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Studies of the antitrypanosomal and toxicological properties of *Anogeissus leiocarpus* (Dc.) Guill. & Perr. (Combretaceae) and *Vitellaria paradoxa* C. F. Gaertn (Sapotaceae) in mice

[Estudios de las propiedades antitripanosómicas y toxicológicas de *Anogeissus leiocarpus* (Dc). Guill. & Perr. (Combretaceae) y *Vitellaria paradoxa* C.F. Gaertn (Sapotaceae) en ratones]

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Abstract: African animal trypanosomosis (AAT) is a disease of concern with ravaging effects on the health of both animals and livestock in tropical Africa. This study investigates the anti-trypanosomal activities of *Anogeissus leiocarpus* (ALE) and *Vitelleria paradoxa* (VPE) stem bark extracts and also determines the toxicological profile of the active plant, with a view to establishing the anti-trypanosomal potential and safety of the plants. Laboratory mice (19 g – 26 g) and rats (140 g – 165 g) obtained from the Animal house, Faculty of Pharmacy, OAU, Ile-Ife were used for the study. The animals were treated according to the standard set criteria for animal use and care. VPE showed neither trypanocidal nor trypanostatic activities while ALE was found to be trypanostatic at 62.5 and 125 mg/kg body weight. However, the partitioned aqueous fraction of ALE was found to demonstrate comparable anti-trypanocidal effect as Diminal (standard agent). In conclusion, the ethanolic extract of *A. leiocarpus* possesses antitrypanosomal effect through the relative suppression or delay in parasite establishment in trypanosome-infected mice. The toxicological study of *A. leiocarpus* stem bark extract revealed that it is relatively safe for use in cattle and other grazing animals.

Keywords: African animal trypanosomosis, Anti-trypanosomal, Safety, *Anogeissus leiocarpus* and *Vitelleria paradoxa* stem bark extracts.

Resumen: La tripanosomiasis africana de los animales es una enfermedad de preocupación que causa estragos sobre la salud de los animales y el ganado en África tropical. Este estudio investiga las actividades anti-tripanosomal de *Anogeissus leiocarpus* (ALE) y *Vitelleria paradoxa* (VPE) del tallo y extractos de corteza. También determina el perfil toxicológico de la planta activa, con el fin de establecer el potencial anti-tripanosomal y la seguridad de las plantas. Ratones de laboratorio (19 g - 26 g) y ratas (140 g - 165 g) obtenidos del Bioterio de la Facultad de Farmacia de la OUA, se utilizaron para el estudio. Los animales fueron tratados de acuerdo con los criterios estándar establecido para el uso y cuidado de animales. VPE mostró actividades no tripanocidas ni tripanostáticas mientras que en ALE se encontró que era tripanostático a 62,5 y 125 mg/kg de peso corporal. Sin embargo, se encontró que la fracción acuosa de ALE demostró un efecto anti-tripanocida comparable como Diminal (agente estándar). En conclusión, el extracto etanólico de *A. leiocarpus* posee efecto sobre tripanosomas a través de la supresión relativa o retraso en la creación de parásitos en ratones infectados con tripanosomosis. El estudio toxicológico del extracto de corteza del tallo *A. leiocarpus* reveló que es relativamente seguro para su uso en el ganado y otros animales de pastoreo.

Palabras clave: Tripanosomiasis africana de los animales, anti-tripanosómica, seguridad, *Anogeissus leiocarpus* y extractos de corteza de *Vitelleria paradoxa*.

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INTRODUCTION

African animal trypanosomosis (AAT) is a potentially fatal disease of animals in tropical Africa. It continues to retard the growth of the livestock industry in the continent (Umar *et al.*, 2010). The economic and devastating effects of AAT have increased dramatically in recent years especially in Sub-Saharan Africa (Welburn *et al.*, 2001). The direct and indirect annual losses are enormous, thus making it an important priority for the scientific community (Aksoy, 2003). The direct and indirect annual losses are enormous, thus making it an important priority for the scientific community (Hursey, 2001, Aksoy, 2003). However, the number of drugs available for the treatment of animal trypanosomosis is very limited. Most importantly, the available trypanocidal drugs are toxic, and have recorded some forms of resistance (Adewunmi *et al*., 2001). Over the last three decades, no significant progress has been made in the development of drugs for AAT (Medical Ecology, 2004). *Anogeissus leiocarpus* (Combretaceae) is a tree plant used in ethno-medicine for the treatment of ascariosis, gonorrhea, general body pains, blood clots, asthma, coughing and tuberculosis (Mann *et al.*, 2003). The crude extract of *A. leiocarpus* has been separated into fractions selectively enriched in ionizable triterpenes, ellagic acid derivatives and flavonoids (Hamzaoui *et al.*, 2013). In another study, sericoside, trachelospero-genin E, an epimer mixture of (+)-gallocatechin and (-)-epigallocatechin,3,3'-di-O-methylellagic acid 4'-O-xylopyranoside, and 3,4,3'-tri-O-methylflavellagic acid 4'-O-glucopyranoside were isolated (Hubert *et al.*, 2014). The presence of known hydrolyzable tannins and some related compounds-with castalagin as the major compound have also been reported from the butanolic fraction of the plant (Shuaibu *et al.*, 2008).

Vitellaria paradoxa (Sapotaceae) is a small to medium-sized tree with numerous medicinal uses. The boiled or ground powder is used for the treatment of dysentery, suppurating wounds and other ailments (Soladoye *et al.*, 1989). Paste from the root bark is also taken orally to cure jaundice, diarrhea, stomach ache in humans and applied topically to treat chronic sores and girth sores in horses (Mallogo, 1989).

