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Maternal Changes in Haematological Profile Following Administration of Ethanol Extract of *Piliostigma thonningii* Leaves on Pregnant Wistar Rats.

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Abstract:*In pregnancy several haematological changes do occur as part of the physiological adaptation of the body to this condition hence some metabolism may be affected. Therefore, this research investigated the effect of extract of Piliostigmathonningii on the haematological parameters of pregnant Wistar rats. The leaves of P. thonningii were collected and air dried for 14 days until constant weight was obtained. Twenty (20) pregnant female albino rats (160-220g) were randomly assigned to groups labeled A-D and treated thus. Animals in group B-D respectively were administered orally 200, 100, 50mg/kg body weight of the extract of Piliostigma thonningii while group A which served as the control received distilled water. The extract administration was done for 14 days consecutively. Thereafter, the animals were sacrificed and blood collected via cardiac puncture for haematological evaluations. The result showed that the extract caused a significant reduction (P< 0.05) on white blood cell count (WBC), monocytes, neutrophil counts and serum platelets compared with the control. However the extract caused a significant increase (P<0.05) in lymphocyte, Red blood cell (RBC),Haemoglobin (Hb) , and packed cell volume (PCV) compared to the control in a dosage dependent manner. It appears when taken during pregnancy, theextract of Piliostigma thonningii leaves may have the potential to act as panacea to anaemic condition or physiological iron deficiency associated with pregnancy.*

Keywords*: Anaemia, Haematological profile, Piliostigma thonningii Physiological iron deficiency, Morbidity, Mortality.*

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I. Introduction

The haematological profile of an individual to a large extent reflects their general health. Many studies have identified the haematological profile of the pregnant women as one of the factors affecting pregnancy and its outcome [1,2,3].During pregnancy, the body undergoes remarkable changes in the cardiovascular, respiratory, renal and gastrointestinal physiology and many studies have also identified the haematological profile of the pregnant woman as one of the factor affecting pregnancy and its outcome [4]. In the absence of illness, the body can generally compensate for these changes, however, in the presence of conditions such as anaemia, clotting/bleeding abnormalities, preeclampsia and trauma, compensation may not be possible. Pregnancy is also influenced by many factors, some of which include culture, environment, socioeconomic status, and access to medical care [5].More so, pregnancy is associated with changes in haematological indices as a result of the physiological haemodilution that occurs in pregnancy, while others are increased, such as the WBC count. There are subtle and substantial changes in haematological parameters during pregnancy and the puerperium, total blood volume increases by about 1.5 litres mainly to supply the needs of the foetus [6,7]. Although, red cell mass increases, a physiologic anaemia occurs in pregnancy as there is an even greater increase in plasma volume. The two most common causes of anaemia in pregnancy are iron deficiency and acute blood loss [8]. The platelet count often drops slightly, with an accepted lower limit of normal range in pregnancy (mild thrombocytopenia), while white cell count increases due to an increase in circulating

neutrophils(neutrophilia). Changes occur in the coagulation system, with increased procoagulant activity, decreased natural anticoagulant activity, and decreased fibrinolysis, resulting in a prothrombotic state [7].Kühnertet al., [9] reported significant increase in total white cell count in pregnant compared to non-pregnant women.

In addition, Lurie and Mamet [10]found that erythropoietin and erythrocyte production are increased during normal pregnancy while erythrocyte mass per unit of body weight remains constant throughout the entire pregnancy, and haemoglobin and haematocrit continuously decrease into the third trimester. Erythrocyte life span is decreased during normal pregnancy due to emergency haematopoiesis in response to elevated erythropoietin levels [11].Each year, ill- health as a result of pregnancy is experienced by more than 20 million women around the world. Furthermore, the lives of eight million women are threatened, and more than 500,000 women are estimated to have died in 1995 as a result of causes related to pregnancy and birth [12]. Over the years, the rate of mortality and morbidity is extremely high possibly due to anaemic conditions, outbreak of malaria or malnutrition in the tropical regions during pregnancy.Therefore, since ethanol extract of *P. thonningii* leaves have been proven for its haematopoietic effect [13]. Paucity demands that this present research investigates the effect of ethanol extract of *P. thonningii* leaves on haematological profile of pregnant Wistar rats.

II. Materials and Methods

2.1 Plant material

Fresh *P.thonningii* leaves were obtained from Igoli /Okuku road, Cross River State, Nigeria in June, 2016.Identification and authentication was done at the Federal College of Forestry Jos, Plateau state, Nigeria, with the voucher number #25.

