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ORIGINAL PAPER

Lactone-rich fraction from *Vernonia blumeoides*: antitrypanosomal activity and alleviation of the parasite-induced anemia and organ damage

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Abstract The anti-*Trypanosoma brucei brucei* activity in vitro and in vivo of a lactone-rich fraction of *Vernonia blumeoides* leaves (VBLF) and its potential in alleviating trypanosome-induced anemia and organ damage were investigated. Gas chromatography–mass spectrometry (GC–MS) analysis of VBLF revealed the presence of a number of lactone-containing compounds. In an in vitro study, VBLF showed concentration-dependent activity and was further used to treat *T. brucei brucei*-infected rats. The VBLF treatments, especially at 300 mg/kg body weight (BW), significantly ($P < 0.05$) kept the parasites reduced during the entire experimental period compared with the infected untreated group. At the end of the experiment, the trypanosome-induced anemia and hepatic damage were significantly ($P < 0.05$) alleviated in all the VBLF treatment groups, but renal damage was only prevented in the 200 and 300 mg/kg BW treatment groups. Furthermore, the trypanosome-induced increase in the relative weights of liver, spleen and kidney were significantly ($P < 0.05$) alleviated by the 300 mg/kg BW VBLF treatment. It was concluded that orally administered VBLF, especially at 300 mg/kg BW, possessed antitrypanosomal activity and could alleviate parasite-induced anemia and organ damage.

Keywords Anemia · Antitrypanosomal activity · Organ damage · Lactones · *Vernonia blumeoides*

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Introduction

African animal trypanosomiasis has re-emerged over the last decade as an important menace to human health and economic development. It is still a major scourge in sub-Saharan Africa and largely accounts for the low livestock productivity of the continent [1], thus making it an important priority for biomedical and public agencies, the agricultural sector and the scientific community [2]. The disease caused by the *Trypanosoma brucei* subgroup is associated with anemia, hepatocellular degeneration and glomerulonephritis [3], which are largely attributed to the large amount of free radicals and superoxides generated by the trypanosomes that attack membrane polyunsaturated fatty acids and proteins, resulting in cellular injuries and consequently affecting vital tissues and organs of the infected animals [4, 5]. The hope for vaccine development against this fatal disease is still elusive, and chemotherapy, as the only available option, remains far from satisfactory [6] due to parasite resistance, cost and toxic side effects of the drugs [7]. Thus, the importance of identifying new lead compounds that could potentially be used for the development of chemotherapeutic drugs against this disease cannot be overemphasized.

The use of medicinal plants for the treatment of various diseases is an important component of a health care delivery system, especially in Africa where more than 5,400 medicinal plants were reported to have over 16,300 medicinal uses [8]. The influence of these medicinal plants and natural products upon drug discovery is impressive because a number of clinically active drugs are either natural products or have a natural product pharmacophore [9].

Vernonia blumeoides (bitter genus) is a perennial herb belonging to the plant family Asteraceae and is commonly used in traditional medicine for the treatment of various

protozoan and other infectious diseases in Nigeria. Plants of the Asteraceae family and especially the genus *Vernonia* are characteristically well known to contain lactone-containing compounds, especially sesquiterpene lactones which have been isolated from several *Vernonia* species [10, 11]. The development of the antimalarial drug artemisinin, which is a sesquiterpene lactone isolated from a Chinese medicinal herb, has attracted renewed interest in the antiprotozoal potentials of this group of phytochemicals. Recently, some researchers from Latin America have investigated the antitrypanosomal activities of sesquiterpene lactones from their indigenous *Ambrosia tenuifolia* and *Anthemis tinctoria* against intracellular parasites, *Trypanosoma cruzi* and *Leishmania mexicana* [12, 13]. However, the biochemistry and pathophysiology of these parasites are distinct from those of African trypanosomes. In this study, we investigated the antitrypanosomal activity in vitro and in vivo of a lactone-rich fraction from *V. blumeoides* leaves (VBLF) against extracellular African animal trypanosomes. Furthermore, the study investigated the ameliorative effects of VBLF towards the trypanosome-induced anemia and organ damage which are key pathogenetic features of the disease.

Materials and methods

Plant material

Vernonia blumeoides was collected in September, 2010 at Dakace village along Jos Road, Zaria, Nigeria. The identity of the plant was confirmed at the herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria (ABUZ) and was deposited with voucher number 1784. The leaves were removed and shade-dried for 2 weeks to a constant weight. The dried leaves were pounded to fine powder with a mortar and pestle, and then stored in dry containers until needed.

