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DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACY

MSC THESIS ON:

ANTITRYPANOSOMAL ACTIVITY OF HYDROMETHANOL EXTRACT OF LEAVES OF *CYMBOPOGON CITRATUS* AND SEEDS OF *LEPIDIUM SATIVUM* USING MICE MODEL

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JULY, 2021

ADDIS ABABA, ETHIOPIA

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This is to certify that the thesis prepared by Ayechew Yetayeh, entitled Antitrypanosomal Activity Of Hydromethanol Extract Of Leaves Of *Cymbopogon Citratus* And Seeds Of *Lepidium Sativum In-Vivo* Mice Model and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of MSc in Pharmacology at Addis Ababa University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

I have read the University's current research ethics guidelines, and accept responsibility for the conduct of the procedures in accordance with the University's guidelines. I have attempted to identify all the risks related to this research that may arise in conducting this research, obtained the relevant ethical and/or safety approval (where applicable), and acknowledged my obligations and the rights of the participants.

July, 2021

ABSTRACT

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

Ayechew Yetayeh Emiru

Addis Ababa University, 2021

Trypanosomiasis is one of the neglected tropical diseases of both humans and animals caused by the protozoa Trypanosomes mainly endemic in Africa. Unavailability of vaccine, difficulty of vector controls, low therapeutic index of the available trypanocidal drugs, and development of resistance lead to the need for research focused on developing alternative safer, more effective and more accessible treatment options especialy from medicinal plants. In Ethiopia, many medicinal plants such as *Cymbopogon citratus* and *Lepidium sativum* are used for treatment of trypanosomiasis as traditional remedies. The objective of the present study was, therefore, to investigate antitrypanosomal activities of leaves of *C. citratus* and seeds of *L. sativum* in *in vivo* mice model. The acute toxicity result showed that the extracts were safe at the dose of 2000mg/kg, and 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} days of *T. congolense* inoculation where the peak parasitemia were achieved. The treatment continues consecutively up to seven days and the parameters were recorded every other day up to the 14^{th} day of treatment commenced. 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 ($P<0.05$), ameliorated 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 (73.2% reduction).

Key words: Antitrypanosomal activity, *Cymbopogon citratus, Lepidium sativum, Trypanosoma congolense*

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LIST OF ABBREVIATIONS/ACRONYMS

AAT	Animal African Trypanosomiasis
BSF	Bloodstream Form
DMSO	Dimethyl Sulfoxide
EMF	Epimastigote Form
FAO	Food and Agriculture Organization
HAT	Human African Trypanosomiasis
MCF	Metacyclic Form
PCV	Packed Cell Volume
PF	Procyclic Form
SIT	Sterile Insect Technique
VSG	Variant Surface Glycoprotein
WHO	World Health Organisation

1. INTRODUCTION

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. Some strains of trypanosome can result 50-100% mortality within months in susceptible animals especially when poor nutrition or other factors contribute to debilitation (Spickler, 2018). Trypanosomiasis is the cause for the death of 3 million heads of cattle annualy, with 50 million animals at risk in sub-Saharan Africa (Chitanga *et al*., 2011). Meta-analysis by leta *et al.*, (2016) concluded that 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.

Human beings as well affected by trypanosome parasites, and which is one of the 13 most neglected tropical diseases recognised by the world health organization (WHO). *Trypanosoma cruzi* which was discovered in 1909 is the cause of American trypanosomiasis (Chagas disease) (Rassi *et al*., 2010). Human African trypanosomiasis (HAT) is common in sub-Saharan Africa, caused devastating epidemics during the 20th century. Regardless of significant reduction of cases because of the WHO involvement, the disease is still endemic in parts of sub-Saharan Africa, with huge burden on rural communities. There are two forms 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 (Büscher *et al*., 2017). The severity of clinical presentations of the disease varies with those forms of the disease. Eventhough the signs and symptoms of the two forms are generally the same, they differ in terms of their frequency, severity, and kinetic appearance. 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 (Franco *et al*., 2014).

In Ethiopia human African trypanosomiasis was first reported in 1967. The disease occurs in south-western Ethiopia bounded by the Baro River, Akobo River and to the east by the

escarpment leading up to the central Ethiopian plateau (Baker *et al*., 1970).

HAT occupies mainly in remote rural areas of sub-Saharan Africa, where there is favourable environmental conditions for the vectors. 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. Because 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. The cost of patient care and the need of seeking medical services to look for a diagnosis and treatment mean that there is an extra cost when the income generation in the family is threatened. In this way, the disease contributes to the sustainability of the poverty cycle in these neglected communities. In areas where HAT is endemic, people are panicked by the infection due to the long lasting suffering and lethality of the disease. 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 (Franco *et al*., 2014). Human African trypanosomiasis (HAT) impacts 70 million people living in sub-Saharan Africa which covers an area of 1.55 million km^2 (Aksoy *et al*., 2017).

Due to the unavailability of vaccines, 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 (Wilkinson & Kelly, 2009 ; Chitanga *et al*., 2011; Feyera *et al*., 2013).

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. Most herbal remedies have traditionally been used for centuries and are still widely used to treat illnesses and other parasitic diseases. Several well-known antiprotozoal drugs such as quinine and artemisinin have been originated from plants. In Ethiopia, many plants are claimed to be useful as traditional remedies for treatment of trypanosomiasis in different parts of the country, but only few of them have been confirmed either by *in vitro* and/or *in-vivo* studies (Solomon,

2017). *Cymbopogon citratus* (lemon grass) and *Lepidium sativum* are among the plants claimed to have antiprotozoal activity and/or antitrypanosomal effect in particular (Yigezu *et al*., 2014; Tchoumbougnang *et al*., 2005; Al-Otaibi *et al*., 2019). But only few studies have been made on them so, in the present study aimed at investigating their antitrypanosomal effect using *T. congolense* infected mice.