2.2 Experimental animals

Twenty (20) virgin female Wistar rats were obtained from animal holding unit, Department of Medical Biochemistry Okuku. The animal was acclimatized for a period of seven (7) days. Each rat was housed in a plastic cage. The animal room was well ventilated and kept at room temperature and relative humidity of 27 ^{o}c and 70% respectively with 12 hours natural light – dark cycle and were allowed free access to standard feed and water. Good hygiene was maintained by constant cleaning and removal of feces and spilled feeds from cages daily. The animals were subcutaneously injected with 0.1mg/kg body weight of diethylstilbestrol in 0.5ml olive oil to ensure the female rats were in estrous. To achieve pregnancy, female Wistar rats were mated with proven male breeder (ratio 2: 1) during their proestrous and the presence of sperm in their vaginal smear was taking as the first day of gestation.

2.3 Preparation of ethanol extract of*P.thonningii* leaves

The leaves of *P. thonningii* were collected and air dried for 14 days until constant weight was obtained. The dried leaves were then pulverized after which 300g was extracted in 1000ml of ethanol for 72 hours with constant shaking using the electric shaker. This was later filtered using Whatman No.1filter paper. The filtrates were the concentrated in water bath at 45^{o}c. The resulting slurry was weighed and reconstituted in coil oil to administer the required dose.

2.4 Animal grouping and administration of extract

Twenty (20) pregnant female albino rats were picked at random and placed into wooden cages labeled A-D. A, B and C were test groups while D-serving as the control groups. The animals in group A were administered orally high dose (200mg/kg body weight) of the ethanol leaf extract. Group B were administered medium dose (100mg/body weight) of the extract, Group C was administered low dose (50mg/body weight) while group D served as the control. The oral administration was done for 14 days. The animals in each group were sacrificed 24 hours after the completion of their respective doses, by cardiac puncture procedure. The animals were handled humanely in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes.

2.5 Blood sample collection

Blood was collected from all the test rats and control by cardiac puncture using disposable syringe and needle draw blood dispensed into tubes containing the anticoagulant ethylenediaminetetra acetic acid (EDTA). The specimens were labeled with the identification alphabets/ number. The EDTA samples were kept at room temperature until processing, which occurred within 30 minutes of collection.

2.6 Laboratory analysis

Full blood count was performed using a KN-21N Haematology Analyzer (Sysmex, Kobe, Japan), a three-part auto analyzer able to test 7 parameters per sample including Hb concentration, PCV, RBC concentration, MCH, MCV, MCHC, WBC count, and PLT count. Standardization, calibration of the instrument, and processing of the samples were done according to the manufacturer's instructions.

2.6.1Procedures

Each blood sample was mixed well and then approximately 20µL was aspirated by allowing the analyzer's sampling probe into the blood serum sample and depressing the start button. Results of the analysis were displayed after about 30 seconds, after which the analyzer generated a paper copy of the results on thermal printing paper.

2.7 Statistical analysis

Data were analyzed using SPSS (v 16; IBM, Armonk, NY, USA). The descriptive data are presented herein as means ± standard deviation (SD). Pearson's Chi-square test and one-way analysis of variance (ANOVA) were used for analytic assessment and the differences were considered statistically significant when the P value obtained was <0.05.

III. Result

The result below shows the effect of ethanol extract of *P. thonnigii* leaves on haematological profile of pregnant Wistar rat.

The extract produced a significant reduction (P< 0.05) on white blood cell count (WBC), monocyte and neutrophil counts for all the experimental groups when compared with the control (Fig .1- 3).

The extract also caused a significant increase (P<0.05) in serum lymphocyte in groups administered with 200mgkg body weight, 100 mg/kg body weight and 50mg/kg body weight when compared with the control (Fig. 4).

Likewise, the administration of the extract of *P. thonnigii* produced a significant increase (P<0.5) on Red Blood Cell (RBC) in the entire experimental groups when compared with the control (Table 1), similar pattern where also show in HCT, HB (Table 1). The extract also produced no significant difference on serum MCH, MCHC, MCV when compared with the control (Table1).

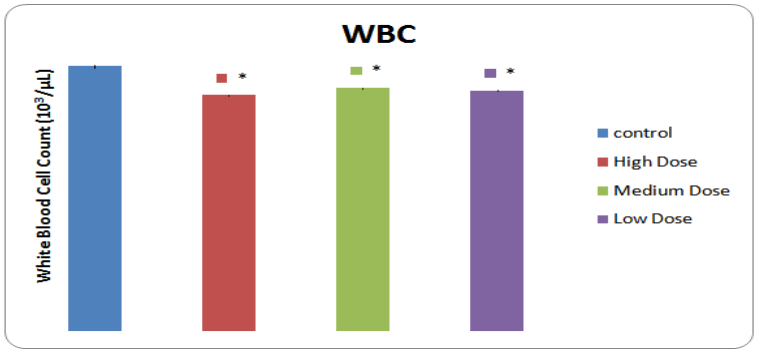

Fig.1. The effect of ethanol extract of P. thonningii leaves on serum White Blood Cell count of pregnant Wistar rat.

