

Full Length Research Paper

Evaluation of haematological and plasma biochemical effects of aqueous extracts of *Parkia biglobosa* seeds in rats

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The effects of sub-acute exposure to aqueous extract of *Parkia biglobosa* seeds on haematology and plasma biochemical parameters were studied. Twenty eight male Wistar albino rats were orally ingested with 0, 200, 400 and 800 mgkg⁻¹ dose of the extract for 21 days and were sacrificed. Haematological parameters were assessed: red blood cell counts, haematocrit and haemoglobin concentration significantly ($p < 0.05$) increased at the 800 mgkg⁻¹ dose. We also observed significant increases in white blood cells, lymphocytes and neutrophils at this dose. Evaluation of effect of seed extract on plasma biochemical values revealed significant increase in total protein, albumin and aspartate amino transferase. However, the extract caused significant decrease ($p < 0.05$) in the levels of blood urea nitrogen and creatinine. Changes observed in plasma levels of globulin, alanine amino-transferase (ALT) and gamma-glutamyl transferase (GGT) were not significantly different from the control at all treatment doses.

Key words: Haematology, biochemistry, aqueous extract, *Parkia biglobosa* seed.

INTRODUCTION

The African locust bean tree, *Parkia biglobosa*, is a perennial tree legume, belonging to the sub-family mimosoidae and family leguminosae. The plant has been used as a source of food, medicinal agents, timber and is of high commercial value. In West Africa, the seeds of *P. biglobosa* provide a rich source of vegetable protein for human food and livestock feed. They are usually fermented to a tasty condiment used as a flavour intensifier for soups and stews (Dike and Odunfa, 2003). The husks and pods are good feed for livestock (Obizoba, 1998). Fetuga et al. (1974) reported the plant to be a good source of tannins, saponins, gums, fuel and wood. The seeds of various species of the genus *Parkia* have been investigated for their protein and amino acid contents. However, the use of African locust bean seeds as protein source is limited by the presence of inherent

anti-nutritional factors that cause poor protein digestibility in man and animals and are capable of precipitating other deleterious effects (Osagie, 1998; Soetan and Oyewole, 2009).

Various workers have shown that haematological investigations could be used to evaluate the physiological status of an animal (Kakade et al., 1974; de Gruchy, 1976) and an assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood. Also, Cells naturally contain enzymes for their functions and damages to cellular membrane normally leads to the escape of the enzymes into the blood where their presence or activities can easily be measured as an index of cellular integrity (Coles, 1989; Coppo et al., 2002). Further, certain plasma biochemistry could be used to identify tissue damage (Patti and Kulkarni, 1993). For instance, aspartate aminotransferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP) were normally found within the cells of the liver, heart, kidneys, muscles and organs. Their increase concen-

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Table 1. Effects of aqueous extract of *P. biglobosa* seeds on erythrocyte values.

Parameter	Control (n=7)	200 mgkg ⁻¹ (n = 7)	400 mgkg ⁻¹ (n = 7)	800 mgkg ⁻¹ (n = 7)
RBC (x10 ¹² /L)	7.21±0.05	7.36±0.06	7.57±0.13	8.19±0.33*
PCV (%)	45.50±1.19	42.75±1.03	46.50±0.88	54.50±2.06*
Hb (g/L)	13.70±0.04	13.10±0.43	14.68±0.39*	17.65±0.61*
MCH (pg)	19.69±0.35	20.53±0.57	19.84±0.57	19.39±0.33
MCV (fl)	60.78±0.78	64.18±1.96	62.03±1.67	60.62±0.55
MCHC (%)	32.39±0.36	32.39±0.36	31.98±0.13	31.98±0.31

*p<0.05. RBC, Red blood cells; PCV, packed cell volumes; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration.

tration in the plasma indicate tissue injury or organ dysfunction (Wells et al., 1986).

Although *P. biglobosa* is used extensively in West Africa for food and medicinal purposes, the effect of the seed or its extract on haematology and plasma biochemistry are not well documented. Therefore, the aim of this study was to evaluate the effects of aqueous extract of *P. biglobosa* seeds on haematological and plasma biochemical parameters in rats in order to confirm its safety or otherwise on these parameters which are useful in evaluating the physiological state of the animal.

