Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Hematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem

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ABSTRACT - The haematological indices (Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Platelets, Neutrophil and Eosinophil) following oral administration of aqueous extract of *Fadogia agrestis* stem at the doses of 18, 50 and 100mg/kg body weight in male albino rats were evaluated progressively on daily basis at 24hrs after 1, 7, 14 and 21days. Extract administration significantly altered ($P<0.05$) WBC count and those relating to it while it produced no significant change on RBC count and its related indices ($P>0.05$). The result suggest that aqueous extract of *Fadogia agrestis* stem has exhibited localized systemic toxicity which will impair the normal functioning of the WBC and its related indices.

KEYWORDS - *Fadogia agrestis,* haematological indices, systemic toxicity.

INTRODUCTION

The indigenous medicinal plants in Nigeria form an important component of the natural wealth of the Country. Most of these plants have been used indiscriminately by many local populations for managing various diseased states without actually knowing how relief is brought about or its safety/toxicity risk. One of such plant is *Fadogia agrestis*.

Fadogia agrestis (Rubiaceae) is an erect, under shrub with yellowish stem and leaves, 1-3feet high (1). It contains alkaloids, saponins, flavonoids and anthraquinones (2). It has been widely used by many localities in Nigeria in the management of male sexual dysfunction most especially erectile dysfunction. The validity of this claim as an aphrodisiac has been scientifically proved (2).

Nowadays, many people are relying on herbal medicines for health care (3), because the other treatment options available are more expensive and are often associated with serious side effects. Therefore, there should be scientific documentation of information on the safety/toxic risk potentials of plants. This study was prompted in view of this and coupled with the fact that there is dearth of information on the effect of its chronic administration on the blood, an important fluid essential for the erection process in males. Therefore this study attempt to investigate the effect of chronic administration of aqueous extract of *Fadogia agrestis* stem (as used in folklore medicine) on haematological parameters using male albino rats as model.

Various workers had shown that haematological investigations among others could be used to evaluate the health status of an animal (4, 5), hence the choice of haematological parameters of Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell Count (WBC), Neutrophils, Lymphocytes and Platelets in this study.

MATERIALS AND METHODS

Laboratory Animals

Male albino rats (*Rattus novergicus*) of Wistar strain weighing between 220-250 were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Science, University of Ilorin, Ilorin, Nigeria.

Plant Material

Fadogia agrestis plant obtained from Herb sellers at Kulende Market, Ilorin, Nigeria during October, 2003 was authenticated at the Department of Horticulture and Landscape Technology, Federal School of Forestry, Jos, Nigeria where voucher specimen was deposited under a voucher number of 2:108.

Preparation of Aqueous Plant Extract

The stem of *Fadogia agrestis* was cut into pieces and

dried in an oven at 40°C to constant weight. The dried pieces were then ground into powder using an electric blender (Blender/Mill Grater III, Model: MS-322, Taiwan). 5g of the powder was extracted in 100ml of distilled water with thorough shaking at regular intervals for 48h at room temperature (26° -28°C). The resulting solution was then filtered using filter paper (Whatman No 1). The distilled water was later evaporated using steam bath to give 0.53g of the residue (brownish black slurry). Calculated amounts of the resulting residue was weighed separately and then reconstituted in distilled water to give the doses of 18mg/kg body weight (value arrived at from ethnobotanical survey – 0.43% w/v in distilled water) and higher doses of 50mg/kg body weight and 100mg/kg body weight used in this study.

Animal Grouping and Extract Administration

A total of 80 male albino rats (*Rattus novergicus*) used for this study were housed individually in plastic metabolic cages of dimensions 33cm x 20.5cm x 19cm, with cleaning of the cages done once daily. They were kept in well-ventilated house conditions (Temperature: 28°C-31°C; photoperiod: 12h natural light and 12h dark; humidity: 50-55%), with free access to rat pellets (Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria) and tap water. The rats were divided into 4 groups: A, B, C and D of 20 rats each. Groups A, B and C were orally administered on daily basis with 1ml of the aqueous plant extract at 18, 50 and 100mg/kg body weight while group D received orally same volume of distilled water (the vehicle). 5 rats each from groups A, B, C and D were sacrificed 24hr after being dosed for days 1, 7, 14 and 21. The extract treated and the controls were studied in parallel with administration done between 0800hr-0900hrs daily. They were allowed free access to rat feed and water before and after their daily administration.