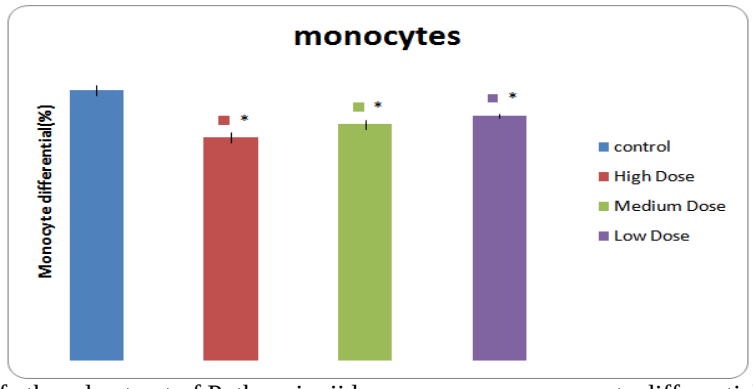

Fig 2: The effect of ethanol extract of P. thonningii leaves on serum monocyte differential concentration of pregnant Wistar rat.*p<0.05 (significant different from control)

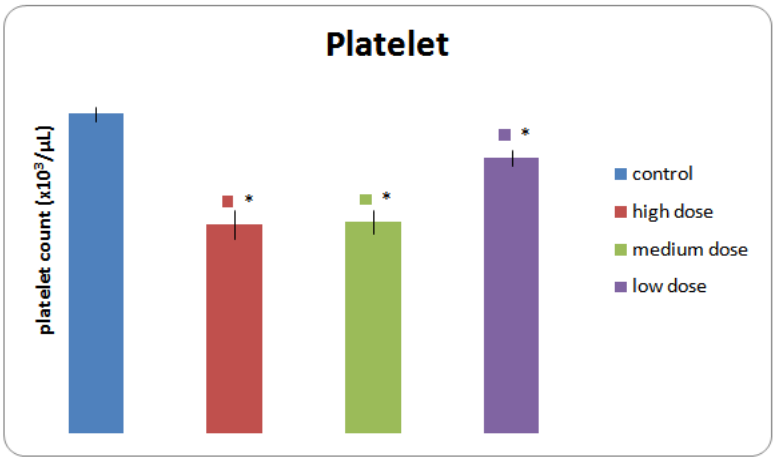

Fig 3:The Effect of ethanol extract of *P. thonningii* leaves on serum platelet concentration of pregnant Wistar rat.*p<0.05 (significant different from control)

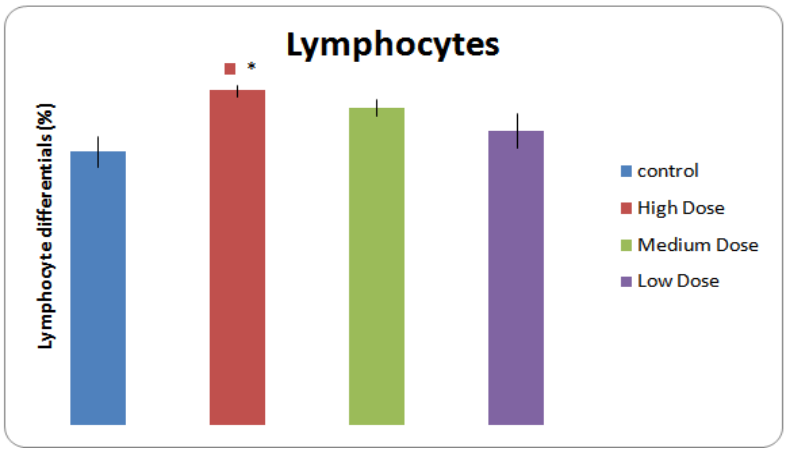

Fig 4: The Effect of ethanol extract of *P. thonningii* leaves on serum lymphocyte concentration of pregnant Wistar rat.*p<0.05 (significant different from control)

Table 1: Effect of the ethanol extract of *P. thonningii* on some haematological parameters of pregnant Wistar rat

PARAMETERS	RBC (10^6/µL)	HCT (%)	HB (g/dl)	MCV (fL)	MCH (pg)	MCHC (g/dl)
Control	6.35±0.649	38.93±4.23	12.40±1.007	61.2±0.681	19.603±0.47	32.1±1.00
HighDose (200mg/kg/bwt)	6.46±0.320	38.43±2.33	12.77±0.638	61.525±0.455	19.80±0.279	32.18±0.397
MediumDose (100mg/kg/bwt)	6.393±0.424	39.3±1.497	12.725±0.256	61.25±1.386	19.65±0.679	32.00±0.410
Low Dose(50mg/kg/bwt)	7.10±0.665	40.83±6.76	13.3±0.945	57.467±0.845	18.80±0.569	32.033±0.273

