IN VITRO AND *IN VIVO* ANTITRYPANOSOMAL EFFECTS OF HYDROMETHANOLIC EXTRACTS OF *SOLANUM ANGUIVI* FRUITS AND *ECHINOPS KEBERICHO* ROOTS

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OCTOBER, 2016

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A thesis submitted to Department of Pharmacology, School of Medicine, Addis Ababa University in partial fulfillment of the requirement for the degree of Master of Science in Pharmacology

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OCTOBER, 2016

This is to declare that the thesis prepared by Debela Abdeta entitled*: In vitro* and *in vivo* antitrypanosomal effects of *E. kebericho* roots and *S. anguivi* fruits against *Trypanosoma congolense* and submitted in partial fulfillment of the requirement for the degree of Master of Science in pharmacology complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

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ACKNOWLEDGEMENTS

First and foremost, I return the glory and earnestly give thanks to Almighty God for his protection and numerous graces.

To my amiable and readily accessible supervisor, Dr. Solomon Mequanente, I express my heartfelt gratitude and appreciation for his unwavering encouragement, keen interest and candid advice in the course of this study.

I would also like to express my deepest gratitude to Dr. Mirutse Giday for his critical comments, suggestions, identification of the plant material and allowing me to work and use the available resources in the lab as well as financial support.

I am delighted to extend my heartfelt thanks to Dr. Nigatu Kebede for his genuine and untiring guidance, advice and critical comments on the manuscript and clearing my difficulties during the course of my research life. The contribution of Dr. Getachew Terefe was also huge especially in providing stock of the parasite.

My special thanks go to W/ro Mahlet Aragie, Yirgalem G/Hiwot, Tsedy Yimenshewa, Ato Jiregna Gemechu, Nega Nugussie, Hailu Getu, Aboma Zewde, Samuel Bekele, Samuel Tolesa, Yohannes Negash and Adane Worku for their unreserved technical and material support as well as their guidance and assistance during my lab work. Their contribution is huge and I never forget them.

I would like to thank School of Graduate Studies, College of Health Sciences and AAU for providing learning environment and finance. I wish to acknowledge the Department of Medical pharmacology and its entire staff for their direction contributed to this work. I am also grateful to Wollega University for sponsoring my Graduate program.

I appreciate my dear friend Ms. Aster Mamo for her love and patience.

Last in my list but never the least, I would like to pass my deepest gratitude to all my parents for their constant encouragements which induced and helped me a lot in all way to complete the program successfully.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAT	African animal trypanosomosis
CNS	Central Nervous System
DA	diminazene aceturate
G	gram
HAT	Human African Trypanosomiasis
IVM	Integrated Vector Management
LD_{50}	Lethal $Dose_{50}$
Mg	milligram
MIC	Minimum Inhibition Concentration
MPS	mononuclear phagocytic system
OCED	Organisation for Economic Cooperation and Development
PBS	phosphate buffered saline
PBSG	phosphate buffered saline glucose
PCV	Packed Cell Volume
RBC	Red Blood Cell
SEM	Standard Error of Mean
SPSS	Statistical Package for Social Science
V/V	volume by volume
WHO	World Health Organization

ABSTRACT

Introduction: Trypanosomiasis is one of the world's most serious infectious diseases caused by *Trypanosoma* parasites. An increased drug resistance to conventional anti-trypanosomal drugs, increasing resistance of mosquito vectors to insecticides, challenge of having effective vaccines and adverse effects of the existing anti- trypanosomal drugs justifies the urgent need for more effective, tolerable and affordable drugs.

Objective: The present study aimed to determine the *in vitro* and *in vivo* antitrypanosomal effect of hydromethanolic extract of *E. kebericho* roots and *S. anguivi* fruits against field isolate of *T. congolense.*

Methods: The 80% methanol extracts of *E. kebericho* roots and *S. anguivi* fruits were prepared by cold maceration technique. *In vitro*, blood incubation infectivity test, curative and prophylaxis tests were done to check the effect of the plant extracts against *T. congolense* in Swiss albino mice. Extracts were administered at doses of 100, 200 and 400 mg/kg for curative and prophylaxis test while 1mg/ml, 2mg/ml and 4mg/ml concentration of the extract were used for *in vitro* and blood incubation infectivity test. Acute toxicity of the extracts at 2000mg/kg was performed according to OECD guide lines. Data obtained from the experiment was analyzed using one way ANOVA followed by Tukey test.

Results: The present study indicated that the extracts did not exhibit any signs of acute toxicity up to the dose of 2000mg/kg. The hydromethanolic extracts of *E. kebericho* roots and *S. anguivi* fruits affected motility at 0.5, 1, 2 and 4mg/ml in *in vitro* tests, and the entire tested group did not develop infection in mice inoculated with infected blood incubated with concentrations of the above extracts. In the prophylactic studies, groups provided with the hydromethanolic extracts before infection got prolonged incubation period with little chemoprophylactic effect at the doses of 100, 200 and 400 mg/kg. In curative test, the extracts reduced parasitemia, prevented drop in packed cell volume and body weight significantly ($p<0.05$), as compared to control. In *in vivo* models, the extracts did not prevent rectal temperature fluctuation. Phytochemical analysis showed the presence of flavonoids, triterpines, steroids, saponins, glycosides, tannins and alkaloids.

Conclusion: The extracts showed *in vitro* effect and a promising curative and prophylactic activities. Further effort is required to isolate and purify specific compounds responsible for the antitrypanosomal activity of the studied plants.

Key words: *Echinops kebericho, Solanum anguivi*, trypanosomiasis, *Trypanosoma congolense*

1. INTRODUCTION

1.1. Overview of Trypanosomiasis

Trypanosomiasis is a parasitic disease caused by haemoprotzoan belonging to the genus, *Typanosoma* of the family *Trypanosomatidae*, that multiply in the blood stream, lymphatic vessels and tissues including the cardiac muscles and the central nervous system. African animal trypanosomiasis (AAT) is most common disease of domestic livestock covering 37 sub-Saharan countries located between latitude 14ºN and 29ºS and about 9 million km^2, an area which corresponds approximately to one-third of the Africa's total land area (Mattioli *et al*., 2004). This highly fatal protozoan disease is virulent, inoculable but not contagious (except dourine, a venereal trypanosomiasis of equines). African trypanosomiasis is responsible for 3 million livestock and 55,000 people deaths annually in agriculture and mixed farming environments thus making it an important priority for the agricultural sector and biomedical and public agencies (Mulumba, 2003, Aliyu *et al*., 2010).

Human African Trypanosomiasis (HAT), commonly known as sleeping sickness, is caused by species specific type of *Trypanosoma* and is an endemic public health threat to Sub-Saharan Africa with an estimated 55 million people at risk. The disease is important yet neglected disease which is a major cause of rural underdevelopment in Sub-Saharan Africa as it mainly affects poor and remote rural regions (Fevre *et al*, 2008). It is estimated that approximately 20,000 people across Africa are infected with HAT with approximately 30 African countries affected by the disease (reviewed Sutherland *et al*, 2015). WHO describes the disease as a neurological breakdown that is caused by the trypanosome parasite in the brain, which eventually leads to a coma or death if a patient is not treated (WHO, 2013).

Current trypanosomiasis control relies on trypanocidal drugs, use of trypanotolerant cattle breeds and control of the vector, namely the tsetse fly. None of these methods have the full potential to work in the long-term control of the disease. Most heavily relied on are the trypanocidal drugs and this has lead to an increasing problem of resistance in the target organisms (Prowse, 2005).

1.2. Epidemology of the disease

African trypanosomiasis is an infectious disease of humans and animals of similar aetiology and epidemiology. The causative agents of the disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies (*Glossina* species). The epidemiology of trypanosomiasis depends on three factors, distribution of the vectors, virulence of the parasite and response of the host (Steverding, 2008).

The vectors of the group of *Glossina* flies, the savannah and riverine varieties are the most important since they inhabit areas suitable for grazing and watering. Although the infection rate of *Glossina* with trypanosomes is usually low, ranging from 1 to 20% of the flies, each is infected for life. Biting flies may act as mechanical vectors but their significance in Africa is still undefined (Leak, 1998). Tsetse fly density is the most variable factor in the transmission of trypanosomiasis. Climate affects tsetse abundance via one or more of four demographically important rates namely birth, mortality, immigration and emigration (Rogers, 1991).

Tsetse fly species differ in their susceptibility to trypanosomes and their subsequent ability, if infected, to transmit trypanosomes. For example, *G. fuscipes* appears to be a better vector of *T.vivax* to cattle than *G.pallidipes*, and *G.pallidipes* is a better transmitter of *T. congolense* than *G.swynnertoni* (Stephens, 1986). Tsetse flies prefer to feed on particular hosts-the bushbuck for example is much preferred whilst the waterbuck is not. Cattle inhabit a medium position. There are also differences within one host species in that trypanosome infected animals attract tsetse more than uninfected hosts (Baylis and Nambiro, 1993). The incubation period for *T. congolense* varies from 4 to 24 days; for *T. vivax*, from 4 to 40 days; and for *T. b. brucei*, from 5 to 10 days.

1.3. Taxonomy and Etiology

Phylum Protozoa
Subphylum Sarcomastigophora
Class Zoomastigophorea
Order Kinetoplastida
Suborder Trypanostomatina
Family Trypanosomatidae
Genus Trypanosoma
Species: *T. congolense*

The pathogenic trypanosomes have been classified either as Stercoraria (posterior station trypanosomes) Typanosoma theleria which is mildly pathogenic to domestic and 20 wild ruminants and Salivaria (anterior station trypanosomes which is pathogenic to both domestic and wild animals Species of the genus, Typanosoma are found in a wide variety of vertebrates. The majority is not pathogenic but some species are of considerable economic importance causing disease in man and animals. These trypanosomes mainly belong to three subgenera namely Trypanozoon, Dutonella and *Nannomonas*. The subgenus Trypanozoon contains T. brucei, two species of which *T. b. gambiense* and *T. b. rhodesiense* are responsible for sleeping sickness in man in Africa and one subspecies *T. b. brucei* for infection in domesticated animals. *T. evansi* which is found in many parts of the world in a wide variety of animals. In the subgenera Nanomas *T. congolense* and *T. simiae* are the important members with *T. equiperdiun* and *T. suis* are also members. The most important African trypanosomes causing diseases in cattle are *T. congolense*, *T. vivax* and *T. brucei* while *T. b. gambiense* and *T. b. rhodesiense* cause infection in humans (Reviewed in Ugochukwu, 2008).

T. congolense resides in the subgenus *Nannomonas*, a group of small trypanosomes with medium-sized marginal kinetoplasts, no free flagella, and poorly developed undulating membranes. In east Africa *T. congolense* is considered to be the single most important cause of AAT. This trypanosome is also a major cause of the disease in cattle in West Africa. Sheep, goats, horses, and pigs may also be seriously affected. In domestic dogs, chronic infection often results in a carrier state (Prowse, 2005).

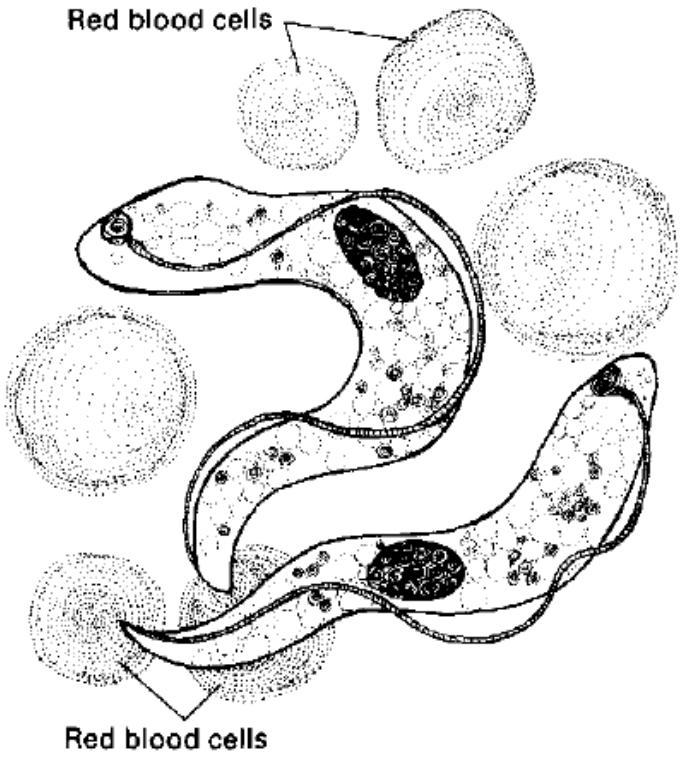

Figure 1: Morphology of *T.congolense*: **Source**: Urquhart G. M. Armour J, Duncan JL, Dunn AM, Jennings FW (2000): Veterinary Parasitology, 2nd edition, pp: 213, black well publishing, Scotland

T. vivax is a member of the subgenus *Duttonella*, a group of trypanosomes with large terminal kinetoplasts, distinct free flagella, and inconspicuous undulating membranes. *T. vivax* is a large (18-26 μm long) monomorphic organism that is very active in wet-mount blood smears. Cattle, sheep, and goats are primarily affected. Although this organism is considered to be less pathogenic for cattle than *T. congolense*, it is nevertheless the most important cause of AAT in West African cattle. This trypanosome readily persists in areas free of tsetse flies (for example, in Central and South America and in the Caribbean), where it is transmitted mechanically by biting flies or contaminated needles, syringes, and surgical instruments (Prowse, 2005).

T. b.brucei resides in the subgenus *Trypanozoon. T. b. brucei* is an extremely polymorphic typanosome occurring as short, stumpy organisms without flagella or long slender organisms with distinct flagella or intermediate forms that are usually flagellated. Horses, dogs, cats, camels and pigs are very susceptible to *T. b. brucei* infection. Infection of cattle, sheep, goats and sometimes pigs results in mild or chronic infection (Prowse, 2005).

T. evansi is a species belonging to the subgenus Trypanozoon and is the causative agent of camel trypanosomiasis. It is hypothesized that *T. evansi* originated from *T. brucei* by adaptation to a non cyclical mode of transmission and loss of ability to undergo growth and differentiation in the fly vector. Camels that came into contact with tsetse flies acquired infections, and when such camels moved to non-tsetse areas, transmission was spread by other haematophagous flies (reviewed in Enwezor and Sackey, 2005).

T. b.rhodesiense (East Africa) and *T. b. gambiense* (West Africa) are transmitted to humans by tsetse flies of the *Glossina morsitans* group (*T. b. rhodesiense*) and of the *G. palpalis* group (*T. b. gambiense*) which are found only in Africa. West African sleeping sickness has almost exclusively a human reservoir, while East African trypanosomiasis is a zoonosis involving antelopes, cattle and humans. Infections by both *T. b. gambiense* and *T. b. rhodesiense* are generally under-reported in humans due to acuteness and lack of specific symptoms at the onset and its rural distribution. *T. b. rhodesiense* is focally endemic in many eastern and southern African countries. It tends to occur in form of epidemic outbursts (reviewed in Gautret *et al*, 2009). *T. b. gambiense* is responsible for 97% of all HAT infection (Nimmo, 2010).

1.4. Transmission

In Africa, the primary vector for *T. congolense*, *T. vivax*, and *T. b. brucei* is the tsetse fly. The trypanosomes replicate in the tsetse fly and are transmitted through tsetse fly saliva when the fly feeds on an animal. The three main species of tsetse flies for transmission of trypanosomes are *G. morsitans*, *G. palpalis* and *G. fusca*, which favors the high, dense forest areas. Trypanosomiasis is also mechanically transmitted by tsetse and other biting flies through the transfer of blood from one animal to another. The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota, Liperosia, Stomoxys*, and *Chrysops* flies have also been implicated. In Africa, both *T. vivax* and *T. b. brucei* have spread beyond the "tsetse fly belts", where transmission is principally by tabanid and hippoboscid flies. The vector for *T. vivax* in the Western Hemisphere remains unknown, but several species of hematophagous (especially tabanid and hippoboscid) flies are believed to serve as mechanical vectors (Roder *et al*. 1984).

1.5. Pathogenesis

Initial replication of trypanosomes is at the site of inoculation in the skin; this causes a swelling and a sore (chancre). The fly bite deposits metacyclic trypanosomes in dermal connective tissue of skin and here a local inflammatory reaction, the 'chancre' develops. All three cyclically transmitted species eventually undergo a chancre (Vickerman & Barry, 1982) although the local skin reaction is less severe with *T. vivax* infections (ILRAD, 1986). From the chancre, the trypanosomes enter the draining lymphatics and then the bloodstream. *T. congolense* multiplies in the tissue of the chancre as a morphologically distinct phase before it invades the bloodstream (Vickerman & Barry, 1982). The chancre phase completed, *T. congolence* and *T. vivax* remain largely intravascular parasites (Losos & Ikede, 1972) the former localizing in small blood vessels where it attaches to the endothelium. Both species are now known to occur also in the lymphatics. *T. brucei* may secondarily escape from the bloodstream into the soft connective tissues and multiply in the tissue fluid (Vickerman & Barry, 1982).

Concurrently there is a variable degree of suppression of immune responses to other antigens such as microbial pathogens or vaccines. The response of antibodies developed to the glycoprotein coat of the trypanosomes kills the parasites and results in the development of immunocomplexes. Antibodies however do not clear the infection since trypanosomes have genes that can code for a number of different surface-coat glycoproteins and therefore changes its surface antigenic makeup to evade the antibodies. Thus there is a persistent infection that results in a continuing cycle of trypanosome replication, antibody production, immunocomplex development and changing surface-coat glycoproteins. Immunologic lesions are significant in trypanosomiasis and it has been suggested that many of the lesions (eg anemia and glomerulonephritis) in this disease may be the result of deposition of immune complexes that interfere with, or prevent, normal organ function. Profound immunosuppression occurs following infection and this lowers the host's resistance to other infections and thus results in secondary disease (Prowse, 2005). Ultimately, in infections of long duration, the lymphoid organs and spleen become shrunken due to exhaustion of their cellular elements.

Anaemia plays the major role in the pathogenesis of bovine African trypanosomiasis and initially it is proportional to the degree of parasitaemia. The development of anaemia is a well recognized sign of trypanosome infection in cattle (Losos & Ikede, 1972). In most early cases, there is an acute onset of anaemia corresponding clearly with the detection of parasites in the bloodstream (ILRAD, 1987). The initial fail in packed cell volume (PCV) value is associated with the first wave of parasitaemia in the blood. During this period the anaemia is extravascular and is possibly the result of increased red cell destruction by erythrophagocytosis in the spleen, lungs, haemal nodes and bone marrow as a result of direct traumatic effect on red cells thereby increasing red cell fragility (Mamo & Holmes, 1975; ILRAD, 1984).

In cattle subjected to a single needle or fly challenge, the PCV progressively decreases up to 50% over the first 4-6 weeks (Morrison *et al*., 1982). During this period the anaemia is haemolytic due to production of toxins like haemolysins; lytic factor associated with protein of low molecular weight which has anaphylatoxin activity and the release by autolysing trypanosomes of endogenous phospholipases (Holmes and Jennings, 1976; Morrison *et al*., 1982).

There is evidence that immune sensitizations of red cells occur in animals infected with trypanosomes. The trypanosomal antigens can attach to red cell membranes and thereby make them susceptible to phagocytosis. The contribution of splenomegally to red cell membrane was suggested that pooling of red cells which may occur in the enlarged extra-sinusoidal compartments having detrimental effect leading to increased osmotic fragility and secondly, enhanced phagocytic activity of reticulo-endothelial system which may lead to clearance of both sensitized and normal red cells (Holmes, 1987). Haemodilution also contributes to the anaemia of African trypanosomiasis by a disproportionate increase in the plasma volume as demonstrated in cattle (Mamo & Holmes, 1975) and laboratory animals (Holmes & Jennings, 1976). This phenomenon was commonly observed to occur during acute phases of the disease. Death may occur within 4-12 weeks after infection (Morrison *et al*., 1982).

Cattle that survive the acute process, progress into a chronic anaemia. This may still result in death, or in either spontaneous recovery or survival with persisting low grade anaemia. This chronic phase is characterized by low and transient parasitaemia or complete absence of detectable parasites in the blood. Death results from congestive heart failure (Morrison *et al*., 1982). Bleeding disorders are commonly associated with trypanosome infections and may become a major aspect of the pathogenesis of trypanosomiasis in cattle with a haemorrhagic syndrome associated with *T. vivax* infection (Holmes, 1987; Wellde *et al*., 1983). In this type of infection, the trypanosomes rapidly reach high numbers in the bloodstream and there is a severe drop in the number of red blood cells and platelets, a dramatic enlargement of the spleen and extensive haemorrhaging, both of the external mucous surfaces and the internal viscera and especially of the gut. Cattle may die of this disease in two weeks (ILRAD, 1984; Wellde *et al*., 1983; Olubayo *et al*., 1985).

The mechanism of anaemia in trypanosomiasis was caused mainly by extra vascular haemolysis in the expanded active mononuclear phagocytic system of the host. This was followed by a drastic reduction of all red blood cell indices during successive waves of parasitaemia. The pattern of anaemia varied, depending on whether the specie of trypanosome was "humoral" or "haemic". Although the mechanism of anaemia is complex and multifactorial, it primarily compromised the cellular integrity of erythrocytes leading to either haemolytic anaemia or enhanced erythrophagocytosis. It is hemolytic in that the red blood cells are removed from the circulation by the expanding mononuclear phagocytic system (MPS). Cell degeneration and inflammatory infiltrates occur in many organs such as skeletal muscle and the CNS, but perhaps most significantly in the myocardium where there is separation and degeneration of the muscle fibers (Urquhart *et al*, 1996).

Injuries sustained by red blood cell (RBC) membranes caused by the flagella and microtubule reinforced body of the organisms greatly enhanced erythrophagocyosis of damaged RBC by the MPS. Similarly, erythrocytes, reticulocytes and platelets that adhered to trypanosomes via sialic acid receptors, caused injuries to erythrocyte membrane at the point of contact (Mbaya, 2009e).

1.6. Clinical Signs

Because simultaneous infection with more than one trypanosome species are very common and simultaneous infection with trypanosomes and other hemoparasites (*Babesia*, *Theileria*, *Anaplasma* and *Ehrlichia*) frequently occurs it is difficult to conclude which clinical signs are attributable to a given parasite. Anaemia is for example seen in a whole series of diseases caused by blood parasites (in particular *Babesia* and *Anaplasma*) as well as in certain gastrointestinal helminthesand it is therefore not typical of trypanosomes by itself (Nyenko *et al*, 1990).

The cardinal clinical sign observed in AAT anaemia. Also invariably present are intermittent fever, edema and loss of condition, mucous pallor, miscarriage, 'petering out', pica, splenomegaly, cachexia, and death (Courtin *et al*., 2008). Abortion is seen and infertility of males and females may be a sequel. The severity of the clinical response is dependent on the species and the breed of the affected cattle and the dose and virulence of the infecting trypanosome. Stress such as poor nutrition, or concurrent disease, plays a prominent role in the disease process (FAO, 1998).

Sleeping sickness is a painful and protracted disease which is almost invariably fatal without adequate treatment; treatment of infected individuals is crucial for reducing the trypanosome reservoir in humans and consequently for controlling the disease. The mostly rural distribution of the disease, civic unrest occurring in many regions affected, the financial and social constraints experienced by endemic countries, and the difficulties in diagnosing and effectively treating HAT, all contribute to make it one of the hardest diseases to control in sub-Saharan Africa (reviewed in Lutje *et al*, 2010).