Ethno-veterinary medical practitioners in the North-eastern and Central Nigeria use the stem bark of both plant as effective treatment of trypanosomosis in cattle. This study is therefore set to investigate the *in- vivo* anti-trypanosomal activities of the plants with a view to establishing their ethnomedicinal uses and relative safety.

Materials and methods

Drugs and Chemicals

Testosterone, follicle-stimulating hormone (FSH), prolactin (PRL) and luteinizing hormone (LH) assay kits were obtained from HySkill Diagnostics (Bahlingen, Germany). Testosterone propionate was a gift from Abeth Consult limited (Ghana), Cyproterone acetate was obtain from Bayer Australia Ltd (Australia). Commercial biochemical kits by Randox Laboratories Limited, United Kingdom.

Plant collection and extraction

Fresh stem barks of *A. leiocarpus* and *V. paradoxa* were collected from Ilesa, Kwara State, Nigeria in November, 2011. The plants were identified by Mr. Bernard Omomoh, Herbarium Unit, Botany Department, OAU, Ile-Ife, Osun State. Voucher specimens IFE 16852 and 16744 were deposited for both plants respectively. The plant materials were washed and chopped into small pieces. *A. leiocarpus* (1.2 kg) was soaked in 70% ethanol (2.5 L) while *V. paradoxa* (1.5 kg) was soaked in 70% ethanol (3 L) separately for seven days with intermittent shaking. The filtrate were concentrated under reduced pressure on a rotary evaporator at 40 °C to a minimum volume and then lyophilized to complete dryness to yield 82.32 g (6.86%) and 105 g (7%) for *A. leiocarpus* (ALE) and *V. paradoxa* (VPE) extracts respectively and stored refrigerated until use.

Solvent partitioning of A. leiocarpus extract

ALE (47 g) was suspended in distilled water and partitioned with solvents of increasing polarity (400 ml each) affording n-hexane (0.320 g, 0.68%), dichloromethane (3.318 g, 7.06%), ethyl acetate (0.649 g, 1.38%), butanol (2.03 g, 4.31%) and aqueous (40.25 g, 85.64%) fractions.

Test Organism

Cultures of *Trypanosoma congolense* were obtained from the Parasitology and Microbiology Unit, Department of Animal Production and Health, Federal University of Technology, Akure. The parasite was maintained in the laboratory in rats by continuous passaging until when needed.

Experimental Animals

Mice (19g – 26 g) and rats (140g – 165 g) were purchased from the Animal house of both Faculties of Pharmacy and Health Sciences, OAU, Ile-Ife and kept in clean and well ventilated small animal cages. They were fed with pelletized growers mash and exposed to 12 hour light and dark cycles. The animals were allowed to acclimatize to laboratory environment for 7 days before use. The animals were treated according to the set criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (National Academy Press, 2000).

Determination of Parasitaemia

Parasitaemia was monitored in the blood obtained from the tail of rats or mice, pre-sterilized with ethanol. The number of parasites was determined following the method described elsewhere (Adelodun *et al.*, 2013).

Acute toxicity study of A. leiocarpus extract

Acute toxicity of ALE was determined both intraperitoneal (ip) (Reed & Muench, 1938) and orally (Lorke's, 1983). For i.p. administration, mice (19 – 24 g) were divided into 5 groups (n = 5/group). Group I (negative control) received the vehicle, groups II, III, IV and V were administered ALE at dose levels of 400, 800, 1600 and 3200 mg/kg respectively. For the phase 1 oral test, twelve (12) mice were divided equally into control group and 3 test groups administered (10, 100 and 1000 mg/kg body weight) doses of the extract. The phase 2 dose selection test was based on the result of the phase 1 test. Animals were administered doses of 1600, 2900 and 5000 mg/kg body weight of the extract. Access to food was withheld but water was allowed freely 12h before the commencement of the experiment.

The animals were closely observed for any signs of toxicity including pattern and rate of respiration, presence or absence of feces, central nervous system stimulation due to hyperactivity or central nervous depression, paralysis of the limbs and positioning of the tail. The number of death(s) per group was recorded at 24, 48 and 72 h.

In vivo antitrypanosomal assays

The 7 days *in-vivo* screening test involved inoculating forty (40) mice via the i.p. route with 10^4 parasites in 0.2 ml of the inoculum. The animals were then divided into 8 groups (n = 5/group each). Group 1 (negative control) were administered the vehicle (normal saline) only. Animals in groups 2, 3 and 4 were treated 24 h after the inoculation with different doses (62.5, 125 and 250 mg/kg) of ALE, while mice in groups 5, 6 and 7 received different doses (50, 100 and 200 mg/kg) of VPE Group 8 (positive control) received the standard drug, Diminazene aceturate (3.5 mg/kg). Partitioned fractions (organic and aqueous fractions) were equally tested. The organic fractions were screened at 2 test dose levels of 31.25 mg/kg and 62.5 mg/kg, while the aqueous fraction was screened at 31.25 mg/kg, 62.5 mg/kg and 125 mg/kg doses.

Sub-chronic toxicity test of A. leiocarpus

Rats were randomly selected and divided into 5 groups (n = 5 per group). Group 1 received the vehicle only (normal saline) and groups 2, 3, 4 and 5 animals were treated with ALE at 85, 170, 340 and 680 mg/kg respectively daily for 28 days. These animals were observed for morbidity and mortality. Body weights were recorded at the beginning, then at 48 h intervals. Water and food consumptions were noted daily.

