Antitrypanosomal Activity of Hydromethanol Extract of Leaves of *Cymbopogon Citratus* and Seeds of *Lepidium Sativum*: in-vivo Mice Model

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Abstract

Background: Trypanosomiasis is one of the neglected tropical diseases of both humans and animals which decreases their productivity and causes death in the worst scenario. Unavailability of vaccine, low therapeutic index of trypanocidal drugs, and development of resistance lead to the need for research focused on developing alternative treatment options especially from medicinal plants. The present study was aimed to investigate antitrypanosomal activities of leaves of *Cymbopogon citratus* and seeds of *Lepidium sativum* in *in vivo* mice model.

Methods: The plant extracts were prepared by maceration using 80% methanol and reconstituted with 10% dimethyl sulfoxide (DMSO) to have the desired concentration. The test doses were adjusted to 100, 200 and 400mg/kg based on the toxicity profile. The Plants extracts were administered to the respective groups of mice after the 12^{th} day of field isolate *T. congolense* inoculation for seven consecutive days. The level of parasitemia, body weight, packed cell volume, and differential white blood cell counts were measured.

Results: The *in vivo* test results revealed that both plant extracts had dose dependent antitrypanosomal activity. Both crude extracts showed a significant reduction in parasite load ($P<0.05$), ameliorate anaemia (increased or prevent the fall of PCV value) ($P<0.05$), decreased lymphocytosis and increased neutrophil counts ($p<0.05$) and improved body weight but significant body weight increment ($P<0.05$) was observed only in *C. citratus* treated mice compared to the negative and positive controls. Comparative results from all tested parameters showed that the best activities were observed with *C. citratus* treated groups of mice.

Conclusion: The present study concluded that the crude extracts of leaves of *C. citratus* and seeds of *L. sativum* had antitrypanosomal effects and can be potential targets for further studies on the development of alternative antitrypanosomal agents.

Background

Trypanosomiasis is a protozoan disease caused by the genus trypanosome. The disease affects both animals and human beings. African animal trypanosomiasis causes severe economic losses in the livestock sector. Morbidity and/or mortality rates for African animal trypanosomiasis are influenced by an animal's general health, as well as the strain and dose of the infecting organisms (1, 29).

Human trypanosomiasis is one of the 13 most neglected tropical diseases recognised by the world health organization. *Trypanosoma Cruzi* which was discovered in 1909 is the cause of American trypanosomiasis (Chagas disease) (2). Human African trypanosomiasis (HAT) is common in sub-Saharan Africa. There are two forms of HAT; slow-progressing form, caused by *Trypanosoma brucei gambiense*, which is endemic in western and central Africa; and faster- progressing form, caused by *Trypanosoma brucei rhodesiense*, originate in eastern and southern Africa including Ethiopia (3, 30). The severity of clinical presentations of the disease varies with those forms of the disease. Rhodesiense HAT is an acute disease that usually progresses to death within six months; whereas gambiense HAT has a more chronic progressive course with an average duration of three years (4).

The disease is considered to have an approximately 100% case fatality rate. Despite the lethality and the individual-level effect, the disease has enormous socio economic impacts. As the disease affects mainly individuals of productive age, and mostly being a chronic disease, it affects the income-generating capacity of the people, worsening the economic situation of impoverished societies resulting in sustainability of poverty cycle in the neglected communities. It is also a stigmatizing disease, primarily because of the neuropsychological impairment entailed, and in many endemic areas, the presence of the disease is hidden and the patients are discriminated or abandoned (4, 28).

Due to the unavailability of vaccine, control and prevention of trypanosomiasis rely on chemo prophylactic and/or curative trypanocidal therapies. Despite their toxicity, most trypanocidal drugs are developing resistance both in AAT and HAT (5, 6, 7, 27).

These factors emphasize the need for research into more comprehensive, formidable and more affordable sources of trypanocidal agents. Medicinal plants have paramount importance in the discoveries and development of new alternative drugs. In Ethiopia, many plants are claimed to be useful as traditional remedies for the treatment of trypanosomiasis in different parts of the country (8). *Cymbopogon citratus* (lemon grass) known by the Amharic name 'Teji sar' in Ethiopia, is used for the treatment of many infectious

and non-infectious diseases including trypanosomiasis. *In vitro* study of citral, the main component of *Cymbopogon citratus*, revealed that the plant has antitrypanosomal activity (9). *Lepidium sativum* 'Fetto' in Amharic is also among the plants claimed to have antiprotozoal activity and/or antitrypanosomal effect in particular. A single dose *in vivo* test reported by Al-Otaibi *et al*., (10) concluded that the plant extract had antitrypanosomal activity against *T. evansi*. Therefore, the present study was targeted to investigate the *in vivo* antitrypanosomal activity of crude extracts of leaves of *C. citratus* and seeds of *L. sativum* at a variable concentration.

Materials And Methods

Experimental animals

Swiss albino mice age of 8–12 weeks and weighing 25 – 35 gm were obtained from Ethiopian public health institute. Animals were housed in polypropylene cages six mice per cage and allowed free access to clean water *ad libitum* and feed (pellet). They were kept at room temperature having 12hrs day and night.

Test organism

Trypanosoma (Nannomonas) congolense is the most prevalent and widespread pathogenic trypanosome in tropical Africa, being found in ruminants, pigs, dogs and other domestic animals throughout the tsetse belt (11). In the mammalian bloodstream, *T. congolense* is a small trypanosome, shorter in length and without a noticeable undulating membrane. In the vector (tsetse fly), initially it develops and multiplies in the midgut; while infective metacyclics develop in the proboscis (12). The field isolates of the organism were obtained from Ghibe valley known with a high prevalence of trypanosomiasis and tsetse fly infestation (13). We had established a temporal field laboratory using generator power. A drop of blood was sampled from the ear vein of trypanosomiasis suspected animals using microscopic slides and examined under the microscope after adding the cover slip. Blood was collected from the jugular vein of trypanosomosis diseased animals using EDTA coated tubes which is then inoculated in to mice which were then used as a donor to the experimental mice.

