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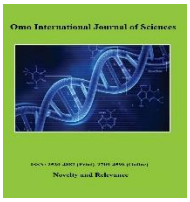

Research Article

Evaluation of *in vivo* anti-trypanosomal activity of methanol and aqueous extracted *Allium sativum* and *Lepidium sativum* against *Trypanosoma evansi* and *Trypanosoma congolense* in Southern Ethiopia

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Abstract

The present study was conducted in Arba Minch town, from September 2022 to April 2023 with the objective of evaluating the anti-trypanosomal activity of aqueous and methanol extract of *Allium sativum* bulbs and *Lepidium sativum* seeds against *T. evansi* and *T. congolense*. This experimental study utilized a total of 195 Swiss albino mice. Mice were assigned into 13 treatment groups, the first group was used as the control group while the rest up to13th groups were infected intraperitoneally with 1*107 trypanosoma and treated with *Allium sativum* bulbs and *Lepidium sativum* seeds extracts at a dose of 150, 250 and 500 mg/kg body weight and 28mg/kg body weight of Diminazine aceturate once a day for a period of five days and observed for up to 42 days. The mouse infected with *T. congolense* and methanol extracted *Allium sativum* at a dose of 250 mg/kg body weight showed a decrease in body weight for a week at post infection, then after their body weight started to increase rapidly. Treating of infected mouse using 250 mg/kg body weight had shown increased body weight, decreased parasitemia, maximizing packed cell volume and improving mean revival survival time of mouse. In addition, intra-peritoneal administration of the extracts to mice at 5,000 mg/kg body weight did not result in deaths during the acute toxicity trial. Therefore, *Allium sativum* and *Lepidium sativum* extracted using methanol and aqueous at 250mg/kg body weight had shown promising effect against *T. congolense* and *T. evansi* and provided a quantitative basis to explain the traditional uses of both medicinal plants.

Keywords: Allium sativum; Aqueous extract; *Lepidium sativum*; Methanol; *Trypanosoma evansi; Trypanosoma congolense*

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1. Introduction

The genus Trypanosoma is a medical and veterinary issue because it causes trypanosomiasis, a disease that is prevalent throughout the world and particularly prevalent in sub-

Saharan Africa (Giordani et al., 2016; Büscher et al., 2017). Trypanosomas in the first group, known as Stercoraria (*Trypanosoma cruzi*, *T. theileri*, and *T. melophagium*), are usually produced in the hindgut and then passed on, while the second group, Salivarian, which includes *Trypanosoma brucei, T. congolense*, and *T. vivax*, are primarily spread by the infected saliva of the tsetse fly vector (Glossina species). Furthermore, this illness continues to have inadequate medical management (Edoga et al., 2013).

Trypanosomiasis has a wide range of complex economic effects in Africa, including direct effects on human health and animal production, weight loss, infertility, decreased milk yield, abortion, and animal mortality, as well as indirect effects on farming, land use, settlement patterns, and animal husbandry (Chanie et al., 2013). The disease is the cause for the death of 3 million heads of cattle annually, with 50 million animals at risk in sub-Saharan Africa (Chitanga *et al*., 2011). According to Leta et al. (2016), there was high level of prevalence in different parts of Ethiopia with all species of the pathogen, with *T. congolese* and *T. vivax* being the dominant species. African farmers spend 35 million US dollar per year on trypanocidal drugs to protect and cure their cattle. A pondered evaluation extrapolated for the total tsetse-infested lands values the total losses, in terms of agricultural Gross Domestic Product (AGDP), at US$ 4.75 billion per year (FAO, 2004).

The primary method of disease control to date has been chemotherapy with commercial trypanocides (*Homidium, Isometamidium, and Diminazene aceturate*), which have been used for over 40 years. However, this approach has made the parasite develop drug resistance and the hosts become toxic (Ajakaiye et al., 2013; Lawal et al., 2013; Faria et al., 2014). The pharmaceutical industry's reluctance towards developing new chemicals due to either the confined character of the disease or an uncertain and unprofitable market further exacerbates the poor prospects for a vaccine caused by the antigenic variation of the parasite. The best option to combat the well-known effect of bovine trypanosomiasis on animal's productivity is to look for novel chemical entities as safe replacements, inexpensive, and effective against all species of trypanosomes in disease-endemic nations (Hoet *et al*., 2004; Samson, 2005).

Using medicinal plants as an alternative treatment was one of the greatest options. Studies reported that about 30 Ethiopian herbs have been tested for their anti-trypanosomal properties using methanol and dichloromethane extracts on the bloodstream strain of *T.b. brucei*. A dozen regularly used medicinal plants have been found by Hana and Ashebir (2018), Mesfin et al. (2016), and Endalachew (2015) as effective against trypanasomiasis in southern Ethiopia, particularly in the Gamo, Gofa, Wolaita, Dawuro zones, and Amaro district. These plants are *Lepidium sativum*

L. (Sibika/feto), *Allium sativum L.* (*Nechshinkurt/Tummo*), *Withania somnifera* (L.) Dunal (Gizawa), *Acmella caulirhiza* Del. (*Gudicho*), *Ranunculus multifidus* Forssk (*Aysmamata*), *Vernonia amygdalina* (buzuwa), *Triumfetta* sp. (*Chyshie*), *Senna occidentalis* (L.) Link (*Kutokwa*), Benth (*Mello*). *Nicotiana tabacum* L. (*Timbaho*), *Milletia ferruginea* (*Hochst*.) Bak. (*Zagiya*) and *Ageratum conyzoides* L. (Zeyisa).

