Original Article

In Vivo Antitrypanosomal Activities of Methanolic Extract of *Lawsonia inermis* Linn. Leaves on *Trypanosome Brucei* Infected Wistar Rat

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ABSTRACT

Background: Trypanosomiasis is a major disease affecting both humans and animals. Nearly 30000 individuals in various countries of sub-Saharan Africa have African trypanosomiasis, which leads to approximately 21000 deaths annually.

Objectives: This study aimed to evaluate the anti-trypanocidal effects of *Lawsonia inermis* (LI) in rats infected with *Trypanosome Brucei*.

Methods: Thirty rats were allotted to groups (1-5), six rats each: Group 1 (negative control), 2 (tryps control), 3 (diminazene [DA] 7 mg/kg), 4 (LI at 200 mg/kg) and 5 (DA+LI). All rats in groups (2-5) were infected with 3×10^6 Trypanosoma brucei per milliliter of blood.

Results: The percentage weight gain of rats in the DA extract combination group showed increased weight gain (6.3%) compared to tryps-control. DA showed significant weight gain compared to the negative control. The survivability rate showed that the DA, LI and DA+LI combinations survived for 14 days without visible relapse. The packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), platelet and mean corpuscular volume (MCV) increased significantly in the extract-treated groups. In contrast, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) decreased significantly. Lymphocytes, monocytes, eosinophils, and basophils showed significantly more growth than those in the control group. Globulin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and blood urea nitrogen increased non-significantly. Creatinine and total bilirubin levels were significantly decreased compared to those in the untreated control. LI significantly increased glutathione (GSH), glutathione S-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD) and

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decreased MDA and inflammatory cytokines (interleukin [IL]-1, 6 and 12) compared to the untreated control groups.

Conclusion: LI reduced parasitemia in the transient phase, and the drug-extract combination cleared parasitemia quickly.

Keywords: Extract, *Lawsonia inermis* (LI), *T. brucei*, Trypanocidal drug, Wistar rats

Introduction

Trypanosomes belong to the Trypanosoma genus, carried by different species of tsetse flies (*Glossina* spp.) that are responsible for causing an intricate illness termed trypanosomosis, affecting both humans and animals (Wamwir & Auma, 2021). *Trypanosoma b. gambiense/T. b. rhodesiense* is the main cause of this disease, affecting more than 30000 individuals across 36 countries in Sub-Saharan Africa (WHO, 2022). Chagas disease has been reported to kill approximately 21000 people in several Latin American countries each year (Abras et al., 2022). Research indicates that Trypanosoma infection is widespread and native to numerous regions in sub-Saharan African countries (Kennedy & Rodgers, 2019) and accounts detailing diverse livestock diseases across African countries highlight trypanosomes as the primary threat to livestock production, resulting in substantial economic losses (Abro et al., 2023). The projected losses in agricultural production caused by trypanosomes exceed three billion USD annually (Abro et al., 2021). The sole approach to combat these threats is through efficient utilization of drugs, employing both chemotherapy and chemoprophylaxis (Sharma et al., 2022). Chemotherapy encounters challenges like a restricted selection of available drugs, elevated costs, toxicity concerns, and the development of drug-resistant strains of the organisms, as documented in many reports (Eghianruwa & Oridupa, 2018).

In less-developed countries, traditional medicine can significantly harm the medicinal potential of plants (Khani & Khorasgani, 2021), playing a substantial role in addressing fundamental health needs (Aremu & Oridupa, 2022; Ghotbitabar et al., 2022). Multiple medicinal plants are employed to treat trypanosomiasis, with reports indicating that over 200 plant species are currently utilized for their anti-trypanosomiasis properties. Leaves have emerged as the most favored plant part, and diverse methods are employed to formulate treatments from these plants (Paré et al., 2020; Hiremath et al., 2024). Most plants possess anti-inflammatory activities (Mojibi et al., 2022; Hakimzadeh & Kosar, 2024). *Lawsonia inermis* (LI) Linn., commonly known as henna, is a highly valued herb utilized worldwide. Powdered leaves are frequently used to stain various body parts, such as hands, nails, and beards, for decorative and aesthetic purposes (Abdulfatai et al., 2022). Various reports indicate that LI leaves are employed to manage several ailments, including diabetes, poliomyelitis, and measles. They are known for their potential medicinal properties in traditional medicine for addressing these health conditions (Aremu et al., 2023). The seeds of the plant have been traditionally utilized in managing reproductive conditions, such as menorrhagia, leucorrhoea, and vagina discharges, due to the believed deodorant properties usually linked to the seeds (Aremu et al., 2023). Undeniably, the powder obtained from roasting the seeds of LI, when mixed with ginger oil, is believed to be effective in treating ringworms. Additionally, a decoction made from the plant leaves is used to clean and promote the healing of infected wounds (Sahoo & Mahalik, 2020).