2. LITERATURE REVIEW

2.1. Aetiology of African Trypanosomiasis and their lifecycle

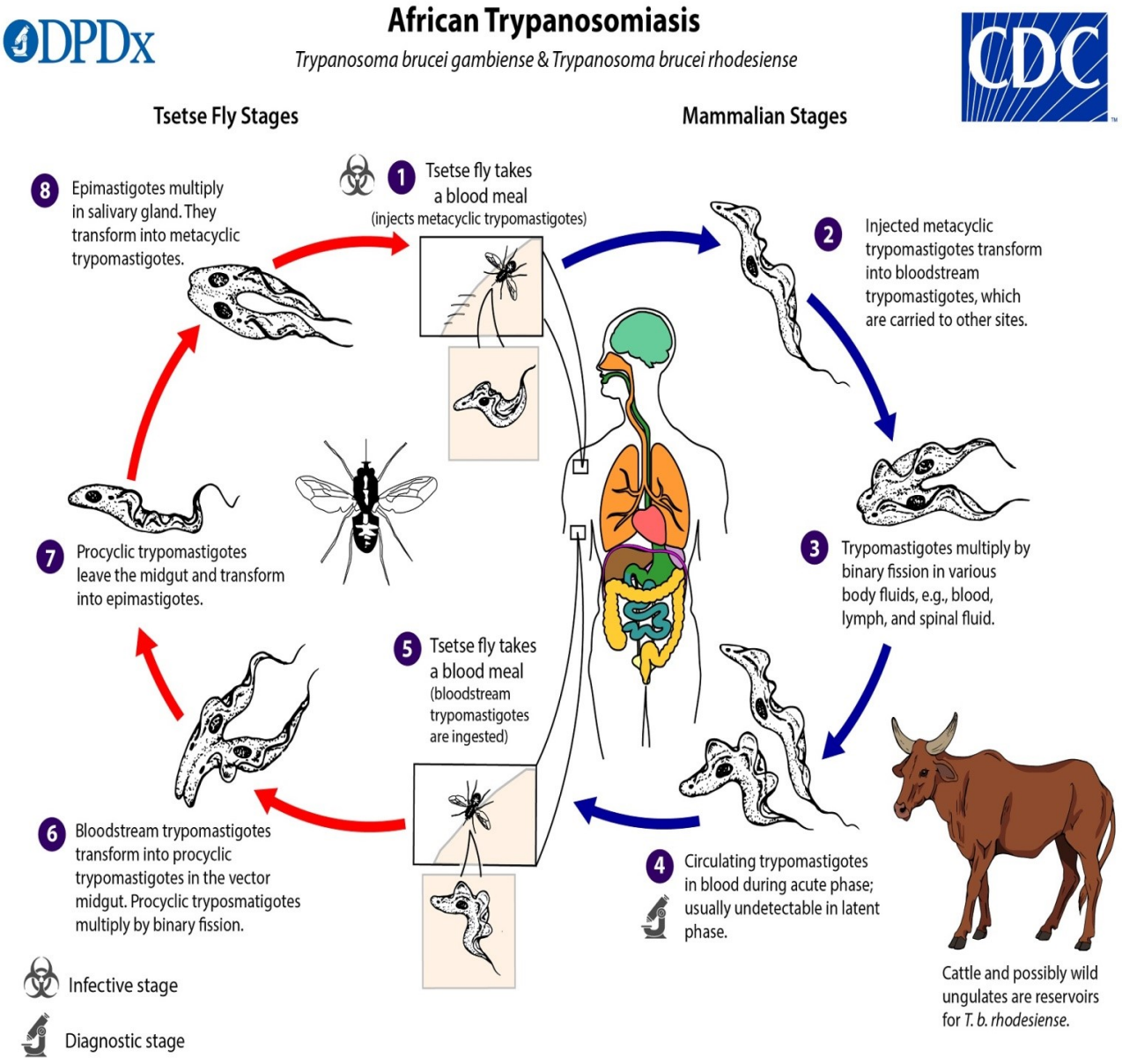
Trypanosomes are single celled protozoan parasites in the family Trypanosomatidae. Trypanosomatids have single flagellum, and similar to other organisms in the order Kinetoplastida, they are characterised by a modified mitochondrial genome called kinetoplast. Trypanosoma is among the genus of trypanosomatids having particular medical and veterinary importance (Cayla *et al*., 2019).

African animal trypanosomiasis is caused by the three most important species trypanosoma namely *Trypanosoma congolense*, *T. vivax* and *T. brucei subsp. Brucei*. In tropical Africa, *Trypanosoma* (Nannomonas) *congolense* is perhaps the most prevalent and widespread pathogenic trypanosome, being found in ruminants, pigs, dogs and other domestic animals throughout the tsetse belt (Peacock *et al*., 2012) . The savannah, forest and kilifi (or Kenya Coast) types are the three variants of *T. congolense*. African animal trypanosomiasis can also be caused by *T*. (Pycnomonas) *Suis, T*. (Nannomonas) *simiae*, *T*. (Nannomonas) *godfreyi* and *T*. (Duttonella) *uniforme*, and possibly by additional unnamed trypanosomes.

Human African trypanosomiasis which is also known as sleeping sickness, is caused by the two related parasites, *T. brucei subsp. gambiense* and *T. brucei subsp. rhodesiense* (Cayla *et al*., 2019).

The genus trypanosoma can be grouped as Stercoraria and Salivaria, based on how the parasites are transmitted from its insect vector to the mammalian host (Uilenberg, 1998). Trypanosomes have complex life cycle involving a vertebrate host and an arthropod Vector mainly tsetse flies. It involves differentiation into several development stages in both the vector and the mammalian host (Itard, 1989). In the Stercoraria section, the metacyclic forms of trypanosomes develop in the hindgut and are transmitted via the faeces of the insect vector. The Salivarian trypanosomes develop into the metacyclic stage in the anterior part of the digestive tract of the tsetse fly and they are inoculated through the saliva into the mammalian host (Stevens & Brisse, 2004).

The African trypanosomes have four main life cycle stages. The procyclic form (PF), epimastigote form (EMF) and metacyclic form (MCF) which develop in vector and bloodstream form (BSF) found in the mammalian host (Peacock *et al*., 2012). The mammalian host are infected when MCF trypanosomes in the saliva of an infected tsetse fly are injected during a blood meal. The MCF trypanosomes proliferate and differentiate into BSF at the site of infection (Roditi and Liniger, 2002). The BSF parasites replicate in the interstitial fluids at the site of infection for 5-9 days before spreading to the circulatory system where they continue to replicate, forming a peak of parasitaemia (Ndung'u *et al*., 2008). The BSF is covered with a dense surface coat of glycosyl-phosphatidylinositol (GPI) on which variant surface glycoprotein (VSG) is anchored. Antigenic variation, allowing the trypanosome population to avoid elimination by the host immune system is due to the presence of these VSG molecules (Wang *et al*., 2003).



Life cycle of *Trypanosome brucei* (Peacock *et al*., 2012, Pays *et al*., 2001).

2.2. Clinical features of trypanosomiasis and diagnosis

Human African trypanosomiasis has two clinical stages. In the first or early stage the parasites dwell in to the lymphatic system and bloodstream (hemo–lymphatic stage). The late or second stage (meningo–encephalitic stage) starts after a variable period, which is much shorter in rhodesiense HAT than in gambiense HAT. In this stage the trypanosomes cross the blood–brain barrier and invade the central nervous system accompanied by progressive neurological damage or meningoencephalitis. Eventhough the severity of clinical presentation varies with the two forms of the disease, HAT is usually considered to be fatal if remained untreated. Patients progress gradually to a coma, severe organ failure and finally death. The signs and symptoms are generally the same for both forms, but they differ in terms of their frequency, severity, and kinetic appearance. Rhodesiense HAT is an acute disease that commonly progresses to death within six months. Gambiense HAT has a more chronic progressive development with an average duration of almost three years. The symptoms and clinical signs are not specific in both forms of the disease, and their appearance varies between individuals and foci. In the first stage, headache, intermittent fever, pruritus and other dermatologic problems, weakness, lymphadenopathies, anemia, cardiac disorders, asthenia, endocrine disturbances, hepatosplenomegaly, and musculoskeletal pains are the main signs and symptoms. Whereas neuropsychiatric signs and symptoms including sleep disturbances are characteristic of the second stage (Büscher *et al*., 2017).

The distinction between the stages based on clinical features is difficult because most of the signs and symptoms of both stages overlap. And misdiagnosis is common with other fever-causing diseases and neuropsychiatric problems (Franco *et al*., 2014).

In AAT, the trypanosomes first affect the biting or inoculation site in the animal skin causing swelling and chancre. The chancre not only forms a site for the establishment of the infection but also is a centre for duplication and persistence of trypanosomes before their propagation into bloodstream. The parasites there after spread to the lymph nodes and blood and continue to reproduce (Emma, 2008). *T. congolense* localizes in the endothelial cells of small blood vessels and capillaries whereas *T. brucei sub sp. brucei* and *T. vivax* localize in tissues such as

lymph nodes (Holmes *et al*., 2000)

AAT is associated with depression, fever, weakness, salivation, anaemia, lacrimation, prominent jugular pulse, nasal discharges and subcutaneous edema of the mandible. The diseased animals have decreased appetite and rapid weight loss which progresses to an extreme emaciation. The death with AAT is often related to severe anaemia and circulatory collapse (Albadrani, 2012). A substantial decline in packed cell volume (PCV) in response to trypanosomosis is a distinctive feature of the disease and a primary criterion for evaluating its severity. Within a week of infection there is usually marked decrease in red blood cells, packed cell volume, haemoglobin, and white blood cells levels (Mersha *et al*., 2012).

