

EFFECT OF *RUELLIA PRAETERMISSA* EXTRACTS ON ERYTHROPOIESIS IN PREGNANT WOMEN

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ABSTRACT

The effect of the extracts of *Ruellia Praetermissa* Schweinf. ex Lindau. on hemoglobin (Hb), Hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red blood cell count (RBC), was investigated in 50 Pregnant women attending prenatal clinic in Belo maternity. The women were assigned to 5 groups of 10 women per group. The first group was the control and the other 4 were the experimental groups. The control was administered daily, 0.5 ml of saline solution while the experimental groups were administered daily oral doses of the plant extract in concentrations of 200 mg/kg, 400 mg/kg, 800 mg/kg and 1,600 mg/kg respectively for 16 days. Blood samples were collected on the 17th day and analyzed. The extracts contain flavonoids aglycones (luteolin and apigenin) and their respective, glycosides and a high concentration of triterpenes (campesterol, stigmasterol, -sitosterol, lupeol) and iridoid glycosides. It was also found to be rich in vitamins and minerals. The extracts increased the values in a dose dependent manner Hb ($P < 0.05$), RBC ($P < 0.05$), hematocrit ($P < 0.05$). It however showed no remarkable effect on the values of MCH and MCHC ($P > 0.05$) but with a dose depending decreasing effect on MCV ($P < 0.05$). The active principles of this plant drug stimulate erythropoiesis which leads to increase in circulating RBCs with slightly microcytic sizes (MCV), normochromic weight, (MCH) normochromic hemoglobin contents (MCHC). The result of this study thus supports the traditional use of *Ruellia Praetermissa* in pregnancies threatened with miscarriage and as a remedy for anemia.

KEYWORDS: *Ruellia Praetermissa*, Pregnant Women, Blood

INTRODUCTION

Gestational problems are a potential source of mortality for both gestation mothers and their developing foetuses in the underdeveloped world in general and Africa in particular. There is great need for effective safe prenatal drugs from natural sources which are available and affordable with respect to modern medicines. Plant drugs have effects haematological parametrs (Nnamdi *et al.*, 2012) which influence reproductive health.

Ruellia praetermissa Schweinf. ex Lindau., is a wild herb and indigenous to central and south eastern Asia and also widespread in tropical and subtropical Africa. In the North West Region of Cameroon it enjoys a folk reputation as blood and pregnancy medicine. In other south Eastern African countries; it is widely applied to relieve pain (Gelfand *et al.*, 1985). The Plant drug exerts estrogenic and cholinergic effects (Salah *et al.*, 2002). The extracts regularize pregnancies threatened with miscarriages in early stages. This is due to its ability to mimic 17β -estradiol. It stimulates the growth of the uterine endometrium. This is by the proliferation and the development of the cells of the uterine endometrium as it up-regulates estrogen, luteinizing hormone LH and progesterone receptors on the uterine muscles at the beginning of gestation and to excite the uterine myometrium at term. (Salah *et al.*, 2002). In addition, the extracts of this plant drug has a stimulatory effect on the motility of the gastrointestinal tract (Salah *et al.*, 2000) and antihypertensive effect by the inhibition of Angiotensin-converting enzyme (ACE) activity (Salah *et al.*, 2001). These biological effects are of particular interest since high blood pressure and indigestion frequently characterize gestation period. This herbal drug is rich in flavonoids such as luteolin, quercetin and apigenin. (Wagner and Bladt, 1998). The favonoids also have an antispasmodic effect on uterine smooth muscle. (Salah 2001). The plant extract has 5-Lipoxygenase inhibition and antispasmodic effects (Salah, 1999). The plant drug promotes implantation, and stabilizes the uterine endometrium in female rats (Salah and Wagner, 2009). The present study is aimed at

finding out the scientific basis of the use of *Ruellia praetermissa* as a prenatal herbal drug in the North-Western region of Cameroon.

MATERIALS AND METHODS

Plant Material

The plant material was collected in Belo, North West Province of Cameroon in September 2011. The specimen was verified and authenticated as the one earlier identified by Kofany of the Cameroon National Herbarium Obili Yaounde under the voucher specimen number 43596 deposited in 1996.

Extraction and sample preparation

Sun dried leaves of the plant were pulverized and 250 g was extracted using the soxhlet for 12 h in each case progressively with 2 l of n-hexane, chloroform, ethyl acetate, and methanol. The extracts (160, 140, 250 and 300 mg, respectively) were recovered by rotavaporization. The chaffs were boiled in water at 85°C for 6 h and 225 mg of extract was recovered by lyophilization. The procedure was repeated 20 times to obtain the plant drug necessary for the whole investigation.