The study was approved by the departmental ethical committee and the high number of rats used was to take care of the progressive monitoring over 21days experimental period and besides, the effect of the plant extract was monitored on other parameters apart from the blood.

Under slight ether anesthesia, about 2ml of blood was collected into EDTA sample bottles by cardiac puncture using needle and syringe and this was carefully mixed with the anticoagulant (EDTA – 10% w/v in distilled water) to prevent clotting. The Automated Haematologic Analyzer, Sysmex, KX-21 (Japan) was used to analyse the haematological parameters of Hb, PCV, RBC, MCV, MCH, MCHC, WBC, Neutrophils, Lymphocytes and Platelets.

Statistical Analysis - Data are mean of 5 replicates ± SD. Statistical analysis was carried out using Duncan Multiple Range Test (6). Data from the test groups were compared with their respective controls and differences were considered significant at $P<0.05$.

RESULTS

The effects of administration of aqueous extract of *Fadogia agrestis* stem at various doses of 18, 50 and 100mg/kg body weight on the haematological parameters of male albino rats for days 1, 7, 14 and 21 are shown in Tables 1, 2, 3 and 4 respectively. Administration of the plant extract at various doses (18, 50 and 100mg/kg body weight) did not produce any significant change ($P>0.05$) on the RBC and factors relating to it (Hb, PCV, MCV, MCH and MCHC) on all the days (1, 7, 14 and 21) investigated (Tables 1, 2, 3 and 4). Interestingly, administration of the plant extract produced significant alterations in the platelets, neutrophils and lymphocytes. Extract administration produced significant decrease ($P<0.05$) in WBC on day 1 (Table 1) which contrast to significant increase ($P<0.05$) on the other days studied (Days 7, 14 and 21) (Tables 2, 3 and 4). By day 1, platelet count decreased significantly ($P<0.05$) following the administration of the least dose (18mg/kg body weight) of the plant extract used while administration at higher doses resulted in significant increase ($P<0.05$) in the blood parameter (Table 1). Extract administration for 7days at the dose of 18mg/kg body weight resulted in significant increase ($P<0.05$) in platelet count while that of 50mg/kg body weight resulted in significant reduction ($P<0.05$). However, administration at 100mg/kg body weight produced values that compared favourably ($P>0.05$) with the control (Table 2). Administration for 14days produced significant increase ($P<0.05$) in platelet count only with 50 and 100mg/kg body weight doses (Table 3). Administration of the extract at 50mg/kg body weight for 21days (Table 4) resulted in significant increase ($P<0.05$) in platelet count whereas higher dose of 100mg/kg body weight produced significant reduction of about half the control value ($P<0.05$) (Table 4).

Administration of the extract at various doses (18, 50 and 100mg/kg body weight) produced significant decrease ($P<0.05$) in the percentage neutrophils on all the days investigated (days 1 – 21). The percentage lymphocytes increased significantly ($P<0.05$) throughout the experimental period for all the doses investigated.

Table 1: Changes in haematological parameters of male rats orally administered with aqueous extract of Fadogia agrestis stem for day 1

