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Effects of *Anogeissus leiocarpus* Root Barks on Rat Liver, Kidney and Haematological Parameters

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Abstract

This study investigated the safety of aqueous root bark extract of *Anogeissus leiocarpus* (DC.) Guill and Perr. in albino rats. Male rats were orally administered the extract (250, 500 and 1000 mg/kg body weight) for a seven-day period. Malondialdehyde concentration and enzyme activities were monitored in liver and kidney as well as haematological, hepatic and renal function parameters. Administration of the extract significantly ($p < 0.05$) altered liver and serum enzymes, decreased liver and kidney malondialdehyde, increased serum total protein, altered serum bilirubin (total and conjugated) and increased serum urea, sodium, potassium, chloride and bicarbonate ions. In contrast, the extract did not significantly ($p > 0.05$) affect kidney alkaline phosphatase activity, serum albumin, creatinine, and haematological parameters. Overall, the findings in this study revealed that the extract is not completely safe at 250, 500 and 1000 mg/kg body weight when consumed repeatedly for a seven-day period, though the adverse effects were mild, selective and not systemic.

Keywords: *Anogeissus leiocarpus*, Combretaceae, Haematology, Liver, Kidney, Toxicity

1. Introduction

The use of various therapeutic agents in the management of several health disorders has been limited by toxic side effects and/or high costs. This has forced many populations in the developing world to turn to cheaper and acclaimed less toxic agents derived from botanicals. Such uses have attracted enormous research interests into study of the safety of plants used in traditional medicine. An example of such plants in folklore use today is *Anogeissus leiocarpus* (DC) Guill and Perr, which belongs to the Combretaceae family.

Anogeissus leiocarpus is called chewing stick tree (English) and *Ayin* (Yoruba, south western Nigeria). The plant is commonly used as chewing sticks[1]. The antibacterial, antifungal, anticancer, free radical scavenging, antioxidant, hepatoprotective and trypanocidal activities of the leaves, stem bark and root bark have been reported[2-9]. The aqueous root bark extract of *A. leiocarpus* contains alkaloids, tannins, flavonoids, phenolics, saponins, phlobatannins and terpenes[6] and, combination with *T. avicennioides*, have been traditionally claimed to be used in the management of tumour in the south western part of Nigeria.

The safety evaluation of this plant has been carried out and reported but such reports remain scanty in the open literature in spite of its various acclaimed and reported uses. Animals were reported to tolerate up to 3200 mg/kg body weight of *A. leiocarpus* leaves orally without physical signs of toxicity[10]. There are no definite reports on the safety of aqueous root bark extract of this plant. The present study therefore aims at investigating the effects of the oral consumption of aqueous root bark extract of *A. leiocarpus* on cellular enzymes, malondialdehyde, function indices of liver and kidney, as well as haematological parameters of rats.

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2. Materials and Methods

2.1 Plant Material

Anogeissus leiocarpus roots were harvested fresh from a farmland in Offa, Kwara State, Nigeria and authenticated at the IFE Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.2 Experimental Animals

Male albino rats (181.98 ± 9.36 g) of Wistar strain were obtained from the animal breeding facility of Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in aluminium cages in a well-ventilated house with optimum conditions (temperature: 22 ± 3°C; photoperiod: 12 hours light and 12 hours dark; humidity: 40-45 %) and were allowed continuous access to rat pellets and water.

2.3 Chemicals and Reagents

Kits for assay of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) were products of Randox Laboratories, County Antrim, UK. All other reagents were prepared with distilled water and of analytical grade.

2.4 Preparation of Extracts

Aqueous root bark extracts of *A. leiocarpus* was prepared as described previously[6]. Briefly, fresh roots of *A. leiocarpus* were oven-dried at 40o C for 3 weeks and pulverised with an AKIRA blender (model: BL-1531, Indonesia). A known amount (300 g) of the powdered sample was extracted in 5 L of distilled water and placed on orbital shaker maintained at 300 rpm for 24 h. This was then filtered with Whatman No. 1 filter paper followed by concentration on water bath to yield 28.5 g (9.5%) of extract.

2.5 Animal Grouping and Extract Administration

Twenty-eight male Wistar albino rats were completely randomized into four groups (A-D) of seven rats each and treated orally with 0.5 ml of extract for a seven-day period as follows: Groups A-D received distilled water, 250, 500 and 1000 mg/kg body weight of aqueous root bark extract of *A. leiocarpus*, respectively. The rats were sacrificed on the 8th day.