Chemicals and Equipment

Chemicals and solvents

Dimethyl sulfoxide (DMSO), Methanol, distilled water, and reference drugs (Diminazene aceturate), phosphate glucose buffered saline solution (PBSG)

Materials

Aluminium foils, cover slide, microscopic slide, mortar and pestle, digital weighing balance, diamond pencil, desiccator, EDTA coated syringe, heparinized capillary tube, Whatman No.1 filter paper, glove, haematocrit reader, microhematocrit centrifuge, oven, refrigerator, petridish, microscope, micropipettes, Rotary evaporator, sterile lancet, syringe 1ml, cristaseal, spatula and flasks were employed in this study.

Plant materials and test organism collection

The plant materials were collected according to a Preliminary Guide to Plant Collection, Identification and Herbarium Techniques, National Herbarium Addis Ababa University. Plant materials were collected from East Gojam zone Debre Elias district. The district is located around 341 km northwest of the capital city of Ethiopia, Addis Ababa. The mean annual temperature of the district ranges from 18–27°C and receives a mean annual rainfall of 1150 mm Hg with an altitude that ranges from 800 to 2200 m above sea level (14). Whereas the test organism, *Trypanosoma congolense* was isolated from Ghibe valley Gurage zone, Abeshige district Borer Tade'le peasant association.

Laboratory where the experiment was carried out

The experiment was conducted in Veterinary pharmacology and Toxicology Laboratory of the College of Veterinary Medicine and Agricuture, Addis Ababa University, Bishoftu Ethiopia.

Study design

Randomized experimental design was employed in which the experimental animals were assigned in to the control or experimental group and also between different experimental subgroups randomly.

Methods

Pre-extraction preparation

Samples of the plant were authenticated by the national herbarium of Addis Ababa University College of Natural Science. The identification was done by Mr. Melaku Wondaferash (Botanist), and the report contained local name, botanical name and its family; Teji sar- *Cymbopogon citratus* (DC.) Stapf- Poaceae and Fetto- *Lepidium Sativum L.*- Brassicaceae, and samples were deposited with specified voucher numbers (AY1 and AY3) respectively.

The plants were planted in the Garden of toxic and medicinal plants established by the author of this thesis in 2016 at the College of Veterinary Medicine and Agriculure, Addis Ababa University, Bishoftu for future use.

After collection, the plant materials were washed with tap water to remove unnecessary particles, dried under shade, and grounded mechanically. The materials were sieved and weighed before subjected to extraction procedures.

Crude extract preparation

The plant materials were extracted by maceration technique using 80%Methanol (LOBA CHEMIE PVT LTD) through mixing the grinded and weighted plant material with 80% Methanol in 1:7 ratios. After 72 hrs maceration with regular shaking, the mixtures were strained using strainer to remove solids and further filtered with whatman filter paper No 1. The filtered solutions were evaporated using Rota vapour (BUCHI Rotavapor R-200) to remove the solvent to the acceptable level. Remnants from the Rota vapour were poured in to petridishes and put in a dry oven at a temperature of 40^{o}C to remove the remained solvent. The prepared solid extracts were stored in a desiccator until the experimental procedures were conducted. The dried solid extracts were weighted to determine the percent yield as indicated in Table 1.

Acute toxicity test

The acute toxicity study was conducted according to the Organization for Economic Co-operation and Development (OECD), 2001 guideline fixed dose (2000mg/kg) toxicity test. A group of six female albino mice were fasted from food but not water for 4hrs before administration and 2hrs after oral administration of the test extract. The mice were critically followed continuously for 1 hr after administration of the extracts; intermittently for 4 hrs over a period of 24hrs for death, gross behavioural changes and other signs of toxicity. The follow up continues for 14days post treatment (15).

Experimental mice inoculation

The donor mice were infected with field isolates of *T.congolense* directly from trypanosome infected cattle at the Ghibe valley. After 12 days of infection, the donor mice reached at its peak level of parasitemia. Blood was collected from donor mice by cardiac puncture and/or drawing through tail veins after mice were anaesthetised using chloroform. Collected blood was diluted with phosphate buffered saline (PBS) to increase the volume of the inoculum. Healthy mice were injected intraperitonneally with 0.2ml of the inoculum containing 10^{7} parasites per millilitre approximately (16). After 9–11 days post infection the experimental mice were tested for the development of parasitemia, and only positive mice were drawn in to the experimental groups.

Dose adjustment, grouping of mice and administration of the plant extracts

Three test doses were adjusted as 100mg/kg, 200mg/kg and 400mg/kg based on the toxicity profile of the extracts. The dried and weighted plant extracts were reconstituted with 10% Dimethyl sulfoxide (DMSO) to have intended concentrations. A total of 60 Mice were then grouped into nine groups having six mice per group (CC100, CC200, CC400, LS100, LS200, LS400, DA3.5, DA28, NC and UU). CC100, CC200 and CC400 are groups of mice treated with 100, 200 and 400mg/kg of *C.citratus* extract, respectively. LS100, LS200 and LS400 were 100, 200 and 400 mg/kg *L.sativum* extract treated mice groups. DA3.5 and DA28 were positive controls took 3.5mg/kg and 28mg/kg standard drug Diminazene aceturate (DA), respectively. Whereas the NC group was the negative control

infected with the parasite but treated only with 1ml of 10%DMSO (vehicle). The UU group was uninfected untreated healthy mice used as a reference. The treatment was started on day 12 post infection. The extracts were administered every morning for seven consecutive days. The control groups (DA3.5, DA28 and NC) were treated once on the first day of the above mentioned doses. All treatments were administered via intraperitoneal route.

