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Haematological evaluation in male rats following sub-chronic administration of aqueous crude extract of *Parkia biglobosa* leaves

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ABSTRACT

Parkia biglobosa is an important multipurpose tree of West African Savannah land used traditionally, to treat a range of human and animal ailments. To evaluate the effects of sub-chronic administration of aqueous extract of the leaves of *Parkia biglobosa* in rats, twenty four male albino rats weighing between 120-200 g were distributed into four groups: A, B, C, and D containing 6 rats each. Aqueous extract of the leaves was administered to rats in groups B, C and D at doses of 100, 200 and 400 mgkg^{-1} respectively for 21 consecutive days, while the rats in group A were administered distilled water (vehicle for extract) and served as control. After the treatment period, results of haematological evaluation revealed a dose dependent significant (p<0.05) decrease in the Packed cell volume of rats at all treatment levels. Likewise, haemoglobin concentration and red blood cell counts were also significantly decreased at the 200 and 400 mgkg^{-1} doses. However, significant increase in total white blood cell count (WBC) was observed at the 400 mgkg^{-1} dose. Changes observed in all analysed plasma biochemical parameters were not significant except for gamma glutamyl tansferase, on which the extract caused a significant (p<0.05) increase at the 400 mgkg^{-1} body weight dose. Moreover, mild portal congestion in the liver, mild renal cortical congestion in the kidney and moderate sub-capsular congestion in the testis were observed at the 400 mgkg^{-1} dose. The study concludes that the extract is relatively safe provided that it is used judiciously at low doses.

Introduction

Haematological investigations among others could be used to evaluate the physiological status of an animal[1,2] and an assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extract on the blood of an animal[3]. Further, haematological assessment can be used to explain physiologic/toxic effects of chemical compounds[4]. Such toxicity testing is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when data are translated from animal studies[5].

Parkia biglobosa, also known as dawadawa (Hausa), African locust beans (English), Igba/Iyere (Yoruba) and Nere (Bambara), has been known to be a native of Africa and is an important multipurpose tree of West African Savannah land. The genus *Parkia* to which *Parkia biglobosa* belongs to is large in the family Fabaceae formerly Leguminosae[6]. Other species of the genus include *Parkia filicoidea*, *Parkia bicolor* and *Parkia clappertoniana*. *Parkia biglobosa* is used for nutritional and medicinal purposes[7,8]. The roots, barks, leaves, stems, flowers, fruits and seeds of *Parkia biglobosa* are all used medicinally to treat a range of ailments including diarrhea, ulcers, pneumonia, burns, coughs and jaundice. The leaves are also used for toothaches as well as for sore eyes[9]. The *in-vitro* anthelmintic effect of the aqueous extracts of the seeds and leaves of the African locust bean (*Parkia biglobosa*) on bovine nematode eggs has also been reported[10].

Various authors have reported the food and nutritive values of *Parkia biglobosa* [11, 12, 13]. *Parkia biglobosa* is one of the plants that form the very basis of the diet of camels, goats and many wild herbivores and play a vital role as a complementary source of proteins, minerals and vitamins for cattle and sheep during the dry seasons[14].

Like many other indigenous medicinal plants, *Parkia biglobosa*, form an important component of the natural wealth of the African continent. The leaves are continually used, indiscriminately, by many local populations for managing various diseases or as food without proper consideration of the safety/toxicity risk on body tissues. This study was prompted in view of the need for documentation of information on the safety/toxicity risk potential of *Parkia biglobosa* leaves extract because there is a dearth of information on the effect of its sub-chronic administration on the blood, an important connective tissue essential for a wide range of physiological processes. The study will investigate the effect of the sub-chronic administration of the extract on haematological parameters using male albino rats as model to provide necessary information on the effect of prolonged oral consumption of the leaves of *Parkia biglobosa* on haematological and plasma biochemical indices.