Preparation of lactone-rich fraction

A powdered leaf sample (300 g) of the plant was exhaustively extracted with 95 % ethanol on a Soxhlet apparatus for 3 h. The extract was concentrated on a Büchi rotary evaporator at 45 °C to afford 42.3 g of crude ethanol extract of *V. blumeoides*. The lactone-rich fraction was obtained by successive partitioning steps as previously described [14]. Briefly, the crude ethanol extract (40 g) was partitioned between equal amounts of chloroform and water. The chloroform fraction (9.10 g) was next partitioned between equal amounts of petroleum ether and 10 % aqueous methanol. Subsequently, the aqueous methanol fraction (8.04 g) was further concentrated and partitioned between equal amounts of water and chloroform. The chloroform fraction was then concentrated to yield a fraction (5.10 g) which was considered as the lactone-rich fraction (VBLF). The fractionation pattern is shown in Fig. 1.

Gas chromatography–mass spectrometry (GC–MS) analysis of VBLF

The GC–MS analysis of VBLF was conducted with an Agilent Technologies 6890 series GC coupled with an Agilent 5973 mass selective detector and driven by Agilent Chemstation software. A HP-5MS capillary column was used (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). The carrier gas was ultra-pure helium at a flow rate of 1.0 ml/min and a linear velocity of 37 cm/s. The injector temperature was set at 250 °C. The initial oven temperature was 60 °C and programmed to 280 °C at the rate of 10 °C/min with a hold time of 3 min. Injection of 1 µl was made in splitless mode with a split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay 4 min and scan range 50–700 amu. Compounds were identified by direct comparison of the retention times and mass spectral data with those in the National Institute of Standards and Technology (NIST) library. The relative percentage of each component was calculated by comparing its peak to the total areas.

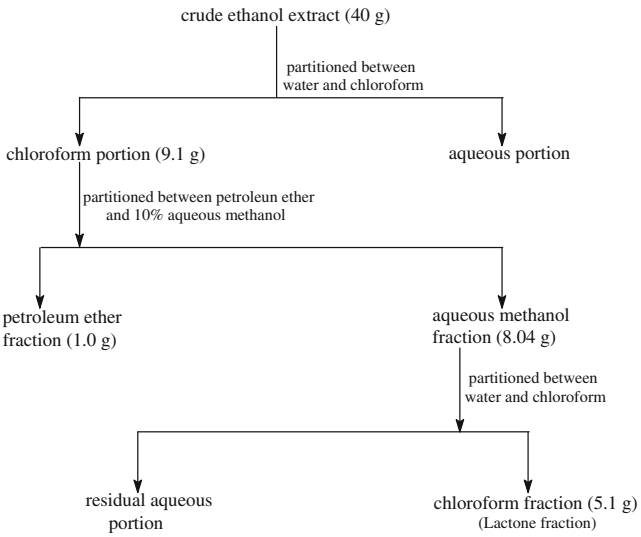

Fig. 1 Preparation of the lactone-rich fraction from *V. blumeoides* leaves

Experimental animals

The protocol employed met the guidelines of the Good Laboratory Practice (GLP) regulations of the World Health Organization and the rules and regulations of the experimental animal ethics committee of ABUZ were also duly followed. Apparently healthy Wistar rats weighing 130–165 g were obtained from the animal house of the National Research Institute for Chemical Research Technology, Zaria. The animals were maintained in polycarbonated laboratory cages (25 ± 2 °C, 12-h light/dark cycle) and fed on a commercial rat chow (ECWA Feeds, Jos, Nigeria) with drinking water ad libitum.

Trypanosome parasites

Trypanosoma brucei brucei (Federe strain) was obtained from the Nigerian Institute of Trypanosomiasis and Onchorcerciasis Research, Vom, Nigeria. Parasites harvested from the blood of a donor rat at peak parasitemia (10^9 parasites/ml) were diluted with phosphate-buffered saline and then used for both the in vitro and in vivo studies.