Plant Drug Analysis

a) TLC

The ethyl acetate extract (5 mg) was co-chromatographed with flavonoid test samples (rutin, chlorogenic acid, hyperoside and isochlorogenic acid), luteolin and luteolin-7-glucoside, quercetin, isoquercetin, delphinidin and caffeic acid using ethyl acetate-formic acid –glacial acetic acid-water (100:11:11:26) as the mobile phase and precoated silica gel 60 F₂₅₄ (20 x 20 thickness 0.25 mm Merck, Darmstadt, Germany) as the stationary phase. The plate was first observed at UV₂₅₄ nm then with natural products-polyethylene glycol reagent (NP/PEG) and evaluated at UV₃₆₆ nm.

b) HPLC

This was carried out with an HP 1090. A liquid chromatography and an HP 1040 photodiode array with a Hewlett Packard detector as a LiChrospher 100 RP 18 (5 µm) column 125 x 4 mm (Merck, Darmstadt,

Germany), and a precolumn LiChrospher RP-18 4-4 mm (5 μ m) (Merck, Darmstadt, Germany) with detection at wavelength 210 nm, 254 nm, 280 nm and 366 nm. The mobile phase used for the separation was HPLC graded water (Solvent A) and acetonitrile (Solvent B), all acidified with 33 μ L of phosphoric acid (85%). It was started at 10% of solvent B and a linear gradient of 10–30% MeCN during 20 min for a total run of 30 min, at a flow of 1 mL/min, and a volume of 12.5 μ L of 1 mg/mL of ethyl acetate extract of *R. praetermissa* was injected.

Experimental Subjects

The study was conducted in Boyo, and Bamenda in the North West Region of Cameroon from December 2011 to April 2012. A total of 50 pregnant women between 20 and 40 years of age attending routine prenatal clinics in Belo Maternity Center were recruited for the study. They gave their informed consent to participate in the study which was approved by the ethics commission of our institution. All participants were interviewed to assess their physical wellness, nutritional factors, gestational history and hematological records. The women were assigned to 5 groups of 10 each. The first group was the control which received daily doses of 0.5 ml of saline solution. The second, third, fourth and fifth groups received 200 mg/kg, 400 mg/kg, 800 mg/kg and 1,600 mg/kg respectively in daily oral doses for sixteen days. Blood samples (0.5 ml) were obtained on the 17th day from each of the women. This was done with needles and syringes in 10 ml tubes using venous puncture by drawing specimens from a superficial vein in the antecubital fossa of the arm. Ethylenediamine tetraacetic acid (EDTA) was used to prevent blood from clotting. These samples were analyzed in the Diagnostic Laboratory in Bamenda for the determination of blood parameters under investigation: Hematocrit (Hct), Hemoglobin Concentration (Hb), and Red blood Cell Counts (RBC)

Blood Analysis

The Hct was measured by the percentage of the total blood volume that is made up of RBCs. The height of the RBCs column was measured after centrifugation and compared to the column of the total whole blood. The ratio of the height of the RBC column compared with the original total blood column was multiplied by 100% and that was recorded as the Hct value. The haemoglobin value and the red blood cell count were done by an automated cell counter. The values of RBC, hematocrit, and haemoglobin test were used to calculate the RBC indices (MCV, MCH and MCHC).

Statistical Analysis

Data was analysed using Sigma Plot 11 for Windows and the significance was set at the 5% level. The results were expressed as mean \pm SEM, differences between means analysed using Student's 't' test. P values of 0.05 or less and 0.001 were taken as being statistically significant.

RESULTS AND DISCUSSION

The results of the chemical analysis of the four extracts revealed that the plant drug contains flavonoids aglycones (luteolin and apigenin) and their respective, glycosides. The extract also contains a high concentration of triterpenes (campesterol, stigmasterol, β -sitosterol, lupeol) and iridoid glycosides.

The effect of various doses of the extract of *R. Praetermissa* on the mean hematological parameters are shown in Tables 1 & 2 below.

Hematocrit (Hct): Increasing the dosage concentration of the plant drug had an effect of increasing the Hematocrit (Hct) in all the treatment groups. Hematocrit (Hct) value at 200 mg/kg 43.90 ± 2.06 % was however not significantly different from that of the control group 43.35 ± 1.82 %. At high doses of the extract, 800 mg/kg and 1,600 mg/kg, there was a significant increase from $47.75 \pm 1.5\%$ to 48.25 ± 1.49 % respectively ($P < 0.05$).

Hemoglobin Concentration (Hb): Increase in hemoglobin concentration occurred with increased concentration of applied extract. The hemoglobin value of 13.26 ± 1.5 g/dL of the control group increased to 15.22 ± 0.19 g/dL at 800 mg/kg and 16.10 ± 0.07 g/dL at 1,600 mg/kg respectively. All these values are significantly different from the control group ($P < 0.05$). Significant change in hemoglobin concentration also occurred between the two high doses i.e. 800 and 1,600 mg/kg.

Red Blood Cell Count: Women that received 200 and 400 mg/kg body weight of the extract had significantly lower red cell value $P < 0.05$ than those who received 800 and 1,600 mg/kg body weight of the extract. There was no significant difference between the red cell count of those that received 200 mg/kg dose of extract and the normal saline in the control group.

