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

Running Title: Antitrypanosomal effect of medicinal plant.

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Abstract

Background: Trypanosomiasis is a major problem affecting both human and animals. Nearly 30,000 individuals in various countries of Sub-Saharan Africa have African trypanosomiasis. It has led to about 21,000 deaths yearly.

Objectives: This experiment was conducted to evaluate the anti-trypanocidal effects of *Lawsonia inermis* in rats infected with *T. brucei*.

Methods: 30 rats were allotted to groups (1-5) of six rats each. Group 1 (negative control), 2 (tryps control), 3 (Diminazene (DA) 7 mg/kg), 4 (*Lawsonia inermis* (LI) at 200 mg/kg), and 5 (DA+LI). All rats in groups (2-5) were infected with $3x10^6$ of *Trypanosoma brucei* per ml of blood.

Result: Percentage weight gain of rats in diminazene extract combination showed increased weight gain (6.3%) when compared to tryps-control. Diminazene showed significant weight gain when compared with the negative control. The survivability rate showed that DA, LI and DA+LI combination survived for 14 days without visible relapse. The PVC, RBC, WBC, platelets and

MCV increased significantly in the extract treated groups while MCH and MCHC decreased significantly. Lymphocytes, Monocytes, Eosinophils and Basophils increased significantly compared to the control. Globulin, AST, ALT, ALP and Blood urea nitrogen increased non-significantly. Creatinine and Total bilirubin significantly decreased compared to untreated control. *Lawsonia inermis* significantly increased GSH, GST, GPx, SOD, while decreasing MDA and inflammatory cytokines (IL-1, 6, and 12) when compared to untreated control groups.

Conclusions: *Lawsonia inermis* reduced the level of parasitemia in a transient phase and drug-extract combination cleared the parasitemia within shortest time.

Keywords: Extract, *Lawsonia inermis*; *T. brucei,* trypanocidal drug, Wistar rats

Introduction

Trypanosomes belongs to the Trypanosoma genus, carried by different species of tsetse flies (*Glossina spp.*) that are responsible for causing the intricate illness termed trypanosomosis, affecting both humans and animals (Wamwir and Auma, 2021). *Trypanosoma b. gambiense*/*T. b. rhodesiense* are the main causa of this disease, affecting more than 30,000 individuals across 36 countries in Sub-Saharan Africa (WHO, 2022). On the other hand, it has been stated that Chagas disease kills about 21,000 people in several Latin American countries each year (Abras *et al.*, 2022). Research indicates that Trypanosoma infection is widespread and native to numerous regions within Sub-Saharan African countries (Kennedy and Rodgers, 2019) and accounts

detailing diverse livestock diseases across African countries highlighted 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 US dollars annually (Abro *et al.*, 2022). The sole approach to combat these threats is through the efficient utilization of drugs, employing both chemotherapy and chemoprophylaxis (Sharma *et al.*, 2022). Chemotherapy encounters challenges like a restricted selection of drugs that are available in the market, elevated costs, toxicity concerns, and the development of drug-resistant strains of the organisms, as documented in many reports (Eghianruwa and Oridupa, 2018).

In the less developed countries, traditional medicine leans significantly on harnessing the medicinal potential of plants (Khani and Khorasgani, 2021), playing a substantial role in addressing fundamental health needs (Aremu and Oridupa, 2022; Ghotbitabar *et al.,* 2022). Multiple medicinal plants are employed in treating trypanosomiasis, with reports indicating over 200 plant species currently utilized for their anti-trypanosomiasis properties. Leaves emerge as the most favored plant part, and diverse methods are employed in formulating treatments from these plants (Paré *et al.,* 2020; Hiremath *et al.,* 2024) and most of these plants

possess anti-inflammatory activities (Mojibi *et al.,* 2022; Hakimzadeh and Kosar, 2024). *Lawsonia inermis* Linn., commonly known as henna, is a highly valued herb utilized worldwide. Its powdered leaves are frequently used for staining various body parts such as hands, nails, and beards, serving decorative and aesthetic purposes (Abdulfatai *et al.,* 2022). Various reports indicate that *L. inermis* leaves are employed in managing 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). Precisely, 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 *L. inermis,* when mixed with ginger oil, is believed to possess properties effective in treating ringworm. Additionally, a decoction made from the leaves of the plant is used for cleansing and promoting healing in infected wounds (Sahoo and Mahalik, 2020).