Haematological Parameters	Control	18mg/kg body weight	50mg/kg body weight	100mg/kg body weight
Hb (g/dl)	12.48±1.17	12.80±0.24	12.94±1.22	12.44±1.43
PCV	40.20±1.64	40.40±1.34	40.20±0.84	40.20±3.03
RBC (X 10^{12}/l)	7.52±1.33	7.45±0.78	7.28±1.99	7.49±0.98
MCV (fl)	53.40±3.29	53.60±3.29	54.20±2.39	54.00±2.45
MCH (pg)	17.40±1.04	16.90±1.40	17.00±0.08	17.30±1.16
MCHC (g/dl)	33.14±0.11	32.92±1.12	32.58±0.58	32.68±0.86
WBC (X 10^{9}/l)	8.34±0.25^{a}	6.38±0.95^{b}	5.22±0.11^{c}	4.90±0.86^{d}
Platelet (X 10^{9}/l)	707.21±2.40^{a}	645.40±8.22^{b}	759.80±9.57^{c}	822.20±16.34^{d}
Neutrophils (%)	51.20±1.64^{a}	45.80±2.17^{b}	43.20±4.38^{b}	33.60±3.21^{c}
Lymphocytes (%)	48.80±3.83^{a}	54.20±2.17^{b}	56.40±4.10^{b}	66.40±3.21^{c}

Values are mean of 5 replicates ± SD; Values with superscripts different from the control and other test groups for each parameter are significantly different (P<0.05)

Table 2: Changes in haematological parameters of male rats orally administered with aqueous extract of Fadogia agrestis stem for day 7

Haematological Parameters	Control	18mg/kg body weight	50mg/kg body weight	100mg/kg body weight
Hb (g/dl)	12.54±0.88	12.78±0.38	12.50±1.51	12.78±0.45
PCV	41.00±2.45	39.00±2.00	41.60±4.51	40.50±0.89
RBC (X 10^{12}/l)	7.42±0.62	6.95±0.56	7.28±0.97	7.59±0.24
MCV (fl)	54.60±1.67	54.20±1.64	53.20±1.64	53.60±2.07
MCH (pg)	17.80±0.84	17.20±1.10	17.80±0.45	17.60±0.89
MCHC (g/dl)	32.20±0.48	33.60±0.55	32.80±0.45	33.20±0.45
WBC (X 10^{9}/l)	8.90±0.39^{a}	12.10±0.00^{b}	11.84±0.68^{b}	11.46±0.95^{b}
Platelet (X 10^{9}/l)	717.00±4.90^{a}	845.20±31.22^{b}	559.40±13.57^{c}	706.20±10.45^{a}
Neutrophils (%)	50.90±1.64^{a}	25.20±1.79^{b}	19.20±1.10^{c}	19.80±0.84^{c}
Lymphocytes %)	49.10±6.15^{a}	73.80±2.05^{b}	76.80±9.44^{b}	79.80±0.84^{b}

Values are mean of 5 replicates ± SD ; Values with superscripts different from the control and other test groups for each parameter are significantly different (P<0.05)

Table 3: Changes in haematological parameters of male rats orally administered with aqueous extract of Fadogia agrestis stem for day 14

Haematological Parameters	Control	18mg/kg body weight	50mg/kg body weight	100mg/kg body weight
Hb (g/dl)	13.20±0.72	13.40±0.82	13.20±0.20	13.30±0.70
PCV	41.10±1.05	41.30±1.25	41.05±0.72	41.25±1.44
RBC (X 10^{12}/l)	7.35±0.61	7.45±0.77	8.15±0.40	7.20±0.30
MCV (fl)	54.20±1.21	54.30±0.88	54.70±2.13	54.40±1.33
MCH (pg)	17.40±0.31	17.00±0.53	17.10±0.55	17.90±0.53
MCHC (g/dl)	32.10±0.44	33.40±1.69	32.64±0.42	32.80±0.61
WBC (X 10^{9}/l)	8.60±1.11^{a}	12.05±0.20^{b}	14.24±0.38^{c}	17.21±1.27^{d}
Platelet (X 10^{9}/l)	693.00±5.30^{a}	685.00±2.00^{a}	748.00±8.25^{b}	896.20±8.52^{c}
Neutrophils (%)	52.24±2.21^{a}	38.36±2.25^{b}	46.80±2.42^{c}	22.70±1.20^{d}
Lymphocytes (%)	47.76±0.11^{a}	61.64±2.84^{b}	53.20±3.63^{c}	77.30±4.35^{d}

