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Some observations in Wistar rats administered ethanolic extracts of the stem barks of Anogeissus leiocarpus

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ABSTRACT

Anogeissus leiocarpus is a plant/ herb used for the traditional treatment of various ailments, including diabetes mellitus by many rural communities in Africa. The active principles of the herb have been defined, but there is no documented report about the effects of extracts of the stem barks of this herb on body fluids. In this study, we report for the first time, the clinical presentation, haematological changes, variation in biochemical profiles and the resistance of the red cell mass of Wister rats administered ethanolic extracts of the stem barks of the herb to osmotic stress (erythrocyte osmotic fragility, EOF). The clinical signs observed following administration of the herb include anorexia, dullness, rough hair coats and huddling. There was no statistically significant difference ($p>0.05$) in mean red blood cell counts of all the experimental groups, compared to the control. Mean packed cell volume (PCV) was statistically significantly elevated ($p<0.05$) in some experimental groups, while mean corpuscular volume (MCV) was elevated in others, compared to the control. Total leucocytes (WBC) did not vary significantly ($p>0.05$) in all the groups, compared to the control. Differential leucocytes were essentially similar ($p>0.05$) in all the experimental groups investigated. Platelet counts statistically significantly decreased ($p < 0.05$) in all but the experimental group administered 500 mg/kg of extract, compared to the control. Mean EOF significantly decreased ($p<0.05$) in some groups, while mean serum glucose levels decreased significantly ($p<0.01$) in all the experimental groups, except the control. The implications of these findings in relation to the use of the herb are discussed. It is concluded that for the herb to be certified safe for clinical use to ameliorate diabetes, it must be administered in combination with an expicient that modulates thrombocytopaenia.

Keywords: Wistar rats; methanolic extracts; stem barks; *Anogeissus leiocarpus*

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1. INTRODUCTION

The reemergence of infectious diseases and the continuous development of multidrug resistance among a variety of disease-causing microorganisms in clinical settings pose a serious threat to public health worldwide. Drug resistance against infectious agents has being on the increase in the last decade and infectious agents have acquired new genes that are resistant to known therapeutic agents (Coppoen et al., 2012). The foregoing makes research on the sourcing of alternative drugs a hot topic of intense research worldwide.

Developing countries, especially those in Africa, are faced with the problems of poverty, malnutrition and disease. Since orthodox medicines are expensive and in most cases not affordable by some people who live in rural areas, herbs have been used to treat ailments. The believe by villagers that herbs are more natural, relatively cheaper and safer compared to manufactured drugs are the reasons for their acceptability and use on a regular basis (Gammaniel, 2000). Although A. leiocarpus is believed to ameliorate high blood sugar levels and some infectious diseases, there is no documented report on the medicinal properties of the stem barks of this herb in body fluids. In this report, we present for the first time, the effects of A. leiocarpus on the haematological and biochemical parameters of Wistar rats administered partition fractions of the stem barks of the herb.

2. Materials and methods

2.1 Plant collection, extraction and preparation

The stem barks of the plant used in this study were collected from the senior staff quarters, Ahmadu Bello University, Zaria, Nigeria in December and identified at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. They were air dried and subsequently ground to powder. The powder materials were weighed and then mixed with ethanol in a ratio of 1:5 w/v in an Erlenmeyer flask for 24 h. The mixture was filtered and the filtrate concentrated in vacuo at 50 ^{o}C in a rotatory evaporator coupled to a thermocirculator. The resultant extract was weighed and reconstituted with distilled water to obtain a 1% stock solution.

2.2 Experimental animals

Twenty five (25) adult Wistar rats of both sexes weighing between 136 and 234 g were obtained from the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. They were housed in rat cages and fed growers marsh mixed with maize offal and groundnut cake in a ratio of -4:2:1. Feed and water were provided ad libitum. The animals were allowed to acclimatize for one week before the commencement of the experiment.