This experiment was conducted to assess the in vivo anti-trypanosomal activity of LI leaves using haemobiochemical parameters, inflammatory cytokines, oxidants and anti-oxidant biomarkers as indicators of the activity of the *Trypanosome Brucei*-induced parasitic rat model.

Materials and Methods

Plant collection, identification and preparation

LI leaves were collected from farmland in Kwara State, Nigeria. The plant samples were taxonomically identified and confirmed at the Botany Department of the University of Ilorin. Subsequently, it was deposited and assigned the voucher number UIL-21210. Following four weeks of air drying, the samples were ground into a fine powder (Figure 1). The powdery leaves of LI Linn were used for crude extract following the standard method as described by Aremu et al., (2022).

Extraction and of the plant material

A total of 10 k/g of powdered LI Linn. leaves were saturated in five liters of methanol for 3 days (72 hours) through a process called maceration. Subsequently, the mixture was carefully poured off using filter paper. The resulting filtrate was then dispersed at 40 ˚C using Rotavap®. The concentrate obtained by this process was dried and stored in a refrigerator at 4 ˚C.

Experimental animal and ethical consideration

Ten-week-old Wistar rats weighing 140-180 g were acquired and housed in the Experimental Animal House of the Department of Vet-Pharmacology and Toxicology, University of Ilorin.

Phytochemical screening

Samples of the crude extract obtained from LI Linn. leaves were analyzed for their phytochemical constituents using the methods outlined by Trase and Evans.

Experimental animals and study design

Thirty Wistar rats weighing 140 and 180 g were procured from the Laboratory Animal Unit of the Department of Biochemistry at the University of Ilorin. The rats were kept in cages at room temperature, ranging from 28 ˚C to 33 ˚C. They were fed standard commercial pelletized feed (vital feed) and had unrestricted access to water. Experimental rats were grouped into 5 (n=6). The extract was given orally to these groups using an oral cannula as shown in Table 1.

T. brucei stock and inoculation

T. brucei was obtained from the Nigeria Institute for Trypanosomosis Research and the Onchocerciasis Research Institute in Kaduna, Kaduna State, Nigeria. Inoculum dose was determined to be 3×10^{-6} *T. brucei*/milliliter of blood following "rapid matching method" described by (Herbert & Lumsden, 1976). *T. brucei* was sustained through successive passages in experimental rats. To inoculate a rat, one millimeter of blood was aspirated from the infected rat and mixed with two milliliters of normal saline. The diluted blood was examined under a light microscope at ×40 to confirm the presence of *T. brucei* and the blood with the inoculum was inoculated intraperitoneally.

Weight measurement

The rats' weights were regularly checked starting on day 1 and then weekly using a scale. To weigh each rat, a circular flexible container was positioned on the scale and zeroed to subtract its weight. The rat was placed inside the container, and its weight was measured as described by Abdulfatai et al. (2017).

Organ weight measurement

Organ weights were measured following the method adopted by (Abdulfatai et al., 2017) and relative organ weight calculated (Equation 1).

1. Relative Organ Weight (%)=(Weight of the Organ×100)/Final Body Weight

Parasitaemia assessment and prepatent period

Starting on the second day after inoculation, the appearance of *T. brucei* in the blood of infected rats was regularly observed. Parasitaemia was estimated following the method outlined by Herbert and Lumsden (1976). A specific quantity (10-15)/field was counted using glass slides under an inverted microscope at ×400 magnification. The average mean trypanosomes count per field was calculated based on these observations.

Determination of haematological parameters

The entire blood sample present in the EDTA bottles was used to assess various hematological parameters. Packed cell volume (PCV), Hb Conc and red blood cells (RBC) were evaluated using Cole's method (Cole, 1986). Additionally, other parameters, including white blood cells (WBC), monocytes, lymphocytes and neutrophils, were also assessed using a fully automatic blood counter (Ehmma® PCE 210).

Serum biochemical parameters

Parameters, such as total protein along with its congener, albumin and globulin, creatinine, blood urea nitrogen, and tissue (liver) enzymes, such as alanine transferase, alanine phosphatase, and aspartate transferase, were assessed and analyzed using a commercial test kit (Randox® Chemicals Netherlands).