This experiment was conducted to assess the *in vivo* antitrypanosomal activities of *L. inermis* Linn. leaves using haemobiochemical parameters, inflammatory cytokines, oxidant and anti-

oxidant biomarkers as an indicator to promotes its activities in *T. brucei*-induced parasitic rat model.

Materials and methods

Plant Collection, Identification and Preparation

Leaves of *Lawsonia inermis* were collected from a farm land in Kwara state, Nigeria. Plant sample was 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 a four-week period of air drying and were later ground into a fine powder. The powdery leaves of *Lawsonia inermis* Linn was used for crude extract following standard method as described by Aremu *et al.*, (2022)

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Figure I: *Lawsonia inermis* leaves and powder

Extraction and of the plant material

A total of 10 kilograms of powdered *L. inermis* Linn. leaves were saturated in 5 liters of methanol for 3 days (72 hours) through a process called maceration. Afterward, the mixture was carefully poured off with filter paper. Resulting filtrate was then dispersed at a temperature of 40°C with a Rotavap®. The concentrate obtained from this process was dried and subsequently stored in a refrigerator at a temperature of 4°C.

Experimental animal and Ethical Consideration

Ten-week-old Wistar rats weighing between 140-180 grams were acquired and kept at the Experimental Animal House within the Department of Vet-Pharmacology and Toxicology, University of Ilorin. Ethical clearance for the usage of experimental animals was obtained from University of Ilorin ethical committee overseeing animal research, with the approval number **UERC/FVM/2021/020** assigned

Phytochemical screening

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

Ethical Consideration

Ethical consent was gotten from the Board in charge of animal use and care at the University of Ilorin Nigeria, under the code **UERC/FVM/2021/020**

Experimental Animals and Study design

For the study, 30 Wistar rats weighing between 140 and 180 grams were procured from the Lab-animal unit of the Department of biochemistry at the University of Ilorin. These rats were kept in cages at a room temperature ranging from 28 to 33 degrees Celsius. They were fed a standard commercial pelletized feed (Vital feeds) and had unrestricted access to water. The experimental rats were grouped into 5 (n=6). The extract was given orally to these groups using an oral cannula

***T. brucei* Stock and Inoculation**

T. brucei was obtained from the Nigeria Institute for Trypanosomosis and Onchocerciasis Research Institute in Kaduna, Kaduna State, Nigeria. Inoculum dose was determined to be $3x10^{-6}$ *T. brucei*/milliliter of blood following "Rapid Matching method" described by (Herbert and

Lumsden, 1976). The *T. brucei* was sustained through successive passages in experimental rat. To inoculate a rat, one millimeter of blood was aspirated from an infected rat and mixed with 2 milliliters of normal saline. This diluted blood was examined with a light microscope at x40 to confirm the presence of *T. brucei*, and the blood with the inoculum was then inoculated intraperitoneally.

Weight measurement

The rats' weights were regularly checked starting from day 1 and then weekly using a scale. To weigh a rat, a circular flexible container was positioned on the scale and zeroed to subtract its weight. 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

$$\text{Relative organ weight (\%)} = \frac{\text{Weight of the organ X 100}}{\text{Final Body weight}}$$

Parasitaemia Assessment and Prepatent period

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

Determination of haematological parameters

The entire blood present in the EDTA bottles was utilized for assessing various hematological parameters. The evaluation of PCV, Hb Conc, and RBC was conducted using Cole's method (Cole, 1986). Additionally, other parameters including Total 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 (TP) along with its congener, Albumin and Globulin, Creatinine, Blood Urea Nitrogen, and tissues (liver) enzymes like 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

The various organs such the liver, brain, heart and kidney were removed and cut from fat and connective tissue. They were all weighed individually and immediately perfused with normal saline. The tissues were homogenized separately with Potassium Phosphate Buffer (KPB) using a homogenizer (Teflon, UK). The tissue homogenate from these various organs were subsequently centrifuged at 10000 rpm for 15 mi using a cold centrifuge (Sipha USA) at 4˚C. Post Mitochondrial Fraction (PMF) was obtained and decanted using a disposable pipette. The PMF supernatant was used for the assaying GSH, SOD, GPx, GST and MDA (Izadi and Ramalakshmi, 2024).

