GC-MS profiling of the phytochemical constituents of the whole plant of Brillantaisia owariensis (P. Beauv) and its anti-inflammatory prospects in the management of African Trypanosomiasis

Sonnie Joshua Oniye (sjoniye@abu.edu.ng)
Ahmadu Bello University
Nafisa Garba Ayawa
Ahmadu Bello University
Mazlina Mazlan
Universiti Putra
Suleiman Babatunde Ramon-Yusuf
University of Abuja
Yunusa Adamu Wada
Ahmadu Bello University
Dalhatu Mukhtari Shehu
Ahmadu Bello University

Research Article

Keywords: Brillantaisia owariensis, GC-MS profiling, Trypanosoma brucei, Induced pathologies, Ameliorative activity, Methanol extract

Posted Date: April 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1509005/v1

Abstract

Brillantaisia owariensis (Acanthaceae) is evident in its traditional use in the forest region of West Africa for the treatment of several ailments, including trypanosomiasis. Despite its widespread use, the plant has not been subjected to pharmacological investigations to ascertain its phytochemical profile and efficacy in folklore medicine. This study was designed to determine the phytochemical and GC-MS profile of the whole plant of *B. owariensis* and elucidate its pharmacological implications in managing trypanosomiasis. The plant was collected from Nigeria's South-western Forest vegetation zone. Thirty grams of the processed powder was extracted using the Soxhlet method using methanol as solvent. The phytochemical and Gas Chromatography-Mass Spectroscopy analyses of the methanol extract were done. Thirty BALB/c mice were randomly allocated into six groups of five mice to test for anti-inflammatory activity. Each mouse in groups 2–6 were infected with 0.1 mL of 10^6 *T. brucei* /mL, while group 1 served as neutral non-infected control. Following the establishment of parasitaemia by 3 days post-infection, mice were either non-treated, treated with standard drug or treated at varying dosages of methanol extracts and sacrificed by day 9 for histopathologic evaluation. High levels of Phyto-constituents were detected, and the GC-MS profiling revealed the presence of twenty-four bioactive compounds. The extracts of *B. owariensis* revealed ameliorative and anti-inflammatory activities on the histology of the vital organs and tissues at varying dosages. The methanol extract of *B. owariensis* is rich in bioactive components with promising potential in managing inflammatory diseases such as African trypanosomosis.

Introduction

The African continent's human health situation is a subject of worry. African Trypanosomosis is one of Africa's most debilitating protozoan infections, straddling human health, cattle health, agricultural production, and rural development (FAO, 2008). Infection with at least one pathogenic *Trypanosoma* haemoflagellate parasite spread by tsetse flies causes a complicated, debilitating, and often deadly sickness in people and animals (WHO, 2005). The disease induces haematological changes that produce severe anaemia, such as decreased packed cell volume, decreased haemoglobin concentration, drop in total protein and leucocyte counts (Wada et al., 2016a). Other clinical outcomes are inflammatory foci in the heart, liver, spleen, and lymph nodes (Wada et al., 2016b).

Chemoprophylactic or chemotherapeutic medicines are commonly utilised to manage African Trypanosomiasis (Antia et al., 2009). However, drug shortages, drug resistance, high costs, unpleasant side effects, and toxicity make treating African trypanosomiasis difficult (Toya, 2010; Barrett et al., 2011). As a result, finding less expensive, more effective, more readily available, and less toxic chemotherapeutic medicines to treat the illness is crucial. Medicinal plants are an essential part of Africa's traditional healthcare system, one of the oldest and most diverse medicinal systems (Mahomoodally, 2013). Medicinal plants include chemicals employed for therapeutic purposes or serve as templates in synthesising beneficial pharmaceuticals in some parts (Sofowora et al., 2013). Fast access to safe and effective medications and animal welfare is the public's primary concern, patients, and customers.

Brillantaisia owariensis, commonly called Bush cow food (fodder), is a fascinating tropical plant that belongs to the Acanthaceae family and is common in the forest regions of western and central Africa. Bush cow fodder is a perennial shrubby plant that grows 20cm to 2m tall. Leaves are ovate-lanceolate, acuminate, gradually narrowing into the petiole, 23 cm long, 7.5 cm wide, glabrous, uniformly serrate on the margins, 23 cm long, 7.5 cm wide, glabrous. The panicle inflorescence is loosely thyrsiform and glandular-pubescent, with lanceolate bracts that fall off early. The segments of the calyx are straight and sharp. The corolla tube is 4 linear inches long, and the lips are 10–11 inches long. Staminodes have glandular structures. 20–24 ovules in pubescent ovary.

In Africa, *B. owariensis* is majorly utilised as a herbal remedy to treat many human health problems, including malaria, anaemia, typhoid fever, stomach ache, chest conditions, yaws, infantile spleen infection, malnutrition, rheumatism, to aid conception and sometimes decocted to ease childbirth and menstrual pains (Akah et al., 2009; Ayawa et al., 2021; Beentje, 2018; Ngbolua et al.,2013). The leaves are boiled in water and taken orally until symptoms disappear. The plants have been proven effective and have relieved so many pains. However, most of the application is anecdotal but lacks detailed scientific elucidation. In mice challenged with *T. brucei brucei*, Ayawa et al. (Ayawa et al., 2021) found that methanol extract had better suppressive and haematinic anti-trypanosomal activity and a good safety margin. However, additional research on this promising plant is needed to understand its pharmacological and medicinal effects better. As a result, the goal of this study was to elucidate further the phytochemistry and anti-inflammatory capabilities of *B. owariensis*.

Materials And Methods

Source of Brillantaisia owariensis, authentication and processing

The whole plant of *Brillantaisia owariensis* (Fig. 1) was collected from the South-western Forest vegetation zone in Ondo state, Nigeria, and authenticated at the Department of Botany, Ahmadu Bello University, Zaria (accession No. ABU01537). As described in our previous study, the whole plant was air-dried before being pulverised and ground to a fine powder (Ayawa et al., 2021).

Hot continuous extraction (Soxhlet) method

Thirty grammes (30 g) of pulverised whole plant of *Brillantaisia owariensis* was placed in a sac-like mesh cloth and transferred to a flask containing 300ml of methanol. After that, the flask was installed on a Soxhlet machine and set to work at 100 degrees Celsius. The extract was collected and transferred to an evaporating dish, then placed in a water bath to allow the methanol to evaporate (at 100 ^{o}C) to obtain a concentrated extract (Ayawa et al., 2021). This extract was used for the phytochemical and GC-MS analysis.

Phytochemical screening

The Phytochemical screening of the plant was done at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria, to detect the presence of alkaloid, anthraquinone, carbohydrate, cardiac-glycoside, unsaturated steroid, triterpenes, saponin glycoside, tannin, flavonoid and anthraquinone of *B. owariensis* according to standard methods and techniques in Trease and Evans (1996).

Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

The Gas Chromatography-Mass Spectrometric (GC-MS) analysis was carried out at the Central Research Laboratory, Federal University of Technology Akure, Ondo State, Nigeria, to determine the bioactive compounds of the whole plant *Brillantaisia owariensis*. Under standard operational conditions, the analysis was carried out on a GC-MS machine (Model: QP2010 plus Shimadzu, Japan). The bioactive compounds with their Retention time (RT), Molecular formula, Molecular weight (MW), and Peak values were determined by comparison of the acquired spectra with the existing National Institute of Standards Technology (NIST) library.

Experimental design, trypanosome inoculation and treatment

Following a safe dose of *Brillantaisia owariensis* established in our previous article (Ayawa et al., 2021), thirty adult BALB/c mice (*Mus musculus*) of both sexes weighing between 19 and 22 grams were randomly allocated to six experimental groups (A to F) in a simple Complete Randomised Design (CRD). Each mouse in groups B to F was

inoculated with 0.1 mL containing10^6 *T. brucei* /mL, while group A served as neutral (non-infected) control. Following patency of 3 days, mice in groups B to F were either non-treated (negative control), treated with standard drug, Diminazene aceturate (positive control) or treated at varying dosages of methanol extracts (100, 75, and 50 mg/Kg). The mice were housed in clean cages throughout the experimental period and fed standard animal feed, with access to clean water *ad libitum. Trypanosoma brucei* was obtained from stabilates maintained at the Department of Veterinary Parasitology and Entomology, ABU. Zaria, Nigeria. The experimental was set up as detailed below:

Group A: Neutral control- non-infected

Group B: Negative control- infected and treated with 1ml normal saline.

Group C: Positive control- infected and treated with Diminazene aceturate 3.5 mg/kg.

Group D: Infected and treated with methanol extract (M-100 mg/kg/day).

Group E: Infected and treated with methanol extract (M-75 mg/kg/day).

Group F: Infected and treated with methanol extract (M-50 mg/kg/day).

Pharmacological evaluation of extracts

Monitoring of parasitaemia

The level of parasitemia was measured daily in blood taken from the infected mice's tails. The number of trypanosomes in infected blood per field was determined microscopically at X 400 (Herbert and Lumsden (1976).

Histopathologic examination of tissues

Following the termination of the experiment on day 9, mice in all the groups were humanely sacrificed for histopathologic evaluation. The heart, lungs, liver, kidney and spleen were excised, and the tissue specimens collected were preserved in 10% buffered neutral formalin (BNF). After 48 hours of fixation, the tissue samples were processed (dehydrated in 50% and 70% alcohol), embedded in paraffin wax and sectioned at 5 microns using a microtome. The sections were mounted on clean, grease-free glass slides and stained with Haematoxylin and Eosin (H&E) stains. Histopathologic lesions were examined microscopically for anti-inflammatory response at X40 objective, and photomicrography was done with a digital camera (Canon 16 Mpx).

Data analyses

The data for daily parasitaemia scores were summarised as the mean for each group and subjected to a one-way analysis of variance (ANOVA) to test for a significant difference in daily mean parasitaemia among treatment groups. Tukey post hoc test was used as post-hoc to separate means where significant, at $P \leq 0.05$. Statistical Package for Social Sciences (SPSS) version 20.0 was used for data analyses.

Results

Phytochemical screening of the extracts

The result of the phytochemical screening revealed the presence of alkaloid, anthraquinone, carbohydrate, cardiac-glycoside, unsaturated steroid, triterpenes, saponin glycoside, tannin, flavonoid and traces of anthraquinone (Table 1).

Table 1
Qualitative phytochemical constituents of the methanol extract of *Brillantaisia owariensis* from Ondo state, Nigeria

Phytochemicals	**Methanol (solvent)**
Alkaloid	++
Anthraquinone	+traces
Carbohydrate	+
Cardiac glycoside	+
Unsaturated steroid /Triterpenes	+
Saponin glycoside	++
Tannin	+
Flavonoid	+
+ = Present ++ = highly present,	

Gas Chromatography-Mass Spectroscopy analyses

The GC-MS analysis of the crude methanol extract of *Brillantaisia owariensis* revealed the presence of twenty-four (24) compounds (Fig. 2). The active compounds with their Retention time (RT), Molecular formula, Molecular weight (MW), and Peak area in percentage (Fig. 2) such as Phytol (23.29%), 9 Octadecinamide Z (8.81%), Hexadecanoic acid methyl ester (8.20%), 7-Hexadecenoic acid, Methyl ester, (Z) (7.62%), 3, 8-Nonadien-2-one, (E) - (7.41%), heptanoic acid (5.07%), are presented (Table 2).

Table 2

GC-MS profile of compounds identified in the crude methanol extract of *Brillantaisia owariensis*.

Peak no.	Retention time (min)	Peak area (%)	Compounds	Molecular formula	Molecular weight (g/mol.)
1	2.688	1.12	1-Methoxy-2,3-cis-dimethylaziridine (anti	$C_5H_{11}NO$	101.15
2	4.822	0.25	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-1,1-Dicyanoethane 2,4 Hexadiyne-1,6-diol	$C_{15}H_{26}$	206.36
3	6.956	5.07	Heptanoic acid	$C_7H_{14}O_2$	130.18
4	7.002	1.49	l-Tyrosinol	$C_9H_{13}NO_2$	167.2
5	7.477	2.75	Methyl 4-(2,4 dinitrophenylhydrazono) valerate	$C_{12}H_{14}N_4O_6$	310.26
6	7.906	1.82	Acetamide, N-(4-hydroxyphenyl)-N-methyl-	$C_9H_{11}NO_2$	165.19
7	8.221	3.14	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24
8	8.599	1.68	Benzisoxazole-2-acetic acid, hydrazide	$C_9H_9N_3O_2$	191.19
9	8.993	1.27	2-p-Nitrobenzoyl-1,3,5-tribenzyl- alpha. -d-ribose	$C_{33}H_{31}NO_8$	569
10	9.817	2.25	3-Methyl-4-nitro-5-(1-pyrazolyl) pyrazole	$C_7H_7N_5O_2$	193.16
11	10.126	1.95	[1,2,3,4] Tetrazolo[1,5-a] pyridine-6-carboxylic acid	$C_6H_4N_4O_2$	164.12
12	11.042	1.26	Cyclododecanone,2-methylene-	$C_{13}H_{22}O$	194.0
13	12.324	8.20	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45
14	13.319	2.14	4-Octenoic acid, 2,3,7-trimethyl-, methyl ester	$C_{12}H_{22}O_2$	198.30
15	14.589	4.42	4-Cyclohexylidene-n-butanol	$C_{10}H_{18}O$	154.25
16	14.670	7.62	7-Hexadecenoic acid, methyl ester,Z)-	$C_{17}H_{32}O_2$	268.40
17	14.818	23.92	Phytol	$C_{20}H_{40}O$	296.50
18	14.990	3.13	Methyl 8-methyl-decanoate	$C_{12}H_{24}O_2$	200.32
19	17.273	1.38	1-(7-Hydroxy-1,6,6-trimethyl-10-oxatricyclo [5.2.1.0(2,4)] dec-9-yl)ethanone	$C_{14}H_{22}O_3$	238.32
20	17.330	8.81	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.50
21	18.183	3.54	1,2,4] Triazolo[1,5-a] pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	$C_8H_9N_5O2$	207.07
22	18.206	7.41	3,8-Nonadien-2-one, (E)-	$C_9H_{14}O$,	138.21
23	18.835	2.79	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37
24	18.950	2.58	Benzenepropanoic acid, alpha. -(1, dimethylethyl)-	$C_{13}H_{18}O_2$	206.28

