

Full Length Research Paper

Haematological profiles in male rats treated with methanolic extract or chromatographic fractions of *Cnestis ferruginea* (de Candolle)

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The current dearth of information on the effect of *Cnestis ferruginea* on haematology necessitated the current study, which is to assess the effects of crude and chromatographic fractions of *C. ferruginea* on the blood values of male rats. Two experiments were carried out in this study. The first was to determine the effect of acute administration of varying doses (500, 1000 and 2000 mgkg⁻¹bw) of methanolic extract of *C. ferruginea* which were given orally to male Wistar rats. In the second experiment, 2000 µgkg⁻¹bw of each of the six pure fractions of *C. ferruginea* was given orally to four male Wistar rats in each of the six groups for 60 days, distilled water was given to the control group. The haematological values (The red blood cell (RBC) counts, packed cell volume (PCV), haemoglobin concentration (Hb), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC), total WBC and lymphocyte were not affected by the methanolic extract of *C. ferruginea*. However, the neutrophil count was significantly lower ($p < 0.05$) in the rats treated with 2000 mgkg⁻¹bw of *C. ferruginea*. RBC, PCV, MCV, MCH and MCHC values of rats treated with pure fractions 1, 2, 5 and 6 were not significantly different ($p > 0.05$) from those of the control group. Rats treated with fractions 3 and 4 had significantly reduced ($p < 0.05$) PCV, Hb and RBC values but the MCV and MCH were significantly increased ($p < 0.05$). MCHC values were not significantly affected ($p > 0.05$) by F3 and F4. Apart from F1 that had similar TWBC value with that of the control group, F2, F3, F4, F5 and F6 had significantly reduced TWBC ($p < 0.05$). All the 6 fractions had significantly reduced neutrophil counts while F2, F5 and F6 had significantly decreased lymphocyte counts. The study revealed that although the methanolic extract did not produce any significant effect on the blood parameters, all the purified fractions caused leucopenia while fractions 3 and 4 caused anaemia. It was therefore concluded that the anaemia observed in rats may be due to the presence of quinolizidine alkaloid in the purified fractions 3 and 4.

Key words: Haematology, *Cnestis ferruginea*, fractions, methanolic extract.

INTRODUCTION

Cnestis ferruginea, belongs to the family *Connaraceae* plants and is highly ubiquitous in the Southern part of

Nigeria. It is known as *omu aja* or *gboyin gboyin* in Yoruba, *amunketa* in Igbo, *Utina bua* in Efik and *Ukpe-ibiaka* in Bini tribes of Nigeria. It was reported to have an excellent antioxidant activity (Oke and Hamburger, 2002). *C. ferruginea* is known to possess powerful antibiotic activities; extract of whole plant of *C. ferruginea* has been used to treat conjunctivitis, syphilis, gum pain, wounds and dysentery and gonorrhoea (Bouquet and Debray, 1974; LeGrand, 1989). The traditional medicine practitioners in Nigeria use the root decoction of the plant to

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Abbreviations: RBC, Red blood cell; PCV, packed cell volume; Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cell; EDTA, ethylene diamine tetraacetic acid.

stabilize pregnancy and to treat ovarian disorder. *C. ferruginea*, has alkaloid as its main bioactive constituent and the preliminary investigation reveals that it has antifertility property.

Many medicinal plants with antifertility activity have been reported to induce anaemia, which is the main toxic effect they produce. Methanol extracts of the plant *Tripterygium wilfordii*, which contains alkaloid and used as an antifertility agent (Quain, 1987) has been reported to induce anemia (Pyatt et al., 2000). Also, *Azadirachta indica*, which was reported to have antifertility activity (Raji et al., 2003), was observed to cause anaemia in Brown Hisex chicks (Ibrahim et al., 1992). Furthermore, *Abrus precatorius*, which has been used as an antifertility agent (Kulsheshtha and Mathur, 1990), was shown to induce anaemia in Lohman broiler chicks (Omer et al., 1992). The thorough search of literature revealed that a dearth of information on effect of *C. ferruginea* on the haematological values. They therefore embarked on this study to assess the effect(s) methanolic and chromatographic fractions of *C. ferruginea* on the haematology of male rats.

MATERIALS AND METHODS

Animals

Adult male Wistar albino rats obtained from the Central Animal House, College of Medicine, University of Ibadan, were used for the experiments. They were acclimated to laboratory conditions (12 h dark-light period), housed five per cage and were fed with rat cubes (Ladokun feeds limited, Ibadan, Nigeria) and water *ad libitum*. The weight range of the rats was 160 - 220 g before the commencement of the study.