2.3 Administration of ethanolic extracts to experimental animals

Wistar rats of both sexes (n=25) were allotted into five experimental groups (n=5). Group 1 was administered 2 mL/kg physiological saline and served as control, while groups 2, 3, 4 and 5 were administered 1000, 500, 350 and 175 mg/kg ethanolic extracts respectively. All the extracts were administered orally on a daily basis for 14 days. Feed and fresh portable water were provided ad libitum throughout the experimental period. Clinical signs accompanying extract administration were observed and recorded. The surviving animals were bled and blood from all the animals in each experimental group was pooled. One (1) mL of the blood was transferred into vacutainers containing EDTA for haematological analysis, while another 1 mL was transferred into vacutainers containing EDTA for erythrocyte osmotic fragility test. About 3 mL of the blood was transferred into anticoagulant-free test tubes and allowed to clot. The clotted blood was centrifuged at 800 g for 10 min and the serum collected for biochemical analysis.

2.4 Determination of variation in weights due to extract

All experimental rats in the various groups were weighed daily during the period of acclimatization and throughout the experimental period.

2.5 Haematological analysis

Erythrocyte, total and differential leucocyte counts were determined using the methods described by Weiss and Wardrop (2010).

2.6 Determination of Erythrocyte Osmotic Fragility

Erythrocyte osmotic fragility (EOF) was determined using the method described by Faulkner and King (1970).

2.7 Determination of serum biochemical changes

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), electrolytes, blood urea nitrogen (BUN), glucose and proteins were determined using the methods described by Davidson et al. (1998).

Statistical analysis. Data obtained from the study were computed as mean ± standard deviation (SD), and analyzed using analysis of variance (ANOVA, Duncan multiple range test). Values of $p<0.05$ were considered statistically significant (Chatfield, 1983).

3. Results

3.1 Clinical signs observed following administration of ethanolic extracts

Varying degrees of anorexia, dullness, rough hair coats and huddling were observed in all the groups investigated, except the control. There was no mortality recorded in any of the experimental groups, including the control.

3.2 Effect of extract on body weight

The weight changes observed in animals administered the ethanolic extracts of A. leiocarpus are shown in Fig. 1. There was a statistically significant increase ($p<0.05$) in mean body weight of all the experimental groups investigated, compared to the control.

3.3 Effects of extract on haematological parameters

The effects of ethanolic extracts of A. leiocarpus on haematological parameters are presented in Table 1. There was no statistically significant difference ($p>0.05$) in mean red blood cell counts of all the experimental groups compared to the control. Mean PCV was statistically significantly elevated ($p<0.05$) in the experimental groups administered 1000 and 175 mg/mg extracts respectively, while mean MCV was elevated in the experimental group administered 175 mg/kg extract, compared to the control. Total leucocytes (WBC) did not vary significantly ($p>0.05$) in all the groups, compared to the control. Differential leucocytes were essentially similar ($p>0.05$) in all the experimental groups investigated. Platelet counts statistically significantly decreased ($p < 0.05$) in all but the experimental group administered 500 mg/kg of extract.

3.4 Changes in erythrocyte osmotic fragility (EOF)

The effects of ethanolic extract of the stem barks of A. leiocarpus on EOF in vitro are presented in Fig. 2. Minimal haemolysis was obtained at 0.9% sodium chloride (NaCl) concentration in all the experimental groups, including the control. Maximum haemolysis was obtained at 0.1% NaCl concentration. There was a statistically significant decrease ($P<0.05$) in % haemolysis in the groups administered 1000, 350 and 175 mg/kg of extract respectively. The experimental group administered 1000 mg/kg extract was the most affected.

3.5 Changes in serum biochemical profile

Mean serum biochemical profile of the experimental animals is presented in Table 2. Mean serum transferases (ALT/ AST) and alkaline phosphatase (ALP) activities fluctuated ($p>0.01$), but were essentially similar in all the experimental groups, compared to the control. There was a statistically significant decrease ($p<0.01$) in serum glucose levels in all the experimental groups investigated, compared to the control.