The diagnosis of AAT based on the identification of trypanosomes by examination of blood smears and sometimes, on lymph node biopsy (Bouteille & Buguet, 2012). It can be done through direct and/or indirect investigation of the parasite. Direct identification of trypanosomes can generally be accomplished with a wet film blood smear with or without concentration (by centrifugation in a haematocrit capillary (HCT)), from stained blood smears as either thin or thick films or dark ground buffy coat technique (DG) (Bouteille & Buguet, 2012; Chappuis et al., 2005; Herbert & Lumsden, 1976). Serological tests like enzyme-linked immune-sorbent assay (ELISA), immune-fluorescence agglutination test (IFAT) and card agglutination test (CAT) can be also used to detect infections with trypanosomes indirectly. It can be possible to characterize trypanosomes both in the vectors and the hosts by using of molecular biological tools particularly the Polymerase Chain Reaction (PCR) (Solano *et al*., 2000; Chappuis *et al*., 2005).

2.3. Epidemiology and economic importance of African trypanosomiasis

2.3.1. Species affected

The organisms that cause African animal trypanosomiasis have been detected in many species of mammals, including all domesticated animals and certain free-living or captive wildlife. Wildlife known to be susceptible to infection include ruminants such as duikers (Cephalophus spp.), South American white-tailed deer/ cariacou (*Odocoileus gymnotis*), antelope and

African buffalo (*Syncerus caffer*), nonhuman primates, as well as wild equids, felids, warthogs (Phacochoerus spp.), elephants, capybaras (*Hydrochoerus hydrochaeris*) and various rodents. Cattle are reservoir hosts for *T. vivax, T. congolense* and *T. b. brucei*, but other animals including pigs, small ruminants and some wildlife (e.g., African buffalo) are also thought to preserve these organisms. Clinical cases have been appreciated in a number of species including cattle, goats, sheep, camels, pigs, donkeys, water buffalo, horses, alpacas, llamas, cats, dogs and captive wild ungulates. Although there could be specific differences in their host preferences or virulence for different species, *T. vivax, T. congolense* and *T. b. brucei* have a wide host range among domesticated animals. Pigs are mainly affected by *T. godfreyi*, *T. simiae* and *T. suis*. *T. simiae* has also been detected in goats, sheep, camels, cattle, horses and wildlife. Eventhough detail information is not currently known about *T. suis*, it is expected to be carried in wild African suids. Attempts to infect goats, sheep, donkeys, calves, dogs, cats, rabbits and laboratory rodents with this organism were unsuccessful. Birds and Reptiles carry their own species of trypanosomes, but *T. vivax* DNA was identified in crocodiles and monitor lizards (*Varanus ornatus*) in Africa by PCR detection (Spickler, 2018).

Human beings are affected by *T. cruzi* (American trypanosomiasis) (Murillo-Godínez, 2018) and the two strains of *T. bruci* sub species, Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense (Human African trypanosomiasis) (Büscher et al., 2017)

2.3.2. Transmission of trypanosomiasis

Trypanosomiasis infection occurrence depends on the interaction of three components within a specific environment; The mammalian reservoirs of trypanosome (animals or humans) that can be also the host suffering from the disease, and which are influenced by their behavioural interactions with the environment; Glossina or Tsetse flies as biological vectors for transmission that are entirely dependent on environmental factors; and the pathogenic parasite or the trypanosome itself (Franco *et al*., 2014).

The cyclical transmission trypanosomes are due to the involvement of Tsetse flies (Glossina spp.) which are mainly found in Africa. They are the vectors of trypanosomes and create a

potent and persistent threat to livestock and humans over much of Sub-Saharan Africa (Abebe, 2005). The main species of tsetse flies can be grouped in to three categories. The fusca group flies, *Glossina austeni* (subgenus Austenina) found in lowland rainforests of West and Central Africa. The palpalis group (subgenus Nemorhina), occupied the riverine galleries of Central and West Africa but can cover the savannah regions between river systems; *G. tachinoides* and *G. palpalis* are important vectors of AAT in this group. The morsitans group (subgenus Glossina) found in a variety of savannah habitats lying between the desert and forest edges and includes several important AAT vectors including *G. morsitans*, *G. longipennis* and *G. pallidipes* (Tadesse and Tsegaye., 2010). During the act of feeding the flies penetrate the skin of the host with its proboscis. Pool of blood is formed in the tissues by the rupture of small blood vessels and the fly injects saliva to inhibit coagulation. At this stage infection of the host takes place, with infective metacyclic trypanosomes in the saliva (Aksoy *et al*., 2003; Franco *et al*., 2014).

The "tsetse belt" inhabited by Tsetse flies covers 8.7 million km^2 of sub-Saharan Africa approximately between latitudes 10°N and 20-30°S. This area represents about a third of the continent. In these tsetse prevalent areas, agricultural output is low because of the risk of African animal trypanosomosis (James, 2007). A few species of tsetse flies have also been identified in parts of the southwestern Arabian Peninsula. Trypanosomes, particularly *T. vivax*, can spread away from the tsetse fly belt due to animal movements and transmission via mechanical vectors. *T. vivax* has become established in parts of Central and South America and the Caribbean, which are free of tsetse flies (Spickler, 2018).

Mechanical transmission occurs by a number of biting flies such as tabanids (horseflies) and stable flies (Stomoxys species) which disseminate the trypanosomes from an infected animal to another in the course of feeding. This mode of transmission has substantiated to be sufficiently effective to sustain *T. evansi* and *T. vivax* in Central and South America (Desquesenes and Dia, 2004).

2.3.3. Economic impact of African trypanosomiasis

The economic influences of trypanosomosis in Africa are complex and diverse, apart from its direct effects on human health and animal production, indirectly affects animal husbandry, settlement patterns, land use and farming (Chanie *et al*., 2013).

Estimates suggested that Africa loses up to US$1.5 billion annually due to African trypanosomiasis (WHO 2017). African trypanosomiasis decreases labour resources, prevents growth of the livestock industry given that high yielding animals are less likely to survive. The disease affects availability of milk and meat and deprives farmers of draught power. The disease commonly occurs in remote rural areas where health systems are fragile or nonexistent and have a tendency to affect economically active people. The consequential burden on the extended family is substantial, not only because diseased individuals come to be unproductive but also because close relatives have to devote time taking them for treatment and looking after them. Money and time spent on pursuing treatment may be a serious drain on the family's resources. If left untreated, the final result of the diseased patient is death, but similarly its devastating effect is on communities, households and quality of life resulted from its insidious and debilitating nature (Bukachi *et al*., 2017).

In Ethiopia, African animal trypanososmosis is one of the most important and costly disease delaying the effort made for food sufficiency (Abebe, 2005). Tsetse infected area covers around 220,000 km^2 in the North West and South West part of the country following the greater river basins of Abay, Ghibe, Omo and Baro, known with high potential for agricultural development (Nigatu and Abebe, 2009; Desta *et al*., 2013).

