Packed Cell Volume.

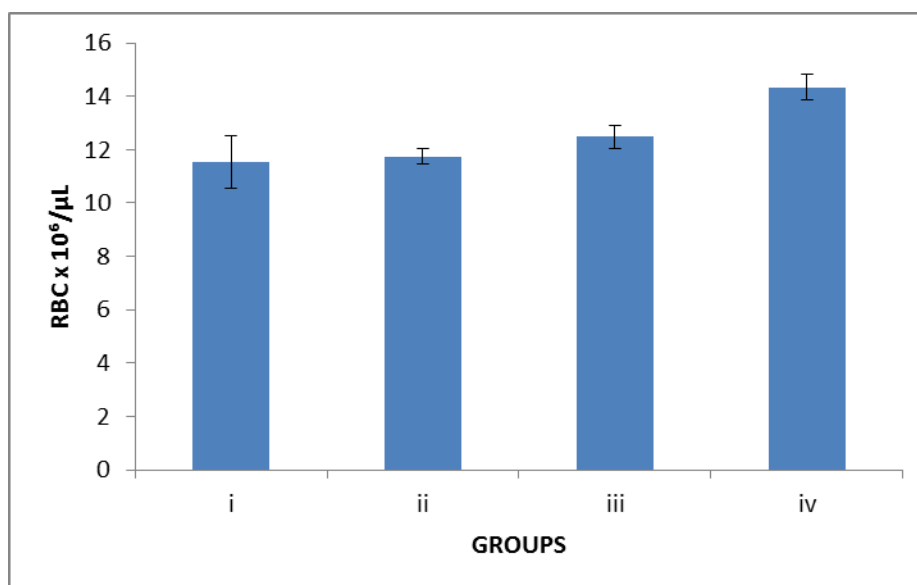


Figure 2: Effect of ethanolic leaf extract of *S.mombin* administration during pregnancy on Red Blood Cell Count.

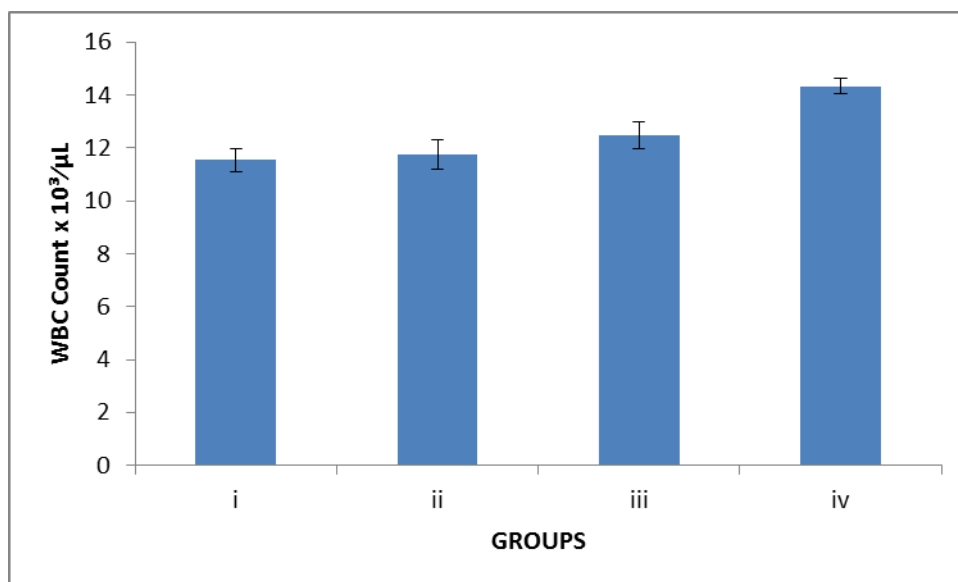


Figure 3: Effect of ethanolic leaf extract of *S.mombin* administration during pregnancy on white Blood Cell Count.

