Available online on 15.10.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Acute and Delayed Oral Toxicity Studies and Observations on Pregnancy, Gestation and Reproductive Performance of Wistar Rats Administered Limit Dose of Purified Extract from Stem Bark of Antidiabetic *Anogeissus leiocarpus* (African Birch Tree)

King Akpofure Nelson Esievo[1,2,3+], Lauretta Oghenekevwe Esievo[4,5], Dahiru Sani[6], Kingsley Oghenerukevwe Esievo[7], Edith Monica Esievo[8,9], Emmanuel Oluwadare Balogun[10,11], John Wassagwa[10]#, Peter Ibrahim Rekwot[4,12], Lushaikyaa Allam[4,13] and Edward Oniovosa Uyobisere[14]

1. *Lecturer/Resource Person, College of Veterinary Surgeons, Nigeria, Pathology Faculty, Samaru, Zaria Study Centre, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.*
2. *Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture, Zuru, Nigeria.*
3. *Consultant, Research and Diagnosis Unit, Kanesco Global Services Limited (RC 829505), Hayin Mallam, Samaru Zaria, Nigeria.*
4. *Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.*
5. *Department of Theriogenology, College of Veterinary Medicine, Federal University of Agriculture, Zuru, Nigeria.*
6. *Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.*
7. *Department of Agronomy, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria.*
8. *Department of Veterinary Surgery, College of Veterinary Medicine, Federal University of Agriculture, Zuru, Nigeria.*
9. *Royal Veterinary College, University of London, Royal College Street, London NW1 0TU, United Kingdom.*
10. *Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.*
11. *African Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology (ACENTDFB), Ahmadu Bello University, Zaria, Nigeria.*
12. *Artificial Insemination Unit, National Animal Production Research Institute, Ahmadu Bello University, Shika, Zaria, Nigeria.*
13. *Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria.*
14. *Department of Soil Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria*

#UNESCO International Centre for Biotechnology, University of Nigeria, Nsukka, Nigeria

Article Info:

Article History:

Received 26 July 2023
Reviewed 02 Sep 2023
Accepted 10 Sep 2023
Published 15 Oct 2023

Cite this article as:

Esievo KAN, Esievo LO, Sani D, Esievo KO, Esievo EM, Balogun EO, Wassagwa J, Rekwot PI, Allam L, Uyobisere EO, Acute and Delayed Oral Toxicity Studies and Observations on Pregnancy, Gestation and Reproductive Performance of Wistar Rats Administered Limit Dose of Purified Extract from Stem Bark of Antidiabetic *Anogeissus leiocarpus* (African Birch Tree), Journal of Drug Delivery and Therapeutics. 2023; 13(10):11-27

DOI: http://dx.doi.org/10.22270/jddt.v13i10.5965

*Address for Correspondence:

King Akpofure Nelson Esievo, Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture, Zuru, Nigeria.

Abstract

Objectives: The maximum purification of the extracts of antidiabetic *Anogeissus leiocarpus* stem bark led to a quest for its lethal dose (LD_{50}) and effects on pregnancy, gestation, pups (kitten) and reproductive performance of Wistar rats.

Design: Extracts of A. *leiocarpus* were subjected to column and thin layer chromatography until final purification.

Five (5) each of adult male and female rats were used for the study. The revised limit test dose of 2000mg/kg.bd.wt. was used to evaluate the acute oral toxicity and effects on reproductive characteristics of the purified extracts on Wistar rats. The rats were monitored for instant death and 24hours later, 3 each of male and female rats were humanely sacrificed. On day 0 and 24hours later biomarkers of liver damage, AST, ALT, and ALP and that of kidney, urea, were assayed. The remaining 4 rats (2 males and 2 females) were monitored for additional 14days and thereafter for any effect of the limit dose on reproductive activities.

Results: The limit dose caused no death within the first 24hours and no hepato-renal damages. Pregnancy, gestation, parturition, pups (kitten) and reproductive performance were normal. The median lethal Dose (LD_{50}) is > 2000mg/kg./bd.wt.

Conclusion: Animal experimentation, using Wistar rats showed that purified extracts of *A. leiocarpus* stem bark as a non-conventional drug for diabetes mellitus, has no effects on pregnancy and reproductive performance. There was no teratogenic effect on pups. The compound has a wide range of safety value.

Keywords: Oral Toxicity, Pregnancy, Gestation, Reproductive Performance, Wistar Rats, *Anogeissus leiocarpus*, Purified Extract

INTRODUCTION

Diabetes mellitus, was very aptly defined as disorders of carbohydrate, protein and lipid metabolism with an existing chronic hyperglycaemia from lack or impairment of insulin secretion, type 1 diabetes mellitus (TIDM), defective insulin action, type 2 diabetes mellitus (T2DM) or both[1]. Accompanying the chronic hyperglycaemia, life threatening complications, such as cardiovascular disease, nephropathy, neuropathy and retinopathy resulting in blindness were reported [2]. The increases worldwide in T2DM without and with microvascular and macrovascular complications of nephropathy and retinopathy implicated DM as a leading cause of death, by WHO, come 2030, with a worse projection for 2035[3]. Thus, DM was reported as a challenging unresolved health problem for the 21st century[4].

This diabetes mellitus, a non-communicable, devastating heterogeneous metabolic disease, reported to afflict well over 425 million people worldwide, caused along its path, enormous economic costs to governments [3,5,6]. In addition, an overview of outpatient visits in United States, alone, between 2007 and 2013, showed that 785 million people appeared diagnosed with diabetes [7,8].

National budgets of countries worldwide were hit severely by the risk factors induced by DM on the progression, prognosis and mortality of COVID-19 patients with pre-existing T2DM [9,10,11].

Unquantifiable economic and social menace to governments and patients alike, could be derived from obesity and excess body weight with physical inactivity [12] which are predisposing factors of diabetes, although debatable in terms of atherosclerotic cardiovascular disease [13]. Delayed or non-healing wounds, diabetic ulcers, induced by chronic hyperglycaemia [7,14,15] which led to limbs amputations at certain instances, create additional unquantifiable worrisome social menace to government and patients alike.

Pet animals [16,17], equine power animals [18] and food animals [19,20] of the human populations were not left out of the DM scourge.

Insulin, the "household name" and conventional treatment for DM, received support from different classes of glucose lowering drugs, such as sulfonyl urea and meglitinides biguanides and thiazolinediones [2,21], enhanced pro-synthetic hypoglycemic drugs which did not effectively control hyperlipidaemia induced by DM, apart from toxicity and resistance experienced by some patients [22]; indeed, the development of these drugs along allopathy principles, increased their costs for the developing world [23,24] and were abandoned by patients. Resistance, side effects and exorbitant costs must be avoided as much as possible during drug development.

Reduced economic resources, associated with developing countries, informed the use of medicinal plants and their phytoconstitutents, traditionally, to manage diabetes mellitus. Strong and numerous advocates for the use of medicinal plants and their phytoconstitutuents such as alkaloids, flavonoids for the treatment of diabetes mellitus exist [4, 12, 22, 24, 25, 26, 27, 28].

Crude ethanolic extract of *Anogeissus leiocarpus* stem bark, administered orally to normal Wistar rats over a 14-day period, reduced blood glucose values and improved blood values in the rats [29]; these results exposed the antidiabetic and haemopoietic activities in the plant. Thereafter, extract of *A. leiocarpus* guill and perr leaf effectively reduced the hyperglycaemia and the dyslipidaemia associated with alloxan-induced diabetic rats [30], while total extracts and fractions of *A. leiocarpus* were antihyperglycaemic in mice [31]. Further support for the efficacy of *A. leiocarpus* as a treatment agent for DM was its sialoglyco conjugate and sialic acids modulating activity on erythrocyte surface and serum sialic acids, which adequately established elevated serum sialic acids as a potent biomarker, predictive and prognostic of alloxan-induced diabetic dogs [32].

In addition, crude ethanolic extract of *A. leiocarpus* stem bark ameliorated hyperglycaemia, hepato-renal damage, deranged electrolytes and acid-base balance in alloxan-induced diabetic dogs [17] in a novel approach as it prevented hyperglycaemic reversal after the extract was withdrawn [17]; its efficacy as a non-conventional treatment agent of DM received more credence as it exhibited antidiabetic and antioxidant activities in alloxan-induced diabetic rats[33] and attenuated dyslipidaemia in alloxan-induced diabetic dogs [34]. In addition, ethanolic extract of *A. leiocarpus* stem bark manifested markedly improved healing activities in surgically-wounded diabetic dogs [35].

