

Full Length Research Paper

Haematological and some biochemical profiles in male rats treated with *Cnestis ferruginea* (de Candolle) root extract and its pure fractions

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The impact of methanolic root extract of *Cnestis ferruginea* on blood and some biochemical profiles was studied in male albino rats. Rats were treated with daily oral administration of the extract (500 mg kg⁻¹ bw) and its pure fractions for 5, 30 and 60 days. Haematological indices and plasma concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were not adversely affected by the extract after 5 days of treatment. However, there were significant reductions ($P < 0.05$) in red blood cell (RBC) and packed cell volume (PCV) after 60 days of treatment with *C. ferruginea*. Haemoglobin concentration and mean corpuscular volume (MCV) were not affected while mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were increased ($P < 0.01$) after 60 days of treatment. Lymphocyte count was higher after 30 days of treatment while the white blood cell counts were not affected by prolonged treatment with the extract and its pure fractions. Plasma levels of ALT increased ($P < 0.01$) while those of AST was not affected after 60 days of treatment. There was a significant restoration of these parameters after withdrawal from treatments. The results suggest that prolonged administration of crude root extract of *C. ferruginea* may induce anaemia.

Key words: *Cnestis ferruginea*, blood indices, aspartate aminotransferase (AST), alanine aminotransferase (ALT), rat.

INTRODUCTION

Cnestis ferruginea belongs to the family of 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-ibieka' in Bini tribes of Nigeria. It was reported to have an excellent antioxidant activity (Oke and Hamburger, 2002). It is also known to possess powerful antibiotic activities. Extract of whole plant of *C. ferruginea* has been used to

treat conjunctivitis, syphilis, gum pain, wounds, dysentery and gonorrhoea (Bouquet and Debray, 1974; LeGrand, 1989). The traditional medicine practitioners in Nigeria use the root decoction of the plant to prevent abortion and to treat ovarian disorder. *C. ferruginea* is rich in bioactive-constituents. Thorough search of the literature revealed a dearth of information on the effect of *C. ferruginea* on haematological parameters. We therefore

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embarked on this study to assess the effect of short and prolonged duration treatment of methanolic extract of roots of *C. ferruginea* and its purified fractions on haematological and some biochemical indices in male rats.

MATERIALS AND METHODS

Animals

Adult male Wistar albino rats obtained from the Central Animal House, College of Medicine, University of Ibadan Nigeria, 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 to 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 Nigeria 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 (Njar et al., 1993). The pulverized root weighing 2,750 g was exhaustively extracted with methanol by means of Soxhlet apparatus and the extract was 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 extract in porcelain dishes in temperature-controlled oven to give a residue weighing 25 g (0.91% yield).

Experimental design

A total of 20 male rats divided into four groups with 5 rats per group were used for each of the crude and pure fractions of *C. ferruginea*. The dose of 500 mg kg⁻¹ bw was administered orally to rats for 5, 30 and 60 days, respectively as a single daily dose using orogastric needle. There was a recovery group that consisted of rats from which extract was withdrawn for 30 days before the rats were sacrificed. The control group received 0.5 ml of distilled water (vehicle for the stock solution of *C. ferruginea*). At the end of each treatment period, rats were sacrificed and blood was withdrawn into bottles containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant. Red blood cell (RBC) and white blood cell (WBC) counts were determined using a haemocytometer. The packed cell volume (PCV) was estimated using the microhaematocrit method and haemoglobin (Hb) concentration by the cyanmethaemoglobin method (Jain, 1986), and the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. The plasma was separated from the blood by centrifugation at 3000 g for 10 min and the plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed with the methods of Mohun and Cook (1957) and Reitman and Frankel (1957).

Statistical analysis

Mean values and the standard deviation (Mean ± SD) were calcu-

lated. The test of significance between two groups was determined by Student's t test (Snedecor and Cochran, 1980) and for more than two groups by the analysis of variance (ANOVA) with Duncan's multiple range test (Duncan, 1975).

RESULTS

Effect of crude and pure fractions of *Cnestis ferruginea* on erythrocyte values

As shown in Table 1, treatment of rats with 500 mg kg⁻¹ bw *C. ferruginea* for 5 days did not adversely affect their erythrocyte indices. However RBC count and PCV values were significantly reduced ($P < 0.05$) in 30 and 60 days treatment with *C. ferruginea*. The values of corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were higher ($P < 0.05$) in the 30 and 60 days of treatment when compared with the control group. Table 2 shows the effect of fractions of the pure extract of *C. ferruginea* on erythrocyte values. The RBC counts, PCV, Hb concentration, MCV, MCH and MCHC of rats treated with 100, 1000 and 2000 µg kg⁻¹ bw of F1 were similar to those of F2, F3, F4, F5 and F6 and were not significantly different ($P > 0.05$) from those of the control group after 30 days of treatments. Hence the results for F1 are presented.