Plant material and extraction procedure

Root of *C. ferruginea* was collected at the botanical garden, University of Ibadan, Ibadan where it was authenticated. A voucher specimen (UIH 22272) was deposited at the herbarium of the Department of Botany and Microbiology, University of Ibadan. The root of *C. ferruginea* was air dried and pulverized before the commencement of the methanolic extraction. The extraction was carried out as earlier described by (Njar et al., 1993). The pulverized root weighing 2,750 g was exhaustively extracted with methanol by means of Soxhlet apparatus and the extract evaporated *in vacuo*. The root extract of *C. ferruginea* was concentrated *in vacuo* using a rotary evaporator. The solvent (methanol) remaining in the extract was finally removed by placing the root extract in porcelain dishes in temperature-controlled oven to give a residue weighing 25 g (0.91% yield).

Separation of column fractions by thin layer chromatography (TLC)

A line was drawn with pencil parallel to, and 2 cm from the bottom of the silica coated plate. The samples were spotted on this line, called the origin, at least 1 cm from each other. The sample or fraction was spotted on the TLC plates using a capillary tube filled with the fraction. The spotted plates were placed in a chamber saturated with Ethylacetate: methanol as mobile phase. Once the

plate had been developed for the predetermined distance, it was removed from the tank and the position of the solvent front was marked. To locate the compounds in the sample, the TLC plate was examined under (354 nm) ultraviolet light and any spots visualized with this procedure were ringed using a pencil. With this procedure fractions with similar retention or retarding factor (Rf) were pooled together. This then reduced the number of fractions to 6 for the root extract.

Administration of crude methanolic extract of *C. ferruginea*

A total of 20 male rats divided into four groups with 5 rats per group were used. Varying doses of 500, 1000 and 2000 mgkg⁻¹bw were given orally to animals in groups 1, 2 and 3, respectively, as a single daily dose using orogastric needle. The control group received volume of distilled water (vehicle for the stock solution of *C. ferruginea*) commensurate with the body weight of rat, average of 0.5 ml. After 5 days of treatment each rat was bled through the orbital sinus for red blood cell count, packed cell volume, haemoglobin estimation, total white cell count and differential white blood cell count

Administration of pure fractions of *C. ferruginea*

The procedure for his experiment was carried out using the six purified fractions of *C. ferruginea*. Four rats per group were used for each fraction. 2000 µgkg⁻¹bw of each fraction of *C. ferruginea* was given orally for 60 days. The control group received volume of distilled water (vehicle for the stock solution of *C. ferruginea*) commensurate with the body weight of rat (average of 0.5ml). After treatment period each rat was bled through the orbital sinus for red blood cell count, packed cell volume, haemoglobin estimation, total white cell count and differential white blood cell count. At the end of each treatment period rats were bled through the orbital sinus. The blood was transferred into bottles containing ethylene diamine tetraacetic acid (EDTA) (2 mg/ml of blood) as anticoagulant. Red blood cell (RBC) and white blood cell (WBC) counts were determined using a haemocytometer. The packed cell volume (PCV) was estimated by the microhaematocrit method and the haemoglobin (Hb) concentration by the cyanmethaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described earlier (Jain, 1986). Blood smears were stained with Giemsa stain for differential WBC counts.

RESULTS

The erythrocyte values (RBC, PCV, Hb, MCV, MCH and MCHC) and leucocyte values (total WBC and lymphocyte count) were not affected by the methanolic extract of *C. ferruginea*. However, the neutrophil count was significantly lower ($p < 0.05$) in the rats treated with 2000 mgkg⁻¹bw of *C. ferruginea* (Table 1). Table 2 shows the effect of the Fractions1, 2, 3, 4, 5 and 6 of the pure extract of *C. ferruginea* on erythrocyte and leucocyte values. The red blood cell (RBC) counts, packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) of rats treated with 2000 µg kg⁻¹bw of F1, 2, 5 and 6 were not significantly different ($p > 0.05$) from those of the control group. Rats

Table 1. Effect of acute administration of the root extract of *C. ferruginea* on the haematological parameters of male albino rats.