The study design was approved by the College of Natural and Applied Sciences, Fountain University, Osogbo, Nigeria (CONAS-FUO) Ethics Committee and the animals were handled following appropriate international guidelines as outlined by the National Institute of Health[11]

2.6 Preparation of Serum and Tissue Supernatants

Serum and supernatants of liver and kidney were prepared as previously described[7]. Briefly, rats were bled into heparinized bottles for haematological analysis following diethyl ether anaesthesia. Blood was also collected into non-heparinized tubes, allowed to clot at room temperature for 10 minutes and centrifuged at 894 x *g* for 15 minutes. Sera were aspirated with Pasteur pipette and kept frozen. The rats were thereafter dissected; the liver and kidneys were removed, cleaned, weighed (after decapsulating kidneys) and homogenized in sucrose (ice-cold; 0.25 M; 1:4 w/v). The homogenates were then centrifuged at 1398 x *g* for 15 minutes to obtain supernatants, which were kept frozen and used within 24 hours for biochemical analyses.

2.7 Determination of Biochemical and Haematological Parameters

Protein, ALP, aminotransferases, GGT and malondialdehyde (MDA) were determined using standard procedures[12–16]. Bilirubin, albumin, creatinine, urea and electrolytes (potassium, chloride, sodium, and bicarbonate ions) were also determined using previously described methods[17–21]. Haematological parameters were determined using a haematology analyzer, Sysmex Haematology Systems (Sysmex Ameria Inc., model no. KX-21N, Kobe, Japan).

2.8 Statistical Analysis of Data

The group mean (n = 7) ± S.D for each analysis was calculated and significant differences were determined by Analysis of Variance (ANOVA) and Tukey's Post Hoc test for multiple comparisons at 95% confidence level.

3. Results

3.1 Cellular Enzymes

All the doses of aqueous root bark extracts of *A. leiocarpus* significantly ($p < 0.05$) increased liver ALP and serum

GGT activities while serum ALP, ALT and AST as well as liver ALT, AST and GGT of rats were significantly ($p < 0.05$) reduced in a manner that was not dose-related (Table 1). The extract did not significantly ($p > 0.05$) alter the kidney ALP activity and liver ALT activity at 500 mg/kg body weight when compared to control (Table 1).

3.2 Malondialdehyde

The entire test doses significantly ($p < 0.05$) reduced the concentration of malondialdehyde (MDA) in both the liver and kidney of the animals with the kidney being dose-dependent (Table 2).

3.3 Liver Function Indices

Serum total protein concentrations were significantly ($p < 0.05$) increased by all the extract doses while 250 and 500 mg/kg body weight significantly ($p < 0.05$) increased conjugated bilirubin (Table 3). Total bilirubin was significantly ($p < 0.05$) decreased by 250 mg/kg body weight of the extract. Serum albumin at all doses, total bilirubin at 500 and 1000 mg/kg body weight, and conjugated bilirubin at 250 and 500 mg/kg body weight of the extract were not significantly ($p > 0.05$) affected when compared with the control (Table 3).

3.4 Kidney Function Indices

Chloride ions at 500 and 1000 mg/kg body weight, bicarbonate ions at 500 mg/kg body weight as well as urea, sodium and potassium at all doses were significantly ($p < 0.05$) elevated in the rat serum when compared with control (Table 4). However, bicarbonate ions at 250 and 1000 mg/kg body weight, chloride ions at 250 mg/kg body weight, and creatinine at all doses were not significantly ($p > 0.05$) affected compared with control (Table 4).

3.5 Haematological Parameters

A. leiocarpus extract at all doses did not significantly ($p > 0.05$) alter the levels of all the haematological indices in the rats when compared with control (Table 5).

4. Discussion

The widespread use of plants in traditional healthcare calls for safety evaluation of these botanicals. This therefore, informed the scientific evaluation of aqueous extract of *A. leiocarpus* root barks that has been used in the management of several diseases.

The increased liver activity of ALP, a biomarker of the plasma membrane[22], suggests enzyme activation and/or increased *de novo* enzyme synthesis as a recovery process in response to extract-induced damage[23–26]. This may lead to increased ALP-mediated hepatic functions[24], leading to abnormally higher rates of phosphate hydrolysis and ion transport. The decreased serum ALP activity might have resulted from inhibition[22], *in situ* inactivation[27], increased plasma clearance[23] and/or

Table 1. Selected serum, kidney and liver enzymes of rats following administration of aqueous root bark extract of *A. leiocarpus*