In vitro screening of VBLF against *T. brucei brucei*

Different concentrations of VBLF ranging from 5 to 20 mg/ml were prepared. The in vitro antitrypanosomal activity was assessed in triplicate in 96-well microtiter plates. In the wells of the microtiter plates, aliquots of 20 µl of each extract concentration were incubated with 40 µl of the infected blood (about 10^9 parasites/ml of blood), achieving effective VBLF concentrations of 6.66, 3.33 and 1.67 mg/ml in the reaction mixtures. The fraction was replaced with phosphate-buffered saline and 6.66 mg/ml of a standard trypanocidal drug (berenil) for control and reference tests, respectively. Parasite count was then monitored under a microscope at 400× magnification. The percentage of motile parasites was counted at 5-min intervals for 1 h. Cessation or drop in motility of the parasites in VBLF- and berenil-treated blood compared to that of parasite-loaded control blood without the fraction was taken as a measure of antitrypanosomal activity [15].

In vivo anti-*T. brucei brucei* activity of VBLF

Thirty-five Wistar rats (130–165 g) of both sexes were randomly allocated into seven groups of five rats each in order to investigate the effect of the fraction on *T. brucei brucei* infection. The rats in each group received the following treatments:

Normal control (NC): neither infected with the parasites nor treated with VBLF.

Fraction control (FC): uninfected but orally treated with 300 mg/kg BW of VBLF daily.

Infected control (IC): Infected and not treated.

Infected + 100 mg/kg BW (ITL): infected and orally treated with 100 mg/kg body weight (BW) of VBLF.

Infected + 200 mg/kg BW (ITM): infected and orally treated with 200 mg/kg BW of VBLF.

Infected + 300 mg/kg BW (ITH): infected and orally treated with 300 mg/kg BW of VBLF.

Infected + berenil (ITS): infected and treated with 100 mg/kg BW of berenil.

The rats in all groups (except NC and FC) were infected by intraperitoneal injection of about 10^5 *T. brucei brucei* per 100 g BW and were daily treated with the respective doses of VBLF or berenil, beginning on day 4 post-infection (PI) when parasitemia reached approximately 10^8 trypanosomes/ml of blood. The parasites were monitored daily using the rapid matching counting method [16] and the experiment was terminated at day 14 PI. The pre-infection and terminal (on day 14 PI) packed cell volumes (PCV) of all groups of rats were determined by the microhematocrit method.

Assessment of the effects of VBLF on trypanosome-induced organ damage

Serum harvested from the blood of all animals after humane decapitation on day 14 PI was used to measure aspartate and alanine aminotransferases (AST and ALT) activities as well as urea concentrations using commercial reagent kits (Randox Laboratories, Ireland). Liver, spleen and kidney of all rats were also collected and weighed to ascertain the relative organ weight for all groups of animals.

Statistical analysis

All data are presented as the mean ± SD of five animals. Data were analyzed with a statistical software package (SPSS for Windows, version 18, IBM Corporation, NY, USA) using Tukey's-HSD multiple range post-hoc test. Values were considered significantly different at $P < 0.05$.

Results

GC–MS analysis and anti-*T. brucei brucei* activity of VBLF

The GC–MS analysis of VBLF showed the separation of 35 components, and 25 of them, constituting 32 % of the lactone-rich fraction, including 4,4-dimethyl benz[c]pyran-1,3-dione (11.13 min); 4,4,7a-trimethyl 5,6,7,7a-tetrahydro

(4H)-benzofuranone (14.62 min); 4,4,7a-trimethyl (3H)-benzofuranone (20.04 min); 4,8,12,16-tetramethylheptadecan-4-olide (20.07 min) and 2-dodecen-1-yl (−)succinic anhydride (21.07 min), were identified through reasonable spectral data matches and found to conform to the NIST spectra database libraries. The fragmentation pattern of the compounds with their retention times are presented in Table 1. VBLF was initially tested for in vitro activity against the bloodstream form of *T. brucei brucei* and the fraction demonstrated a concentration-dependent in vitro activity against the parasites. There was no significant difference ($P > 0.05$) in the number of motile *T. brucei brucei* between VBLF and berenil at 6.66 mg/ml each during most of the incubation period (Fig. 2). Subsequently, the effects of different oral doses of VBLF on the course of *T. brucei brucei* infection in rats were investigated and the results are presented in Fig. 3. The parasites were first detected in the bloodstream of all infected animals on day 4 PI but the VBLF treatments suppressed the multiplication of the parasites during the entire 14-day experimental period. In fact, a significantly higher ($P < 0.05$) in vivo antitrypanosomal activity was observed in the ITH group than the ITS group, indicating that 300 mg/kg BW of VBLF is more trypanocidal than berenil. There was a progressive increase in parasitemia in the IC group up to day 14 PI.