Preparation of serum for biochemical analysis

Following the sub-chronic test, animals were subjected to 24 h fast, weighed and sacrificed by cervical dislocation before dissection. Blood samples were collected by cardiac puncture into EDTA containing bottles and plain bottles. Non-anticoagulant blood samples were allowed to stand for about 30 min for complete clotting. The clotted blood was then centrifuged at 3000 rpm for 15 min and the serum utilized for biochemical test. Blood collected into the EDTA bottles was used for hematological study.

Determination of relative organ weight

Body organs (heart, lung, liver and kidneys) were excised, washed with ice-cold normal saline and weighed individually. The relative organ weight was determined thus:

$$\text{Relative organ weight} = \frac{\text{bsolute organ weight (g)}}{\text{Body weight of rat on day of sacrifice (g)}} \times 100$$

The organs were then wrapped in clean foil and kept frozen until needed.

Estimation of serum and liver total cholesterol, triglycerides, HDL and LDL cholesterol

The blood samples were centrifuged at 3000 rpm for 15 min and serum was collected and stored at -20º C until assayed. Tissue homogenate were prepared in 10% buffer solution. Liver (1 g) was first allowed to attain to room temperature before homogenizing.

Serum and liver total cholesterol, triglycerides, HDL and LDL cholesterol concentrations were estimated using Randox commercial assay kits. Assays were carried out according to manufacturer's instruction.

Histological evaluation

Liver, kidney, heart and lung tissues of control and treated animals were removed, weighed, washed in normal saline and fixed immediately in 10% buffered formal saline for histological studies. After dehydration in two changes of 70%, 80%, 90% and 99% alcohol, tissues were embedded in paraffin. Sections were cut at 3µm thick with Leica Rotary Microtome and stained in Heamatoxylin and eosin (H & E) solutions for microscopy/photomicrograph analysis.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was employed for between and within group comparison while Duncan's multiple range test was used for paired comparison. Ninety five per cent (95 %) level of significance ($p < 0.05$) was used for the statistical analysis (SAS, 2008).

RESULTS

Acute Intraperitoneal Toxicity Test

Group II animals showed some behavioral changes such as restlessness, increase in respiration rate and loss of appetite. However, the toxic effects were transient and the animals soon restore to normal behavioral functions after 72 h. Mortality of about 20% and over 50% were observed in groups III and IV respectively with more profound behavioral changes. Total mortality (100%) was observed in group V animals. The LD_{50} for ALE via the i.p. route was found to be 1281.7 mg/kg.

Acute Oral Toxicity Test

In the phase 1 test, ALE was found to demonstrate a low to no toxicity when administered orally at doses of 10, 100 and 1000 mg/kg over a period of 72 h. The LD_{50} value was estimated to be > 5000 mg/kg body weight of mice. Our findings showed that the polar extract is non-toxic and could be considered relatively safe for administration to animals. However, increase in respiration, coarse body tremor and loss of appetite were some effects observed in the animals. The adverse reactions lasted a few hours, thereafter the animals were soon restored to normal activities.

In vivo Antitrypanosomal Studies

The *in-vivo* antitrypanosomal effects of the both extracts and the partitioned fractions of ALE in particular are presented in Tables 1 and 2 respectively. Parasites were not completely cleared following treatment with the extracts. However, ALE was found to show some trypanostatic properties by delaying the establishment of parasitaemia in the experimental animals. Interestingly also, the aqueous fraction (62.5 mg/kg) significantly lowered ($p < 0.05$) the parasitaemia level of animals until day 4 of the experiment. Also some of the animals in this group survived up to day 12 of the experiment, compared with animals treated with 125 mg/kg of the same aqueous fraction in which all died after the third dose.

Table 1
Effect of VPE and ALE on blood forms of *T. congolense*

	Days of treatment and mean parasitaemia count						
Dose of extract (mg/kg)	2	4	6	8	10	12	14
VPE							
50	0	5.46±0.06b	8.48±0.26a	-	-	-	-
100	0	0	5.46±0.06c	7.62±0.26b	8.70±0.00a	-	-
200	0	0	6.06±0.41b	7.70±0.27a	-	-	-
ALE							
62.5	0	0	0	5.40±0.00c	6.30±0.35b	7.80±0.00a	7.80±0.00a
125	0	0	0	5.40±0.00	7.20±0.63a	7.20±0.00a	-
250	0	-	-	-	-	-	-
Diminal® (3.5 mg/kg)	0	0	0	0	0	0	0
Infected but untreated	0	5.46±0.60c	7.44±0.22b	8.10±0.17a	-	-	-

Legend: a, b, c - values are significantly different (P < 0.05), − indicates death, 0 indicates total clearance of parasite, note: antilog of these values gives the absolute no. of parasites per ml of blood

Table 2
Parasitemia profile-effect of partitioned fractions of ALE on blood forms of *T. congolense*