4. Discussion

Globally, millions of people in the developing world rely on medicinal plants for primary health care, income generation and livelihood improvement (WHO, 2002). Between 50,000 and 70,000 plant species are known to be used in traditional and modern medicinal systems throughout the world (Schippmann et al., 2006). According to World Health Organization (WHO), the international market of herbal products is estimated to be US $ 62 billion, and is poised to grow to US $ 5 trillion by the year 2050 (WHO, 2002). The present study suggest that A. leiocarpus is safe for use, with minimal side effects. Although inapettance was observed throughout the period of treatment of the rats, it was observed that repeated dosing of rats with the herb produced higher weight gains in the administered groups, compared to the control. The high weight gains observed is an indication that the plant may contain some phytochemical principles that increase basal metabolic rate (BMR) in rats and by extrapolation in human beings too.

Extracts of the herb showed no significant effect on the mean red blood cell (erythrocyte) counts and haemoglobin concentration. However, mean packed cell volume increased ($p<0.05$), following administration of the herb. There were slight variations in the RBC values, which increased, though not significantly. This indicates that the stem bark extracts, rather than destroy the red cell mass, have a stimulatory effect on erythropoeisis. Erythropoeisis develops through several mechanisms, when certain chemical agents are used (Searcy, 2001). No dosage regimen of the extract affected the disposition of haemoglobin concentration negatively, implying that the herb does not cause anaemia. All these taken together may imply that the herb could have a stimulatory effect on erythropoeisis. The main hormone responsible for erythropoeisis is erythropoietin (EPO), produced by the kidney, which regulates the production of RBC (Sanchez-Elsner et al., 2004). In cases of hypoxia, EPO acts on the erythroid precursors of the bone marrow and causes rapid maturation of the immature cells to mature erythrocytes. This effect rapidly leads to a rise in RBC counts and the oxygen carrying capacity of the blood is improved (Sanchez-Elsner et al., 2004). Some active principles reported in this herb like flavonoids are active oxygen scavengers (Heijnen et al., 2001; Chun et al., 2003). It is therefore possible that they may compete favourably with haemoglobin for the oxygen, resulting in hypoxia, which in turn stimulates the release of EPO.

Although the ratio of oxygen delivery to oxygen requirements is the primary physiologic regulator of EPO production, there are other factors like androgenic and anabolic steroids (Basaria et al., 2001). The steroids

reported in this plant could also be responsible for the increased red blood cell production (increased PCV) observed following administration of particular dosage regimens. It has been hypothesized that steroid metabolites may act as depressor agents for the structural gene coding for 5-aminolevulinic acid synthetase, permitting it to transcribe more actively, leading to enhanced formation of this enzyme and thus to increased production of haem. The mechanisms for this regulatory effect of steroid metabolites on haemoglobin synthesis in the erythropoietic cells of the mouse could be a direct effect on young red cell precursors, an indirect effect mediated through another factor such as EPO, or a combination of the above (Gorshein and Gardner, 1970).

Repeated dosing of the rats with ethanolic extract of A. leiocarpus, showed a slight decrease in the mean total white blood cell counts that was not statistically significant ($P>0.05$), but should not be ignored. This may suggest leucopaenic activity. Excessive ingestion of a wide variety of some other plants or their products has been reported to cause hypoproliferative or non-regenerative anaemia, which is a stem cell disorder, characterized by reduced bone marrow production of all blood components, in the absence of a primary disease process infiltrating the bone marrow or suppressing haematopoiesis (Olson et al., 1984). It can be speculated, based on our report, therefore, that continuous administration of these extracts or ingestion of the leaves of this herb may produce these effects in animals. Thus, this plant needs some expicients to modulate its biological activity and usage in clinical situations, especially in the management of diseases that are known to cause immunosupression or in individuals that have myeloproliferative disorders.