IV. Discussions

Pregnancy is associated with physiological and biochemical changes that are relevant in the nurturing and survival of the foetus. Biochemical and haematological parameters may reflect these adaptive changes which become very important in the event of complications hence considered one of the factors that affect pregnancy and its outcome. The lives of eight million women are threatened, and more than 500,000 women are estimated to have died as a result of causes related to pregnancy and childbirth complications [14]. In fact, anaemia is the most common haematological problem in pregnancy, followed by thrombocytopenia and

leukocytosis accordingly. Haematological indices are measured all over the world to estimate general health, because it is a reliable indicator and is a simple, fast and cost-effective test [15]. In the present research, HB concentration was found to decrease significantly in pregnancy which may be attributed to an increased demand for iron as pregnancy progresses. More iron is requirement for iron to meet the expansion of maternal HB and the needs for foetal growth. During pregnancy, an increased plasma volume with the lack of an adequate increase in erythrocytes mass results in a decrease in haemoglobin level and the development of anaemia, which is defined as dilution anemia [15]. In addition, the general decrease in the other blood indices is more likely explained by increased needs during pregnancy. Therefore, the increase in these blood indices could be a reflection of adequate iron metabolism resulting in increased haemoglobin production following the administration of the extract. Also, the additional progesterone and estrogen that are secreted by the placenta during pregnancy cause a release of renin from the kidneys. Renin stimulates the aldosterone-renin-angiotensin mechanism, leading to sodium retention and increased plasma volume. The increase in plasma volume is relatively greater than the increase in red cell mass, which results in a fall in maternal HB. Hence, the physiological anaemia that occurs in pregnancy, Contrastingly, the treated groups mitigate this effect suggesting that the extract contain some bioactive substances possibly saponin, flavonoid or alkaloid which synergistically enhances hematopoietic or hematopoietic system during pregnancy.

Likewise, the significant increase observed on the Red blood cells (RBC following the administration of the extract may be an indication that it improve the rate of production of red blood corpuscles (erythropoietin). This may imply that the extract was capable to release erythropoietin in the kidney, which is the hum oral regulator of RBC production. Since MCHC, MCH and MCV relate to individual red blood cells while HB, RBC, PCV are linked to the total population of red blood cells, the significant increase following the administration of the extract of the extract on these indices may imply the incorporation of hemoglobin into red blood cells or the morphology and osmotic fragility of the red blood cells was increased this is in accordance with our earlier report [13].

Therefore, it is likely that the extract affect the oxygen-carrying capacity of each of the RBC and the total population. In addition, since the anaemia diagnostic indices of MCV, MCH and MCHC were altered by the extract, it may be logical to infer that the extract had effect on the average size of RBC (microcytes) as well as the weight of haemoglobin per RBC but a contrary effect was observed in the untreated group which displayed a dis hematopoietic effect, hence exhibiting an evidence of physiological anaemia associated with pregnancy.

White blood cell differentials are indicators of the ability of an organism to protect itself against infection. A rising WBC count in pregnancy is not a reliable indicator of infection in subclinical chorioamnionitis; rather, clinical methods of detection such as maternal pyrexia, offensive vaginal discharge, and foetal tachycardia are better indicators, especially of preterm labor and membrane rupture [1].Leukocytosis occurring during pregnancy may be due to the physiologic stress induced by the pregnant state. In this context, [7] explained this change as a result of the body building the immunity of the foetus and it is achieved by a state of selective immune tolerance, immunosuppressant and immunomodulation in the presence of a strong antimicrobial immunity. There is also down-regulation of potentially dangerous T-cell-mediated immune responses, while activating certain components of the innate immune system, such as neutrophils which are the major type of leukocyte on differential count [16,17]. Therefore, the decrease of WBC count in all the treated groups following the administration of the extract suggests that the extract might not enhance systemic immunity or prevents predisposition to opportunistic diseases and infections during pregnancy.

Neutrophilia observed following the administration of the extract to the pregnant rats may be likely due to impaired neutrophilic apoptosis in pregnancy [18,19]. Neutrophil chemo taxis and phagocytic activity are depressed, especially due to inhibitory factors present in the serum of a pregnant female [20,21]. The above unique deregulation between different components of the immune system plays a central role in the maternal adaptation to pregnancy or help in preventing fetal allograft rejection by infiltrating the decidua tissue possibly, through Prostaglandin E2 mediated immunosuppressant.

V. Conclusions

It is logical to conclude that extract of *Piliostigma thonningii* leaves may have the potential to act as panacea to anaemic condition or physiological iron deficiency associated with pregnancy

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