1.7. Chemotherapy and Chemoprophylaxis

The common trypanocides in use for treatment are Homidium bromide (Novidium), Isometamedium chloride (Samorin) anddiaminazene diaceturate (Berenil). Treatment of second-stage HAT relies on melarsoprol, eflornithine, or nifurtimox; currently the only anti-trypanosomal compounds that can reach therapeutic levels in the central nervous system. Apart from the side effects of these drugs, the expensiveness and absence of new trypanocidal drugs

leading to over dependence on the old drugs; there is growing concern that their future effectiveness may be curtailed by wide spread drug resistance (Onyiah, 1997; Geerts and Holmes, 1998). Although the drugs are quite effective, the circumstances in which they are used, and the fact that many of them closely related chemically, have led to the development of drug resistance in many countries (Radostitis *et al.*, 2000).

These drugs have been in use for many years and there *in vivo* efficacy against HAT has been extrapolated after animal studies or, in the case of nifurtimox, after being used to treat American trypanosomiasis (Chagas disease). Also, the use of any of these drugs is complicated by multiple factors including the increasing incidence of therapeutic failures, painful administration, severe adverse reactions, availability, and high production costs (reviewed in Lutje *et al*, 2010). Control strategies in trypanosomiasis concentrate on vector control, parasite control with chemotherapy and chemoprophylaxis and use of the inherent trypanotolerant trait in some breeds of animals (PATTEC, 2001).

Drug resistance is very common in trypanosome even though resistance is possible to all drugs. The best for avoiding drug resistance is the alternate use of two drugs, which are not chemically related. Example Berenil and Trypandium may be used alternatively. The development of drug resistance occurs with time under the following conditions; under dosage due to either dilution or reduced volume given or underestimation of the weight of the animal, too long intervals between prophylactic treatments beyond the manufacturers order, heavy challenge of tsetse fly, illegal and unprofessional handling of drugs by smugglers, traders pharmacies and formation of absess and cysts at the site of injection (Alemu *et al*., 2007).

The development of drug resistance can be recognized whenever there is high incidence of infections in recently treated animals or a gradual change in the prevalence of circulating trypanosome species. Collection of trypanosome strains in laboratory animals and screening them for the laboratory drug sensitivity is very vital (Radostitis *et al.*, 2000). Either removing animals from affected area, or changing the drug or increasing the drug level can minimize drug resistance. Drug resistance could be prevented by correct assessment of the tsetse risk, avoiding under dosage, maintaining correct and regular treatment schedules, use of drugs in correct

sequence, conducting drug sensitivity tests before initiating scheme and establishing national drug use policy (NTTICC, 2006).

1.8. Trypanosomiasis Control measures

1.8.1. Control Measures Against the Vector

Vector control is the most reliable means of disease control since it removes the threat of trypanosomiasis on a permanent basis. Many vector control methods including woody vegetation clearance to remove tsetse shelter and large scale application of insecticides by air (non-persistent and persistent formulations) and ground spraying (only persistent insecticides) and large wild life elimination to deny tsetse its source of food i.e. blood(SIT, 1996).

Removal of vegetation in savannah areas causes larviposition to occur in shaded places. This method was used quite extensively with success in the past but is labor intensive and requires that there be reslashing of vegetation on an annual basis. However, removal of vegetation for firewood and urbanization has sometimes achieved the same effect. Also, killing of wild animals with the objective to remove reservoirs of infection in the wild animal populations was used extensively in the past (Robertson, 2004). However, these methods are now unpopular on environmental and on biodiversity grounds (SIT, 1996).

Instead of the destruction or contamination of the environment of the tsetse, they depend on the attraction of the fly from its surroundings to some introduced object, which may be insecticidal, but which can if necessary be removed later; this may be an artifact (e.g. trap), or a live host, treated with insecticide. Although not new, several developments have come together over the last 10-15 years to render bait techniques more practicable, over a wider range of tsetse habitats, than ever before. Bait systems are inherently of low environmental impact, and are relatively low-technology. It is also claimed that they are logistically less demanding than other approaches, and are capable of being adopted by local communities on self-help basis and seems increasingly likely that these techniques will form the basis for tsetse fly control in the short to medium term (Green, 1994).

Although attempts were made to control tsetse using targets impregnated with insecticide many years ago, successful application of this technique followed the production of the second-generation synthetic Pyrethroid insecticides (deltamethrin, cypermethrin, cyfluthrin) and the development of potent odour attractants(Leak, 1999). Traps and targets are a more acceptable means of controlling tsetse than either ground or aerial spraying of insecticides in terms of the direct ecological and environmental impact they might have (Leak, 1999). These approaches offer the prospect of cheaper alternatives, although they are not a simple panacea as competent supervision and management are still essential (Jordan, 1986). Most development of this method of tsetse control has concentrated on improved and cheaper designs of the target and odour attractants in order to attract as many tsetse as possible and to increase the number of tsetse actually landing on a target (Radostitis *et al.,* 2000).

Another approach is to use a 'pour-on' (or spot-on) formulation, in which the insecticide is applied to the back of the animal and spreads over the body surface (Green, 1994; Leak *et al*., 1995). Application of Deltamethrin spot-on appeared to effectively control *G. m. submorsitans* and *G. tachinoides* in the upper Didessa Valley where reinvasion pressure was not high (Lemecha, 1994).

One of the latest methods of control is the Sterile Insect Technique (SIT) involving continuous release of sterile insects among the indigenous insect population at rates sufficient to result in a reduction in biotic potential of the target population. The mating of released sterile male insects with indigenous fertile female insects causes infertility in the target population (SIT, 1996).

1.8.2. Control measures against the host

Vector eradication and prophylactic use of drugs have not succeeded in eradicating Trypanosomiasis while hopes of vaccination appear to be hampered by the antigenic variability of trypanosome. Tolerance to trypanosomiasis is determined by at least three main characteristics; namely the ability to control parasitamia, the ability to develop of an effective immune response and the ability to resist the development of anaemia (Murray, 1987 and Radostitis *et al.,* 2000).

1.8.3. Use of Innate Resistance (Trypanotolerance) of breeds

Innate resistance, or trypanotolerance, has been recognized since 1906 when the ability of indigenous taurine cattle in West Africa to survive and be productive under trypanosomiasis risk was observed. Both acquired and innate resistance to African trypanosomiasis can occur in cattle. The two most important trypanotolerant breeds are the *Bos taurus* subtypes, N'Dama and Baoule, whilst a degree of trypanotolerance has also been shown to occur in some *B. indicus* zebu breeds- for example, the Orma Boran (Leak,1999). The use of trypanotolerant cattle had been limited in availability (account only for 17% of the total cattle population of the continent), was a potential alternative strategy for coping with the problem (Erkelens *et al*., 2000).

1.9. Significance of the disease

The incidence and severity of the disease in different regions are dependent upon local conditions. In some areas virtually no economic livestock development is achievable due to the disease. Tryanosomiasis has direct impact on livestock productivity; increase calf mortality by 20%, decreases both lamping and kidding rates, reduce meat and milk production by 20%, affects livestock management especially the number of livestock kept by farmers, the breed and species composition of the livestock herd, the way the livestock are grazed, cost of trypanocidal drugs and cost of insecticides (reviewed in Prowse**,** 2005; Swallow, 2000). Livestock producers and consumers lose is estimated $1.34 billion annually to trypanosomiasis in Africa. HAT remains a major public health problem and occurs in 36 countries of sub-Saharan Africa. It always been an exceptional travel-associated disease (reviewed in Gautret *et al*, 2009 and Lamy *et al*. 2012).

1.10. The current trypanosomiasis situation in Ethiopia

Six species of trypanosomes, including *T. b. rhodesiense* have been recorded in Ethiopia and these are the most important parasites from both medical and veterinary point of view because they include the causative agents of human sleeping sickness and the trypanosomiasis in livestock. In tsetse-infested areas, trypanosomiasis is a problem, and against the background of reduced productivity and other animal health problems occur too. Trypanosomiasis cannot therefore, be viewed in isolation. In many parasite conditions, host and parasite establish

equilibrium, and the presence of the parasite does not necessarily affect the health of its host seriously. This is true in animal trypanosomiasis and is best exemplified by the wild animal hosts of tsetse, which from a huge reservoir of trypanosomal infections, and they are often to be found in areas where other breeds cannot survive. Even though, there is evidence that trypanosuscetible animals can establish a delicate balance with potential pathogens (Delia *et al*., 2009).

Trypanosomal infections are often protracted, and chronic trypanosomiasis is much more common than acute disease in enzootic areas. In all animals, disturbance of the equilibrium precipitates or exacerbates disease. The disturbance takes the form of stress, which may be due to the occurrence of another disease, malnutrition, water deprivation, heat stress or production stress such as pregnancy, location or work. The significance of this point is that management determines to a large extent the well-being of animals and may do much to mitigate the effects of trypanosomisis. However, the pathogenesis of the different species of trypanosomes in the different species of livestock varies and within a trypanosomes species there is a range of virulence (Omamo and D'ieteren, 2003).

1.11. Recent advances and future perspectives

The most important recent development has been the completion of the *T. brucei* genome which will greatly facilitate the discovery of new drug targets and genetic markers. Correct staging of the disease is of key importance for treatment. The analysis of sleep patterns is a promising new method to this end and has advanced enough to begin thorough clinical trials. In terms of novel drug candidates, dicationic molecules show the most promise with one oral diamidine in phase 3 clinical trials. New targets and classes of molecules which show in vitro trypanocidal activity are also described. Two new methods – MGE-PCR and microsatellites – allow analyses without parasite cultivation, eliminating a major impediment to efficient sampling for population studies. The finding that several wild animal species harbour *T. b. gambiense* and that parasite transmission is efficient even from very low parasitaemias sheds a new light on the importance of animal reservoirs (Bruna and Balmer, 2006).

There is an urgent need for new drugs for the chemotherapy of trypanosomosis. Progress has been made in the identification and characterization of novel drug targets for rational chemotherapy and inhibitors of trypanosomatid glycosomal enzymes, trypanothione reductase, ornithine decarboxylase, S-adenosylmethionine decarboxylase, cysteine proteases and of the purine and sterol biosynthetic pathways (reviewed in Gutiérrez, 2013).

Trypanosomes have the capacity for antigenic variation, which is the basis of their ability to escape the host immune response, and because of this, prospects for the development of a vaccine against trypanosomosis have been considered poor. However, the most effective and sustainable way of controlling trypanosomosis should be a safe and cost effective vaccine. Therefore, it is necessary to develop a vaccine against trypanosomes based on other potential target proteins of these organisms, as the proteins of the tubulin family (Li *et al*, 2007, Kurup and Tewari, 2012).

1.12. Herbal medicine and trypanosomiasis

Recently, Musuyu Muganza *et al.* (2012) reported *in vitro* antiprotozoal and cytotoxic activity of 33 ethonopharmacologically selected medicinal plants from Democratic Republic of Congo. It was found that most of the tested extracts exhibited pronounced or good antiprotozoal activity against one or more of the selected protozoa. Atawodi *et al* (2002) documented several plants which are used by Fulani herdsmen and other livestock farmers to treat trypanosomiasis. In folkloric medicine of Idoma people of North Central Nigeria, *H. acida* is used alone or in combination to treat trypanosomiasis and other fever related diseases (Ada & Claffey, 2003).

The extracts of *H. acida* stem bark exhibited significant trypanocidal activity whereas *G. erubescens* and L. *lanceolata* were also effective (Abu *et al.*, 2009). Nigerian plants evaluated *in vitro* for trypanocidal activity against *T.b. brucei* and *T. congolense* at concentrations of 4 mg/ml, 0.4 mg/ml and 0.04 mg/ml. It was found that extracts of *K. senegalensis, P. reticulatum, S. longepedunculata* and *T. avicennides* were strongly trypanocidal to both organisms while extracts of *A. differmis, Cassytha spp, L. kerstingii, P. clappertioniana, Striga spp, A. digitata* and *P. africana* were trypanocidal to either *T. b. brucei* or *T. congolense* (Justina *et al*, 2015). *K. africana* from Kenya was evaluated *in vivo* and was found that the dichloromethane fruits extract

of *K. africana* tested at a dose of 2000 mg/kg was effective, curing 60% of the Swiss white mice that had previously been inoculated with *T. b. rhodesiense* (Atawodi *et al.*, 2003; Peter *et al.*, 2009). Nsekuye *et al* (2006) evaluated some medicinal plants from Mali for their *in vitro* and *in vivo* trypanosomal activity and established that out of 165 extracts of the plants screened for *in vitro* antitrypanosomal activity 24 were active.

1.13. Overview of the studied plants

A number of medicinal plants and their secondary metabolites have been screened for anti-trypanosomal activity. Active natural products include several groups of alkaloids, phenolics, saponins, cardiac glycosides, other terpenoids, and polyacetylenes. Although some natural products are active in the submicromolar range and show good selectivity, only few have been studied *in vivo* in an animal model. The mode of action of some drugs from plants with anti-trypanosomal activities has been explored with a major target on glycolysis as blood forms can only gain energy by converting glucose to pyruvate or glycerol (reviewed in Wink, 2012).

The **genus Echinops** that belongs to the family Asteraceae is reported to comprise over 120 species, of *E. kebericho* mesfin (local Amharic name: kebercho) known only in Ethiopia, is an erect massive root stock-bearing perennial herb or shrub that grows up to a height of 1.2 m with leafy stems (Getachew *et al*, 2011). It is among the most important medicinal plants of the country, valued primarily for its root parts (Getachew *et al*, 2011). Its varied medicinal applications are documented in the ancient medico-religious pharmacopoeia and are well-recognized by modern-day traditional professionals (Hareya, 2003).

Figure 2: Stem bark of *E. kebericho* mesfin, photo captured by Debela Abdeta from Tibe Café kebele of Jimma Arjo woreda

In Ethiopia, root powder of Kebercho (*E. kebericho Mesfin*) is sprinkled on burning charcoal and smoke is inhaled for evil eye (possession by evil spirits in Ethiopian folk religion) (Tilahun and Mirutse, 2007). The smoke is inhaled to fight typhus and fever, to relieve headache, and is known to be used as a fumigant for mosquitoes and as a snake repellent. The traditionally used preparation varies among societies. Inhalation for pain relief and oral chewed preparation for malaria, diarrhoea and stomach ache among the different preparation used in traditional medicine. Extracts and essential oils of the roots of *E. kebericho* have been assessed for their antimicrobial, antihelmintic and molluscicidal activities (review in Shiferie and Shibeshi, 2013).

Although the plant is in use for the treatment of trypanosomiasis in Ethiopian society, there is no laboratory-based evidence for the effectiveness and safety of the plant (Shilema *et al*, 2013). Reports and ethnobotanical surveys also evidence long traditional use of this plant for preparation of medicines against migraine, mental illness, heart pain, lung tuberculosis TB, leprosy, kidney disease, malaria, billharzia, syphilis and amoebic dysentery (Abebe and Ahadu, 1993). *E. kebericho* exerted strong antileishmanial activity that was even higher than that of amphotericin B with with moderate cytotoxicity and weak haemolytic effects (Tariku *et al*, 2011).

Its antihelmintic, antitumor, antimutagenic, antibacterial and fungicidal properties have also been reported (Abegaz *et al*, 2007; Tariku *et al*, 2011). The root is burned to be used as a fumigant, mainly after childbirth, toward off mosquitoes and as a snake repellant in the house (Hymete *et al*, 2007). The root is also chewed to alleviate stomach ache, as a taenicidal and a decoction from it is used to cure intestinal diseases in cattle (Hymete *et al*, 2007). Further more in laboratory, the toxic properties of *E. kebericho* have been exploited in the control of insect pests of medical, veterinary and agricultural importance (Hymete and Kidane, 1991 and Jemal *et al*, 2011).

The bioactive extract of the species also has been shown to have antimicrobial effects equal to or better than penicillin, especially against *S. beta-haemolyticus, E. coli* and *Klebsiela aerogenes* (Belachew, 1993). Alkaloid extract of the roots of *E. kebericho* has been shown to have a very strong lethal activity against earthworm (Hymete and Afifi, 1997). In the oil of *E. kebericho*, 43 compounds representing 92.85% of the total essential oil constituents were identified. The tubers

are extremely rich in sesquiterpene amounting to about10% of the dry weight of the tubers (Abegaz, 1996).

Solanum anguivi lam (local Afan Oromo name: 'Hiddii seexanaa') is a rare ethnomedicinal herb that belongs to the family *Solanaceae* and can be found throughout the non- arid parts of Africa. *S. anguivi* have been recognized to possess medicinal properties and their use in traditional systems of medicine has been on record for a long time. It is highly polymorphic and variable in its plant structure, fruits and leaf characters (Adanlawo and Akanji, 2003).

Figure 3: picture of *S. anguivi* Lam, photo captured by Debela Abdeta from Wayu Tuqa woreda

It is a nourishing vegetable that is eaten raw or cooked as soups and medically used to control high blood pressure and diabetes (Schipper, 2000). The roots are carminative and expectorant useful in coughs, catarrhal, colic, nasal ulcers, asthma, tooth ache, nervous disorder and fever (Zhu *et al*., 2000). In Nigeria, the fruits are used as sources of food and drugs. Although, there are reports abound on biological activities of *S. anguivi* fruits like, cholesterol lowering activity, hypolipidemic property, and *in vivo* antioxidant activity (Elekofehinti and Kade, 2012). *S. anguivi* fruit is one of the richest sources of edible saponin in south western and south eastern part of Nigeria. Saponins which exhibited a variety of biological and pharmacological properties including antioxidant (Hung *et al*, 2009, Elekofehinti *et al*, 2012), hypolipidemic potential (Elekofehinti *et al*, 2012) and inhibition of erythropoesis in Rattus novergicus (Elekofehinti *et al*, 2012). Although the plant is in use for the treatment of trypanosomiasis in Ethiopian there is no laboratory-based evidence for the effectiveness and safety of the plant (Megersa *et al*, 2013).

1.14. Statement of the problem

Trypanosomoses is protozoan diseases, affecting both human and animals, and mainly found in tropical Africa, Latin America and Asia. In Africa, trypanosomes produce serious diseases in human beings such as West and East Sleeping Sickness caused by *T. b.gambiense* and *T. b. rodensia* respectively; while in the Americas *T. cruzi* causes the Chagas disease. Other species of Trypanosoma affect animals and produce enormous economical impact in the endemic areas. Those species could be classified as those transmitted by tsetse flies- *(T.a vivax, T. congolense* and *T. b. brucei*) producing a disease known as nagana and those non-transmitted by tsetse (*T. evansi* –surra, *T. equiperdum* –dourine) (Gutiérrez *et al*, 2013).

More than 90 percent of crop production in Ethiopia is dependent on animal draught power on ploughing oxen which are mainly infected by animal trypanosomiasis, which in turn, worsens food supply and living conditions in affected areas (reviewed in Fromsa *et al*., 2011). Due to antigenic variation shown by the trypanosome, prophylaxis of these diseases using vaccines is challenging; for that, most of the control and eradication programs against animal trypanosomes carried out in the infected areas in the world are based on therapeutic and prophylactic measures, using trypanocidal drugs or combining both measures. However, only six compounds are available in the market (isometamidium chloride, homidium –bromide and chloride- and diminazene aceturate), pentamidine, melarsoprol and eflornithine and all of them have been on the market for over 40 years. One of the most important risks for the future use of these existing trypanocides is the development and dissemination of resistances and, for that, new drugs have been developed in the recent past and are available in the market to treat *T. evansi* (melarsomine) (Steverding, 2010; Gutiérrez *et al*, 2013).

In the search for new trypanocides, a wide range of medicinal plants have been screened for antitrypanosomal activity and quite a number of them have been reported to have significant antitrypanosomal activity (Atawodi *et al*, 2003; Ogbadoyi *et al.*, 2007; Nathan *et al*., 2011).This is due to the fact that these plants contain phytochemicals which are responsible for this activity. A lot of plants belonging to different families have been known to have antitrypanosomal activity both *in vitro* and *in vivo* (Atawodi *et al.,* 2004; Wurochekke *et al.,* 2004; Ene *et al*.,

2009; Abu *et al.,* 2009; Olukunle *et al.,* 2010; Umar *et al.,* 2010, Feyera *et al*, 2014; Kifleyohannes *et al*, 2014; Mergia *et al*, 2014, Tadesse *et al*, 2015; Mergia *et al*, 2016) due to the fact that they've been used for the treatment of trypanosomiasis traditionally.

Therefore, the search for new chemical entities that should be effective against all species of trypanosome, safe and affordable for disease-endemic countries like our country is the best choice left without option to fight against the notorious impact of bovine trypanosomiasis on cattle productivity (Fromsa *et al*, 2011) and to reduce human loss due to human trypanosomiasis. These are found in plants which are potential sources of new drugs since they contain countless number of molecules that have pharmacological effects (Newman *et al*., 2003)

1.15. Significance of the study

Trypanosomiasis is well known for its debilitating, anemia, edema, decreased fertility and abortions, loss of milk and meat production and work capacities and coma and death in human and therefore, exploration of the possible control mechanisms is central to addressing these losses, with the final aim of finding a novel drug which can help combat it. So this study tries to scientifically prove that the traditional plants claimed to be used for trypanosoma control actually has the effect. Given the numerous problems associated with the few trypanocidal drugs, the current research interest in the treatment and control of trypanosomiasis aims at developing less toxic, cheaper, efficacious and readily available alternative medicine of plant origin. Such information, on the other hand, helps to speculate the possible mechanisms of action of active principle of the plant responsible for antitrypanosomal effects. Furthermore, the study will also give us a clue about the preliminary bioactives. The study will also provide evidence that encourages the traditional use of the plant especially by communities which reside in areas where modern health facilities did not reach due to geographic and socio-demographic challenges.

2. OBJECTIVES

The study was carried out to determine the *in vitro* and *in vivo* antitrypanosomal effect of hydromethanolic extract of *E. kebericho* root and *S. anguivi* fruit against mice experimentally infected with a field isolate of *T. congolense*

2.1. Specific objectives

- To study the *in vitro* activity of crude hydroalcoholic extracts of the plants against *T. congolense* based on motility the parasite
- To evaluate blood incubation infectivity test of the extracts against *T. congolense*
- To investigate the curative effects of the extracts against *T. congolense*
- To determine prophylactic activity of the hydromethanolic extracts of the plants against *T. congolense*.
- To assess acute toxicity of the crude hydroalcoholic extracts of the plants.
- To determine the phytochemical constituents of the crude hydroalcoholic extracts of the plants

3. MATERIALS AND METHOD

3.1. Chemicals and instruments

Analytical grade solvents and reagents used in this work were phosphate buffered saline(PBS), methanol (MOH), diminazene aceturate (DA), Giemsa stain, anticoagulant (Na EDTA), glycerol, 0.9% saline solution, distilled water. Instruments like mortar and pestle, automatic weight balance, heater, refrigerator, microscope, slides, syringe, centrifuge, microhaematocrit reader, digital rectal thermometer, aluminium foil, Whatman no1.filter paper, sealer, gloves, and parafilm, microtiter was used.