Values are mean of 5 replicates ± SD ; Values with superscripts different from the control and other test groups for each parameter are significantly different (P<0.05)

Table 4: Changes in haematological parameters of male rats orally administered with aqueous extract of Fadogia agrestis stem for day 21

Haematological Parameters	Control	18mg/kg body weight	50mg/kg body weight	100mg/kg body weight
Hb (g/dl)	12.50±0.41	13.28±0.11	13.20±0.00	13.10±0.55
PCV	41.60±2.07	41.60±1.52	41.00±0.00	41.80±1.64
RBC (X 10^{12}/l)	7.37±0.35	7.59±0.22	7.27±0.90	7.31±0.25
MCV (fl)	54.60±0.55	54.60±0.89	54.20±1.64	55.60±0.24
MCH (pg)	17.20±0.45	17.00±0.00	17.60±0.55	17.00±0.00
MCHC (g/dl)	32.20±0.45	32.40±0.89	32.00±0.00	33.00±0.00
WBC (X 10^{9}/l)	8.70±1.12^{a}	17.26±2.52^{b}	20.00±0.00^{c}	24.30±2.57^{d}
Platelet (X 10^{9}/l)	694.20±5.61^{a}	692.00±5.85^{a}	743.00±0.00^{b}	316.00±0.00^{c}
Neutrophils (%)	51.60±2.51^{a}	23.00±0.00^{b}	26.00±0.00^{c}	30.00±0.00^{d}
Lymphocytes (%)	48.40±2.51^{a}	77.00±0.00^{b}	74.00±0.00^{c}	70.00±0.00^{d}

Values are mean of 5 replicates ± SD; Values with superscripts different from the control and other test groups for each parameter are significantly different (P<0.05)

DISCUSSION

The administration of a chemical compound may bring about significant changes in the structure, function, metabolic transformation and concentration of biomolecules, enzymes and even metabolic pathways. These alterations which may be rapid or slow may lead to different biochemical mechanism producing similar pathological, clinical and laboratory findings (7). Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extract on the blood. It can also be used to explain blood relating functions of chemical compound/plant extract.

The non-significant effect of the extract at various doses (18, 50 and 100mg/kg body weight) on the RBC and indices relating to it (Hb, PCV, MCV, MCH and MCHC) throughout the experimental period is an indication that there was no destruction of matured RBC's and no change in the rate of production of RBCs (erythropoiesis). It further shows that the extract does not have the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production (8, 9). The non-significant effect on the RBC and Hb also implies that there was no change in the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following the extract administration since RBC and Hb are very important in transferring respiratory gases (5). The calculated blood indices MCV, MCH and MCHC have a particular importance in anaemia diagnosis in most animals (10). The non-significant effects on these indices relating to RBC suggest that there was no effect on the average size of RBC (microcytes) and also in the haemoglobin weight per RBC. This implies that the aqueous plant extract does not possess any potential of inducing anaemia throughout the 21days period of administration.

Other indices that relate to white blood cells were significantly altered; an indication of pathological conditions which may imply challenge on the immune system by the plant extract. The decrease in WBC observed on Day 1 (Table 1) may imply reduction in the ability of the body to respond to infection. However, the significant increase in WBC following the administration of the plant extract indicates a boost in the immune system. Such effects may also be due to increase in vascular permeability. The increase in platelet (Tables 3 and 4) may be due to stimulatory effect on thrombopoietin (11, 12). The significant reduction in the percentage neutrophils on all the days investigated (Days 1 – 21) (Tables 1 – 4) may be adduced to impairment in the ability of the neutrophils to phagocytose (cellular ingestion of offending agents) (13). Lymphocytes are the main effectors cells of the immune system (14). The administration of the plant extract appears to exhibit stimulatory effect on the effectors cells of the immune system.

CONCLUSION

In conclusion, administration of aqueous extract of *Fadogia agrestis* stem at the doses investigated has brought about alterations in the WBC and factors relating to it without any significant effect on red blood cells and factors that relate to it. This may be an indication of local systemic toxicity.

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