2.4. Prevention and control of African trypanosomiasis

2.4.1. Vector control

Vector control is the most reliable method of disease control since it eliminates the risk of trypanosomosis on a long-lasting basis. Many vector control means such as woody vegetation clearance to remove tsetse shelter, and large scale application of insecticides by ground and air spraying could be applied (Adamu *et al*., 2011). One of the latest methods of vector

control is the Sterile Insect Technique (SIT). SIT is a genetic population suppression approach and comprises continued, systematic release of irradiated sterile male insects among the wild vector population. Males are sterilized by irradiation and then taken to the selected area and released by air in high numbers over a period of 3–4 generations, after having condensed population density by other methods (insecticide spraying, trapping, etc.), the target population can be exterminated (Rogers and Randolph, 2002; Feldman, 2004). African Union suggested continent-wide Sterile Insect Technique. However, the practicability of the application of this technique continent-wide has come into question given the variety of species that can spread the parasite, its high cost, and reliance on major infrastructure such as irradiation facilities, large insectaries and airplanes (Akosy *et al*., 2017).

Alternative technique of control of African animal trypanosomiasis is use of trypanotolerant breeds of cattle. Trypanotolerance or innate resistance has been known since 1906 when the capacity of indigenous taurine cattle in West Africa to survive and be productive in danger of trypanosomosis was detected. In cattle both acquired and innate resistance can occur for African trypanosomosis. It is well documented that trypanotolerance has a genetic foundation. Bos taurus subtypes N'Dama and Baoule, are the two most important trypanotolerant breeds whilst a degree of trypanotolerance has also been shown to occur in certain breeds of Bos indicus zebu. Apart from limited in availability (account only for 17% of the total cattle population of the continent), the use of trypanotolerant cattle was a potential alternative approach for managing the problem (Vinhaes and Schofield, 2003).

2.4.2. Chemotherapy and chemoprophylaxis

The fight against the vector (tsetse fly) has not been very successful, and the chemicals used as part of the controlling activities pollute the environment. Because of the problem of trypanosomes' antigenic variation, immunization against the disease has not been possible. Therefore, chemotherapy continues to play a major role in the treatment and control of trypanosomosis. This is vital because deprived of treatment; the result of African trypanosomosis is almost all the time fatal (Legros *et al*., 2002).

Trypanocides are employed for the control of the disease in the thirty seven African countries where animal trypanosomosis is endemic. Diminazene aceturate, Homidium salts (homidium bromide and homidium chloride) and Isometamidium chloride are three compounds used in the treatment of AAT (Dolan *et al*., 1990). Diminazene aceturate is only used for the treatment purpose whereas homidium salts and Isometamidium chloride the have both curative and prophylactic capacities (Docampo and Moreno, 2002).

Treatment of HAT is dependent on the subspecies and stages the disease. If the parasites are limited to the blood/lymphatic system, suramin is used against *T. b. rhodesiense* and pentamidine against *T. b. gambiense*. Neither of the two compounds can cross the blood brain barrier efficiently hence these drugs have little use against cerebral disease. The treatments available for this lethal (cerebral) stage are restricted to eflornithine and melarsoprol. Once the central nervous system (CNS) has been affected, melarsoprol is the only drug active against both subspecies of *T. brucei*. However, it is very toxic arsenical, and also the development of drug resistance is a major issue (Matovu *et al*., 2001). In contrast eflornithine is relatively safe, but this drug is effective solely against West African trypanosomiasis and the cost of treatment is challenging in poor countries. Clinical trials are assessing the efficacy of a combination therapy containing eflornithine and nifurtimox against the cerebral stages of West African trypanosomiasis. Findings have proved very effective and in recent times it has been recommended by the World Health Organization (WHO) as a front-line treatment for infections with *T. b. gambiense* (Checchi *et al*., 2007, Priotto *et al*., 2006, Priotto *et al*., 2009).

The main challenge in the treatment of trypanosomiasis is the development of drug resistance and narrow therapeutic index of trypanocidal drugs. Resistance looks to develop in a step-wise manner with trypanosomes resistant to a low dose of antitrypanosomal agents being removed by a higher dose of the same drug. Both *T. congolense* and *T.vivax* develop resistance against Isometamidium and Diminazine Aceturate (Chaka and Abebe, 2003, Mulugeta *et al*., 2014, Tewelde *et.al*., 2004, Afewerk *et.al*., 2014, Kebede and Abebe, 2010, Shimelis *et al*., 2017) and also *T. brucei* develops resistance against Isometamidium (Tewelde *et.al*., 2004). The difficult is that, there is only limited scope to overcome resistance by increasing the dosage because of the narrow therapeutic indices of the trypanocides.

2.5. Overview of study plants

2.5.1. Cymbopogon citratus

Cymbopogon is a genus contains about fifty five species, which are native in tropical and semi-tropical areas of Asia and are cultivated in Central and South America, Africa and other tropical countries. These grasses are tufted perennial C 4 grasses with numerous stiff stems arising from a short, rhizomatous rootstock (Figure 1) (Shah *et al*., 2011) having citrus flavour, and it can be used as fresh or dried and powdered form. The term Cymbopogon is derivative of two Greek words "kymbe" (boat) and "pogon" (beard), referring to the flower spike arrangement.

Cymbopogon citratus, Stapf (Lemon grass) is a broadly used herb in tropical countries, particularly in Southeast Asia. The essential oils of the plant are used in aromatherapy. The major compounds identified in *Cymbopogon citratus* are terpenes, alcohols, ketones, aldehydes and esters. Several reports about phytoconstituents of the plant mentioned that the plant contains essential oils that contain Citral, Citronellal, Nerol, Terpinolene, Geraniol, Myrecene, Geranyl acetate and Terpinol Methylheptenone. The plant also contains flavonoids and phenolic compounds, which consist of isoorientin 2'-O-rhamnoside, luteolin, kaempferol, quercetin and apiginin (Shah *et al*., 2011).

Fig1: *Cymbopogon citratus* stapf. (Ayechew Yetayeh, Garden of toxic and medicinal plants, AAU, CVMA, 2016)

C. citratus has wide range of therapeutic applications in traditional and Ayurvedic medicine in several countries. It is used as a herbal medicine for a wide range of applications because of its antibacterial (Wannissorn *et al*., 2005), antifungal (Nikos et al., 2007, Cristiane et al., 2008), antiprotozoal (Monzote *et al*., 2012), anti-carcinogenic (Vinitketkumnuen *et al*., 1994), anti-inflammatory (Abe *et al*., 2004), treatment of diabetes (Mansour *et al*., 2002), dyslipidemia, gastrointestinal disturbances (Carlini *et al*., 2002), and malaria (Tchoumbougnang *et al*., 2005). On top of its therapeutic uses, *C. citratus* is also consumed as a tea, added to non-alcoholic drinks and baked food, and used as a flavouring agent and preservative in confections and cuisines. In cosmetics industry, its essential oils are used as fragrance in the manufacture of soaps, detergents, perfumes and creams (Lorenzetti 1991, Shah *et al*., 2011). *Invitro* study of citral, the main component of *cymbopogon citratus,* revealed that the plant has antitrypanosomal activity. At the concentrations higher than 60 ug/ml citral causes 100 % cell death both epimastigote and trypomastigote forms of trypanosome (Cardoso & Soares, 2010).

2.5.2. *Lepidium sativum*

Lepidium sativum belongs to the family Bassicaceae (cruciferae) is famous garden cress. Some scientists say the origin plant started from Ethiopia and then distributed to different parts of the world (Wadhwa *et al*., 2012). In Ethiopia, it is known as "fetto" in Amharic which is cultivated for its medicinal value; edible oil is also obtained from its seeds. *L. sativum* is usually grown in Ethiopia as a garden plant and found in the market, though commonly in small quantities. It is an annual herb, stem finely striate, branched and glabrous (hairless, smooth); its leaves are long at the bottom of the stem and small green feather-like leaves arranged on opposite side of its stalk at the top (figure 2). They could differ a bit in shape but not in taste. The flowers are bisexual, regular, merous, pedicel, ascending; sepals are ovate; petals are spatulate with short claw, white or pale pink; whereas anthers are usually purplish. The fruits are globose, 1.2 cm across, and purple black with hard ribbed endocarp. Seeds are oval-shaped, small, pointed and triangular at one end, smooth, about 2-3 mm X 1-1.5 mm, reddish brown to almost black, Seedling with epigeal germination; leaflets spatulate, lateral ones smaller than central one. The powder of the seeds looked creemish yellow in color (Wadhwa et al., 2012, Sheel and Nidhi, 2011).