Partitioned fractions	Days of treatment and mean parasitaemia counts					
(Test doses)	2	3	4	5	6	7
n-hexane						
(31.25 mg/kg)	6.42±0.20abcde	6.96±0.11ab	7.38 ±1.20ab	8.16 ±0.15a	8.63±0.08ab	8.85±0.87ab
(62.50 mg/kg)	6.12±0.15bcde	6.24±0.11cd	6.36±0.15de	7.20±0.21de	7.60±0.10c	-
Dcm						
(31.25 mg/kg)	6.48±0.26abcd	7.38±0.20a	7.74±0.15a	7.92±0.23ab	8.60±0.27ab	-
(62.50 mg/kg)	6.66±0.18ab	7.14±0.18a	7.56±0.15a	7.92±0.15ab	8.25±0.15ab	8.63±0.29b
EtoAc						
(31.25 mg/kg)	6.78±0.20a	7.32±0.28a	7.74±0.22a	8.25±0.15a	8.63±0.75ab	9.00±0.00a
(62.50 mg/kg)	6.60±0.13abc	7.20±0.10a	7.68±0.12a	8.28±0.23a	8.60±0.20ab	9.00±0.00a
n-BuOH						
(31.25 mg/kg)	6.60±0.13abc	7.08±0.74a	7.44±0.11a	8.34±0.11a	8.60 ±0.10ab	6.60±2.21ab
(62.50 mg/kg)	6.36±0.20abcde	6.60±0.10bc	6.96±0.06bc	7.38±0.12bc	7.80±0.23bc	8.60±0.10b
Aqueous						
(31.25 mg/kg)	5.88±0.15e	6.30±0.13cd	6.72±0.18cd	7.14±0.18cd	7.86±0.35abc	5.70±1.90c
(62.50 mg/kg)	6.12±0.12bcde	6.18±0.74cd	6.06±0.11ef	6.48±0.15e	6.96±0.37c	6.75±0.87d
(125 mg/kg)	6.00±0.13de	5.94±0.18d	6.12±0.14ef	-	-	-
Diminal ®	6.30±0.19cd	6.9±0.16cd	5.82±0.20ab	5.40±0.00a	-	-
-ve cont.	6.48±0.12abcde	7.26±0.18d	8.16±0.11f	8.78±0.14f	8.85±0.15f	-

Following table 2

Partitioned fractions	Days of treatment and mean parasitaemia counts				
(Test doses)	8	9	10	11	12
n-hexane					
(31.25 mg/kg)	-	-	-	-	-
(62.50 mg/kg)	-	-	-	-	-
Dcm					
(31.25 mg/kg)	-	-	-	-	-
(62.50 mg/kg)	9.00±0.00a	-	-	-	-
EtoAc					
(31.25 mg/kg)	-	-	-	-	-
(62.50 mg/kg)	-	-	-	-	-
n-BuOH					
(31.25 mg/kg)	-	-	-	-	-
(62.50 mg/kg)	8.90±0.31a	-	-	-	-
Aqueous					
(31.25 mg/kg)	8.10±0.30ab	8.55±0.15	-	-	-
(62.50 mg/kg)	7.05±0.87bc	7.73±0.26b	8.00±0.36ab	8.55±0.15a	8.70±0.00a
(125 mg/kg)	-	-	-	-	-
Diminal ®	-	-	-	-	-
-ve cont.	-	-	-	-	-

Legend: a, b, c - values are significantly different (P<0.05), − indicates death, 0 indicates total clearance of parasite, (note: antilog of these values gives the absolute no. of parasites per ml of blood), DCM - dichloromethane, EtOAc - Ethylacetate, BuOH - Butanol, -ve cont: - negative control

Phytochemical investigation

Preliminary phytochemical investigation of the partitioned fractions revealed the presence of terpenoidal spots (Rf 0.65, 0.76 and 0.81) developing purple to bluish colours as the major constituents of the dichloromethane and ethyl acetate fractions. The butanolic and the aqueous fractions showed similar TLC profile and contained majorly phenolic compounds (reaction to $FeCl_3$ spray) and antioxidant (DPPH spray), with spots (Rf 0.1, 0.14) reacting yellow to vanillin/H_2SO_4 spray using ethylacetate: MeOH: H_2O (10:1.5:1) as the developing system.

Sub-chronic toxicity studies

The daily administration of ALE during the 28 days test provoked a dose dependent decrease in food consumption and eventual decrease in body weight of the treated animals in the first week of the experiment (Figure 1). However, rate of food intake improved gradually during the course of the study (Figure 2). Similarly, a dose dependent decrease in water consumption in all the treated groups was also noted to the fourth week. There was no significant variation in the relative weight of excised organs such as liver, kidneys, heart, and lungs of treated animals compared with the control untreated animals (Figure 3).

Estimation of Biochemical parameters

The levels of biochemical agents were estimated using RANDOX commercial kits. The hepatic ALT and AST of treated animals were not significantly different ($p > 0.05$) compared with the control (Figure 5) whereas a significant decrease ($p < 0.05$) was observed in activity of serum ALT and AST (Figure 4). The lipid profile (Cholesterol, HDL, LDL, and Triglycerides) of all the treated groups also showed no significant differences ($p > 0.05$) when compared with the control group (Figure 6).

Histological studies

Histology of the heart, lung, kidney and liver of the animals in treatment and control groups were compared. Daily administration of ALE for 28 days did not cause remarkable distortion to the histology of the studied organs after excision (plates I-IV). Sections of the heart tissues of all the treated animals showed normal cardiac muscle and cardiac myocytes (Plate I) while sections of the lung tissue showed normal morphological arrangement of the tissue structures *i.e*. the bronchus, bronchiole and alveolus (Plate III). The renal tissues showed normal arrangement of the glomerulus, glomerular capsule, tubules and interstitium tissue structures (Plate II) while sections of the liver tissues also shows a normal arrangement of the central vein, hepatocytes, and portal triad (Plate IV). All the organs of the treated animals examined showed normal general tissue structures from H&E stained microscopic assessments when compared to the untreated wistar rats (Plates I, II, III and IV).

