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Stechnolock Veterinary Science
Research Article

In Vivo Evaluation of Trypanocidal Drugs Resistance and Activity of Hypoxis Villosa and Eriosema Montanum (African Potato) Extracts on Field Isolates of Trypanosoma Congolense

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Citation: Abebe Bulcha, Takele Beyene Tufa, Sultan Suleman, Fufa Abunna, Fikru Regassa (2021) In Vivo Evaluation of Trypanocidal Drugs Resistance and Activity of Hypoxis Villosa and Eriosema Montanum (African Potato) Extracts on Field Isolates of Trypanosom Congolense. Vet Sci 1: 1-13

ABSTRACT

Ethno-pharmacological relevance: Bovine trypanosomosis is the major problem of sub-Saharan Africa where trypanocidal drugs have been extensively used to treat the disease and drug resistance is quite common. Ethno-veterinary medicine has always been an integral part of livestock rearing community of Ethiopia in the treatment of different illnesses including trypanosomosis. However, though there could be potentially effective against drug resistance trypanosomes, we lack proper documentation and activity evaluation of medicinal plants. Hence, a trypanocidal activity of plants traditionally used to treat trypanosomosis should be well documented and validated for potential future use.

Aim of the study: To determine trypanocidal drugs resistance of trypanosomes species and in vivo anti-trypanosomal activities of methanol and chloroform extracts of Hypoxis villosa and Eriosema montanum (African potato), in T. congolense infected mice.

Materials and Methods: Forty mice were randomly allocated to eight groups of five mice per cage. Group I and II were given 3.5mg/kg and 7mg/kg BW of Diminazene aceturate (DA) and Group III and V 0.5mg/kg and 1mg/kg BW of Isomethamidium chloride (ISM), respectively. The other groups, V and VI, and VII and VIII were receiving, 600mg/kg BW of methanol and chloroform extracts of Hypoxis villosa and Eriosema montanum, respectively. All treatments were given orally consecutively for seven days. The mean PCV, body weight, survival period and the level of parasitaemia were measured every two days intervals.

Results: All mice that received herbal extracts of 300mg/kg and 600mg/kg BW of Hypoxis villosa and Eriosema montanum showing no clinical toxicity signs of the extracts and indicated it is relatively safe. Among experimental mice receiving Hypoxis villosa chloroform extracts were significantly increased in body weight and showing a low mortality rate. The parasitaemia load of the extract treated by profoundly decrease in Hypoxis villosa chloroform extracted treated group and relatively, in the other herbal treated groups. Those groups, treated with trypanocidal drugs of Diminazene aceturate and Isomethamidium chloride at different doses, were initially clear the parasites and relapse occur in all groups starting from 14 days of treatment given.

Conclusions: In this study the chloroform extracts of Hypoxis villosa treatment groups showing superior results for the treatment of trypanosomosis in mice model. The finding from this study could contribute to the development of efficacious trypanosomosis treatment options.

Keywords: Hypoxis Villosa; Eriosema Montanum; Trypanosomosis; Trypanocidal; Parasitaemia

Background

The menace of African Animal Trypanosomosis (AAT), still constitutes a significant obstacle to food security despite previous attempts towards chemotherapy and tsetse control. Parasite control currently relies on a small group of trypanocidal compounds, and new compounds are unlikely to become available soon [1].

In trypanosome endemic areas, trypanocidal drugs; both prophylactic (Isometamidium chloride) [2] and curative (Diminazene aceturate), are the most widely used methods of animal trypanosomosis control [5]. Trypanosomosis is also controlled either by vector or parasite control, or a combination of both. Various efforts to control the disease and the associated economic losses have been directed mainly against the parasite through trypanocidal drugs and against the vector through odor- baited, insecticide-impregnated targets/traps/ and insecticide-treated cattle [3, 4].