Allium sativum and *Lepidium sativum* were selected because they are the most abundant and easily accessible of these therapeutic plants in the South Ethiopia Regional State, especially in Arba Minch, Gamo zone. *Allium sativum*, a member of the Liliaceae family, has been used medicinally for thousands of years. Antioxidant, antimicrobial, antihelminthic, anti-protozoal (anti-trypanosomal), antifungal, insecticidal, antitumor, antithrombotic, anti-cancer, anti-arthritic, hypolipidemic, and hypoglycemic qualities are among the many diverse functions of *Allium sativum* (Ross et al., 2001; Krstin et al., 2018). Ethiopia and Eritrea are believed to be the original locations for the polymorphous species *Lepidium sativum* (Ahmad et al., 2015) that grows fast in any climate, at any elevation, and in any type of soil. It requires little technical expertise, and its capacity to withstand a variety of environmental circumstances has allowed it to expand throughout the world. Despite the economic significance of seeds, leaves, and roots, the crop is mostly grown for its seeds.

Arba Minch, the current study area, is an endemic place for tsetse fly and other biting flies (Teka *et al*., 2012) and therefore existence of tsetse fly and tsetse transmitted trypanosomosis was also reported from this area (Sheferaw et al., 2019; Tora *et al*., 2022). The city is found in low land. Tsetse flies are concentrated in such low land area as climatic conditions are more favorable for them (Getachew et al., 2014). There also many other favorable conditions for the fly to spread and reproduce in the study area, which can have both direct and indirect consequences on cattle. The cost of commercial medications which is used to treat trypanosomiasis has indeed dramatically increased from time to time without any apparent reason. The accessibility and availability of the drug have also been an issue. Due to these and other factors, farmers in the current study area preferred to use the medicinal plants mentioned earlier to treat trypanosomiasis.

Allium sativum and *Lepidium sativum* were plants frequently used by the farmers in the current study area as a treatment for trypanosomiasis. However, the dosage, frequency, and chemical makeup of *Allium sativum* and *Lepidium sativum* were not taken into consideration. Therefore, we planned to conduct the current study with the objective to evaluate In vivo Anti-trypanosomal activity of methanol and aqueous extracted *Allium sativum* and *Lepidium sativum* against *Trypanosoma evansi* and *Trypanosoma congolense*.

2. Materials and Methods

2.1. Description of study area

The study was conducted in Arba Minch city in the South Ethiopia Regional State. Arba Minch city is the capital of Gamo zone, located 500 kilometers southwest from Addis Ababa, the capital city of Ethiopia and geographically found in between 37°28′ 54″E to 37°36′ 45″E and 5°55′16″N to 6°05′14″N. It is surrounded by the two rift valley lakes, Abaya and Chamo, the dense ground water forest of Nech Sar National Park, and the forty springs. The major economic activity of the city is trade. Tropical fruit plantations and cotton production, reliant on irrigation from the nearby lakes and rivers are also common in the region. Traditional fishing is also a major livelihood of Arba Minch residents. The map of the city and its surrounding is displayed in Figure 1. With a mean annual temperature of 30 °C, with temperatures ranging between 10 °C and 37 °C, the weather alternates between a long summer rainy season (June to September) and a winter dry season (December to March). The annual rainfall varies between 750 to 930 millimeters (Seyoum et al., 2022).

