

Original Article

Effects of 1:1 Mixture of *Anogeissus leiocarpus* and *Terminalia avicennioides* Root Bark Extracts on Haematological Parameters, Liver and Kidney Function Indices of Male Rats

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ABSTRACT

Background: This study evaluated the effects of 1:1 mixture of aqueous root bark extracts of *Anogeissus leiocarpus* (DC) Guill & Perr (Combretaceae) and *Terminalia avicennioides* Guill & Perr (Combretaceae) in male rats.

Methods: Male rats were orally administered a 1:1 mixture of both extracts (250, 500 and 1000 mg/kg body weight) for seven days. Liver and kidney function indices, haematological parameters and the levels of malondialdehyde were evaluated in the animals at 7 days post-administration of the mixture of the extracts.

Results: Administration of mixture of the extract significantly ($p < 0.05$) increased the activities of liver and kidney alkaline phosphatase and reduced the serum alkaline phosphatase, liver and serum aspartate aminotransferase, alanine aminotransferase and gamma glutamyl transferase activities. The mixture also decreased the levels of serum chloride ions, liver and kidney malondialdehyde. Furthermore mixture significantly ($p < 0.05$) increased the serum total protein concentrations whereas the levels of serum albumin, creatinine, urea, potassium, sodium and bicarbonate ions, red blood cells, white blood cells and their related indices were not significantly ($p > 0.05$) altered.

Conclusions: The present study revealed that the mixture caused functional toxicity of the liver and kidney of male rats without any evidence of haematotoxicity. The consumption of the 1:1 mixture of the plant extracts at 250, 500 and 1000 mg/kg body weight has some toxic implications in male rats.

Keywords: *Anogeissus leiocarpus*, Combretaceae, Functional Toxicity, Haematotoxicity, *Terminalia Avicennioides*.

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INTRODUCTION

Plants from various families and species have been traditionally acclaimed and scientifically reported to possess various pharmacological activities. One of such plant family is the Combretaceae, which include *Anogeissus leiocarpus* (DC) Guill & Perr and *Terminalia avicennioides* Guill & Perr. *A. leiocarpus* is commonly known as chewing stick tree (English) and *Ayin* (Yoruba, south western Nigeria). *A. leiocarpus* has been shown to have trypanocidal, antibacterial and antifungal activities [1,2]. On the other hand, *T. avicennioides*, also known as *Baushe* (Hausa, northern Nigeria), *Idi* (Yoruba) and *Edo* (Igbo, south-east Nigeria), has also been reported to have cytotoxic, wound healing, trypanocidal, gastroprotective, anticancer, antioxidant, hepatoprotective, antibacterial and antifungal properties [1, 3-11]. The secondary metabolite constituents as well as the cytotoxic activity of the aqueous extract of the root barks of the 1:1 mixture of *A. leiocarpus* and *T. avicennioides* roots against Ehrlich Ascite carcinoma cells have been documented [9]. Furthermore, it has also been reported that rats tolerated the oral administration of the extract of *A. leiocarpus* leaves up to 3200 mg/kg body weight without any visible signs of clinical toxicity [12].

In spite of the myriads of pharmacological studies on the plants, toxicological information on the 1:1 mixture of *A. leiocarpus* and *T. avicennioides* root barks remains scanty in the open scientific literature. Therefore, the present study aims at providing information on the toxicity of the 1:1 mixture of both plants using toxicological parameters like hepatic and renal function parameters and haematological indices. This study is a follow-up on the efficacious study of the 1:1 mixture of the extracts against the Ehrlich Ascites carcinoma cells earlier reported by Salau et al [9].

MATERIALS AND METHODS

Plant Materials

Fresh samples of *A. leiocarpus* and *T. avicennioides* roots were obtained from a farmland in Offa, Nigeria, and authenticated at the IFE Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. *A. leiocarpus* and *T. avicennioides* roots were assigned voucher numbers 13775 and 15428, respectively.

Experimental Animals

Healthy male albino rats (181.98±9.36 g) were obtained from the Animal Breeding Unit of the Department of Biochemistry, University of Ilorin, Ilorin,

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Nigeria. The animals were housed, fed and treated humanely at optimum conditions of temperature and humidity.

Chemicals and Reagents

Gamma-glutamyltransferase (GGT), the aminotransferases (alanine aminotransferase, ALT and aspartate aminotransferase, AST) and alkaline phosphatase (ALP) assay kits were products of Randox Laboratories, County Antrim, UK. All other chemicals and reagents were of analytical grade and prepared in distilled water.