For a proper and detailed understanding of the mechanisms involved in an effective drug delivery, hundred percent extract purification, of *A. leiocarpus* to the point of crystallization, was accomplished taking cognizance of the environmental soil fertility requirements of the different sources of *A. leiocarpus*.

This study was designed to investigate the median lethal dose (LD_{50}) of the purified extract of *A. leiocarpus* as a non-conventional treatment for DM and any side effects on pregnancy, gestation, the pus (Kitten) and reproductive performance in Wistar rats.

MATERIALS AND METHODS

Plant Collections:

Stem bark of *Anogeissus leiocarpus* (family, combretaceae) was harvested from two distinctly different terrains; 2.372kg from a shrub-like plant, (batch 1) and 3.410kg from a tree-like plant, (batch 2) respectively, both in Samaru, Zaria on the 8th and 10th December, 2021 respectively, declared the 6th warmest month on record (National Oceanic and Atmospheric Administration [NOAA], USA), and the warmest month in 40 years in Nigeria (Nigerian Meteorological Agency (NiMeT). Samaru, Zaria is located in the north of Nigeria with GPS coordinates of Longitude 7° 38′E and Latitude 11° 11′ N. situated at an altitude of 686m above sea level in the Northern Guinea Savanna ecological zone of Nigeria.

The environmental conditions for 2021, the rainfall distribution pattern of the NGS was monomodal with total rainfall of 1150.3mm, spread over 7 months from April to October. Relative humidity (RH) for the December was similar to the long term average of 17% and average maximum and minimum air temperatures were 29.0°C and 17.6°C respectively, against the year's average of 31.7°C and 21.0°C respectively; these criteria evaluated 2021as a normal year, though the harmattan season was just slightly warmer and dusty.

The plant was authenticated in the herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, with a Voucher sample number ABU01756. The harvested stem bark samples were air dried for about four weeks under shade at room temperature until constant weights were obtained. The dried samples were pulverized with mortar and pestle.

Fertility assessment of tree grown soils on *A. leiocarpus*

Soil samples were collected from the base of the tree trunk, 30 to 40 cm around the tree stem, from multiple sampling points at two depths, 0 – 10 cm and 10 – 15 cm, considered to be the

root zone for nutrient uptake. A composite sample was thereafter derived from these multiple points for each depth and tree soil and processed by air drying for 3 days, sieved using the 2mm mesh sieve to remove the larger diameter stones and organic materials and analyzed for basic properties in the laboratory using standard procedures.

Particle size distribution for soil textural classification was determined by the Hydrometer method [36]. Soil pH was determined in 1:2.5 soil and solution ratio in distilled water and 0.01M $CaCl_2$ [37]. Organic carbon as described by Juo [38] and total nitrogen as described by Bremner [39]. Available phosphorus by the Bray No. 1 method [40]. Exchangeable bases (Ca, Mg, K and Na) by the NH_4OAc saturation and extraction [41], cation exchange capacity (CEC) by ammonium acetate saturation method [42]. Extractable micronutrient elements (Fe, Mn, Cu, Zn and Co) in solution were estimated using the Atomic Absorption Spectrophotometer. Exchangeable acidity (H^+ and Al^{3+}) was determined by the KCl method [42] and electrical conductivity was determined by water saturation using the Conductivity Bridge instrument, Metrohn E587 conductometer [41].

Ethanolic Extraction of *A. leiocarpus* Stem Bark

The pulverized sample was subjected to a cold maceration method using 95% v/v ethanol to obtain the plants ethanolic extract, as detailed previously [17,29,32].

Qualitative phytochemical screening of ethanolic extract of *A. leiocarpus*

The different phytochemical constituents in the ethanolic extract were detected by applying standard procedures and protocols, as applied earlier [17,29,32].

Quantitative phytochemical screening of ethanolic extract of *A. leiocarpus*

Standard procedures were applied to determine the yields (expressed in percentages) of alkalids, flavonoids, saponins, tannins and phenols in the ethanolic extract, as described earlier [17,32].

Partition of Ethanolic Extracts into Fractions

The ethanolic extracts of the stem barks, batches 1 and 2 were mixed for this purpose. The ethanolic extract was partitioned with ethyl acetate [43]. About 50 -100 mL of water was placed into a separating funnel to form a crude suspension, and 500 mL of ethyl acetate was added into the separating funnel; thereafter the mixture was vigorously shaken 3-4 times at 2-3 min intervals. The funnel was kept standing and undisturbed for 1 hr. Following the separation of the mixture into layers, the upper layer of ethyl acetate was removed with caution. This was repeated severally so as to ensure that all ethyl acetate soluble compounds were released. The ethyl acetate fraction was concentrated with the aid of the rotary evaporator, while the aqueous fraction was concentrated on water bath before being stored at 20°C.

Column Chromatography

The ethyl acetate fraction was subjected to column chromatography packed with silica gel (70-230 mesh). Briefly, silica gel (500 g) was mixed with 95% n-hexane and 5% ethyl acetate; the homogenous suspension/slurry formed was stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then poured into a glass column. The sample loaded on the column was prepared by dissolving 20 g of the ethyl acetate fraction in 100 mL of 9:1 of n-hexane and ethyl acetate, to which 30g of silica was added and mixed by stirring with a glass rod. The mixture was allowed to dry at room temperature and the dried silica extract mixture was layered on the column layer bed. The column was first eluted with n-hexane as the mobile phase with the polarity increasing by 5 % increments of ethyl acetate. After getting to 100 % ethyl acetate, the polarity was further increased by 5 % increments of methanol. For each eluent system, the fractions were collected in vials. The fractions collected were concentrated to dryness by evaporation at 40 ^{0}C [44].

Thin Layer Chromatography

Thin layer chromatography (TLC) of the concentrated fractions collected from the column chromatography was carried out. Briefly, a spot of each fraction was carefully applied with capillary tube onto a thin layer chromatographic plate (coated with silica) and left to dry. After about five minutes, the plate was dipped in a suitable solvent which allowed the compounds in the spot to move upwards by capillary attraction. The plate was then removed from the solvent and left to dry. The positions of different compounds were observed by fluorescence under UV-light and was sprayed with sulfuric acid for the presence of specific compounds. Fractions with similar TLC profiles were pooled [45].

Bioassay for Active Ingredients

Acute oral toxicity studies

The revised limit dose test, up and down procedure by Dixon [46] was used to evaluate the acute oral toxicity of the purified extract of *Anogeissus leiocarpus* in adult male and female rats as described earlier [47]. Five (5) adult male and female rats respectively were selected at random, for the experiment. They were labelled and housed in cages in the laboratory for 7 days to allow for acclimatization to the laboratory conditions. The rats were fasted overnight but allowed free access to water prior to dosing on each occasion. A single rat was picked, weighed and dosed orally with a limit dose of 2000 mg/kg body weight of the freshly prepared extract. Another animal from the same gender was given the same dose of the extract until all the 5 animals were sequentially fed with the same dose of the extract. Same procedure was done for the other gender. The male and female rats were administered the purified extract on the 18th May, 2022, using 18G cannula oral gavage. Each animal was observed each time for instant death and then monitored for the successive 24hr for the short-term outcome and finally for the next 14 days for any delayed toxic effects [48, 49].

Constitution of the purified extract of *A. leiocarpus*

Two thousand and five hundred (2,500) mg. of the purified extract was dissolved in 5ml of distilled water to produce a concentration of 500mg/ml.

Determination of biomarkers of Liver and Kidney damages (Liver and Kidney function tests).

At the 24th hour post administration (p.a) of the purified extract, 3 males and 3 females were humanely sacrificed, blood samples were collected for liver and kidney function tests [50] to investigate any toxic effect on these organs.

The remaining 4 rats comprising of 2 males and 2 females were left and observed for the next 14days for any delayed effects of the limit dose and on pregnancy, gestation, pups (kitten) and reproductive performance. Their feeds (diets) were changed to growers' mash.

The 2 remaining female rats now relabeled RF1 and RF2 were allowed to undergo a minimum of 2 cycles of pregnancy, gestation and parturition of pups (kitten).

Hormonal Assays

Progesterone and Testosterone

Repeated and multiple blood samplings of the pregnant rats were avoided, not to induce additional risk factors.

Haematology and Reticulocyte counts: Haematological parameters were enumerated [50] on day 0 and 24hrs post-administration. Reticulocytes were identified [20], enumerated 24hrs post administration, 5 and 14 days later.