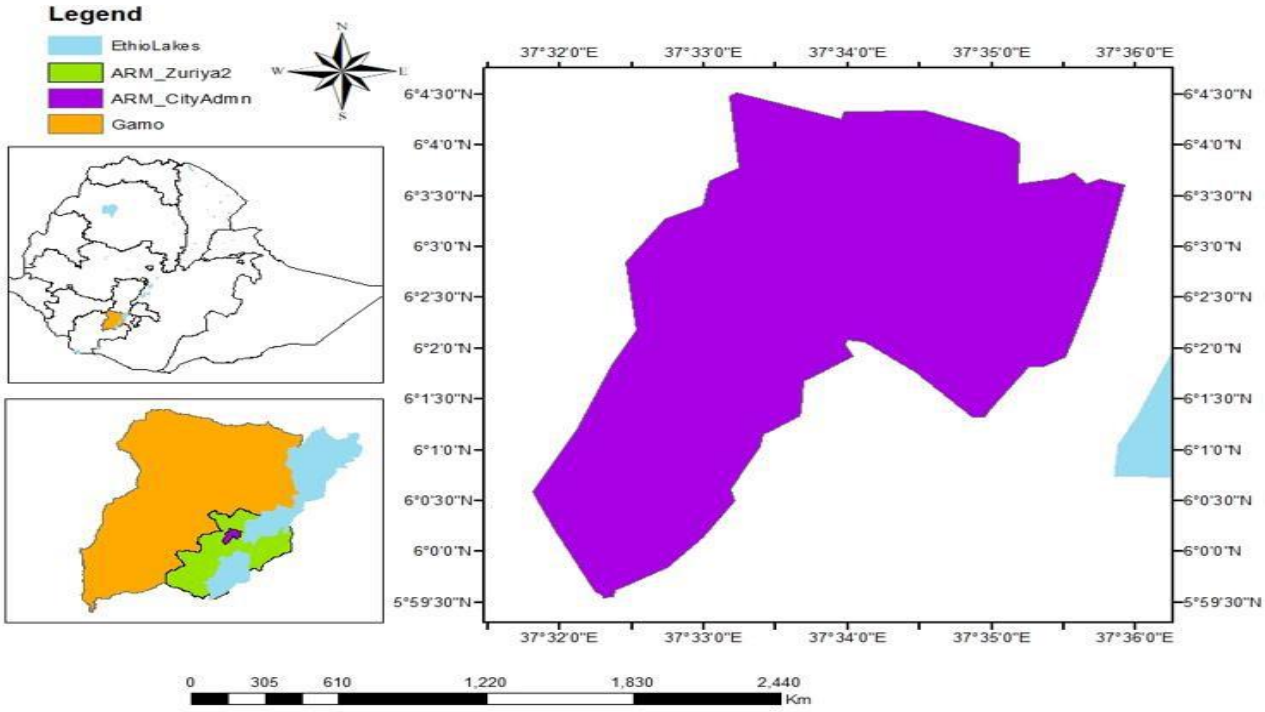

Figure 1. Map of the study area, Arba Minch town administration.

2.2 Study populations

A total of 195 apparently healthy Swiss albino mice of either sex (weighing 20-30 g and age of 8-12 weeks) were obtained from National Veterinary Institute (NVI), Debre zeit, Ethiopia. They were housed in polypropylene cages (6 mice per cage) and allowed free access to pellet diet

and clean water ad libitum. They all were kept in Arba Minch agricultural research center parasitology laboratory with standard twelve hours light and twelve hours dark schedule where room temperature, humidity and ventilation were controlled during the acclimatization period of one week in which they were accustomed to handling through regular weighing and Phlebotomy.

2.3 Study design and sampling

For the current study, experimental study design was applied with randomly assigning of study population (experimental mice) in to thirteen (13) different treatment groups. The study was conducted in Arba Minch agricultural research center from September 2022 to April 2023.

2.4 Preparation and extraction of *Allium sativum* and *Lepidium sativum* for anti-trypanosomial efficacy testing

2.4.1. Collection of plants and processing

Fresh white garlic (*Allium sativum*) bulbs locally named Tumuwa/Nech shinkurt and seed of *Lepidium sativum* (Sibika/feto) were purchased from local market of Arba Minch city and Arba Minch zuriya districts by veterinarian in collaboration with the purchasing expert teams of the research center. The bulb was separated into individual garlic cloves, manually peeled off and any damaged ones were discarded while the seed of *Lepidium sativum* washed thoroughly with tap water to remove debris, dirt, foreign materials. Air dried under a shade at room temperature for 7 days (one week). Dried plant materials were grounded into course powder using a mortar and pestle. The course powdered material was put into polyethylene bags and stored in the moisture free place for further processes (Ilić et al., 2017; Ratti et al., 2007; Yohannes et al., 2018).

2.4.2. Crude extraction

Crude extraction was done by using two extraction solvents. They are aqueous/water and methanol.

2.4.3 Aqueous extraction

To obtain methanol extract, 100 g of powder was macerated in 1000ml of methanol. The residue left after maceration was successively extracted twice with the same medium separately. For each of the two solvents extracts were collected in separate flasks and left undisturbed before being filtered through a sterile filter paper (Whatman No. 1) into a clean conical flask. The filtrate was dried by evaporating the solvents using hot oven. The dried extracts were weighed and placed in refrigerator at 4 °C (Yohannes et al., 2018).

2.4.4 Methanol extraction

To obtain Methanol extract 100g of powder was macerated in 1000ml of Methanol. The residue left after maceration was successively extracted twice with the same medium separately. For each of the two solvents extracts were collected in separate flasks and left undisturbed before being filtered through a sterile filter paper (Whatman No. 1) into a clean conical flask. The filtrate was dried by evaporating the solvents using oven. The dried extracts were weighed and placed in refrigerator at 4 °C (Yohannes et al., 2018).

2.5 Phytochemical screening of study plants

Tables 1 and 2 summarize the phytochemical screening of both *Lepidium sativum* and *Allium sativum* and these results were obtained from previous researches conducted on those plants.

Table 1 Results of phytochemical screening of *Lepidium sativum* seeds

Constituents	Alcoholic	Aqueous
Saponins	+	+
Steroids	+	+
Alkaloids	-	-
Tannins	-	+
Cadenolide	+	-
Carbohydrates	-	+
Cardiac glycosides	+	+
Flavonoid	+	-
Anthraquinone	+	-

Table 2. Results of phytochemical screening of Allium sativum cyanogenic glycoside

Constituents	Alcoholic	Aqueous
Alkaloids	+	+
Carbohydrates	-	+
Phenolic compounds	+	-
Flavonoid	+	+
Proteins and amino-acids	+	+
Saponins	+	+
Mucilage	+	-
Resins	+	-
Lipids / Fats	+	-

- = Absent, + = Present

2.6. Identification of test organisms and infection

The presence of *T. congolense* in the screened cattle was detected from 5ml blood samples collected from the ear vein by veterinarians and laboratory assistants with properly sterilized 5ml syringes and needle in to vacutainer tube with EDTA, ant-coagulant. First wet blood sample slide

was prepared, examined for presence of *trypanosoma* based on its type of motility and characteristics features in the microscopic field with 40x objective and again confirmations was made by preparation of Giemsa stained thin smear and examination under a light microscope using oil immersion 100x objectives (Murray et al., 1977). After microscopic identification, the parasite was maintained in mice by serial passage of blood from infected mice to non-infected ones on weekly basis.