Determination of parasitaemia and body weight

Parasitaemia was monitored every other day starting from the first day of treatment and continues until the 14th day. These were done by microscopic examination of blood obtained from the tail of each mouse. The tail was cut to extrude blood, and a drop of blood was placed on a microscope slide and a wet smear was prepared by covering the drop by cover slips. The smears were examined microscopically at 400X total magnification (camera aided Olympus microscope). The degree of parasitaemia was determined using the “Rapid Matching” method of Herbert and Lumsden. Smears were prepared in triplicates from each animal and the mean value of slide counts was taken per sample examined microscopically. Logarithm values of these counts were obtained by matching with the table and the charts given by Herbert and Lumsden (17).

The body weight (in gram) of each mouse in all groups was taken on the day of treatment was commenced (day 0) and every other day (on Days 2, 4, 6, 8, 10, 12 and 14) up to the 14th day.

Determination of packed cell volume (PCV) and differential white blood cell count

PCV was measured to predict the effectiveness of the test extracts in preventing hemolysis resulting from increasing parasitaemia associated with trypanosomosis. It was monitored on Days 0, 7 and 14. Blood was collected from tail of each mouse in heparinized microhaematocrit capillary tubes filled up to 3/4th of their length. The tubes were then sealed immediately with crystal seal and centrifuged in a micro haematocrit centrifuge (Hawksley micro haematocrit centrifuge, England) for 5 min. After centrifugation the packed cell volume was measured using haematocrit reader (Hawksley micro haematocrit reader, England). The effect of extracts in improving PCV of treated animals was compared with the controls.

The white blood cell count (WBC) was determined on thin blood films stained with Giemsa stain obtained from each mouse on the 14th day post extract administration.

Data Analysis

Data analysis was performed using Statistical Packagefor Social Science SPSS version 20. Data were expressed as mean ± standard error of mean. One way ANOVA followed by Tukey’s multiple comparison tests was performed to determine statistical significance. P values less than 0.05 were considered significant.

Results

Percent yield of plant extracts

The test plants were weighed before and after the extraction process and the percentage yields were calculated. The result showed that hydro methanol extraction produced 15.03% yield for the plant *C. Citratus* leaves and 16.48% from the seeds of *L. sativum*.

Acute toxicity test

The acute toxicity test result concluded that all mice taking both groups of the extract did not show any sign of toxicity at a dose of 2000mg/kg.

The effect of plant extracts on parasitemia level

Effect of 80% methanol extract of leaves of Cymbopogon citratus on the level of parasitemia

The result showed that 80% methanol extract of *C. citratus* reduced the level of parasitemia in mice treated with different concentrations of the extract. The onset of the decrement in the level of parasitemia varied with concentrations. At a dose of 100mg/kg, a reduction started to be observed on the 8th day; a statistically significant reduction was, however, observed on the 10th day ($P < 0.05$ compared with the negative control and standard drug (3.5mg/kg). At a dose of 200mg/kg, the reduction in parasite

load started to be observed on day 6 though significant reduction was observed on the 10th day ($P < 0.05$ compared with the negative control and standard drug (3.5mg/kg). The highest dose (400mg/kg) reduced the level of parasitemia starting from the 6th day and significantly reduced along with day14, ($P < 0.05$) compared with those of negative control and at both doses (3.5mg/kg and 28mg/kg) of the standard drug (Table 1).

Table 1
Effect of 80% methanol extract of *Cymbopogon citratus* leaves on the level of parasitemia

Group of mice	Log value of parasitemia/ ml							
	D0	D2	D4	D6	D8	D10	D12	D14
CC-100	6.3 ± 0.11	7.5 ± 0.10	7.60 ± 0.13	8.0 ± 0.17	7.80 ± 0.21	7.50 ± 0.09^{ab}	7.35 ± 0.05	7.05 ± 0.05
CC-200	7.1 ± 0.46	7.8 ± 0.40	8.20 ± 0.31	8.0 ± 0.09	7.68 ± 0.20	7.40 ± 0.10^{ab}	7.13 ± 0.04	6.80 ± 0.05
CC-400	6.7 ± 0.42	6.83 ± 0.40^{a}	7.93 ± 0.23	7.5 ± 0.28	6.85 ± 0.06^{ab}	5.60 ± 0.06 ^{abc}	3.60 ± 1.13^{abc}	1.80 ± 1.13^{abc}
DA-3.5	7.38 ± 0.18	7.73 ± 0.18	8.10 ± 0.18	7.93 ± 0.32	8.16 ± 0.15	8.23 ± 0.20	8.26 ± 0.21	8.53 ± 0.13
DA-28	7.8 ± 0.20	5.53 ± 0.05	5.96 ± 0.29	7.35 ± 0.14	7.46 ± 0.07	7.80 ± 0.21	8.10 ± 0.12	8.40 ± 0.10
NC	7.2 ± 0.13	7.95 ± 0.06	7.65 ± 0.20	8.17 ± 0.10	8.25 ± 0.25	8.55 ± 0.06	8.55 ± 0.06	8.70 ± 0.13

Data are expressed as mean ± SEM; n = 6; D = day; D0 = the day treatment commenced; SEM = standard error of mean; CC = Cymbopogon citratus; NC = negative control taking 1ml 10% DMSO; 100, 200, 400, 3.5, and 28 are doses in mg/kg; DA = Diminazene aceturate; All superscripts indicate significance at $p < 0.05$ (a = compared to negative control; b = compared to 3.5 mg/kg DA; c = compared to 28mg/kg DA

Effect of 80% methanol extract of seeds of Lepidium sativum **on the level of parasitemia**

Eventhough there was a fluctuation of values, infected mice treated with *L. sativum* extract showed a reduction in parasite load from Days 2 to 8 as shown in Table2. After the 8th day, the level of parasitemia increased as a relapse but the rate of increment was less than that of the negative control. At 100 and 200 mg/kg doses of the extract, a significant reduction was observed on the 8th day ($P < 0.05$) compared with negative control (1ml 10%DMSO) and standard drug, diminazene aceturate (3.5mg/kg). At 400mg/kg, the extract showed a significant reduction from days 6 to 14 compared with the negative control and diminazene aceturate (3.5mg/kg); while significant reduction was observed only on the 14th day compared with diminazene aceturate (28mg/kg).