Statistical Analysis

Paired t-test student t: samples for means was applied to all data and *p* values less than 0.05 were considered significant.

RESULTS

Batches 1 and 2 of the harvested stem bark of *A. leiocarpus* had lost 42.7 and 43.6% of water by the first week of the drying process, while minimal water losses occurred between the first and second weeks, reaching 42.9 and 44.5% for batches 1 and 2, respectively. Complete dryness was achieved by the second week and the harvested stem bark had constant weights of 1.355 and 1.892kg between the second and fourth weeks for batches 1 and 2, respectively (Table 1).

Table 1: Locations, weight changes (and percentage water loss) of stem bark of *A. leiocarpus* pre-ethanolic extraction

Batch	1	2
Location of collection	Area BZ, Main Campus ABU, Zaria	Area C, Main Campus, ABU, Zaria
Date of collection	Wednesday, 8th Dec., 2021	Friday, 10th Dec., 2021
Weight of harvested sample	8th Dec. 2021	10th Dec., 2021
	2.372kg	3.410kg
Weight, 1 week after collection (and percent water loss)	15th Dec., 2021	17th Dec., 2021
	1.360kg (42.7%)	1.923kg. (43.6%)
Weight, 2 weeks after collection (and percent water loss)	22nd Dec., 2021	24th Dec., 2021
	1.355kg (42.9%)	1.892kg (44.5%)
Weight, 3 weeks after collection (and percent water loss)	29th Dec., 2021	31st Dec., 2021
	1.355kg (42.9%)	1.892kg (44.5%)
Weight, 4 weeks after collection (and percent water loss)	05th Jan., 2022	07th Jan., 2022
	1.355kg (42.9%)	1.892kg (44.5%)
Final weight (and percent packaging and transportation loos) pre-pulverization and ethanolic extraction	17t Jan., 2022	17th Jan., 2022
	1.345kg (0.7%)	1.879kg (0.7%)

The characteristics of soils of both tree sites are presented in Table 2. The soil textural class of both tree locations was loamy. The pH of both locations were 6.90 and 6.59 for surface soil (0 – 10 cm depth) for soils 1 and 2 respectively. The subsurface soil (10 – 15 cm, also within the plow layer and root zone of plants) had pH values of 6.92 and 6.75 for both locations respectively. The OC contents of the soils 1 and 2 were low, but much higher than the average for savanna soils. Total nitrogen (TN) contents for the soils were also low as characteristic for savanna soils. The available phosphorus content of the soils was classified as medium and adequate for these loamy soils. The exchangeable potassium content was good for soil 1 and low for soil 2, with reference to the accepted critical value of 0.2 cmol/kg. The CEC values of the soils were low, the micronutrient contents of the soils were good, with soil 2 being better supplied with the elements in comparison. Electrical conductivity (EC) was lower for soil 2 and values for both soils were far below the 4 dSm^{-1} considered as critical value for saline soils. A summary assessment placed soil 2 as the more fertile and productive, but only marginally so.

Table 2: Some Basic Chemical and Physical Properties of the Soils Sampled from the Sites of the Growing *A. leiocarpus* Plants.

	Soil 1		Soil 2	
Parameters	**0 – 10cm**	**10 – 15cm**	**0 – 10cm**	**10 – 15cm**
Particle Size Distribution:				
Clay %	10	16	14	16
Silt %	44	44	46	42
Sand %	46	40	40	42
Textural Class	Loam	Loam	Loam	Loam
Chemical Properties				
pH (H_2O)	6.90	6.92	6.59	6.75
pH (0.01M $CaCl_2$)	6.17	5.77	5.50	5.60
Org. Carbon %	1.413	0.655	1.602	0.524
Total N %	0.630	0.420	0.385	0.350
Avail. P mg/kg	8.75	7.55	8.23	6.52
EC dSm^{-1}	0.24	0.10	0.07	0.08
Exchangeable Bases, Acidity and CEC ($cmol^+/kg$)				
Ca^{2+}	2.78	2.36	2.84	2.02
Mg^{2+}	1.48	1.46	1.51	1.49
K^+	0.32	0.37	0.17	0.13
Na^+	0.26	0.35	0.17	0.14
$H^+ + Al^{3+}$	0.60	0.80	0.80	0.60
CEC	5.80	5.50	5.60	4.80
Micronutrients (mg/kg)				
Fe	166.15	156.34	145.63	128.18
Mn	31.34	24.42	44.54	28.88
Cu	2.18	2.49	1.17	2.38
Zn	2.14	4.04	5.16	5.14
Co	0.93	0.43	1.38	0.42

EC = Electrical Conductivity, CEC = Cation Exchange Capacity.

Yields from Ethanolic Extraction

From batch 1 a total of 224.2gm (16.67%) was obtained while batch 2 yielded a total of 227.38gm (12.10%). This is a total (combined) yield of 451.58gm (14.01%).

Phytoconstituents:

The phytoconstituent of the ethanolic extracts of *A. leiocarpus* are presented in Table 3.

Table 3: Qualitative Phytochemical Screening of Batches 1 and 2

S/No	Phytoconstituents	Test	Batch 1	Batch 2
1.	Alkalids	Dragendorff test	+	+
2.	Cardiac Glycosides	Keller-Kiliani test	+	+
3.	Saponins	Frothing test	+	+
4.	Phenolic compounds	Lead acetate test	+	+
5.	Tannins	Ferric Chloride test	+	+
6.	Steroids	Salkowiski test	+	+
7.	Carbohydrates	Molisch test	+	+
8.	Flavonoids	Shinoda test	+	+
9.	Terpeniods	Liebermann Burchard test	+	+
10.	Anthraquinones	Bontragers test	-	-

KEYS + = Present, - = Absent

Column chromatography, Ethyl Acetate and Aqueous Fraction:

A total of 150 ethyl acetate fractions and 57 aqueous fractions were collected. The interface and three ethyl acetate fractions were observed promising and are presented in Table 4.

Table 4: Ethyl Acetate Fractions and Yields (weights) from Column Chromatography

S/No	Fractions	Weights (gm)
1	Interface	5.10
2	28 – 37	0.70
3	38 – 58	0.60
4	59 – 80	0.70

Total yields at this level is 7.10gm, a percentage yield of 1.6% (7.10/451.58)

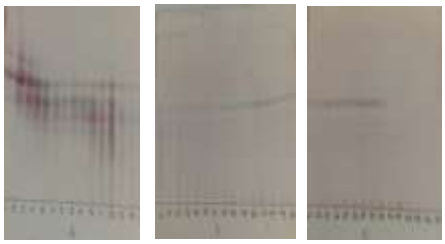
Plate 1: Thin Layer Chromatograms of Ethyl Acetate Fraction (In support of Table 4)

Final purifications of sub-fractions from column chromatography and thin-layer chromatograph of four crystals of pure compounds were produced, for NMR, structural determinations and analyses.

Fraction A, B & C under UV

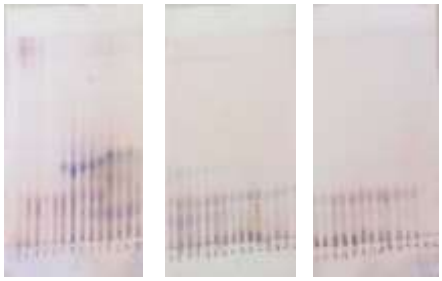
Fraction A, B & C sprayed with H_2SO_4

Thin layer chromatogram of the pooled fractions of ethyl acetate from the first column chromatography: A (28 – 37), B (38 – 58), C (59 – 80)

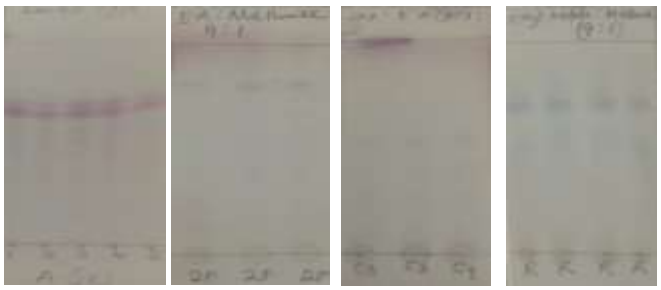


Sub-fraction A (1 – 65)

Sub-fraction B (38 – 58)



Sub-fraction C (1 – 44)



Sub-fraction A Sub -fraction B Sub-fraction C Interface

TLC of compounds isolated from column chromatography of sub-fraction A, B, C and interface

Plate 2: Purified crystals of sub-fractions A,B,C and interface

Bioassay: Table 5-7

The blood glucose levels of the rats before extract administration (at day zero) ranges between 116 and 135mg/dl.