Preparation tissues homogenate (brain, heart, kidney and liver) and evaluation of oxidative stress markers

Various organs, such as the liver, brain, heart and kidneys were removed and cut from the fat and connective tissue. They were weighed individually and immediately perfused with a normal saline solution. Tissues were homogenized separately in potassium phosphate buffer us-

Table 1. Experimental protocol

	Groups	Treatment
1	Negative control	Uninfected and untreated
2	Positive control	Infected and untreated
3	DA	DA aceturate (7 mg/kg)
4	LI	*LI* (200 mg/kg)
5	DA+LI	*LI* (200 mg/kg) and DA at (7 mg/kg)

DA: Diminazene; LI: *L. inermis*.

ing a ho post mitochondrial fraction (PMF) mogenizer (Teflon, UK). The tissue homogenates from these organs were centrifuged at 10000 rpm for 15 minutes using a cold centrifuge (Sipha, USA) at 4 °C. PMF was obtained and decanted using a disposable pipette. The PMF supernatant was used for the assaying glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST) and malondialdehyde (MDA) (Izadi & Ramalakshmi, 2024).

Evaluation of inflammatory cytokines

Interleukin (IL)-1, IL-6 and IL-12 levels were determined using a commercial Elisa test kit following the standard method and procedure as stated by the manufacturer. Briefly, this assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit was pre-coated with antibodies specific for IL-1, IL-6 and IL-12. Standards or samples were added to the appropriate microtiter plate wells containing Biotin-conjugated IL-1, IL-6 and IL-12. A competitive inhibition reaction is initiated between IL-1, IL-6 and IL-12 (standards or samples) and biotin-conjugated IL-1 with a pre-coated antibody specific for IL-1. The greater the amount of IL-1, IL-6 and IL-12 in the samples, the less antibody was bound by biotin-conjugated IL-1, IL-6 and IL-12. After washing, avidin-conjugated horseradish peroxidase is added to each well. Substrate solution was added to the wells, and the color developed in contrast to the amounts of IL-1, IL-6 and IL-12 in the sample. Color development was stopped and the intensity of the color was measured. The detection range was 12.5-200 pg/mL.

Data analysis

All data collected during the study are shown as Mean±SD. Analysis of variance (ANOVA) was conducted, followed by Dunnett's post-hoc multiple comparison analysis. This was ensured using the GraphPad Prism statistical package. Probability values ≤0.05, (P≤0.05), 0.01, (P≤0.01) was regarded as significant.

Results

Phytochemical analysis

Phytochemical analysis of the LI Linn extracts typically revealed the presence of various phytoconstituents. These include alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, and glycosides. These constituents often contribute to the plant's medicinal properties and potential therapeutic effects (Table 2).

Weight gain

Rats treated with the combination of diminazene (DA) and the extract showed a weight gain of 6.3% compared to the untreated control. The group treated with only DA exhibited a significant weight gain of 8.7% by day 14 compared to the other treatment and negative untreated groups. All the treatment groups, including those treated with the extract alone and the combination, showed improved weight gain after the 14-day treatment compared to untreated control (positive) (Table 3).

Relative organ weight

Kidney weight increased significantly (P<0.01) compared to both control groups. Liver weight did not exhibit significant changes compared to normal control. Spleen weight significantly increased (P<0.001) in the LI-treatment group compared to uninfected rats. The positive untreated control also significantly increased spleen weight compared to the other treatments and untreated control. The weight of the testes remained unaltered in all treatment groups, as well as in the untreated control (Table 4).

Table 2. Phytochemical screening of *LI* Linn

Test	*Methanolic Extract*
Saponins	Abundantly present
Tannins	Abundantly present
Flavonoids	Abundantly present
Cardiac glycosides	Abundantly present
Terpenoids	Present
Steroids	Present
Anthraquinones	Present
Alkaloids	Present

Level of the parasites in the blood

T. brucei was detected in the blood of all inoculated rats. They were visible 3 days post-inoculation in all experimental rats. Parasitaemia increased in all rats, attained (25×10^8 trypanosomes/mL). DA+LI presented significantly decreased parasitaemia, similar to DA, after 72 hours of treatment. One week post-treatment showed that the two combinations (DA+LI) cleared the parasites compared to DA alone. All other treatment groups showed decreased parasitaemia. On day 14, a considerable reduction in parasitaemia was observed in all the treatment groups. The extract combined with DA also cleared parasitaemia, while the extract only significantly reduced the level of parasitaemia (Table 5).

Survivability and mortality rate

The survivability and mortality rates of rats infected with *T. brucei* were notably affected by the treatments administered. Rats treated with DA aceturate alone, LI alone, and the DA-LI combination showed complete survival for up to 14 days without evident relapse until sacrifice. In contrast, the mortality rate of the infected untreated rats reached 60%. This demonstrates the potential of these treatments in enhancing survival rates and reducing mortality among the experimental subjects compared to the untreated infected group (Table 6).