	Alkaline phosphatase					
	Kidney		Liver		Serum	
A	780.50 ± 97.45a		319.81 ± 50.10a		4.18 ± 0.10a	
B	924.61 ± 201.01a		516.09 ± 48.34b		3.92 ± 0.08b	
C	756.28 ± 197.50a		636.00 ± 79.03c		2.67 ± 0.45c	
D	767.83 ± 199.49a		511.34 ± 23.45b		3.09 ± 0.38c	
	Alanine aminotransferase		Aspartate aminotransferase		Gamma glutamyltransferase	
	Liver	Serum	Liver	Serum	Liver	Serum
A	7.82 ± 0.38a	0.77 ± 0.15a	12.09 ± 0.99a	2.05 ± 0.04a	37.23 ± 2.50a	0.33 ± 0.00a
B	6.28 ± 0.41b	0.48 ± 0.09b	9.23 ± 1.47b	1.22 ± 0.00b	6.46 ± 0.75b	4.62 ± 0.09b
C	7.44 ± 0.44a	0.38 ± 0.04c	9.68 ± 0.79b	1.10 ± 0.14b	9.56 ± 0.03c	3.29 ± 0.10c
D	4.18 ± 0.11c	0.40 ± 0.00c	7.49 ± 0.85b	1.11 ± 0.01b	10.62 ± 0.01d	1.46 ± 0.05d

A = Control, B = 250 mg/kg body weight, C = 500 mg/kg body weight, D = 1000 mg/kg body weight. Values are mean ± SD; n = 7. Values carrying different superscripts down the column for each parameter are significantly different ($P < 0.05$). Specific activities of enzymes are expressed as U/mg protein.

decreased bioavailability of specific activator(s)[28] of this enzyme; the activator(s) could be bound by tannins in the extract[6,29].

The decreased activities of serum and liver aminotransferases may be attributed to decreased synthesis, inactivation/inhibition, and/or increased plasma clearance of these enzymes[23]. Such hepatocellular toxicity[30], may lead to impaired carbohydrate and amino acid metabolism[31]. Decreased liver and serum aminotransferases by plant extracts has also been previously reported[32,33]. The decreased liver GGT activity may be due to decreased synthesis and/or inhibition/*in situ* inactivation. This may lead to reduced transfer of amino acids and peptides across the hepatocyte membrane. The corresponding increase in serum activity could result from activation and/or decreased plasma clearance[23]. It may be inappropriate to speculate leakage from liver due to loss of membrane components because alkaline phosphatase, also membrane-bound, did not follow this trend but rather decreased in the serum as earlier discussed. The ordered lipid membrane bilayer is likely to be preserved as indicated by the reduced liver MDA discussed later. Decreased liver GGT and increased serum GGT have been previously attributed to liver damage[32]. The effects observed here could have been mild, local distortions.

Malondialdehyde indicates lipid peroxidation[9]; the decreased liver and kidney MDA suggests hepatoprotective and nephroprotective activity, thereby maintaining the integrity of the membrane lipids. This is supported by previous studies[7-9].

Table 2. Liver and kidney malondialdehyde concentrations of rats following administration of aqueous root bark extract of *A. leiocarpus*

	Malondialdehyde concentration (nmol MDA/mg protein)	
	Liver	**Kidney**
A	1.95 ± 0.01a	3.90 ± 0.02a
B	1.34 ± 0.03b	3.28 ± 0.05b
C	1.70 ± 0.02c	3.02 ± 0.04c
D	0.82 ± 0.03d	1.62 ± 0.02d

A = Control, B = 250 mg/kg body weight, C = 500 mg/kg body weight, D = 1000 mg/kg body weight. Values are mean ± SD; n = 7. Values carrying different superscripts down the column for each parameter are significantly different ($P < 0.05$)

Table 3. Selected liver function parameters of rats following administration of aqueous root bark extract of *A. leiocarpus*

	Total protein (mg/mL)	Albumin (mg/mL)	Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)
A	78.00 ± 7.04a	57.75 ± 0.21a	32.74 ± 2.85a	13.00 ± 1.47b
B	90.60 ± 7.39b	57.82 ± 0.27a	27.54 ± 1.33b	18.00 ± 1.74a
C	102.30 ± 1.96b	58.19 ± 0.24a	33.49 ± 2.63a	16.00 ± 1.24a
D	99.30 ± 5.13b	58.37 ± 0.28a	32.00 ± 2.23a	11.00 ± 1.24b

A = Control, B = 250 mg/kg body weight, C = 500 mg/kg body weight, D = 1000 mg/kg body weight. Values are mean ± SD; n = 7. Values carrying different superscripts down the column for each parameter are significantly different ($P < 0.05$). All parameters were determined in serum.