Mean platelet counts were significantly decreased ($p < 0.05$) with the use of extracts of A. leiocarpus in all the experimental groups, compared to the control. Reduced platelet counts is one of the problems associated with the use of some herbal remedies (Cheesbrough, 2000). Zhang et al. (1999) studied the effect of six steroidal saponins from Anemarrhena asphodeloide and reported that they had an inhibitory effect on platelet aggregation and at least one of them had a strong side effect of haemolysis. Our finding on thrombocytopaenia associated with ethanolic extracts of this herb is in agreement with Zang et al. (1999). This shows that the plant may have an adverse effect on haemostasis, thereby prolonging the bleeding time to interfere with blood clotting. Herbs that decrease platelet aggregation also inhibit platelet-activating factor, and those that contain salicylates may increase the risk of bleeding (Chavez, 2005). This herb needs to be refined before use, to abolish its thrombocytopaenic tendency, especially in animals and human beings with bleeding disorders, septicaemic infections, vitamin B12 deficiency, aplastic anaemias and leukaemias. However, it may be beneficial in the treatment of post myocardial infarcts as an antithrombotic agent.

Mean serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities increased slightly, though not statistically significant ($p>0.01$). This could indicate a small response of the liver to some of the phtyochemical principles of the plant, which is not sufficient to cause severe liver damage. Slight increases are also seen in cases of fatty liver disease. Mean alkaline phosphatase (ALP) activity of the ethanolic extract showed slight variation that was not statistically significant ($p>0.01$). Malnutrition has been listed as one of the causes of decreased serum levels of ALP (Ophardt, 2003). In this study, inapettance observed throughout the course of the study could result to malnutrition in the long run. It is therefore safe to assume that the fluctuations in mean serum levels of ALP may be partly attributed to malnutrition occasioned by inapettance. The inappetance observed in the experiment may be due to the presence of tannins in the plant extracts. Tannins are anti-nutritive factors in plant extracts. They are strong astringents and have the ability to precipitate proteins, thereby reducing their availability to animals (Brooks, 2000). They are also reported to cause malaise in animals, thus reducing feed intake (Alldredge, 1993).

Mean serum levels of glucose decreased remarkably ($p<0.01$) in all the experimental groups, compared to control. It thus indicates that this plant may have a hypoglycaemic effect. It has been reported that herbal remedies may reverse the catabolic features of insulin deficiency (e.g. Gymnema Sylvestre), decrease the release of glucagon or increase that of insulin (e.g. Panax ginseng), stimulate glycolysis in peripheral tissues (e.g. Panax ginseng), increase glucose removal from blood or reduce glucose absorption from the gastrointestinal tract (Musabayane et al., 2006). The hypoglycaemic effects of A. leiocarpus observed in the present study could possibly be due to increased peripheral glucose utilization.

Mean serum blood urea nitrogen (BUN) and total proteins were not markedly affected. The slight variations observed may be as a result of the steroid content of the plant. Urea is excreted by the kidneys and to some extent enteric microorganisms in the gastrointestinal tract of ruminants (Cullen, 2007). The overall result indicates that both extracts do not cause renal toxicity.

It is concluded that A. leiocarpus has the potential for ameliorating increased sugar levels clinically and this

finding requires further investigation. The future use of the herb in combination with expicients that modulate thrombocytopenia will abolish possible bleeding disorders that could occur as side effects if used alone.