Mean daily parasitemia

The parasitemia pattern is depicted in Fig. 3. On day three after infection, all the infected groups developed parasitaemia. Mice treated with diminazene aceturate (positive control) cleared all parasites in their blood by day 6 (i.e., 3 days after treatment commenced). In contrast, those treated with methanol plant extracts remained parasitaemic throughout the experiment with a significant level of suppression ($P = 0.027$) compared to the negative control (non-treated group) by day 7 (i.e., 4 days post-treatment). The experiment was terminated on day 9, and all mice were sacrificed for histopathologic evaluation.

Histopathology

Heart

Photomicrograph of tissue sections of the heart of mice infected and treated with methanol extract of *B. owariensis* showed normal heart architecture in the positive control group, M100 and M75. However, mild myocardium necrosis was observed in the group treated with M50 compared to the negative control revealed moderate myocardium necrosis (Fig. 4).

Lung

Photomicrograph of lung tissue sections showed normal alveoli, while the positive control treated with Diminazene aceturate shows alveoli congestion. In comparison, the groups treated with M100 and M50 showed normal alveoli. The M75 showed lymphocyte hyperplasia (Fig. 5).

Liver

Photomicrograph of liver tissue sections revealed normal hepatocytes in the normal control. There was moderate to severe hepatic necrosis in the negative control group. Mild hepatic necrosis and normal hepatocytes were observed in M100 and M75, respectively, compared to the positive control that showed mild hepatic necrosis (Fig. 6).

Kidney

Photomicrograph of tissue sections of the kidney (Fig. 7). Normal control showed normal tubules and glomerulus. There was moderate to slight tubular adhesion, and necrosis was observed in the positive control group, M100, M50, and M75, in comparison to the positive control., while the negative control group presented tubular distortion and tubular necrosis.

Spleen

Photomicrograph of the spleen of mice infected and treated with methanol extract of *B. owariensis* are presented in Fig. 8. Normal spleen architecture with normal red and white pulp distribution was observed in the positive control group, M100 and M50, while M75 had Slight lymphocyte hyperplasia, while those in the negative control group had mild and red pulp necrosis.

Discussion

The phytochemical constituents in *B. owariensis* include alkaloids, anthraquinone, carbohydrate, cardiac-glycoside, unsaturated steroid/triterpenes, saponin glycoside, tannin and flavonoid. The presence of phytochemicals such as

alkaloids, flavonoids, tannin, anthraquinone, and saponin, as revealed by the phytochemical screening of methanol extract of *B. owariensis* has been reported in other medicinal plants such as *Severinia buxifolia* (Truong et al., 2019), in *Brillantaisia patula* (Faparusi et al., 2012), in *Cassia arereh* (Abbas et al., 2017), and *Abrus precatorius* seeds (Nwodo and Nwodo, 2012). Phytochemical investigations of the whole plant extract of *B. owariensis* by Akuru and Amadi (2018) revealed high concentrations of spartein, anthocyanin, oxalate, phenol, epicatechin, lunamarin, saponin, ribalinidine, phytate, rutin, kaempferol, catechin, sapogenin and also the presence of antioxidants properties.

The GC-MS analysis of the concentrated methanol extract presented many compounds with diverse use. The most abundant bioactive compounds are Phytol, 9 Octadecinamide Z, Hexadecanoic acid methyl ester, 7-Hexadecenoic acid, methyl ester (Z), 3, 8-Nonadien-2-one, (E), Heptanoic acid. These six primary bioactive metabolites have been reported to act as an anti-trypanosomal (Saad et al., 2020), anti-inflammatory compound (Phatangare et al., 2017; Basile et al., 1999), anti-schistosomal (Eraky et al., 2016), antioxidant (Santos et al., 2013), anti-bacterial and antifungal, nematicidal, anti-bacterial and antifungal (Chandrasekaran et al., 2011; Idan et al., 2015) and hypnotic property (Idan et al., 2015). New compounds, Iridolactone and Owariensisone, together with six known compounds (Nepetin-7-*O*-glucoside, choline, sucrose, mannitol, xylitol, and 1-O-palmitoyl-2eicosanoyl-3-O-(6-amino-6-deoxy)-β-D glucopyranosyl glycerol) were isolated from the whole plant extract of *B. owariensis* (Perrin et al., 2016). Therefore, these findings suggest the presence of multiple compounds in *B. owariensis*, which could be used as possible potential additives in treating several ailments of animals and plants.

The *extracts' in-vivo anti-trypanosomal activity revealed* no cessation or complete elimination of parasites from the bloodstream of infected mice when administered methanol extract of *B. owariensis*, but only reduced parasitaemia. The extract was unable to eliminate parasitaemia has been reported to demonstrate a significant haemanitic activity capable of reversing anaemia, which is a typical characteristic of African Trypanosomiasis (Akah et al., 2009; Ayawa et al., 2021). *Trypanosoma brucei brucei* is one of the parasites that show tissues tropism to the heart, skeletal muscle, liver, spleen, brain, lungs, kidney, testes, and adipose tissue (Silva et al., 2019). Tissues affected have characteristic lesions from extensive inflammation, which are mediated by levels of increased cytokines such as Tissue Necrotic Factor (TNF-a) and Interleukin 6(IL-6), with cellular infiltrations primarily by lymphocytes accompanied by plasma cells, macrophages and neutrophils (Abenga, 2014). Lymphocyte hyperplasia is generally a reactive or immune response and immunological protection to the tissue. It is not considered a preneoplastic lesion in the lymph node (Elmore, 2006). The Lymphocyte proliferation in tissues observed in this study is also reported by Wada et al. (2016b) in 'Yankasa *T. brucei brucei*, and *T. evansi* infected Rams.

The normal myocardium of the heart observed in this study could be that the parasites might not have extravasated into the extracellular matrix, just as reported by McCarrol *et al*. (2015). Slight necrosis of the myocardium could be associated with stress, where cardiomyocytes undergo apoptotic responses (Woodcock and Matkovich, 2005).