L. sativum contains proteins, pats, carbohydrate, mineral matter, calcium, phosphorous and trace elements such as; iron, nickel, cobalt and iodine. Vitamin A, thiamine, riboflavin, niacin and volatile oil are also found in the herb (Gokavi et al., 2004 Fulwah et al., 2019).

Figure 2: *Lepidium sativum* (Ayechew Yetayeh, Garden of toxic and medicinal plants, AAU, CVMA, 2016)

Ethinobiological studies repetitively mentioned that *L. sativum* has wide range traditional medical value. It used for the treatment of Amoebiasis and diarrhoea, Gland TB and malaria (Solomon *et al*., 2015). The plant is used for treatment and insect repellent effect for trypanosomiasis. The antitrypanosomal effect of methanol extract the plant was performed at a single dose (200mg/kg) on *T. evansi* infected mice (Abiy *et al*., 2013, Al-Otaibi *et al*., 2018).

OBJECTIVES OF THE STUDY

The general objective of this study was to investigate the *invivo* antitrypanosomal activity of 80%methanol extracts of leaves of *Cymbopogon Citratus* (lemon grass) and seeds of *lepidium sativum* against field isolates of *T. congolense* in mice model.

Specific objectives

- ✓ To evaluate the acute toxicity profile of crude extracts of the plants;
- ✓ To evaluate the antitrypanosomal activity of crude extracts of the plants in mice infected with *T. congolense*;
- ✓ To evaluate the effect of the crude extracts of the plants on packed cell volume of mice infected with *T. congolense*;
- ✓ To evaluate the effects of crude extracts of the plants on body weight of mice infected with *T. congolense*; and
- ✓ To investigate the effect of the crude extracts on differential white blood cell count of mice infected with *T. congolense*

3. MATERIALS AND METHODS

3.1. Experimental animals

Swiss albino mice age of 8–12 weeks and weighing 25 –35 gm were obtained from Ethiopian public health institute (EPHI). 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.

3.2. 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 (Stephen, 1986). 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 (Peacock, 2012). The field isolates of the organism were obtained from Ghibe valley known with high prevalence of trypanosomiasis and tsetse fly infestation (Rowlands *et al*., 1993). 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.

3.3. Chemicals and Equipment

Chemicals and solvents

Dimethyl sulfoxide (DMSO), methanol, distilled water, and reference drugs (Diminazene aceturate), phosphate glucose buffered saline solution (PBSG) were used in the study.

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.

3.4. Plant materials and test organism collection

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 (Achenef and Admas, 2012). The red soil is the dominant soil type and it is moderately fertile (Debre Elias Woreda Agriculture and Rural Development office report, 2012). Whereas the test organism, *Trypanosoma congolense* was isolated from Ghibe valley Gurage zone Abeshige district Borer Tade'le peasant association. Abeshige is one of the 15 districts in the Gurage zone. The district is located 185 km southwest of Addis Ababa.

3.5. Laboratory where the experiment was carried out

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

3.6. Study design

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

3.7. Methods

3.7.1. Pre-extraction preparation

Samples of the plant were authenticated coded by the national herbarium 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 preserved with specified voucher numbers (AY1 and AY3) respectively.

The plants have 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 Agriculture, Addis Ababa University, Bishoftu for future use (annex I).

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.

3.7.2. 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.

3.7.3. *In vivo* tests

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 14 days post treatment (OECD, 2001).

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 intraperitoneally with 0.2ml of the inoculum containing 10^7parasites/ml approximately (Feyera *et al*., 2014). 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 of either sex 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 the intraperitoneal route.

3.7.4. Measuring *in-vivo* activity parameters

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 (Herbert and Lumsden, 1976, Annex II).

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.

$$\%\ \text{Change in parasitemia DAY } 14-0 = \frac{\text{Mean parasitemia on DAY } 14 - \text{Mean Parasitemia on DAY } 0}{\text{Mean Parasitemia on DAY } 0} \times 100$$

$$\%\ \text{Change in body weight Day } 14-0 = \frac{\text{Mean body weight on Day } 14 - \text{Mean body weight Day } 0}{\text{Mean body weight on Day } 0} \times 100$$

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 14^{th} day post extract administration.

3.8. 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.

4. RESULTS

4.1. 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*.

4.2. Acute toxicity test

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

4.3. The effect of plant extracts on parasitemia level

4.3.1. 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 8^{th} day; a statistically significant reduction was, however, observed on the 10^{th} 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 day6 though significant reduction was observed on the 10^{th} day ($P< 0.05$) compared with the negative control and standard drug at a dose 3.5mg/kg. The highest dose (400mg/kg) reduced the level of parasitemia starting from the 6^{th} day and significantly reduced (73.2%) along with day14, ($P< 0.05$) compared with those of negative control and at both doses (3.5mg/kg and 28mg/kg) of standard drug (Table 1). The regression coefficient (-0.006) indicated that the antitrypanosomal activity of the plant was dose dependent in which a unit increase in the dose of the extract would resulted in decrease in parasite count by 0.006 unit.

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)

4.3.2. 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 that of the negative control (1ml 10%DMSO) and standard drug, diminazene aceturate (3.5mg/kg). At 400mg/kg, the extract showed significant reduction from days 6 to 14 compared with the negative control and diminazene aceturate (3.5mg/kg); while a significant effect was observed only on the 14th day compared with diminazene aceturate (28mg/kg). The regression coefficient (-0.002) indicated that the antitrypanosomal activity of the plant was dose dependent in which a unit increase in the dose of the extract would resulted in decrease in parasite count by 0.002 unit.

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 of plant extracts

As shown in figure 1, the comparative values of the highest doses of the extracts and control groups realized that methanol extract of *C. citratus* showed better activity (73.2% reduction in level of parasitemia) compared with *L. sativum* extract and control groups.