Table 2
Effect of 80%methanol extract of seeds of *L. sativum* on the level of parasitemia

Group of mice	Log value of parasitemia/ ml							
	D0	D2	D4	D6	D8	D10	D12	D14
LS-100	8.08 ± 0.08	7.98 ± 0.10	7.83 ± 0.12	7.61 ± 0.10	7.30 ± 0.08^{ab}	7.85 ± 0.08^{a}	8.13 ± 0.09	8.33 ± 0.04
LS-200	8.30 ± 0.08	8.33 ± 0.04	8.45 ± 0.04^{a}	7.98 ± 0.03	7.80 ± 0.10^{ab}	7.85 ± 0.02^{a}	8.00 ± 0.06^{a}	8.26 ± 0.05^{a}
LS-400	6.90 ± 0.40	6.43 ± 0.46^{ab}	7.36 ± 0.14	6.93 ± 0.40^{ab}	7.43 ± 0.04^{ab}	7.53 ± 0.02^{ab}	7.73 ± 0.04^{ab}	7.96 ± 0.05^{abc}
DA-3.5	7.38 ± 0.18	7.73 ± 0.18	8.10 ± 0.18	7.93 ± 0.32	8.16 ± 0.21	8.23 ± 0.20	8.26 ± 0.21	8.53 ± 0.13
DA-28	7.80 ± 0.2	5.53 ± 0.05	5.96 ± 0.29	7.35 ± 0.14	7.46 ± 0.20	7.80 ± 0.21	8.10 ± 0.12	8.40 ± 0.10
NC	7.20 ± 0.13	7.95 ± 0.06	7.65 ± 0.20	8.17 ± 0.10	8.25 ± 0.06	8.55 ± 0.06	8.55 ± 0.06	8.70 ± 0.13

Data are expressed as mean ± SEM; n = 6; D = day; D0 = the day treatment commenced; SEM = standard error of mean; LS = *Lepidium sativum*; NC = negative control taking 1ml 10% DMSO; 100, 200, 400, 3.5, and 28 are doses in mg/kg; DA = Diminazene aceturate; All superscripts indicate significance at $p < 0.05$ (a = compared to negative control; b = compared to 3.5 mg/kg DA; c = compared to 28 mg/kg DA;

Comparison of parasitemia level between groups

As shown in Fig. 1, the comparative values of the highest doses of the extracts and control groups realized that methanol extract of *C. citratus* showed better activity compared with *L. sativum* extract and control groups.

Effect of plant extracts on body weight

The effect of 80% methanol extracts of Cymbopogon citratus **leaves on body weight**

The present finding showed that extract improved the body weight of mice. As shown in Table 3, at all dose levels of the extract significantly increased ($P < 0.05$) body weight on the 12th and 14th days compared with the negative control and standard drug at both concentrations (3.5 and 28mg/kg).

Table 3
Effect of *C. citratus* leaves extract on body weight

Group of mice	Body weight in grams							
	D0	D2	D4	D6	D8	D10	D12	D14
CC-100	33.6 ± .13	32.6 ± .13	35.0 ± .30	34.0 ± .51	35.6 ± .92	36.0 ± .48^{c}	36.2 ± .44^{c}	37.8 ± .43^{abc}
CC-200	33.4 ± .77	32.6 ± .55	34.9 ± 1.28	33.3 ± .83	36.2 ± 1.33^{c}	36.0 ± 1.15^{c}	37.6 ± 2.34^{abc}	36.8 ± 1.17^{abc}
CC-400	32.8 ± 1.55	31.0 ± 1.42	31.5 ± 1.10	33.2 ± 1.24	33.5 ± .98	34.0 ± 1.01	34.5 ± .98^{abc}	35.3 ± 1.17^{abc}
DA-3.5	31.1 ± .78	31.9 ± .81	32.0 ± 1.01	32.3 ± 1.18	33.1 ± 1.06	33.1 ± .98	33.1 ± .93	33.2 ± .80
DA-28	31.5 ± .92	31.9 ± .93	30.9 ± 1.54	30.6 ± 1.36	31.2 ± 1.37	31.0 ± 1.28	30.9 ± 1.18	31.6 ± .93
NC	34.7 ± .74	34.3 ± .94	34.7 ± .84	34.4 ± .85	33.2 ± .70	33.0 ± .88	31.7 ± 1.13	31.0 ± .98

Data are expressed as mean ± SEM; n = 6; D = day; D0 = the day treatment commenced; SEM = standard error of mean; CC = *Cymbopogon citratus*; NC = negative control taking 1ml 10% DMSO; 100, 200, 400, 3.5, and 28 are doses in mg/kg; DA = Diminazene aceturate; All superscripts indicate significance at $p < 0.05$ (a = compared to negative control; b = compared to 3.5 mg/kg DA; c = compared to 28 mg/kg DA;

Effect of 80% methanol extracts of Lepidium sativum **seeds on body weight of mice**

As presented in Table 4, methanol extract of seeds of *L. sativum* increased the body weight of treated mice though all values were not statistically significant compared with both groups of controls.