Preparation of Extract Mixture

Aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides* were prepared as described in a previous study [9]. Briefly, fresh roots of both plants were separately oven-dried at 40 °C for three weeks and pulverized with an AKIRA blender (model: BL-1531, Indonesia). A 300 g portion of each powder was extracted in 5 L of distilled water, placed on orbital shaker at 300 rpm for 24 h, and filtered. The filtrate was concentrated on water bath of to yield 28.5 g *A. leiocarpus* (9.5%) and 30 g of *T. avicennioides* (10%). A 1:1 mixture of both extracts was then prepared for use in the present study.

Animal Grouping and Administration of Extracts

Twenty-eight albino rats were selected into four groups (I – IV; using completely randomized design) of seven rats each. They were treated orally with 0.5 ml of the extract mixture on daily basis for seven days as follows: Rats in group I received distilled water; groups II – IV received 250, 500 and 1000 mg/kg body weight, respectively, of the extract mixture. The experimental rats were sacrificed 24 hours after the seventh administration.

The experimental design was approved by the Ethical Committee on the Care and Use of Experimental Animals of the College of Natural and Applied Sciences, Fountain University, Osogbo, Nigeria. The experimental rats were also handled in accordance with the Guidelines of the National Institute of Health (NIH; Bethesda, Maryland, USA) and Guide for the Care and Use of Laboratory Animals [13].

Preparation of Serum and Tissue Supernatants

Liver and kidney supernatants as well as serum were prepared as previously described [11]. Briefly, rats were anaesthetized by placing them in a glass jar containing cotton wool soaked with (97%) w/v of diethyl ether and bled into heparinized bottles for haematological analyses. Blood (5ml) was also collected into clean, dry test tubes, centrifuged at 503 x g for 15 minutes and the resulting serum was used within 12 hours of preparation. The liver and kidneys were removed from each rat, homogenized in ice-cold 0.25 M sucrose solution and

the resulting supernatants were used within 24 hours of preparation.

Determination of Biochemical and Haematological Parameters

Determination of protein, malondialdehyde (MDA), ALP, AST, ALT, GGT, albumin, bilirubin, urea, creatinine, and electrolytes (Na⁺, K⁺, Cl⁻ and HCO₃⁻) were carried out using previously described procedures [14-23]. Haematological indices were determined with the aid of the analyzer, Sysmex Haematology Systems (Sysmex America Inc., model no. KX-21N, Kobe, Japan).

Statistical Analysis of Data

The mean ± SD for each analysis (n = 7) was computed and significant differences were determined by one-way Analysis of Variance (ANOVA). Tukey's *post hoc* test was used for multiple comparisons at p<0.05.

RESULTS

All the doses of the extract mixture significantly (p<0.05) elevated the activities of ALP in the liver and kidney of the animals (Table 1). Activities of the aminotransferases, serum ALP and GGT were significantly reduced (p<0.05) in the tissues at all doses of the mixture except for the liver ALT at the 250 mg/kg body weight and serum AST at 1000 mg/kg body weight, which were not (p>0.05) significantly different from the control. All the alterations in the activities of the enzymes by the extract mixture were not dose-dependent (Table 1).

The MDA concentrations in the rat liver and kidney after the administration of the extract mixture were significantly lowered (p<0.05). The reduction in the levels of MDA were not dose-dependent (Table 2).

The 1:1 extract mixture significantly (p<0.05) increased the concentration of serum total protein of the male rats whereas the serum albumin concentration was not significantly (p>0.05) altered (Table 3). Serum total and conjugated bilirubin levels were also significantly (p<0.05) reduced (Table 3).

The extract mixture at 250 and 1000 mg/kg body weight significantly decreased (p<0.05) the levels of serum K⁺ whereas the Cl⁻ concentration was decreased by all the doses of the extract mixture (Table 4). Furthermore, the levels of K⁺ at 500 mg/kg body weight as well as those of urea, creatinine, Na⁺ and HCO₃⁻ were not significantly (p>0.05) altered by the extract mixture (Table 4).

The levels of haemoglobin, red blood cells, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cells, lymphocytes, platelets, and neutrophils were not significantly (p>0.05) altered by all the doses of the extract mixture (Table 5).

Table 1. Activity of some enzymes in the liver, kidney and serum of male rats after oral administration of 1:1 mixture of aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides*.

	Alkaline phosphatase					
	Kidney		Liver		Serum	
I	780.50±97.45 ^a		319.81±50.10 ^a		4.18±0.10 ^a	
II	1870.90±234.42 ^b		431.48±10.64 ^b		2.62±0.37 ^b	
III	1942.10±89.99 ^b		656.99±17.09 ^d		2.76±0.71 ^b	
IV	2413.78±154.55 ^c		560.89±35.11 ^c		3.32±0.51 ^b	
	Alanine aminotransferase		Aspartate aminotransferase		Gamma glutamyltransferase	
	Liver	Serum	Liver	Serum	Liver	Serum
I	7.82±0.38 ^a	0.77±0.15 ^a	12.09±0.99 ^a	2.05±0.04 ^a	37.23±2.50 ^a	0.33±0.00 ^a
II	7.45±0.50 ^a	0.32±0.04 ^c	8.09±0.19 ^b	1.09±0.11 ^b	22.12±0.11 ^c	0.20±0.01 ^c
III	3.2±0.19 ^b	0.31±0.05 ^c	5.71±0.05 ^c	1.00±0.14 ^b	24.62±0.95 ^b	0.16±0.04 ^d
IV	1.77±0.13 ^c	0.50±0.01 ^b	7.08±1.42 ^b	1.99±0.06 ^a	23.72±0.79 ^b	0.26±0.01 ^b