Haematology result

PCV, RBC, WBC, platelets and MCV increased significantly ($P<0.05$) in the LI treatment group across the days. Additionally, the MCH and MCHC decreased significantly. Moreover, differential WBC counts, lymphocytes, monocytes, eosinophils, and basophils increased significantly ($P<0.05$) compared to the control (Table 7).

Serum chemistry

Total protein, globulin, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST)

Table 3. % Weight gain of rats infected with *T. brucei* and treated with LI and DA aceturate

Days/Group	Mean±SD (%)		
	Day 0	Day 7	Day 14
Negative control	178.6±18.28	185.2±19.43 (3.7)	191.0±21.61 (6.5)
Positive control	170.4±5.68	174.2±8.701 (2.1)	147.0±16.08 (-15.9)*
DA	160.6±19.51	167.6±22.23 (4.1)	175.8±27.23 (8.7)
LI	156.6±14.52	158.8±17.51 (1.4)	149.2±23 (4.9)*
DA+LI	175.0±6.519	179.0±8.515 (2.2)	186.8±9.365 (6.3)

*Significantly ($P<0.05$), **Significantly ($P<0.01$).

Table 4. Relative organ weight of *T. brucei* infected rats treated with methanol extract of LI and DA aceturate

Organs/Groups	Mean±SD				
	Kidney	Liver	Spleen	Heart	Testes
Negative control	0.49±0.11	3.41±0.74	0.33±0.1	0.33±0.18	1.65±0.44
Positive control	0.67±0.03	3.6±0.7	1.28±0.06***	0.5±0.06	1.83±0.06
DA	0.55±0.13	3.3±0.34	0.34±0.09	0.33±0.04	1.55±0.07
LI	0.58±0.17*	3.7±0.61	0.49±0.33	0.37±0.06	1.72±0.63
DA+LI	0.62±0.18	2.94±0.39	0.42±0.1	0.35±0.09	1.80±0.08

***Significantly lower (P<0.001).

and blood urea were not significantly different (P>0.05) in rats treated with DA alone or in combination with LI compared to the control. This indicates no statistically significant variation in these parameters between the treated and the control groups. There exists a significant-difference (P<0.05) in creatinine and total bilirubin levels in the untreated infected group (group B) compared to non-infected control (Table 8).

Glutathione (GSH)

The GSH levels in the brain increased significantly (P<0.01) after LI treatment. Other treatments and infected controls decreased significantly compared to uninfected rats. In the heart, the GSH levels increased significantly after LI treatment. The infected control decreased non-significantly compared to the negative control. GSH levels

Table 5. Parasitaemia clearance level

Days (Infected)	Negative	Positive	DA	LI	DA+LI
1	0	0	0	0	0
2	0	5.3	5.0	5.0	5.0
3	0	6.3	6.6	6.3	5.7
4	0	6.6	6.6	6.6	6.6
5	0	6.9	6.3	6.6	6.9
6	0	7.5	5.4	6.0	6.0
7	0	7.8	<5.4	<5.4	<5.4
8	0	8.1	0	5.3	5.3
9	0	8.7	0	5.2	5.2
10	0	8.7	0	5.2	5.2
11	0	8.7	0	0	0
12	0	8.7	0	0	0
13	0	9.0	0	0	0
14	0	9.0	0	0	0

DA: Diminazene; LI: *L. inermis*.

Note: Data are expressed as mean Log_{10} value for Conc of the parasites per millimetre of blood for reference.

Table 6. Survivability and mortality rate of rats infected with *T. brucei* and treated with LI and DA aceturate

Group/Days RX	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Negative control	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Positive control	0/6	0/6	0/6	0/6	0/6	1/5	0/5	1/4	0/4	0/4	0/4	0/4	1/3	1/3
DA	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
LI	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
DA+LI	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5	0/6	0/6	0/6	0/6	0/6

in the liver were significantly (P<0.01) higher in all the treated rats than in the infected untreated rats (Table 9).

Glutathione s-transferase (GST)

GST levels in the brain decreased significantly (P<0.01) in infected untreated mice compared to the treatment and negative control groups. GST (heart) showed the same trend as most brain treatments and the uninfected controls. Kidneys GST also decreased significantly in the positive untreated controls. Liver GST also decreased significantly (P<0.05) in the positive untreated group compared to all treatment groups and the negative control group (Table 10).