Significant histopathology of the lungs was alveoli congestion and lymphocyte hyperplasia. The lungs' congestion might be an inflammatory response to the parasite, thus leading to vasodilatation and exudation. Bal et al. (2012) made a similar observation in the lungs of mice experimentally infected with *Trypanosoma evansi*.

The spleen is essential to the lymphatic tissues, playing a defensive role during parasite invasion. Spleen damage varies in parasitic infections (Biswas et al., 2001). The report in this study presents normal spleen histology with normal red and white pulp distribution. It could be that trypanosome organisms are not able to destroy the erythrocytes, which is in contrast with that of Aremu et al. (2018), where photomicrographs of the spleen in the study showed marked congestion of the splenic sinuses and sinusoids, suggesting marked splenic depletion *in T. brucei brucei*-infected Rats treated with the methanolic extract of *Moringa oleifera*.

The kidney is vulnerable to blood diseases. Structural impairment and kidney malfunction are usually from toxins produced by parasites and the accumulation of immune complexes (Biswas et al., 2001). In this study, Glomerular and tubular necrosis and tubular distortion of the kidney are similar to the pathological changes observed by Ghaffar et al. (2016) in *T. evansi*-infected mice.

Histopathology of the liver sections of mice reveals normal hepatocytes to moderate necrosis; this could be a result of the presence of Methionine (essential amino acid) in the plant extracts, which can protect the liver from damage by poisons such as carbon tetrachloride, arsenic and Chloroform (Akuru et al., 2018). The presence of flavonoids and alkaloids in the plant extracts might have prevented further liver cell necrosis and inflammation, as their hepatoprotective /anti- hepatotoxic ability is similar to previous studies (Cheedella et al., 2013; Meharie et al., 2020). This result contrasts with Biswas et al. (2001) reports, where severe liver cell necrosis was observed in the bandicoot rat infected with *T. evansi*.

The prolonged survival period of the extract-treated mice could be due to the presence of flavonoids as it reduces trypanosomiasis-induced inflammatory reaction by aiding the antioxidant defence system (Chen et al., 2004). For instance, flavonoids' ability to transport electrons to free radicals, chelate metals, activate antioxidant enzymes, diminish alpha-tocopherol radicals or inhibit oxidases are all antioxidant effects in biological systems (Akuru and Amadi, 2018). Further infiltration of leucocytes which might have led to neutrophils infiltration in tissues stimulated by oxidative stress, has been managed/minimised by the extract in this study, attributable to the presence of 9-Octadecinamide Z, Hexadecanoic acid methyl ester, Phytol which is highly abundant in the plant extracts. For instance, Hexadecanoic acid methyl ester and phytols have been reported to reduce inflammation by inhibiting neutrophils proliferations caused by interleukin (IL)-1β, TNF-α and oxidative stress (Silva et al., 2013; Obaseki et al., 2016).

Furthermore, Phytol can also be presented as a redox monitoring compound when it is not redox-active. Its anti-inflammatory effects can be seen in its ability to trigger reactive oxygen species production (ROS) from the phagocyte NADPH oxidase (NOX2) complex. Contrary to the fact that ROS has been reported to cause damage in tissues and cells, it is suggested that increased ROS production could ameliorate tissues (Olofsson et al., 2003; Hultqvist et al., 2006; Gelderman et al., 2007; Olofsson et al., 2014). From these antecedents, the extracts of *B. owariensis* may have promising anti-inflammatory potentials in managing inflammatory diseases such as trypanosomosis.

Conclusion

The phytochemical screening of B. owariensis indicates the presence of alkaloids, flavonoids, tannin, anthraquinone, saponin, and cardiac glycoside. Further GC-MS profiling of the methanol extract of B owariensis revealed twenty-four bioactive compounds were identified, with Phytol (23.29%), 9-Octadecenamide, Z (8.81%), Hexadecanoic acid methyl ester (8.20%), 7-Hexadecenoic acid methyl ester (7.62%), 3,8-Nonadien-2-one (7.41%) and Heptanoic acid (5.07%). In the BALB/c mice trypanosomosis model, methanol extracts of *B. owariensis* had ameliorative and anti-inflammatory effects at an optimal dose of 100 mg/Kg against *T. brucei* induced pathologies on the liver, kidney, spleen, lungs, and heart. As a result, additional research is needed to extract the most prevalent secondary metabolites for pharmacological testing against African Trypanosomes.

Declarations

Funding

This study received no specific support from public, private, or non-profit funding bodies. The authors entirely sponsored the research.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NGA, YAW, SJO, and DMS conceptualised and designed the study. NGA experimented, and data was collected. SBR-Y, YAW, SJO, and DMS oversaw the experiment in the laboratory. YAW was in charge of data analysis and manuscript writing. SBR-Y, MM, DMS and SJO revised the manuscript. The final version of the manuscript has been read and approved by all authors.

Availability of data and materials

The datasets utilised and analysed in this study are available on reasonable request.

Ethics approval

The Ethical Committee on Animal Usage and Care, A.B.U., Zaria, Nigeria, gave their clearance to use animals (Approval Number: ABUCAUC/2018/062). All appropriate international, national, and institutional animal care and usage guidelines were followed in the letter.