Evaluation of inflammatory Cytokines

IL-1, IL-6 and IL-12 were determined using commercial Elisa test kit following 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 has been pre-coated with an antibody specific to IL-1, IL-6 and IL-12. Standards or samples are added to

the appropriate microtiter plate wells with Biotin-conjugated IL-1, IL-6 and IL-12. A competitive inhibition reaction is launched between IL-1, IL-6 and IL-12 (Standards or samples) and Biotin-conjugated IL-1 with the pre-coated antibody specific for IL-1. The more amount of IL-1, IL-6 and IL-12 in samples, the less antibody bound by Biotin-conjugated IL-1, IL-6 and IL-12. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Substrate solution is added to the wells and the color develops in opposite to the amount of IL-1, IL-6 and IL-12 in the sample. The color development is stopped and the intensity of the color is measured. Detection range is 12.5-200 pg/mL.

Data Analysis

All data collected during the study were showed as mean ± SD (standard deviation). ANOVA was conducted, followed by Dunnett's Post-hoc multiple comparison analysis. This was ensured with GraphPad Prism statistical package (www.GraphPad.com). Probability values equal to/less than 0.05, ($P \leq 0.05$), 0.01, ($P \leq 0.01$) was regarded as significant

Results

Phytochemical analysis

Phytochemical analysis of the extracts of *L. inermis* Linn typically reveals the existence of various phytoconstituents. This includes alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, glycosides, and other compounds. These constituents often contribute to the plant's medicinal properties and potential therapeutic effects (Table 2)

Weight gain

The rats treated with the combination of diminazene and the extract showed an increased weight gain of 6.3% compared to the untreated control. The group treated with only diminazene exhibited a significant weight gain of 8.7% by day 14 in comparism to other treatment and negative untreated. 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 ($P<0.01$) significantly when compared to both controls. Liver weight did not exhibit significant changes compared to normal control. Spleen weight showed a notable increase ($P<0.001$) in the LI-treatment in comparism to the uninfected rats. The positive untreated control also demonstrated a significant increase in spleen weight compared to other

treatments and untreated control. The weight of testes remained unaltered in all treatment groups as well as in the untreated control as shown in table 4.

Level of the parasites in the blood

T. brucei were detected in the blood of all inoculated rats. They were visible after 3 days post inoculation in all the experimental rats. Parasitaemia increased in all the rats and attained (25 $X10^8$ trypanosomes/mL). DA+LI presented a significant decreased parasitaemia just like diminazene after 72 hours treatment. 1 week post treatment showed that the two combinations (DA+LI) cleared the parasites than when compared with diminazene alone. All other treatment group showed decreased parasitaemia. At day 14, there is a considerable reduced parasitaemia in all the treatment groups. The extract combination with diminazene also cleared the parasitaemia while extract only significantly reduced the level of parasitaemia (Table 5).

Survivability and mortality rate

The survivability and mortality rates among rats infected with *T. brucei* were notably affected by the treatments administered. Rats treated with diminazene aceturate alone, *Lawsonia inermis* alone, and the diminazene-LI combination showed complete survival up to 14 days without

evident relapse until their sacrifice. In contrast, the mortality rate among 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

PVC, RBC, WBC, platelets and MCV increased significantly ($P<0.05$) in *Lawsonia inermis* treatment across the days. Additionally, MCH and MCHC decreased significantly. Moreover, differential WBC counts; lymphocytes, monocytes, eosinophils, and basophils) were shown to increase significantly ($P<0.05$) in comparism to the control as shown in table 7.