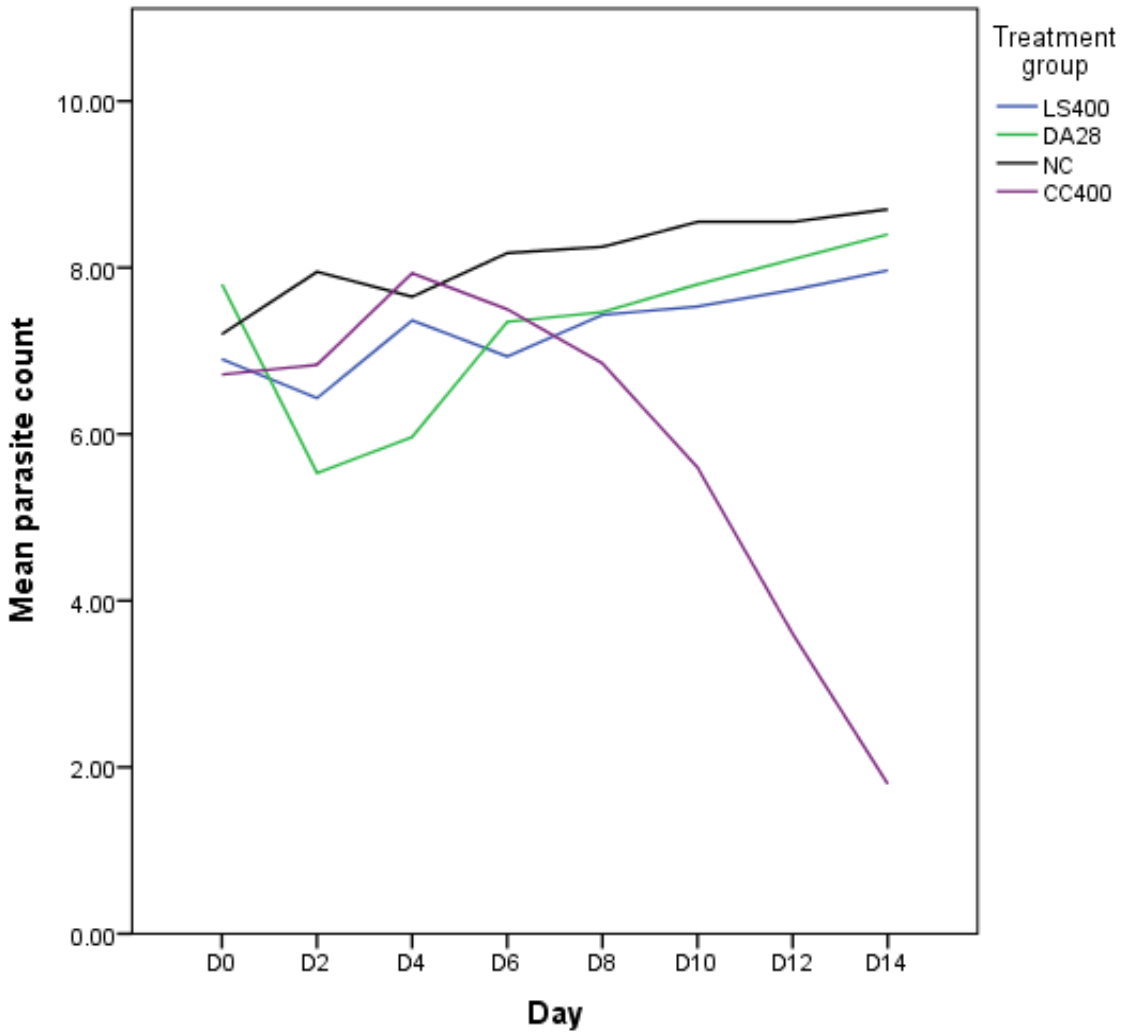


Figure 3: Comparison of the level of parasitemia treated with *L.sativum* and *C. citratus* with both control groups. Mean parasitemia count= log value per ml of blood; CC= *C. citratus*; LS= *L. sativum*; NC= negative control (1ml 10% DMSO); DA= deminazene aceturate. 400, 28 = are doses in mg/kg.

4.4. Effect of plant extracts on body weight

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

The present finding showed that the 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 12^{th} and 14^{th} 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;

4.4.2. Effect of 80% methanol extract of *Lepidium sativum* seeds on body weight of mice

As presented in table 4, methanol extracts 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

4.5. Effect of plant extracts on packed cell volume (PCV)

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

Figure 4 showed reduction in PCV values on day 7 though the rate of reduction was less than the negative control and DA (3.5mg/kg). From day 7 to 14, all dose levels of the extract (100, 200, 400mg/kg) significantly ($P< 0.05$) increased the PCV value (22.4%, 20.4% and 31% increment respectively) compared to the negative control taking 1ml 10%DMSO, and Diminazene aceturate at doses of 3.5mg/kg and 28mg/kg.

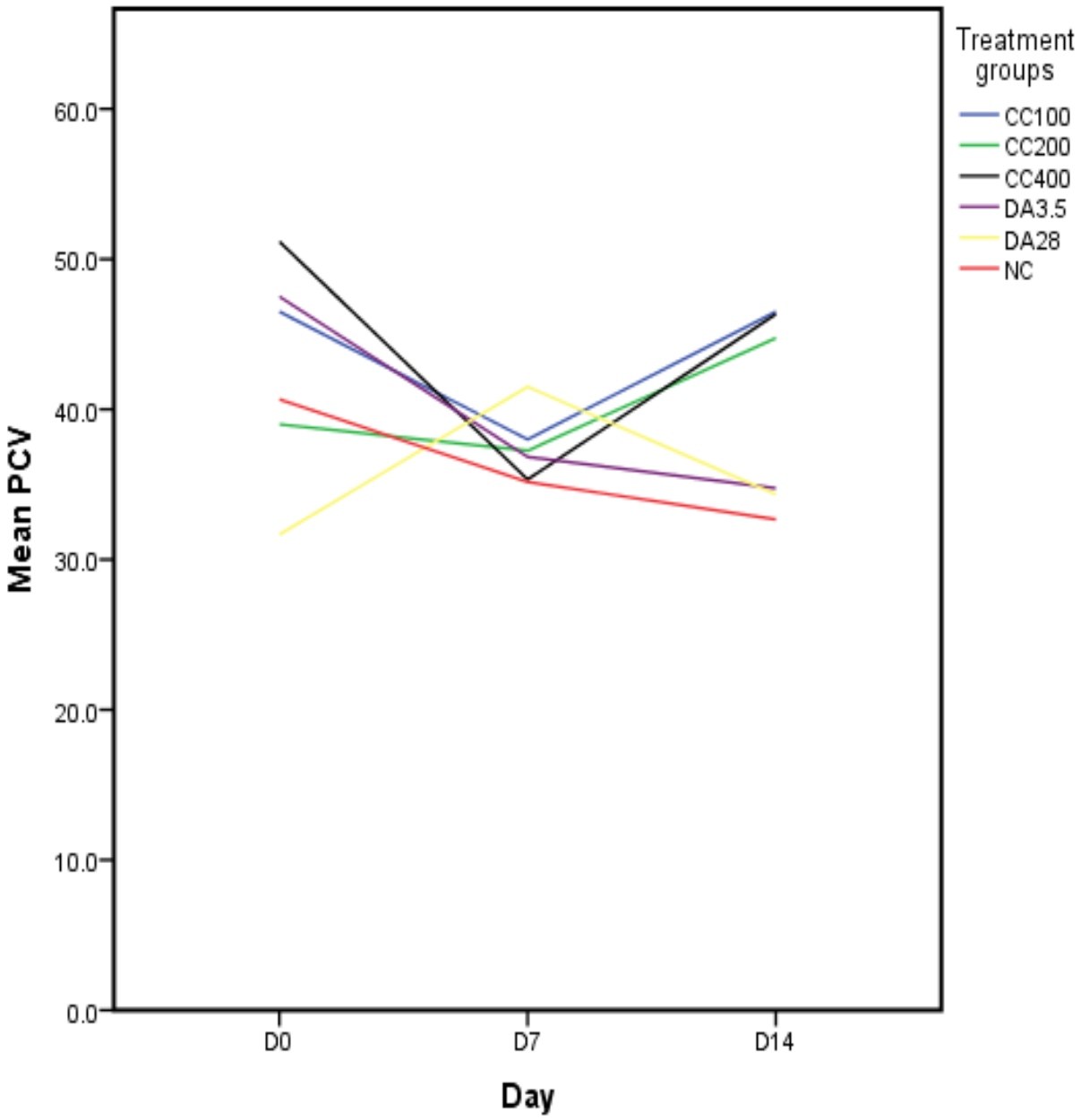


Figure 4: Effect of 80% methanol extract of *C. citratus* leaves on packed cell volume.

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.