3.2. Plant collection and authentication

The roots of *E.kebericho* and fruit of *S. anguivi* were collected in November, 2015 in Jimma Arjo and Wayu Tuqa Woredas of Eastern Wollega, respectively for the experiments. Spacemen of the plants were collected, identified and authenticated by Dr. Mirutse Giday, at Addis Ababa University, Akililu Lema Institute of Pathobiology and the vouchers were deposited at the National Herbarium of Addis Ababa University with numbers DA 01 for *E. kebericho* and DA 02 for *S. anguivi.*

3.3. Preparation of plants extracts

The air dried powdered plant materials were macerated in an Erlenmeyer flask with 80% methanol, allowed to stand at room temperature for a period of 72 hours with occasional shaking using mini orbital shaker. It was then filtered two times with gauze then through whatman filter paper (Number 1). The supernatant part of agitated materials was separated from the un-dissolved portion of the plant. The residue was re macerated two times for 6 days in order to obtain a better yield and the combined liquid was further filtered. The filtrate was concentrated using rotary evaporator to remove MOH. Then the concentrated filtrate was lyophilized to remove water.

3.4. Experimental animals

Swiss albino mice, purchased from Ambo University were maintained at the animal house of Akililu Lemma Institute of Pathobiology (ALIPB) bred for experimental animals. Swiss albino mice of either sex, weighing 30-35 g (age 10-12 weeks) bred in the laboratory animal unit of ALIPB was used. They were fed with standard animal feed and watered *ad libitum* at room temperature 23-25°C with relative humidity of 60-65%. All procedures complied with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

3.5. Test organism and its maintenance

T. Congolense was obtained from Addis Ababa University, Faculty of Veterinary Medicine and Agriculture, Department of Veterinary Parasitology which originally were brought from Arbaminch area by infecting white albino mice by intraperitoneal inoculation.

Mice were screened for development of infection using the buffy coat technique or Murray method (Murray *et al*, 1977). Blood from tail vein was drawn and fixed by methanol and stained with Giemsa stain and read using an oil immersion (100×) objective lense for identification of trypanosomes. The morphology of the trypanosome in the stained field was compared with that of reference species (WHO, 1998).

Blood from the tail was used for the estimation of parasitaemia in wet mount. The trypanosome count was determined by examination of the wet mount microscopically at x40 magnification using the "rapid matching" method of Herbert & Lumsden (1976). This method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted (1:1) with PBS, pH 7.2. Passage was considered necessary when parasitemia were in the range of 16-32 parasites per field i.e at 10^{th} day's pos infection. During passaging, $5*10^5$ parasites were introduced intraperitonially into mice in 0.2 ml blood/PBS solution (V/V) obtained by cardiac puncture into 1 ml syringe(Ene *et al*, 2009).

3.6. Evaluation of *in vitro* anti trypanosomal activity

The *in vitro* test was performed to detect any motile trypanosomes and it was done in triplicates in a 96 well microtitre plate. Original concentration of the extract solution was prepared in glass test tubes and it was closed with the aid of rubber stoppers. Twenty micro liter of blood containing about 16-32 organisms per field were mixed with 5 micro liter of each of the test substance in concentrations of 2.5, 5, 10, 20mg/ml to produce test concentrations of 0.5, 1, 2, 4.0 mg/ml respectively.

All the crude extracts were freshly prepared. Dilutions of infected mice blood was made using phosphate glucose buffered saline solution (PBSG). The parasitic load of the diluted blood was estimated to be 1 x 10^5 parasites/ml (Murray *et al*., 1983). Two set of controls containing the parasite (20 µl of infected blood) suspended in 10% PBSG (pH7.2) only, and a similar concentration of standard trypanocidal drug DA was included to serve as untreated control and treated controls, respectively.

The solution was allowed to be incubated at 37°C for up to three hours. During the period, the motility of the parasites in the solution was checked at 20 min intervals using light microscopy (x 40 objective lens), about 2 µl of test mixtures was placed on separate microscope slides and covered with cover slips and the parasites observed. Reduced motility or complete cessation of motility was taken as effect of the extracts.

3.7. In vivo experimental design

Thirty mice of either sex for each plant extracts was randomly grouped into five groups (I, II, III, IV and V) of 6 animals per-group for all *in vivo* models. For infectivity test group I was inoculated with 0.3ml infected blood diluted in PBSS while groups II, III, IV and V were inoculated with infected blood diluted in PBSS which were incubated respectively with Diminazine aceturate (3.35mg/kg) and extract mixtures at 1mg/ml, 2mg/ml and 4mg/ml.

For prophylactic test Groups I and II were administered orally with vehicle (distilled water) 0.3 ml and diminazine aceturate (3.35mg/kg) intrapretoneally while groups III, IV and V administerd extracts at daily doses of 100, 200 and 400mg/kg body weight respectively for 5 days before parasite inoculation.

For curative test groups I and II were administered orally distilled water 0.3 ml and diminazine aceturate (3.35mg/kg) intraperitoneally respectively to serve as untreated and treated controls, while groups III, IV and V administered extracts at daily doses of 100, 200 and 400mg/kg body weight respectively for 7 consecuative days intraperitoneally from 10^{th} days of parasite inoculation.

Dose of extracts were selected based on the results of pilot study and acute toxicity results. Three mice per group were used for each dose of both plants. Three groups were administered extracts at 100, 200 and 400mg/kg for 7 consecutive days, one group was treated with standard drug and the last group used as untreated control; checked for parasite suppression effects. This dose was deduced from acute toxicity; one tenth of the limit dose was used as medium dose, half of the medium dose as lowest dose and twice the medium dose for higher dose. For all models parasitemia, PCV, body weight, rectal temperature and mean survival time of each group was measured.

3.8. Experimental Procedures

3.8.1 Blood Incubation Infectivity Tests

A blood incubation infectivity test was performed, as follows: parasite suspension incubated in the presence of the hydromethanol extract of root of *E. kebericho* and fruit of *S. anguivi* at 4 mg/ml, 2 mg/ml and 1 mg/ml as described for *in vitro* studies. After an incubation period of 2 hrs, 0.2 ml of each preparation was injected into healthy mice. On the 10^{th} day of parasite and extract mixture inoculation, wet blood films were prepared from tail blood of each mouse as for prophylaxis and curative tests. Parasitemia, PCV, body weight and rectal temperature were measured to predict the effectiveness of the test extracts. Each mouse was followed till day 30, and their survival recorded (Abedo *et al*, 2013).

3.8.2. Evaluation of the prophylactic activity of the extracts

The test for prophylactic activity was done using Peters, 1967 and Igwe and Onabanjo 1989 methods with slight modification. Thirty Swiss albino mice of either sex for each plants extracts were divided into respective groups with six mice per-group. Mice in the test groups were administered with the crude hydromethanolic extracts of *E. keberich*o roots and *S. anguivi* fruits at 100mg/kg, 200mg/kg and 400 mg/kg intraperitoneally for five consecuative days prior to infection. Mice in the positive and untreated control groups were treated with diminazine aceturate (3.35 mg/kg), and vehicle (0.2mL distilled water), respectively. On day 6, a standard inoculum of *T. congolense* infected erythrocytes was administered by intraperitoneal route to each mouse. On 10^{th} day of infected blood inoculation, wet blood films were prepared from tail blood of each mouse. The animals were observed for 21 days post infection. Parasitemia, PCV, body weight and rectal temperature were measured every 4 days. For each mouse their survival time was recorded (Peters1967, Igwe and Onabanjo 1989).

3.8.3. Evaluation of curative activity of extracts

Thirty mice of either sex were randomly grouped in to five groups (I, II, III, IV and V) of 6 animals for each plant extracts. All the Groups (I-V) were intraperitoneally infected with *T. congolense (*$5*10^5$ parasites/ml). Group I and II were administered orally vehicle 0.2 ml and Diminazine aceturate(3.35 mg/kg) intraperitoneally to serve as untreated and treated controls, respectively, while Groups III-V received the extract intraperitoneally at daily doses of 100, 200 and 400 mg/kg body weight respectively for 7 consecutive days. Parasitemia and PCV were observed every 4 days for 21 days while body weight and rectal temperature was monitored every 2 days for each plant extracts. Mean survival time was observed for 6 weeks (Ene *et al*, 2009).

3.9. Parameters studied during the experiments

3.9.1. Determination of parasitemia

On the tenth day post infection in all *in vivo* experimental model (infectivity test, phrophylactic assay and curative test) and then after every four days, the parasitemia level of mice were

checked. Parasitemia was monitored by examination of blood drawn from the tail of mice and examining microscopically at × 40 magnifications using the "Rapid Matching" method of Herbert and Lumsden (1976). Briefly, the method involves microscopic counting of parasites per field in blood without dilution. Logarithm values of these counts were obtained by matching with the table of Herbert and Lumsden (1976). Monitoring of parasitemia was performed every four days until the 20th day post-treatment initiation (Maikai, 2011, Obah *et al.,* 2013).

3.9.2. Determination of Packed Cell Volume

PCVwas determined using microhaematocrit centrifuge and microhaematocrit tube reader. The heparinized capillary tube was filled ¾ with blood samples obtained from the tail vein of the mice. The end of the tube was sealed with crystal seal and excess cleared off using cotton wool. The filled tubes were placed in a slot in the centrifuge head with sealed end outward. The tubes were centrifuged in microhaematocrit centrifuge at room temperature for 5 minutes at a revolution of 13 000 rpm to determine and record the PCV for each sampled to assess anaemia. A special scale, the microhaematocrit reader was used to obtain the PCV percentage. The length of the packed red blood cells column was expressed as a percentage of the total volume of blood. PCV was monitored on day of treatment initiation and every 4 days until 21th day post treatment initiation (Tasew and Duguma, 2012; Ngulde *et al*, 2013).

3.9.3. Determination of body weight

The body weight of each mouse in all groups were recorded on the day of parasite challenge, day of treatment initiation and every other day for 21days by a sensitive digital weighing balance (Nweze *et al*, 2011).

3.9.4. Determination of rectal temperature

Rectal temperature was measured by digital rectal thermometer (Mettler Toledo, Switzerland) per rectum on the day of parasite inoculation, day of treatment commencement and every other day thereafter for 21days (Ngulde *et al*., 2013).

3.9.5. Determination of mean survival time

The average survival rates of individual mouse was calculated after recording of specific dates of death for each mouse post-infection and compilation of data done for each treatment group and control groups throughout the follow up period for 6 weeks post-inoculation of the parasite in case of infectivity and curative test whereas 4 weeks post parasite inoculation for prophylactic assay (Ngure et *al*, 2009; Feyera *et al*, 2014).

3.10. Phytochemical Screening

The crude hydromethanol extracts of the plants were tested for the presence of bioactive compounds by using standard tests (Yadav & Agarwala, 2011; Iqbal, 2012) and others as described below.

Saponins: About 2g of the sample was boiled in 20ml of distilled water in a water bath and filtered. A 10ml portion of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth.

Glycosides: This was carried out as described by Evans, 1996
Keller Killiani test: the extract test solution was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Formation of lower reddish brown layer and upper acetic acid layer which turns bluish green was taken as an indication for presence of glycosides.

Steroids:
Two ml of acetic anhydride added to 0.5g of the extracts with 2ml of H2SO4. The color changes from violet to blue indicating the presence of steroids.

Phenols: About 0.5 g of each extract was treated with few drops of neutral ferric chloride solution 5%, deep bluish green solution formed indicates the presence of phenol.

Flavonoids: This was carried out as described by Dermarderosian and Liberti (1988)

A: ferric chloride test**:** Few drops of ferric chloride were added to the extract test solution. Formation of blackish red color indicated the presence of flavonoids.

B: alkaline reagent test**:** ml of 10% sodium hydroxide (NaOH) was added to the extract test solution followed by 3 ml of 10% HCl. The formation of a yellow color on addition of NaOH, which disappeared on addition of the HCl indicated the presence of flavonoids.

C. Lead acetate solution test: formation of yellow precipitate after addition of few drops of lead acetate (10%) solution to the extract solution indicated the presence of flavonoids.

Terpenoids: About 0.2g extracts was mixed with 2ml Chloroform and 3ml of concentrated H2SO4 was carefully added to form a layer. A reddish brown coloration of the interface formed indicating the presence of terpenoids.

Anthroquinones: This was carried out as described by Tyler *et al,* 1988

a. Free Anthraquinones: 5 gm of each plant extract was shaken with 10 ml of benzene and filtered. A 10% ammonium hydroxide solution (5 ml) was added to the filtrate, and the mixture was shaken. The presence of a pink, red or violet color in the ammonia phase was taken as an indication of the presence of anthraquinones.

b. Combined Anthraquinones: 5 gm of plant extract was boiled with 10 ml of 1% HCl and filtered while hot. The filtrate was shaken with 5 ml of benzene. The benzene layer was removed and 10% ammonium hydroxide (equal to half the volume of benzene) was added to it. A pink, red or violet color in the ammonia phase indicated the presence of anthraquinone derivatives.

Alkaloids: This was carried out as described by (Rafauf, 1970) and (Sofowora, 1982).

a. Dragendorff's test: 1 ml of Hydrochloric acid (HCl) and 3 drops of Dragendorff's reagent were added to the extract solution. The formation of orange precipitates indicated the presence of alkaloids.

b. Wagner's test: 1 ml of HCl and 3 drops of Wagner's reagent were added to the extract solution. The formation of a brown precipitate indicated the presence of alkaloids.

c. Meyers test-few drops of Meyers reagent wasadded to sample of the extract in a test tube. Cream precipitate indicate the presence of alkaloids

Tannins:

Three grams of the sample was boiled in 50ml distilled water for 30minutes on a hot plate. The mixture was filtered and a portion of the filtrate was diluted with sterile water in a ratio of 1:4 and 3drops of 10% ferric chloride solution was added. A blue or green colour indicates the presence of tannins.

Test for phlobatannins - Two millilitres of 0.5 M HCl was added to 5 ml of the extract and heated for 10 minutes. Red precipitate indicates presence of phlobatannins.

3.11. Determination of acute toxicity of crude extracts

The median lethal dose (LD_{50}) in mice were determined according to the method of Lorke (1983) and Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 420 (OECD, 2001) on Swiss albino mice of female sex, weighing 30-35 g (age 10-12 weeks). The limit test dose of 2000 mg/kg was orally administered sequentially to five female mice and observed for 24 hrs and then for 14 days. They were observed for toxicity signs like changes in physical appearance, behavioral change and feeding activities, hair erection, lacrimation, reduction in motor and other signs of acute toxicity and mortality were observed and recorded.

3.12. Statistical analysis

Statistical Package for Social Science (SPSS) version 20 was use for data analysis. Analysis of variance (ANOVA, one way) was employed to test statistical difference within all groups; Tukey test used for significant difference between two groups' means. Significance was determined at 95% confidence interval (CI) and all results expressed as mean ± SEM. P values less than 0.05 were considered statistically significant

4. RESULTS

4.1. Percentage Yield of Extracts.

After the extraction; the percentage yield was calculated as 11.7% and 23.3 % for *E .kebericho* roots and *S. anguivi* fruits respectively (table1).

Table 1: Percentage yields of roots of *E .kebericho* and *S. anguivi*

Plant name	Percentage yield
Hydromethanolic extract of *E. kebericho* roots	11.7%
Hydromethanolic extract of *S. anguvi* fruits	23.3%

4.2. *In vitro* antitrypanosomal activity

Assessment of *in vitro* trypanocidal activity was performed intriplicates in wells of 96 micro titer plates. Infected mice at high parasitemia state were sacrified and blood collected in EDTA coated tubes mixed with phosphate buffered saline glucose (PBSG). Twenty micro litre of blood containing about 16-32 organisms per field obtained were mixed with 5 microlitre of each of the test substances at final concentrations of 0.5, 1.0, 2.0, and 4.0 mg/ml.

As shown in Tables 2 and 3 below the hydromethanol extracts of *E.kebericho* and *S.anguivi* had ceased motility of the trypanosomes within 40 min at 4 and 2 mg/ml concentration. However the positive control diminazine aceturate immobilized motility of trypanosomes with the first 20 min whereas in the untreated control (distilled water) motility continues for 180 minutes. In lowest dose of the experiment 0.5 mg/ml of the hydromethanol extracts of *S.anguivi* fruits and *E.kebericho* root the motility stays for 60- 100 minutes respectively after which motility of the parasite is completely ceased. The motility of parasites ceased at 40 minutes in *S.anguivi* fruits extract and 60 minutes for *E. kebericho* roots extract at dose of 1mg/kg.

Table 2: *In vitro* activity of crude hydromethanolic roots of *E. Kebericho*

Duration	*E. kebericho*				DA	Control
in minutes	0.5mg/ml	1mg/ml	2mg/ml	4mg/ml	3.35mg/ml	
0	+	+	+	+	+	+
20	+	+	+	+	-	+
40	+	+	-	-	-	+
60	+	+	-	-	-	+
80	+	-	-	-	-	+
100	-	-	-	-	-	+
120	-	-	-	-	-	+
140	-	-	-	-	-	+
160	-	-	-	-	-	+
180	-	-	-	-	-	-
200	-	-	-	-	-	-

Control= distilled water; DA= diminazine aceturate, += motility of parasite present, -= no parasite motility.

Table 3**:** *In vitro* activity of crude hydromethanolic fruits of *S. anguivi*

Duration in	*S. anguivi*				DA	Control
minutes	0.5mg/ml	1mg/ml	2mg/ml	4mg/ml	3.35mg/ml	
0	+	+	+	+	+	+
20	+	+	+	+	-	+
40	+	-	-	-	-	+
60	+	-	-	-	-	+
80	-	-	-	-	-	+
100	-	-	-	-	-	+
120	-	-	-	-	-	+
140	-	-	-	-	-	+
160	-	-	-	-	-	+
180	-	-	-	-	-	+
200	-	-	-	-	-	-

Control= distilled water; DA= diminazine aceturate, += motility of parasite present, -= no parasite motility.

From the table 2 and 3 above *S. anguivi* fruits extract have better *in vitro* antitrypanosomal effects than *E. kebericho* roots extract based on reducing the motility of the parasites.

4.3. Impacts of hydromethanolic extracts of *E. Kebericho* roots and of *S. anguivi* fruits on infectivity of *T. congolense*

Infectivity tests were performed in order to know any remaining parasites after infected blood was incubated in the presence of extracts and standard drug. For the validation of the *in vitro* antitrypanosomal activity, similar concentrations of extracts as used in the *in vitro* test were assessed for blood incubation infectivity test. All the control mice became parasitiemic and died within 5.4 ±. 0.21 (mean ± SEM) for *E. kebericho* groups and 5.17 ±.0.48 for *S. anguivi* mean days while all mice inoculated with inoculum mixtures and standard drug did not developed infection during the 30 days of observation.

4.3.1. Impacts of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits on packed cell volume of *T. congolense* infected mice

Mice in control group showed gradual decrease in PCV values while all mice on extract inoculums and standard drug showed slightly constant value of normal range and survived throughout study period. There is statistical significant association ($p<0.05$) in PCV values between those in untreated control (infected blood without any chemical) when compared with *E. kebericho* and *S. anguivi* at doses of 1mg/ml, 4mg/ml, 2mg/ml and DA3.35mg/ml containing infected blood through day 0 to day 4 post observation(figure 4 and 5).

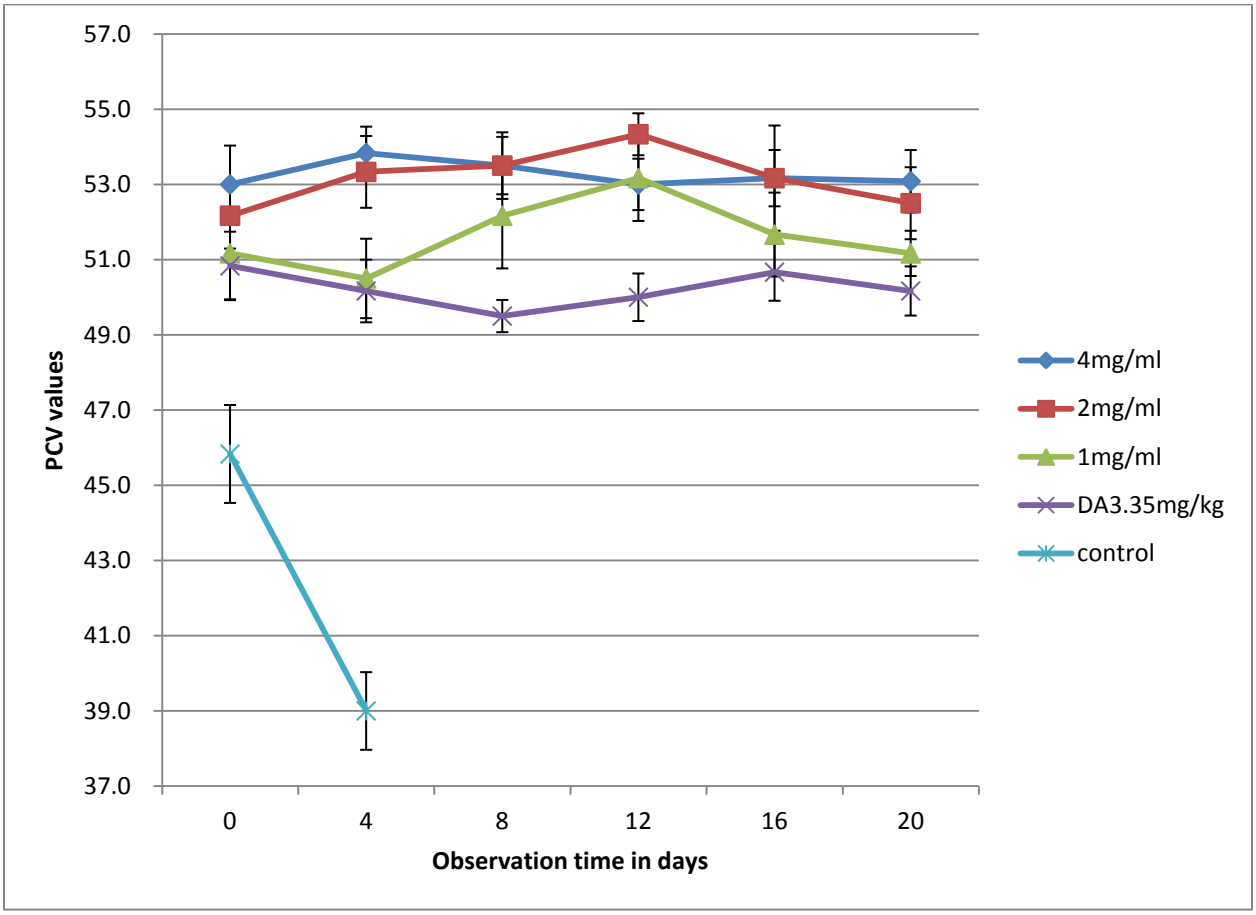

Figure 4: Impacts of *E. kebericho* root extract on PCV of *T. congolense* infected mice

Values are expressed as mean ± SEM, Day 0 = the 10th day after extract and standard drug incubated infected blood inoculation