Table 4. Selected kidney function parameters of rats following administration of aqueous root bark extract of *A. leiocarpus*

	Urea (mmol/L)	Creatinine (mmol/dL)	Na+ (mmol/L)	K+ (mmol/L)	Cl- (mmol/L)	HCO_3- (mmol/L)
A	3.94 ± 0.70a	29.52 ± 3.05a	144.07 ± 11.72a	3.83 ± 0.47a	305.87 ± 27.22a	120.00 ± 15.39a
B	10.84 ± 1.03b	28.83 ± 0.91a	233.28 ± 1.10b	8.10 ± 0.60b	320.00 ± 30.95a	110.00 ± 12.36a
C	10.56 ± 0.90b	28.46 ± 0.42a	226.37 ± 7.42b	8.64 ± 0.35b	456.22 ± 34.36b	190.00 ± 20.36b
D	10.58 ± 1.17b	27.32 ± 1.11a	246.78 ± 5.96c	8.26 ± 1.07b	534.29 ± 43.05c	110.00 ± 22.36a

A = Control, B = 250 mg/kg body weight, C = 500 mg/kg body weight, D = 1000 mg/kg body weight. Values are mean ± SD; n = 7. Values carrying different superscripts down the column for each parameter are significantly different ($P<0.05$). All parameters were determined in serum.

Table 5. Haematological parameters of rats following administration of aqueous root bark extract of *A. leiocarpus*

	Hb (g/dL)	RBC (x106/µl)	PCV (%)	MCV (fL)	MCH (pg)
A	13.37 ± 0.21a	7.85 ± 0.30a	47.70 ± 0.89a	60.83 ± 1.53a	17.03±0.40a
B	12.73 ± 0.31a	7.37 ± 0.22a	43.53 ± 1.76a	59.07 ± 0.67a	17.27±0.32a
C	14.30 ± 1.87a	8.43 ± 0.66a	48.80 ± 3.30a	57.93 ± 2.60a	16.97±0.67a
D	14.43 ± 1.40a	8.45 ± 0.29a	49.50 ± 2.18a	58.60 ± 2.34a	17.10±0.20a
	MCHC (g/dL)	**WBC (x103/µL)**	**LYM (%)**	**PLT (x103/µL)**	**NEU (%)**
A	28. 00 ± 0.26a	14.40 ± 2.09a	89.07 ± 4.45a	690. 00 ± 39.40a	11.12±1.11a
B	29.23 ± 0.81a	15.33 ± 2.06a	84.40 ± 3.58a	712. 00 ± 64.47a	11.63±1.32a
C	29.30 ± 0.26a	15.77 ± 2.02a	77.33 ± 4.06a	707.67 ± 51.68a	10.90±1.51a
D	29.20 ± 0.89a	15.47 ± 2.52a	80.10 ± 1.91a	761.33 ± 68.08a	12.09±1.09a

A = Control, B = 250 mg/kg body weight, C = 500 mg/kg body weight, D = 1000 mg/kg body weight, Hb = haemoglobin concentration, RBC = red blood cells, PCV = packed cell volume, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, WBC = white blood cells, LYM = lymphocytes, PLT = platelets, NEU = neutrophils. Values are mean±SD; n = 7. Values carrying different superscripts down the column for each parameter are significantly different ($P < 0.05$)

The increased total protein may indicate increased protein synthesis, decreased protein catabolism and/or osmotic imbalance (supported by increased serum Na^+ discussed later), while the normal albumin concentration suggests normal synthetic activity[32,34]. Total protein increase was also reported for *Ficus exasperata* leaf extract[35]. The decreased serum total bilirubin (250 mg/kg body weight) and altered conjugated bilirubin levels indicate impaired secretory function of the liver[32].

The increased serum urea and normal creatinine levels indicate selective impairment of glomerular filtration[23]. The increase in serum sodium may be an indication of decreased sodium reabsorption suggestive of renal tubular dysfunction. This may affect cellular homeostasis due to impaired cellular hydration and osmotic gradients of the body fluids.

The absence of any effect on all the blood parameters suggests that the extract has no observable effect on erythropoiesis and blood cell turnover, indicating local and not systemic toxicity in the animals. The effects of the extract on hepatic and renal functions are attributable to the presence of some toxic forms of alkaloids, tannins and saponins in the extract[32,36–39].

In conclusion, this study has shown that *A. leiocarpus* extract affected some enzymes and function indices of liver and kidney, while kidney ALP, some hepatic and renal function indices, and blood indices were not affected. Reduced MDA also indicated hepatic and renal protection. Overall, the extract has been shown to exhibit mild, local and selective toxicities in rats.

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6. Conflicts of Interests

The authors declare that there are no conflicts of interests. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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