MATERIALS AND METHODS

Preparation of plant extract

The seeds of *P. biglobosa* obtained from the Botanical Garden, University of Ibadan, were air dried, pulverized and subjected to cold aqueous extraction. The extraction was carried out as described by the method of Njar et al. (1993) and Raji (1995). The pulverized seeds weighing 1000 g poured into a muslin bag were soaked in 4.5 L of distilled water, for 72 h. The extract was obtained after filtration of the supernatant with Whatman No.1 filter paper. Subsequently, complete removal of the solvent from the supernatant, was achieved with a water bath placed in a fume cupboard because of the pungent smell emanating from it. A sticky, dark brown coloured extract weighing 154.61 g (a yield of 15.46%) was obtained after boiling on the water bath for about 12 h. The resulting extract was reconstituted in distilled water to give final concentrations of 100, 200 and 400 mg/ml by dissolving 10, 20 and 40 g of the extract in 100 ml of distilled water, respectively.

Experimental animals

Twenty eight healthy male white Wistar strain albino rats (200 to 240 g) of both sexes, obtained from the Animal House, Faculty of Veterinary Medicine, University of Ibadan, were used for the study. The rats were fed with rat cubes (Ladokun Feeds Limited, Ibadan, Nigeria) and water *ad libitum*, acclimated to laboratory conditions for one week, and randomly divided into four groups (control, groups 1, 2 and 3). A varied dosage of the seed extract at 200, 400 and 800 mg/kg, dissolved in distilled water was administered orally, by means of bulbed steel needle to rats in groups 1, 2 and 3, respectively. The rats in the control group were administered distilled water, orally. Rat was chosen as the experimental animal for the study because toxic substances readily produce demonstrable effects in rats (Farris and Griffith, 1949).

Haematology and plasma biochemical studies

At the end of the 21 days of treatment, each rat was bled through the orbital sinus into heparinised bottles for haematological studies. Packed cell volume (PCV) and haemoglobin concentration were determined by the microhematocrit and cyanmethaemoglobin methods, respectively as described by Jain (1986). Erythrocyte count was determined by the haematocytometry method as described by Jain (1986). Total white blood cell (WBC) count was 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 (Coles, 1989). Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined from the values obtained from red blood cells (RBC) count, haemoglobin concentration and PCV values (Duncan et al., 1994).

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 (BCG). Globulin was obtained from difference of total protein and albumin. Aspartate and alanine aminotransferases were determined using a photoelectric colorimeter as described by Duncan et al. (1994). Blood urea nitrogen and creatinine levels were also determined using photoelectric colorimeter as described by Coles (1989). Gamma-glutamyl transferase (GGT) was measured using the kinetic photometric method (Szasz, 1969).

Statistical analysis

All values were expressed as mean ± standard error of mean (S.E.M). The means and standard error of means as well as one-way analysis of variance (ANOVA) for statistical significance was determined using GraphPad Prism, version 5.0, San Diego, CA. P values at 5% were regarded as significant.

RESULTS

The results of the effects of the aqueous extract of *P. biglobosa* seeds on haematological parameters are presented in Tables 1 and 2. Changes induced by the extract on erythrocyte values of treatment groups were not significantly different (p>0.05) from the control, except for increases in red blood cell count and packed cell volume, which are significant (p<0.05) at 800 mgkg⁻¹ dose and haemoglobin, which is significant (p<0.05) at

Table 2. Effects of aqueous extract of *P. biglobosa* seeds on leukocyte values.

Parameter	Control	200 mgkg ⁻¹	400 mgkg ⁻¹	800 mgkg ⁻¹
WBC	4.76±0.47	5.53±0.57	5.86±0.33	6.77±0.46*
Lymphocyte (x10 ⁹ /L)	3.18±0.06	3.35±0.60	3.84±0.29*	4.17±0.39*
Neutrophil (x10 ⁹ /L)	1.68±0.08	1.57±0.23	1.79±0.15	2.64±0.24*
Eosinophil (x10 ⁹ /L)	0.10±0.02	0.12±0.04	0.07±0.04	0.08±0.02
Monocyte (x10 ⁹ /L)	0.10±0.02	0.13±0.04	0.08±0.04	0.08±0.02

*p<0.05.