To infect the mice, blood sample was collected through cardiac puncture of an experimental mouse with a rising parasitaemia. Then, the blood with test organisms was diluted in normal saline; so that each mouse was passaged with 0.2 ml of the infected blood containing about $1x10^7$ trypanosomas parasitized red blood cells via intra-peritoneal route (Adebayo and Motunrayo, 2018). Then, the test organism infected mouse was strictly followed in the research center parasitology laboratory for about 12 days without any treatment with ant-Trypanosomal drugs.

2.7. In vivo trial of the experiment

The plant extracts were administered 12 days post-infection at doses of 125, 250 and 500mg/kg by intraperitoneal injection once daily for seven (7) days. Parasitaemia, packed cell volume (PCV), mean survival time and change in body weight were used as indices for monitoring the efficacy of the extracts by comparing with the positive control (28mg/kg body weight dose of Diminazen aceturate based on previous reports (Moti et al., 2012; Feyera et al., 2014) and negative control (distilled water) treated groups. After administering plant's extract, the experimental mice were followed by animal health researchers and laboratory assistants in Arba Minch agricultural research center, parasitology laboratory for acute toxicity effect of the plants. The acute toxicity was conducted in accordance with the Lorke's method. All mice were kept under strict observation for behavioral, neurological, autonomic or physical changes such as alertness, motor activity, restlessness, convulsions, coma, diarrhea and lacrimation for 24 hours. These observations were continued for further 14 days for any signs of overt toxicity (Lorke, 1983).

To estimate the lethal dose of the extracts, this study followed two phases. In the first phase, nine mice were assigned into 3 groups (n=3). Each group was given 10, 100, and 1000 mg/kg body weight of the test substance respectively. In the second phase, further specific doses (1600, 2900, and 5000 mg/kg) of each extract was administered to nine mice. Then the lowest dose which killed one mouse (minimum toxic dose) and the highest dose which had not killed any mouse (maximum tolerated dose) were noted, and the geometric mean of these two doses gave LD_{50}. Every six mice

were administered by each of the same dose and the same solvent extracted crude plant and six mice was also be treated with *Diminazine acceturate* as a positive control, and six mice was remaining untreated as negative control.

2.7.1 Determination of parasitaemia

Parasitaemia was monitored every other day by microscopic examination of blood obtained from the tail of each mouse that was pre-sterilized with methylated spirit. The tail tip was cut to extrude blood and drop of blood was placed on microscope slide and covered with a cover-slide. The blood was examined microscopically at 400x total magnification. The degree of parasitaemia was determined using the "Rapid Matching" method of Herbert and Lumsden (1976). Wet smear was prepared in triplicates from each animal and the mean value of slide counts were taken per sample examined microscopically. A logarithm value of these counts was obtained by matching with the table given by Herbert and Lumsden (1976).

2.7.2. Determination of packed cell volume

Packed Cell Volume was measured (Wintrobe and Landsberg, 1970) to predict the effectiveness of the test extracts in preventing hemolysis resulting from increasing parasitaemia associated with trypanosomiasis. It was monitored before infection and three times till the 14th day (on day 0, 7 and 14). Briefly, blood was collected from tail of each mouse by laboratory technician into heparinized micro haematocrit capillary tubes filled up to 3/4th of their length. The tubes then sealed immediately by crystal seal and centrifuged in a micro-haemtocrit centrifuge (Hettich Haematokrit, Germany) for 5 min at 12,000 rpm. After centrifugation, the height of the red blood cell column was measured by use of haematocrit reader and it was compared to the total height of the column of the whole blood (Wernery, 2001). The effects of extracts in improving PCV of treated mouse were compared with the controls.

2.7.3. Determination of body weight

The body weight of each mouse in all treatment groups was measured before infection, on the day the treatment commenced (day 0) and every other day up to day 30.

2.7.4. Determination of mean survival time

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow up period for six weeks.

2.7.5. Determination of toxicity on mouse

In the present study, two experimental phases were employed for the determination of the acute toxicity (LD50) of the garlic aqueous extract, using the Lorke's method (Lorke, 1983; Madaki *et al*., 2019). In the first phase, nine mice were grouped into three each and were given the *Allium sativum* and *Lepidium sativum* extract intra-peritonially at a dose of 10, 100 or 1000mg/kg, i.e., groups 1, 2 & 3, respectively. After 24 hours of the extract administration, no death occurred among the animals. Subsequently, the second experimental phase was run in three groups of three mice each that received the extract at a dose of 1,600, 2900 or 5000 mg/kg, i.e., groups 1, 2 and 3, respectively.