Table 4
Effect of 80% methanol extract of *L. Sativum* seeds on body weight

Group of mice	Body weight in grams							
	D0	D2	D4	D6	D8	D10	D12	D14
LS100	35.4 ± 1.5	34.7 ± 1.53	34.3 ± 1.86	35.1 ± 1.98	34.5 ± 1.72	34.5 ± 1.72	34.5 ± 1.74	35.0 ± 1.79
LS200	31.8 ± 0.38	33.9 ± 0.73	32.9 ± 0.81	32.4 ± 1.05	33.3 ± 0.85	33.3 ± 1.01	33.3 ± 1.15	34.6 ± 1.40
LS400	33.7 ± 0.61	32.1 ± 0.54	32.2 ± 0.67	32.3 ± 0.65	33.1 ± 0.67	33.6 ± 0.68	34.2 ± 0.69	35.2 ± 0.82
DA3.5	31.1 ± 0.78	31.9 ± 0.81	32.0 ± 1.01	32.3 ± 1.18	33.1 ± 1.06	33.1 ± 0.98	33.1 ± 0.93	33.2 ± 0.80
DA28	31.5 ± 0.92	31.9 ± 0.93	30.9 ± 1.54	30.6 ± 1.36	31.2 ± 1.37	31.0 ± 1.28	30.9 ± 1.18	31.6 ± 0.93
NC	34.7 ± 0.74	34.3 ± 0.94	34.7 ± 0.84	34.4 ± 0.85	33.2 ± 0.70	33.0 ± 0.88	31.7 ± 1.13	31.1 ± 0.98

Data are expressed as mean ± SEM; n = 6; D = day; D0 = the day treatment commenced; SEM = standard error of mean; LS = *Lepidium sativum*; NC = negative control taking 1ml 10% DMSO; 100, 200, 400, 3.5, and 28 are doses in mg/kg; DA = Diminazene aceturate

Effect of plant extracts on packed cell volume (PCV)

The effect of 80% methanol extract of C. citratus **on packed cell volume**

Figure 2 showed reduction in PCV values on day 7 though the rate of reduction was less than the negative control and DA (3.5mg/kg). On the 14th day, all dose levels of the extract (100, 200, 400mg/kg) significantly ($P < 0.05$) increased the PCV value compared to the negative control taking 1ml 10%DMSO, and DA at doses of 3.5mg/kg and 28mg/kg.

Effect 80% methanol extract of Lepidium sativum **seeds on packed cell volume (PCV)**

As shown in Fig. 3, L. *sativum* seeds extract increased the PCV value on day 7 at concentration of 200 mg/kg though not significant ($P > 0.05$). On the 14th day all dose levels of the extract significantly reduced ($P < 0.05$) rate of reduction in PCV value compared with all groups of controls.

Effect of plant extracts on differential white blood cell counts

As shown in Figs. 4 and 5, the differential white blood cell counts showed that both plant extracts significantly decreased lymphocyte count and increased neutrophils at 200 and 400mg/kg compared with infected-untreated and Diminazene aceturate (3.5mg/kg) groups. The highest percentage of lymphocytes was observed in mice that were infected with *T. congolense* but treated with 1ml 10%DMSO (NC). Mice treated with 400mg/kg *C. citratus* extract had a comparable proportion of cell counts with a pronounced increment in neutrophils compared with healthy mice (uninfected untreated).

Discussion

The main objective of this study was to evaluate the *invivo* antitrypanosomal effect of hydro methanolic extracts of leaves of *Cymbopogon Citratus* (lemon grass) and seeds of *Lepidium sativum* against field isolates of *T. congolense* in mice model. The results of the present study showed that both plant extracts significantly reduced the parasite load though they did not completely clear parasites from the blood stream of the infected mice. The reduced level of parasitemia observed by methanol extract of *Cymbopogon citratus* leaves was in a dose dependant manner. Administration of the extracts was continued for seven consecutive days but the reducing effect of parasitemia was noticed starting on the 8th day and continued up to the 14th day. This implies that *C. citratus* extracts may have slow onset of action and/or the effect might be due to the cumulative effect of the repeated administration. Very pronounced anti trypanosomal activity of *C. citratus* extract was observed on the 14th day at a dose of 400mg/kg at which the mean logarithmic value of parasites per ml was less than 5.4, i.e., no trypanosome organism was observed in 20 fields of the microscopic slide (17). At this concentration, significant effects ($P < 0.05$) were observed from days 10 to 14 compared with all controls (negative control, 1ml 10%DMSO and positive control (Diminazene aceturate at a dose of 3.5 and 28mg/kg). These results are in line with the findings of a previous *in vitro* study which reported a dose dependent antitypanosomal activity of the plant (9). The antitrypanosomal effect of the plant might be attributed to the major component citral and/or the additive or synergistic effects of other components.

Methanolic extract of *Lepidium sativum* seeds also had antitrypanosomal activity reducing the level of parasite load in mice infected with *T.congolense* in a dose dependent manner. At 400mg/kg of the extract, a reduction in parasitemia was observed on Day 6, there after the load slightly increased as a relapse. But the rate of increment in parasitemia level was significantly very low ($P < 0.05$) compared with that of the controls. The present study results are in agreement with those of the previous study by Al-Otaibi *et al*., (10), which reported antitrypanosomal activity of *L. sativum* against *T. evansi*. The antitrypanosomal activity of the extract might be attributed to its antioxidant and/or its immune boosting properties (10).