Materials and methods

Plant materials

Fresh leaves of *Parkia biglobosa* were collected from the Botanical Gardens, University of Ibadan, Ibadan, Nigeria and were identified at the Forestry Research Institute of Nigeria (voucher no: 109574). The leaves were air dried, pulverized and subjected to cold aqueous extraction. 1000 g of dry, pulverized leaves were poured into a muslin bag and soaked in 4.5 litres of distilled water, for 72 h. A gelatinous dark brown coloured extract weighing 116 g (a yield of 11.6 %) was obtained after filtration with WATMAN no.1 filter paper and subsequently

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evaporating the filtrate to dryness, using a water bath. The extract was then stored in a refrigerator for the study.

Animals

A total number of 24 young adult male rats whose average weight ranged between 140 g and 160 g (2–2.5 months old) were used for the study. The animals were obtained from the Animal House, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. They were housed in steel cages, and maintained under standard conditions (12 h light/12 h dark). The rats were allowed to acclimatize in the animal house for a period of 2 weeks before the commencement of the study. Feed from Ladokun Feeds Nigeria Ltd., Ibadan, Nigeria, and water were provided *ad libitum*.

Haematology and plasma biochemical studies

At the end of the 21days of treatment, each rat was bled through the orbital sinus into heparinised bottles for haematological studies. Packed cell volume (PCV) and haemoglobin concentration (Hb) were determined by the microhematocrit and cyanmethaemoglobin methods, respectively, as previously described[15]. Red blood cell (RBC) count was determined by the haematocytometry method as previously described[15]. Total white blood cell (WBC) counts were made in a haemocytometer using the WBC diluting fluid and differential leucocytes counts were made by counting the different types of WBC from giemsa stained slides viewed from each of the 30 fields of oil immersion objective of a microscope. Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined from the values obtained from RBC count, Hb and PCV values[16]. From the plasma, total protein was measured using biuret reaction, while albumin was measured by colorimetric estimation using sigma diagnostic reagent (Sigma Diagnostic, UK.), which contained bromocresol green. Globulin was obtained from the difference of total protein and albumn. AST and ALT were determined using a photoelectric colorimeter as previously described[16], blood urea nitrogen (BUN) and creatinine levels were also determined using photoelectric colorimeter as previously described[17].

Histopathology

All the animals from each of the experimental groups A, B, C and D were sacrificed 24 h after their respective daily doses. The rats were thereafter quickly dissected to remove the liver, kidney and testis, which were then transferred into 10% buffered formalin. The organs were dehydrated in ethanol (70 to 100%), cleared in xylene and embedded in paraffin. Tissue sections were examined under a light microscope after staining with haematoxylin and eosin (H and E)[18].

Statistical analysis

All the values were expressed as mean ± standard deviation. Statistical analysis was carried out by using PRISM software package (version 5.0). Statistical significance was assessed by the student t-test and values of probability less than 5% were considered statistically significant.

Results

Effects of extract on erythrocyte parameters

The result of the effect of the extract of *Parkia biglobosa* on red blood cells and haematometric indices is presented in table 1. The extract caused dose dependent decreases in the values of packed cell volume (PCV), haemoglobin concentration (Hb), and red blood cells (RBC) count. These decreases were significant (p<0.05) at the 200 mgkg^{-1} and 400 mgkg^{-1}doses. However, changes in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Effect of* aqueous extract of *Parkia biglobosa* leaves *on total and differential white blood cell count

The result of the effects of the aqueous extract on total and differential white blood cell (WBC) counts is presented in table 2. The extract caused significant (p<0.05) increases in the WBC counts and platelet values at the 400 mgkg^{-1} dose. In contrast, no significant changes were observed in the values of lymphocytes, neutrophils and monocytes.

Effects of the extract on plasma biochemical parameters of rats

The result of the effect of extract of *Parkia biglobosa* leaves is presented in Table 3. The extract did not cause significant changes in all the analysed plasma biochemical parameters, except for GGT, on which the extract caused a significant (p<0.05) increase at the 400 mgkg^{-1}.