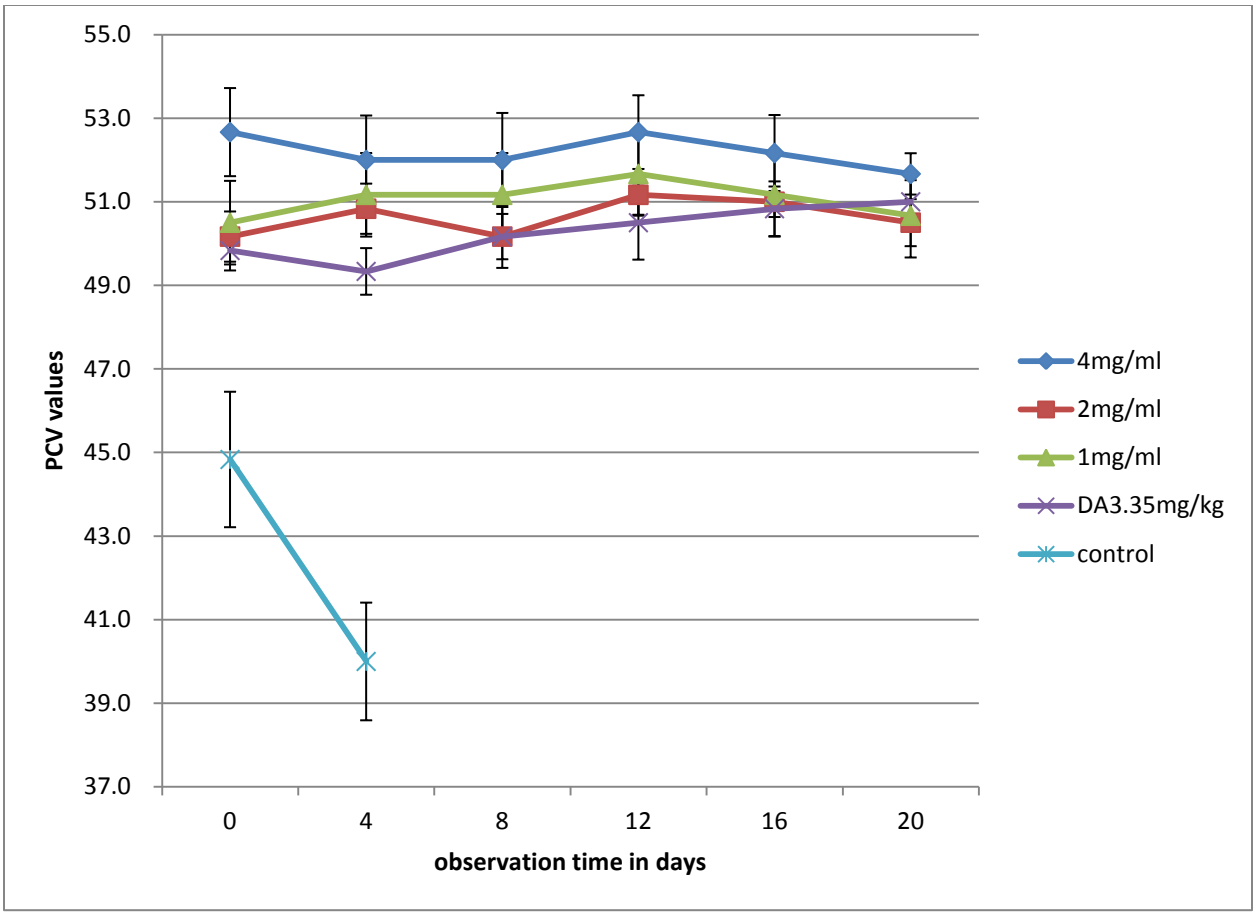

Figure 5: Impacts of *S. anguivi* fruit extract on PCV of *T. congolense* infected mice

Values are expressed as mean ± SEM, Day 0=the 10th day after extract and standard drug incubated infected blood inoculation

4.3.2. Impacts of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits on body weight of *T. congolense* infected mice

All animals in untreated control group showed gradual decrease in body weight while all animals on extract inoculums and standard drug showed increase in body weight and survived throughout study period. There is statistical significant association ($p<0.05$) in body weight between those in untreated control (infected blood without any chemical) when compared with *E. kebericho* and *S. anguivi* at doses of 1mg/ml, 2mg/ml and 4mg/ml and DA3.35mg/ml containing infected blood on day 4 post observation(figure 6 and 7).

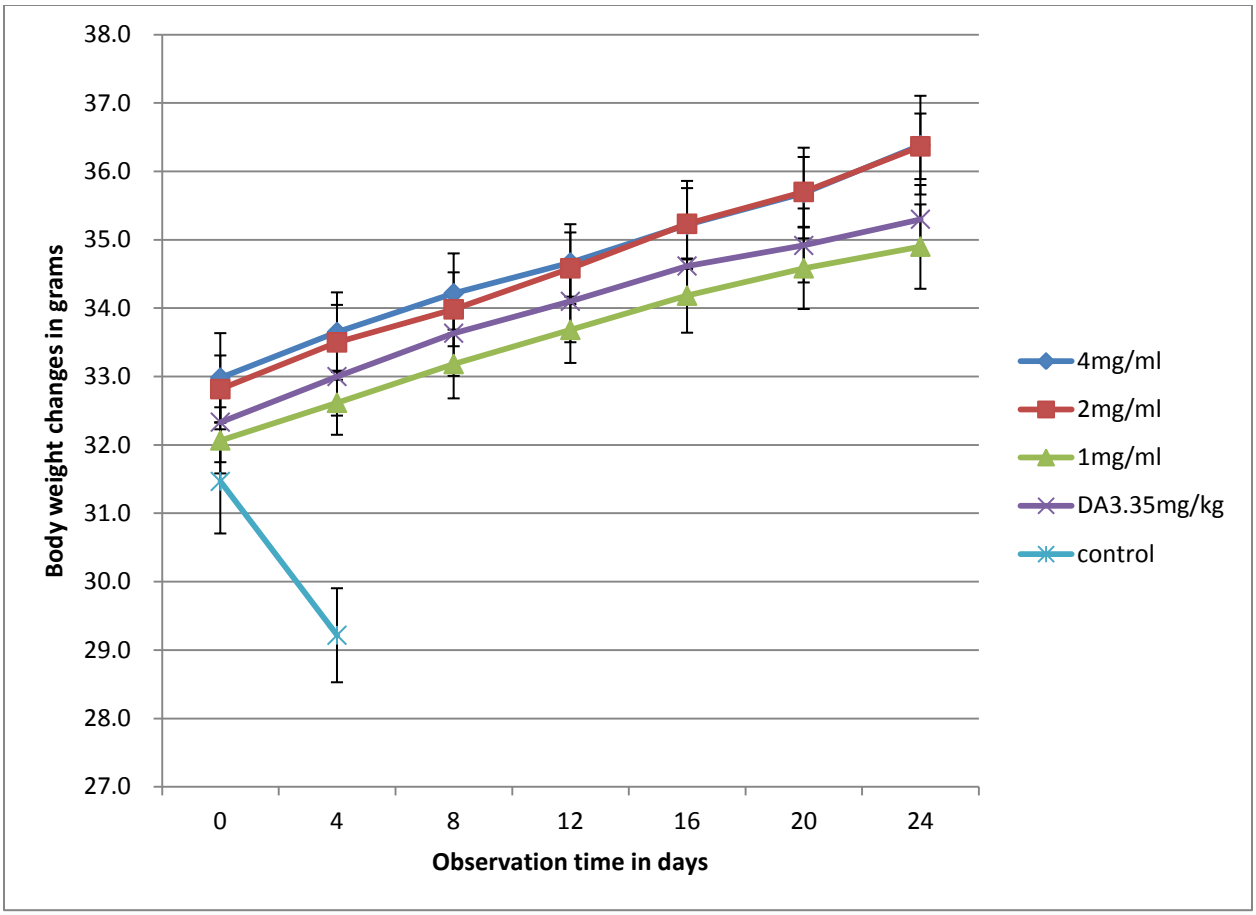

Figure 6: Impacts of *E. Kebericho* roots extract on body weight of *T. congolense* infected mice

Values are expressed as mean ± SEM, Day 0=the 10th day after extract and standard drug incubated infected blood inoculation

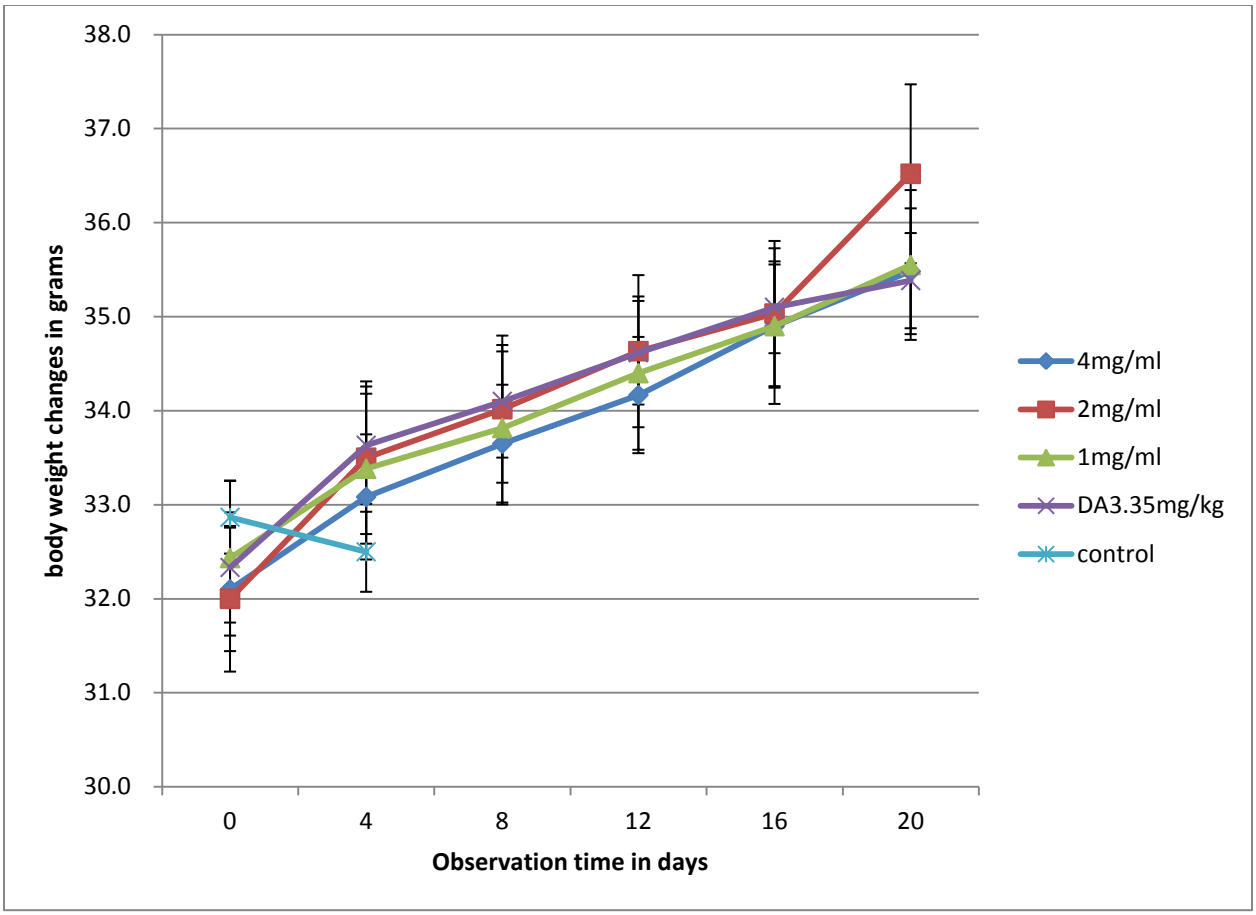

Figure 7: Impacts of *S. anguivi* fruit extract on body weight of *T. congolense* infected mice

Values are expressed as mean ± SEM, Day 0=the 10th day after extract and standard drug incubated infected blood inoculation

4.3.3. Impacts of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits on rectal temperature of *T. congolense* infected mice

The pretreatment mean rectal temperature was 34.32±0.26. The temperature showed relatively constant values in all groups throughout study period indicating the extract could not modify the temperatures of infected mice. There is no observed difference throughout the follow up period (figure 8).

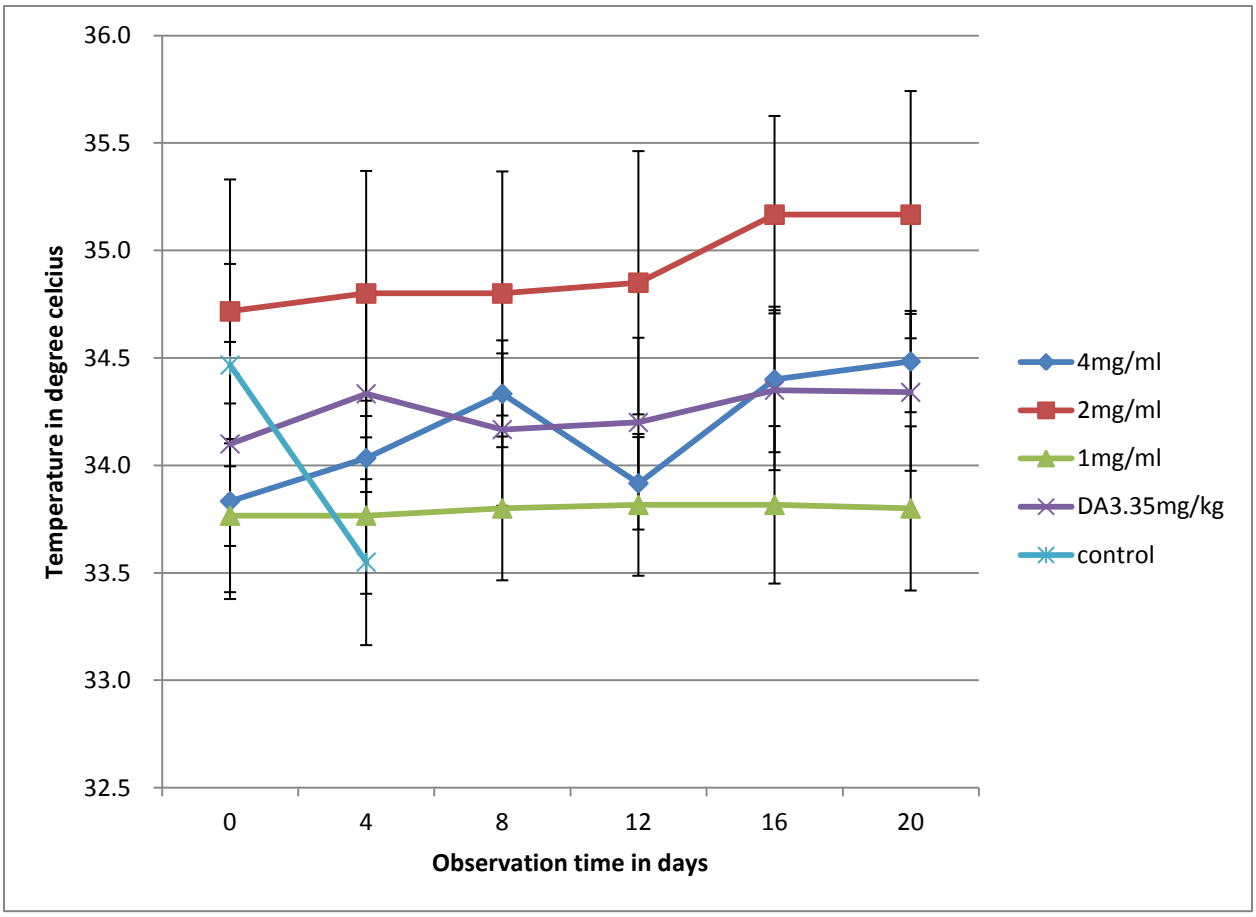

Figure 8: The effect of crude hydromethanolic extracts of *E. kebericho* root on rectal temperature of mice challenged with mixture of extract and infected blood.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after extract and standard drug incubated infected blood inoculation

The pretreatment mean rectal temperature was 34.12±0.56. The temperature value shows fluctuation in all groups throughout study period (figure 9).

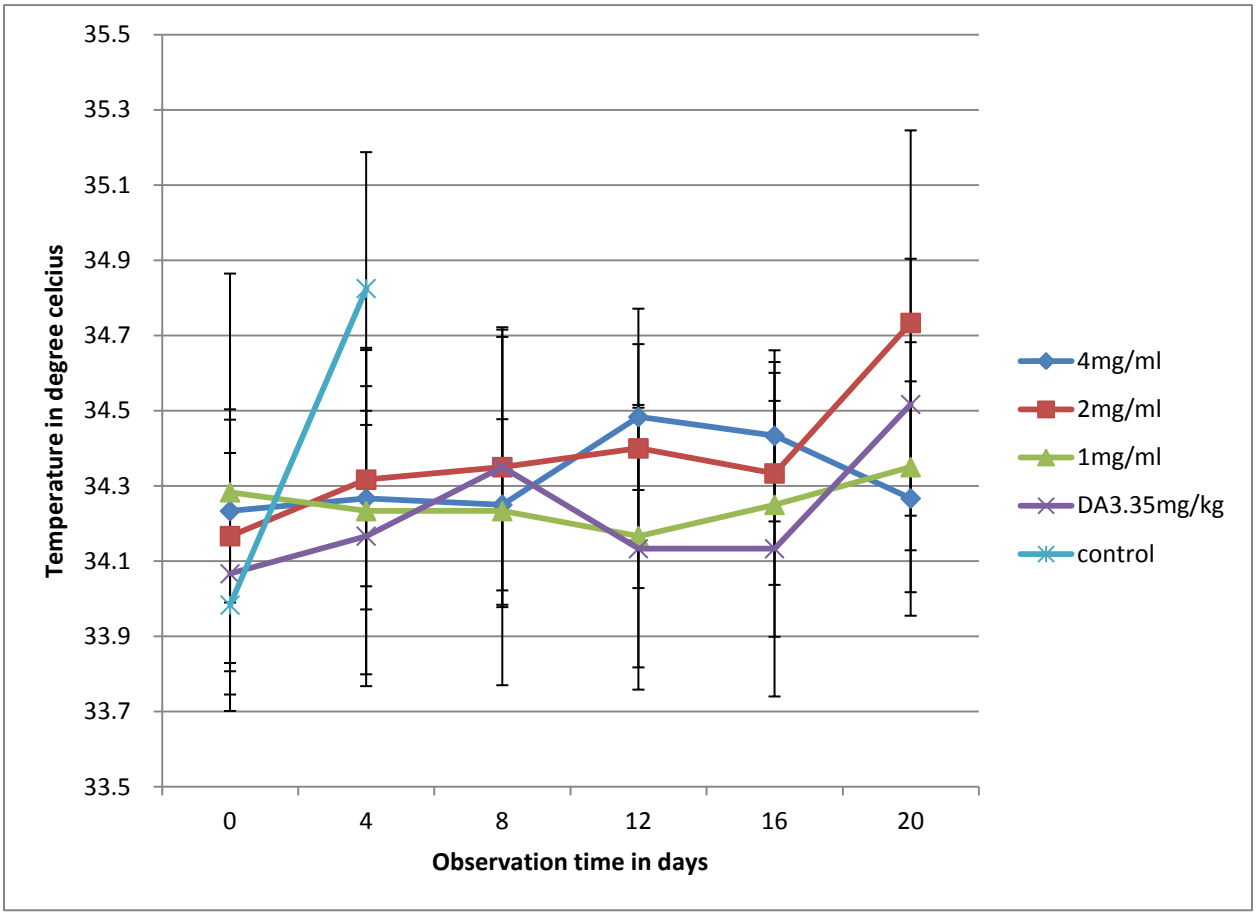

Figure 9: The effect of crude hydromethanolic extracts of *S. anguivi* on rectal temperature of mice challenged with mixture of extract and infected blood.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after extract and standard drug incubated infected blood inoculation

4.4. Prophylactic Activity of Extracts

A group of 60 mice were divided into 10 groups for each plant extracts (A to E). A, B, C respectively received extracts at 100mg/kg, 200mg/kg and 400mg/kg; group D received DA3.35 mg/ kg/body weight for 5 day. Group E that served as control was not given any drug or extract. All the five groups were challenged with parasite on 6th day. Infected mice were then routinely monitored microscopically for parasitaemia from tail blood smear.

4.4.1. Impacts of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits prechallenge on parasitemia of *T. congolense* infected mice

All animals in untreated control (group E) and standard drug group(group D) died before day 7 post observations showing an increase in number of parasites while all animals on extracts showed slightly prolonged survival time showing the extract has some chemoprophylactic effects. There is statistical significant association ($p<0.05$) between those in untreated control and standard drug prechallenge when compared with extracts of *E. kebericho* 400mg/kg, 200mg/kg, 100mg/kg on frist day of observation (10th day of inoculation) and between the two controls and extracts 400mg/kg, 200mg/kg on day 14 post parasite challenge. There is statistical significant association ($p<0.05$) between those in untreated control and standard drug prechallenge when compared with extracts of *S. anguivi* 400mg/kg on 10th day of inoculation and 400mg/kg and 200mg/kg day 14th days of post parasite challenge(figure 10 and 11).