Effect of crude and pure fractions of *Cnestis ferruginea* on leucocyte values

As shown in Table 3, leucocyte values (total WBC, lymphocyte, and monocyte counts) were similar ($P > 0.05$) in the control and the different test groups (days 5, 30 and 60 treatment groups). However, lymphocyte count after 30 days of treatment was higher ($P < 0.01$) than the control. Total white blood cell (TWBC) count in 100 and 1000 µg kg⁻¹ bw F1 to F6 treated rats were similar and significantly lower ($P < 0.01$) than those of the control group (Table 4).

Effect of crude and pure fractions of *Cnestis ferruginea* on plasma AST and ALT in albino rats

As shown in Figure 1, the plasma levels of AST and ALT were not affected after 5, 30 and 60 days of treatment of the rat with 500 mg kg⁻¹ bw of *C. ferruginea*. Similarly, AST and ALT were not adversely affected in rats treated with the pure fractions of *C. ferruginea* as shown in their representative Figure 2.

DISCUSSION

In the present study, all the erythrocyte and leucocyte

Table 1. Effect of different duration of treatment of 500 mg kg⁻¹ bw methanolic extract of *Cnestis ferruginea* on erythrocyte values of male rats (n = 5).

Parameter	Control	5 days	30 days	60 days	Recovery
RBC ($\times 10^{12}/L$)	14.97 \pm 2.67	15.28 \pm 2.77	11.70 \pm 1.50*	8.84 \pm 1.16*	10.84 \pm 2.48*
PCV (%)	53.40 \pm 2.61	49.20 \pm 3.56	51.20 \pm 1.10	42.22 \pm 9.15*	51.00 \pm 3.00
Hb (g/dl)	11.52 \pm 2.85	9.58 \pm 0.10	12.46 \pm 0.77	12.90 \pm 2.51	13.64 \pm 0.43
MCH (pg)	7.99 \pm 2.66	6.53 \pm 1.39	10.87 \pm 2.31	14.68 \pm 2.95*	13.12 \pm 2.99*
MCHC (g/dl)	21.42 \pm 4.26	19.70 \pm 1.64	24.33 \pm 1.27	30.85 \pm 2.73*	30.85 \pm 2.73*
MCV (fl)	36.77 \pm 7.87	32.88 \pm 4.84	34.21 \pm 16.29	47.65 \pm 9.05	48.89 \pm 11.08

Value significantly different from control at *P < 0.05.

Table 2. Effect of the fraction 1 of *Cnestis ferruginea* on erythrocyte values of male albino rats (n = 5).

Parameter	Control	100 μ g kg ⁻¹ bw	1,000 μ g kg ⁻¹ bw	2,000 μ g kg ⁻¹ bw
RBC ($\times 10^{12}/L$)	11.32 \pm 0.90	10.86 \pm 0.44	10.32 \pm 1.54	11.27 \pm 2.72
PCV (%)	54.25 \pm 2.06	55.50 \pm 1.10	52.25 \pm 4.19	48.50 \pm 6.35*
Hb (g/dl)	16.75 \pm 0.05	17.15 \pm 0.24	16.70 \pm 1.04	15.95 \pm 0.19*
MCH (pg)	14.67 \pm 1.84	15.78 \pm 5.31	16.72 \pm 2.55	15.02 \pm 4.81
MCHC (g/dl)	30.41 \pm 1.30	31.05 \pm 2.73	31.64 \pm 1.03	33.28 \pm 4.00
MCV (fl)	48.68 \pm 4.60	51.06 \pm 3.48	53.79 \pm 9.41	46.54 \pm 19.59

Value significantly different from control at *P < 0.05 on the same row.

Table 3. Effect of different duration of treatment of 500 mg kg⁻¹ bw methanolic extract of *Cnestis ferruginea* on leucocyte values of male rats (n = 5).