Consent for publication

Not applicable

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Figures

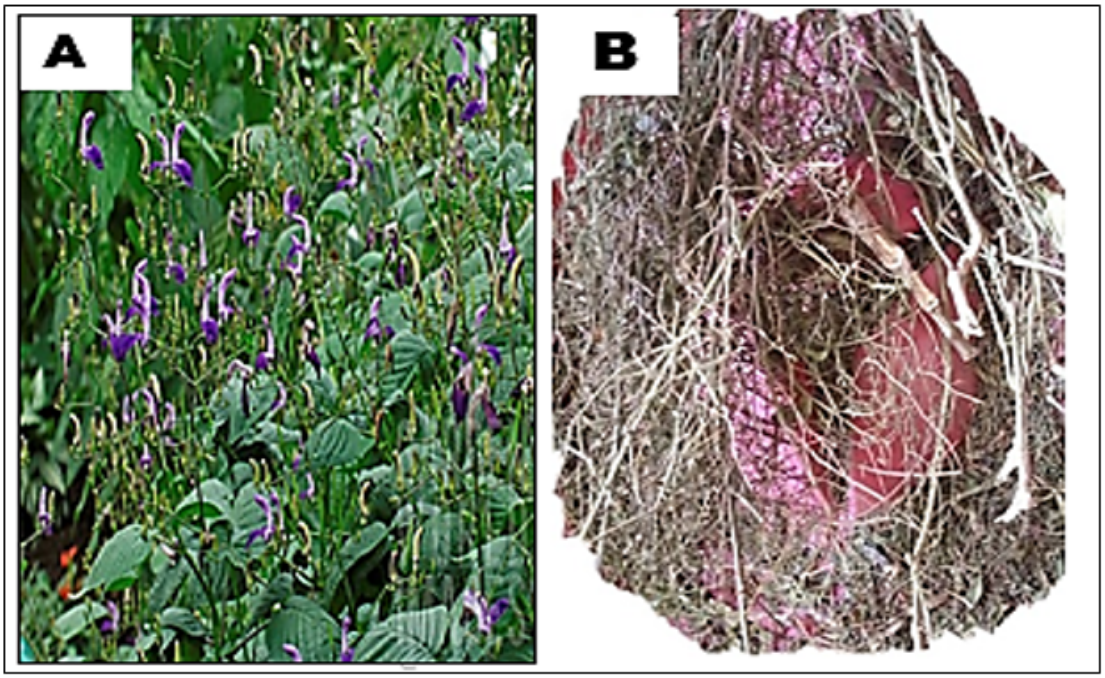

Figure 1

Brillantaisia owariensis, **A-** Whole fresh plant **B**-Air-dried whole plant

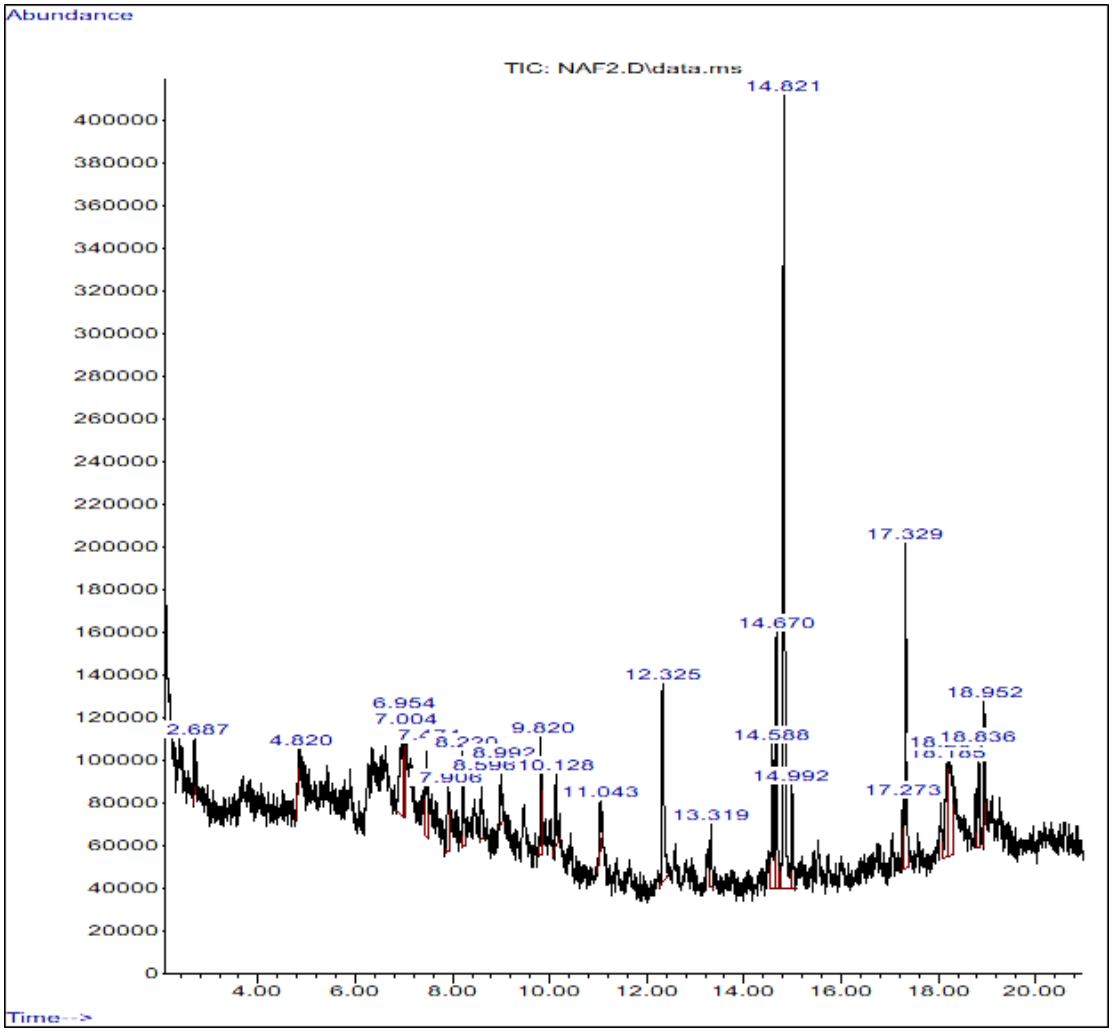

Figure 2

GC-MS spectral chromatogram of compounds identified in the crude methanol extract of *Brillantaisia owariensis*.

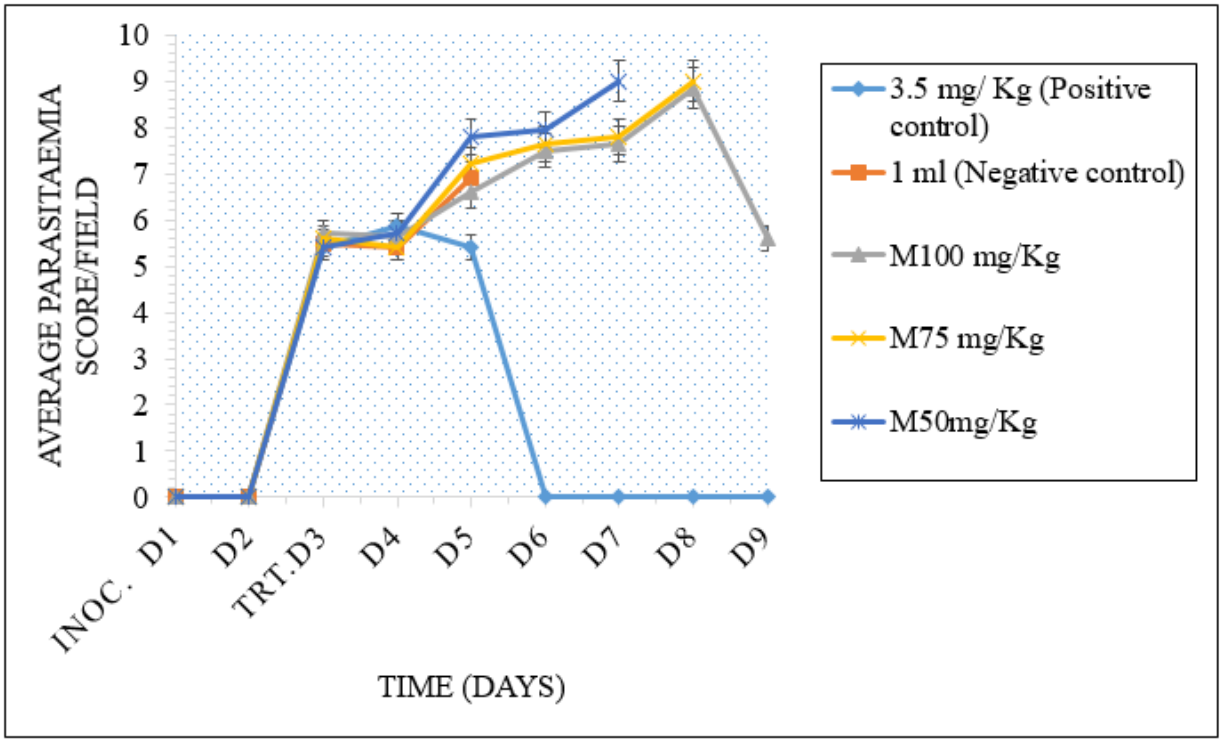

Figure 3

Effect of methanol extracts of *Brillantaisia owariensis* on the mean daily parasitemia score of control and treated BALB/c mice against *Trypanosoma brucei* infection.