2.8. Data management and analysis

Data was first entered into Microsoft offices excel and analyzed by gen stat. The data obtained from the study were summarized as means ± standard deviation and the differences between and within the means were analyzed using two-way ANOVA followed by Tukey's multiple comparison tests was performed to determine statistical significance. P values less than 0.05 was considered significant.

3. Results and Discussion

3.1. Effect of extracts on body weight of mouse

According to our results, body weight of the mouse infected with *T. congolense* and treated by 250 mg/kg dose body weight garlic extracted by methanol and positive control group treated by Diminazene aceturate at dose 28mg/kg body weight had shown a decrease in the first 7 days post infection (PI) but starts to increase their body weight from 8th days PI onward (Figure 2).

This result agrees with the finding of Mohammad *et al*. (2022), who stated that significant increase in the body weight of the mice treated with 250 and 500mg/kg of the extract compared to those in the control groups, which was not treated with extracts. This body weight reduction of the mouse up to 7th day PI may be due to that the parasite inoculated within the blood cell of the mouse duplicated rapidly, the blood cell lysed, feed and water intake reduced due to low appetite as the mouse was at anemic condition and reason for the mouse starts to increase their body weight 8th days PI was due to that the crude extract had the ability to treat and kill the parasites with the dose given. Therefore, current results were in disagreement with the report by Eghianruwa (2012) who stated that the garlic extract significantly prevents weight loss in the mice during the trypanosoma infection.

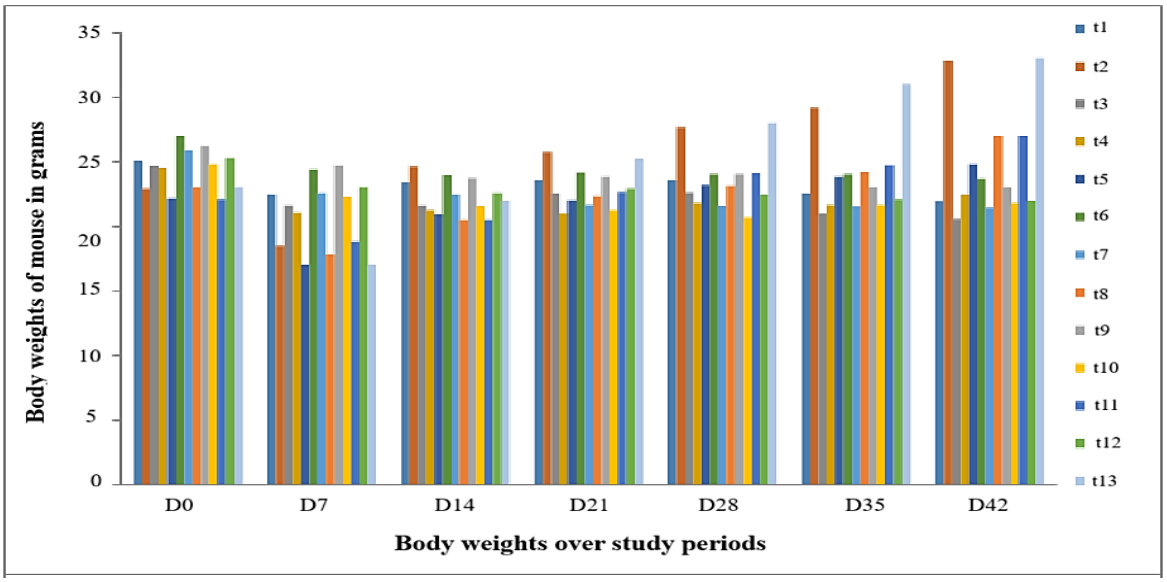

Figure 2. Average body weight of mouse during the study periods

3.2. Effect of extracts on PCV of mouse

Average Packed Cell Volume (PCV) of mouse inoculated with *Trypanosoma congolense* and treated by garlic (*Allium sativum*) at dose of 250mg/kg body weight and *Diminazine aceturate* 28mg/kg of body weight had showed rapid reduction of PCV value up to 7th days PI and starts to increase the PCV value at 9th days PI (Figure 3), continued in the whole study periods.