Histopathology

The result of the histopathology of rats in the control group and those administered with the aqueous extract the leaves of *Parkia biglobosa* is presented in Figures 1-6. No visible lesion was observed in the liver (Figure 1), kidney (Figure 3) and testis (figure 5) of the control group. However, the extract induced mild portal congestion, moderate periportal fibroplasia and mild cellular infiltration in the liver (Figure 2) as well as mild renal cortical congestion in the kidney (Figure 4) and reduction of germinal epithelial length in the testis (Figure 6).

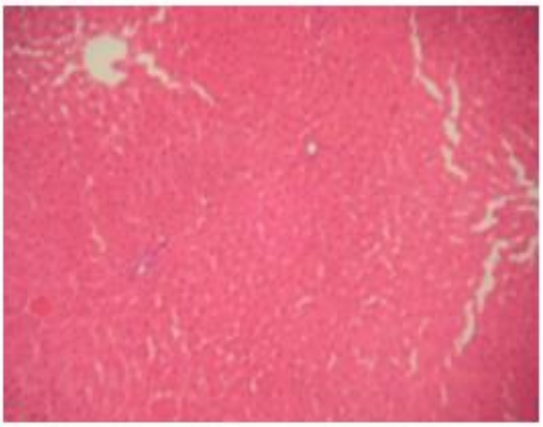

Figure 1. Liver of the control group with no visible lesion. Magnification x100 H&E

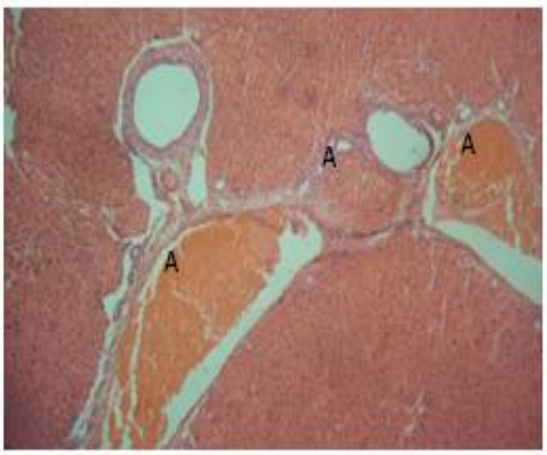

Figure 2. Liver of 400 mgkg-1 group showing portal congestion with moderate fibroplasias at the periportal area with mild cellular infiltration (A). Mag. X 100. H&E

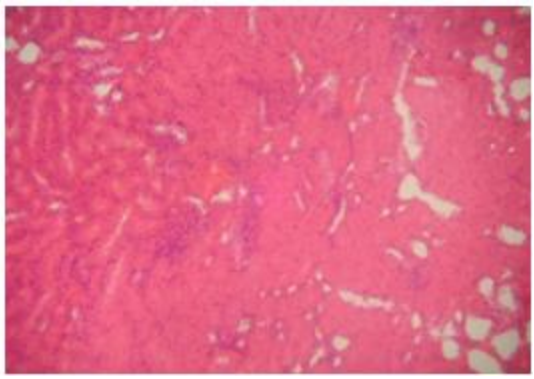

Figure 3. kidney of the control group with no visible lesion. Magnification x100 H&E

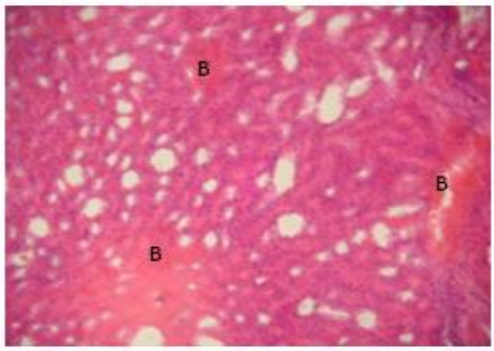

Figure 4. Kidney of 400 mgkg-1 group showing mild renal cortical congestion (B). Mag x100. H&E