Parameter ($\times 10^9/L$)	Control	5 days	30 days	60 days	Recovery group
Total WBC	19.68 \pm 2.69	16.24 \pm 5.44	25.48 \pm 6.92	17.68 \pm 3.67	16.04 \pm 3.89
Lymphocyte	12.25 \pm 3.17 (62.20 \pm 13.48) ^a	10.25 \pm 4.57 (61.60 \pm 17.54) ^a	22.95 \pm 9.03* (82.40 \pm 7.13) ^a	11.94 \pm 2.30 (68.40 \pm 11.22) ^a	12.93 \pm 4.92 (79.00 \pm 11.64) ^a
Neutrophil	7.35 \pm 2.95 (37.40 \pm 13.94) ^a	5.99 \pm 3.12 (38.40 \pm 17.54) ^a	4.52 \pm 2.29 (17.60 \pm 7.13) ^a	5.52 \pm 2.87 (30.20 \pm 12.44) ^a	3.06 \pm 1.55 (20.60 \pm 11.59) ^a
Eosinophil	0	0	0	0	0
Monocyte	0.19 \pm 0.03 (1.00 \pm 0.00)	0	0	0.26 \pm 0.19 (1.75 \pm 1.50)	0

^aValue expressed as a percentage of total WBC count. Values significantly different from control at *P < 0.01 on the same row.

values were not affected when rats were treated for 5 days with *C. ferruginea*. However, the values of RBC and PCV were significantly reduced when rats were treated for prolonged period with *C. ferruginea*. This means that *C. ferruginea*, which is rich in alkaloids, tannins and anthraquinones, does not induce systemic toxicity when administered for a short duration. It could nevertheless cause systemic toxicity such as anaemia during chronic

treatment.

Adedapo et al. (2004) reported that alkaloids obtained from *Euphorbia balsamifera*, *Euphorbia heterophylla*, *Euphorbia hirta*, *Euphorbia hyssopifolia*, and *Euphorbia lateriflora* caused anaemia and leucopenia in rats. However, contrary to the present finding, Raji et al. (2003) reported that extracts of *Azadirachta indica*, which also has alkaloids as its main bioactive constituent, caused

Table 4. Effect of Fraction 1 of *Cnestis ferruginea* on leucocyte values of male albino rats (n = 5).

Parameter ($\times 10^9/L$)	Control	100 $\mu g\ kg^{-1}\ bw$	1,000 $\mu g\ kg^{-1}\ bw$	2,000 $\mu g\ kg^{-1}\ bw$
Total WBC	19.68 \pm 2.69	13.20 \pm 0.46*	14.40 \pm 3.39*	16.80 \pm 3.39
Lymphocyte	12.25 \pm 3.17 (62.20 \pm 13.48) ^a	8.44 \pm 1.40 (64.25 \pm 12.26) ^a	10.13 \pm 3.35 (70.50 \pm 18.79) ^a	12.97 \pm 4.04 (76.25 \pm 8.18) ^a
Neutrophil	7.35 \pm 2.95 (37.40 \pm 13.94) ^a	4.76 \pm 1.77 (35.75 \pm 12.26) ^a	4.20 \pm 3.26 (29.00 \pm 18.24) ^a	3.74 \pm 0.93* (23.25 \pm 7.63) ^a
Eosinophil	0	0	0	0
Monocyte	0.08 \pm 0.10 (0.60 \pm 0.55) ^a	0	0	0.85 \pm 0.17 (0.50 \pm 1.0) ^a

^aValue expressed as a percentage of Total WBC count. Values significantly different from control at *P < 0.01 on the same row.