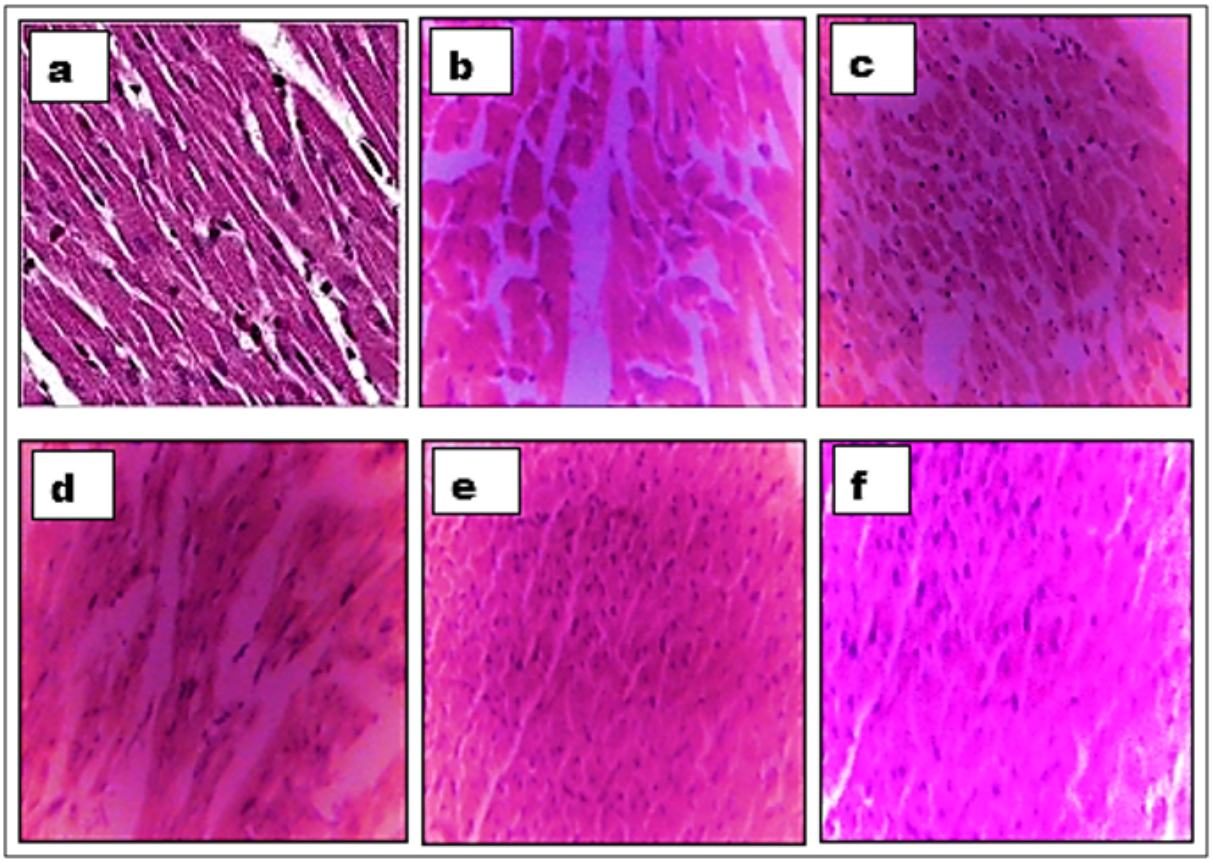

Figure 4

(**a-f**): Photomicrograph of heart sections of BALB/c mice: **a-** Normal control showing normal myocardium. **b-** Treated with normal saline, moderate myocardium necrosis. **c**- Control; treated with Diminazene aceturate, normal myocardium), **d**- treated with methanol 100mg/ kg showing normal myocardium, **e-** M75mg/kg showing normal myocardium. **f**- M50mg/kg showing mild myocardium necrosis. (H and E, X 400)

