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Research Article

Effect of Extract (Interface) from Stem Bark of Antidiabetic *Anogeissus leiocarpus* (African Birch Tree) on Random Blood Glucose Levels of Adult Female Wistar Rats: Optimisation for Therapeutic Hypoglycaemic Dose

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Abstract

Objectives: The lethal dose (LD_{50}) of the interface portion of ethyl acetate and n-hexane extract from antidiabetic *Anogeissus leiocarpus* stem bark was greater than 2000 mg/kg.bd.wt., with a wide range of safety value. In addition it caused no death 24hours and thereafter and had no effects on pregnancy, gestation, parturition, reproductive performance with no teratogenic effects on pups of Wistar rats. This led to investigation of the effects of the extract on the ovarian functions of adult female Wistar rats but strategically optimized to obtain an appropriate therapeutic hypoglycaemic dose.

Design: On day 0, twenty (20) adult female Wistar rats weighing between 155 and 235 g were separated, at random, into 4 cages (A-D) of 5 rats each, unadministered A (normal control), administered B (5 mg/kg.bd.wt.), C (10 mg/kg.bd.wt.) and D (20 mg/kg.bd.wt.); to each group, an adult male Wistar rat was added. They were on feeds (superstarter chow) and water *ad libitum*. Groups B and C were reduced to 4 rats each due to exclusion criteria. On day 0, baseline parameters, were collected from all female rats. Thereafter, from day 0 to day 12, each female rat received the optimized dose of the interface and samples were collected for random blood glucose (RBG), vaginal cytology and assay for oestrogen and progesterone. Blood samples were collected after day 12 for clinical biochemistry analyses. Weights were taken at intervals.

Results: At day 1, mean RBG values were significantly ($P<0.05$) higher (129.5±57.7 mg/dl) in group B than values of groups A, C and D which were 99.0±13.2, 101.8±3.2 and 102.0±11.9 mg/dl, respectively.

From days 2 to 12, the mean RBG values significantly ($P<0.05$) decreased to 98.3±15.6 in group B. The mean bodyweights of group B decreased from day 2 to day 14. In group D, mean values of body weights equally decreased on day 14, but after an increase on day 7.

Conclusion: Daily administration of 5 mg/kg/bd.wt. of the extract (interface) of *A. leiocarpus* stem bark is therapeutically hypoglycaemic as it reduced RBG significantly ($P<0.05$) up to day 12. The clinical application of the lower doses is suggested to maintain normoglycaemia for a while after "crashing" down the hyperglycaemia of DM with a much higher therapeutic dose.

The shelf life/expiry date of the extract (interface) of *A. leiocarpus* stem bark is greater than seventeen (17) months, when stored at room temperature.

Keywords: *A leiocarpus*; extract; Interface; Therapeutic hypoglycaemic dose; Diabetes Mellitus.

INTRODUCTION

Worldwide increases in type 2 diabetes mellitus (T2DM) without and with microvascular and macrovascular complications of nephropathy and retinopathy implied that DM may become a leading cause of death, by WHO, come 2030 but a worse situation was projected for 2035[1], thus creating challenging unresolved health concerns for the 21st century[2]. This has impacted on the global economy and the national budgets of numerous countries worldwide; the direct consequences of the risk factors induced by DM on the progression, prognosis and mortality of COVID-19, obesity, as a predisposing factor of DM and delayed wound healing or diabetic ulcers that led to amputation of limbs with their accompanying social menace had been highlighted earlier[3,4,5,6,7].

Several reports[2,8,9,10,11] emphasized on the benefits of medicinal plants and their phytochemical constituents for the treatment of diabetes mellitus; this fits the situation in countries with reduced economic growth, which influenced traditional methods for the treatment of diabetes mellitus.