The standard drug, Diminazene aceturate was tested as a positive control at its recommended normal dose (3.5mg/kg) and the highest dose (28mg/kg). Diminazene aceturate is one of the most frequently employed trypanocidal drugs used to treat animal trypanosomiasis. At a dose of 3.5mg/kg the drug did not show significant effect on the parasite load throughout the study period; the level of parasitemia continued to increase from D0 to D14 in the same way as the negative controls which were infected but treated with the vehicle. The highest dose (28mg/kg) of diminazene aceturate employed in the present study, however, significantly reduced parasitemia ($P < 0.05$) between days 2 and 4 compared with lower dose (3.5mg/kg), all doses of both extracts and the negative control. Starting on day 6, the level of parasite count increased rapidly up to the end of the follow up period, i.e., day 14. These findings might suggest that the drug is at an alarming level of resistance. The results of the present study are in line with those of previous studies carried out on the development of drug resistance by trypanocidal agents (18, 19, 20, 21, 22, 23).

In the present study, *Cymbopogon citratus* leaves showed better activity in reducing the parasite count than *Lepidium sativum* seeds.

The antitrypanosomal activity of the plant extracts could also be deduced from their effect in improving body weight. Both extracts increased body weight throughout the study period. The weight gain in mice treated with all doses of *C. citratus* extract was statistically significant between days 12 and 14 compared with negative and both positive controls. Eventhough mice treated with *L. sativum* extract seems to increase their body weight, the values were not statistically significant. As AAT is associated with anaemia, decreased appetite and a rapid weight loss which progresses to extreme emaciation (24), the observed effect on body weight by the extracts can be associated with a reduction in parasite load in the blood stream.

Severe anaemia is one of the causes of death associated with trypanosome infection. In the present study, the test extracts specially that of *C. citratus* significantly ameliorated the level of anaemia. Packed cell volume (PCV) improvement and prevention of further drop observed with both extracts mainly at the second week of treatment where parasitemia levels were very low could show antitrypanosomal effect of the extracts. The increment in PCV could be attributed to the reduction of the proliferating parasite load, neutralization of the toxic metabolites produced by trypanosomes or scavenging the trypanosome associated free radicals. Diminazene aceturate at a dose of 3.5 mg/kg was not able to improve the PCV of infected mice, but it sharply increased the PCV value in the first week of treatment at 28mg/kg concentration, and the values fell rapidly on the second week along with the relapse of parasitemia.

Lymphocytes are the main effector cells of the immune system in mice (25). Lymphocytosis has been implicated in trypanosomiasis and usually resulted from wax and wear syndrome in the animal immune system caused by the ever-changing variable surface glycoprotein of the infecting trypanosomes (26) which demands the immune system to continuously produce antibodies and hence keep the level of lymphocytes high. The present results of differential white blood cell counts revealed that both plant extracts decreased the percentage of lymphocytes compared with the negative controls in a dose dependant manner. These findings are in agreement with those of the previous study done by Feyera *et al.*, (16), which reported that plant extracts which decreased parasite load also decreased lymphocytosis and improved the immune system of mice by increasing the levels of other sets of white blood cells mainly neutrophils and monocytes.

Conclusions

From the present study, it can be concluded that both plant extracts can reduce the level of parasitemia, improve weight and prevent anaemia in a dose dependent manner suggesting their antitrypanosomal activity. The study also showed *C. citratus* leaves had better activity than *L. sativum* seeds extract and even better than the standard drug suggesting its high potential to be developed as an effective antitrypanosomal drug.

Abbreviations

DA; Diminazene aceturate, *C. citratus*; *Cymbopogon citratus*, *L.sativum*; *Lepidium sativum*, PCV; Packed cell volume, WBC; White blood cell, *T. congolense*; *Trypanosoma congolense*, AAT; Animal African Trypanosomiasis, HAT; Human African Trypanosomiasis

Declarations

Ethical approval and consent to participate

The study was carried out in compliance with the Animal Research: Reporting of *In- Vivo* Experiments (ARRIVE) guidelines. All Ethical clearance protocol, guidelines and principles of the Animal Care and Use followed and obtained for this study from Addis Ababa University College of Veterinary Medicine and Agriculture Minutes of Animals Research Ethics and Review Committee. The project leader presented for an explanation of the purpose of carrying out the studies and all possible care planned to reduce animals from suffering due to sampling was given to the committee. After the committee evaluated the significance of this research, an ethical approval certificate was given (VM/ERC/01/13/12/2020).

The pant collection was done according to A Preliminary Guide to Plant Collection, Identification and Herbarium Techniques, National Herbarium Addis Ababa University.

Consent for publication

Not applicable

Availability of data and materials

All data included in the manuscript are the original data obtained from the research. Data are kept by the corresponding author as a backup

Competing interests

The authors declare that they have no financial and/or nonfinancial competing interests

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Authors’ contributions

A.Y.E comprehended the study, designed and conducted all laboratory experiments; analysed and interpreted experimental results. T.B.T. and F. R.G. participated in the conception of the project. F. R.G. participated in project conception and guiding plant processing procedures. A.Y.E and T.B.T participated in isolation of the parasite from field. T.B.T supplied all laboratory inputs and monitored the whole research and participated in manuscript preparation. E.M.E. participated in the proposal development, study design and manuscript preparations. All authors read and approved the final manuscript.