Table 5a: Effect of administration of purified extract of *Anogeissus leiocarpus* at a limit dose of 2000mg/kg body weight on blood glucose levels (mg/dl) of normal male rats

	Blood glucose level (mg/dl)		
Rat Label	**Pre**	**Post (24 hours)**	**% Decrease**
I	135	116	14.1
II	133	118	11.3
III	127	109	14.2
IV	124	105	15.3
V	116	109	6.0

n=5 p<0.002

Table 5b: Effect of administration of purified extract of *Anogeissus leiocarpus* at a limit dose of 2000mg/kg body weight on blood glucose levels (mg/dl) of normal female rats

	Blood glucose level (mg/dl)		
Rat Label	**Pre**	**Post (24 hours)**	**% Decrease**
I	131	117	10.7
II	127	105	17.3
III	118	99	16.1
IV	125	107	14.4
V	128	123	3.9

n=5 p<0.01

Table 5c (Summary of males and females combined)

	Pre	**Post (24 hours)**	
Male	127 ± 3.39a	111.4 ± 2.42b	P<0.002
(Min-Max)	(116 – 135)	(105 – 118)	
Female	125.8 ± 2.18a	110.2 ± 4.32b	p<0.01
(Min-Max)	(118 – 131)	(99 – 123)	
Male & Female	126.4 ± 1.91a	110.8 ± 2.34b	p<0.0001
(min-Max)	(116 – 135)	(99 – 123)	

Mean (±SEM) values with different superscript values in the same row differ significantly at p<0.05 using paired t-test.

Table 6a: Haematological analysis of male rats following oral administered of limit test dose of 2000 mg/kg of purified extract of *Anogeissus leiocarpus*

Parameters (units)	Control (day 0)	24hr-post administration
HEMATOCRIT (%)	52.0 ± 4.8[a]	40.0 ± 0.8[b]
HAEMOGLOBIN (g/dl)	14.5 ± 0.8[a]	11.7 ± 0.5[b]
TRBC ($^{x}10^{9}$/L)	7.8 ± 0.3[a]	4.5 ± 1.4[b]
TWBC ($^{x}10^{9}$/L)	15.7 ± 0.6	4.7 ± 0.8[b]
MCV	65.3 ± 0.2[a]	62.0 ± 2.7[a]
MCH (%)	18.3 ± 0.7[a]	18.2 ± 0.8[a]
MCHC (%)	27.9 ± 1.1[a]	29.3 ± 0.7[a]
Platelet	440 ± 27[a]	631.3 ± 28.5[b]

Data are expressed as mean ± SD, n=5. Means with different superscript across each row are statistically significant (p<0.05). **Keys:** TWBC (Total White Blood Cells), TRBC (Total Red Blood Cells).

Table 6b: Haematological analysis of female rats following oral administered of purified extract of *Anogeissus leiocarpus* at limit test dose of 2000 mg/kg

Parameters (units)	Control (day 0)	24hr-post administration
HEMATOCRIT (%)	54.8 ± 0.9[a]	30.8 ± 0.05[b]
HGB (g/dl)	15.5 ± 0.3[a]	9.7 ± 0.05[b]
TRBC ($^{x}10^{9}$/L)	8.4 ± 0.1[a]	4.9 ± 0.04[b]
TWBC ($^{x}10^{9}$/L)	14.2 ± 2[a]	9.8 ± 1.7[b]
MCV	65.5 ± 0.2[a]	62.3 ± 0.7[b]
MCH (%)	18.5 ± 0.06[a]	19.6 ± 0.05[a]
MCHC (%)	28.2 ± 0[a]	31.4 ± 0.2[a]
Platelet	558.5 ± 48.5[a]	620 ± 5.0[b]

Data are expressed as mean ± SD, n=5. Means with different superscript across each row are statistically significant (p<0.05). **Key:** TWBC (Total White Blood Cells), TRBC (Total Red Blood Cells).

Table 7: Reticulocyte Counts (in percentages %)

Twenty-four (24) hour post administration of limit test dose of 2000mg/kg of purified extract of *A. leiocarpus* stem bark.

Male rats	Female rats
M_1 = 10.7	F_1 = 20.6
M_2 = 17.8	F_2 = 25.0
M_3 = 19.2	F_3 = 30.6
M_4 = 18.6	F_4 = 20.9
M_5 = 11.8	F_5 = 16.6

22.74±2.37 (P<0.05 significant difference mean, significantly higher in female than in male)

Five (5) days post administration of limit test dose of 2000mg/kg of purified extract of *A. leiocarpus* stem bark.

Male rats	Female rats
M_2 = 12.0	F_2 = 15.0
M_4 = 17.0	F_4 = 15.0

Fourteen (14) days post administration of limit test dose of 2000mg/kg of purified extract of *A. leiocarpus* stem bark

Male rats	Female rats
M_2 = 14.0	F_2 = 14.0
M_4 = 19.0	F_4 = 14.0

Acute and Delayed Oral Toxicity Studies

No death of the rats occurred 24hours and 14days post administration of the limit dose, 2000mg/kg body weight of the purified extract; both males and females were alert and very active (Tables 8a and 8b).

Table 8a: Acute and delayed oral toxicity of purified extract of *Anogeissus leiocarpus* at a limit dose of 2000mg/kg body weight of male rats

Rat Label	Dose (mg/kg)	Short-term effect (24 hours)	Delayed effect (14 days)
I	2000	No death	No death
II	2000	No death	No death
III	2000	No death	No death
IV	2000	No death	No death
V	2000	No death	No death

LD_{50}>2000mg/kg.bd.wt.

Table 8b: Acute and delayed oral toxicity of purified extract of *Anogeissus leiocarpus* at a limit dose of 2000mg/kg body weight of female rats

Rat Label	Dose (mg/kg)	Short-term effect (24 hours)	Delayed effect (14 days)
I	2000	No death	No death
II	2000	No death	No death
III	2000	No death	No death
IV	2000	No death	No death
V	2000	No death	No death

LD_{50}>2000mg/kg.bd.wt.

From the liver and kidney function tests, the values of their biomarkers of any liver or kidney damage after 24hours post administration of the purified extract are presented in Tables 9a and 9b, respectively.

No significant (P> 0.05) difference occurred in AST in both male and female rats. ALT and ALP liver enzymes were significantly (P<0.05) reduced between day 0 and 24hrs p.a respectively (Tables 9a and 9b).

The situation was different for blood urea by 24hrs as blood urea was significantly (P<0.02) higher in females than males.

Table 9a: Biomarkers of Liver and Kidney damage following the limit dose administration of purified extract of *Anogeissus leiocarpus* in male rats

Parameters	Pre (0 h)	Post Treatment (24 h)
AST (u/l)	58.3 ± 4.2^a	57.3 ± 2.1^a
ALT (u/l)	44.0 ± 1.0^a	32.3 ± 5.5^b
ALP (u/l)	238.3 ± 37.1^a	158.2 ± 61.3^b
BUN (mg/dl)	16.7 ± 5.5^a	1.3 ± 0.6^b

Data are expressed as mean ± SD. Means with different superscript across each row are statistically significant (*p<0.05*). **Key:** AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), ALP (Alkaline Phosphatase), BUN (Blood Urea Nitrogen).

Table 9b: Biomarkers of Liver and Kidney damage following the limit dose administration of purified extract of *Anogeissus leiocarpus* in female rats

Parameters	Pre (0 h)	Post Treatment (24 h)
AST (u/l)	51.3 ± 4.0^a	54.3 ± 5.9^a
ALT (u/l)	44.0 ± 1.0^a	34.7 ± 4.2^b
ALP (u/l)	218.0 ± 17.2^a	227.2 ± 67.6^a
BUN (mg/dl)	19.0 ± 7.0^a	11.7 ± 5.7^b

Data are expressed as mean ± SD. n=5. Means with different superscript across each row are statistically significant (*p<0.05*). **Key:** AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), ALP (Alkaline Phosphatase), BUN (Blood Urea Nitrogen).

[By 24h, mean urea concentrations were significantly (p<0.02) higher in female than in male rats]

Pregnancy and Gestation

Remaining female rat one (RF1): First parity

On the 15th June, 2022, one of the two remaining females (RF1) left for observation, developed protruding, developing mammary glands (28days post dosing of the 2000mg/kg.bd.wt. of the purified extract) (Fig. 1.1a).