From numerous studies[3,4,5,6,7,12,13] one medicinal plant, *Anogeissus leiocarpus* stand out. Crude ethanolic extracts of *A. leiocarpus* stem bark modulated sialic acids of plasma glycoproteins and red blood cells and revealed elevated serum sialic acids as a potent biomarker, predictive and prognostic in alloxan-induced diabetic dogs[3]. In addition, the crude ethanolic extracts ameliorated hyperglycaemia, hepato-renal damages, deranged electrolytes, acid-base balance and enhanced haematopoiesis in alloxan-induced diabetic dogs, further exhibiting a promise to prevent progression to type 2 diabetes mellitus since there was no reversal to hyperglycaemic state following withdrawal of administration of the extracts[4].

The antioxidant activities coupled with the antidiabetic properties of the crude ethanolic extract of *A. leiocarpus* in alloxan-induced diabetic Wistar rats[5] provided a boost to the plants efficacy in the treatment of DM. in addition, the dyslipidaemia produced in alloxan-induced diabetic dogs was attenuated by crude ethanolic extracts of *A. leiocarpus* stem bark[6]. The latter accelerated healing of surgically-induced deep skin wounds in alloxan-induced diabetic dogs[7] showing landmarks of enhanced inflammatory responses of healing processes[7]. The guill and perr leaf of *A. leiocarpus* were effective on the hyperglycaemia and the associated dyslipidaemia in alloxan-induced diabetic rats[12] while total extract and fractions exhibited antihyperglycaemic activity in mice[13]. The attenuation of dyslipidaemia on alloxan-induced diabetic dogs[6] and rats[12] by crude ethanolic extracts of *A. leiocarpus* is very important, laudable and requires more attention, since different classes of glucose lowering drugs, such as sulfonyl urea and meglitnides biguanides and thiazoliniediones[14,15] controlled the hyperglycaemia but did not effectively control the hyperlipidaemia associated with the DM, apart from the toxicity and resistance some patients experienced[16] and the exorbitant costs[17,18] for developing countries.

Due to the above-listed endowed medicinal activities and properties of *A. leiocarpus*, under the context of a non-conventional treatment for DM, the ethanolic extract of its stem bark was purified to the point of crystallization and four components, an interface and three fractions were produced[19] and the interface had a much higher proportion than the three other fractions[19]. Bioassay and toxicity studies with the interface in Wistar rats at a limit dose of 2000 mg/kg.bd.wt. caused no death within 24hours, no hepato-renal damages and pregnancy, gestation, parturition and reproductive performance were normal, with no teratogenic effects on pups[19].

It became imperative to have an insight into the effect of the antidiabetic compound of extract (interface) of *A. leiocarpus* stem bark on the ovarian functions of adult female Wistar rats and this aspect is strategically designed to investigate an appropriate and hence the optimization for therapeutic hypoglycaemic dose.

MATERIALS AND METHODS

Anogeissus leiocarpus Stem Bark Harvest and All Purification Processes:

The harvest of stem bark from *A. leiocarpus*; its authentication; the fertility assessment of the tree grown soils, the ethanolic extractions, along with the qualitative and quantitative phytochemical screenings; partitioning of the ethanolic extracts into fractions and the final purification processes with column chromatography and thin layer chromatography were adequately described with details[19]. The bioassay and toxicity studies, using the interface component of the four different purified components were performed[19]. The yields of the interface and the other three fractions in grams were reported earlier.

All purified extracts were stored in sample bottles, at room temperature (plate 1) and are reconstituted freshly when required. Storage of the purified *A. leiocarpus* stem bark extracts commenced on June, 2022.

Plate 1: Sample bottles that contained purified extracts of interface and fractions A, B and C

Constitution of purified extract

Five hundred (500) mg. of the extract, interface was dissolved in 1ml of distilled water to produce a concentration of 500 mg/ml.

Experimental Animals and experimental design

Twenty (20) adult female Wistar rats that weighed between 155 g and 235 g commenced the experiment on day 0. They were separated, at random, into 4 groups (cages) of 5 female rats each. Based on the major objective of the study, which is the effect of the antidiabetic compound of *A. leiocarpus* stem bark on ovarian functions, an adult male Wistar rat was placed into each of the 4 groups (cages), on day 0 and labelled groups A, B, C and D.

Group A (n=5): Served as control and received normal saline daily for 12 days.