Serum chemistry

Total protein, globulin, ALT, ALP AST and blood urea showed a non-significant difference ($P>0.05$) in rats treated with DA alone or when combine with *Lawsonia inermis* compared to the control. This indicates no statistically significant variations in these parameters between the treated groups and the control. There exists a significant difference ($P<0.05$) in creatinine and

total bilirubin levels in the untreated infected group (group B) when compared with non-infected control (Table 8)

GSH: Glutathione

GSH of the brain increased significantly ($p<0.01$) in LI treatment. Other treatment and infected control decreased significantly when compared to uninfected rats. In the heart, GSH increased significantly in LI treatment. Infected control decreased non-significantly compared to negative control. GSH of the liver significantly ($p<0.01$) in all the treated rats when compared to infected untreated (Table 9).

GST: Glutathione s-transferase

GST of the brain decreased significantly ($p<0.01$) in infected untreated when compared to treatment groups and the negative control. GST (heart) showed the same trend like brain most treatment and uninfected control. Kidneys GST also decreased significantly in the positive untreated control. Liver GST also decreased significantly ($p<0.05$) in the positive untreated group compared to all treatment groups and negative control (Table 10).

SOD: Superoxide Dismutase SOD

In the brain of the infected control SOD decreased significantly compared to the treated rats and uninfected control. Heart (SOD) decreased significantly in untreated infected rats compared to all treated rats. In the kidney SOD decreased significantly in the positive control rats when compared to all treatment groups and 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 Malondialdehyde

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

Expression of interleukins

Interleukin 1

IL-1 showed a significant increase level ($p<0.001$) in untreated infected control while LI and DI showed non-significantly reduction when compared to uninfected control (Figure 1).

Interleukin 6

Expression of IL-6 showed a non-significant increase ($p<0.001$) in infected untreated control, while LI treatment increased significantly compared to uninfected control. Other treated rats presented non-significant alterations in the expression of IL-6 (Figure 2).

Interleukin 12

Interleukin-12 (IL-12) appeared as an important pathway for chronic inflammation associated with trypanosomiasis. In untreated infected control, IL-12 increased significantly compared to LI, DI and uninfected control (Figure 3

Discussion

Phytochemical analysis of *Lawsonia inermis* Linn leaves used in this research revealed the existence of various compounds: Flavonoid Tannins, Alkaloids, Glycosides, and Saponins and Glycosides. These constituents are commonly found in plants and contribute to their medicinal properties and potential therapeutic effects. This observation conforms with previous reports of (Aremu and Oridupa, 2022) who reported that *Lawsonia inermis* possesses various phytochemical compounds as shown in this study.

It appears that the outcome of the percentage weight gain in this experiment demonstrated that *Lawsonia inermis* 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 percentage weight gain might be credited to the existence of saponins and tannins in the plant, that can produce anti-nutritive activities, potentially reducing feed consumption. This aligns with findings from (Kemboi *et al.,* 2023) which reported that plants containing tannins and saponins can indeed reduce feed consumption.

The relative organ-body weight ratio serves as a vital indicator of inflammation, atrophy, and hypertrophy according to (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 did not show significant alterations compared to uninfected rats. These findings suggest that the administration of the extract led to notable increased relative weights of spleen and kidney, while other organs like the liver, heart, and testes did not display significant changes in their relative weights compared to the normal uninfected rats.

The study revealed that *Lawsonia inermis* leaves exhibited anti-trypanosomal activity against *T. brucei* infected rats. This was evident through a significant decrease in parasitemia levels in the extract-treated rats compared to the untreated positive control. This finding aligns with prior reports by (Wurochekke *et al.,* 2004) which also highlighted the trypanocidal properties of *Lawsonia inermis*. Moreover, combining diminazene with the extract enhanced efficacy, resulting in the clearance of parasites in a shorter duration (by day 5) compared to when either was used alone (taking until day 7). The combination of diminazene with *Lawsonia inermis* exhibited improved synergistic effects, positively impacting weight, survivability rates, and decreasing mortality among the experimental subjects. The result also showed that the extract-diminazene combination have a significant advantage from the therapeutic point of view with increased efficacy during the course of the parasites clearance.