Table 3. Effect of aqueous extract of *P. biglobosa* seeds on plasma chemistry.

Parameter	Control (n=7)	200 mgkg ⁻¹ (n=7)	400 mgkg ⁻¹ (n=7)	800 mgkg ⁻¹ (n=7)
Total Protein	8.33±0.06	8.13±0.11	8.70±0.07*	7.75±0.03*
Albumin	4.75±0.02	4.15±0.25	4.07±0.14*	3.72±0.08*
Globulin	3.90±0.04	3.77±0.04	3.85±0.06	4.05±0.01
Albumin:Globulin	1.28±0.03	1.00±0.09	1.15±0.03*	0.90±0.04*
AST	34.25±0.75	42.50±1.04	36.25±0.85	41.50±0.50*
ALT	26.75±0.85	29.25±0.75	25.75±0.63	29.50±0.96
BUN	12.00±0.40	11.25±0.47	11.25±0.43	10.50±0.29*
Creatinine	1.25±0.10	0.90±0.09	0.50±0.09**	0.25±0.03*
GGT	1.08±0.03	1.05±0.02	1.03±0.03	1.02±0.03

*p<0.05. AST, Aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen.

400 and 800 mgkg⁻¹ dose. Also, the extract induced significant (p<0.05) increase in total white blood cell count and neutrophil at 800 mgkg⁻¹, and 400 and 800 mgkg⁻¹ dose caused significant (p<0.05) increase in lymphocyte values. Changes produced in eosinophil and monocyte values of the treatment groups are not significantly different from the control.

Effects of extract on plasma biochemistry of rats

The result of the effects of the aqueous extract of the seeds of *P. biglobosa* on plasma biochemical values is presented in Table 3. The extract caused significant (P<0.05) increase in total protein and albumin at the 400 and 800 mgkg⁻¹ dose. Aspartate amino transferase (AST) also significantly increased at the 800 mgkg⁻¹ dose. The extract, at the 800 mgkg⁻¹ dose caused significant decrease in the level of blood urea nitrogen (BUN), and at 400 and 800 mgkg⁻¹ dose caused significant decrease in the level of creatinine. However, changes observed in plasma levels of globulin, alanine amino transferase (ALT) and GGT were not significantly (P>0.05) different from the control at all treatment doses.

DISCUSSION

The main function of red blood cells is the transportation

of oxygen into tissues of the body. Any pathological condition that affects the red blood cell alters its function and this may be detrimental to the body (Agbor et al., 2005). In this study, there were no obvious haemolytic changes on red blood cells, haemoglobin, packed cell volume, mean corpuscular volume and mean corpuscular haemoglobin concentration of rats treated with the *P. biglobosa* seed extract. Rather, the extract significantly increased some of these parameters. The absence of significant decrease on the erythrocyte indices suggests that the extract does not possess toxic substances that can cause anaemia and the induction of significant increases in erythrocyte count, packed cell volume and haemoglobin strongly suggests that *P. biglobosa* seed extracts have haematinic or haematopoeitic properties. Substances that demonstrate significant effect on these parameters would have effects on bone marrow, kidney and also haemoglobin metabolism (Young and Maciejewski, 1997). Plant extracts with oxygen scavenging activity may compete with haemoglobin in erythrocytes for oxygen resulting in hypoxia which then stimulates haemoglobin synthesis and erythrocytes production (Song et al., 1987). It is also possible that the end product of metabolism of the seed extract, *in vivo*, stimulates the kidney directly to cause formation and secretion of erythropoietin which converts erythropoietinogen to erythropoietin, a potent stimulator of the bone marrow.