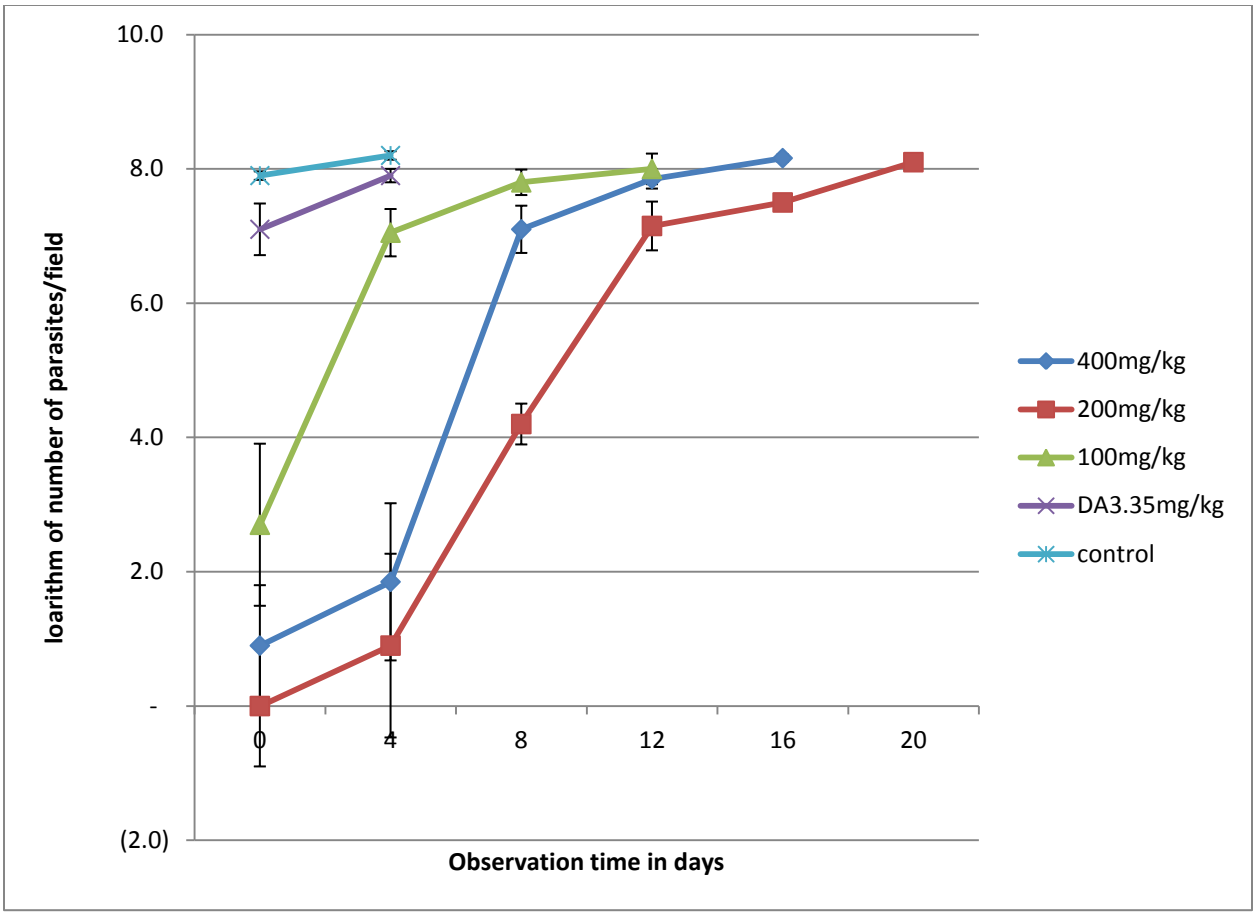

Figure 10: The effect of crude hydromethanolic extracts of *E. kebericho* roots on number of parasite in milliliter of blood of pre etract challenged infected mice.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after extract and standard drug challenged mice inoculated with infected blood

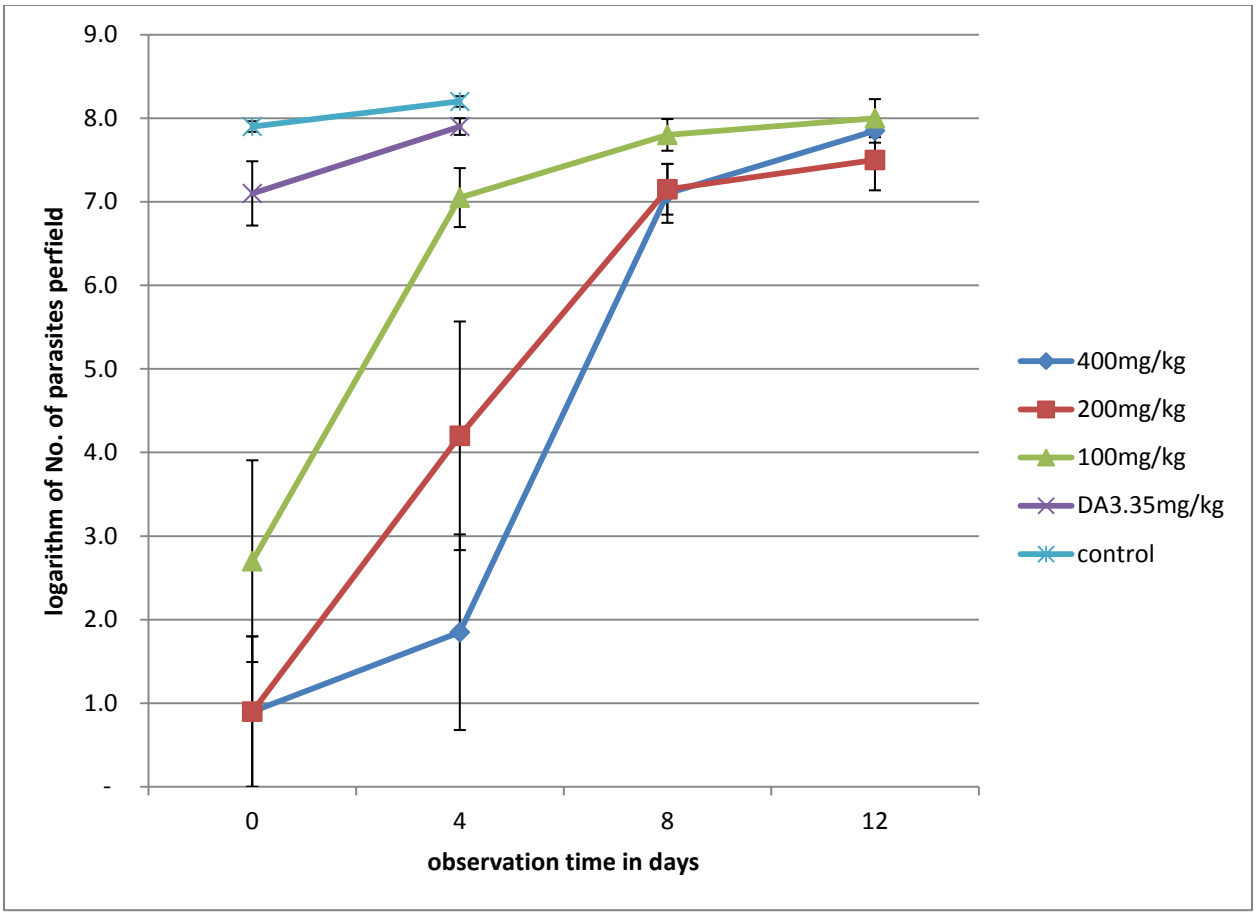

Figure 11: The effect of crude hydromethanolic extracts of *E. kebericho* on number of parasitemia in milliliter of blood of pre extract and challenged infected mice.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after extract and standard drug challenged mice inoculated with infected blood

4.4.2. Impacts of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits prechallenge on packed cell volume of *T. congolense* infected mice

All animals in un treated control and standard drug group died before day 7 post treatment showing gradual decrease in PCV values while all animals on extracts showed slightly prolonged time showing the extract has some chemoprophylactic effects. There is statistical significant association ($p<0.05$) in PCV values between those in untreated control and standard drug prechemical challenge when compared with extracts of *E. kebericho* at 400mg/kg, 200mg/kg, 100mg/kg through day 0 to day 4 post commencement of observation. There is statistical

significant association ($p<0.05$) in PCV values between those in untreated control preparasite challenge when compared with extracts of S. *anguivi* at 400mg/kg, 200mg/kg, 100mg/kg on day the first day of observation(day10th) of infection(figure 12 and 13).

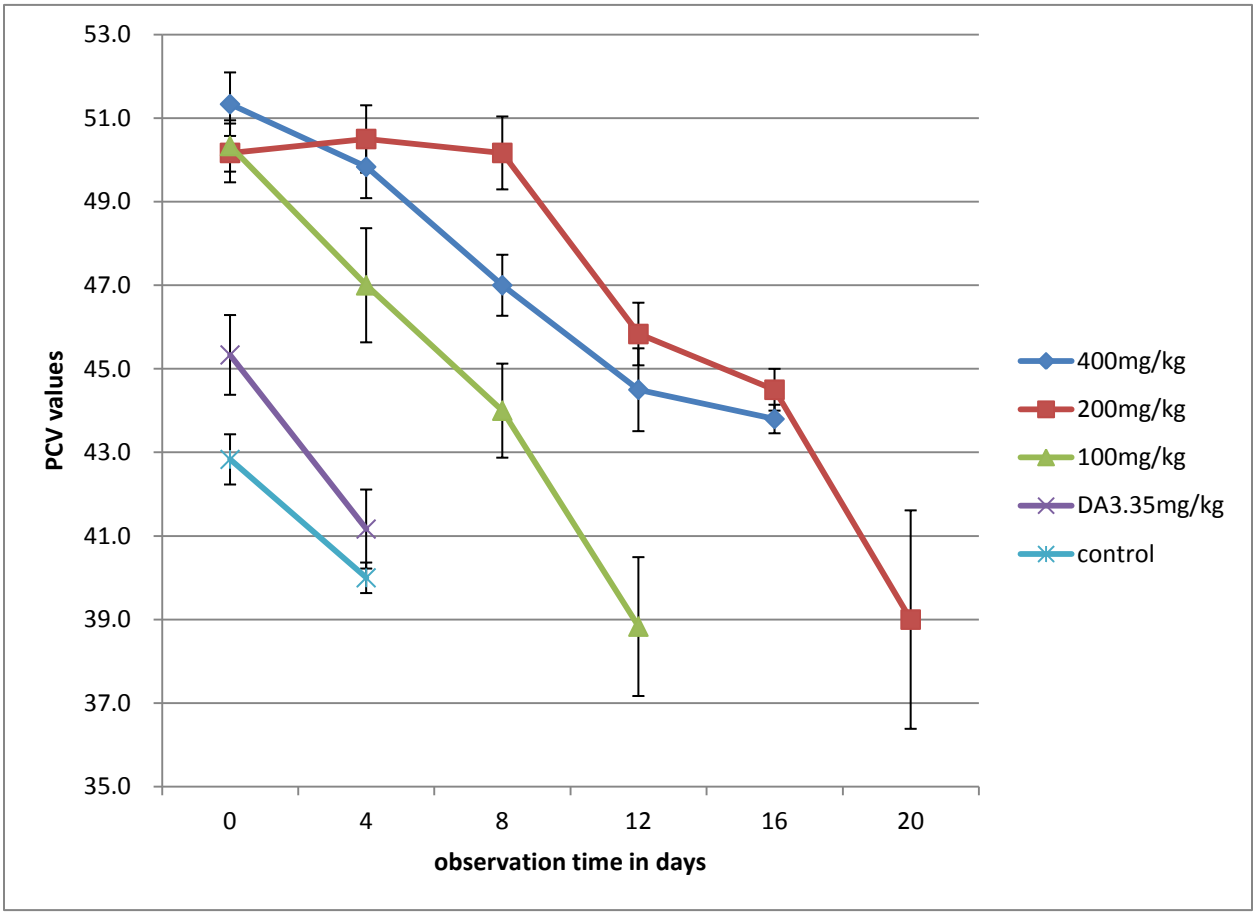

Figure 12: Effect of *E. kebericho* root extract challenged *T. congolense* infected mice on PCV

Values are expressed as mean ± SEM, Day 0=the 10th day after extract and standard drug challenged mice inoculated with infected blood

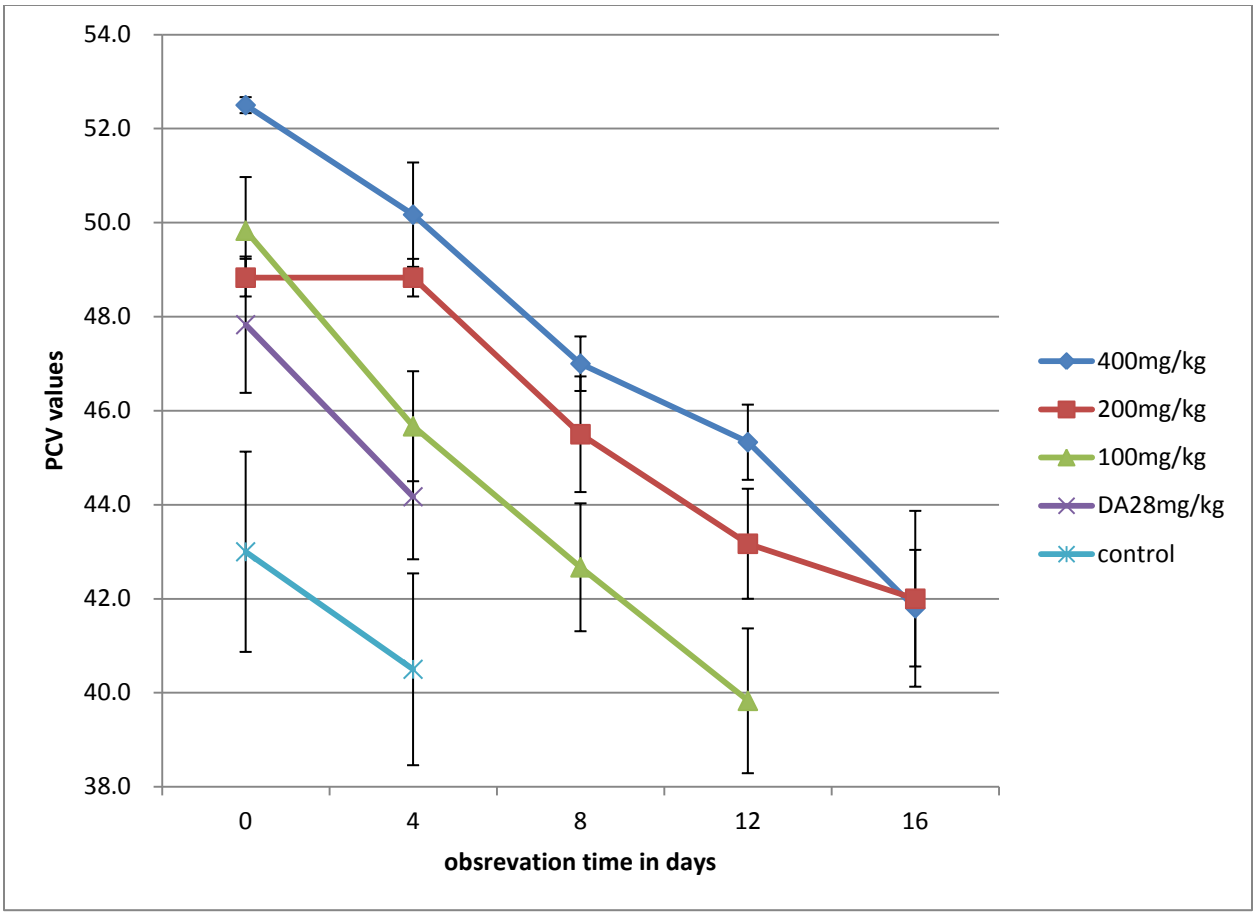

Figure 13: Effect of *S. anguivi* fruit extract challenged *T. congolense* infected mice on PCV

Values are expressed as mean ± SEM, Day 0=the 10th day after extract and standard drug challenged mice inoculated with infected blood

4.4.3. Impacts of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits prechallenge on body weight of *T. congolense* infected mice

All animals in untreated control and standard drug group died before day 7 post follow up showing gradual decrease in body weight values while all animals on extracts showed slightly prolonged time showing the extract has some chemoprophylactic effects. There is statistical significant association ($p<0.05$) in body weight between those in untreated control and standard drug prechemical challenge when compared with extracts of *E. kebericho* at 400mg/kg, 200mg/kg challenged at day 4 and between untreated control and 100mg/kg on the same day. There is statistical significant association ($p<0.05$) of body weight between those in untreated

control and pre chemical challenge when compared with those extract of *S. anguivi* challenged at 400mg/kg on day 4 post observation(figure 14 and 15).

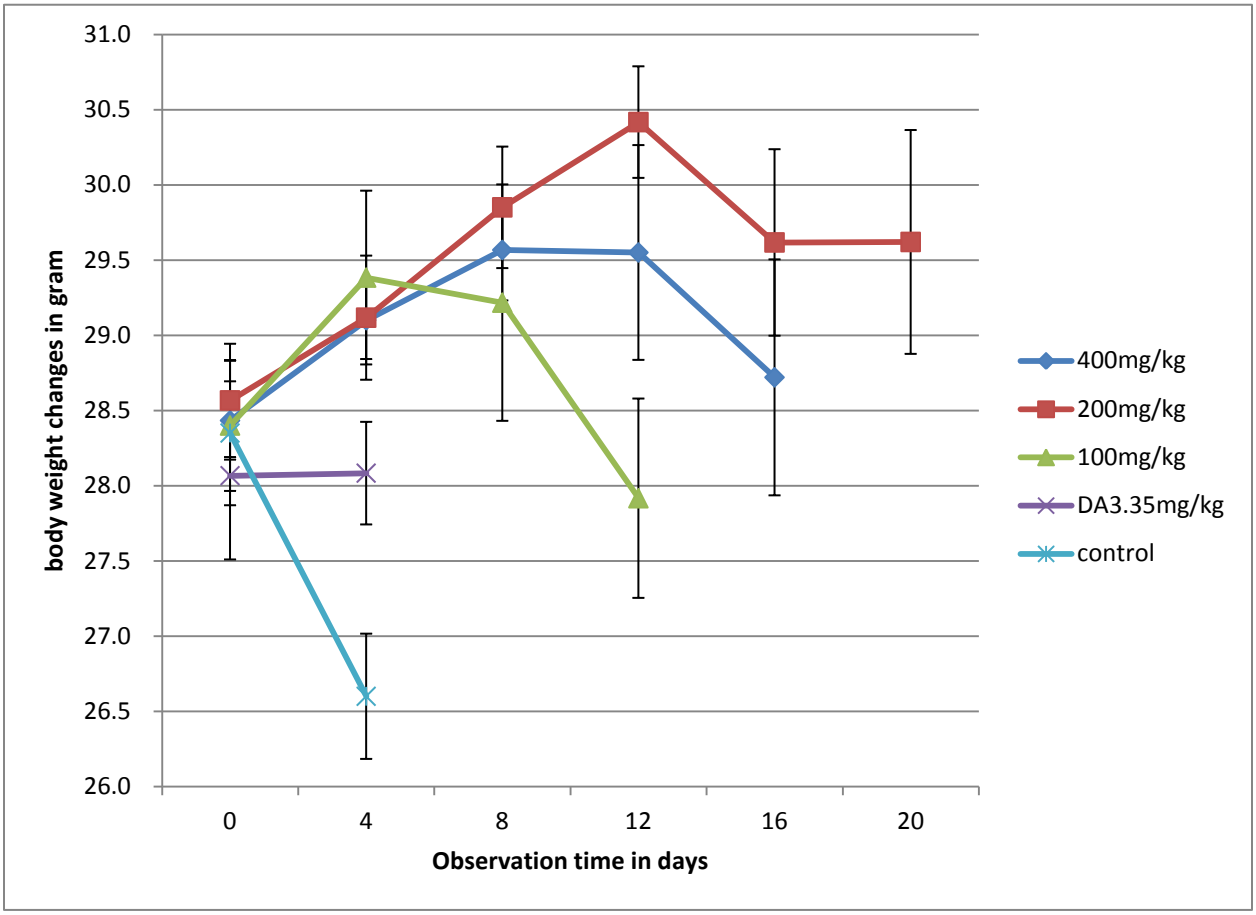

Figure 14: Effect of *E. kebericho* root extract challenged *T. congolense* infected mice on body weight

Values are expressed as mean ± SEM, Day 0=the 10th day after extract and standard drug challenged mice inoculated with infected blood

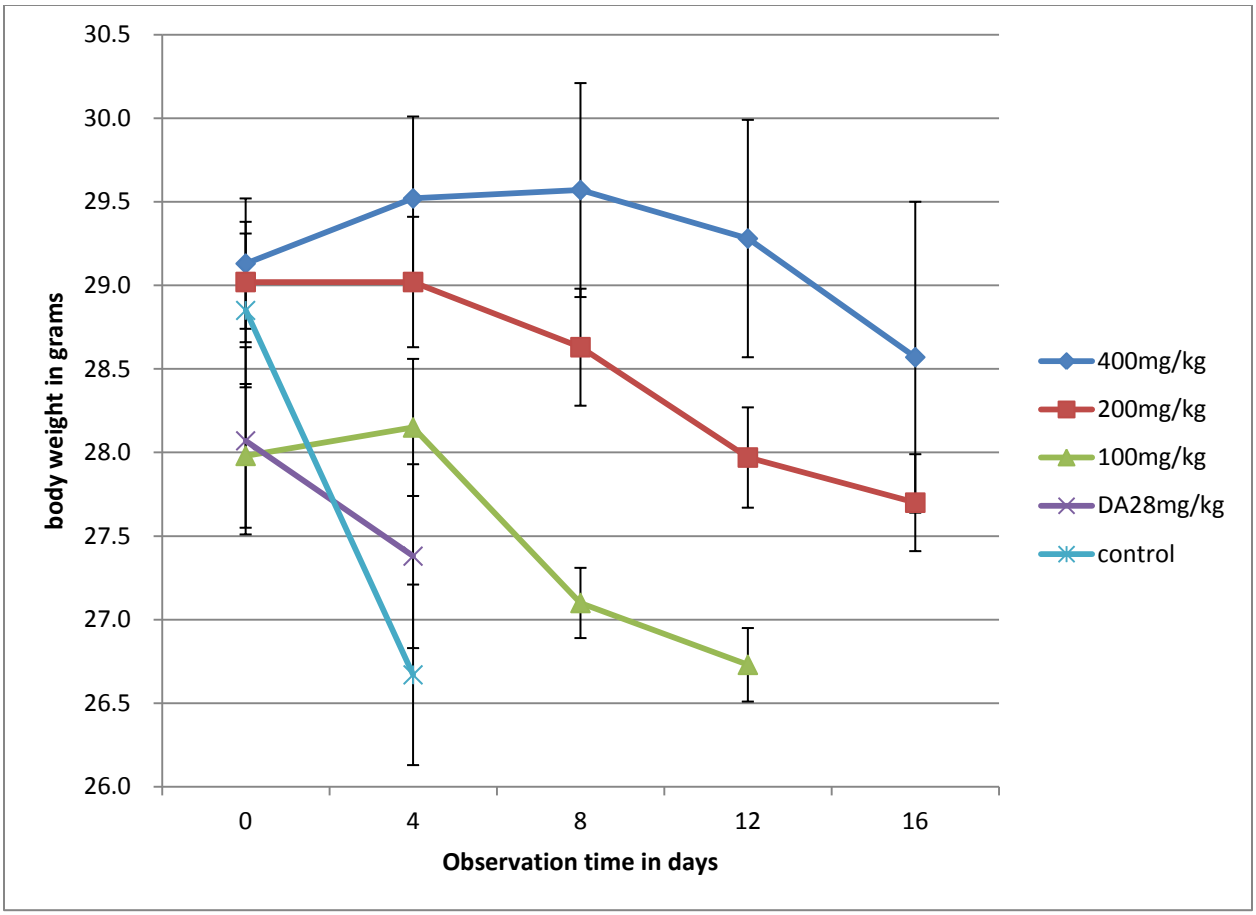

Figure 15: Effect of *S. anguivi* fruit extracts challenged *T. congolense* infected mice on body weight

Values are expressed as mean ± SEM, Day 0=the 10th day after extract and standard drug challenged mice inoculated with infected blood

4.4.4. Impacts of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits prechallenge on rectal temperatures of *T. congolense* infected mice

The pretreatment mean rectal temperature was 34.10±0.36. The temperature value shows fluctuation in all groups throughout study period (figure16)**.**

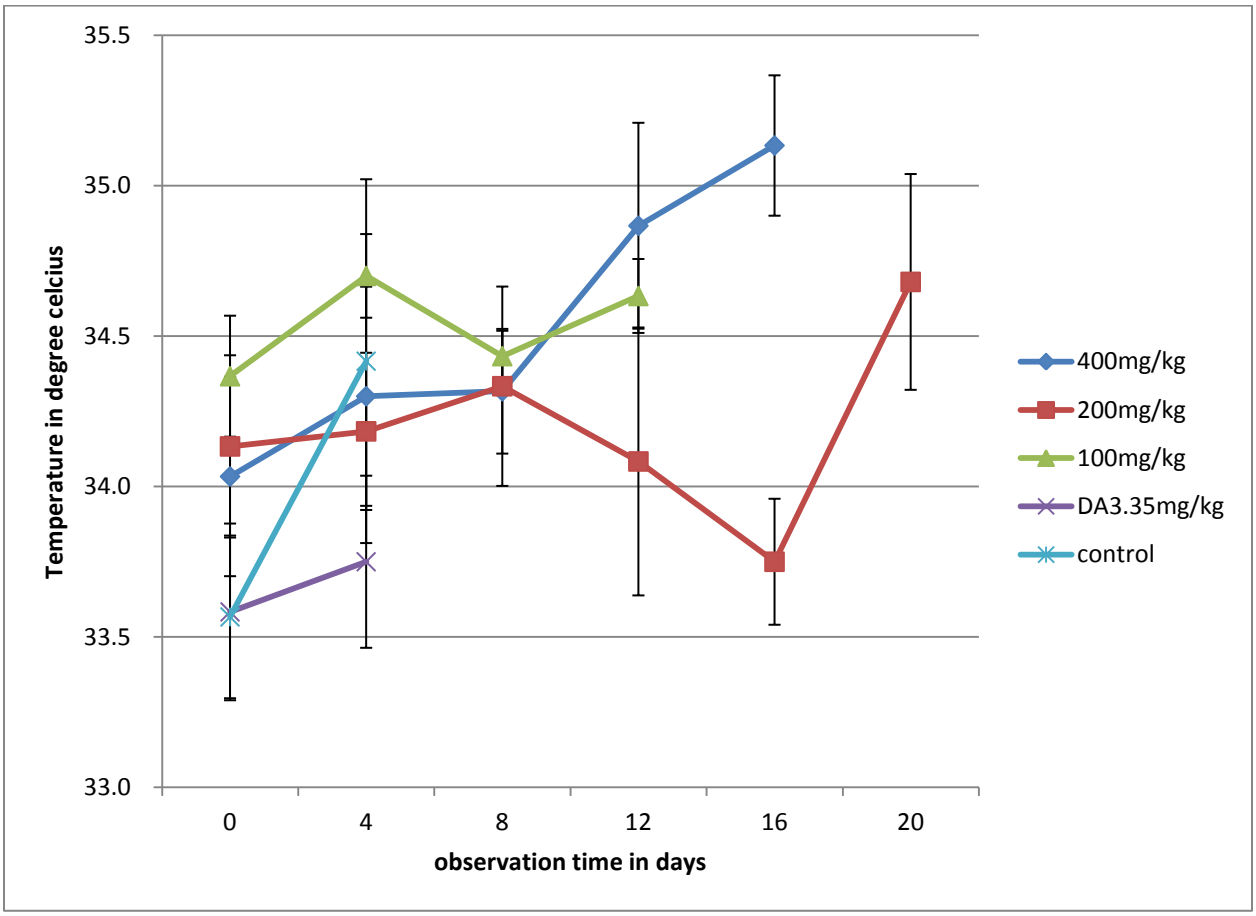

Figure 16: The effect of crude hydromethanolic extracts of *E. kebericho* on rectal temperature of pre extract and chemical challenged infected mice.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after extract and standard drug challenged mice inoculated with infected blood

The pretreatment mean rectal temperature was 34.22±0.35. The temperature value shows fluctuation in all groups throughout study period (figure17).