The findings of this study indicated that *Lawsonia inermis* improved the blood indices of infected rats. Anaemia in trypanosomiasis is a complex condition caused by several factors like haemolysis of the RBC, haemodilution and erythrophagocytosis by the organisms (Stijlemans *et al.,* 2018, Chikhaoui *et al.,* 2023). *Trypanosoma brucei* induces anaemia by disrupting the integrity of erythrocyte membranes as reported by (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,* according to (Neves *et al.,* 2021).

This study demonstrated significant improvements in hematological parameters (PCV, RBC, Hb, MCV, MCH, and MCHC) in *Lawsonia inermis* treatment when compared to infected and untreated rats, where these parameters were notably lower. This outcome aligns with findings from (Aremu *et al.,* 2018). However, differential WBC counts showed lymphocytopenia, neutrophilia, and monocytopenia, particularly in groups not treated with *Lawsonia inermis.* Notably, there were non-significant changes in several biochemical values in *T. brucei*-infected rats. Hypoproteinemia was observed in groups not treated with *Lawsonia inermis* extract, suggesting that the extract prevented hypoproteinemia in infected rats, as previously reported by (Siddiqui, 2023).

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

Trypanosomiasis poses severe neurological (Asadi-Rizi *et al.,* 2024), cardiac, and hematological risks if not addressed promptly. Oxidative stress biomarkers serve as crucial indicators in understanding the disease's mechanisms, shedding light on potential therapeutic targets (Ukwueze *et al.,* 2022; Satarzadeh *et al.,* 2024). The study's findings indicated that *Lawsonia inermis* 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 untreated control groups. This aligns with findings from (Salifu *et al.,* 2022) who reported improved oxidative stress markers in goats infected with *T. evansi* and treated with artemether-lumefantrine. These results suggest that *Lawsonia inermis* might possess antioxidative and anti-inflammatory properties, potentially contributing to mitigating the effects of trypanosomiasis

Conclusion

The study's findings suggest that *Lawsonia inermis* exhibits appreciable trypanocidal activities against *T. brucei* infected rats. Additionally, the extract appears to enhance weight gain and survivability rates among the experimental subjects. Furthermore, it demonstrates the ability to enhance antioxidant biomarkers while reducing oxidant levels and inflammatory cytokines. Notably, there seems to be a positive synergistic interaction between diminazene aceturate and *Lawsonia inermis*, indicating potential cooperative effects in combating trypanosomiasis.

Further Investigation

The acute and chronic toxicity study on the *Lawsonia inermis*-diminazene combination could be explored since the result obtained from this study showed optimal efficacy in the treatment of *T. brucei* infected rats.

Ethical approval: This work was ethically approved by Unilorin ethical committee who is the regulatory body in charge of animal use at University of Ilorin (Nigeria). Full approval with the assigned number: **UERC/FVM/2021/020** date 30/07/2022

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Statements and Declarations/Competing interest .1

None .2

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Table 1: Experimental protocol

Group 1 (Negative control)	Uninfected and untreated.
Group 2 (Positive control)	Infected and untreated.
Group 3 (DA)	Diminazene aceturate (7 mg/kg).
Group 4: (LI)	*L. inermis* (200 mg/kg).
Group 5: (DA+LI)	*L. inermis* (200 mg/kg) and DA at (7 mg/kg)

Table 2: Phytochemical screening of *L. inermis* 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

Table 3: % Weight gain of rats infected with *T. brucei* and treated with *Lawsonia inermis* and Diminazene aceturate

Days/Group	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%)
Diminazene (DA)	160.6±19.51	167.6±22.23 (4.1%)	175.8±27.23 (8.7%)
Lawsonia inermis (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	186.8±9.365

	(2.2%)	(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 *Lawsonia inermis* and diminazene aceturate