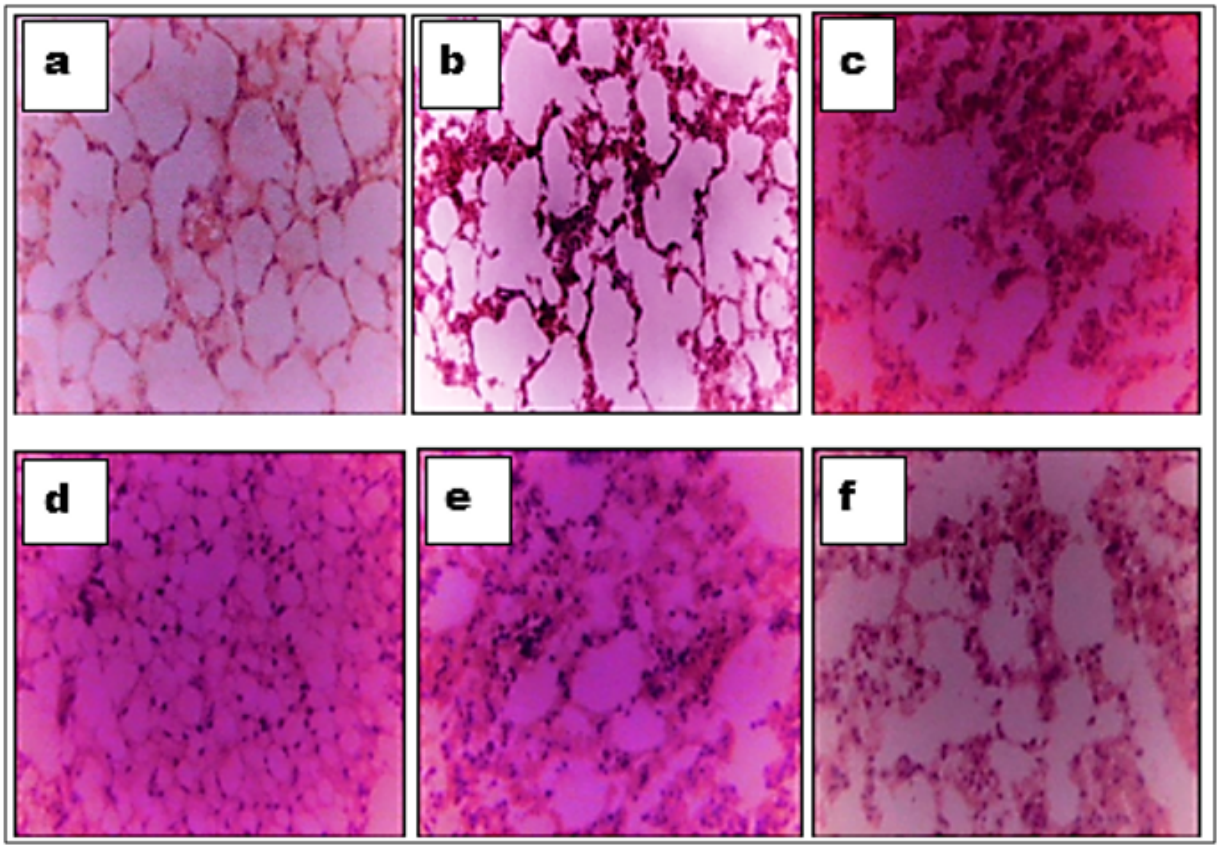

Figure 5

(**a-f**): Photomicrograph of lung sections of BALB/c mice: **a-** Normal control showing normal alveoli. **b**- Treated with normal saline with Lymphocyte hyperplasia. **c**- Control; treated with Diminazene aceturate shows alveoli congestion. **d**- methanol 100mg/kg with normal alveoli. **e**- M75mg/kg with Lymphocyte hyperplasia. **f**- M50mg/kg with normal alveoli. (H and E, X 400)

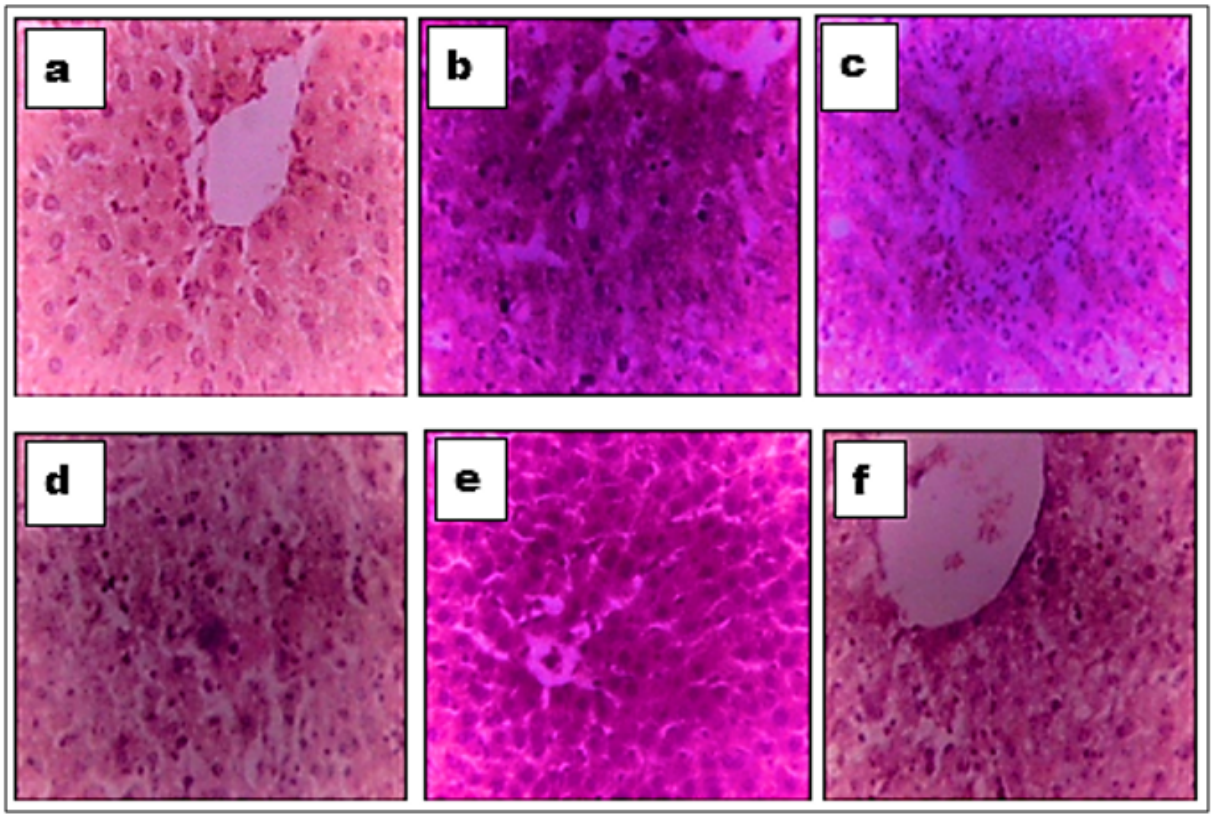

Figure 6

(**a-f**): Photomicrograph of liver sections of BALB/c mice: **a-** Normal control showing normal hepatocytes. **b**- Treated with normal saline moderate to severe hepatic necrosis. **c**- Control; treated with Diminazene aceturate showing mild hepatic necrosis. **d**- methanol 100mg/kg with mild hepatic necrosis. **e**- M75mg/kg showing normal hepatocytes. **f**- M50mg/kg showing mild hepatic necrosis. (H and E, X 400)