Superoxide dismutase (SOD)

In the brain of the infected control rats, SOD decreased significantly compared to the treated rats and uninfected controls. The heart SOD decreased significantly in un-

Table 7. Haematology result of infected rats treated with methanolic LI and DA aceturate

Parameters	Mean±SD				
	Control		DA	LI	DA+LI
	Negative	Positive			
PCV	42.2±0.86	36.0±1.05	40.62±1.29	42.62±1.29	42.62±1.29
RBC (×10⁶/µL)	7.47±0.15	6.42±0.22	6.99±0.2	7.04±0.18	7±0.23
Hb (g/dL)	15.02±0.16	12.9±0.34*	13.5±0.34	14.3±0.38	13.5±0.34
PLT (×10⁹)	145.2±8.9	164±15.2	138±63	158±6.9	143±4.3
MCV (fl)	53.6±5.6	60.9±0.59	60.4±0.47	60.2±0.89	60.2±0.98
MCH (pg)	19.8±0.25	20.1±0.24	20.1±0.25	19.7±0.21	19.9±0.25
MCHC (g/dL)	33.2±0.34	27.7±0.22*	33.3±0.27	32.8±0.21	27.7±2.7*
WBC (×10³)	5.66±1.03	4.82±0.62	4.95±0.39	5.14±0.44	5.09±0.33
L (×10³)	3.38±0.57	2.06±0.30	3.35±0.31	2.82±0.29	2.82±0.26
N (×10³)	2.19±0.48	1.59±0.35	1.37±0.07	1.16±0.14*	1.1±0.11*
M (×10³)	0.13±0.04	0.12±0.03	0.06±0.02	0.06±0.02	0.09±0.01
E (×10³)	0.16±0.05	0.17±0.01	0.13±0.02	0.08±0.02	0.06±0.01

*Significantly lower (P<0.05)

Abbreviations: PCV: Packed cell volume; RBC: Red blood cell; Hb: Haemoglobin; PLT: Platelet; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cell.

Table 8. Serum chemistry of infected rats treated with methanolic extract of LI and DA aceturate

Parameters	Mean±SD				
	Negative	Positive	DA	LI	DA+LI
Total. P	82.4±1.6	84.4±1.3	84.2±1.2	78.2±1.5	82.5±2.8
Albumin	33±1.8	34.6±1.3	33±0.71	28±1.3*	32±2.7
Globulin	49.4±0.68	49.8±2.06	52.6±0.68	50.2±0.92	52.5±0.87
AST	45±0.63	39.8±1.43	42±1.23	42.8±2.39	37.5±1.4
ALT	32.8±0.37	29.2±0.97	31±1.27	31.8±1.16	29±0.82
ALP	106±7.01	111±4.36	99.8±7.07	111±4.01	102±5.56
BUN	16.54±0.27	17.12±0.19	16.96±0.19	17±0.35	16.28±0.24
Creatinine	0.9±0.06	0.82±0.09	0.78±0.07	0.82±0.05	0.7±0*
Total BL	0.42±0.08	0.42±0.08	0.52±0.16	0.38±0.11	0.35±0.05

*Significantly lower (P<0.05).

Abbreviations: ALP: Alkaline phosphatase; AST: Aspartate transaminase; ALT: Alanine transaminase; BUN: Blood urea nitrogen; BL: Bilirubin; DA: Diminazene; LI: *L. inermis*.

treated infected rats compared to all treated rats. In the kidneys, SOD decreased significantly in the positive control rats compared to all treatment groups and the negative control. Kidney SOD levels in all treated rats decreased non-significantly compared to negative uninfected rats. SOD (liver) increased significantly in DA+LI treated rats (Table 11).

MDA

The MDA (brain, heart, kidney and liver) increased significantly in the infected untreated group compared to the treated and uninfected control groups (Table 12).

Expression of IL

IL-1

IL-1 showed a significant increase (P<0.001) in the untreated infected control, while LI and DI showed non-significant reductions compared to the uninfected control (Figure 2).

IL-6

Expression of IL-6 showed a non-significant increase (P<0.001) in the infected untreated control, while LI treatment increased it significantly compared to the un-

Table 9. GSH (µmol) of *T. brucei* infected rats treated with LI and DA aceturate on of different organs

Group/Organ	Mean±SD			
	Brain	Heart	Kidney	Liver
Control	81.66±5.16	94.05±25.16	91.57±4.02	122.98±11.18
Infected untreated	71.23±23.44	81.45±11.12	80.07±17.01	89.93±22.45
DA	118.801±34.29	98.25±43.71	89.39±2.21	118.53±64.95
Lawsonia inermis	122.98±29.51*	91.59±12.81	88.52±11.02	101.8±14.96
DI+LI	151.73±73.68**	96.38±13.03	95.94±19.57	100.09±36.5

*Significant (P<0.05), **Significant (P<0.01).