According to [6], nowadays toxicity and resistance to the commonly used trypanocidal drugs haven been emerged in sub-Saharan Africa and interferes with the effective veterinary management of trypanosomosis. The problem of drug resistance in trypanosomes chemotherapy appears to be widely spreading geographically to different regions, in which trypanosomosis occurs. At present, there are twenty-one African countries in which trypanocidal drug resistance has been reported. In addition, the occurrence of multiple (one or more of the commonly used trypanocidal Drugs), trypanosomes parasites resistance has been reported in eleven Sub-Saharan African (SSA), countries: (Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, Sudan, Tanzania, Uganda, and Zimbabwe) [7, 8]

Even if, the amount of trypanocidal used in Africa is very high for the pharmaceutical industries interest in developing new products remains low and leaving farmers to rely on the existing drugs for many decades. As reported by privatization of veterinary services in most parts of Africa easily access to farmers and this has resulted in rampant misuse and under-dosage of the medications.

Thus, drug resistance in trypanosomes poses a serious problem to livestock productivity unless checked and brought under control [1]. Some of the research conducted reveals that limited success had been achieved, despite enormous efforts by several workers in the field of chemotherapy and allied disciplines to discover or develop an 'ideal' trypanocide. In Ethiopia, the appearance of drug-resistant trypanosomes has been reported by several authors [9]

In every effort to discover new drugs for infectious diseases, plant materials are the main focus of the researchers: which contain diverse chemical substances with biological and physiological properties (Maikai, 2010). Different research studies have shown that plants are used in traditional medicine of Africa to treat trypanosomes in humans and animals [10].

It is the major component of traditional medicine, including 40,000-70,000 medicinal plants, out of which 20% of them are higher-plant species [11]. Thus, the search for medicinal plants with trypanocidal activities continues to generate a lot of research interest [12]. Although recent reports indicate anti-trypanosomal activity exists in some medicinal plants [13], the potentials of many other plants used in folkloric medicine in Ethiopia are yet to be investigated [14]. Reports by [15], stated that close to half the world' best-selling pharmaceuticals were either natural products or their derivatives. So, it is a vital task to identify and investigate those plants (natural remedies) suitable for use as a source of new drugs, focusing on efficacy and toxicity. Therefore, the study was conducted to evaluate *in vivo* trypanocidal activities of methanolic and chloroform extracts of *H. villosa* and *E. montanum* in mice infected with *T. congolense*.

Materials and Methods

Ethical Consideration

All Ethical clearance protocol, guidelines and principles of the Animal Care and Use followed and obtained for this study from Addis Ababa University College of Veterinary Medicine and Agriculture Minutes of Animals Research Ethics and Review Committee. A seven-page request for an explanation of the purpose of carrying out the studies and all possible care planned to reduce animal reduced from suffering due to sampling was given to the committee. After the committee evaluated the significance of this

research, approval was given (*Minutes No. and date of review:* VM/ERC/08/05/10/2018).

Plant materials

The two plants materials (*Eriosema montanum* and *Hypoxis villosa*) were collected together with traditional healers from a forest in Benishangul Gumuz regional state, Ethiopia. The plants were selected based on information gathered from local traditional healers on their curative effect to treat animal trypanosomosis and other internal parasites of animal and human in the study area. They were botanically identified at the National Herbarium Identification Unit, College of Natural Sciences, Addis Ababa University, Ethiopia where voucher specimens were deposited (Collection EM/001 and 002). These plants were identified as *Eriosema montanum* and *Hypoxis villosa.*

Eriosema montanum belongs to the family Fabaceae, which erect, rarely prostrate or climbing, herbs or subshrubs. It is known by its local name as; ''Qambu'', inhabiting in lowland areas of the Ethiopia. The chemical composition of this traditional herbal plant medicine was not identified. This plant is locally known by its vernacular name of ''Africa potato'', inhabiting lowland areas and with rare distribution.

Preparation of plant extract

The whole plant sample, of each plant after collection were washed, sliced and transported to Addis Ababa University College of veterinary medicine, Bishoftu, Ethiopia; to pharmacology laboratory for extraction of the shade dried at room temperature for 14 days. The dried parts were ground to a powder using a mortar and pestle. The powder was collected in polyethene bags and stored at room temperature until it was passed through the extraction procedure.