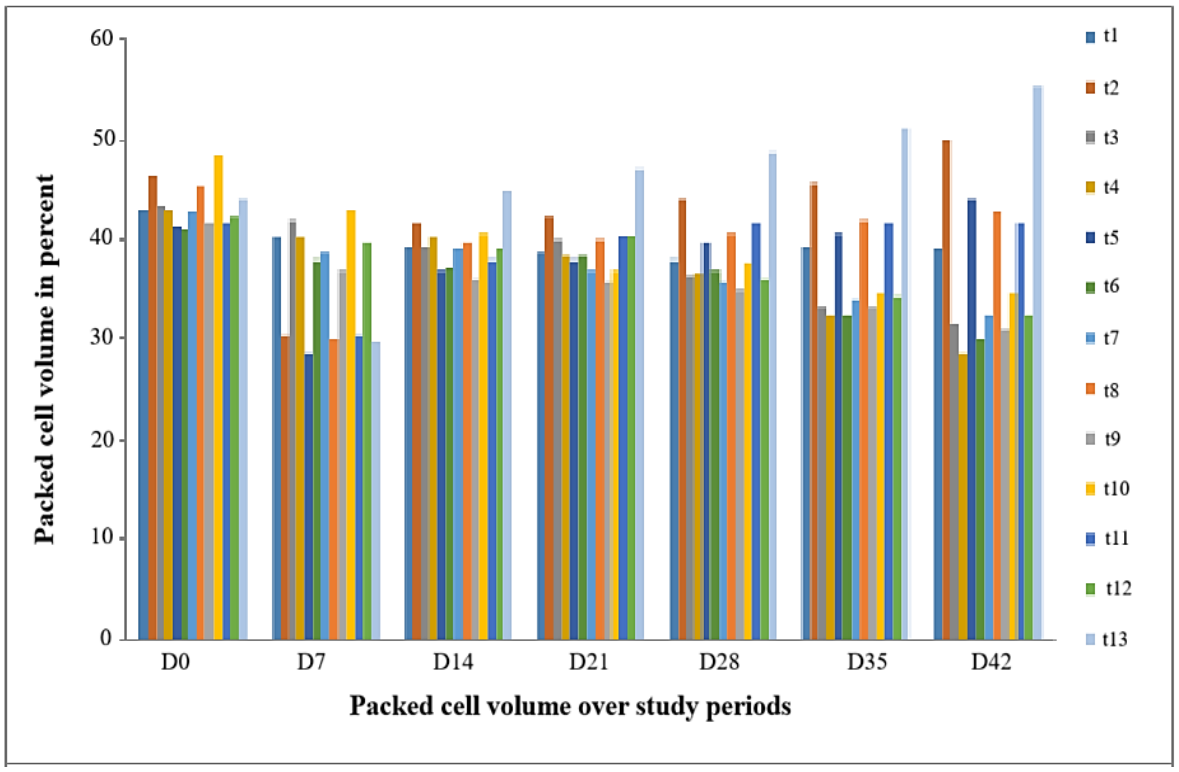

Figure 3. Average packed cell volume of mouse infected and treated by different dose level both *Allium sativum* and *Lepidium sativum*

The PCV level of the groups administered 250mg/kg body weight of garlic extract was significantly higher than those given other dose level of the extract compared to the control group (treated with *Diminazine aceturate*) ($P < 0.05$). Anemia is induced by trypanosoma species through generation of free radicals, which attack the RBC membrane, leading to oxidation and hemolysis (Karori et al, 2008). The significant increase in PCV level observed in methanol extracted from *Allium sativum* (MEAS) at the extract dose of 250mg/kg indicates that the extract acts as an anti-trypanosomal agent and inhibits the parasite while being able to generate red blood cells in the bone marrow more than other dose level of the extract. The current result is in disagreement with the finding of Madaki et al. (2019), who stated that PCV level of the group administered 500 mg/kg of the extract was significantly higher than those given 3.5 mg/kg Diminazene aceturate compared to the normal control group. The mean PCV between standard drug and different doses of the *Allium sativum* and *Lepidium sativum* extract were relatively comparable and this result was in consistence with the findings of Tadesse et al. (2015).

All the rest groups had shown reduced PCV value during the study periods. Mouse treated by water extracted *Lepidium sativum* at dose of 150mg/kg, methanol extracted *Allium sativum* at dose of 150 mg/kg and methanol extracted *Lepidium sativum* at dose of 150 mg/kg body weight had started to increase their PCV value from the 21st day PI. This may be due to the crude extracts were low in dose, therefore, started their action of killing the parasite at 21st days PI. The observed rise in the PCV level indicates that the extract possesses anti-trypanosomal property by suppressing or eliminating the parasites thus preventing the generation of free radicals by the trypanosomes. This also implies better transportation of oxygen and dissolved nutrients (Isaac *et al.*, 2013). The ability to improve PCV is the reason for the long-time survival of treated mice. This is possibly by reducing parasite load or in activating the toxic metabolites produced by try-panosome or enhancing resistance of erythrocyte hemolysis (Inabo & Fathuddin, 2011; Maikai, 2008).

3.3. Effect of extracts on parasitemia of mouse

The current trial concluded that the mouse infected with Trypanosomas species and treated by *Allium sativum* extracted by methanol at the dose of 250 mg/kg body weight and 28 mg/kg body weight of *Diminazine aceturate* had shown rapid and approximately similar reduction in the number of parasite (trypanosoma) within the blood cell and this revealed significant differences ($P < 0.05$) for the mean parasitemia counts among the treated mice. This finding is inconsistent

with the finding reported that the relapse in parasitemia was detected in rats treated with 40 mg/kg/body weight of *Allium sativum* bulb extracted with methanol.

In other groups treated by aqueous extracted *Lepidium sativum* at dose 250 mg/kg body weight, methanol extracted *Lepidium sativum* 150 mg/kg body weight and methanol extracted *Lepidium sativum* 250 mg/kg body weight had shown relatively slight reduction of trypanosoma with in the mouse blood cell at 21st days PI and rapid reductions of the parasite (trypanosoma) within the blood cell of mouse had been observed in between 14th and 35th days PI (Figure 4), then after the mouse survive with very few numbers of parasites in their blood. This may due to effectiveness of the garlic crude extracts against trypanosoma at dose level of 250mg/kg body weight. A constant rise of the parasite in the blood samples was noted in the negative control mice that were infected but not treated until the 14th day and they were all died after 14 days survival. This finding was consistent with those of a former study conducted by Maikai *et al*. (2008) where all of the untreated mice died on the 10th day of exposure to the parasite.