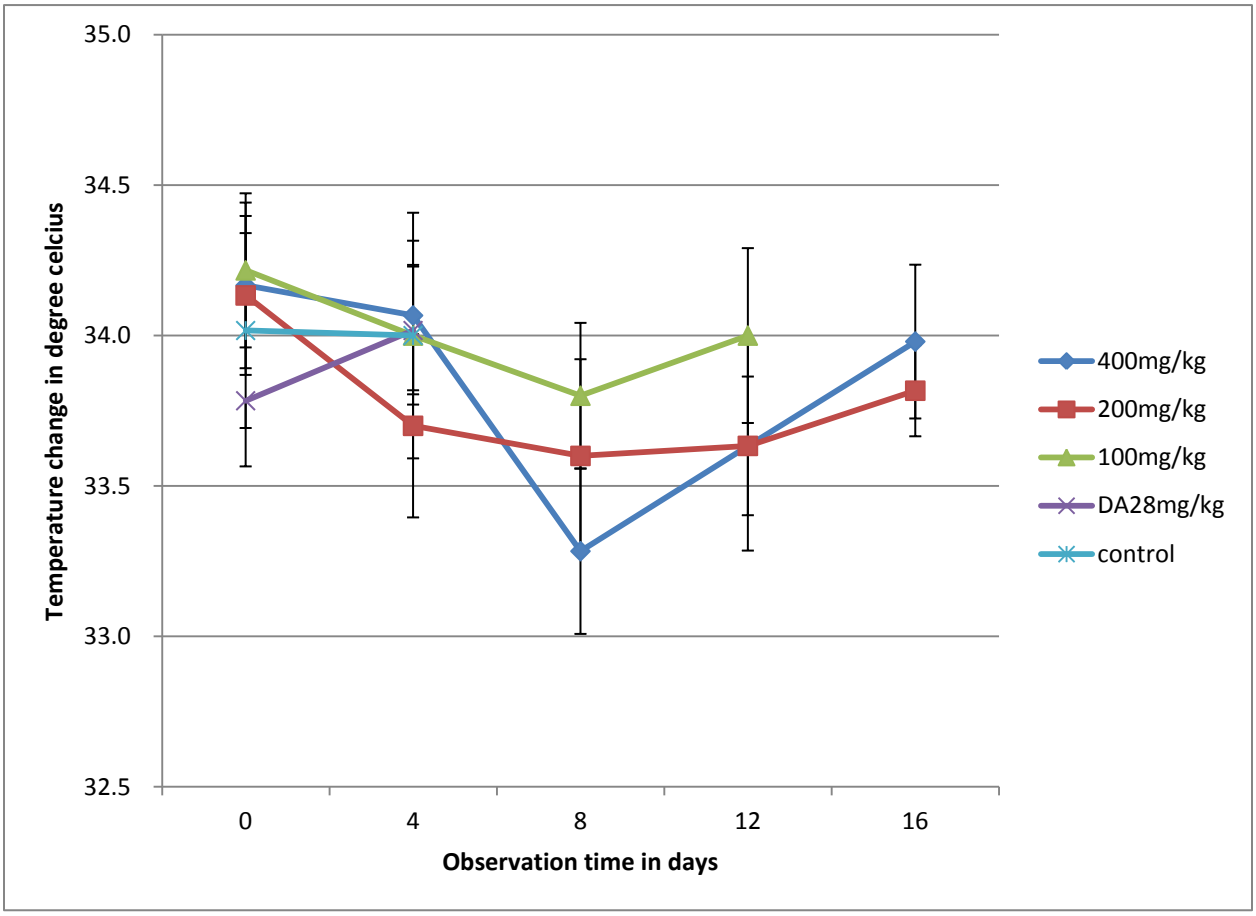

Figure 17: The effect of crude hydromethanolic extracts of *S. anguivi* on rectal temperature of pre extract and chemical challenged infected mice.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after extract and standard drug challenged mice inoculated with infected blood

4.4.5. Impacts of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits prechallenge on survival periods of *T. congolense* infected mice

Animals challenged with hydromethanolic extracts of *E. kebericho* 400mg/kg had shown higher mean survival time (27.33±.96) days while lowest survival time was noticed by hydromethanolic extracts of *S. anguivi* 100mg/kg (19.83±1.62) days from extracts. There were significance association ($p<0.05$) in survival days of infected mice beween those extracts challenged and diminazine aceturate compared with distilled water challenged groups (table 4).

Table 4: Effect of *S. anguivi* fruit and E. *Kebericho* root extracts challenged *T.congolense* infected mice on mean survival time in the prophylactic model

Dose	**Mean survival time in days**	
	E. kebericho	*S. anguivi*
100mg/kg	21.17±.83[a](19.02-23.31)	19.83±1.62[a](15.67-24.00)
200mg/kg	25.00±.78[a](23.01-26.99)	20.67±1.31[a](17.30-24.03)
400mg/kg	27.33±.96[a](24.88-29.79)	24.83±1.22[a](21.69-27.98)
DA 3.35mg/kg	16.33±.76(14.38-18.29)	17.33±1.02(14.71-19.96)
Control	14.00±.73(12.12-15.88)	14.83±.48(13.61-16.06)

Values are expressed as mean ± SEM and 95% CI (values in bracket), the mean difference is significant at the 0.05 level, control=distillled water, DA= diminazine aceturate, [a]= compared to untreated control, [c]=compared to 100 mg/kg

4.5. Evaluation of curative activity of extarcts

A curative test was performed on a total of sixty mices of either sex divided in to ten groups of six mice.

4.5.1. Effects of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits treatment on parasitemia of *T. congolense* infected mice

Preliminary screening for antitrypanosomal activity of hydomethanolic extracts of *E.kebericho* and *S. anguivi* revealed none of them completely cleared trypanosoma from blood of infected mice. The pretreatment mean parasite count for all groups was around antilog 8.01 parasites/ml of blood. The changes observed in the level of parasitemia of infected treated mice were shown in figures 18 and 19 below.

There is statistical association ($p<0.05$) in parasitemia between untreated control groups and those on the three doses of *E. kebericho* extract and the standard drug at day 4 post treatment. Treatment with hydromethanolic extract of *E. kebericho* at 200 mg/kg and 400 mg/kg and DA3.35mg/kg showed statistically significant ($p<0.05$) reduction in the level of parasitemia on day 8 to day 12 post treatments when compared to 100mg/kg body weight. Treatment with hydromethanolic extract of *S. anguivi* 100 and 200 mg/kg showed statistically significant ($p<0.05$) reduction in the level of parasitemia within day 8 to day 12 days post treatments when compared to 400 mg/kg post treatment.

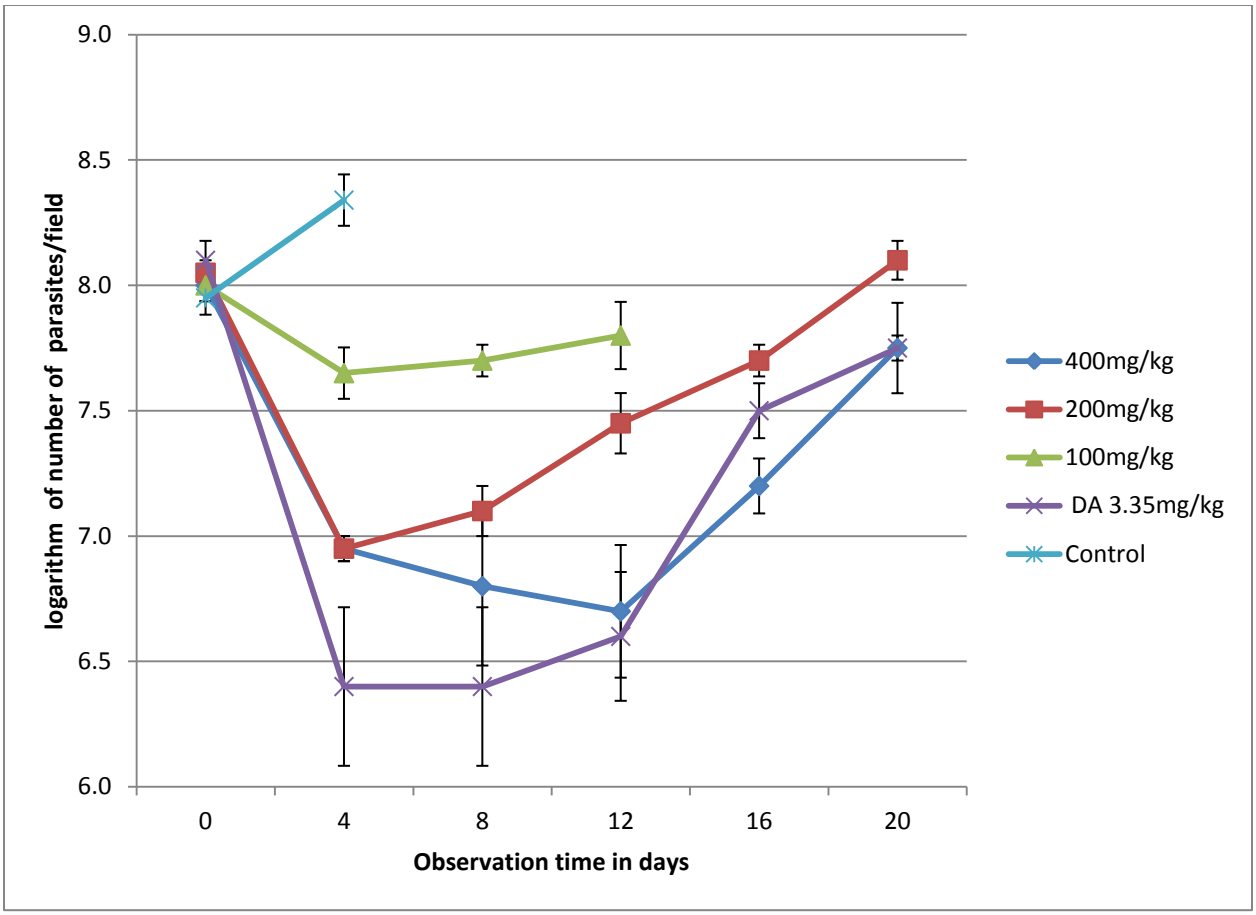

Figure 18: Effect of E. *Kebericho* root extract treatment on parasitemia of *T. congolense* infected mice

Values are expressed as mean ± SEM, Day 0=the 10th day after infected blood inoculation

Relapse was recorded with all treatment and positive control groups in which parasites started to be highly detected in blood of infected mice. There is statistical significant association ($p<0.05$) in parasitemia between those in 100mg/kg of *S. anguivi* compared with all existing groups on day8 and 12 post treatment.

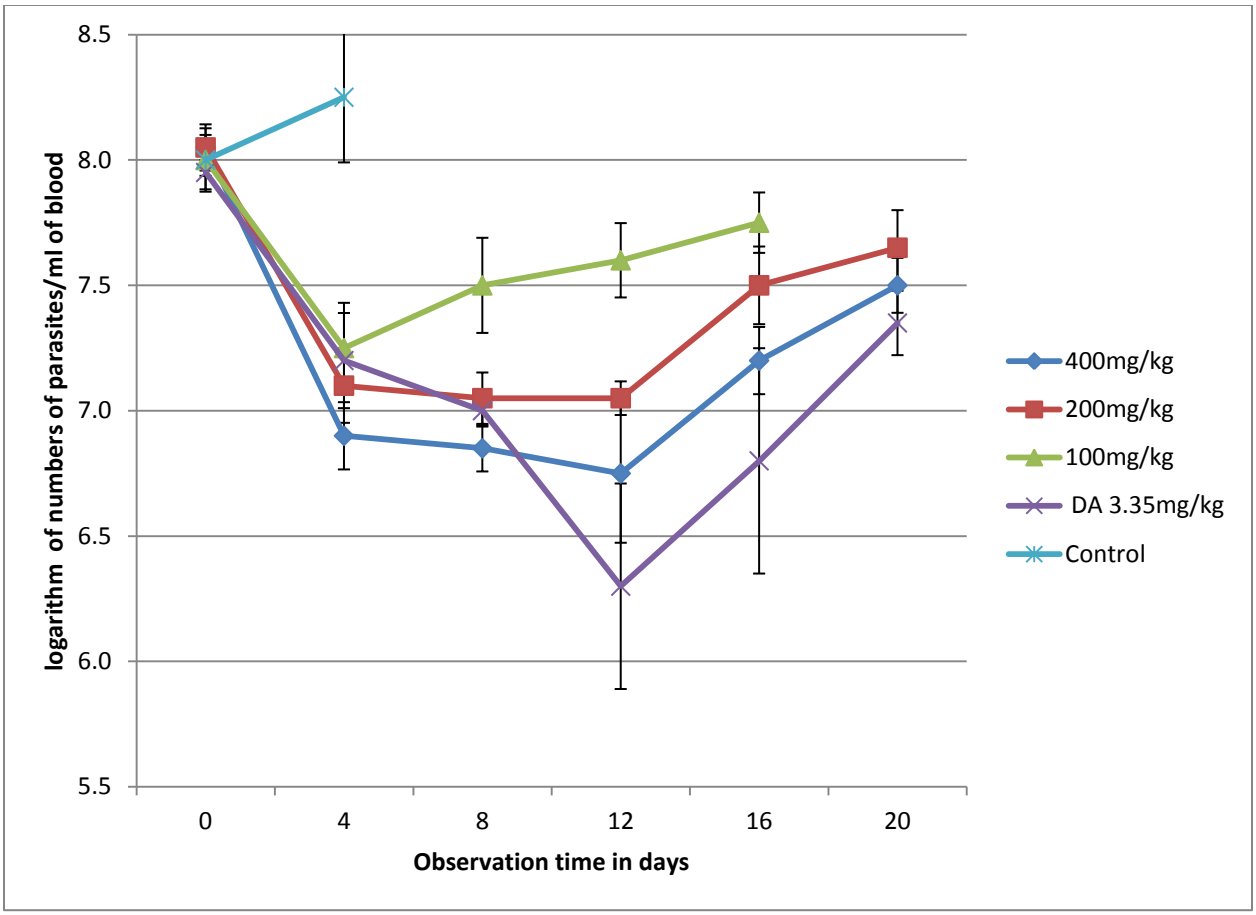

Figure 19: Effect of *S. anguivi* fruit extract treatment on parasitemia of *T. congolense* infected mice

Values are expressed as mean ± SEM, Day 0=the 10th day after infected blood inoculation

4.5.2. Effects of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits treatment on packed cell volume of *T. congolense* infected mice

While the mean PCV in the untreated control group continued to decrease until all the animals died due to infection, 200mg/kg and 400mg/kg of *E. kebericho* and DA 3.35mg/kg shows an increase in mean PCV values from day 0 to day 12 post treatment while treatment with 100mg/kg shows slight decrease until the animals died. PCV measurement in blood of infected mice treated with *E. kebericho* at dose of 200mg/kg and400mg/kg and DA 3.35mg/kg showed statistical significance ($p< 0.05$) improvement compared with those not treated (untreated control) on day 4 post treatment initiation(figure 20)

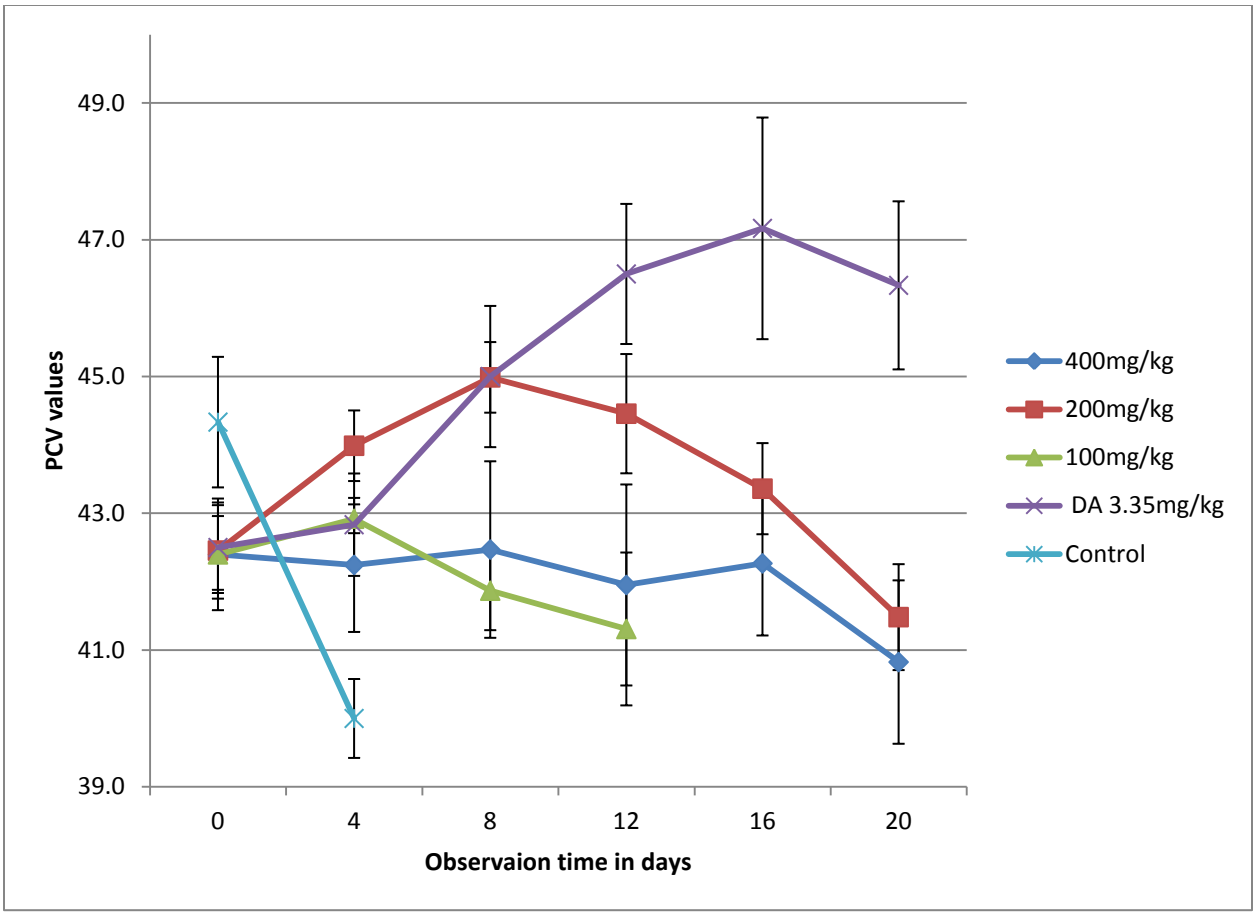

Figure 20: Effect of *E. kebericho* root extract treatment on PCV of *T. congolense* infected mice

Values are expressed as mean ± SEM, Day 0=the 10th day after infected blood inoculation

Groups of mice treated with 100, 200, 400mg/kg of *S. anguivi* and DA 3.35mg/kg showed statistical significance ($p< 0.05$) improvement in PCV measurement on day 4 post treatment initiation compared with untreated control. Groups of mice treated with 200 and 400mg/kg of *S. anguivim* and DA 3.35mg/kg showed statistically significant($p< 0.05$) improvement in PCV measurement on day 12 to day 16 post treatment initiation compared with groups treated with 100mg/kg body weight(figure 21).