Group B (n=5): Received oral administration of constituted extract, interface at a dose of 5 mg./kg.bd.wt. daily for 12 days.

Group C (n=5): Received oral administration of constituted extract, interface at a dose of 10 mg./kg.bd.wt. daily for 12 days.

Group D (n=5): This group received oral administration of constituted extract, interface at a dose of 20 mg/kg.bd.wt. daily for 12 days.

This design was aimed at investigating an appropriate, hence optimization for therapeutic hypoglycaemic dose for the antidiabetic *A. leiocarpus* and the choice of 12 days was due to the short oestrous cycle of the rats.

All the rats were fed commercial feeds (superstarter chow) and water was supplied *ad. libitum*.

Exclusion Criteria:

Exclusion criteria were applied to groups B (5 mg/kg.bd.wt.) and C (10 mg/kg.bd.wt.) which reduced the Wistar rats in groups B and C to 4 adult females each. (These are addressed under Results of this manuscript).

In group B, one female Wistar rat exhibited naturally acquired type 2 diabetes mellitus, noticeable on days 0 and 1. The exciting management of this naturally occurring type 2 diabetes mellitus in a female Wistar rat, as a case report, is part of another manuscript.

For group C, a female Wistar rat died from an unusual and unexpected cage related accident, three days after the commencement of the study.

Oral Administration of extract, interface of *A. leiocarpus* stem bark:

This was performed using 18G cannula oral gavage.

Samples Collections:

RBG:

On day 0, blood samples were collected from all female rats, through the ocular vein and baseline RBG levels were measured by placing a drop of blood directly onto an Accu chek® test strip inserted into the portable glucometer (Accu chek® Active, Roche, Roche Diabetes Care, Middle East, FZCO).

Thereafter all female rats were orally administered the extract, interface at the optimized dose corresponding with the designated group (as enumerated above) 5, 10 or 20 mg/kg.bd.wt. using 18G cannula oral gavage. Blood sample collections for RBG continued daily for the 12 days and to avoid traumatizing the ocular vein, blood samples were alternatively collected, that is, every other day, by cutting the rats tail tips.

Hormonal Assays:

Oestrogen and Progesterone:

Blood samples for the assay of oestrogen and progesterone were collected every other day post (daily) administration of the extract, by cutting the rats tail tips. Oestrogen and progesterone were measured using the human ELIZA kits.

Vaginal Cytology:

Vaginal swabs were collected every other day post (daily) administration of the extract, with the use of cotton balls.

Body Weights: The body weights of all female rats were measured in grams, at intervals, during the 12 day study.

Statistical analysis: The results obtained from this study were presented in charts and tables of means ± standard error of means (SEM). The data were subjected to ANOVA using the GraphPad Prism® statistical package with Tukey's post-hoc tests to analyze the difference between groups. Values of $P<0.05$ were considered significant.

RESULTS

Random Blood Glucose (RBG)

On day 0 the mean ± SD of the RBG levels showed no significant ($P>0.05$) difference in all groups, but the values were nonsignificantly higher in groups B and D compared to groups A and C.

At day 1, the mean ± SD of the RBG values were significantly ($P<0.05$) increased in group B than in groups A, C and D.

From day 2 upto day 12, the mean ± SD values of RBG significantly ($P<0.05$) decreased in group B; non-significant fluctuations occurred in all other groups. (Table 1 and Fig. 1)

Random Blood Glucose (RBG) values (mg/dl.) of adult female Wistar rats of control and groups administered the optimized doses of 5, 10 and 20 mg/kg.bd.wt. of extract (Interface) of *A. leiocarpus* stem bark