Female RF1 had successful parturition with 10 pups (kitten) on the 27th June, 2022 (12days post identification of developing mammary glands and 40days post dosing of the extract) (Fig. 1.1b).

Records of the monitoring of RF1 and her 10 pups (kitten) are presented on Fig. 1.1c (14-day-old) Fig. 1.1d (20-day-old) until the pups (kitten)were weaned at 33-day-old (fig. 1.1e).

All 10 pups (kitten) were alert and very active from parturition date of 27th June, 2022 up to weaning date of 2nd August, 2022. No teratogenic effect of the administration was observed.

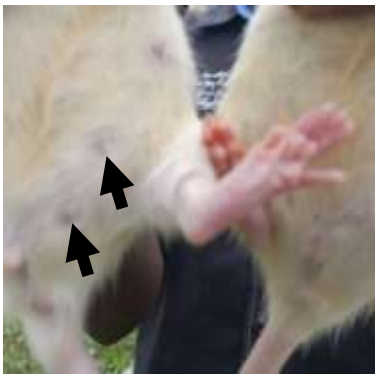


Fig. 1.1a. Female Wistar rat, left (RF1) with protruding/developing mammary glands, arrowed (June 15th, 2022)

RFig. 1.1b. RF1 with 10 day-old pups (kitten) (June 28th, 2022; parturition was evening of June 27th, 2022) No external abnormalities observed

Fig. 1.1c. RF1 with the 10, 14-day-old (2 weeks old) pups (kitten), very alert and active. No external abnormalities observed (July 12th, 2022)

Fig. 1.1d. (RF1) with the 10, 20-day-old pups (kitten); very alert and active. No external abnormalities observed. (July 18th, 2022)

Fig. 1.1e. The 10, 33-day-old pups (kitten) were weaned, remaining alert and very active with no external abnormalities observed. (Five males and five females) **RF1 was re-introduced to a male for another cycle. (August 2nd, 2022)**

Second Parity

RF1 showed developing mammary glands on the 30th August, 2022 and had a successful parturition, producing 7, day old pups (kitten) on September 8th, 2022 with no external abnormalities observed. Monitoring continued and the 34-day-old pups (kitten), made up of six males and one female, very active and alert with no external abnormalities, were weaned on October 12th, 2022. RF1 was re-introduced to a male for another cycle of parity (see Figures 1.2a – 1.2e).

RF1: Second Parity

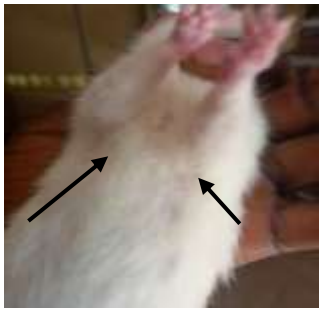


Fig 1.2a. Female Wistar rat (RF1) with protruding/developing mammary glands, arrowed (August 30th, 2022).

Fig 1.2b. RF1 with 7 day-old pups (kitten) (September 8th, 2022). No external abnormalities observed.

Fig 1.2c. Female Wistar rat (RF1) with 7, 12-day-old pups (kitten), very alert and active. No external abnormalities observed. (September 20th, 2022).

Fig 1.2d. Female Wistar rat (RF1) with 7, 26-day-old pups (kitten), very alert and active. No external abnormalities observed (October, 4th, 2022) [6 males and 1 female].
The pups (kitten) were weaned on October 13th, 2022, when they were 35 days old.

Fig 1.2e. Female Wistar rat (RF1) with 34-day-old pups (kitten), very alert and active. No external abnormalities observed (October, 12th, 2022).
(Six males and one female)
The pups (kitten) were weaned the next day October 13th, 2022 and RF1 was re-introduced to a male for another cycle (October 13th, 2022).

Third Parity

RF1 showed signs of developing mammary glands in December 16th, 2022 and had a successful parturition at 5:30pm, on December 26th, 2022, producing 7, day-old pups (kitten) with no external abnormalities observed (see Figures 1.3a and 1.3b).

RF1: Third Parity

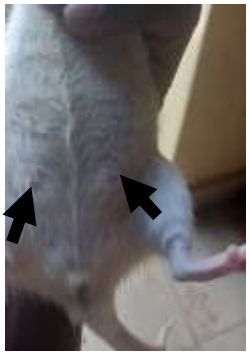


Fig 1.3a. Female Wistar rat (RF1) with protruding/developing mammary glands, arrowed.
(December 16th, 2022).

Fig 1.3b. RF1 with 7 day-old pups (kitten) (December 27th, 2022) parturition, at 5:30pm, December 26th, 2022. no external abnormalities observed.

Fourth Parity

Protruding mammary glands were identified on March 23rd, 2023 and successful parturition occurred on April 8th, 2023 with 7-day-old-pups (kitten). No external abnormalities were observed. (see figs 1.4a and 1.4b).

RF1: Fourth Parity

Fig. 1.4a: RF1; protruding mammary glands (arrowed). March 23rd, 2023

Fig. 1.4b: RF1 with 7-day-old pups (kitten). Parturition, 8th April, 2023. No external abnormalities observed

"Second Generation"

RF1/2.

First Parity

A successful parturition occurred on March 5th, 2023 with 6-day-old-pups (kitten). No external abnormalities were observed. (see figs 1.1/2a and 1.1/2b). [RF1/2, a female wistar rat from RF1]

RF1/2: First Parity

Fig. 1.1/2a. RF1/2 March 6th, 2023. Parturition, March 5th 2023 with 6-day-old pups (kittens). No abnormalities were observed.
[RF1/2, a female wistar rat from RF1; see Figs 1.2d and 1.2e].

Remaining female rat two (RF2): First parity

RF2 had delayed conception and only displayed suspicious size and movements of pregnancy on October 31st, 2022 before a successful parturition on November 1st, 2022, producing 8, day-old pups (kitten). No external abnormalities were observed (see Figures 2.1a and 2.1b).

Remaining Female Rat 2 (RF2): First Parity

Fig.2.1a. RF2 a day pre-parturition. (October 31st, 2022)

Fig.2.1b. RF2 with 8 day old pups (kitten) at parturition (November 1st, 2022). No external abnormalities observed.

Second Parity

RF2 had a successful parturition on December 16th, 2022 producing 7, day-old pups (kitten) with no external abnormalities. The pups were monitored and weaned in January 25th, 2023 at 40-day-old with no external abnormalities observed (see Figures 2.2a, 2.2b and 2.2c).

RF2: Second Parity

Fig 2.2a. RF2 with 7 day-old pups (kitten) at parturition (December 16th, 2022). No external abnormalities observed.

Fig 2.2b. RF2 with 7, 11 day-old pups (kitten) (December 27th, 2022). No external abnormalities observed.

Fig 2.2c. RF2 with 7, 29-day-old pups (kitten), No external abnormalities observed. (January 25th, 2023). The pups were weaned and RF2 was re-introduced to a male for another cycle.

"Second Generation"

First Parity

RF2/2

Protruding mammary glands were identified on March 30th, 2023 and a successful parturition occurred with 2-day-old pups (kitten). No external abnormalities were observed. (see fig. 2.1/2).

[RF2/2, a female Wistar rat from RF2, see figs 2.2a, 2.2b and 2.2c]

"Second Generation"

First Parity

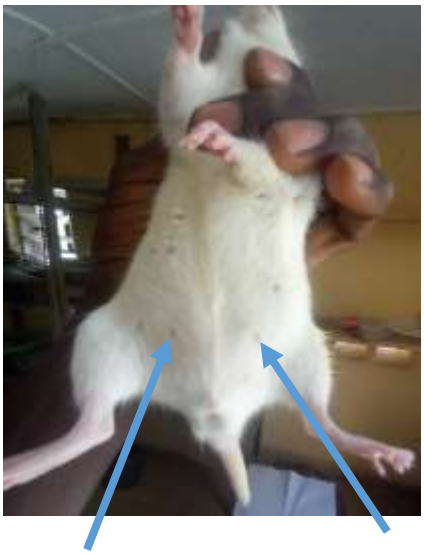


Fig. 2.1/2a. RF2/2. Protruding mammary gland (arrowed) March, 30th 2023. Parturition with 2-day-old pups (kitten).