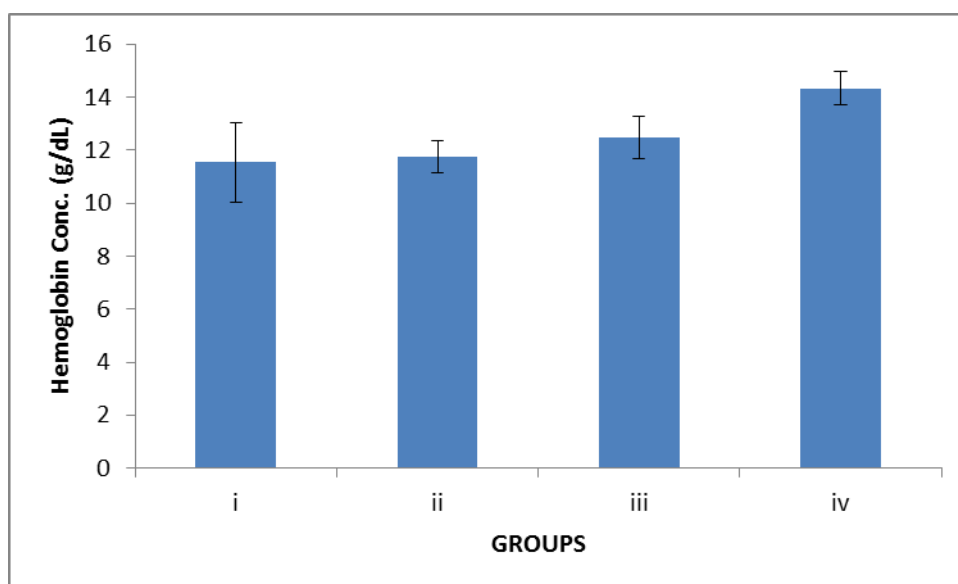


Figure 4: Effect of ethanolic leaf extract of *S.mombin* administration during pregnancy on Hemoglobin Concentration.

DISCUSSION

Hematological indices provide physiological information on the blood picture and reticulo-endothelial system. It could also be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals.

This study has revealed increase in the hematological parameters with increase in concentration. This indicates that the extract may possess the ability to induce erythropoiesis even during pregnancy. The significant increase in PCV in this study may be indicative of the normal functioning of the bone marrow in the process of erythropoiesis.

The increase in RBC observed in this study suggests that the extracts may have the potential to stimulate erythropoietin release in the kidney which is the humoral regulators of RBC production.^[26] Indicating that this extract may probably possess properties that stimulate the bone marrow to produce more blood cells when orally administered during pregnancy.

Furthermore, the observed increase in blood parameters could be related to the reported constituents of the extract which are flavonoids and anthraquinones that have been shown to have hematopoietic properties.^[27, 28] The increases in the blood indices could also be related to the chemical composition of the leaves of *S. mombin* which includes protein, fat, ascorbic acid, calcium, iron, vitamin A, thiamine, riboflavin and nicotinamide and

other substances with antioxidant properties^[23], which may help in red cell membrane stabilization.^[29] Most of these constituents are well-known hematological factors that have direct influence on the production of blood from the bone marrow.^[30, 31] Antioxidant phytochemicals have also been reported to protect lymphocytes and decrease oxidative DNA damage in WBCs^[27], hence the observed increase in WBC.

The observed increase in Hemoglobin concentration indicates that the oxygen-transporting potential of blood of the extract-administered animals were not detrimentally affected. This could be attributed to the iron and other constituents contained in the leaf extract.^[32] Iron plays an important role in the production of hemoglobin. The increase in haematological parameters with increase in the concentration of the extract observed above is in agreement with previous works^[16, 21] but not consistent with the work of Igwe *et al.*^[20] who reported no significant change in the levels of the blood parameters as compared to control when the ethanolic extract was administered to albino rabbits. This may be due to difference in concentration or geographical area.

CONCLUSION

The increased maternal hematological parameters at the highest administered doses showed that *Spondias mombin* may be useful in ameliorating the burden of dilutional anaemia in pregnancy. Therefore, this result supports the use of leaf extract of *Spondias mombin* during pregnancy for stabilization and as a hematinic given to pregnant women during and after childbirth to combat anaemia induced during pregnancy.

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