Table 10. GST (µmol) of *T. brucei* infected rats treated with LI and DA aceturate on of different organs for 14 days

Group/Organ	Mean±SD			
	Brain	Heart	Kidney	Liver
Control	5.22±1.42	4.62±1.35	6.89±0.59	6.44±1.98
Infected untreated	3.39±2.07**	2.03±0.51**	3.01±2.37**	3.48±2.21*
DA	5.12±0.80	3.51±0.99**	3.93±1.59	5.52±5.11**
Lawsonia inermis	5.14±1.48	4.29±0.88**	5.97±1.05	7.14±0.97**
DI+LI	4.54±1.13	3.68±0.84**	6.65±1.78	7.90±2.45**

*Significant (P<0.05), **Significant (P<0.01).

Table 11. SOD (µmol) of *T. brucei* infected rats treated with LI and DA aceturate on of different organs for 14 days

Group/Organ	Mean±SD			
	Brain	Heart	Kidney	Liver
Control	8.3±1.5	4.8±1.1	6.3±0.5	2.3±0.12
Infected untreated	6±2.6*	2±0.80**	3.±0.6**	1.8±0.2
DA	10±2.7	4.4±1.2	4.2±0.6	3±1.5
Lawsonia inermis	10±2.4	5±1.5	3.7±0.8	2.5±0.4
DI+LI	8.4±1.9	5±1.3	4.8±0.8	2.4±0.6

*Significant (P<0.05), **Significant (P<0.01).

infected control. The other treated rats showed non-significant alterations in the expression of IL-6 (Figure 3).

IL-12

IL-12 appears to be crucial for chronic inflammation associated with trypanosomiasis. In untreated infected controls, IL-12 increased significantly compared to LI, DI, and uninfected controls (Figure 4).

Figure 1. *LI* leaves and powder

Table 12. MDA (µmol) of *T. brucei* infected rats treated with LI and DA aceturate on of different organs for 14 days

Group/Organ	Mean±SD			
	Brain	Heart	Kidney	Liver
Control	0.63±0.1	0.75±0.47	0.27±0.07	0.2±0.08
Infected untreated	1.83±0.37	1.74±0.17*	1.39±0.01**	0.85±0.06
DA	0.81±0.27	0.97±0.68	0.37±0.1	0.39±0.28
LI	0.3±1*	0.69±0.24	0.32±0.15	0.32±0.18
DI+LI	0.79±0.17	0.82±0.36	0.47±0.21	0.3±0.31

*Significant (P<0.05), **Significant (P<0.01).

Discussion

hytochemical analysis of the LI Linn leaves used in this study revealed the existence of various compounds, including flavonoids, alkaloids, glycosides, and saponins and glycosides. These constituents are commonly found in plants and contribute to their medicinal properties and therapeutic effects. This observation conforms to previous reports by Aremu and Oridupa (2022), who reported that LI contains various phytochemical compounds, as shown in this study.

The outcome of the percentage weight gain in this experiment demonstrated that LI leaves improved weight gain by 15.1% after 14 days of treatment compared to the negative control, which showed an improvement of 26%. This decrease in weight gain might be attributed to saponins and tannins in the plant, which can produce antinutritive activities, potentially reducing feed consumption. This aligns with the results of Kemboi et al. (2023), who reported that plants containing tannins and saponins can reduce feed consumption.

The relative organ-body weight ratio is a vital indicator of inflammation, atrophy, and hypertrophy (Soren et al., 2019). In this study, the relative organ weights indicated a significant increase in the spleen and kidney in the extract-treatment groups. The weights of the liver, heart, and testes were not significantly altered compared to uninfected rats. These findings suggest that the administration of the extract led to notably increased relative weights of the spleen and kidney. At the same time, other organs, such as the liver, heart, and testes, did not display