Estimated total number of white blood cells in the blood

varies according to age, sex and more importantly, the physiological conditions of the body. 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 (Bochner et al., 1991). In this study, the extract of the seeds of *P. biglobosa* caused significant increase in total white blood cell count, neutrophil and lymphocyte values at 800 mgkg⁻¹ dose. Therefore, it can be inferred that the extract has immunoprotective effect because white blood cells are involved in the cellular and humoral defense mechanisms of the body and are responsible for fighting against foreign agents. The significant rise in lymphocytes also points to the potential usefulness of this plant as an immune system stimulating agent (Kinney et al., 1999).

Estimation of plasma albumin is a good criterion for assessing the function and secretory capacity of the liver (Yakubu et al., 2005) because albumin is the protein with the highest concentration in plasma and it transports many small molecules in the blood (for example, bilirubin, calcium, progesterone and drugs). It also prevents the fluid in the blood from leaking out into the tissues (Duncan et al., 1994). The significant decrease of extract of *P. biglobosa* on the levels of albumin at 200 and 800 mgkg⁻¹ and total protein at 400 and 800 mgkg⁻¹ may suggest that the extract has ability to inhibit *in vivo* protein biosynthesis. However, the extract caused significant increase in total protein at 400 mgkg⁻¹. Therefore, the dose is an important factor. Alanine and aspartate transferases (ALT and AST) are well known transaminases used as biomarkers to predict possible toxicity in the blood of sick animals (Akdogan et al., 2003). These enzymes are only released into the blood in significant amounts from the cytosol and subcellular organelles when hepatic injuries occur (Lu, 1996). It is known that an increase in concentration of ALT, AST and alkaline phosphatase in the serum directly reflects a major permeability, congestion or cell rupture (Pieme et al., 2006; Tedong et al., 2008). However, ALT is more hepatospecific than AST because it is more sensitive to hepatic damage (Herfindal and Gourley, 2000). Elevations in ALT levels are rarely observed except in chronic liver disease (Gad, 2001). Therefore, the lack of significant effect of the extract on ALT implies that oral administration of the extract did not cause serious impairment to liver function. The non-significant effect on GGT further attests to this fact. It is however possible that the extract could have deleterious effects on other organs at high doses, or when used for longer duration.

Conclusion

Aqueous extract of the seeds of *P. biglobosa* does not contain deleterious chemical substances capable of altering haematological values or cause blood dys-

functions in rats. The seeds may be helpful as immunomodulating agent because of the effects observed on white blood cells. These findings justify the use of the seeds of *P. biglobosa* for nutritional and medicinal purposes.