Figure 5. Testis of the control group with no visible lesion. Mag x 100 H & E

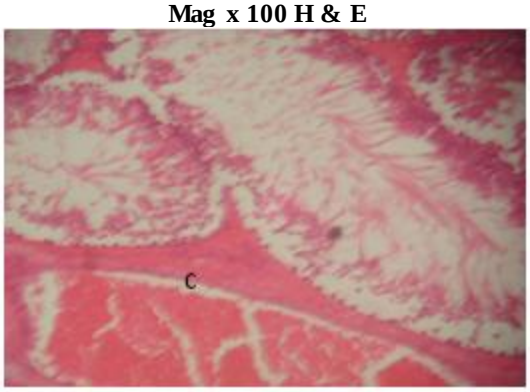

Figure 6. Testis of 400 mgkg-1 group showing moderate subcapsular congestion (C). Mag. X100. H&E

Discussion

In this study, a dose dependent significant decrease was observed in the values of packed cell volume (PCV), haemoglobin concentration (Hb), and red blood cell (RBC) count at the 200 and 400 $mgkg^{-1}$doses. These haematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts/botanicals in living systems[19]. The observed decreases in the values of PCV, Hb and RBC suggest that the plant extract may have adverse effects on the haematology of rats and cause anaemia in animals that feed on the leaves. The reduction in these parameters may be the result of toxic factors present in the leaves acting together to inhibit erythropoiesis. There are two general mechanisms that lead to true anemia – either decreased production or increase destruction of erythrocytes. Evaluation of a peripheral blood sample can provide evidence for the underlying mechanism of anemia[20] and increased destruction is usually accompanied by an increase in reticulocytes which are easily enumerated using appropriate stains[21].

Further, anaemia can result from lack of production of various erythropoietic factors, inactivation of erythropoietic factors (as by antibodies) or a failure of the bone marrow to respond to erythropoietin[22]. Since there was no significant change in the values of MCHC and MCV, the anaemia observed in this study can be said to be normocytic and normochromic. It has been reported that *Parkia biglobosa* contain different secondary metabolites like saponins, tannins, and flavonoids which are known to be responsible for antimicrobial activity[23]. However, saponins have been reported to have pronounced haemolytic properties[24] and this may be one of the contributing factors to the development of the anemia observed in this study.

A significant increase was observed in the total white blood cell (WBC) counts of rats administered with 400 $mgkg^{-1}$ of the extract. Although, in most instances, increase in WBC count reflects a general presence of harmful substances in the body, several reports about WBC counts have suggested that medicinal plants that increase WBC count could be helpful in boosting immune system[25,26] and a reduction in white blood cell, neutrophil and lymphocyte counts is positively correlated with susceptibility to infection, leukaemia, and possible compromise of cellular and humoral mediated immunity[27]. Therefore, the extract of *Parkia biglobosa* leaves may have immunoprotective effect because white blood cells are involved in the cellular and humoral defense mechanisms of the body and are responsible for fighting against invading foreign agents[28].

Significant changes were not observed in the values of ALT and AST, Albumin, Globulin, BUN and Creatinine of the rats, but a significant increase was observed in the level of GGT of rats to which 400 $mgkg^{-1}$ of the extract was administered. GGT is a cell-surface protein contributing to the extracellular catabolism of glutathione. The enzyme is produced in many tissues but most GGT in serum is derived from the liver[29]. Increased concentration of GGT in plasma has been suggested to be associated with increased risk of artherosclerotic cardiovascular disease[30].

The histopathologic lesions observed in this study are mild to moderate in severity and include mainly congestion, fibroplasia and cellular infiltration. It has been reported that toxic irritant substances and or the metabolites can cause degenerative changes in various tissues of the body when transported via the blood circulation to the tissues[31]. In a similar study that evaluated the effects on the aqueous extract of *Parkia biglobosa* stem bark in rats, severe histopathological changes such as dilation of glomerular capillaries in the kidneys and fatty degeneration in the liver were observed[6]. Therefore the results from this study corroborate the facts that different parts of *Parkia biglobosa*, like most other plants with medicinal and nutritional uses, may have deleterious effects when excessive amounts are consumed by animals.