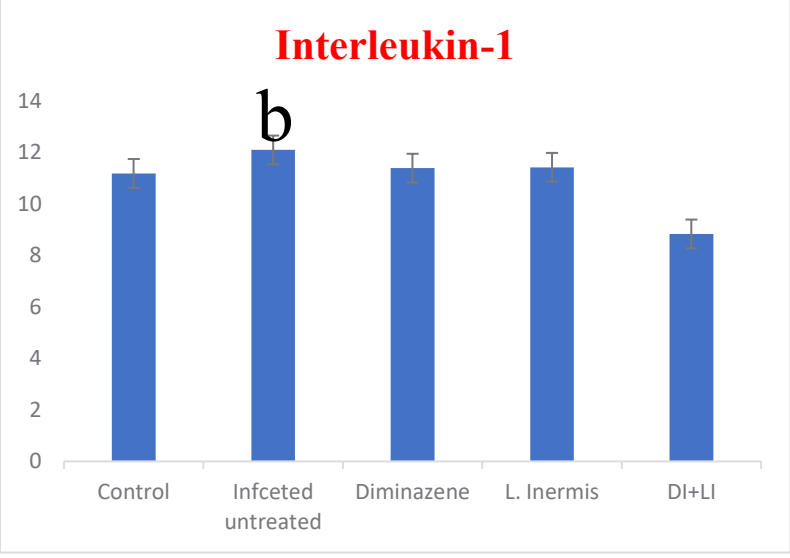

Figure 2. IL-1 (µmol) of *T. brucei* infected rats treated with LI and DA aceturate for 14 days

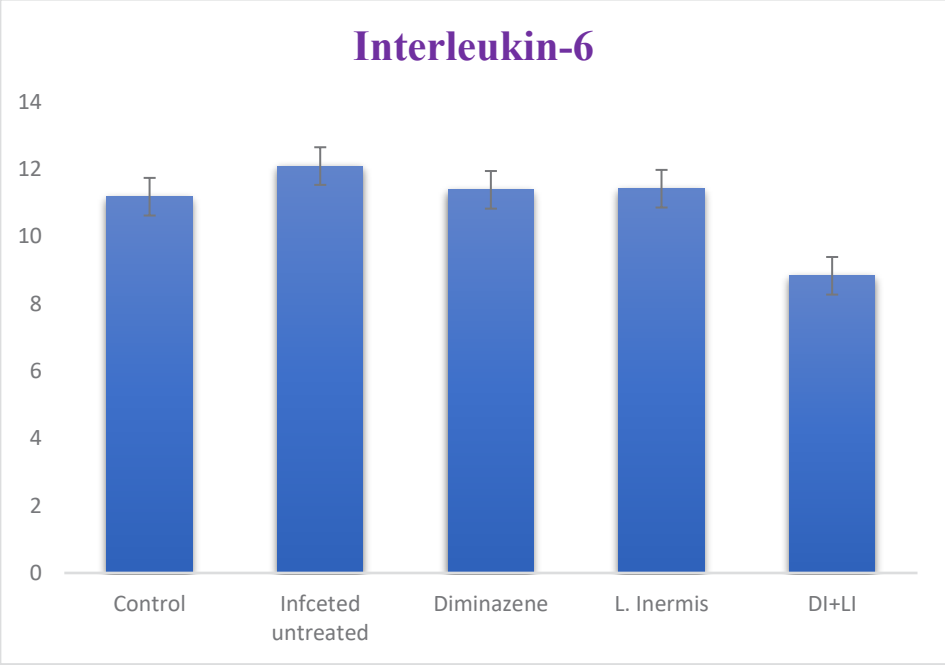

Figure 3. IL-1 (µmol) of *T. brucei* infected rats treated with LI and DA aceturate treated rats for 14 days

significant changes in their relative weights compared to normal uninfected rats.

This study revealed that LI leaves exhibited anti-trypanosomal activity against *T. brucei*-infected rats. This was evident from a significant decrease in parasitemia levels in the extract-treated rats compared to the untreated positive control. This result aligns with prior reports by Wurochekke et al. (2004), who also highlighted the trypanocidal properties of LI. Moreover, combining DA with the extract enhanced efficacy, resulting in the clearance of parasites in a shorter duration (by day 5) than