Parameters	Control (n = 5)	500 mgkg ⁻¹ bw (n = 5)	1000 mgkg ⁻¹ bw (n = 5)	2000 mgkg ⁻¹ bw (n = 5)
RBC($\times 10^{12}$ /L)	14.97 \pm 2.67	15.28 \pm 2.77	14.10 \pm 3.69	13.60 \pm 0.64
PCV (%)	53.40 \pm 2.61	49.20 \pm 3.56	54.80 \pm 4.09	52.00 \pm 2.00
Hb (g/dl)	11.52 \pm 2.85	9.58 \pm 0.10	11.30 \pm 1.73	11.00 \pm 0.00
MCH (pg)	7.99 \pm 2.66	6.53 \pm 1.39	8.67 \pm 3.18	8.04 \pm 0.04
MCHC (g/dl)	21.42 \pm 4.26	19.70 \pm 1.64	20.56 \pm 2.37	20.15 \pm 0.20
MCV (fl)	36.77 \pm 7.87	32.88 \pm 4.84	41.59 \pm 13.64	39.46 \pm 0.15
Total WBC ($\times 10^9$ /L)	19.68 \pm 2.69	16.24 \pm 5.44	17.72 \pm 4.19	15.72 \pm 3.87
Lymphocyte ($\times 10^9$ /L)	12.25 \pm 3.17	10.25 \pm 4.57	10.34 \pm 3.11	11.45 \pm 3.50
Neutrophil ($\times 10^9$ /L)	7.35 \pm 2.95	5.99 \pm 3.12	7.35 \pm 1.37	4.25 \pm 0.37*
	(62.20 \pm 13.48) ^a	(61.60 \pm 17.54) ^a	(57.60 \pm 6.19) ^a	(71.40 \pm 6.95) ^a
	(37.40 \pm 13.94) ^a	(38.40 \pm 17.54) ^a	(42.20 \pm 6.05) ^a	(28.60 \pm 6.95) ^a

Value not significantly ($p > 0.05$) different from control on the same row. ^a Value expressed as a percentage of Total WBC count.

Table 2. Effect of the 6 Fractions of *Cnestis ferruginea* at 2000 $\mu\text{g kg}^{-1}\text{bw}$ on the haematological values of male albino rats.

Parameters	Control (n = 4)	F1 (n = 4)	F2 (n = 4)	F3 (n = 4)	F4 (n = 4)	F5 (n = 4)	F6 (n = 4)
RBC ($\times 10^{12}$ /L)	11.32 \pm 0.90	11.27 \pm 2.72	9.79 \pm 0.70	6.06 \pm 0.76*	6.94 \pm 0.85*	9.57 \pm 2.73	9.57 \pm 2.73
PCV (%)	54.25 \pm 2.06	48.50 \pm 6.35	54.75 \pm 3.50	48.25 \pm 0.50*	50.00 \pm 2.31	53.50 \pm 0.57	55.00 \pm 2.00
Hb (g/dl)	16.75 \pm 0.05	15.95 \pm 0.19	16.22 \pm 0.45	14.50 \pm 1.00*	15.60 \pm 0.69*	16.15 \pm 0.98	16.30 \pm 0.60
MCH (pg)	14.67 \pm 1.84	15.02 \pm 4.81	16.62 \pm 1.04	25.23 \pm 2.01*	22.64 \pm 1.79*	18.25 \pm 6.48	20.15 \pm 6.33
MCHC (g/dl)	30.41 \pm 1.30	33.28 \pm 4.00	29.69 \pm 1.21	31.46 \pm 1.28	31.20 \pm 0.06	30.17 \pm 1.51	29.68 \pm 1.74
MCV (fl)	48.68 \pm 4.60	46.54 \pm 19.59	56.14 \pm 5.31	80.49 \pm 9.25*	72.57 \pm 5.61*	61.00 \pm 23.82	67.40 \pm 18.46
TOTALWBC ($\times 10^9$ /L)	19.68 \pm 2.69	16.80 \pm 3.39	10.20 \pm 1.20*	12.00 \pm 0.92*	10.70 \pm 0.60*	14.00 \pm 1.53*	10.90 \pm 2.34*
LYMPHOCYTE ($\times 10^9$ /L)	12.25 \pm 3.17	12.97 \pm 4.04	5.26 \pm 0.59*	5.81 \pm 0.37	8.87 \pm 0.90	11.42 \pm 0.72	5.49 \pm 1.11*
	(62.20 \pm 13.48) ^a	(76.25 \pm 8.18) ^a	(51.75 \pm 4.86) ^a	(48.50 \pm 0.58) ^a	(75.25 \pm 11.50) ^a	(82.00 \pm 5.89) ^a	(50.50 \pm 3.00) ^a
NEUTROPHIL ($\times 10^9$ /L)	7.35 \pm 2.95	3.74 \pm 0.93*	4.92 \pm 0.93*	6.18 \pm 0.54*	3.33 \pm 2.71*	2.59 \pm 1.08*	5.41 \pm 1.30*
	(37.40 \pm 13.94) ^a	(23.25 \pm 7.63) ^a	(48.00 \pm 4.76) ^a	(51.50 \pm 0.58) ^a	(24.75 \pm 11.50) ^a	(17.50 \pm 5.26) ^a	(49.50 \pm 3.00) ^a