Effects of VBLF on trypanosome-induced pathological changes

There were no significant ($P > 0.05$) differences in the pre-infection PCV of all groups of rats (Fig. 4), but all infected groups developed anemia as the infection progressed, as indicated by significant ($P < 0.05$) drops in PCV. However, the disease-induced anemia was significantly ($P < 0.05$) ameliorated by all the VBLF treatments.

The results of the indices of hepatic and renal functions analyzed in this experiment are presented in Table 2. The *Trypanosoma brucei brucei* infection caused significant increases ($P < 0.05$) in serum AST and ALT levels from the normal level and VBLF treatment significantly ($P < 0.05$) ameliorated the trypanosome-induced increase in these parameters at all the doses tested. The rats in the FC group also recorded a significant increase ($P < 0.05$) in the level of ALT but not AST. Furthermore, serum urea concentration was also significantly ($P < 0.05$) elevated in the IC group (Table 2), but the parasite-induced elevation was significantly ($P < 0.05$) prevented in the ITM and ITH groups. Data for the relative organ weights are presented in Table 3. Hepatomegaly and splenomegaly were observed as increase in liver:BW and spleen:BW ratios, respectively, in the infected animals. However, VBLF treatment significantly ($P < 0.05$) ameliorated the trypanosome-induced hepatomegaly and non-significantly ($P < 0.05$) ameliorated the splenomegaly in the ITH group only. Relative kidney weight was also significantly ($P < 0.05$) increased in the IC group and was only significantly ($P < 0.05$) prevented in the ITH group.

Discussion

Previous studies have demonstrated the potentials of a number of African medicinal plants in the treatment of trypanosomiasis [17–19], but the groups of phytochemicals responsible for the observed antitrypanosomal activity have not been investigated for most of these plants. In the present study, we evaluated the in vitro and in vivo anti-*T. brucei brucei* activity of a lactone-rich fraction of *V. blumeoides* leaves and also the ameliorative effects of the fraction on the disease-induced anemia and organ damage.

Analyses of the GC–MS data for VBLF revealed that a number of the compounds among the 25 identified components contain lactone derivatives. Interestingly, other components with a sesquiterpene carbocyclic skeleton such as eudesma-4(14),11-diene and its oxygenated derivatives including caryophyllene oxide, bacchotricuneatin C and *cis*-Z-alpha-bisabolene epoxide were also identified. This chemical feature suggests the possible occurrence of sesquiterpene lactones in the fraction because the carbocyclic components are derived from sesquiterpene lactones. In addition, the major compound among other compounds with no lactone moiety is chrysin; although chrysin is a flavonoid, it has an oxygenated heterocyclic system, a chemical feature of a lactone moiety.

Parasite motility is a relatively reliable indicator of viability of most zooflagellate parasites [20]. Cessation or drop in motility of trypanosomes therefore serves as a measure of antitrypanosomal potential of an agent when compared to the control. Our in vitro studies revealed that VBLF was effective in reducing *T. brucei brucei* motility. Since a phytochemical with high in vitro activity may show no in vivo antitrypanosomal activity and vice versa, due to xenobiotic metabolism that may convert active therapeutic molecules to inactive ones, we further tested VBLF for in vivo activity so that a definite statement could be made on its antitrypanosomal effects.

VBLF in vivo antitrypanosomal activity indicates that the fraction is therapeutically active in reducing the number of *T. brucei brucei* in the bloodstream of infected animals and could be relevant in the treatment of African animal trypanosomiasis. Although the exact mechanism for the observed in vivo antitrypanosomal activity is not yet known, previous studies have reported the anti-cancer activity of a number of lactone-containing compounds, especially sesquiterpene lactones from the *Vernonia* genus [21]. This is exciting because the currently available drugs