I = Control, II = 250 mg/kg body weight, III = 500 mg/kg body weight, IV = 1000 mg/kg body weight. Values are mean±SD; n=7. Values with different superscripts down the column for each enzyme are significantly different (P<0.05). Specific activities of enzymes are expressed as U/mg protein.

Table 2. Liver and kidney malondialdehyde concentrations of male rats after oral administration of 1:1 mixture of aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides*.

	Malondialdehyde concentration (nmol MDA/mg protein)	
	Liver	Kidney
I	1.95±0.01 ^a	3.90±0.02 ^a
II	1.27±0.02 ^c	1.54±0.04 ^d
III	0.73±0.05 ^d	1.84±0.01 ^c
IV	1.68±0.01 ^b	1.99±0.05 ^b

I = Control, II = 250 mg/kg body weight, III = 500 mg/kg body weight, IV = 1000 mg/kg body weight. Values are mean±SD; n=7. Values with different superscripts down the column are significantly different (P<0.05)

Table 3. Some liver function indices of male rats after oral administration of 1:1 mixture of aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides*.

	*Total protein	*Albumin	**Total bilirubin	**Conjugated bilirubin
I	78.00±7.04 ^a	57.75±0.21 ^a	32.74±2.85 ^a	13.00±1.47 ^a
II	186.00±20.74 ^b	54.82±0.56 ^a	28.52±2.07 ^{ab}	10.14±1.44 ^b
III	226.40±40.31 ^b	58.06±1.28 ^a	25.94±2.74 ^b	9.19±0.27 ^c
IV	210.00±23.45 ^b	57.89±2.25 ^a	25.59±1.75 ^b	10.67±0.62 ^b

I = Control, II = 250 mg/kg body weight, III = 500 mg/kg body weight, IV = 1000 mg/kg body weight. Values are mean±SD; n=7. Values with different superscripts down the column for each parameter are significantly different (P<0.05). All parameters were measured in the serum. *expressed in mg/mL, ** expressed in mg/dL

Table 4. Some kidney function indices of male rats after oral administration of 1:1 mixture of aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides*.

	*Urea	**Creatinine	*Na ⁺	*K ⁺	*Cl ⁻	*HCO ₃ ⁻
I	3.94±0.70 ^a	29.52±3.05 ^a	144.07±11.72 ^a	3.83±0.47 ^a	305.87±27.22 ^a	120.00±25.39 ^a
II	3.03±0.30 ^a	26.97±3.74 ^a	145.81±2.14 ^a	3.30±0.1 ^b	232.17±11.74 ^b	160.50±24.37 ^a
III	3.69±0.25 ^a	29.42±2.54 ^a	153.37±19.59 ^a	3.91±0.2 ^a	221.79±24.24 ^b	120.00±16.35 ^a
IV	3.80±0.24 ^a	25.58±2.18 ^a	147.39±2.35 ^a	2.93±0.23 ^c	249.50±20.56 ^b	139.50±15.99 ^a

I = Control, II = 250 mg/kg body weight, III = 500 mg/kg body weight, IV = 1000 mg/kg body weight. Values are mean±SD; n=7. Values with different superscripts down the column for each parameter are significantly different (P<0.05). All parameters were measured in the serum. * expressed in mmol/L, ** expressed in mmol/dL

Table 5. Haematological parameters of male rats after oral administration of 1:1 mixture of aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides*.

	Hb (g/dL)	RBC (x10 ⁶ /μl)	PCV (%)	MCV (fL)	MCH (pg)
I	13.37±0.21 ^a	7.85±0.30 ^a	47.70±0.89 ^a	60.83±1.53 ^a	17.03±0.40 ^a
II	13.62±1.30 ^a	7.76±1.23 ^a	46.95±5.35 ^a	61.10±2.52 ^a	17.64±1.11 ^a
III	14.81±1.60 ^a	8.72±1.04 ^a	51.256.14 ^a	59.10±1.11 ^a	16.97±0.54 ^a
IV	12.83±0.67 ^a	7.48±0.96 ^a	45.32±2.37 ^a	61.20±4.33 ^a	16.78±1.23 ^a
	MCHC (g/dL)	WBC (x10 ³ /μL)	LYM (%)	PLT (x10 ³ /μL)	NEU (%)
I	28.00±0.26 ^a	14.40±2.09 ^a	89.07±4.45 ^a	690.00±39.40 ^a	11.12±1.11 ^a
II	29.01±0.82 ^a	14.87±1.86 ^a	90.81±3.04 ^a	758.25±107.18 ^a	10.45±1.21 ^a
III	28.92±0.56 ^a	14.50±1.45 ^a	88.35±0.65 ^a	755.75±79.83 ^a	11.58±1.00 ^a
IV	28.28±0.35 ^a	14.24±0.74 ^a	86.33±4.94 ^a	733.50±105.66 ^a	12.59±1.29 ^a