Preparation of crude plant extract

The powdery plant materials (50g) were extracted by methanol and additional 50g were extracted by chloroform. The powder of each remedy was extracted using methanol and chloroform, at 65^0C and 62^0c respectively, by Soxhlet extraction method, and the time taken for one extraction range from 3-4 hr. A five-fold (250 ml) quantity of solvent in relation to the herbals material was used for the extraction. All extracts were concentrated on a rotatory evaporator (BüCHI Rota-vapor R-200, Switzerland), at low pressure coupled to a thermoregulatory device in order to obtain dry extracts. The dried herbal remedies extracted by methanol where dissolved in Phosphate buffer solution and those extracted with chloroform was dissolved in DMSO (Dimethyl methanol Sulfoxide). The residue (solvent-free extracts) was weighed (i.e., 1.2gm., 2.2gm., 2.5gm.and and 2.6 gm), stored at +4^{o}C till use, and used in the preparation of the stock extract for administration.

Trypanosome isolation and stock preparation

T. congolense parasite isolated from a field where traditional healers used the above-mentioned medicinal plants to treat trypanosomosis used in this study. Depending on parasitological examination blood sample was collected, isolation of *T. congolense* parasites were carried out during sampling period from January –February 2018; the positive blood samples from naturally mono-infected zebu cattle with *T. congolense* using a parasitological technique were collected and mixed with cryomedium solution in 1:1 ratio for the preparation of stabilates [16], and stored in liquid nitrogen (-196 ^{o}C). The cryomedium used for the preparation of stabilates has 14% glycerol. The stabilates prepared were tested with PCR [17], to confirm pure *T. congolense* parasite and passage into mice to diagnosis trypanocidal drug resistance of the *T. congolense* and to evaluate the effectiveness of *Hypoxis villosa* and *Eriosema montanum* herbal remedies

Experimental Animals

A total of forty (40) adult Swiss white albino mice weighing 25 –35 gm. and age of 8–12 weeks were used. Mice used were obtained from the laboratory of the animal house; The Ethiopian Public Health Institute (EPHI), Addis Ababa University. The mice were housed under standard hygiene in polypropylene cage, five mice per cage with wood shavings as bedding, which was changed

every five days. The mice were kept at the ambient temperature of 24 – 26 ^{0}c and relative humidity of 70 – 80 %, with 12 h/ daylight and dark cycles and allowed free access to growers feed (pellet) and clean water ad libitum. They were classified either as donors or experimental [18]. The experiment was conducted in compliance with internationally accepted principles for laboratory animal use. Animal care guidelines on animal use protocol review (CCAC, 1997). The animals were allowed a 14-day period of acclimatization before they were divided into groups for experiment.

Donor and experimental mice parasite inoculation

The donor mice infected with a field isolate of *T. congolense* from the study area were followed up to peak parasitaemia. The mice were anesthetized using chloroform reagent and immediately blood was collected by cardiac puncture with an EDTA coated syringe and diluted with physiological saline PSG solution to increase the blood volume and number of animals injected with the infected blood collected from the donor mice. Each mouse was inoculated intra-peritoneal with 0.2 ml of diluted infected blood which contains~10^{5} trypanosomes [19].

Experimental design

The efficacy of trypanocidal drugs [Diminazene aceturate (DA) and Isomethamidium chloride (ISM)] was tested against *T. congolense* field isolates in experimentally infected mice based on previously established protocols [20, 21]. And the effectiveness of locally used herbal remedies collected from the traditional healers, used for the treatment of bovine trypanosomosis, was assessed. The experimental mice were randomly assigned to eight groups of five mice per group for this experiment.

Extract administration

Both extracts were administered orally using a nasogastric tube to the experimental mice. The dosage of the prepared extract given to each experimental mouse was calculated to their body weight (mg/kg), administered orally for seven days consecutively at a dose rate of 600mg/kg body weight for each group of mice, of different extract of plant material powder. Parasitaemia level was monitored every two days under the microscope slide until the disappearance of trypanosomes or end of the experiment. Animals were euthanized immediately at the end of the experiment.