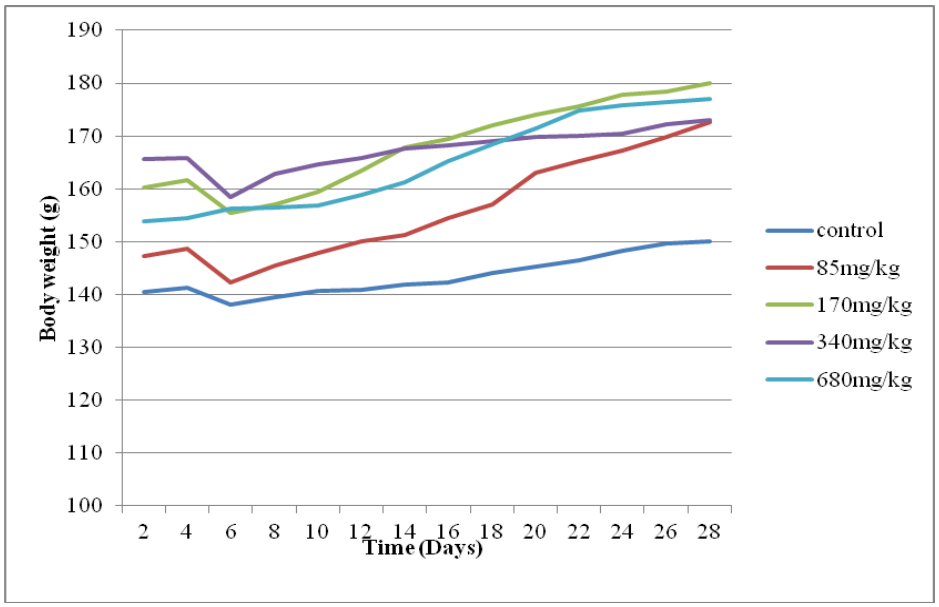
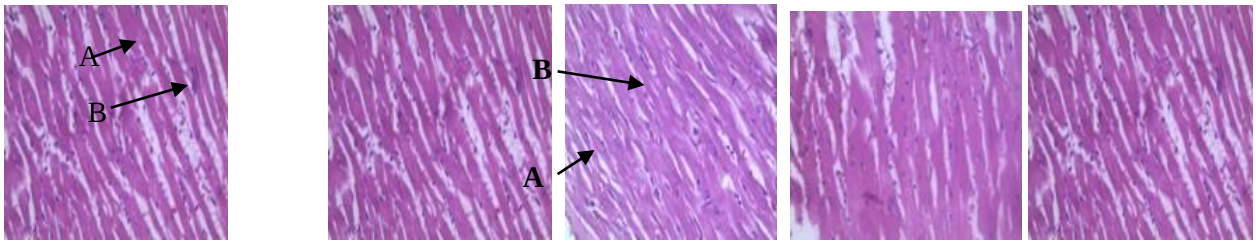


Figure I
Effect of daily oral doses of *A. leiocarpus* extract on the body weight of rats treated for 28 days



85 mg/Kg **170 mg/Kg** **340 mg/kg** **680 mg/kg** **Control**

Plate I
Photomicrograph of section of the heart of control and animals treated with daily oral doses of ALE for 28 days, stained in H&E (MgX400). (A: cardiac muscle stained salmon pink; B: cardiac myocytes stained blue)

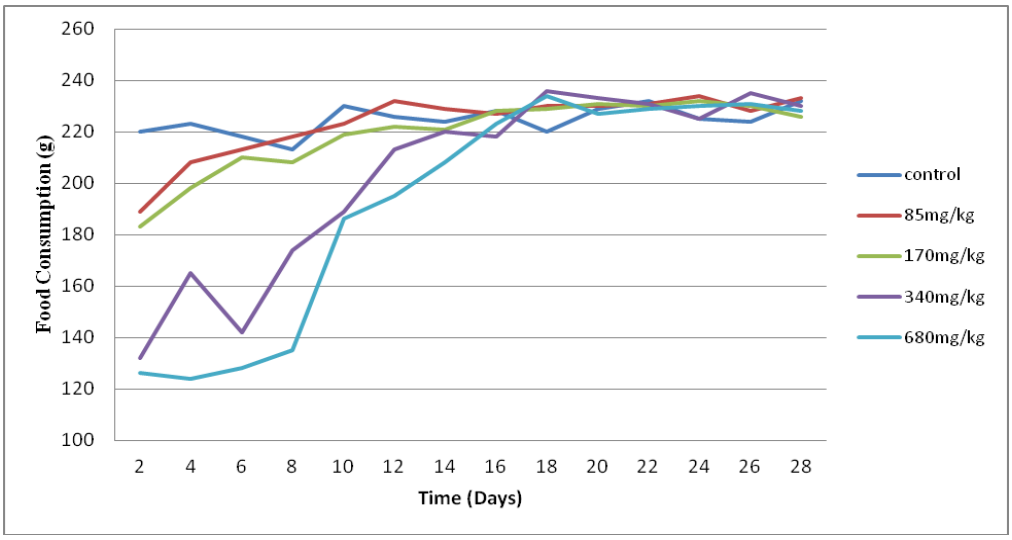
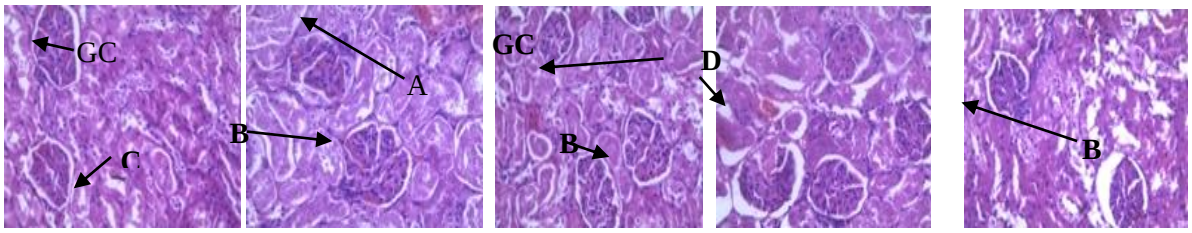
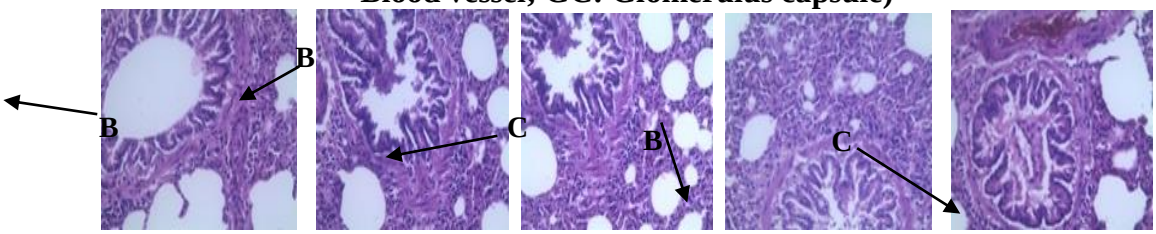