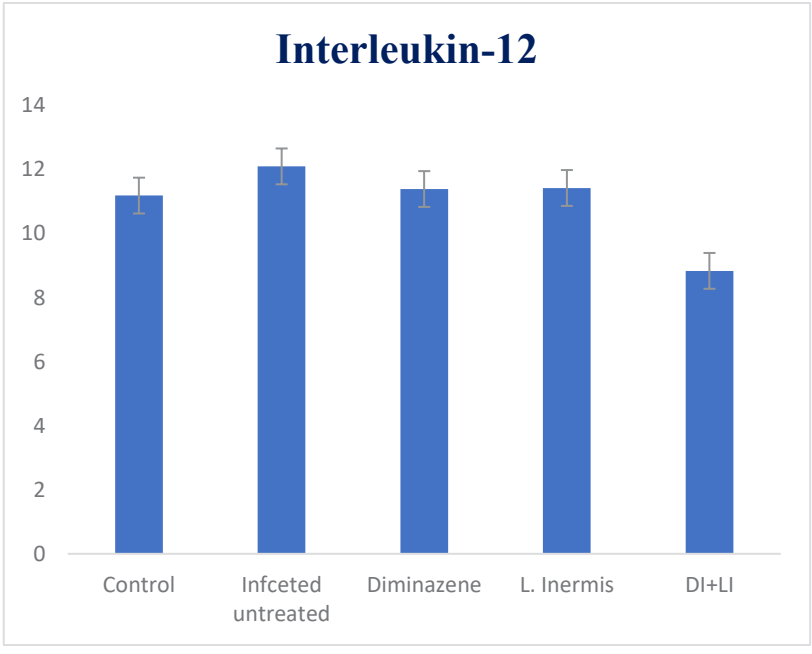

Figure 4. IL-12 (µmol) of *T. brucei* infected rats treated with *LI* and DA aceturate treated rats for 14 day

either treatment alone (until day 7). The combination of DA with LI exhibited improved synergistic effects, positively impacting weight, and survival rates, and decreasing mortality among the experimental subjects. The results also showed that the extract-DA combination has a significant therapeutic advantage with increased efficacy during parasites clearance.

The results of this study indicated that LI improved the blood indices of infected rats. Anaemia in trypanosomiasis is a complex condition caused by several factors, such as haemolysis of the RBC, haemodilution, and erythrophagocytosis by organisms (Stijlemans et al., 2018, Chikhaoui et al., 2023). *Trypanosoma brucei* induces anaemia by disrupting erythrocyte membrane integrity (Oula et al., 2023). Additionally, erythrocyte peroxidation has been identified as another factor contributing to the pathogenesis of anaemia in mice infected with *T. brucei* (Neves et al., 2021).

This study demonstrated significant improvements in hematological parameters (PCV, RBC, Hb, MCV, MCH and MCHC) in LI treated rats compared to infected and untreated rats, where these parameters were notably lower. This outcome aligns with the results reported by Aremu et al. (2022). However, differential WBC counts showed lymphocytopenia, neutrophilia and monocytopenia, particularly in groups not treated with LI. No significant changes were observed in several biochemical values in *T. brucei*-infected rats. Hypoproteinemia was observed in the groups not treated with LI extract, suggesting that the extract prevented hypoproteinemia in infected rats, as previously reported (Siddiqui, 2023).

The evaluation of hepatocellular damage induced by trypanosomes often involves assessing serum activities of enzymes, such as AST and ALT, which leak from hepatic tissues (Dkhil et al., 2020). In this study, an increase in ALP and AST was observed in the infected control group, consistent with earlier reports by Aremu et al. (2022). This elevation in enzyme levels has been associated with inflammation and necrosis in infected hosts, affecting organs, such as the liver, kidneys, muscles, and heart (Renu et al., 2020). The invasion of soft tissues, particularly major organs, by *T. brucei* potentially leads to enzyme release from damaged tissue (Aremu et al., 2018). Renal damage due to trypanosomiasis was evident due to increased urea and creatinine levels. This result demonstrated a decrease in blood urea nitrogen, creatinine, and total bilirubin levels across all the groups. This result coincides with Aremu et al, who also noted decreased serum biochemical values in *T. brucei*-infected rats.

If not addressed promptly, trypanosomiasis poses severe neurological (Asadi-Rizi et al., 2024) cardiac, and hematological risks. Oxidative stress biomarkers serve as crucial indicators for understanding the disease's mechanisms, shedding light on potential therapeutic targets (Ukwueze et al., 2022; Satarzadeh et al., 2024). The study's results indicated that LI significantly increased antioxidant markers, such as GSH, GST, GPx and SOD, while concurrently reducing MDA levels and inflammatory cytokines (IL-1, IL-6 and IL-12), compared to the untreated control groups. This aligns with the results of Salifu et al. (2022), who reported improved oxidative stress markers in goats infected with Trypanosoma evansi and treated with artemether-lumefantrine. These results suggest that LI might possess antioxidative and anti-inflammatory properties, potentially contributing to mitigating the effects of trypanosomiasis.

Conclusion

The study's results suggest that LI exhibits appreciable trypanocidal activity against *T. brucei*-infected rats. Additionally, the extract enhanced the experimental subjects' weight gain and survival rates. Furthermore, it demonstrated the ability to enhance antioxidant biomarkers while reducing oxidant levels and inflammatory cytokines. A positive synergistic interaction between DA aceturate and LI exists, indicating potential cooperative effects in combating trypanosomiasis.

Further investigation

The acute and chronic toxicity study on the LI-DA combination could be explored since the results obtained from this study showed optimal efficacy in treating *T. brucei*-infected rats.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of University of Ilorin, Ilorin, Nigeria (Code: UERC/FVM/2021/020).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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