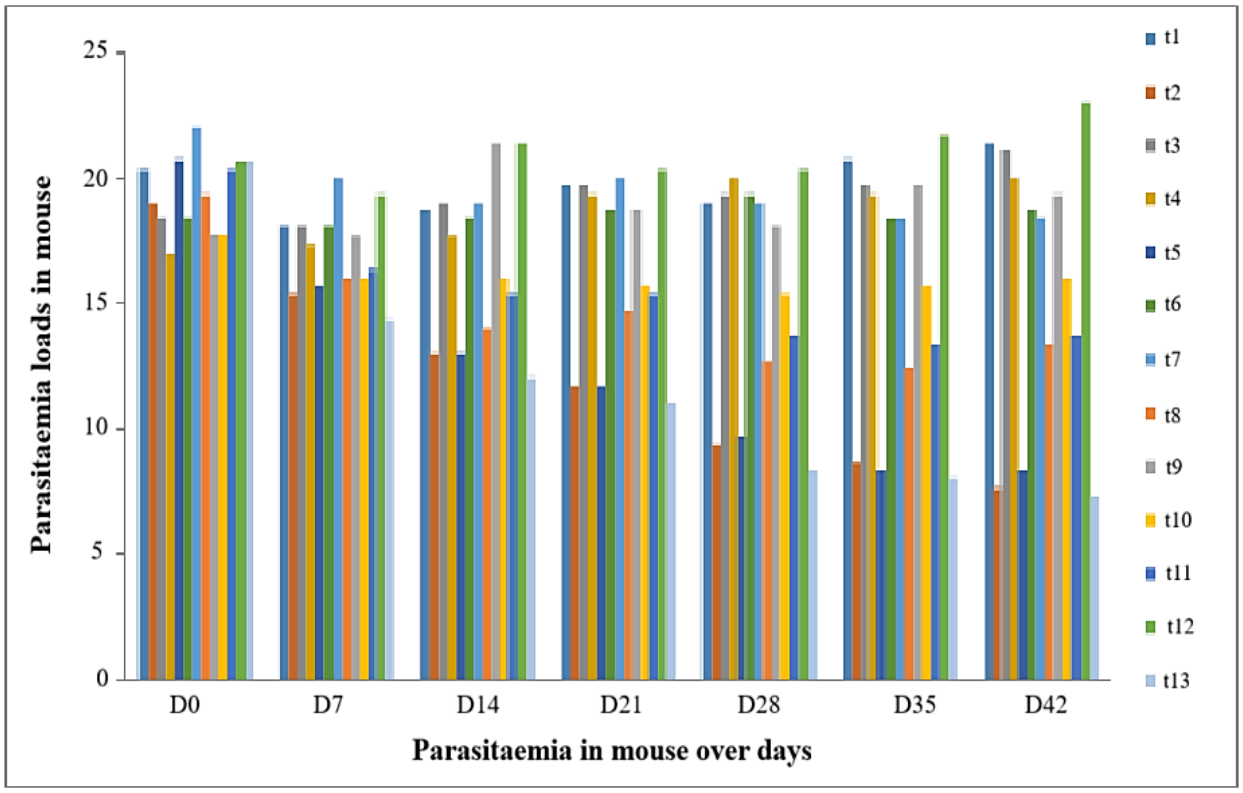

Figure 4. Average number of parasites within the blood cell before and after treatment

3.4. Effect of extracts on mean survival time of mouse

The result of the current trial is presented in Figure 5 and showed that mean survival time of mouse infected with trypanosomes species and treated by 28 mg/kg body weight of *Diminazine aceturate* (standard drug) had relatively highest survival time than mouse treated by

crude extracts of both *Allium satvum* and *Lepidium sativum* during study periods. Yet, methanol extracted *Allium sativum* at dose 250 mg/kg body weight had shown relatively moderate mean survival time during study periods. Mouse allocated in the rest of all treatment groups resulted in a decreased survival time.

Mouse treated by methanol extracted *Allium sativum* 250 mg/kg body weight and *Diminazine aceturate* at dose level of 28 mg/kg had shown moderate mean survival time during study period. This may be due to that crude extract *Allium sativum* with this dose level was effective against trypanosoma within the blood cell, as the treatment gives approximately similar result with that of *Diminazine aceturate* 28 mg/kg body weight. The reason for the decrease of the parasite in the blood could be access of the extract to the parasites in the blood. Though the extract fails to eliminate all the Trypanosoma species from blood of infected mice, it reduced level of parasitaemia. This finding is in consistent with the report of previous studies (Obah et al., 2013; Justina et al., 2015; Mergia et al., 2014; Abdeta et al., 2020).