Table 1: Mean ± SD glucose level

Dates (and Day)	Group A Control	Group B 5 mg/kg	Group C 10 mg/kg	Group D 20 mg/kg
11/09/23 (0)	68.8 ± 14.0	89.5 ± 5.2[1]	77.8 ± 8.3	83.2 ± 7.6
12/09/23 (1)	99.0 ± 13.2[a]	129.5 ± 57.7[b,2]	101.8 ± 3.2[a]	102.0 ± 11.9[a]
13/09/23 (2)	76.8 ± 8.7	81.5 ± 5.8[1]	84.0 ± 5.4	90.4 ± 15.0
14/09/23 (3)	87.2 ± 6.8	85.5 ± 6.1[1]	91.8 ± 11.5	92.2 ± 8.3
15/09/23 (4)	98.6 ± 12.5	91.0 ± 14.3[1]	76.3 ± 9.8	90.8 ± 23.8
16/09/23 (5)	90.4 ± 3.8	86.8 ± 5.1[1]	90.3. ± 12.8	86.6 ± 11.7
17/09/23 (6)	91.8 ± 16.1	83.0 ± 2.5[1]	84.5 ± 7.6	88.8 ± 17.0
18/09/23 (7)	93.8 ± 13.5	100.0 ± 5.4[1]	94.0 ± 9.8	101.0 ± 7.7
19/09/23 (8)	90.4 ± 14.5	86.3 ± 11.2[1]	111.3 ± 4.8	106.2 ± 18.7
20/09/23 (9)	103.6 ± 17.8	115.8 ± 10.8[1]	107.3 ± 12.5	106.2 ± 13.0
21/09/23 (10)	95.8 ± 13.9	107.8 ± 11.9[1]	100.8 ± 11.8	106.2 ± 20.5
22/09/23 (11)	93.2 ± 15.9	99.3 ± 8.3[1]	99.0 ± 18.6	103.2 ± 11.3
23/09/23 (12)	86.6 ± 10.0	98.3 ± 15.6[1]	73.3 ± 22.2	87.0 ± 10.2

Mean ± SD values with different superscript alphabets in the same row differ significantly at P < 0.05; values with different superscript numbers in the same column differ significantly at P < 0.05

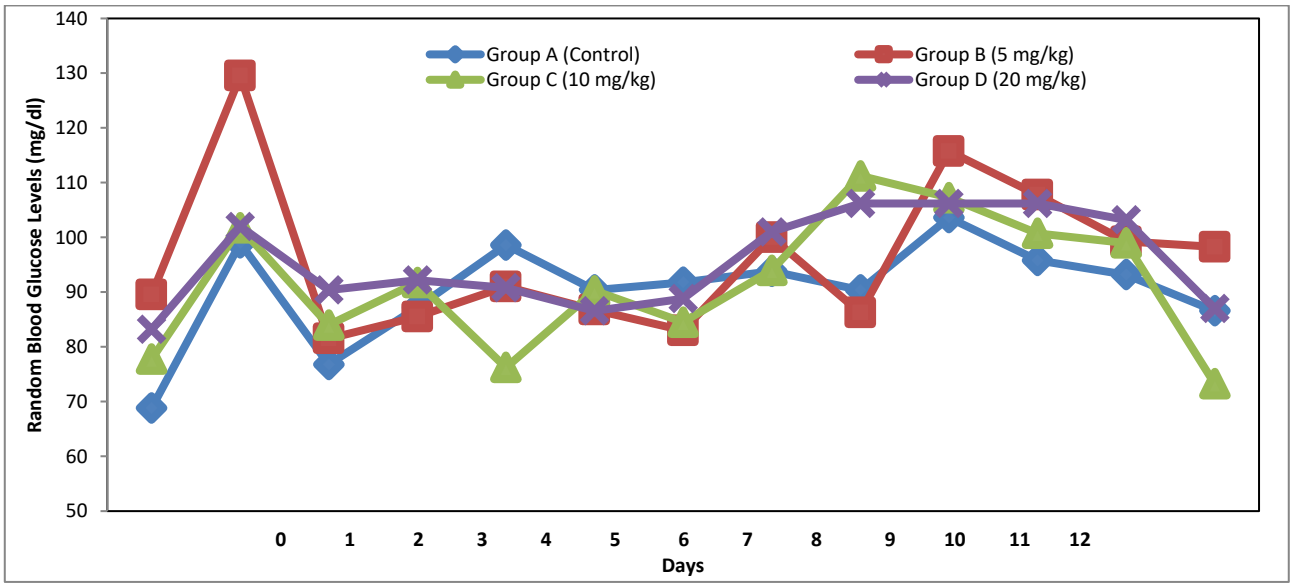

Figure 1: Line graph showing the pattern of changes in glucose levels

The variations of the RBG values of all the adult female Wistar rats under the study are presented in Table 2.