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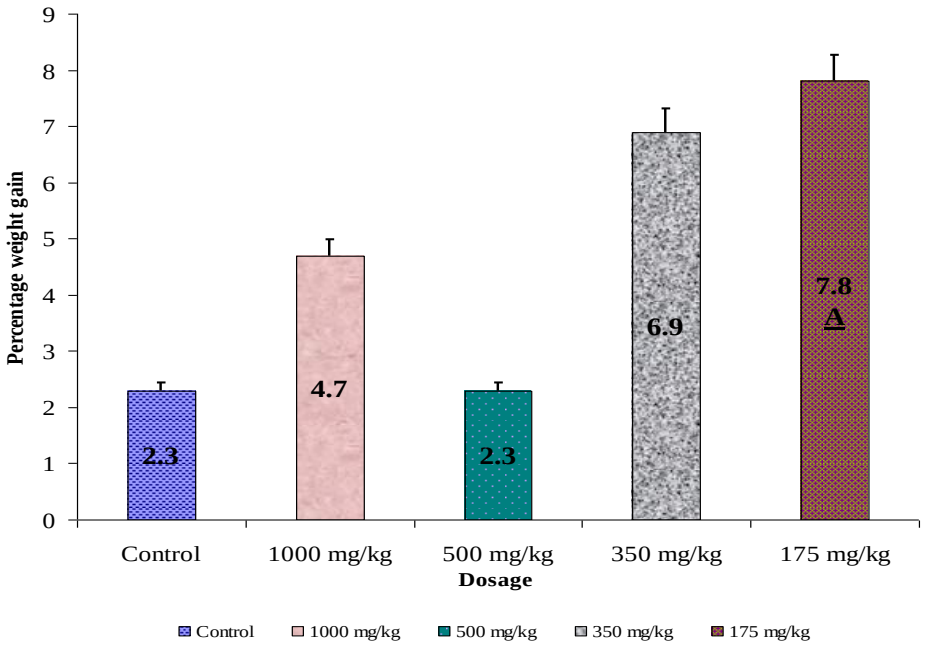

Fig. 1: Subacute effect of ethanolic extracts of *A. leiocarpus* on body weight changes of wistar rats

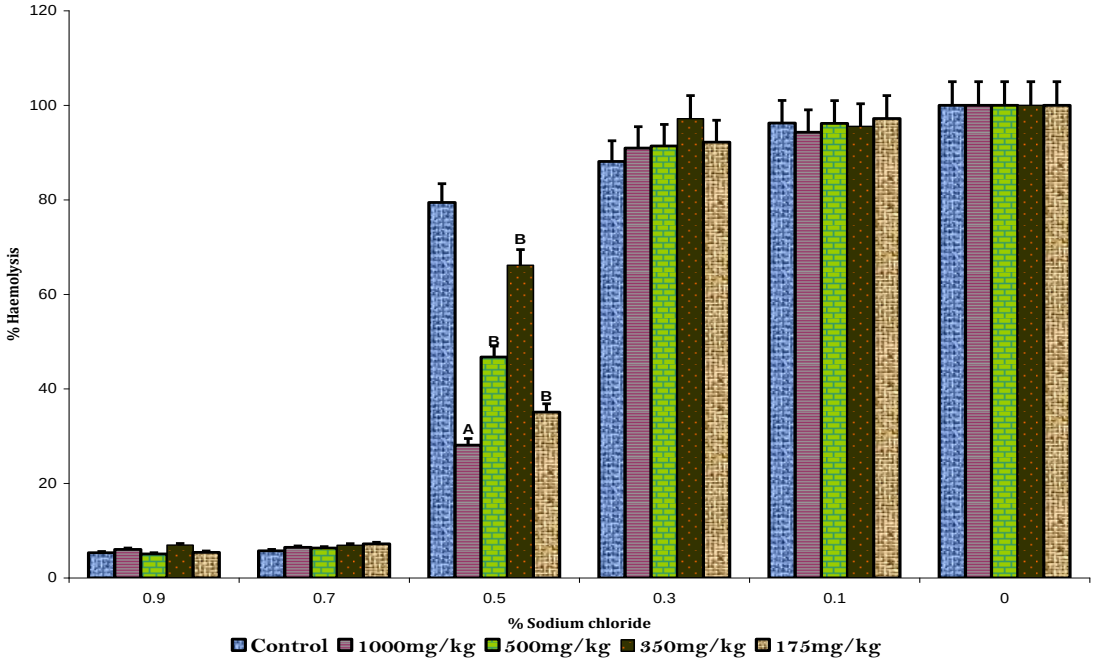

Fig. 2. Mean percent haemolysis (EOF) of erythrocytes of wistar rats administered different doses of the ethanolic extract of *A. leiocarpus*