Determination of acute toxicity

The study was conducted for each extract in Swiss albino mice after adaptation of unfamiliar environment; acute toxicity study was investigated following the Lorke (1983) method. To investigate whether the plant is toxic or not, mice were divided into eight groups and each group contains 3 mice for *E. montanum* methanol and chloroform extracts solution, were given orally at a dose of 300mg/kg and 600mg/kg of body weight using a nasogastric tube. Similarly, for *H. villosa* both methanol and chloroform extracts also given at a dose of 300mg/kg and 600mg/kg orally.

All animals were kept under strict observation of behavioral, neurological, autonomic, or physical changes such as alertness, motor activity, restlessness, convulsions, coma, diarrhea, and lacrimation. These observations continued for further 14 days for any signs of overt toxicity. During the period of observation, mice were showing no clinical signs of toxicity. The mortality rate was recorded as long as the experimentation ended.

Parasitological and observation of the clinical signs of the experimental

Starting from the day of experimental study, animals were controlled daily for clinical signs and parasitological findings (parasite load, PCV and body weight measurement) using direct microscope examination (buffy coat technique), by collecting blood from the tail of mice in to paired heparinized capillary tube [22] and digital balance every two days for two weeks. The number of parasite seen per field under microscope was counted using a rapid matching method as described by [23].

Data Analysis

All results obtained during laboratory activities were entered and stored in the Microsoft office excel data sheet. The data collected was analyzed using Stat-12 version and presented using percentages, mean ± standard error. Statistical significances of differences in PCV, between levels and combination in the survival duration for the experimental studies, were analyzed using (ANOVA) one-way analysis of variance. Variation in survival duration was compared using between different groups and the p values less than 0.05 were considered as the statically significant difference between considered variables [24].

Results

Experimental trial results

All mice inoculated with fresh blood containing *T. congolense* isolates taken from the field were parasitological positives. Parasitaemia was proven after 15 days of inoculation in donor mice and peak parasitaemia was detected 20 days after inoculation. Then after transferred to experimental mice, parasitaemia was detected ten days of post-infection in all groups and blood samples were collected five days after treatment was given to check for the clearance of parasitaemia. In this studies bleeding and wound in the abdominal areas of three mice, one from Isomethamidium treated group with 1mg/kg BW, one from Diminazene treated group at a dose 7mg/kg BW and the other one from *H. villosa* chloroform extract product at a dose 600mg/kg BW on the fourth days of treatment commenced by the extract. During experimental period three mice died from Isomethamidium 1mg/kg BW on the second day of treatment, Diminazene 7mg/kg BW on the fourth day of treatment and *H. villosa* methanol extract on the seven days of treatment, which accounts 20% of each of the treated groups by the products.

Sensitivity status of trypanocidal and extracts of E. montanum and H. villosa

Isomethamidium chloride and Diminazene aceturate of different doses used for the comparison of cattle doses in mice have initially cleared the parasites. Fast apparent clearance observed in Diminazene aceturate used at both doses (3.5mg /kg) for the experiment after two days of treatment given and three days post-treatment for Isomethamidium chloride used at both doses intervals, with comparative therapeutic doses in mice. The parasitaemia loads in those treated by herbal remedies were not clear the parasites totally, but there was a decrease in parasitic load and increased in the body weight of treated animals.

The clinical finding and development of parasitaemia in experimental mice

The measured mean parasitaemia load of the infected mice with *T. congolense* and treated by extracts of *E. montanum* and *H. villosa* herbal remedies were significant ($P < 0.05$) as compared to each other (Table 1).

Extracted plant types	Mean value of parasitaemia load				
	1st day	2nd day	3rd day	4th day	5th day
E. montanum chloroform ext.	29.2± 4.17	31.2±4.96	31.6±5.15	30.4±4.9152	33.2±5.16
E. montanum methanol ext.	35.6± 7.38	38.4±7.54	40±8.62	43.2±10.36	43.2±9.26
H. villosa methanol ext.	35.2± 4.23	39.5±1.65	32.5±4.34	36.75±1.49	34±2.94
H. villosa chloroform ext.	25.2± 6.34	24±6.84	26±7.53	25.6±7.87	24.6±8.45

Ext: extracted, *E. montanum: Eriosenama montanum, H. villosa: Hypoxis villosa*

Table 1: The average parasitaemia load recorded during laboratory activities in mice treated with *E. montanum* and *H. villosa*

The parasitaemia loads during the experimental study were measured at different days of the experimentation and were recorded, and the result obtained was compared and computed as the logarithmic values described in by Herbert and Lumsden (1976)(Figure 1).