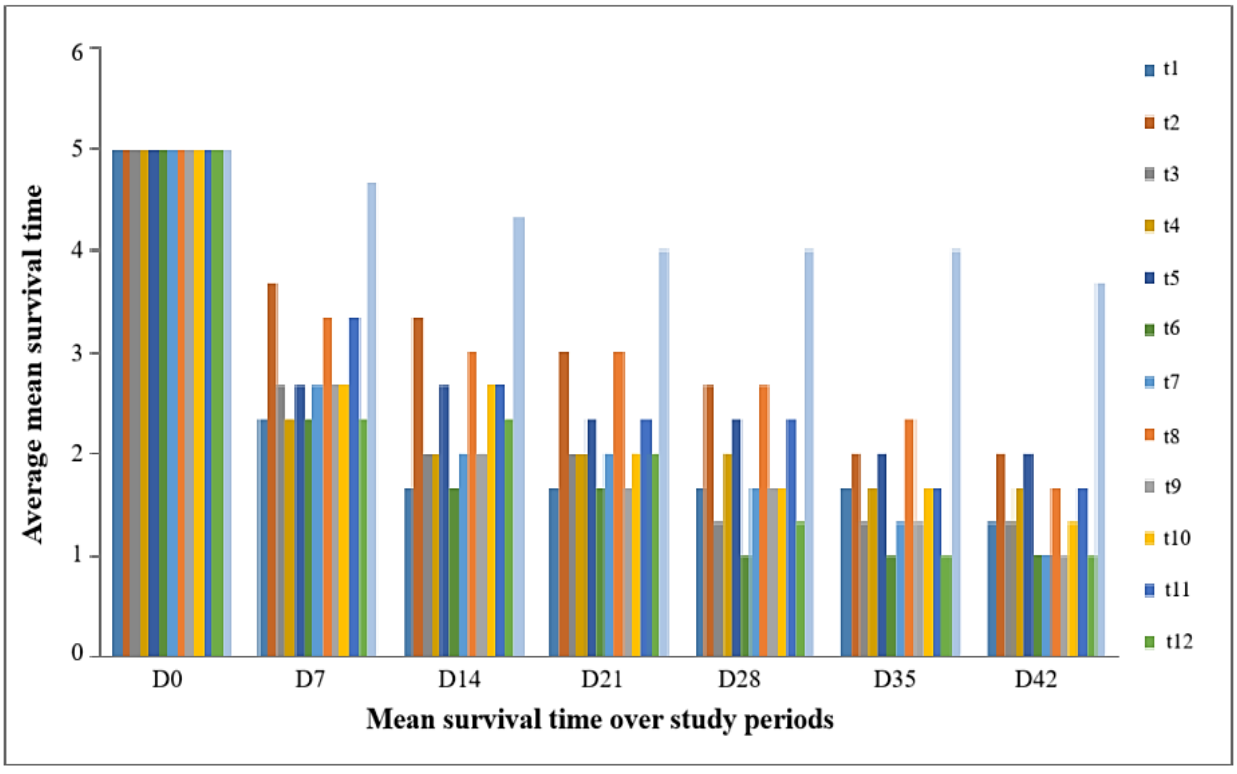

Figure 5. Mean survival time of mouse infected and treated by different dose level of *Allium sativum* and *Lepidium sativum*

This study further demonstrated that the extract is safe since the acute toxicity test of the extracts at doses from 10 to 5000 mg/kg body weight showed no abnormal physical changes or death of the mouse up to this dose level. This is consistent with a previous study conducted by Raphael et al. (2009), who stated that no lethal outcome was recorded at doses up to

5000mg/kg body weight in mice, although the animals were inactive for few hours. These mice were also observed for death and behavioral changes as the signs of toxicity over 24 hours and the result signified that the estimated LD_{50} was more than 500 0mg/kg of the body weight since no mortality was recorded in the mouse by the current high dose of the plants extract.

3.5. Limitations of this study

The current study was conducted on in vivo anti-trypanosomal efficacy of both *Allium sativum* and *Lepidium sativum* only on Swiss albino mice of both sexes. For comparison purposes in positive control, only one standard drug which is *Diminazine aceturate* has been used. Therefore, their effect on large as well as small ruminants has not been addressed currently due to budget shortage of the institute. During the study, the toxicity effects of both plant extracts on the study population (mice) was below 5000 mg/kg body weight and the study period was short, that is only up to 42 days (one and half a month).

4. Conclusions

In this study the efficacy of methanol and aqueous extracted bulb of *Allium sativum* and seed of *Lepidium sativum* against trypanosomiasis were evaluated using an experiment on mice. The efficacy of both *Allium sativum* and *Lepidium sativum* against trypanosoma was found to be satisfactory at dose 250 mg/kg body weight. It was effective on improving final average body weights of the mouse, reducing parasite number within the blood cells, increasing PCV, improving mean survival time. This study also indicated that the methanol and aqueous extracted *Allium sativum* and *Lepidium sativum* at the dose level from 10 mg/kg to 5000 mg/kg of body weight was not toxic to the mouse as no death were recorded during the study period. Therefore, *Allium sativum* and *Lepidium sativum* at dose of 250 mg/kg body weight is recommended to treat Trypanosoma within cells as it was found effective against trypanosoma species. However, further study on methanol and aqueous extracted bulbs of *Allium sativum* and seeds of *Lepidium sativum* against *T. congolense* and *T. evansi* infected large as well as small ruminants using currently effective dose level will be needed.

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Conflicts of Interest

The authors declared no conflict of interests.

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