Table 1: Effect of aqueous extract of *Parkia biglobosa* leaves extract on erythrocyte and haematometric indices

Parameters	Control n=6	100mg/kg n=6	200mg/kg n=6	400mg/kg n=6
PCV	47.5±1.52	46.33±2.66	45.67 ± 1.75*	43.00 ± 1.90*
Hb (g/L)	15.72 ± 0.41	14.88 ± 1.33	14.98 ± 0.87*	14.02 ± 0.86*
RBC ($x10^{12}$/L)	7.89 ± 0.49	7.39 ± 0.40	7.49 ± 0.37	7.13 ± 0.32*
MCV (fl)	60.43 ± 31.02	62.69 ± 66.50	60.97 ± 47.30	60.30 ± 59.38
MCH (pg)	11.3±1.0	10.2±2.1	10.3±2.7	10.9±1.3
MCHC (g/dl)	33.09±26.97	32.12±0.5	32.80±4.9	32.60±45.26

Superscripted items indicate significant values (p<0.05) from control

Table 2. Effect of extract of *Parkia biglobosa* leaves extract on total and differential white blood cell counts

Parameters	Control n=6	100mgkg^{-1} n=6	200mgkg^{-1} n=6	400mgkg^{-1} n=6
WBC ($x10^9$/L)	7.18±1.3	7.85±0.1	8.11±0.2	8.87±0.3*
Lymphocytes (%)	67.67 ± 7.66	67.33 ± 8.26	67.33 ± 7.50	63.3 ± 4.37
Neutrophils (%)	24.67 ± 6.77	28 ± 7.85	27.83 ± 8.64	29.5 ± 4.28
Monocytes (%)	3.5 ± 1.38	1.83 ± 1.17	2.67 ± 1.50	3.17 ± 0.98
Platelet(µL)	8.7±2.1	8.5±1.9	8.7±0.1	10.3±1.9*

Superscripted items indicate significant values (p<0.05) from control

Table 3. Effects of *Parkia biglobosa* leaves extract on serum biochemical parameters

Parameters	Control n=6	100 mgkg^{-1} n=6	200 mgkg^{-1} n=6	400 mgkg^{-1} n=6
Total Protein (g/dl)	8.23±0.44	8.03 ± 0.27	7.92 ± 0.18	7.97±0.60
Albumin(g/dl)	4.13 ± 0.55	4.08 ± 0.71	3.9 ± 0.54	4.37 ±0 .36
Globulin (g/dl)	4.1 ± 0.36	3.95 ± 0.51	4.02 ± 0.38	3.6 ± 0.36
A : G ratio	0.97 ± 0.23	1.02 ± 0.29	0.95 ± 0.21	1.18 ± 0.13
AST(U/L)	21.7±3.2	22.3±4.4	26.4±1.6	28.3±7.3
ALT(U/L)	27.83 ± 2.23	29.5 ± 1.64	29.33 ± 3.50	30.0 ± 4.90
Urea (mg/dl)	13.67 ± 1.75	14.33 ± 1.50	14.67 ± 1.86	14.17 ± 1.17
Creatinine (mg/dl)	1.13 ± 0.31	1.35 ± 0.23	1.12 ± 0.12	1.32 ± 0.31
GGT (U/L)	1.43 ± 0.14	1.55 ± 0.24	1.62 ± 0.32	1.65 ± 0.17*

Superscripted items indicate significant values (p<0.05) from control

Conclusion

The decrease in haematological parameters; packed cell volume, red blood cell count and haemoglobin concentration, observed in this study, suggests the ability of *Parkia biglobosa* leaves to induce anaemia when consumed in large quantities, by animals that graze on it. The mild-to-moderate congestion observed in the testis, liver and kidney may be responsible for the observed anaemia. Ordinarily, liver cell damage is characterized by a rise in plasma enzymes (AST, ALT) and since there was no significant increase in these plasma enzymes in this study, it can be concluded that the extract of the leaves of *P. biglobosa* did not induce serious tissue injury or cell death. Therefore the leaves appear to be safe for consumption and may be exploited for their possible role in boosting the immune system.

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