[RF2/2, a female Wistar rat from RF2; see Figs. 2.2a, 2.2b and 2.2c]

DISCUSSION

The complete loss of water and the accompanying full dryness of the harvested stem bark of *A. leiocarpus* at the end of the second week agrees with the two week drying period (under shade) applied by others [17,29,32]. Important factor that facilitated the fast drying period of the stem bark of *A. leiocarpus* are the environmental meteorological indices enumerated earlier and the cold but dry harmattan season of the study area. A summary assessment of the soils in comparison placed soil number 2 as more fertile and productive, but only marginally. It may be recalled that plant 1 is shrub-like while plant 2 is tree-like. However, the overall value met the recommended standards for non-deficient soils [51]. The plants, if taken as ecotypes can rely on structures developed through adaptation to their habitats to perform

better; these structures include better root proliferation, hence stronger soil nutrient acquisition which invariably translates to higher nutrient uptake.

The phytochemical screenings of the two batches of ethanolic extracts of *A. leiocarpus* stem bark were identical and agree with earlier reports and as calculated previously [17,29,32]. These identical phytoconstituents of the two batches and the very apparent similarities of the fertility assessment of the neighbouring soils of the two batches informed the mixing of the ethanolic extracts from the two batches for their partitioning into fractions. The interface had the greatest yield 5.10gm strongly suggesting that most of the active compounds exist in the interface. This speculation is derived from the notion that 451.58gm of the ethanolic extract of the stem bark, partitioned with ethyle acetate fractions and subjected to column chromatography may not be expected to yield 0.70gm, 0.60gm and 0.70gm respectively (Table 4). More supports for this speculations are; crude ethanolic extracts of *A. leiocarpus* had hypoglycaemic and haemopoietic effects when administered orally to normal Wistar rats over a two-week period [29] which adequately betrayed the antidiabetic and haemopoietic activities of this plant; extracts of *A. leiocarpus* reduced blood levels and expressed antioxidant effect in alloxan-induced diabetic rats [30]; total extract and fractions of *A. leiocarpus* exhibited antihyperglycaemic activity in mice [31]. In addition, ethanolic extracts of *A. leiocarpus* ameliorated hyperglycaemia, hepato-renal damage, deranged electrolytes and acid-base balance in alloxan-induced diabetes in dogs [17], an indication of an efficacy on organic damages associated with T2DM; it expressed antidiabetic and antioxidant activities in alloxan-induced diabetes in Wistar rat [33] including its attenuation of dyslipadaemia in alloxan-induced diabetes in dogs [34]. In addition, recently, ethanolic extracts of *A. leiocarpus* exhibited sialoglycoconjugate and hence sialic acids modulating effect which very effectively demonstrated that elevated serum sialic acids as a potent biomarker of alloxan-induced diabetes in dogs [32], with elevated serum sialic acids serving as additional DM predictive and prognostic values with *A. leiocarpus* [32] in addition to its accelerated wound-healing activity [35]. It is therefore, incomprehensible, scientifically, that 451.58gm of the ethanolic extract of *A. leiocarpus*, with the above-listed health benefiting activities, would yield only 0.60gm or 0.70gm of active compounds after maximum purification. This justifies the use of interface for bioassay, although the 0.60gm or 0.70gm fractions were insufficient for bioassay.

Much credence and greater support for these speculation and justification were provided when Wistar rats used for lethal dose (LD_{50}) and bioassay studies exhibited highly significant ($p<0.002$; $p<0.01$ and $p<0.0001$) reduction of blood glucose levels in males, females and male/female combined, respectively only 24hours after the oral administration of the purified extract of *A. leiocarpus* stem bark, using the interface. Normocytic normochromic anaemia developed within 24hours of administration of the lethal high limit does but reticulocyte counts, increased simultaneously and sequentially in the rats, an indication of erythropoiesis. The reduction of blood glucose levels and the reticulocysis in this normal rats agree with earlier report [17,32]. The anaemia over 24 hours was normocytic normochromic suggestive of a dilution effect on the blood (hydraemia) from the large dose of the purified extract and the marked reticulocytic response implied erythropoietic and thrombopoitic activities in the purified extract due to the thrombocytosis exhibited. The justification for the use of both males and females as experimental model had been clarified [17,32].

Acute oral toxicity and delayed toxicity studies with the purified extract of *A. leiocarpus* showed a lethal dose (LD_{50}) greater than 2000mg/kg of body weight which strongly suggests that this non-conventional treatment has a wide margin of safety.

The global threat of T1DM and T2DM to the human population worldwide their pet animals, their equine power animals and their food animals strongly supports the research for a non-conventional drug for T1DM and T2DM, the risk factors and the unquantifiable accompanying social menace and the biomarker aspect [32] is supported by the review to detect pre-diabetes using biomarkers [52].

During the process of final purifications through column chromatography and supported with thin-layer chromatography, crystallization from the sub-fractions and interface is an indication of purity of each of the four different compounds.

The administration of the purified extract of *A. leiocarpus* stem bark at a limit dose of 2000mg/kg.bd.wt. did not cause death of any of the rats within 24hourse and subsequent 14 days post administration. Indeed, the remaining 4, 2 male and 2 female rats survived 3 parities and uptil April 30th, 2023. This is in line with earlier study that reported the one median lethal dose of aqueous leaf extract of *Anogeissus leiocarpus*; the oral LD_{50} was observed to be greater than 2000mg/kg.bd.wt. in rat [53], and for aqueous and methanolic extract, it was greater than 5,000mg/kg.bd.wt. in rats [54].

The normal ranges of AST, and the significant reductions of ALT and ALP activities 24hrs thereafter, confirmed absence of functional defects in the hepatocytes of the livers of the rats. Since the limit dose of the purified extract of *A. leiocarpus* stem bark was administered orally, the liver, usually exposed to toxic substances, through the hepatic portal vein, did not manifest signs of toxic assault. Hydraemia may have contributed, partly, to the significant reduction of ALT and ALP at 24hours, while the non-reduction of AST at 24hours may be associated with AST presence in other sources, such as skeletal muscles [50]. In addition, the excretion of the purified extract of *A. leiocarpus* stem bark, either intact or as metabolites, had no deleterious effect on the kidney parenchyma, as kidney damage biomarker, urea concentrations, were normal in the present study. Therefore, administration of the limit dose of the purified extract of *A. leiocarpus* stem bark has no adverse effect on the liver and kidney of Wistar rats, within the first 24 hours. Further evaluation of these biomarkers showed no delayed toxicity in the liver and kidneys, 14days post administration of the limit dose of the purified extract of *A. leiocarpus* stem bark.

However, a remarkable observation showed a significant and a highly significant reduction of blood urea concentrations in the female, rats between day 0 and 24 hours post administration and the male rats against the female rats, also 24 hours post administration, respectively, of the limit dose. These significant reductions in the concentrations of blood urea concentrations in the females and the highly significant reduction of same, in the males against the females, 24hours post administration suggest accelerated or enhanced clearance of urea from the blood, which is highly suggestive of a detoxifying effect of *A. leiocarpus.* Furthermore, hydraemia may have contributed to urea reduction. The highly significant reduction of blood urea in the male rats against the female rats, 24 hours post administration further suggests a markedly reduced production of urea in the liver of male rats, against the female rats. A possible factor responsible for this significant reduction of blood urea in the male rats may be due to a reduced production of toxic ammonia in the liver of the male rats. In humans and other mammals, urea production was ascribed to liver detoxification of large amounts of ammonia generated from the deamination of amino acids [50] in

contrast to the Dalmatian breed of dogs, birds and reptiles with uric acid as end-product of detoxification of ammonia [50].

The remaining female rats, RF1 and RF2, equally administered the limit dose of *A. leiocarpus* stem bark got pregnant, and exhibited signs of existing pregnancies, such as enlarged mammary glands. Delayed conception occurred in RF2, a phenomenon that is not unusual in humans and animals. From the observations of a minimum of two parities each, successful parturitions occurred within the short 3-4 days oestrous cycle and the 21-23 days of gestation in rats. Therefore, it is being suggested that progesterone was elevated on these pregnant female rats to maintain these cycles of pregnancies during these different parities.

The pups (kitten) from the normal parturitions of all the first, second and third parities are of reasonable numbers, 10 7 and 7 for RF1 and 8 and 7 for RF2, respectively in the present study,

The pups (kitten) were very alert, active and had no external abnormities from parturition to weaning ages ranging from 33 to 35 days, hence no teratogenic manifestation.

The data from the present study imply that the limit does of 2000mg/kg.bd.wt of the purified extract of *A. leiocarpus* stem bark has no deleterious effects on pregnancy, gestation, parturition, the pups (kitten) and the reproductive performance of Wistar rats. In addition, purified extract of *A. leiocarpus* stem bark as a non-convential treatment for diabetes mellitus has a wide range of safety value. Indeed, 100mg/kg.bd.wt of the purified extract of *A. leiocarpus* stem bark was very effective in treating alloxan-induced diabetic rats (part of another manuscript).