Table 2: Variations of random blood glucose levels (mg/dl) of all adult female Wistar rats under study.

Table 2a Group A: Control (n=5)

Dates (and Days)	11/9/23 (0)	12/9/23 (1)	13/9/23 (2)	14/9/23 (3)	15/9/23 (4)	16/9/23 (5)	17/9/23 (6)	18/9/23 (7)	19/9/23 (8)	20/9/23 (9)	21/9/23 (10)	22/9/23 (11)	23/9/23 (12)
S/No													
1a	44	89	62	79	114	88	84	71	75	76	75	67	72
2a	76	119	78	86	95	88	80	92	85	105	93	93	86
3a	72	105	84	83	80	95	82	104	87	99	94	101	99
4a	78	95	78	96	103	87	119	101	91	121	110	96	92
5a	74	87	82	92	101	94	94	101	114	117	107	109	84

Table 2b Group B: Administered 5mg/kg.bd.wt. (n=4)

Dates (and Days)	11/9/23 (0)	12/9/23 (1)	13/9/23 (2)	14/9/23 (3)	15/9/23 (4)	16/9/23 (5)	17/9/23 (6)	18/9/23 (7)	19/9/23 (8)	20/9/23 (9)	21/9/23 (10)	22/9/23 (11)	23/9/23 (12)
S/No													
1b	85	216	85	85	77	92	81	106	81	104	103	96	95
3b	97	98	80	93	89	90	81	96	73	113	96	89	91
4b	88	100	74	78	111	81	84	95	95	130	124	107	86
5b	88	104	87	86	87	84	86	103	96	116	108	105	121

Table 2c Group C: Administered 10mg/kg.bd.wt. (n=4)

Dates (and Days)	11/9/23 (0)	12/9/23 (1)	13/9/23 (2)	14/9/23 (3)	15/9/23 (4)	16/9/23 (5)	17/9/23 (6)	18/9/23 (7)	19/9/23 (8)	20/9/23 (9)	21/9/23 (10)	22/9/23 (11)	23/9/23 (12)
S/No													
1c	70	99	79	106	68	91	79	91	111	106	91	79	59
3c	81	105	90	95	79	101	77	98	109	116	93	95	104
4c	72	99	80	79	69	97	92	105	107	117	102	124	75
5c	88	104	87	87	89	72	90	82	118	90	117	98	55

Table 2d Group D: Administered 20mg/kg.bd.wt. (n=5)

Dates (and Days)	11/9/23 (0)	12/9/23 (1)	13/9/23 (2)	14/9/23 (3)	15/9/23 (4)	16/9/23 (5)	17/9/23 (6)	18/9/23 (7)	19/9/23 (8)	20/9/23 (9)	21/9/23 (10)	22/9/23 (11)	23/9/23 (12)
S/No													
1d	73	110	79	94	84	74	85	94	100	102	95	107	82
2d	84	90	78	79	68	76	75	104	91	97	91	91	102
3d	79	88	115	92	82	102	75	97	91	99	102	92	79
4d	87	111	92	94	89	93	116	97	114	104	101	109	79
5d	93	111	88	102	131	88	93	113	135	129	142	117	93

Body Weight

Body weights increased in groups A, B and C, but decreased in group D, from day 0 to day 2. Further increases in body weights occurred in groups A and C as against group B which experienced further decreases up to day 14. Group D experienced increased body weights at day 7 and then decreased at day 14. (Table 3 and Fig. 2). The variations in body weights of all adult female Wistar rats are in Table 4.