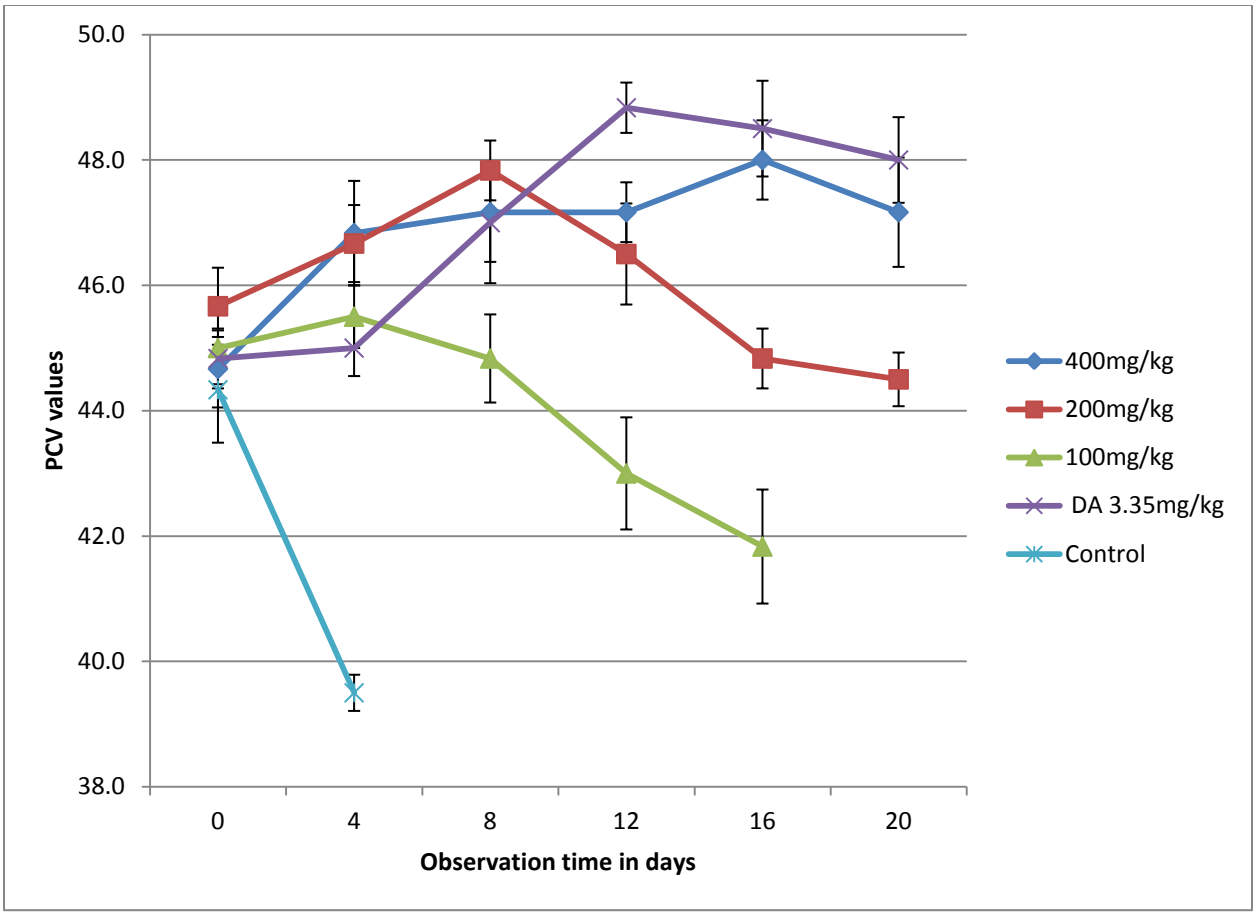

Figure 21: Effect of *S. anguivi* fruit extract treatement on PCV of *T. congolense* infected mice

Values are expressed as mean ± SEM, Day 0=the 10th day after infected blood inoculation

4.5.3. Effects of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits treatment on body weight of *T. congolense* infected mice

The patterns of the groups on mean body weight changes are presented in figures below. The mean body weight of the groups at day zero (during commencement of treatment) for all groups was 32.08. This mean body weight decrease for 100mg/kg *E. kebericho* and untreated control until all the animals in the two groups end around 14 mean days post treatment whereas the value shows an increase for 200mg/kg, 400mg/kg and diminzine aceturate during follow up period. There is statistical significance ($p<0.05$) in body weight changes between 200mg/kg, 400mg/kg and DA 3.35mg/kg compared with 100mg/kg through day 4 to day 6 post treatment initiation and with untreated control on day 4 post treatment(figure 22).

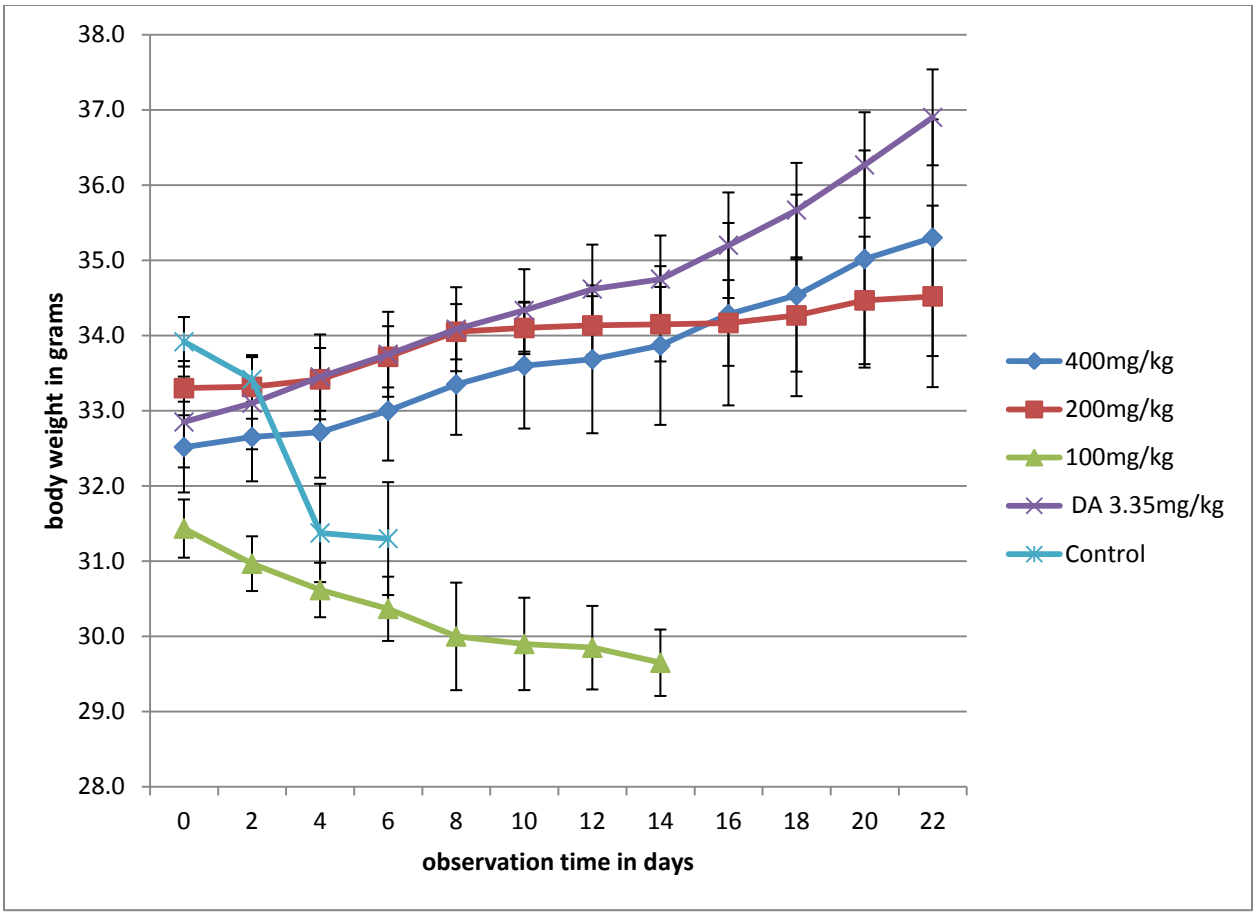

Figure 22: The effect of crude hydromethanolic extracts of *E. kebericho* root on body weight of *T. congolense* infected mice.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after infected blood inoculation

The mean body weight for *S. anguivi* indicates there is a gradual increase trough day 0 to day 12 post treatment for all treatments with gradual decrease for untreated control at which all animals in the last group end before day 7 post treatment. The result indicates that groups treated with higher doses and DA shows higher mean body weight than that of 100mg/kg body weight in which the last groups' shows slight decrease in body weight until all animals of the group died at day 18 post treatment (figure 23).

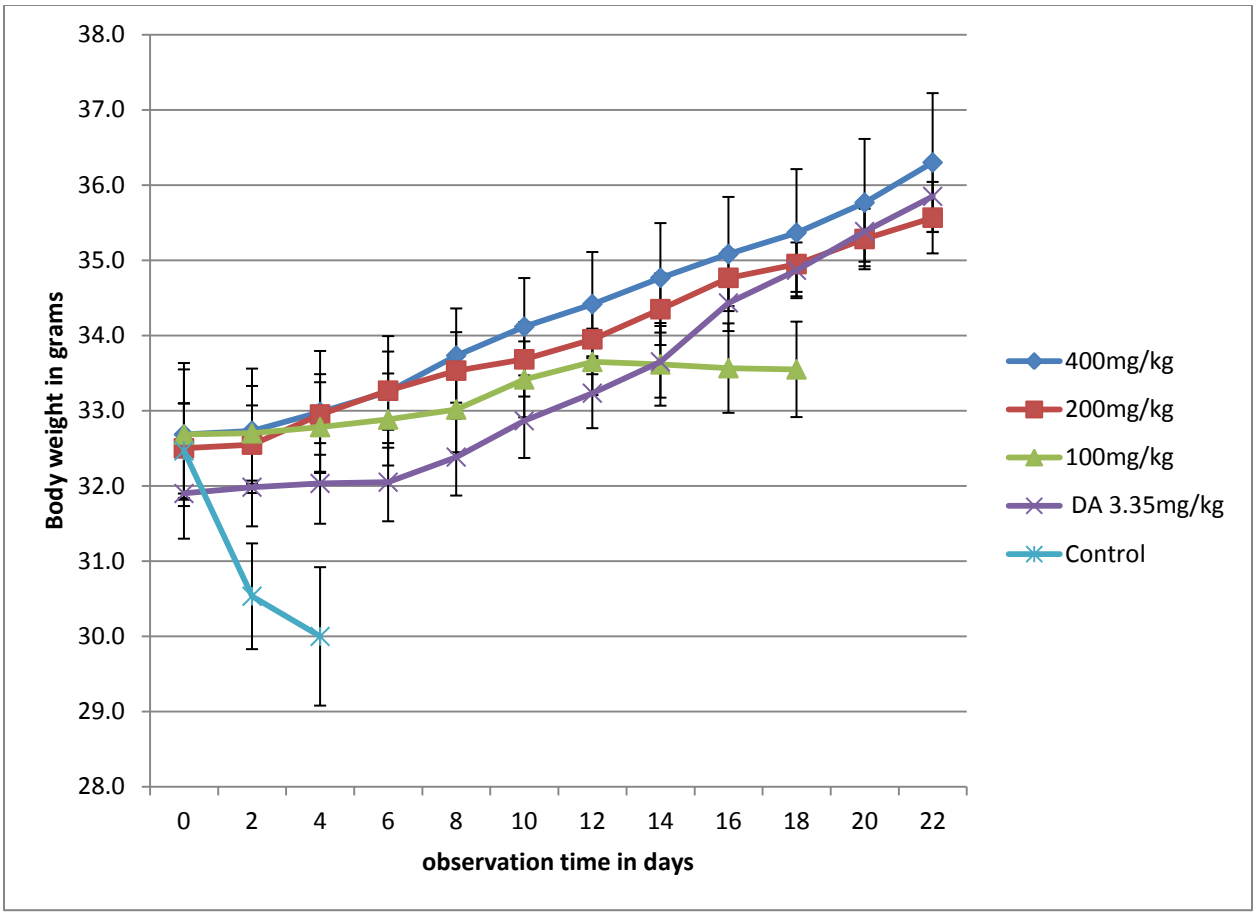

Figure 23: The effect of crude hydromethanolic extracts of *S. anguivi* fruit on body weight of *T. congolense* infected mice.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after infected blood inoculation

4.5.4. Effects of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits treatment on rectal temperature of *T. congolense* infected mice

The rectal temperatures of the animals were fluctuating throughout the experiment. There is no observed difference throughout the follow up period (figure 24 and 25).

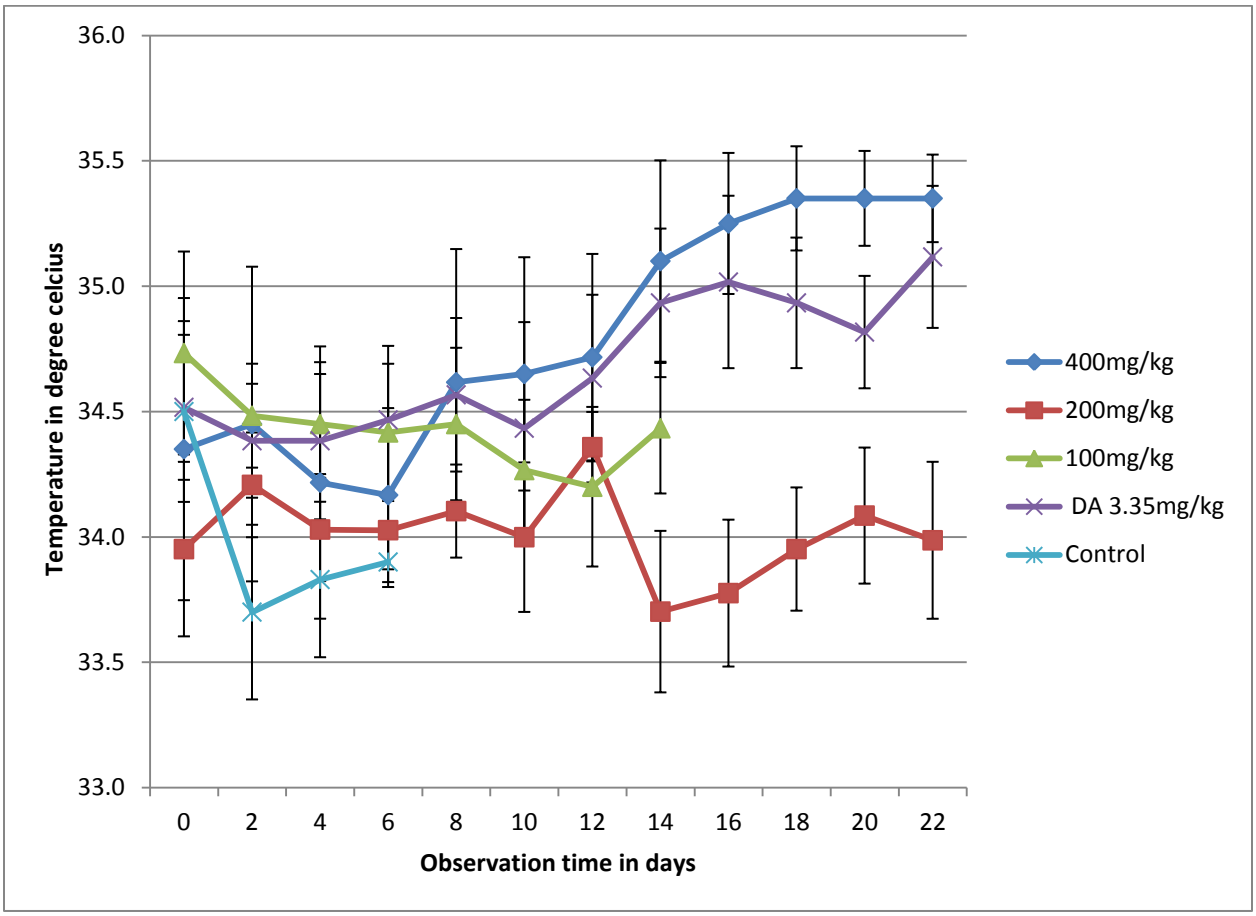

Figure 24: The effect of crude hydromethanolic extracts of *E. kebericho* root on rectal temperature of *T. congolense* infected mice.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after infected blood inoculation

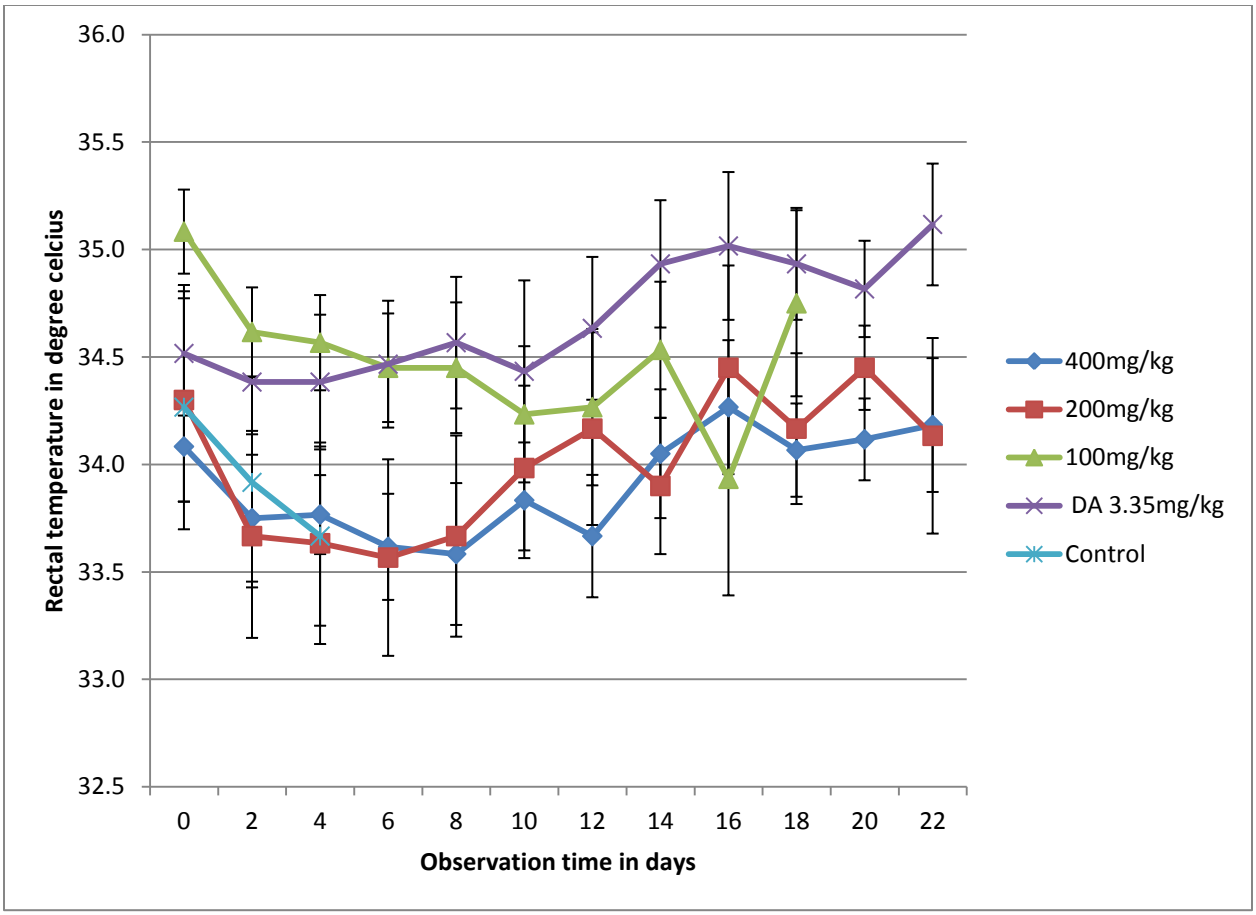

Figure 25: The effect of crude hydromethanolic extracts of *S.anguivi* fruits on rectal temperature of *T. congolense* infected mice.

Values are expressed as mean ± SEM, n=6, Day 0=the 10th day after infected blood inoculation

4.5.5. Effects of hydromethanolic extracts of *E. Kebericho* roots and *S. anguivi* fruits treatment on survival periods of *T. congolense* infected mice

Death in vehicle (non treated treated mice) started 2 days post treatment initiation with all mice end at 14.00±.73 and 14.83±.48 for *E. kebericho* root and *S. anguivi* fruits groups 44.83±.91 and 41.83±1.17 days respectively. Maximum survival period was recorded with DA3.35 mg/kg and *S. anguivi* fruits 400mg/kg body weight. The mean survival time of all groups of both extracts and DA 3.35mg/kg showed statistical significance ($p< 0.05$) increase in days compared with those not treated (untreated control) and the higher dose of both extracts and DAtreated mice

also showed statistical significance ($p< 0.05$) increase survival days as compared with groups treated with 100mg/kg body weight(table5).

Table 5: Effects of hydromethanolic extracts of *E. Kebericho* root and *S. anguivi* fruit treatment on mean survival time of *T. congolense* infected mice

Dose	**Mean survival time in days**	
	E. kebericho	*S. anguivi*
100mg/kg	25.83±.60[a](24.29-27.38)	31.83±1.14[a](28.91-34.76)
200mg/kg	30.00±.86[a](27.80-32.20)	39.67±.88[a](37.40-41.93)
400mg/kg	39.83±2.4[ac](33.63-46.04)	41.83±1.17[ac](38.83-44.83)
DA 3.35mg/kg	44.83±.91[ac](42.50-47.17)	43.00±1.21[ac](39.89-46.11)
Control	13.50±.22(12.93-14.08)	14.67±.88(12.40-16.93)

Values are expressed as mean ± SEM and 95% CI (values in bracket), the mean difference is significant at the 0.05 level, control=distillled water, DA= diminazine aceturate, [a]= compared to untreated control, [c]=compared to 100 mg/kg

4.6. Phytochemical Screening

Phytochemical screening revealed the presence of various phytochemicals in the extracts. Hydromethanol extracts of *E. kebericho* and *S.anguivi* were found to contain all the phytochemicals tested such as saponins, tannins, phenol, terpenes, flavonoids, glycosides and alkaloids were found in the extract(table 6).

Table 6. Phytochemicals in hydro methanolic extracts of *E. kebericho* roots and *S. anguivi* fruits

Secondary Metabolites	***E. kebericho***	***S. anguivi***
Glycosides	+	+
Anthraquinone	+	+
Saponins	+	+
Steroids	+	+
Tritepenes	+	+
Flavonoids	+	+
Tanins	+	+
Alkaloids	+	+
Phenols	+	+
Phlobatannins	+	+

+ = present, - = absent

4.7. Acute Toxicity

There was no death recorded at a dose of 2000mg/kg for both plant extracts. More over there was no gross behavioral changes and signs of toxicity during the observation period monitored according to OECD, 2001 guide line. The results were presented in table 7

Table 7: Acute toxicity for hydromethanol extracts of *E. kebericho* roots and *S. anguivi* fruits.

Name	***S. anguivi***		***E. kebericho***	
Type of response	Before	After	Before	After
Alertness	N	N	N	N
Convulsion	A	A	A	A
Corneal reflux	N	N	N	N
Hyper activity	A	A	A	A
Lacrimation	A	A	A	A
Salivation	N	N	N	N
Skin colour	N	N	N	N
Torch response	P	P	P	P
Tremors	A	A	A	A
Urination	N	N	N	N

N=normal, A= absent, P= present, before= before extract administration, after= after extract adminstration

5. DISCUSSIONS

For several decades trypanosomiasis has continued to contribute adversely to economic and social well being of sub Saharan Africans (WHO, 2006). Despite the enormity of health and economic implication of African trypanosomiasis current chemotherapeutic options are very limited and far from ideal for both human and livestock (Legroos *et al*, 2002; Mattoli *et al*, 2004). So the need for safer, cheaper, available sources of medication cannot be overemphasized. Literature studies and field survey have shown that plants are used in Africa and Ethiopia for management of trypanosomiasis (Mbaya and Ibrahim, 2011; Shilema *et al*, 2013, Megersa *et al*, 2013). In this regard the objectives of this study were to screen and evaluate the *in vitro* and *in vivo* antitrypanosomal activity of crude hydromethanol extracts of *E. kebericho* roots and *S. anguivi* fruits which were previously reported with its traditional use (Shilema *et al*, 2013; Megersa *et al*, 2013).