The rats were able to carry their pregnancies to term and they littered successfully. This implies that the limit dose of 2000 mg/kg.bd.wt. of the purified extract of *A. leiocarpus* stem bark had no deleterious effect on their pregnancies. However, work is ongoing to determine ovarian function and reproductive hormonal levels before, during and after pregnancy of rats that are fed the plant.

CONCLUSION

Oral administration of a limit dose, (2000 mg/kg/bd.wt.) of purified extracts of *A. leiocarpus* stem bark is relatively safe as it caused no death and no hepato-renal damage in Wistar rats. It had no deleterious effects on pregnancy, gestation parturition, pups (kitten) and reproductive performance. It did not produce teratogenic effect. *A. leiocarpus* has a wide range of safety value as a drug for diabetes mellitus.

Acknowledgement

The Authors acknowledge the technical supports of Adamu Mohammed, Kabiru Ibrahim, Department of Pharmacognosy and Drug Development, Dennis Otie, and Sunusi Musa Department of Pharmacology and Toxicology, ABU, Zaria.

Authors contribution

Esievo, KAN: Conceptualisation; Supervision; Investigation; Writing, Editing; Correspondences

Esievo, LO: Investigation; Ethanolic Extraction and Purification, Pregnancy; Gestation; Writing

Sani, D: Supervision; Investigation; Bioassay; Writing, Editing

Esievo, KO: Investigation; Soil Composition; Ethanolic Extraction and Purification, Writing

Esievo, EM: Investigation; Soil Composition; Ethanolic Extraction and Purification, Writing

Balogun, EO: Supervision; Investigation; Writing; Editing

Wassagwa, J: Investigation; Purification; Writing; Editing

Rekwot, PI: Supervision; Investigation; Pregnancy; Gestation; Writing; Editing

Allam, L: Supervision; Investigation; Pregnancy; Gestation; Writing; Editing

Uyovbisere, EO: Supervision, Invsetigation; Soil composition; Weather conditions; Writing; Editing

Funding source

The funding was partly funded by the Research and Diagnosis Unit of Kanesco Global Services Limited (RC 829505) with additional funds from supervising Authors.

Conflict of interest

The Authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national and institutional guidelines for the care and use of animals were followed. This article contains studies with animal subjects performed by the authors under an existing ethical approval by the committee on Animal use and care. (Ethical clearance No. ABUCAC/2019/16).

REFERENCES

1. Punthakee Z, Goldenberg R, Katz P, Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome, Canadian Journal of Diabetes, 2018; 42:1S10-5. https://doi.org/10.1016/j.jcjd.2017.10.003 PMid:29650080

2. Qureshi M, Gammoh E, Shakil J, Robbins R, Update on management of type 2 diabetes for cardiologists, Methodist DeBakey Cardiovascular Journal, 2018; 14(4): 273-280. https://doi.org/10.14797/mdcj-14-4-273 PMid:30788013 PMCid:PMC6369620

3. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE, Global estimates of diabetes prevalence for 2013 and projections for 2035, Diabetes Research and Clinical Practice, 2014; 103(2):137-49. https://doi.org/10.1016/j.diabres.2013.11.002 PMid:24630390

4. Amjad S, Jafri A, Sharma AK, Serajuddin M. A, novel strategy of nanotized herbal drugs and their delivery in the treatment of diabetes: Present status and future prospects, Journal of Herbal Medicine, 2019; 17:100279. https://doi.org/10.1016/j.hermed.2019.100279

5. World Health Organization, Global report on diabetes: executive summary, World Health Organization; 2016.

6. International Diabetes Federation, IDF Diabetes Atlas.8th ed. 2017.

7. Armstrong DG, Boulton AJ, Bus SA, Diabetic foot ulcers and their recurrence, New England Journal of Medicine, 2017; 376(24):2367-75. https://doi.org/10.1056/NEJMra1615439 PMid:28614678

8. Skrepnek GH, Mills Sr JL, Lavery LA, Armstrong DG, Health care service and outcomes among an estimated 6.7 million ambulatory care diabetic foot cases in the US, Diabetes Care, 2017; 40(7):936-42. https://doi.org/10.2337/dc16-2189 PMid:28495903

9. Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, Qin R, Wang H, Shen Y, Du K, Zhao L, Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes/Metabolism Research and Reviews, 2020; 36(7):e3319. https://doi.org/10.1002/dmrr.3319 PMid:32233013 PMCid:PMC7228407

10. Hussain A, Bhowmik B, do Vale Moreira NC, COVID-19 and diabetes: Knowledge in progress. Diabetes Research and Clinical Practice, 2020; 162:108142. https://doi.org/10.1016/j.diabres.2020.108142 PMid:32278764 PMCid:PMC7144611

11. Zhu L, She ZG, Cheng X, Qin JJ, Zhang XJ, Cai J, Lei F, Wang H, Xie J, Wang W, Li H, Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes, Cell Metabolism, 2020; 31(6):1068-77. https://doi.org/10.1016/j.cmet.2020.04.021 PMid:32369736 PMCid:PMC7252168

12. Kumar V, Ahmed D, Gupta PS, Anwar F, Mujeeb M, Anti-diabetic, anti-oxidant and anti-hyperlipidemic activities of Melastoma malabathricum Linn. leaves in streptozotocin induced diabetic rats, BMC Complementary and Alternative Medicine, 2013; 13(1):1-9. https://doi.org/10.1186/1472-6882-13-222 PMid:24010894 PMCid:PMC3847142

13. Vega GL, Grundy SM, Current trends in non-HDL cholesterol and LDL cholesterol levels in adults with atherosclerotic cardiovascular disease, Journal of Clinical Lipidology. 2019; 13(4):563-7. https://doi.org/10.1016/j.jacl.2019.05.012 PMid:31248654

14. Ponnanikajamideen M, Rajeshkumar S, Vanaja M, Annadurai G, In vivo type 2 diabetes and wound-healing effects of antioxidant gold nanoparticles synthesized using the insulin plant Chamaecostus cuspidatus in albino rats, Canadian Journal of Diabetes, 2019; 43(2):82-9. https://doi.org/10.1016/j.jcjd.2018.05.006 PMid:30413371

15. Blakytny R, Jude E, The molecular biology of chronic wounds and delayed healing in diabetes, Diabetic Medicine, 2006; 23(6):594-608. https://doi.org/10.1111/j.1464-5491.2006.01773.x PMid:16759300

16. Ihedioha JI, Enahoro G, Prevalence of diabetes mellitus and reference values for the fasting blood glucose levels of locally available breeds of dogs in Warri, Nigeria, Comparative Clinical Pathology, 2019; 28:1107-12. https://doi.org/10.1007/s00580-019-02938-7

17. Num-Adom SM, Adamu S, Aluwong T, Ogbuagu NE, Umar IA, Esievo KA, Ethanolic extract of Anogeissus leiocarpus ameliorates hyperglycaemia, hepato-renal damage, deranged electrolytes and acid-base balance in alloxan-induced diabetes in dogs, Scientific African, 2022; 16:e01183. https://doi.org/10.1016/j.sciaf.2022.e01183

18. Meier H, Diabetes Mellitus in Animals: A Review, Diabetes, 1960; 9(6):485-9. https://doi.org/10.2337/diab.9.6.485

19. Jayalakshmi K, Selvaraj P, Veeraselvam M, Yogeshpriya S, Venkatesan M, Diabetic ketoacidosis in a buck: a case report, Iranian Journal of Veterinary Research, 2019; 20(3):213.

20. Sharma GU, Diabetes mellitus in a buffalo-cow, Indian Veterinary Journal, 1941; 17:370.

21. Hui H, Zhao X, Perfetti R, Structure and function studies of glucagon-like peptide-1 (GLP-1): the designing of a novel pharmacological agent for the treatment of diabetes, Diabetes/Metabolism Research and Reviews, 2005; 21(4):313-31. https://doi.org/10.1002/dmrr.553 PMid:15852457

22. Raj RR, Sahay SS, Tripathi JT, Medications of diabetes mellitus and antidiabetic medicinal plants: a review. International Journal of Indigenous Herbs and Drugs, 2016; 31:19-28.