Organs / Group	Kidney	Liver	Spleen	Heart	Testes

Negative control	0.49±0.11	3.41±0.74	0.33±0.10	0.33±0.18	1.65±0.44
Positive control	0.67±0.03	3.60±0.70	1.28±0.06***	0.50±0.06	1.83±0.06
Diminazene (DA)	0.55±0.13	3.30±0.34	0.34±0.09	0.33±0.04	1.55±0.07
***Lawsonia inermis* (LI)**	0.58±0.17*	3.70±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±0.10	0.35±0.09	1.80±0.08

All values are express in mean ± standard deviation

*** Significantly lower (P<0.001)

Table 5: Parasitaemia clearance level

Days	Negative	Positive	DA	LI	DA+LI
1day(infected)	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

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 *Lawsonia inermis* and diminazene aceturate

GROUP/DAYS R_X	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
Diminazene (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
Lawsonia inermis (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

Table 7: Haematology result of infected rats treated with methanolic *Lawsonia inermis* and Diminazene aceturate

Parameters	Negative (Control)	Positive (Control)	DA	LI	DA+LI
PCV	42.2±0.86	36.0±1.05	40.62±1.29	42.62±1.29	42.62±1.29
RBC(x10^6/µl)	7.47±0.15	6.42±0.22	6.99±0.20	7.04±0.18	7.00±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 (x10^9)	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 (x10^3)	5.66±1.03	4.82±0.62	4.95±0.39	5.14±0.44	5.09±0.33
L(x10^3)	3.38±0.57	2.06±0.30	3.35±0.31	2.82±0.29	2.82±0.26
N(x10^3)	2.19±0.48	1.59±0.35	1.37±0.07	1.16±0.14*	1.1±0.11*
M(x10^3)	0.13±0.04	0.12±0.03	0.06±0.02	0.06±0.02	0.09±0.01
E(x10^3)	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)

Table 8: Serum chemistry of infected rats treated with methanolic extract of *Lawsonia inermis* and Diminazene aceturate

Parameters	**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±.19	17±0.35	16.28±.24
Creatinine	0.9±0.06	0.82±0.09	0.78±0.07	0.82±0.05	0.7±0.0*
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)

Table 9: Glutathione (GSH) (µmol) of *T. brucei* infected rats treated with *Lawsonia inermis* and diminazene aceturate on of different organs

Grp/organ	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
Diminazene	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	101.80±14.96
DI + LI	**151.73±73.68****	96.38±13.03	95.94±19.57	100.09±36.50

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

Table 10: Glutathione transferase (GST) (µmol) of *T. brucei* infected rats treated with *Lawsonia inermis* and diminazene aceturate on of different organs for 14 days

Grp/organ	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***
Diminazene	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: Superoxide Dismutase (SOD) (µmol) of *T. brucei* infected rats treated with *Lawsonia inermis* and diminazene aceturate on of different organs for 14 days

Grp/organ	Brain	Heart	Kidney	Liver
Control	8.30±1.50	4.80±1.10	6.30±0.50	2..30±0.12
Infected untreated	**6.00±2.60***	**2.00±0.80****	**3.00±0.60****	1.80±0.20
Diminazene	10.00±2.70	4.40±1.20	4.20±0.60	3.00±1.50
Lawsonia inermis	10.00±2.40	5.00±1.50	3.70±0.80	2.50±0.40
DI + LI	8.40±1.90	5.00±1.30	4.80±0.80	2.40±0.60

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

Table 12: Malondialdehyde (MDA) (µmol) of *T. brucei* infected rats treated with *Lawsonia inermis* and diminazene aceturate on of different organs for 14 days

Grp/organ	Brain	Heart	Kidney	Liver
Control	0.63±0.10	0.75±0.47	0.27±0.07	0.20±0.08
Infected untreated	1.83±0.37	**1.74±0.17***	**1.39±0.01****	0.85±0.06
Diminazene	0.81±0.27	0.97±0.68	0.37±0.10	0.39±0.28
Lawsonia inermis	**0.30±1.0***	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	0.30±0.31

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