Table 1: Effect of the Extract of *Ruellia Praetermissa* on the Hemtaocrit (Hct) haemoglobin concentration and red blood cell count in the pregnant women blood

Treatment with <i>Ruellia</i> Extract	(Hct) (%) \pm S.E.	Hb(g/dL) \pm S.E.	RBC(million/mm ³) \pm S.E.
0.5 ml Saline	43.35 ± 1.82	13.26 ± 1.5	4.10 ± 0.23
200 mg/kg	43.90 ± 2.06	13.34 ± 1.7	4.34 ± 0.18
400 mg/kg	46.62 ± 1.60	14.75 ± 0.14	4.80 ± 1.03
800 mg/kg	47.75 ± 1.50	15.22 ± 0.19	5.33 ± 0.03
1600 mg/kg	48.25 ± 1.49	16.10 ± 0.07	5.53 ± 1.1

Table 2: Effect of the Extract of *Ruellia Praetermissa* on the Blood indices (mean corpuscular volume [MCV], Mean corpuscular haemoglobin [MCV], and Mean corpuscular haemoglobin concentration [MCHC]).

Treatment with <i>Ruellia</i> Extract	MCV \pm S.E. (mm ³)	MCH \pm S.E. (Pg)	MCHC \pm S.E. (g/dL)
0.5 ml Saline	105.68 ± 2.92	32.34 ± 1.4	30.58 ± 1.1
200 mg/kg	101.15 ± 0.50	30.73 ± 1.73	30.38 ± 1.7
400 mg/kg	96.26 ± 1.06	36.56 ± 0.25	31.63 ± 1.5
800 mg/kg	89.58 ± 1.72	28.55 ± 0.53	32.87 ± 2.43
1600 mg/kg	87.25 ± 1.35	29.11 ± 1.39	33.36 ± 1.14

MCV, MCH AND MCHC

Women in the control group who received only normal saline had significantly higher MCV values ($P < 0.05$) than those recorded for all the groups of women who received the extract in various doses. The MCV values decreased in the dose dependent manner. The MCH and MCHC showed no significant effect ($P > 0.05$) as the plant drugs were administered in different concentrations.

The result of this study showed that the aqueous extract of the leaf of *Ruellia Praetermissa* increased the overall hemoglobin concentration, red cell count and packed cell volume in Pregnant women in a dose dependent manner. Extract of sorghum has been demonstrated to present similar effects on rats. (Ogwumike, 2002). However, during pregnancy, there is a decrease in hematocrit, red blood cell count and hemoglobin values due

to increase ECF volume that increase plasma volume resulting in hemodilution (Sembulingam and Prema, 2010). This presents a potential source of anaemia for the gestation mother which can result in miscarriage. Gestational state also causes physiological polycythemia due to the increased emotional conditions and demand for proton buffering. Hypoxia due to the high demand of respiratory gases for both the gestation mother and the developing fetus also stimulates erythropoietin secretion (Strand, 1998). Erythropoietin or erythrocyte stimulating factor is the main hormone responsible for erythropoiesis in bone marrow, liver and spleen. The plant extract inhibits Angiotensin converting enzymes (ACE) activity and consequently stimulates the secretion of erythropoietin by peritubular capillaries of the nephron. (Vander *et. al.*, 1994). *Ruellia* is a vegetable rich in Vitamin B₁₂ (cyanocobalamin) and minerals such as Iron and copper. (Duke, J., 2010). Cyanocobalamin is the major maturation factor necessary for erythropoiesis, iron is necessary for the formation of the heme part of the haemoglobin and copper is important for the absorption of iron from the gastrointestinal tract. (Sembulingam and Prema, 2010). The iridoid glycosides isolated from this plant drug exert an inotropic effect of the myocardial muscles (Salah, 1999) and hence increase the cardiac output during pregnancy to satisfy the demands of the developing fetus (Constanzo, 2004). Increase cardiac output increases

erythropoietic activity resulting in more than usual number of erythrocytes. The active principles of this plant drug stimulate erythropoiesis which leads to increase in circulating RBCs with slightly microcytic sizes (MCV), normochromic weight, (MCH) normochromic hemoglobin contents (MCHC). The increase in numbers of erythrocytes containing the same amount and concentration of haemoglobin will fulfil the physiological needs of the pregnant mother with the growing fetus.

CONCLUSION

This study suggests that administration of *Ruellia praetermissa* enhances the process of erythropoiesis in gestation mothers and hence counters the anemic effects of pregnancy in a dose dependent manner. In combination with our previous studies on the effects of *Ruellia* on ovulation, growth of the uterine endometrium, implantation and the estrogenic effects. These findings provide the pharmacological basis for the traditional use of this plant for prenatal care in the North West Region of Cameroon.

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