Table 1 Components of VBLF identified by GC–MS

No	Retention time (min)	Name of compound	Molecular formula	Molecular weight	Peak area (%)
1	10.12	Vanillin	$C_7H_7O_3$	139	0.617
2	11.13	Benz[c]pyran-1,3-dione, 4,4-dimethyl-	$C_{10}H_{10}O_3$	178	0.295
3	13.09	2-(3-Hydroxybutyl)cyclooctanone	$C_{12}H_{22}O_2$	198	0.411
4	13.98	2(1H)-Naphthalenone, octahydro-4a, 7,7-trimethyl-, *trans*-	$C_{13}H_{21}O$	193	0.157
5	14.23	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	1.086
6	14.62	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{17}O_2$	181	0.428
7	15.73	1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]oxiren-5(1aH)-one, 2,7,9,10-tetrakis(acetyloxy)decahydro-3,6,8,8,10a-pentamethyl-	$C_{27}H_{38}O_{11}$	538	2.280
8	16.00	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.401
9	16.01	Eudesma-4(14),11-diene	$C_{15}H_{21}$	201	2.498
10	16.25	Bacchotricuneatin C	$C_{20}H_{22}O_5$	342	0.545
11	16.71	3,5-Dimethoxy-4-hydroxycinnamaldehyde	$C_{11}H_{12}O_4$	208	0.867
12	16.81	Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl)-	$C_8H_{10}O_4$	170	3.933
13	20.04	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-	$C_{11}H_{18}O_2$	182	1.429
14	20.07	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	1.260
15	21.07	2-Dodecen-1-yl (−)succinic anhydride	$C_{17}H_{26}O_3$	278	0.480
16	21.24	*Cis*-Z-alpha-bisabolene epoxide	$C_{15}H_{34}O_2$	220	1.429
17	21.43	Sandaracopimar-15-ene-6-beta, 8-beta-diol	$C_{20}H_{34}O_2$	306	0.910
18	21.53	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	$C_{16}H_{22}O_4$	298	0.601
19	22.16	2(1H)-Pyridinone	C_5H_5NO	95	0.980
20	22.19	9,10-Cyclolanostan-3-ol, 24,24-epoxymethano-, acetate	$C_{33}H_{54}O_3$	498	0.598
21	22.55	Rhododactynaphin-jc-1	$C_{30}H_{27}O_{12}$	578	0.980
22	22.72	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-	$C_{17}H_{24}$	228	1.420
23	22.90	Chrysin	$C_{15}H_{10}O_4$	222	6.754
24	23.25	5H-Dibenz[c,e]azepine, 6,7-dihydro-	$C_{15}H_{12}N$	206	0.576
25	24.54	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2ylester	$C_{12}H_{20}O_2$	196	1.911

Grouped components	Percentage (%)	Total peak area (%)
Lactone-containing compounds	32	7.60
Sesquiterpene and sesquiterpenoids	16	4.88
Phenolics	24	11.21
Terpenoids and others	16	7.46
Fatty acids and esters	12	3.11
Total	100	34.26

used clinically for the treatment of trypanosomiasis are known to have some levels of anti-cancer activities and the only antitrypanosomal drug that has entered the market in recent times, difluoromethylornithine (DFMO), was originally developed as an anti-cancer drug [22]. On the other hand, sesquiterpene lactones have been reported to inhibit cancer growth through selective alkylation of thiol groups in key regulatory enzymes which control cell division [23]. While not discounting other possible mechanisms of action and involvement of other components, it is reasonable to speculate that the lactone-containing components of VBLF might be interfering with the cell cycle progression of the parasites, causing cell cycle arrest and subsequently halting cell proliferation.

Anemia with concomitant increase in the levels of serum AST and ALT as well as urea concentration have all been reported in experimental animal trypanosome infections [3–5], partly due to the generation of free radicals and superoxides by the parasites which causes degenerative changes in vital tissues and organs of infected animals [24], subsequently leading to death. Thus, the presence and severity of these pathological alterations are good indicators of the disease status and their control is an integral part of the disease management. Consequently, any drug used

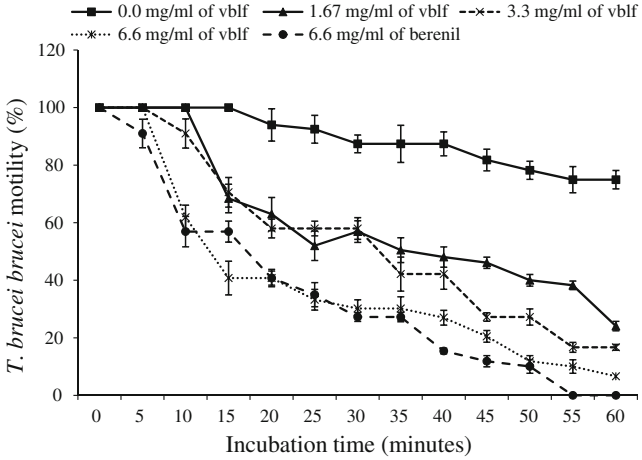

Fig. 2 In vitro anti-*T. brucei brucei* activity of various concentrations of VBLF. All data are shown as mean ± SD