REFERENCES

- Agbor GA, Oben JE, Ngogang JE (2005). Haematinic activity of *Hibiscus cannabinus*. Afr. J. Biotechnol. 4(8):833-837.
- Akdogan M, Kilinc I, Oncu M, Karaoz E, Delibas N (2003). Investigation of biochemical and histopathological effects of *Mentha piperita* L. and *Mentha spicata* L. on kidney tissue in rats. Hum. Exp. Toxicol. 22:213-219.
- Bochner BS, Lusinskas F W, Gimbrone MA, Newman W, Sterbinsky S A, Derse-Anthony CP, Klunk D, Schleimer RP (1991). Adhesion of human basophils, eosinophils and neutrophils to Interleukin 1-activated human vascular endothelial cells: Contribution of endothelial cell adhesion molecules. J. Exp. Med. 173:1553-1556.
- Coles EH (1989). Veterinary Clinical Pathology. 4th Edn. W.B. Saunders Co. USA pp. 130-148.
- Coppo JA, Mussart NB, Fioranelli SA (2002). Physiological variations of enzymatic activities in blood of Bullfrog, *Rana catesbeiana* (Shaw, 1802). Rev. Vet. 12(13):22-27.
- de Gruchy GC (1976). Clinical Haematology in Medical Practice. Blackwell Scientific Publication, Oxford, London, pp: 33-57.
- Dike EN, Odunfa SA (2003). Microbiological and biochemical evaluation of a fermented soyabean product-soyadawadawa. J. Food Sci. Technol. 40:606-610.
- Duncan JR, Prasse KW, Mahaffey EA (1994). Veterinary Laboratory, Medicine (Clinical Pathology). Iowa State University Press: Ames. pp. 94-96.
- Farris EJ, Griffith JQ (1949). The rat in laboratory investigation. Hofner publishing company, New York.
- Fetuga BL, Babatunde GM, Oyenuga VA (1974). Protein quality of some unusual protein foodstuffs. Studies on the African locust-bean seed. Br. J. Nutr. 32:27-36.
- Gad SC (2001). Statistics for Toxicologists. Principles and methods of toxicology, Taylor and Francis, Philadelphia, pp. 351-353.
- Herfindal DR, Gourley ET (2000). Textbook of therapeutic drug and disease management. 7th ed. Philadelphia: Lippincott Williams and Wilkins p. 83.
- Jain NC (1986). Schalm's veterinary haematology 4th Ed. Lea and Febiger Philadelphia, USA.
- Kakade ML, Rackis JJ, Mcghee JE, Puski G (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem. 51:376-382.
- Kinney TR, Ronald W, Helms EE, O'Branski KO (1999). Safety of Hydroxy Urea in children with sickle cell anaemia: Results of the HUG-KIDS study, a phase I/II trial. Blood 5:1550-1554.
- Lu FC (1996). Basic toxicology fundamentals, target organs and risk assessment. 3rd ed. Philadelphia, USA: Taylor and Francis; pp. 184-251.
- Njar VCO, Alao TO, Okogun JI, Holland HL (1993). 2 methoxy cathin-6-one: A new alkaloid from the stem wood of *Quassia amara*. Planta Med. 59:259-261.
- Obizoba IC (1998). Fermentation of African locust bean. Text on nutrition quality of plant fruits. (Edn) Osagie, Eka (2000). Post Harvest Research Unit. Dept. Biochem. UNIBEN. Nigeria pp. 160-198.
- Osagie AU (1998). Anti-nutritional factors. In: Osagie AU and Eka OU (Eds.), Nutr. Qual. Plant Foods pp. 221-244.
- Patti M, Kulkarni RS (1993). Ovarian and hepatic biochemical response to Sumaach (a crude form of HCG) in fish, *Notopterus notopterus* pallas, under pesticide treatment. Geobios 20:255-259.
- Pieme CA, Pendlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX, Ngogang J. (2006). Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L) Roxb Ceasalpinaceae. Afr. J. Biotechnol. 5(3):283-289.

- Raji Y (1995). Hormone and Drug stimulation of testosterone secretion in rat leydig cell. PhD Thesis. Department of Physiology, University of Ibadan XXVIII +227.
- Soetan KO, Oyewole OE (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *Afr. J. Food Sci.* 3(9):223
- Song KY, Schwartz F, Maeda N, Smithies O, Kucherlapati RS (1987). Accurate modification of a chromosomal plasmid by homologous recombination in human cells. *Proc. Natl. Acad. Sci.* 84:6820-6824.
- Szaszi G (1969). A kinetic photometric method for serum gamma-glutamyl transpeptidase. *J. Clin. Chem.* 15:124-126.
- Tedong L, Dzeufiet PD, Dimo T, Asongalem EA, Sokeng SN, Flejoy J, Callard P, Kamtchouing P (2008). Acute and subchronic toxicity of *Anacardium occidentale* Linn Anacardiaceae leaves hexane extract in mice. *Afr. J. Trad. Comp. Alt. Med.* 4(2):140-147.
- Wells RM, McIntyre RH, Morgan AK, Davie PS (1986). Physiological stress responses in big gamefish after exposure: Observations on plasma chemistry and blood factors. *Comp. Biochem. Physiol.* 84:565-571.
- Yakubu MT, Akanji MA, Oladiji AT (2005). Aphrodisiac potentials of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Heirn) stem in male albino rats. *Asian J. Androl.* 7(4):399-404.
- Young NS, Maciejewski J (1997). The pathophysiology of acquired aplastic anemia. *N. Engl. J. Med.* 336:1365-1371.