Figure 2
Rate of food consumption of animals treated with daily oral doses of *A. leiocarpus* extract



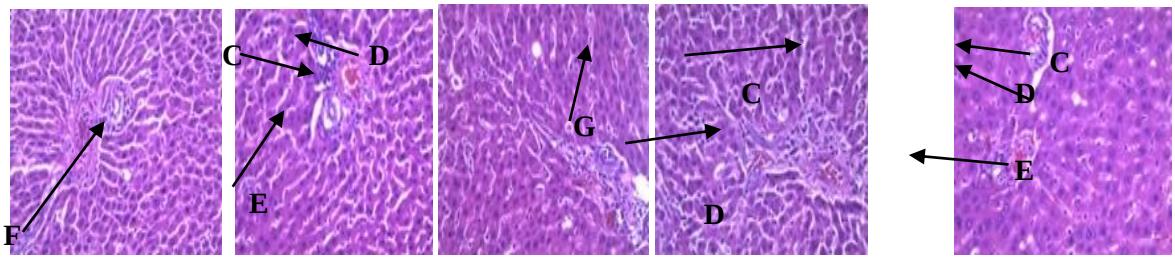
85 mg/kg **70 mg/kg** **340 mg/kg** **680 mg/kg** **Control**

Plate II
Photomicrograph of section of the kidney of control and animals treated with daily oral doses of ALE for 28 days, stained with H&E (MgX400). (A: interstitium; B: glomerulus; C: Tubules; D: Blood vessel; GC: Glomerulus capsule)



85 mg/kg **170 mg/kg** **340 mg/kg** **680 mg/kg** **Control**

Plate III
Photomicrograph of section of the lung of control and animals treated with daily oral doses of ALE for 28 days, stained with H&E (MgX400). (B: bronchiole; C: alveolus)



85 mg/kg **170 mg/kg** **340 mg/kg** **680 mg/kg** **Control**

Plate IV

Photomicrograph of the section of the liver of control and animals treated with daily oral doses of ALE for 28 days, stained with H&E. (MgX400) (Triads; C: portal artery; D: portal vein; E: portal duct; F: sinusoid; G: hepatocyte)

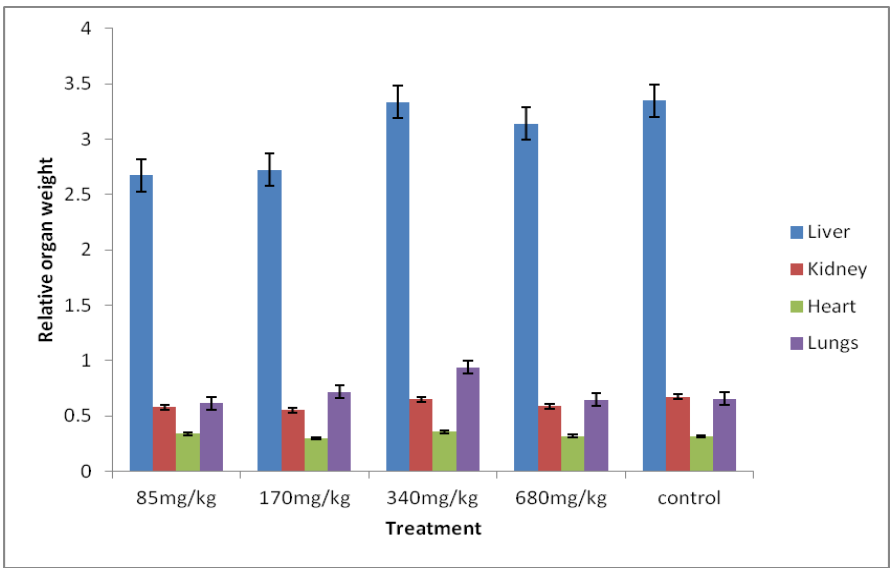


Figure 3
Relative weight of the liver, kidneys, heart and lungs of animals treated orally with *A. leiocarpus* extract for 28 days