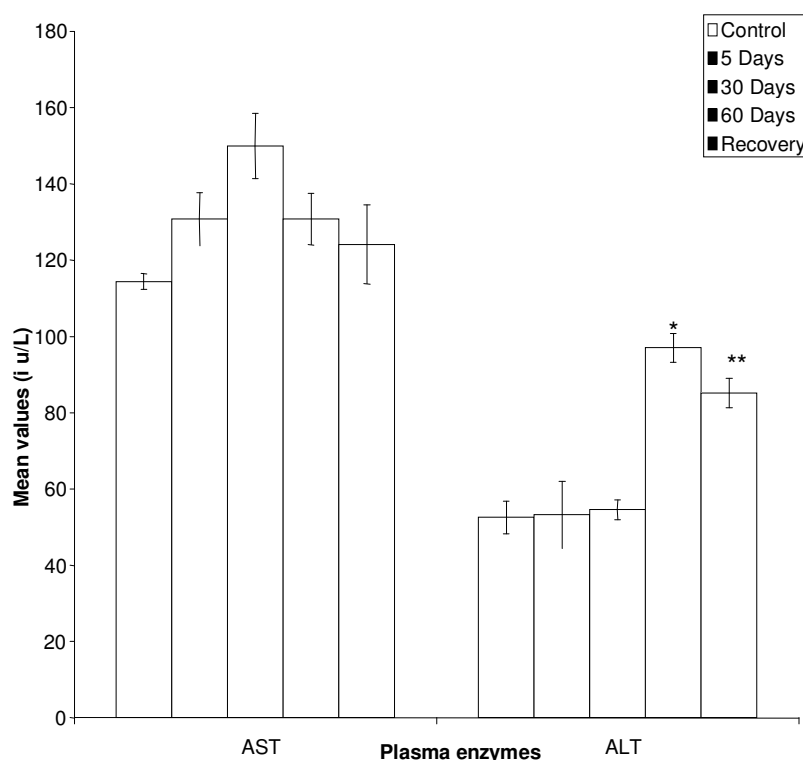


Figure 1. Effects of 5, 30 and 60 days administration of *Cnestis ferruginea* on plasma levels of AST and ALT in male rat. Values are expressed as Mean \pm SEM of five (5) rats. Values significantly different from control value at *P < 0.001 and **P < 0.01.

significant increases in RBC, PCV and Hb concentration. Furthermore, chloroform extract of *Carica papaya* seed with concomitant alkaloid bioactive-constituent did not have any effect on haematological parameters of Langur

monkey (Lohiya et al., 2002).

Similarly, *Calotropis porcera* which caused testicular degenerative changes (Akinloye et al., 2002) was reported not to affect the haematological values in rats during

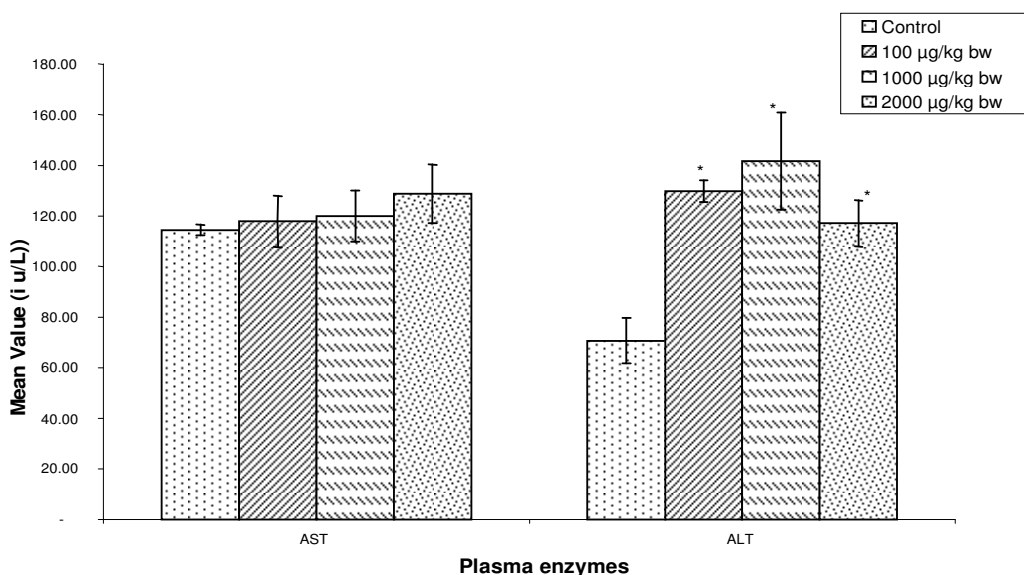


Figure 2. Effect of Fraction 1 of *Cnestis ferruginea* on the plasma levels of AST and ALT of male rats. Values are expressed as Mean \pm SEM. Values significantly different from control (* $P < 0.01$).

both short and prolonged administration (Dada et al., 2002). Therefore it is probable that treatment of infections with *C. ferruginea* as it is done in traditional medicine for prolonged periods could be detrimental as this might result in anaemia.

There was a significant increase in plasma ALT after prolonged treatment of rat with *C. ferruginea*. However, the level of AST was not affected at both the short and prolonged administration. Reports have indicated that an increase in value of ALT might suggest that there is liver damage (Doxey, 1971). AST on the other hand is widespread in different tissues of the body, with the highest concentration in muscles, liver and intestine and an increase AST is mainly seen in heart disease. It therefore means that high dosage and prolonged dosing periods with *C. ferruginea* can be injurious to the liver.

Conclusion

Taken together, the results suggest that while low doses with short duration of administration may be recommended, prolonged treatments of *C. ferruginea* might be contraindicated as it might be deleterious to red blood cell and liver functions.

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