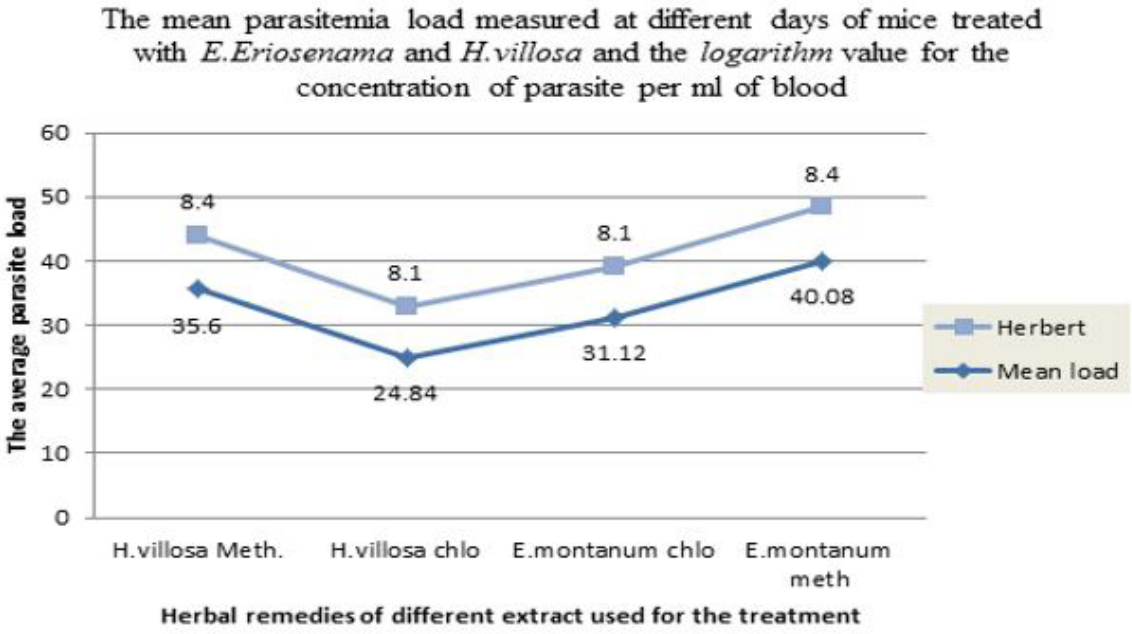


Figure 1: The effect of the extracts *E. montanum* and *H. villosa* on the parasitaemia load. As compared the mean body weight of those experimental mice treated by herbal remedies extracts and convectional trypanocidal drugs were highly significant ($p < 0.05$).

The PCV of infected mice with *T. congolense* and treated with *E. montanum* and *H. villosa*

The mean PCV value of experimental mice infected with *T. congolense* and treated with extracts of *E. montanum* and *H. villosa* herbal remedies were significant ($P < 0.05$) as compared each other (Table 2), because of there is decrease PCV value of the experimental mice.

Groups	Rx	Packed cell volume in mice				
		Day 1	Day 2	Day 3	Day 4	Day 5
H. villosa chloroform ext.	5	34.8±0.86	34 ±0.70	33±0.70	32.2±0.66	32+0.70
H. villosa methanol ext.	5	37.6±0.5	37.2±0. 73	36±0.89	35.2±0.86	34.4+0.92
E. montanum chloroform ext.	5	38.25±0.47	37.75±0.47	37.5±0.6	36.75±0.8	36+0.40
E. montanum methanol ext.	5	39.4±0.5	39.2±0.37	37.6±.50	38±0.70	37.8+0.58