DISCUSSION

Vitellaria paradoxa stem bark extract showed neither trypanocidal nor trypanostatic activities at the tested concentrations. However, previous study reported that the ethanolic extract was able to effect a complete suppression or delay in parasite establishment and thereby reduce the severity of the resultant disease in trypanosome-infected rats after being treated daily against *T. brucei brucei* and *T. congolense* with a dose of 200 mg/kg intraperitoneal for 7 days (Mbaya *et al.*, 2007). The differences in the outcome of the studies could not be ascertained, however, seasonal variation and difference in geographical locations are some of the possible factors. *A. leiocarpus* stem bark extract at 62.5 and 125 mg/kg body weight was found to be trypanostatic since parasites were not detected between day 1 (after infection) until day 8. All of the animals in untreated control group died before tenth day of the study while animals treated with 62.5 mg/kg survived beyond the experimental period. Bioactivity-guided partitioning of *A. leiocarpus* extract using solvents of increasing polarity showed that the aqueous fraction at 62.5 mg/kg prolonged the survival of the animals when compared with other fractions. In a similar study involving the oral treatment of experimental rats

infected with *T. brucei brucei* with 200 mg/kg body weight of *A. leiocarpus* stem bark methanolic extract showed that the rats survived longer than the control animals (Wurochekke & Anyanwu, 2012).

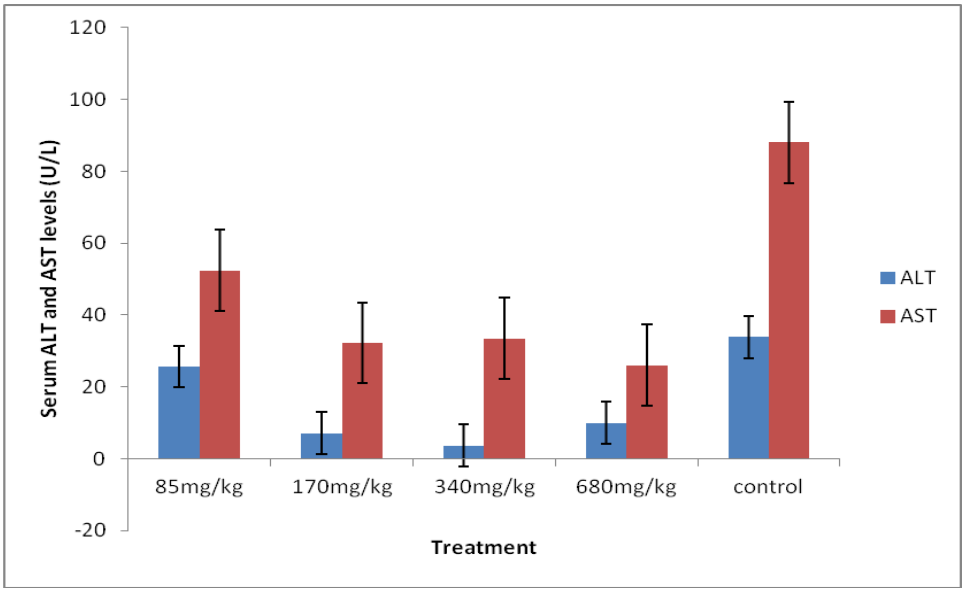


Figure 4
Effects of *A. leiocarpus* extract on the levels of serum ALT and AST in rats treated orally for 28 days

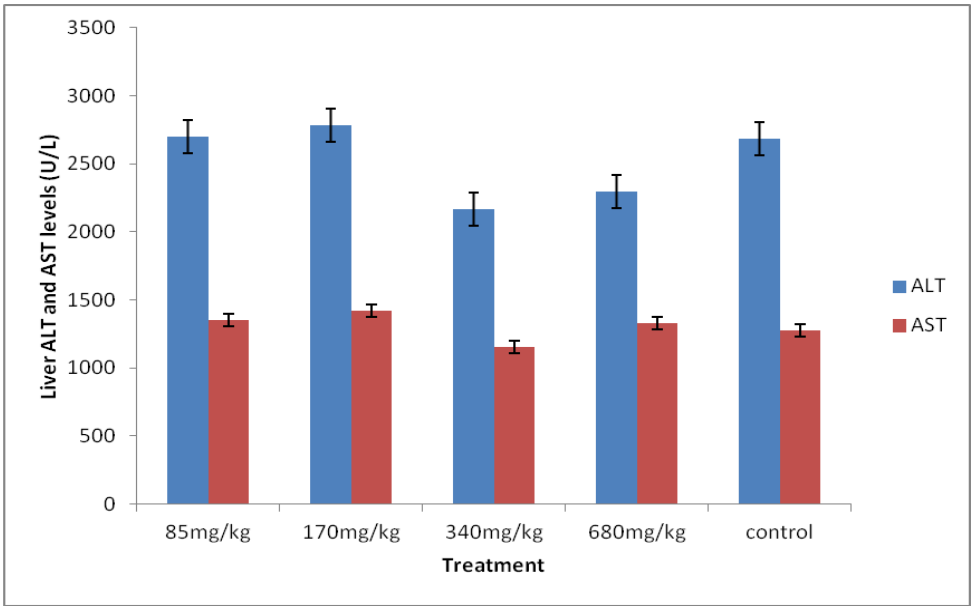


Figure 5
Effects of *A. leiocarpus* extract on the levels of hepatic ALT and AST in rats treated orally for 28 days