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

As shown in figure 5, *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 14^{th} day all dose levels of the extract significantly reduced ($P<0.05$) rate of reduction in PCV value compared with all groups of controls.

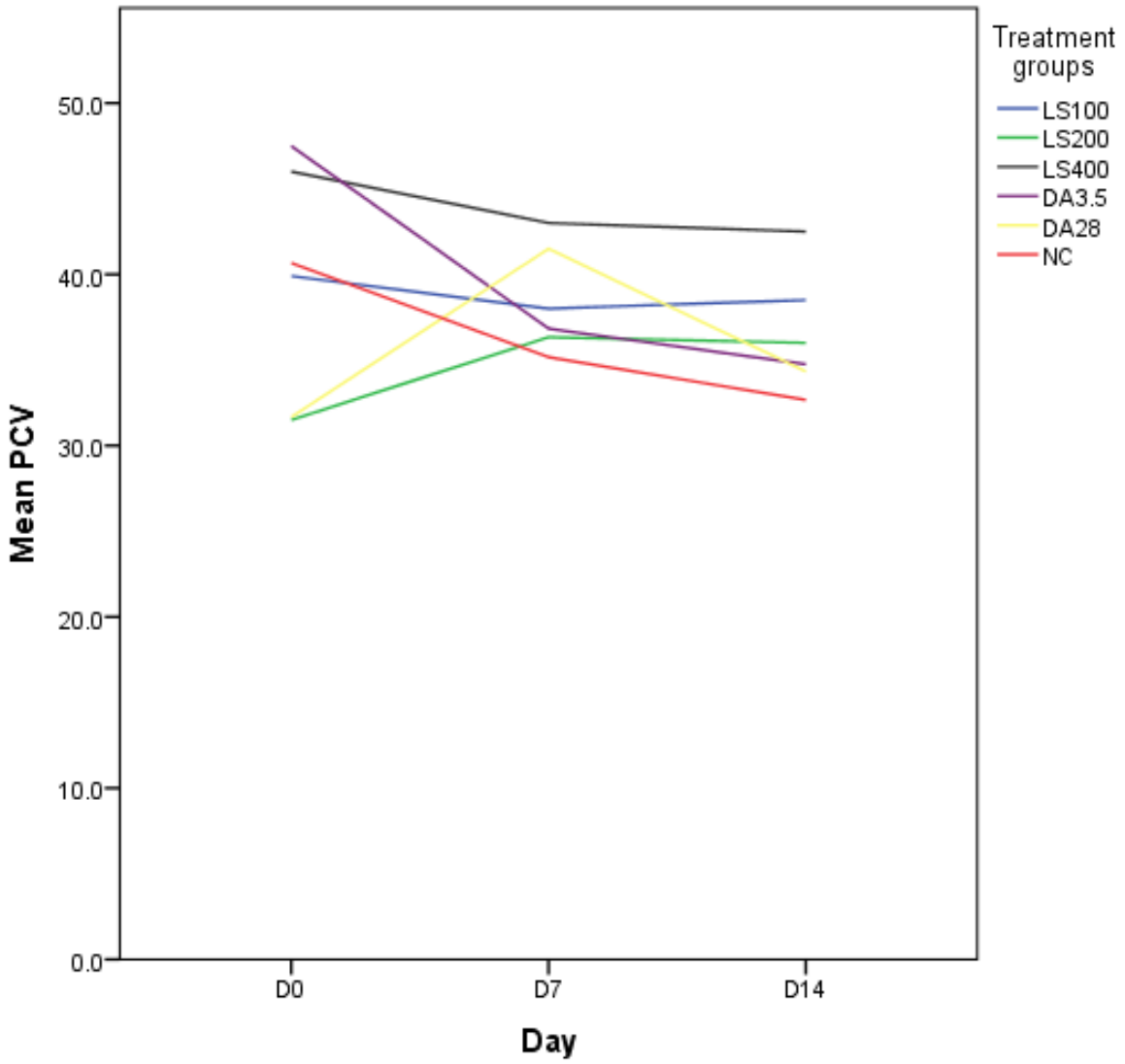


Figure 5: The effect of 80% methanol extract of *L. sativum* on packed cell volume.

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.

4.6. Effect of plant extracts on differential white blood cell counts

As shown in figures 6 and 7, 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 which 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).

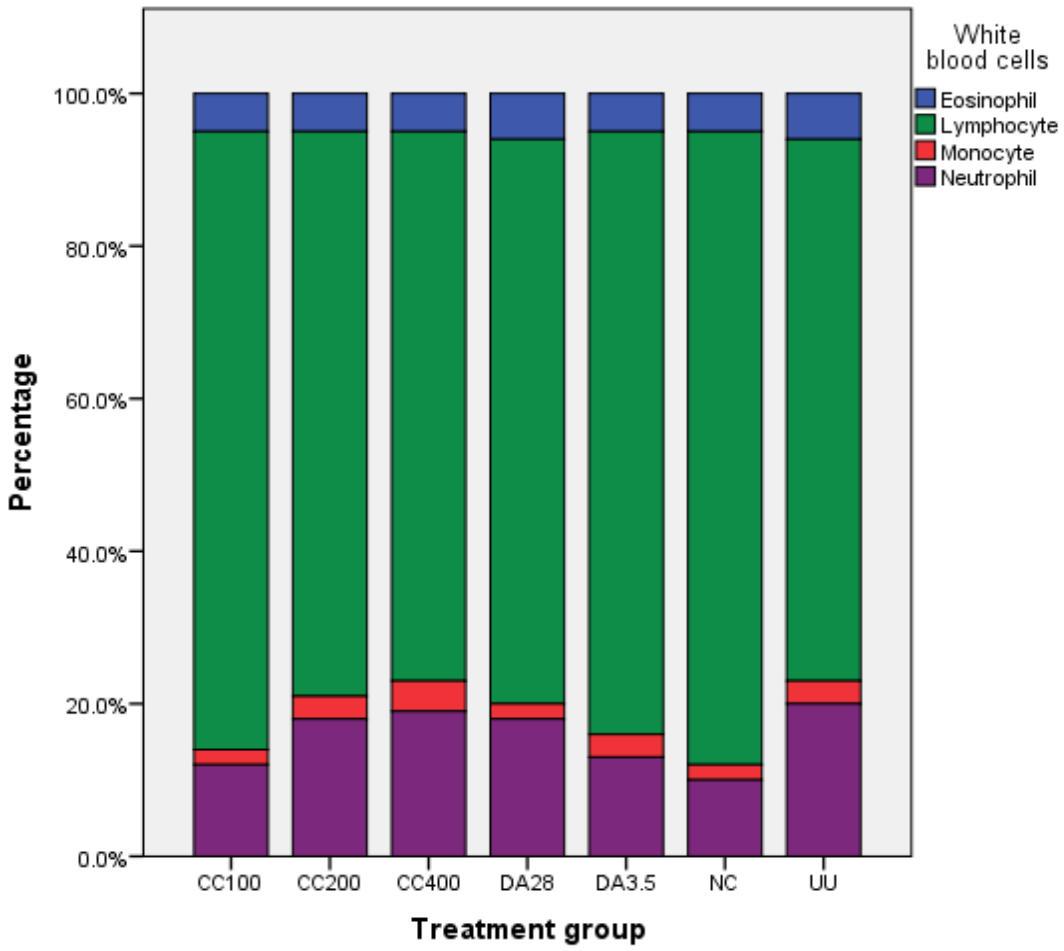


Figure 6: Effect of *C. citratus* leaves extract on differential white blood cell counts

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.