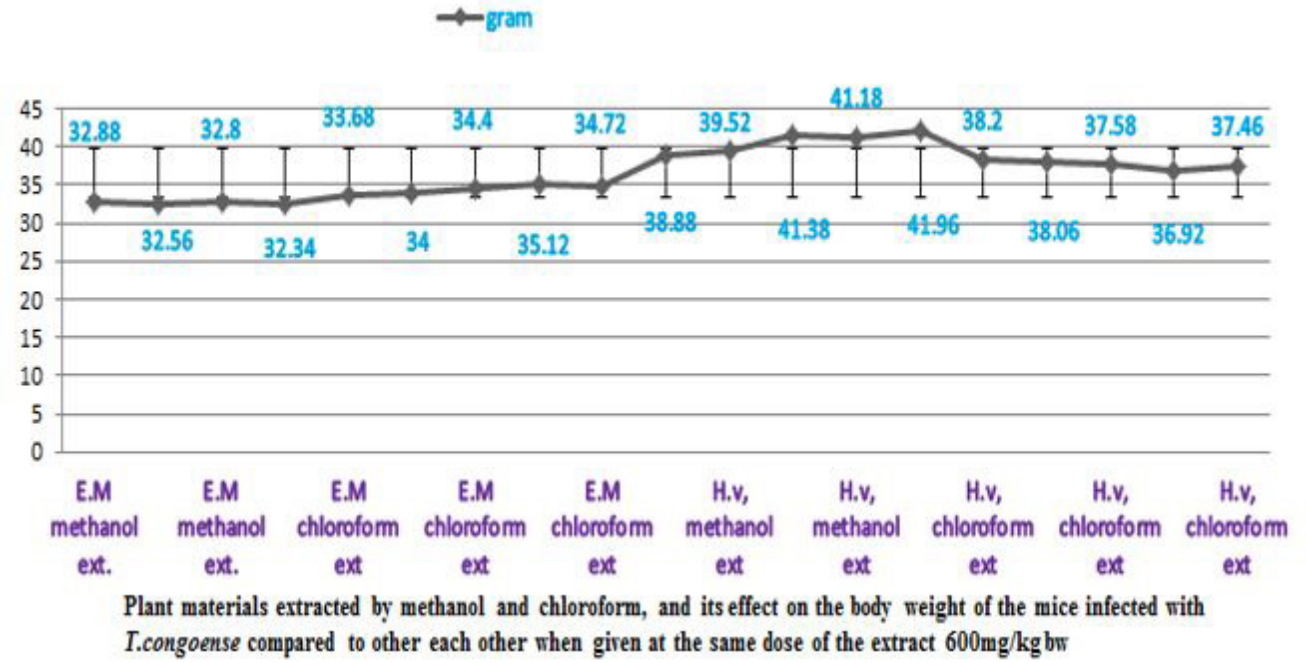
ext.: extract, Rx: treatment

SE: standard error, n = 5, even though the mean PCV values were slightly changed as gone through the row of the table, and the p-value ($p < 0.05$) indicates that there is a significant difference in the mean PCV in a group of mice infected with *T. congolense* and treated with methanol and chloroform extracts of *E. montanum* and *H. villosa.*

Table 2: Impact of methanolic and chloroform extracts of medicinal plants on PCV of the *T. congolense* infected mice

Effects of *E. montanum* and *H. villosa* extracts on body weight of mice infected with *T. congolense*

The extracts of *E. montanum* and *H. villosa* prepared by methanol and chloroform extract were orally administered at a maximum dose rate of 600mg/kg for each group of mice containing five animals per group, and as the time progresses the animals become getting decreased in body weight (Figure 2).