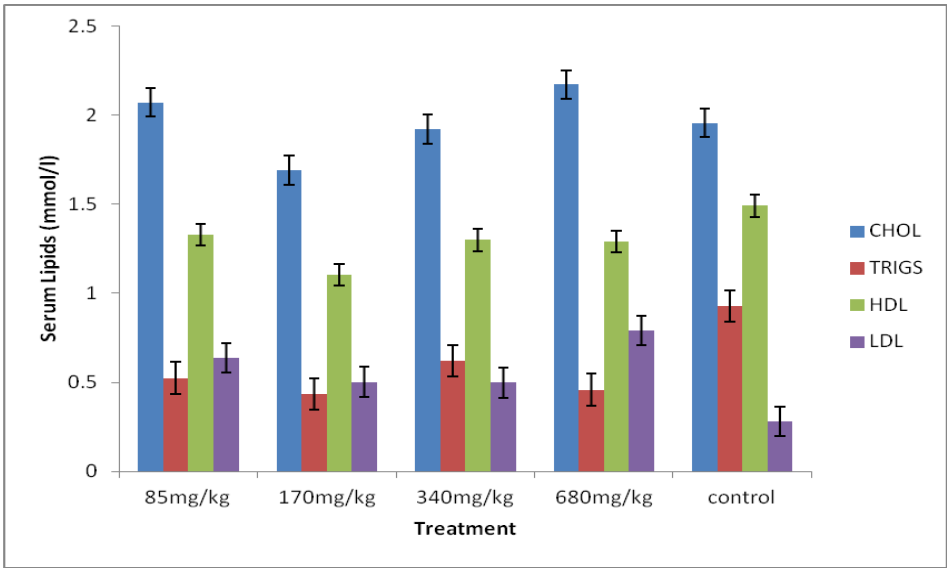


Figure 6
Effects of *A. leiocarpus* extract on the serum levels of Cholesterol, Triglycerides, HDL cholesterol, LDL cholesterol in rats treated orally for 28 days

The toxicological study of *A. leiocarpus* stem bark extract revealed that it is relatively safe for use in cattle and other grazing animals. In acute intraperitoneal toxicity test, all the animals treated showed behavioral changes such as restlessness, increase in respiration rate and loss of appetite, but all the animals treated at a concentration of 400 mg/kg body weight survived and their behaviors restored back to normal. There was 20% and over 50% mortality in animals treated at concentrations of 800 mg/kg and 1600 mg/kg respectively with more profound behavioral changes while total mortality was observed in animals treated at a concentration of 3200 mg/kg. The LD_{50} for *A. leiocarpus* via the intraperitoneal route was determined to be 1281.7 mg/kg. In acute oral toxicity test, there was no record of death at the end of 14 days of observation, while the LD_{50} via the oral route is > 5000 mg/kg. This finding was supported by previous study (Agaie *et al.*, 2007), in which no mortality was recorded in the animals administered the leaf aqueous extract up to the dose of 3200 mg/kg body weight. However, the rats showed signs of depression and lack of appetite. When administered by the i.p. route, the rats showed dose-dependent signs of toxicity ranging from lack of appetite, depression, unsteady gait, tremors, and respiratory distress to death. The intraperitoneal LD_{50} was 1400 mg/kg body weight. No gross pathological changes were observed in the organs of rats that died following extract administration. Histopathological lesions were also not observed in all the organs except the lungs, which showed congestion, edema and bronchitis. The ethanolic extract of stem barks *of A. leiocarpus* as observed in this study could be regarded as relatively safe, although it was found to induce some physical behavioral changes such as restlessness, increase in the respiration rate, and aggressiveness. However, the animals overcame the changes and returned to normal conditions after 48 hours.

Sub-chronic toxicity study

This was a repeated dose experiment. Albino rats (25) were randomly divided into 5 groups of 5 animals each (n = 5 per group). Animals of group 1 served as control and received the vehicle only (normal saline) while the animals in groups 2, 3, 4 and 5 were treated daily with the ethanolic extract of *A. leiocarpus* at 85, 170, 340 and 680 mg/kg orally for 28 days. All the animals survived the period of treatment.

Biochemical analysis

Aminotransferases are chemicals the liver uses to help make the energy-storage molecule, glycogen. Because these enzymes are found in liver cells, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) can leak into the blood if the hepatocytes are damaged. In this study, the impact of *A. leiocarpus* extract on the liver was evaluated by

determining the levels of serum and hepatic ALT and AST of the treated and control animals. ALT and AST which are important biomarkers of the syndrome of cytolysis or for estimating the extent of destruction of the hepatocyte cells (Brissot *et al.*, 1998). Our result showed that the extract is devoid of prominent toxicity. Both ALT and AST serum levels were significantly lower ($p < 0.05$) in the treated groups compared with the control (Figure 5), suggesting the non-hepatotoxic effect of the extract. The extract may be demonstrating its protective effect by stabilization of plasma membrane and consequently preserving the structural integrity of cell as well as protection against hepatic tissue damage (Pari & Murugan, 2004).

Histological studies

The excised organs (liver, kidney, heart and lung tissues) were subjected to histological studies. The liver and kidney constitute the principal organs of detoxification and so represent the first targets of all substances in the body. Thus, these two organs are very often affected in the event of toxicity. The relative weight of all the organs examined did not vary significantly for all the treated animals when compared with the animals in the control group (Figure 4). The histological appearance of the four organs show no remarkable distortion (ulceration) and damaging effects in all the tested doses (85, 170, 340 and 680 mg/kg) when compared with the control group, in which also no evidence of tissue damage could be found (Plates 1-4).

Our preliminary phytochemical investigation of the partitioned fractions revealed the presence of terpenoids as the major constituents of the dichloromethane and ethyl acetate fractions, while the butanolic and the aqueous fractions showed similar TLC profile and contained majorly phenolic and antioxidant compounds.

In conclusion, the polar extract of *A. leiocarpus* was found to possess some anti-trypanosomal effect through the relative suppression or delay in parasite establishment in trypanosome-infected mice at 62.5 mg/kg tested dose.

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