I = Control, II = 250 mg/kg body weight, III = 500 mg/kg body weight, IV = 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).

DISCUSSION

Safety evaluation of plant extracts has become necessary in view of the numerous uses to which medicinal plants have been put. *A. leiocarpus* and *T. avicennioides* root barks have been combined in the folklore medicine of Nigeria (south-west) for the management of tumours. This 1:1 extract mixture of *A. leiocarpus* and *T. avicennioides* root barks, which showed promising results against Ehrlich Ascites cell carcinoma [9] necessitated the present investigation on the toxic implications on the normal functioning of the liver, kidney and the haematological parameters.

ALP is involved in the transportation of ions across membranes and hydrolysis of phosphate. It is a biomarker of the integrity of the membranes of the cells and endoplasmic reticulum integrity [24]. The increase in the liver and kidney ALP activities may be due to its activation and/or increased *de novo* synthesis caused by the extract mixture as a consequence of increased activity of the organs. The decreased serum ALP activity in the present study may be attributed to inhibition and/or *in situ* inactivation as a result of the presence of secondary metabolites in the extract mixture, prominent among which may be saponins and tannins; some of which are known to chelate metal ions and precipitate proteins [9, 24, 25]. The reduction in the activity of ALP in the serum of the animals may also be due to increased plasma clearance of the enzyme [26], which is consistent with the findings of a previous report [25].

The aminotransferases are markers of liver cytolysis since they are cytosolic enzymes while GGT is the most sensitive enzyme marker of hepatobiliary dysfunction. The decrease in ALT, AST and GGT activities in the liver and serum could be attributed to decreased enzyme synthesis, enhanced inhibition of the enzyme molecules, and/or increased plasma clearance. This may adversely affect amino acid and energy metabolism, because both processes are linked via the aminotransferases [27]. These findings in the present study are similar to that from a previous study, which reported a decrease in the activities of liver GGT and the aminotransferases [28].

MDA is one of the products of lipid peroxidation and is increased as a result of oxidative stress. The decrease in liver and kidney MDA may be an indication that the extract mixture did not prone the cell membranes of the animals to oxidative stress. This school of thought is also reinforced with the increase in alkaline phosphatase activities in the liver and kidney of the animals.

Serum total protein and albumin are useful indices of the synthetic function of the liver [28]. The increase in the level of total protein, without a corresponding alteration in the albumin, may indicate an imbalance in the rates of protein synthesis and degradation. The extract mixture might have selectively affected protein catabolism in the liver without a commensurate effect on its synthetic function. The increase in total protein in this study is consistent with a similar trend reported previously after the administration of aqueous extract of *Ficus exasperata* to rats [29]. Bilirubin levels reflect the secretory activity of the liver [28]. The decrease in serum bilirubin levels (total and conjugated) in this study suggests that some aspects of the secretory functioning of the liver of male rats might have been compromised.

Determination of the levels of some biochemical parameters such as serum urea, creatinine and electrolytes (Na⁺, K⁺, Cl⁻ and HCO₃⁻) can indicate the functioning of kidney of animals. The kidneys are responsible for the excretion of urea and reabsorption of electrolytes into the blood. Therefore, the decrease in the levels of K⁺ and Cl⁻ among all the electrolytes investigated in the present study suggests selective effect on the tubular reabsorption without concomitant effect on the glomerular function. This may lead to impaired cellular hydration and osmotic gradients of the body fluids.

Assessment of haematological parameters can be used to provide information on the health status of animals, since blood flows throughout the body, carrying nutrients and waste products. The absence of an effect on the haematological parameters of male rats after the administration of the extract mixture suggests that the mixture of aqueous root bark extracts of *A. leiocarpus* and *T. avicennioides* did not adversely affect the synthesis and destruction of the blood indices. This is an

indication that the components of the mixture at these doses were not haematotoxic to the male rats the male rats.

CONCLUSION

This study has shown that the 1:1 mixture at the doses investigated has adversely affected of the extracts normal functioning of the liver and kidney of the male rats without any evidence of haematotoxicity. Further studies involving long-term exposures to the extract mixture be carried out.

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