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Figures

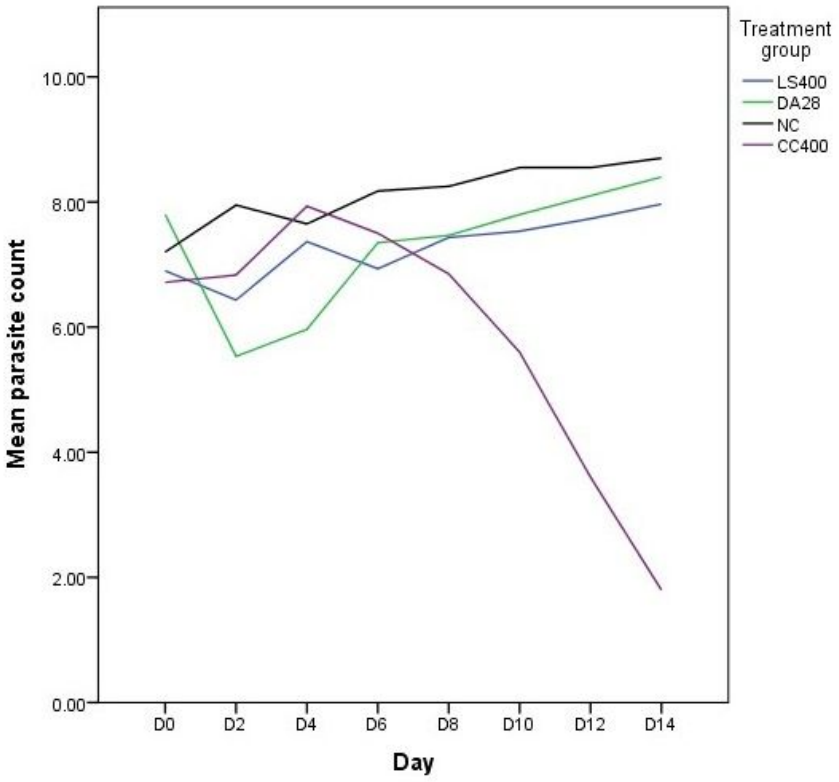

Figure 1

Comparison of the level of parasitemia treated with L.sativum and C. citratus with both control groups. Mean parasitemia count= log value per ml of blood; CC= C. citratus; LS= L. sativum; NC= negative control (1ml 10% DMSO); DA= deminazene aceturate. 400, 28 = are doses in mg/kg.

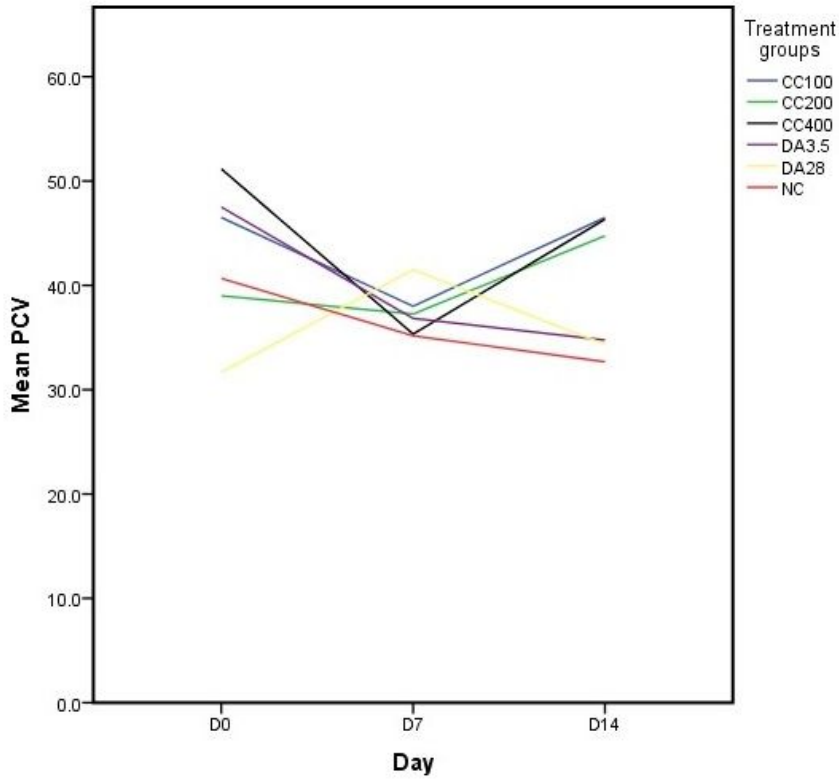

Figure 2

Effect of 80%methanol extract of C. citratus leaves on packed cell volume. Data are expressed as mean ± SEM; n = 6; D = day; D0 = the day treatment commenced; SEM = standard error of mean; CC= Cymbopogon citratus; NC= negative control taking 1ml 10% DMSO; 100, 200, 400, 3.5, and 28 are doses in mg/kg; DA = Diminazene aceturate; PCV= packed cell volume.

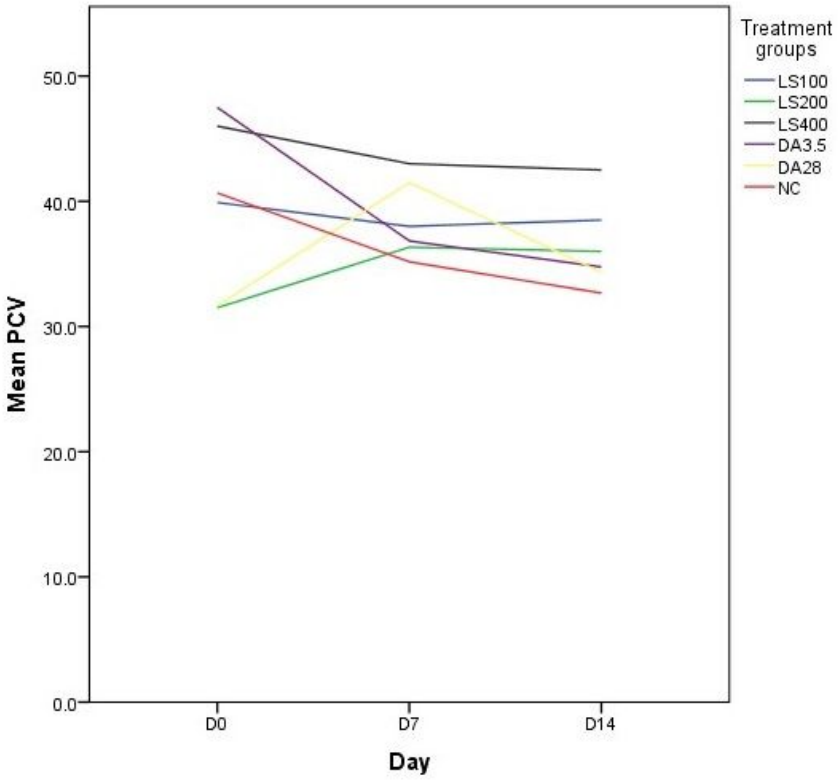

Figure 3

The effect of 80%methanol extract of L. sativum on packed cell volume. Data are expressed as mean ± SEM; n = 6; D = day; D0 = the day treatment commenced; SEM = standard error of mean; LS= Lepidium sativum; NC= negative control taking 1ml 10% DMSO; 100, 200, 400, 3.5, and 28 are doses in mg/kg; DA = Diminazene aceturate; PCV= packed cell volume.