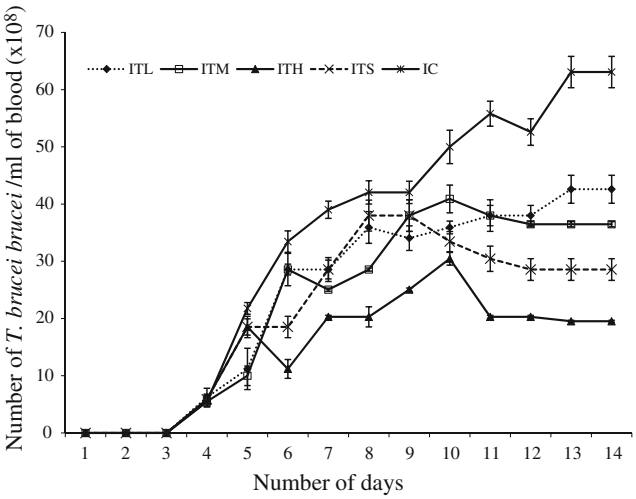

Fig. 3 Effects of different doses of VBLF on the course of *T. brucei brucei* infection in rats. All data are shown as mean ± SD. *ITL*, *ITM* and *ITH* are groups of rats infected with *T. brucei brucei* and orally treated with 100, 200 and 300 mg/kg BW of VBLF, respectively. *ITS* is a group that was infected and treated with 100 mg/kg BW of berenil. *IC* is the infected untreated control group

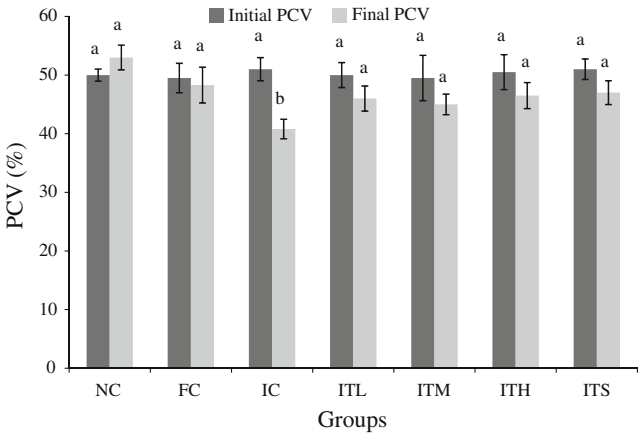

Fig. 4 Effects of different doses of VBLF on the PCV levels of *T. brucei brucei* infected rats. All data are shown as mean ± SD. [a,b]Values with different letters over the bars for a given group are significantly different from each other (Tukey's-HSD multiple range post-hoc test, $P < 0.05$). *NC* is an uninfected untreated (normal) control group while *FC* is a VBLF-treated uninfected control group. *ITL*, *ITM* and *ITH* are groups of rat infected with *T. brucei brucei* and orally treated with 100, 200 and 300 mg/kg BW of VBLF, respectively. *ITS* is a group that was infected and treated with 100 mg/kg BW of berenil. *IC* is the infected untreated control group

for the treatment of African trypanosomiasis should also be effective in controlling the associated pathological changes. The alleviation of the trypanosome-induced anemia, hepatic and renal damages by VBLF is remarkable because only a few plant materials have been previously reported to possess such activities in addition to in vivo antitrypanosomal activity [5, 25]. It is thus possible that VBLF also possesses some antioxidant activities that could scavenge the *T. brucei*-generated free radicals and thus spare the erythrocytes as well as hepatic and renal tissues from oxidative damage. The amelioration of trypanosome-induced anemia and organ damage by some antioxidant vitamins [3, 4, 26] and the antioxidant activity of some lactone-containing compounds have been reported [27, 28].