23. Balogh E, Toth M, Bölcsházi G, Abonyi-Toth ZS, Kocsis E, Semjen G, Oral hypoglycaemic drugs in alloxan-induced diabetes mellitus in dogs, Acta Veterinaria Brno, 2008; 77(3):363-71. https://doi.org/10.2754/avb200877030363

24. Rao MU, Sreenivasulu M, Chengaiah B, Reddy KJ, Chetty CM, Herbal medicines for diabetes mellitus: a review. International Journal of Pharmaceutical Technology Research, 2010; 2(3):1883-92.

25. Gaikwad S, Krishna Mohan G, Sandhya Rani M, Phytochemicals for diabetes management, Pharmaceutical Crops, 2014; 5(1). https://doi.org/10.2174/2210290601405010011

26. Govindappa M, A review on role of plant (s) extracts and its phytochemicals for the management of diabetes, Journal of Diabetes Metabolism, 2015; 6(7):1-38.

27. Bacanli M, Effects of phytochemicals against diabetes, Advances in Food and Nutrition Research (2019): 1-28. https://doi.org/10.1016/bs.afnr.2019.02.006 PMid:31351526

28. Mursiti S, Matsjeh S, The Hypoglycemia Effect of Alkaloid Compounds from Oil Free Mahagony Seeds (Swietenia macrophylla, King), European Journal of Medicinal Plants, 2016; 16(4):1-5. https://doi.org/10.9734/EJMP/2016/18574

29. Num SM, Oladele SB, Esievo KA, Useh NM, Some observations in Wistar rats administered ethanol extracts of the stem barks of Anogeissus leiocarpus, Asian Journal of Pharmacology and Toxicology, 2014; 2(03):4-10.

30. Onoja US, Ugwu CC, Uzor PF, Nweze IE, Omeje EO, Nnamani PO, Nwachukwu E, Njoku I, Effiong EJ, Effect of Anogeissus leiocarpus guill and perr leaf on hyperglycaemia and associated dyslipidaemia in alloxan-induced diabetic rats, Dhaka University Journal of Pharmaceutical Sciences, 2018; 17(1):65-72. https://doi.org/10.3329/dujps.v17i1.37120

31. Motto EA, Lawson-Evi P, Kantati Y, Eklu-Gadegbeku K, Aklikokou K, Gbeassor M, Antihyperglycemic activity of total extract and fractions of Anogeissus leiocarpus, Journal of Drug Delivery and Therapeutics, 2020; 10(3):107-13. https://doi.org/10.22270/jddt.v10i3.4078

32. Esievo KA, Num-Adom SM, Adamu S, Ogbuagu NE, Aluwong T, Umar IA, Elevated serum sialic acids, a potent biomarker of alloxan-induced type 1 diabetes in dogs by ethanolic extract of Anogeissus leiocarpus, Journal of Diabetes & Metabolic Disorders. 2021; 20:179-86. https://doi.org/10.1007/s40200-021-00726-1 PMid:34178829 PMCid:PMC8212296

33. Num-Adom SM, Adamu S, Aluwong T, Ogbuagu NE, Umar IA and Esievo KAN, Antidiabetic and antioxidant activities of ethanolic extract of Anogeissus leiocarpus in alloxan induced type 1 diabetes mellitus in Wistar rats, EC Diabetes and Metabilic Research., 2022b; 6.1.

34. Num-Adom SM, Ogbuagu NE, Aluwong T, Adamu S, Umar IA and Esievo KAN "Dyslipidaemia of alloxan-induced type 1 diabetes in dogs is attenuated by ethanolic extracts of A. leiocarpus stem bark". Diabetes & Obesity International Journal. 7.3: (2022c). https://doi.org/10.23880/doij-16000260

35. Anefu EO, Ogbuagu NE, Adamu S and Esievo KAN, Healing activities of ethanolic extract of Anogeissus leiocarpus on surgically-induced skin wounds in alloxan-induce diabetic dogs". EC Diabetes Metabolic. Research 2022; 6.3.

36. Gee GW, Bauder JW, Particle-size analysis. Methods of soil analysis: Part 1 Physical and mineralogical methods, 1986; 5:383-411. https://doi.org/10.2136/sssabookser5.1.2ed.c15

37. McLean E O, "Soil pH and lime requirement. In methods of oil analysis". Part 2. Page, A.I., Miller, R.H., Keeny, D.R. (Eds). America Society of Agronomy, Madison, Wisconsin. 1982; 199-224. https://doi.org/10.2134/agronmonogr9.2.2ed.c12

38. Juo ARS, Selected methods of soils and plant analysis, Institute for Tropical Agriculture. Manual, Series 1. 1979

39. Bremner JM, Nitrogen-total. Methods of soil analysis: Part 3 Chemical methods, 1996; 5:1085-121. https://doi.org/10.2136/sssabookser5.3.c37

40. Udo EJ, Ibia TO, Ogunwale JA, Ano AO, Esu IE, Manual of soil, plant and water analysis. Lagos: Sibon Books Limited, 2009:4-8.

41. Knudsen D, Peterson GA, Pratt PF, Lithium, sodium, and potassium. Method Soil Analysis, Part. In America Society, 1982.

42. Anderson JM, Ingram JS, TSBF: A Handbook of Methods of Analysis: CAB International, 1989: 39.

43. Ebada SS, Edrada RA, Lin W, Proksch P, Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates, Nature Protocols. 2008; 3(12):1820-31.https://doi.org/10.1038/nprot.2008.182 PMid:18989260

44. Harvey D, Modern analytical chemistry. (1st Ed). McGraw-Hill, 2000; Pp 547-589.

45. Mbaveng AT, Ngameni B, Kuete V, Simo IK, Ambassa P, Roy R, Bezabih M, Etoa FX, Ngadjui BT, Abegaz BM, Meyer JM, Antimicrobial activity of the crude extracts and five flavonoids from the twigs of Dorstenia barteri (Moraceae), Journal of

Ethnopharmacology, 2008; 116(3):483-9. https://doi.org/10.1016/j.jep.2007.12.017 PMid:18280679

46. Dixon JL, Furukawa S, Ginsberg HN, Oleate stimulates secretion of apolipoprotein B-containing lipoproteins from Hep G2 cells by inhibiting early intracellular degradation of apolipoprotein B, Journal of Biological Chemistry, 1991; 266(8):5080-6. https://doi.org/10.1016/S0021-9258(19)67758-6 PMid:1848237

47. Ebbo AA, Sani D, Suleiman MM, Ahmad A, Hassan AZ, Acute and sub-chronic toxicity evaluation of the crude methanolic extract of Diospyros mespiliformis hochst ex a. Dc (ebenaceae) and its fractions, Toxicology Reports, 2020; 7:1138-44. https://doi.org/10.1016/j.toxrep.2020.08.028 PMid:32983901 PMCid:PMC7493130

48. Sani D, Abdu PA, Mamman M, Jolayemi KO, Yusuf PO, Andamin AD, Research Note: Evaluation of acute oral toxicity of povidone-iodine in cockerels using the up-and-down procedure, Poultry Science, 2021; 100(2):631-4. https://doi.org/10.1016/j.psj.2020.11.002 PMid:33518116 PMCid:PMC7858085

49. Sani D, Sanni S, Ngulde SI, Hypoglycaemic effect of aqueous stem extract of Anisopus mannii in normal rats, African Journal of Pharmacy and Pharmacology, 2009; 3(10):481-4.

50. Esievo KAN, "Veterinary Clinical Pathology", 1st Edit Spectrum Books, Ibadan. United Kingdom Limited, Jersey, Safari Books (Export) 37: pp. 37, 118-112, 149, 157-158.

51. Federal Ministry of Agriculture and Natural Resources (FMANR). "Literature review on soil fertility investigations in Nigeria" in five volumes, 1990; 280: 20-25.

52. Gupta A, Behl T, Sehgal A, Sharma S, Singh S, Sharma N, Garg M, Unmasking the therapeutic potential of biomarkers in type-1 diabetes mellitus, Biointerface Research of Applied Chemistry, 2021; 11:13187-201. https://doi.org/10.33263/BRIAC115.1318713201

53. Adejoh IP, Mark-Maria AU, Ojochegbe AB, Mayowa OJ, Boniface MT, Effect of aqueous leaf extract of Anogeissus leiocarpus against experimental models of pain and pyrexia. International Journal of Advanced Biological and Biomedical Research, 2019; 6(6):181-8.

54. Namadina MM, Nuhu A, Aliyu BS, Sani A, Aliko AA, Hayatu M, Babura SR, Muhammad MI, Umar A, Anas A. Physicochemical screening and acute toxicity study of Anogeissus leiocarpa stem bark, Dutse Journal of Pure and Applied Science, 2019; 5(1b):32-40.