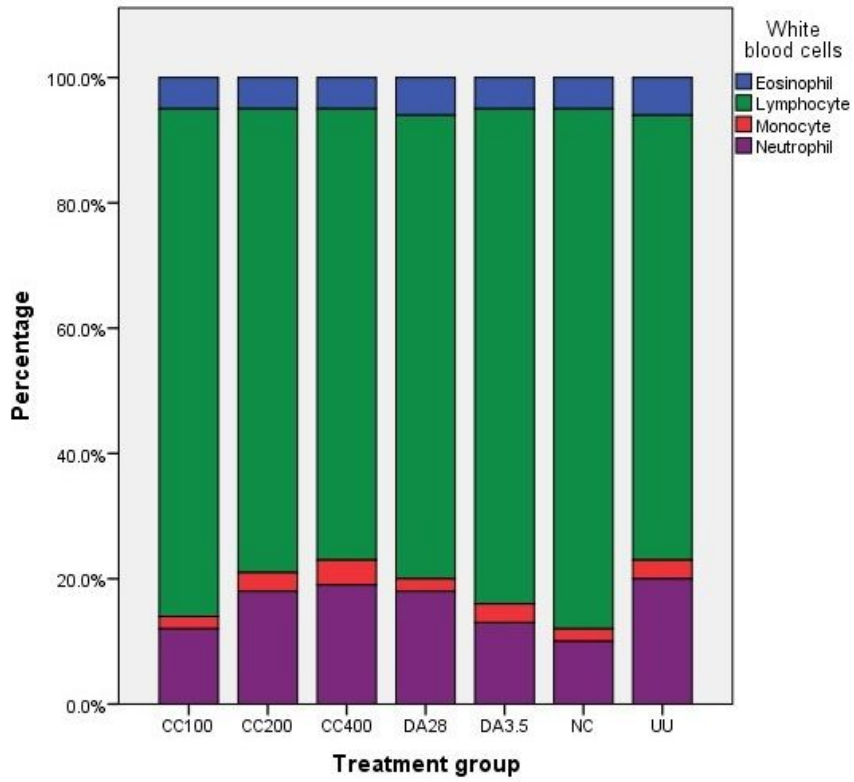

Figure 4

Effect of C. citratus leaves extract on Differential white blood cell counts CC= Cymbopogon citratus; NC= negative control taking 1ml 10% DMSO; 100, 200, 400, 3.5, and 28 are doses in mg/kg; DA = Diminazene aceturate; UU= uninfected untreated group; PCV= packed cell volume.

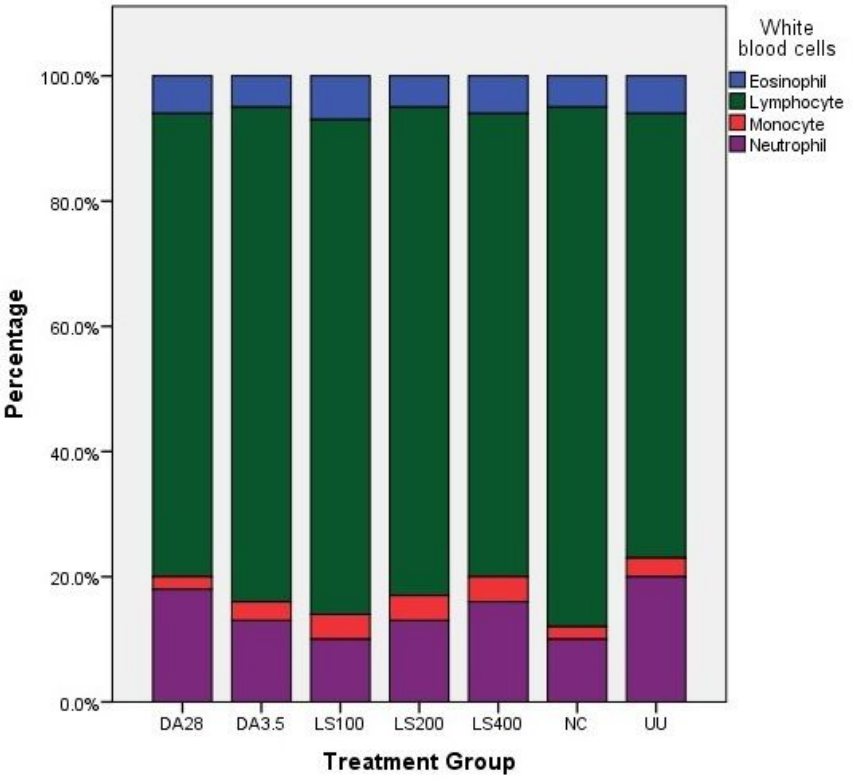

Figure 5

Effect of L. sativum seed extract on Differential white blood cell count LS= Lepidium sativum; NC= negative control taking 1ml 10% DMSO; 100, 200, 400, 3.5, and 28 are doses in mg/kg; DA = Diminazene aceturate; UU= uninfected untreated group; PCV= packed cell volume.