Haematological parameters	Experimental groups				
	Control	1000 mg/kg	500 mg/kg	350 mg/kg	175 mg/kg
RBC (10^{12}/L)	7.46 ± 0.46[a]	7.23 ± 0.85[a]	7.46 ± 0.47[a]	7.26 ± 0.48[a]	7.91 ± 0.48[a]
Hb (g/L)	14.66 ±1.06[a]	14.68 ± 1.00[a]	14.80 ± 1.31[a]	14.56 ± 0.89[a]	15.12±0.55[a]
PCV (%)	43.56± 2.80[a]	44.78 ±4.11[b]	44.80 ± 4.15[a]	43.22 ± 3.29[a]	46.58 ± 1.67[b]
MCV (Fl)	58.40± 2.32[a]	59.58 ± 3.38[a]	60.00 ± 2.48[a]	58.00 ± 2.09[a]	62.20 ± 4.68[b]
MCH (Pg)	19.66±0.70[a]	20.42±1.60[a]	19.80±0.59[a]	20.08 ±0.78[a]	19.26 ± 0.74[a]
MCHC (g/L)	33.66± 1.23[a]	32.88 ±1.07[a]	33.06 ± 0.76[a]	33.74 ± 1.12[a]	32.86 ± 0.85[a]
WBC (10^9/L)	16.22± 5.78[a]	13.54 ±2.99[a]	13.90 ±3.44[a]	12.74±3.60[a]	15.74 ± 7.73[a]
Neut (10^9/L)	4.31 ±0.96[a]	3.78 ±0.38[a]	3.67±0.90[a]	3.84±0.47[a]	4.54 ±1.37[a]
Lymp (10^9/L)	11.91 ±5.28[a]	10.09± 3.41[a]	10.23 ±2.85[a]	9.66 ±3.84[a]	11.20±6.58[a]
Platelet(10^9/L)	845.6±107.2[a]	606.2 ± 53.18[b]	838.8 ± 197.8[a]	700.4 ± 185.9[b]	655.4 ± 59.15[b]

Table 1: Mean ± standard deviation (SD) of haematological parameters of Wistar adult rats of both sexes treated with various doses of ethanolic extracts of *Anogeissus leiocarpus*

[a,b] p values in the same roll with different superscripts are statistically significant

Serum biochemical parameters	Experimental groups				
	Control	1000mg/kg	500mg/kg	350mg/kg	175mg/kg
AST (mU/mL)	14.00±3.16[a]	22.60±6.31[a]	20.40±6.84[a]	18.40±6.23[a]	21.20±4.15[a]
ALT (mU/mL)	38.20±13.54[a]	37.20±9.01[a]	41.80±13.61[a]	51.20±11.48[a]	43.00±8.80[a]
ALP (mU/mL)	70.20±5.12[a]	60.20±5.12[a]	65.20±7.60[a]	55.00±7.05[a]	64.60±13.53[a]
Glu (mg/dL)	5.44±0.45[a]	4.12±0.47[b]	4.08±0.28[b]	3.86±0.17[b]	4.32±0.27[b]
Na (mEq/L)	138.2±1.79[a]	139.6±2.07[a]	140.2±2.86[a]	137.6±3.51[a]	139.6±1.82[a]
K (mEq/L)	3.92±0.30[a]	3.76±0.47[a]	4.18±0.33[a]	4.00±0.19[a]	3.88±0.50[a]
Cl (mEq/L)	98.80±1.79[a]	99.60±2.97[a]	99.60±3.85[a]	96.00±2.45[a]	99.60±2.19[a]
HCO_3 (mEq/L)	24.00±3.16[a]	22.80±2.28[a]	25.00±2.24[a]	24.60±2.61[a]	25.60±1.52[a]
Urea (mg/dL)	3.74±0.92[a]	4.42±0.85[a]	4.66±0.76[a]	3.76±0.68[a]	4.04±0.25[a]
Total proteins (g/dL)	69.40±4.88[a]	67.40±4.10[a]	70±10.35[a]	64.20±3.27[a]	70.40±6.95[a]

Table 2: Mean (±SD) of serum biochemical parameters of adult Wistar rats of both sexes treated with various doses of ethanolic extracts of *Anogeissus leiocarpus*

[a,b] p values in the same roll with different superscript are statistically significant

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