With the assumption that some of the active ingredients for the claimed antitrypanosomal activity might not adequately be soluble in water, 80% hydromethanolic extracts were selected for this particular study. The extracts gave 23.3 and 11.7% for *S. anguivi* fruits and *E. kebericho* root respectively. The results were comparable with Pascaline, 2011 and Mergia *et al*, 2016 who obtained 23.6 and 18.13 % w/w respectively.

The hydromethanol extracts of *E.kebericho* roots and *S.anguivi* fruits had ceased motility of the trypanosomes within 40 min at 4 and 2 mg/ml concentration. However the positive control diminazine aceturate immobilized motility of trypanosomes with in the first 20 min whereas in the untreated control distilled water motility continues for 160 minutes which is in agreement with finding of Nagagi *et al*, 2016 by *C. swynnertonii*. In the lowest dose of the experiment 0.5mg/ml of the hydromethanol extracts of *S. anguivi* fruits and *E. kebericho* roots the motility stays for 60- 100minutes respectively after which motility of the parasite was ceased. The extracts were able to clear the parasite in less than 100 minute at all concentrations. This finding was in agreement with the finding of (Nwosu, 2006) in which all concentration of stem bark methanolic extract of *H. acida* against *T. evansi* stop parasite motility.

Parasites motility constitutes a relatively reliable indicator of viability of most trypanosomes (Freiburghaus *et al*, 1996) and a complete elimination or reduction in motility of trypanosomes when compared to the control could be taken as index of trypanocidal activity (Wurochekke and Nok, 2004). Roots of *E. kebericho* and fruits of *S. anguivi* crude hyrdromethanolic extracts had shown considerable *in vitro* antitrypanosomal activity. The current finding of extracts at dose of 4 mg/ml which ceased motility of parasites within the 40 minutes is not in agreement with *in vitro* antitrypanosomal activity of *P. kotschi* which immobilized motility of *T. congolense* at 55 min of incubation (Atawodi *et al*, 2003).

Freiburghaus *et al*., 1997 have shown that the mean minimum inhibitory concentration value of common trypanocidal drugs is 10.7 mg/ml and that agent with minimum inhibitory concentration value between 5 – 20 mg/ml could be regarded as very active. In this study both extracts were found to be active at 2 and 4 mg/ml, this is comparable to the value reported for standard trypanocidal drug. The present finding is not in agreement with Habila *et al*, 2007 who examined the antitrypanosomal potentials of *A. indica* seeds methanolic extract against *T. evansi* at 25, 50 and 100 mg/ml and immobilized the parasites within 14 min, 8 min and 3 min respectively.

Moreover, a plant with high *in vitro* trypanocidal activity may have no *in vivo* activity and vice versa, because of peculiarities in the metabolic disposition of the plant's chemical constituents. Nevertheless and for practical purposes bioactive screening *in vitro* remains a useful method for preselection of plant for anti-trypanosomal activity (Freiburghaus *et al*., 1996). Therefore, plants found to be active in *in vitro* investigations must be tested *in vivo* before a definite statement can be made on their trypanocidal potentials (Atawodi *et al*., 2003). Umar *et al*. (2010) evaluated the *in vitro* and *in vivo* anti *T. evansi* activity of the stem bark ethanolic extract of *K. senegalensis* which supports earlier reports that some plant extracts possess *in vivo* activities against trypanosomes (Nok *et al.*, 1993).

The *in vitro* antitrypanosomal activities of both extracts were confirmed by blood incubation infectivity. All the untreated control mice dead within mean of 5 days post observation. While all mice inoculated with inoculum mixtures and standard drug did not develop infection during the observation period. This result was in agreement with finding of Tewabe *et al*, 2014 who

reported that test substances inhibited healthy mice from developing infection for more than 30 days. Yusuf *et al*, 2012, suggested that complete immobility of the parasites *in vitro* may not necessarily indicate that the parasites were dead, but rather the parasites may have lost their infectivity.

The present result might not be in agreement with Mergia *et al*., 2014 and Nagagi *et al*, 2016 who reported that the mice which received the test concentrations containing 4 and 2 mg/ml methanol extract of *V. sinaiticum and C. swynnertonii* lost infectivity to some of the animals and had only prolonged establishment of infection. Loss of infectivity may be by abrogating some vital metabolic processes in the parasites or could be due to some morphological changes on the parasites induced by the extract that render them more susceptible to the mice immune systems. The complete absence of infection from *in vitro* mixtures of both extracts at the tested concentration showed better promise than finding of Feyera *et al*, 2011 and Yusuf *et al*, 2012 who reported prolongation of the pre patent period of animals inoculated with the *in vitro* mixtures for more than 16 days.

The results of this current study have also indicated that the trypanocidal activity of the hydromethanolic extract which was expressed in the *in vitro* compared to the *in vivo* study. The observed antitrypanosomal activity of the plant extracts confirm earlier *in vivo* and *in vitro* studies which suggest that plant extracts could contain potent trypanocidal constituents (Atawodi *et al*, 2003; Owolabi *et al*, 1990; Igweh and Onabanjo, 1989; Freiburghaus *et al*, 1997; Freiburghaus *et al*, 1998; Asuzu and Chineme, 1990). However, it is not possible to compare many of current results with those of earlier reports because the plant investigated here was not previously studied for trypanocidal activity, although the use of the plant in the traditional management of trypanosomiasis have been reported (Shilema *et al*, 2013; Megersa *et al*, 2013).

The present study also showed that prophylactic effect of hydromethanol extracts of of *E. kebericho* roots and *S. anguivi* fruits show significant ($P<0.05$) parasitemia chemosuppression effect at day 0 and day 4 as compared to positive and control group. Prophylactic tests which evaluate the chemosuppression capability of the candidate extract prior to infection to prevent the upcoming disease are not commonly used for antitrypanosoma drug screening.

In the prophylactic studies of the hydromethanolic extract of roots of *E. kebericho* and fruits of *S. anguivi* against *T. congolense* it was observed that the groups previously injected with the extract had got prolonged life before expressing parasitaemia for up to 13 days as compared to non chemical challenged group which developed parasitemia at 5-8 days. This finding is in line with findings of Abedo *et al*, 2013 who reported that administering extracts of *P. guineense* for Trypanocidal activities against *T. b. brucei* for five days to mice at 50 mg/kg and 100 mg/kg intraperitoneally and orally to groups respectively did not lead to the development of parasiteamia. He also stated the groups that were given 20 mg/kg of extracts of *P. guineense* intraperitoneally and 50 mg/kg orally came down with the disease on the 6th and 4th day respectively (Abedo *et al*, 2013). This may be due to the fact that the animals in the group after receiving the extracts developed a slight resistance state for *T. congolense* even before they were challenged with the parasite. The prolonged administration of the extract for 5 days may have increased the immune response mechanism of the animals, which enable the immune system of the animals to combat and resist the expression of *T. congolense.*

In these experiments, the observed prepatent period of *T. congolense* in mice was 5-8 days. Experimental inoculation of *T. congolense* in mice recorded a prepatent period of 3 days (Nok, 2002) where as a prepatent period of 4-5 days in mice infected with *T. brucei* (Umar *et al*, 2007). Generally, *T. congolense* has a longer prepatent period than *T. brucei*. For example, mice infected intraperitoneally with *T. b. bruceiand T.congolense* organisms were parasitaemic after two days post infection (Kubata *et al*., 2005 and Kobo *et al*, 2014). Length of prepatent period is determined by the strain of the parasite in question and host immune status. Different strains of *T. congolense* differ in their pathogenicity (Bengaly *et al*., 2002). The parasitemia observed in the current experiment rose progressively without any period of drop in untreated control groups, which indicates an acute phase of the disease.

In vivo anti trypanosomal effect showed that at 200 and 400mg/kg doses of both extracts and 100mg/kg of *S. anguivi* after highly decreasing the parasite, the survival days of the animals had increased compared to untreated control and 100mg/kg of *E. kebericho*. Preliminary screening for antitrypanosomal activity of both extracts revealed none of them completely cleared trypanosome from blood of infected mice but highly reduce parasite load and prolong survival

time. The reason why the parasite is decreased in the blood could be as a result of access of the extract to the parasites in the blood. Mergia *et al*, 2014 reported the plant extracts did not completely eliminate parasites from the blood stream of infected mice, but only reduced the level of parasitaemia which is also in line with finding of Justina *et al*, 2015. The parasites load was reduced in extract treated groups especially during treatment week as compared with untreated control. This is in agreement with finding of Obah *et al.*, 2013 who reported significant reduction in the parasitemia of treated mice compared to that of untreated ones. Mergia *et al*, 2016 also concluded reduction of parasitaemia showed variation among the administered doses of the extracts of *V. sinaiticum* with the animals treated at 100, 200 and 400 mg/kg had kept parasitaemia at a significantly low level as compared with the untreated control.

This current finding is in line with finding of Tadesse *et al*, 2015 who reported that treatment with the extracts of *D. abyssinica* resulted in reduction in the level of parasitemia and Kifleyohannes *et al*, 2014 who reported animals treated with extracts of *A. absinthium* and *M. stenopetala* reduce parasite count approximately half that of the non-treated control group. Ene *et al*, 2009 and Ngure *et al*, 2009 reported active ingredients from *A. maciverae and A.indica* significantly reduce parasitemia with a dose-dependent effect.

The consistent parasitaemic suppression combined with prolonged time of survival of the experimental mice displayed by the *in vivo* test might be due to the ability of the extract to inhibit glycolysis, which is the major source of energy for the blood stream forms of trypanosome. However, the inability of the extracts at 100 mg/kg to produce curative effects might have resulted from inadequate tissue concentrations of the active components of the extracts or the high distribution of the parasite in animals (Fairlamb, 1982).

The mean PCV in the untreated control group continued to decrease until all the animals in the group died due to infection which is in agreement with findings of Obah *et al.*, 2013 who reported significant reduction in the parasitemia of treated mice compared to that of untreated groups. The decrease in value for untreated control may be as a result of anaemia which is the most outstanding clinical and laboratory feature of African trypanosomiasis (Suliman and

Fieldman, 1989) and the primary cause of death (Losos and Ikede, 1972; Mamo and Holmes, 1975).

The PCV of the mice treated with the extracts and DA stayed with in constant range and showed statistically significant ($P<0.05$) as compared to untreated control. The result shows that the crude hydromethanolic extract of both plants has the capacity to improve the PCV even if it decline after relapse of parasites. The finding is in agreement with finding of (Abubakar *et al.*, 2005) who reported *M. balsamina* and *S. longipenduculata* possess the highest antitrypanosomal potential since they are able to control anemia by resisting sudden drop in PCV values. Inabo and Fathuddin, 2011 and Feyera *et al*, 2014 also reported extracts to improve the PCV possibly by reducing the parasite load or inactivating the toxic metabolites produced by trypanosomes. The observed antitrypanosomal effect of the extracts in this study was accompanied by prevention of further drop in PCV suggesting that they have potentials to ameliorate anaemia though their was relapse.

The mean PCV between standard drug and different doses of the extract were relatively comparable which is in agreement with Kifleyohannes *et al*, 2014 and Tadesse *et al*, 2015 who studied on *A. absinthium* and *D. abyssinica* respectively in which PCV level is significantly improved with comparable potential to diminazene aceturate since they are able to control anemia, especially at the mid stages of the infection, by minimizing drops in PCV values.

The prevention of drop in PCV is dependent on the dose of the extract in those groups which survived upto the end of experiment. This finding is in agreement with Ngure *et al*, 2009 and Mergia *et al*, 2014 who reported *A. indica* and *C. abyssinica* show significant improvement from decrease in the drop of PCV which is dose-dependent. This could possibly be by reducing the proliferating parasite load, neutralizing the toxic metabolites produced by trypanosomes or scavenging the trypanosome associated free radicals (Ogoti *et al*, 2009, Ekanem *et al*, 2009, Mpiana *et al*, 2007). Animals treated with a higher dose (400 mg/kg) of the aqueous and methanol extract of *V. sinaiticum* and had a higher PCV value as compared to the untreated control groups at the end of the observation period (Mergia *et al*, 2016). The activities of both extracts are in agreement with the findings of some other works that have shown that extracts of

different plants may exhibit antitrypanosomal activity (Freiburghaus *et al.,* 1996; Atawodi *et al.,* 2003). The antitrypanosomal effect shown by these extracts might be attributed to the presence of phytochemicals (Asres *et al.,* 2001).

The mean body weight for *S. anguivi* treated mice indicates there is a gradual increase trough day 0 to day 12 post treatment for all treatments with gradual decrease for untreated control at which all animals in the last group end before day 7 post treatment. The result indicates that groups treated with higher doses and DA shows higher mean body weight than that of 100mg/kg body weight in which the last groups' shows slight decrease in body weight. This is in agreement with finding of Kobo *et al,* 2014, who reported slight increase in body weight and Feyera *et al,* 2014 who reported treatment with the crude extracts of *Z. officinale* prevented loss of weight associated with parasitaemia. Kifleyohannes *et al,* 2014 also reported that the weight in the untreated infected mice group started to decrease after 12 days post infection till all the mice died by day18where as those standard drug and extract of *A. absinthium* and *M. stenopetala* treated mice generally showed a gradual increase in mean weight until the end of the experimental period. The finding is also in agreement with finding of Tadesse *et al,* 2015 who reported, treatment with the crude extracts prevented loss in body weights particularly at higher doses but the report is not agreement with reports of Ngure *et al,* 2009 who reported extact of *A. indica* and suramin-treated groups had significant decline in body weight. The aqueous and methanol extracts of *V. sinaiticum* were capable of improving body weight of treated animals on days 8–14 as compared to the untreated control group (Mergia *et al,* 2016)

In blood incubation infectivity model all animals in untreated control group showed decrease in body weight while all animals on both extract inoculums of different doses and standard drug showed normal body weight gain indicating the extracts have good *in vitro* effects. In prophylactic tests the body weight of animals on extracts of *E. kebericho* and *S. anguivi* at 200 and 400 mg/kg showed prevention of sudden drop in body weight compared to untreated control indicating extracts have some chemoprophylactic effects. The mean body weight of 32.08g for all groups during commencement of treatment decrease for 100mg/kg *E. kebericho* and untreated control but show normal patterns for 200mg/kg, 400mg/kg and DA 3.35mg/kg in curative tests indicating the extracts have curative values. Generally there is no statistical observable

difference in weight gain between the extract and standard drug treated groups which are in agreement with finding of Feyera *et al*, 2014 who reported no detectable differences in preventing weight loss associated with parasitaemia between the extracts as well as between the extracts and standard drug.

The result of study indicates that there is significant ($p<0.05$) increase in mean survival time of groups treated 100mg/kg, 200 mg/kg and 400 mg/kg hydromethanolic extract of *E. kebericho* and *S. anguivim* and DA3.35mg/kg compared with untreated control. The mean survival time was shortest with untreated control and higher with standard drug and *S. anguivi* extracts at 400mg/kg. This finding was in agreement with results of Tadesse *et al*, 2015 and Kifleyohannes *et al*, 204 who reported similar survival time for extracts and standard drug. Olukunle *et al*, 2010 reported rats treated with *M. morindiodes* root bark extracts had the highest mean survival time of 12.60±0.7 days which is lower life than present finding. The prolongation of lives of treated animals may therefore be associated with the ability of the extract to improve the PCV possibly by reducing the parasite load or inactivating the toxic metabolites produced by trypanosomes (Inabo and Fathuddin, 2011; Maikai, 2011; Wurochekke and Anyanwu; 2012).

The hydromethanol extracts of *E. kebericho* roots and *S. anguivi* fruits were found to contain saponins, tannins, phenol, terpenes, flavonoids, glycosides and alkaloids. Preliminary phytochemical screening of potent plants against trypanosome showed the presence of these known bio-active compounds in the crude plant extracts tested (Freiburghaus *et al.*, 1996). The presence of these secondary metabolites in of roots of *E. kebericho* was agreement with the report of Johnson *et al* 2010 in the same plant. The stem, fruits, roots, flowers and leaves of *S. anguivi* contain alkaloids, solamargine and solasoline (Chopra *et al*, 1994).

It has been known that flavonoids and flavonoid-derived plant natural products are effective antitrypanosomal substances against different trypanosome species (Hoet *et al*, 2004; Harborne and Williams, 2000). Phenolics and polyphenols have been reported in the literature to have antitrypanosomal potential by inhibiting the trypanosome alternative oxidase (Yabu *et al*, 2003). Similarly Adeyemi *et al.*, 2009 have showed that *Psidium guajava* leaf extract has trypanocidal properties and has attributed these effects in parts to the broad antimicrobial and iron chelating activity of flavonoids and tannins respectively. They have also proposed that iron chelation is an

effective way of killing trypanosomes and the prime target is the enzyme ribonucleotide reductase whose activity is central to DNA synthesis prior to cell division as depicted in trypanosomiasis infection. Saponins from *S. anguivi* fruits exhibit free radical scavenging activities that possess reducing power, potent antioxidant and iron chelating ability making it an excellent candidate in the treatment of diseases in which reactive oxygen species has been implicated (Elekofehinti *et al*, 2013).

Numerous *in vitro* and *in vivo* studies conducted on the antitrypanosomal activities of the class of compounds listed above reported the potential of each class of compounds in killing or inhibiting the growth of wide ranges of trypanosomes (Wurochekke *et al*, 2014). Hence, further in-depth investigations should be carried out to resolve this issue. In the treatment of trypanosomiasis, although the particular pure compound responsible for the antitrypanosomal activity was not purified, the anti trypanosomal properties may be as a result of these compounds acting individually or synergistically to bring about the observed trypanosuppression activity (Hoet *et al,* 2004). Therefore, the observed *in vitro* and *in vivo* antitrypanosomal activity of roots of *E. kebericho* and fruits of *S. anguivi* might be attributed to either the individual class of compounds present in each plant or to the synergistic effect that each class of compounds exert to give the observed biological activity.

Nonetheless, several investigations tend to suggest that it is often difficult to probe the exact mode of action by which these plants extracts exhibit their trypanocidal action. Indeed, the possible mechanisms by which these plant extracts and phytochemicals there in carry out this role remain a subject of great speculations and debate in the scientific community. However, accumulated evidence (Supulveda–Boza and Cassels, 1996, Atawodi *et al*, 2003; Antia *et al*, 2009; Joseph *et al*, 2011) suggest that many natural products exhibit their trypanocidal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite. Respiration of trypanosomes is obligatory for their motility as well as for managing the energy reserve required

for the synthesis of the variable surface glycoproteins. The inhibition of cellular and mitochondrial respiration by any chemotherapeutic agent will obviously compromise all the energy dependent processes. This was confirmed by the microscopy of the trypanosomes, which showed a cessation or reduction in motility after incubation with different concentrations of extracts (Nok, 2002).

It has also been reported that existing human trypanocidal drugs exert their therapeutic action through a variety of mechanisms while arsenic compounds poison the cells by acting on glucose catabolism through glutathione, suramine targets glycolysis in the glycosomes, while pentamidine and other diamines disrupt the kinetoplast, which may also interfere with polyamine synthesis, other drugs like eflornithine are selective inhibitors of ornithine decarboxylase, depleting the biosynthesis of polyamines such as spermidine, a precursor of trypanthione (Nok *et al.,* 1993; Atawodi, 2004).

The results of the acute toxicity study revealed that hydromethanolic extract of roots of *E. kebericho* and fruits of *S. anguivi* are non-toxic since no treatment-related signs of toxicity were noticed in the animals throughout the observation period at dose of 2000mg/kg. The present finding is in agreement with results of Shiferie and Shibeshi, 2013 who reported oral administration of the aqueous root extract of *E. kebericho* produced neither significant toxic signs nor death during the observation period of 14 days after a single administration of 2000 mg/kg with oral LD50 greater than 2000 mg/kg in mice where as the administration of graded doses of hydro alcoholic extract of *E. kebericho* (up to a dose of 5,000 mg/kg) did not produce sign of toxicity with LD_{50} greater than 5 g/kg body weight in mice(Toma *et al* , 2015).

Clarke and Clarke (1977) had reported that a substance has low toxicity if its LD50 is 1000 mg/kg body weight. Similarly, Lorke (1983) classified substances as slightly toxic if their LD50 range from 100 to 1000 mg/kg body weight. According to Garner and coworkers, any compound or drug with an oral LD50 estimate greater than 1,000 mg/kg could be considered low toxic and safe (Garner and Clarke, 1977).

6. CONCLUSIONS

The present study provides strong evidence of the potential beneficial effects of phytotherapy in the traditional management of trypanosomiasis, which need to be subsequently developed into a cost effective alternative medicine to complement treatment of trypanosomiasis and hence reduce its economic and health impacts. The hydromethanolic extracts of both plants have a good safety of margin suggesting their ethnopharmacological usefulness and phytochemical screening reveals the presence of important secondary metabolities.

The observed *in vitro* and *in vivo* anti tyrpanosomal activity of *E. kebericho* roots and *S. anguivi* fruits supports earlier reports that some plant extracts possess activity against trypanosomes which also supports the scientific basis for the traditional use of both plants in the management of trypanosomiasis. In conclusion considering the effects of the plant extracts in this study and the need for appropriate control measures against trypanosomiasis the studied plants can be said to have anti trypanosomal activity on *T. congolense* and said to be a potential source of new drug in the treatment of trypanosomiasis.

7. RECOMMENDATIONS

- Further work is needed to be done in order to isolate and purify specific compounds responsible for the antitrypanosomal activity of the studied plant.
- The crude hydromethanolic extract and solvent fractions of roots of *E. kebericho* and fruits of *S. anguivi* should be tested on other trypanosomes species.
- Although the plants are safe under preliminary studies further investigation on toxicological issues have to be confirmed in repeated administration of the extracts beyond this
- The effects of the extracts on blood parameters other than PCV need to be investigated.

8. LIMITATIONS OF THE STUDY

The main aim of this study was to evaluate the use of selected ethnomedicinal plants to treat trypanosomiasis in traditional settings. In traditional settings, plant extracts preparation is a complex process that involves performance of rituals and the combination of different plant species to develop effective treatment concoctions. This study was limited to the therapeutic activity of two plant species and it disregards other aspects that are used in traditional settings to mix extracts. For these reasons, the conclusions drawn from this study about the efficacy of the selected plants are not a representation of the whole traditional healing system. Secondly, crude extracts comprises a mixture of compounds. Hence; the conclusions about the efficacy of the selected plants do not reflect their activity in clinical settings.

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10. ANNEX

Annex 1: Table of Rapid "Matching"Method for Estimating the Host's Parasitemia

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				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
20 fields	10 fields	5 fields			
			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		

[a] The base 10 logarithm value for the concentration of organisms per milliliter of blood for the reference point (the circle containing 32 "organisms") was obtained from the regression line in Fig. 2.

Statement of declaration

I, the undersigned, declare that this MSc thesis is my original work, it has not been presented for a degree in this and any other University and that all sources of materials used for this thesis have been duly acknowledged. I affirm that I have cited and referenced all sources used in this document. Every effort has been made to avoid any plagiarism in the preparation of this thesis.

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