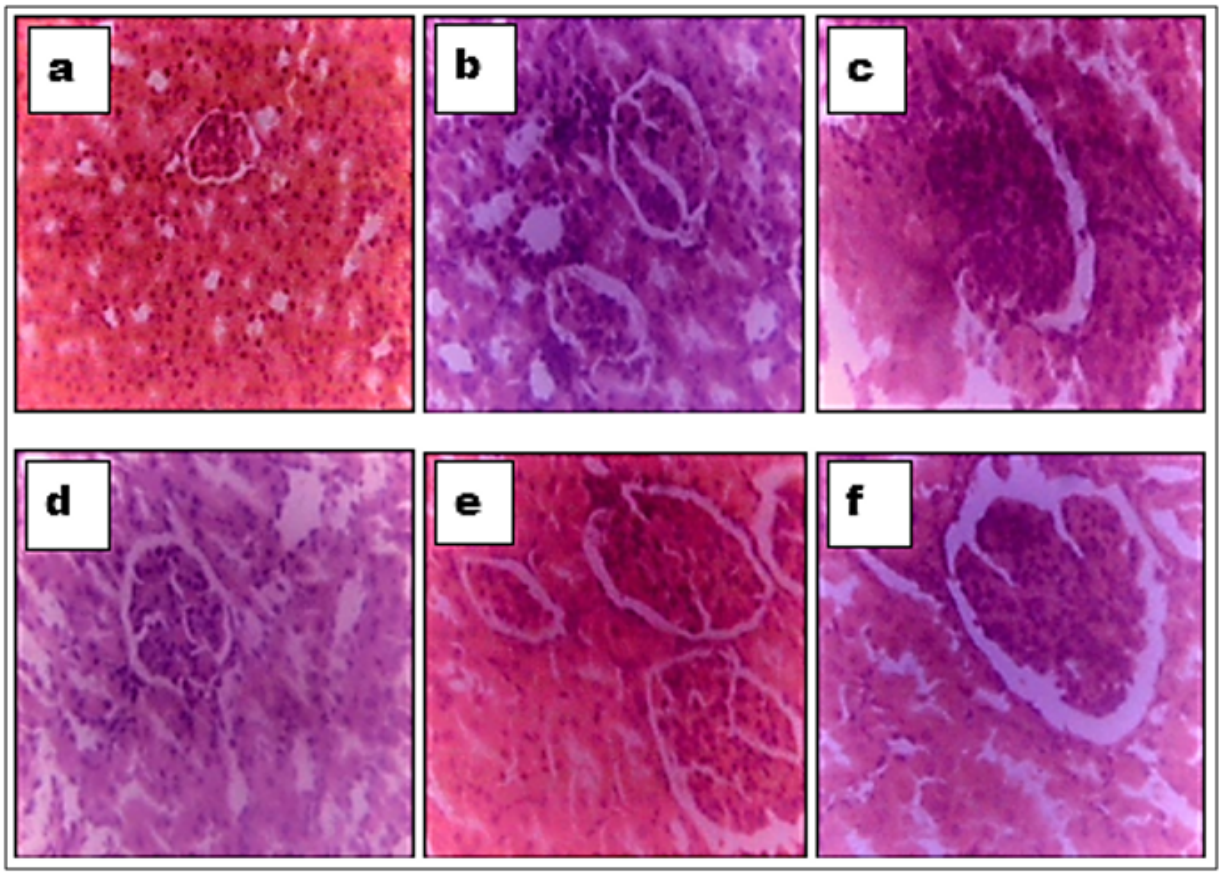

Figure 7

(**a-f**): Photomicrograph of kidney sections of BALB/c mice: **a-** Normal control showing normal tubules and glomerulus. **b**- Treated with normal saline with tubular distortion and tubular necrosis. **c**- Control; treated with Diminazene aceturate showing moderate tubular adhesion and necrosis. **d**- Methanol 100mg/kg with mild tubular necrosis. **e**- M75mg/kg normal tubules and glomerulus. **f**- M50mg/kg moderate tubular distortion and tubular necrosis. (H and E, X 400)

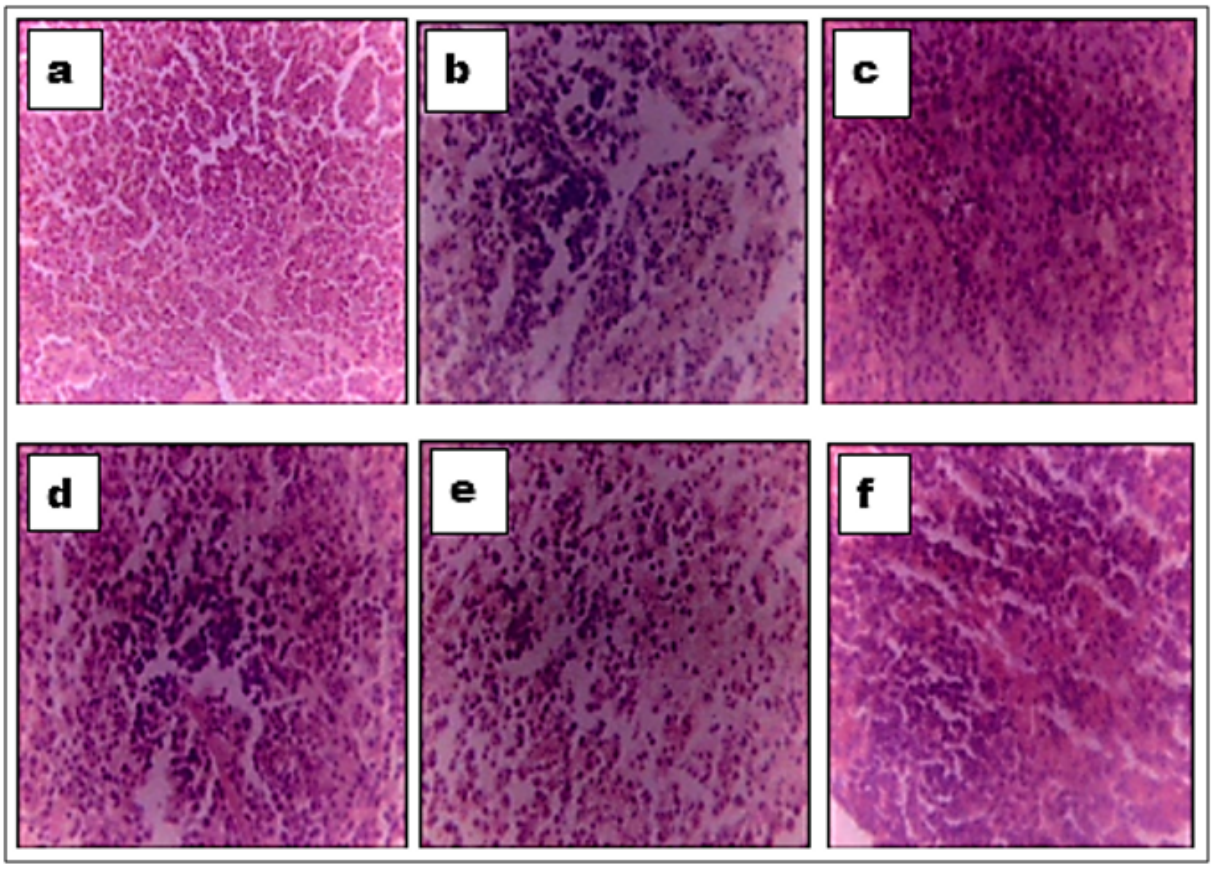

Figure 8

(**a-f**): Photomicrograph of spleen sections of BALB/c mice: **a-** Normal control with normal red and white pulp distribution. **b**- Treated with normal saline shows slight necrosis. **c**- Control; treated with Diminazene aceturate showing normal red and white pulp distribution. **d**- Methanol 100mg/kg with Normal red and white pulp distribution. **e**- M75 mg/kg Slight lymphocyte hyperplasia. **f**- M50mg/kg Normal red and white pulp distribution. (H and E, X 400)