Table 3: Mean ± SD body weight in gram

Days	0	2	7	14
Group A - Control	209.6 ± 8.3	212.4 ± 15.1	217.2 ± 16.1	217.8 ± 12.9
Group B - 5 mg/kg	215.0 ± 14.7	223.8 ± 14.2	216.0 ± 21.1	210.8 ± 19.2
Group C - 10 mg/kg	178.5 ± 15.7	183.0 ± 12.7	193.0 ± 14.0	197.3 ± 17.2
Group D - 20 mg/kg	174.2 ± 15.8	164.8 ± 21.5	167.8 ± 10.9	165.2 ± 2.6

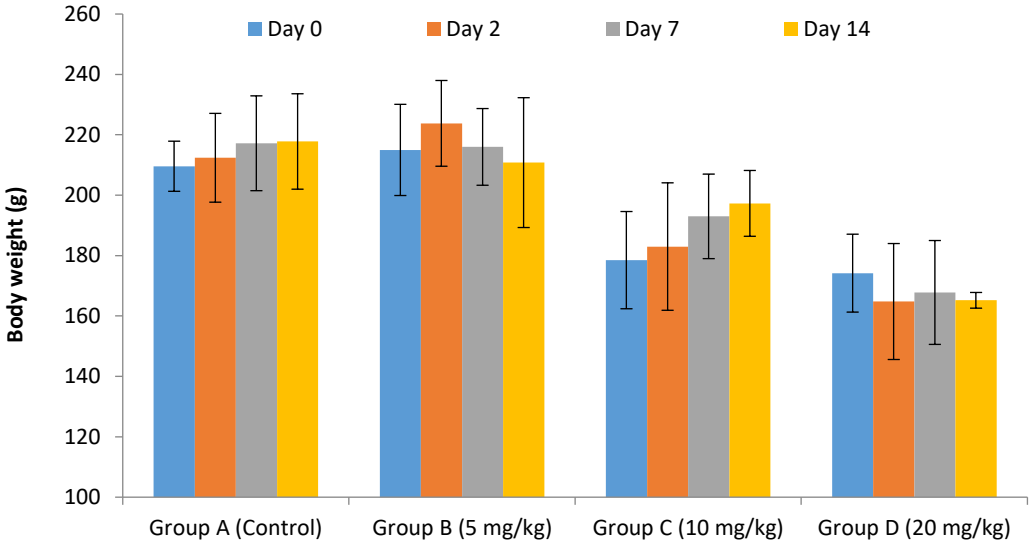

Figure 2: Chart showing the changes in body weights

Table 4: Body weight changes (gm) of adult female Wistar rats of control and groups administered the optimized doses of 5, 10 and 20mg/kg.bd.wt. of extract (interface) of *A. leiocarpus* stem bark

Table 4a = Group A: Control (n=5)

Dates (and Days)	**11/9/23 (0)**	**13/9/23 (2)**	**18/9/23 (7)**	**25/9/23 (14)**
S/No				
1a	219	234	240	230
2a	206	200	208	212
3a	209	217	221	224
4a	198	196	197	198
5a	216	215	220	225

Table 4b = Group B: Administered 5mg./kg.bd.wt. (n=4)

Dates (and Days)	**11/9/23 (0)**	**13/9/23 (2)**	**18/9/23 (7)**	**25/9/23 (14)**
S/No				
1b	235	245	246	238
3b	216	217	204	209
4b	208	215	215	202
5b	201	218	199	194

Table 4c = Group C: Administered 10mg/kg.bd.wt. (n=4)

Dates (and Days)	**11/9/23 (0)**	**13/9/23 (2)**	**18/9/23 (7)**	**25/9/23 (14)**
S/No				
1c	185	189	204	210
3c	188	190	205	214
4c	186	189	187	185
5c	155	164	176	180

Table 4d = Group D: Administered 20mg/kg.bd.wt. (n=5)

Dates (and Days)	**11/9/23 (0)**	**13/9/23 (2)**	**18/9/23 (7)**	**25/9/23 (14)**
S/No				
1d	163	154	160	162
2d	169	160	165	168
3d	171	154	169	167
4d	202	203	186	166
5d	166	153	159	163

Correlation between RBG levels and body weights at days 0, 2 and 7

There was a significant strong positive correlation between RBG levels and body weights at days 0, 2 and 7 in groups A and C. while in group B, a non-significant strong negative correlation existed between the RBG levels and body weights at days 0, 2 and 7. In group D, the correlation was non-significant and moderately negative (Table 5).