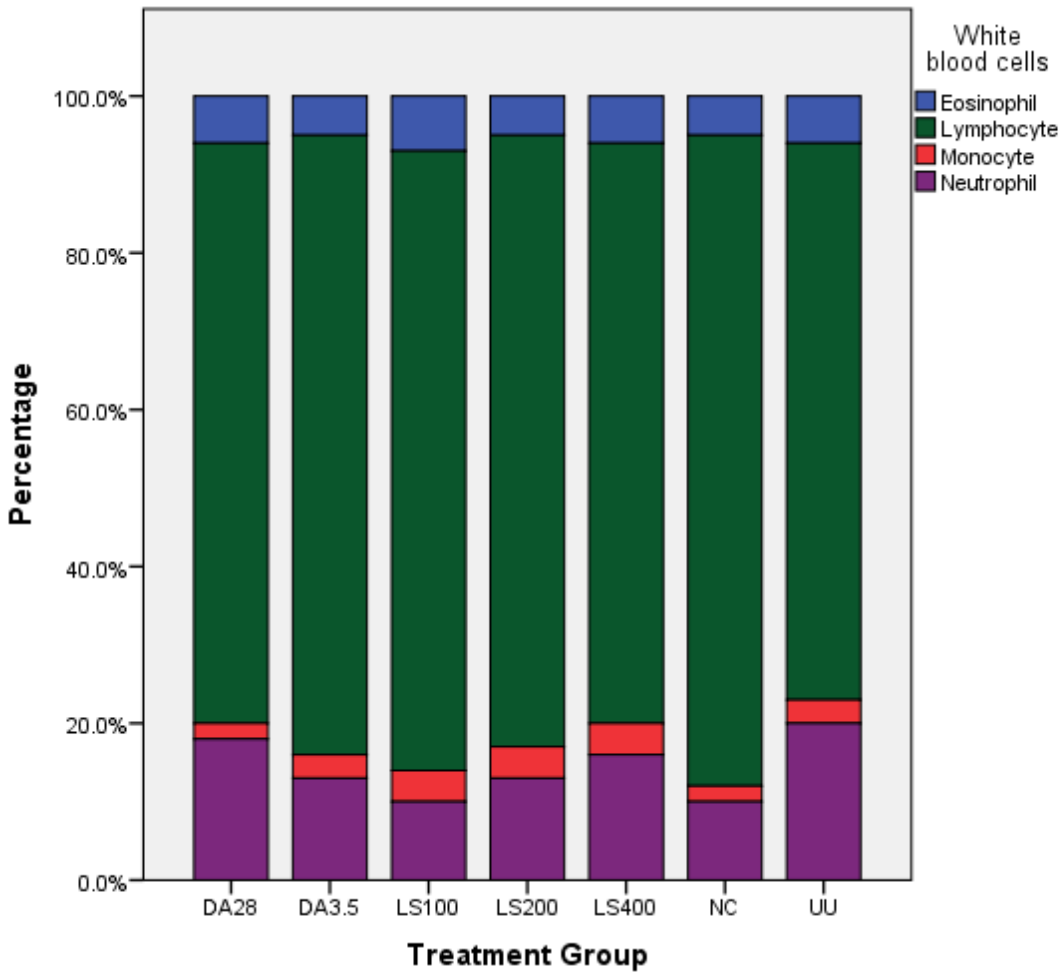


Figure 7: Effects of *L. sativum* seeds extract on differential white blood cell count

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.

5. 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 didn't 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-dependent manner (regression coefficient of -0.006). Administration of the extracts was continued for seven consecutive days but the reducing effect of parasitemia was noticed starting on the 8^{th} day and continued up to the 14^{th} 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 14^{th} 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 (Herbert and Lumsden, 1976). At this concentration, significant effects (73.2% reduction) ($P<0.05$) 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 (Cardoso, Soares, 2010). 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 (regression coefficient of -0.002). 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*., (2018), 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 (Al-Otaibi *et al*., 2018, Raish *et al*., 2016).

The standard drug, Diminazene aceturate was tested as a positive control at its recommended normal dose (3.5mg/kg) and at the highest dose (28mg/kg). Diminazene aceturate is one of the most frequently employed trypanocidal drugs used to treat animal trypanosomiasis. A dose of 3.5mg/kg 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 the 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 (Chaka and Abebe, 2003, Mulugeta *et al*., 2014, Tewelde *et. al*., 2004, Afewerk *et. al*., 2014, Kebede and Abebe, 2010, Shimelis *et. al*., 2017). 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 the 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 (Albadrani, 2012), 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 the drug 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 (Alli *et al*., 2011). 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 (Abubakar *et al*., 2005) 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.

6. CONCLUSION AND RECOMMENDATIONS

6.1. 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 (73.2%) than *L. sativum* seeds extract and even better than the standard drug suggesting its high potential to be developed as an effective antitrypanosomal drug.

6.2. Recommendations

Based on the present results the following recommendations are forwarded for future directions

- The acute toxicity test was done only at a single dose and resulted safe but further investigations are needed for both acute and chronic toxic profiles of the plants at a variable concentration and repeated administrations.
- Further researches aimed at identification, isolation active compounds of *C. citratus* and *L. sativum* and evaluating the activity of individual components against trypanosomiasis

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8. ANNEXES

Annex I: Garden of Toxic and Medicinal plants

Annex II: herbert and lumisden rapid matching table and chart

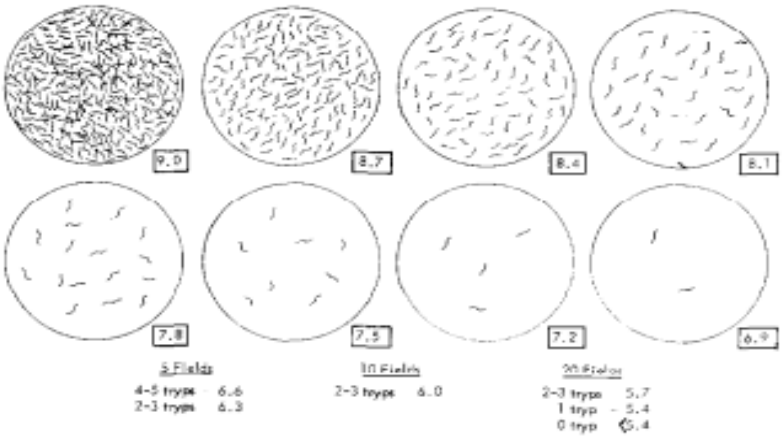

FIG. 1. Chart and table for estimating trypanosome parasitemias. The circles are used for matching when more than one organism per microscope field is present, the tables for lower concentrations. The values in the boxes in the charts and in the tables indicate the logarithm of the number of trypanosomes per milliliter as computed for *Trypanosoma brucei* infections in mouse blood inspected under ×400 magnification. For viewing at 25 cm, the circles are drawn with a diameter of 6.5 cm. They contain representations of trypanosomes (6 mm) that decrease in number by twofold steps.

Chart Showing Rationale of Computation of Values for the Circles and Tables in Fig. 1 for Wet Films of Blood from Mice Infected with *Trypanosoma brucei* Viewed under ×400 Magnification[a]

Organisms in 20 fields	Organisms in 10 fields	Organisms in 5 fields		Organisms per field	Equivalent log number of organisms per milliliter of blood
				>256	>9.0
			M	256	9.0
			A	128	8.7
			T	64	8.4
			C	32	8.1 Reference point
			H	16	7.8
			I	8	7.5
			N	4	7.2
			G	2	6.9
			C		
		4–5	O	1	6.6
		2–3	U	0.5	6.3
	2–3		N	0.25	6.0
2–3			T	0.125	5.7
1			I	0.0625	5.4
0			N	<0.0625	<5.4
			G		