Values significantly different from control at * $P < 0.05$ on the same row. ^a Value expressed as a percentage of Total WBC count.

treated with 2000 $\mu\text{g kg}^{-1}\text{bw}$ of F3 and F4 had significantly reduced ($p < 0.05$) PCV, Hb and RBC but the MCV and MCH were significantly increased ($p < 0.05$) and MCHC values were not significantly affected ($p > 0.05$). Also, apart from F1 that had similar TWBC value with that of the control group, F2, 3, 4, 5, 6 significantly reduced TWBC. The 6 fractions also significantly reduced ($p < 0.05$) neutrophil counts while F2, F5 and F6 significantly decreased lymphocyte counts.

DISCUSSION

In the present study, all the erythrocyte and leucocyte values were not affected when the rats were treated for a short period with *C. ferruginea*. This means that *C. ferruginea*, does not induce anaemia when given for a short duration. The bioactive pure fractions (F3 and F4) nevertheless induce anaemia. These were also the pure

fractions that were observed to possess antifertility male effects (Olayemi, 2007). Similarly, Ibrahim et al. (1992) reported that *A. indica*, a plant which has antifertility property, caused anaemia. Also, addition of *A. precatorius* seed to livestock feed has been shown to induce anaemia (Omer et al., 1992). *A. precatorius* which also has male antifertility effect has been shown to induce anaemia (Kulshreshtha and Mathur, 1990). The chloroform extract of *Carica papaya* seed, which has been reported to have antifertility effect, was not observed to have any effect on haematological parameters of Langur monkey (Lohiya et al., 2002). There were significant reductions ($p < 0.05$) in the values of PCV, RBC and Hb but significant increases in the values of MCV and MCH in the 2000 $\mu\text{g kg}^{-1}\text{bw}$ treatment group of fractions 3 and 4 of *C. ferruginea*, which contain quinolizidine alkaloid (Olayemi, 2007). It seems the anaemia by the pure fractions is macrocytic and regenerative (Increased MCV and an increase in MCH caused by F3

and F4).

It is well established that one of the toxic effects of alkaloid in animals is anaemia. *Crotalaria spectabilis* which contains three pyrrolizidine alkaloids (fluvine, monocrotaline and crispatine) has been implicated in pyrrolizidine toxicosis. Animals with this pyrrolizidine toxicosis have severe haemorrhage which results in anaemia (Smith et al., 1974). However, contrary to the present finding, Raji (1995) reported that extracts of *Quassia amara*, which has alkaloids as its main bioactive constituent like *C. ferruginea*, caused significant increases in RBC, PCV and Hb concentration. Toxins from plants have been known to induce regenerative anaemia (Aiello, 1998). Regenerative anaemia is seen as an increase in MCH. It was observed that when RBC precursors mature in the bone marrow their volume decreases as the Hb content increases. However, reticulocytes which are released into circulation during regenerative anaemia have a higher MCH (Aiello, 1998). The acute administration of methanolic extract of *C. ferruginea* did not have any effect on the WBC value. Nevertheless, the chromatographic fractions (2 - 6) of *C. ferruginea* significantly reduced the TWBC counts. Jain (1986) reported that toxic substances caused decrease in TWBC through either bone marrow depression or competition with folic acid utilization to cause leucopenia. It implies that, the toxic action of *C. ferruginea* on leucocytes could be by any of these two mechanisms. It seems the purified fractions are toxic to leucocytes. It therefore means that, the antibacterial property of *C. ferruginea* may not be by enhancing the immune status of the animal but probably by destruction of the microbes.

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