Correlation between RBG and body weight mean values of group B was non-significant and strongly negative on days 0, 2 and 7.

Table 5: Correlation between glucose level and body weight at days 0, 2 and 7

	r	P-value
Group A - Control	0.999	0.034*
Group B - 5 mg/kg	-0.770	0.440
Group C - 10 mg/kg	0.997	0.046*
Group D - 20 mg/kg	-0.566	0.617

* Levels of significance

DISCUSSION

This aspect of the current study has shown that 5mg/kg.bd.wt. of the extract (interface) of *A. leiocarpus* stem bark significantly reduced mean RBG levels in adult normal female Wistar rats (group B) by day 12 post administration. In addition, it has provided reasons to suggest that the 5 mg/kg.bd.wt. can be therapeutically hypoglycaemic in adult female Wistar rats. Further observation revealed that 10 mg.bd.wt. of the extract (interface) of *A. leiocarpus* stem bark non-significantly reduced the mean RBG levels in the adult female Wistar rats (group C) by day 12 post administration, when compared to the mean RBG levels of day 1. While a dose dependent glucose reduction response was expected, it is paradoxical that 20 mg/kg.bd.wt. of the extract (interface) of *A. leiocarpus* stem bark equally produced a non-significant reduction of the mean RBG levels in the adult female Wistar rats (group D) by day 12 post administration; for the latter, (groups C and D) further observation to exceed 12 days did not occur due to the original design of the overall study on ovarian functions.

Crude ethanolic extract at 1000 mg/kg.bd.wt. reduced blood glucose levels and enhanced haematopoiesis after a 14-day oral administration in adult Wistar rats[20]; the crude ethanolic extracts contained all the fractions, prior to its purification. The limit dose of 2000 mg/kg.bd.wt. of the extract (interface) produced an overwhelming and a highly significant ($P<0.0001$) reduction of blood glucose levels in normal adult male and female Wistar rats combined over a 24 hour period[19]. In a related study to investigate the molecular basis of the activities of antidiabetic *A. leiocarpus*, 100 mg/kg.bd.wt. of the extract (interface) reduced blood glucose levels in alloxan-induced diabetic Wistar rats, 3 days after the development of marked hyperglycaemia (part of another manuscript).

In the current study, mean RBG levels of all the groups increased on day 1 post administration of the extract (interface) with the increase in the same group B (5 mg/kg.bd.wt.) being significant when compared with groups A, C and D. The observed increase that occurred in control group A (without extract administration) an increase that is similar to those of groups C and D [administered with the extract (interface)], albeit, non-significant, strongly suggest that the extract oral administration may not be implicated for the increases of the mean RBG values on day 1. It is being suggested that the increased mean RBG values of all groups on day 1 was in part, caused by the unusual and initial handling of these Wistar rats, accompanied by excitement, fright and secretion of epinephrine with expected increases of glucose in the peripheral circulation[21] of these Wistar rats, until the rats got used to the daily routines of samples collections.

From days 2 to 14, group B rats experienced decreases in mean body weights; indeed a non-significant strong negative correlation existed between the mean RBG levels and body weights.

CONCLUSION

Daily oral administration of a minimal dose, 5 mg/kg.bd.wt of extract (interface) of *A. leiocarpus* stem bark is therapeutically hypoglycaemic, as it significantly reduced mean RBG levels by day 12. Clinical application of these lower doses is maintenance of normoglycaema, for a while, after "crashing" down the hyperglycaemia of DM, with a much higher therapeutic dose.

The shelf life/expiry date of the extract (interface) of *A. leiocarpus* stem bark is greater than seventeen (17) months when stored at room temperature.

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Author's contribution

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

Esievo, LO: Investigation; Ethanolic Extraction and Purification; Writing.

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

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

Esievo, EM: Investigation; Ethanolic Extraction and Purification; Writing.

Balogun, EO: Supervision; Investigation; Writing; Editing.

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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).

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