Table 2 Effects of different oral doses of VBLF on some serum biochemical parameters of *T. brucei brucei*-infected rats

	Groups						
	NC	FC	IC	ITL	ITM	ITH	ITS
AST (U/l)	26.06 ± 1.27^{a}	25.48 ± 1.18^{a}	62.52 ± 5.08^{b}	37.97 ± 3.82^{c}	28.26 ± 6.60^{ac}	20.09 ± 5.66^{a}	32.52 ± 4.00^{c}
ALT (U/l)	9.24 ± 0.57^{a}	13.56 ± 1.32^{b}	18.07 ± 0.68^{c}	11.02 ± 1.77^{ab}	11.82 ± 0.94^{ab}	9.88 ± 1.27^{a}	9.64 ± 0.26^{a}
Urea (mM)	4.20 ± 0.95^{a}	4.30 ± 0.89^{a}	6.20 ± 0.75^{b}	5.20 ± 1.26^{b}	4.90 ± 0.48^{a}	4.60 ± 0.55^{a}	5.60 ± 0.65^{b}

Data are presented as mean ± SD values. Values with different letters within a row are significantly different from each other (Tukey's-HSD multiple range post-hoc test, $P < 0.05$). *NC* is an uninfected untreated (normal) control group while *FC* is a group uninfected but orally treated with 300 mg/kg BW of VBLF. *ITL*, *ITM* and *ITH* are groups of rat infected with *T. brucei brucei* and orally treated with 100, 200 and 300 mg/kg BW of VBLF, respectively. *ITS* is a group that was infected and treated with 100 mg/kg BW of berenil. *IC* is the infected untreated control group

Table 3 Effects of different oral doses of VBLF on the relative organ weights of *T. brucei brucei*-infected rats

Groups	Liver:BW ($\times 10^{-2}$)	Spleen:BW ($\times 10^{-2}$)	Kidney:BW ($\times 10^{-2}$)
NC	3.73 ± 0.50^{a}	0.71 ± 0.05^{a}	0.66 ± 0.03^{a}
FC	4.91 ± 0.16^{b}	0.94 ± 0.03^{b}	1.01 ± 0.05^{b}
IC	7.80 ± 1.32^{c}	2.33 ± 0.08^{c}	1.48 ± 0.08^{c}
ITL	6.37 ± 0.17^{c}	3.04 ± 0.21^{d}	1.44 ± 0.24^{c}
ITM	6.61 ± 0.11^{c}	2.70 ± 0.06^{e}	1.40 ± 0.03^{c}
ITH	4.33 ± 0.94^{ab}	1.22 ± 0.04^{f}	1.02 ± 0.01^{b}
ITS	4.52 ± 0.22^{b}	1.81 ± 0.09^{g}	1.05 ± 0.02^{b}

Data are presented as mean ± SD. Values with different letters within a column are significantly different from each other (Tukey's-HSD multiple range post-hoc test, $P < 0.05$). *NC* is an uninfected untreated (normal) control group while *FC* is a group uninfected but orally treated with 300 mg/kg BW of VBLF. *ITL*, *ITM* and *ITH* are groups of rat infected with *T. brucei brucei* and orally treated with 100, 200 and 300 mg/kg BW of VBLF, respectively. *ITS* is a group that was infected and treated with 100 mg/kg BW of berenil. *IC* is the infected untreated control group

T. brucei brucei caused hepatomegaly and splenomegaly, which is consistent with our previous report [5]. However, the trypanosome-induced hepatomegaly and splenomegaly were ameliorated in the ITH group and this observation might not be unconnected with the lowest level of parasitemia recorded in this group during the experimental period, because the severity of the disease-induced hepatomegaly and splenomegaly is often associated with the onset and degree of parasitemia [3]. On the other hand, the increase in the relative kidney weight by the infection was quite surprising to us because we had not recorded such changes in any previous study, and the virulence of the strain of the parasite used in this study could account for this observation. Interestingly, VBLF was able to reverse the relative kidney weight changes, at least in the ITH group. Thus, considering liver, kidney and spleen as representative organs in infected animals, it can be suggested that VBLF is therapeutically active in alleviating most of the organ weight alterations caused by *T. brucei brucei*.

Data from this study suggest that lactone-rich fraction from *V. blumeoides* leaves possesses anti-*T. brucei brucei* activity in vitro and in vivo and tends to ameliorate the disease-induced anemia and organ damage. Evaluating the in vivo anti-*T. brucei brucei* activity of some isolated lactone-containing compounds from this plant will be the subject of our further studies.

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Conflict of interest The authors declare that they have no competing interest.

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