E.M: *Eriosenama montanum*; H.V: *Hypoxis villosa*; ext.: extract

Figure 2: The effect of *E. montanum* and *H. villosa* on the weight of experimental mice

The effect of E. montanum and H. villosa extract on the survival time of mice infected with T. congolense

The group of experimental mice infected with *T. congolense* and treated with similar dose of 600mg/kg of BW; methanol and chloroform extracts of *E. montanum* and *H. villosa.*

Type of Rx	Condition of experimental mice					Mean survival time (days±SE)
	Rx	Cured	Survived	Death	relapse	
*E.M.*600mg/kg, meth. Ext.	5	Initially cleared	5	-	Not cured	30±00
*E.M.*600mg/kg, chlor. Ext.	5	Initially cleared	4	1	Not cured	28±2
H.v. 600mg/kg, meth. Ext.	5	Initially cleared	4	1	Not cured	27±3
*H.v.*600mg/kg, chlor. Ext.	5	Initially cleared	5	-	Not cured	30±00

E.M.: Eriosenama Montunam, H.V.: Hypoxis villosa, chlor: chloroform, *Meth*: methanol

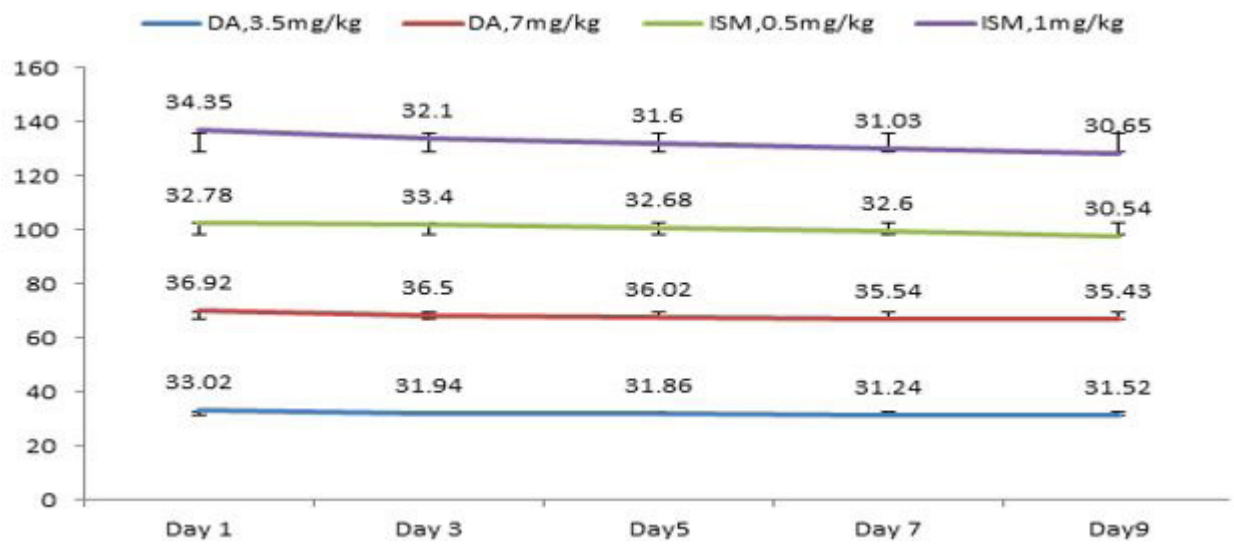
Table 3: Mean survival time of mice infected with *T. congolense* and treated by *E. montanum* and *H. villosa*

Type of Rx	Condition of experimental mice					Mean survival time (days ±SE)
	Rx	Cured	Survived	Death	relapse	
DA 3.5mg/kg bw	5	Initially cleared	3	2	3	30±00
DA 7mg/kg bw.	5	Initially cleared	3	2	2	26±2.75
ISM 0.5mg/kg bw	5	Initially cleared	5	-	2	30±00
ISM 1mg/kg bw	5	Initially cleared	4	1	2	28±2

DA: Diminazene, *ISM:* Isomethamidium, *Rx:* treated, *SE:* standard error

Table 4: Mean survival time of mice with *T. congolense* and treated by Diminazene and Isomethamidium

The body weight of those infected experimental mice and treated by Diminazene aceturate and Isomethamidium chloride as indicated in the (Figure 3) there is a slight decrease from time to time in their body weight.



ISM; Isomethamidium chloride, **DA**: Diminazene aceturate

Figure 3: The average body weight of experimental mice

Discussion

Experimental trails in mice were conducted to assess trypanosomes resistance status to trypanocidal drugs and the effectiveness of locally used herbal remedies for the treatment of bovine trypanosomosis with emphasis on *T. congolense* isolates from the study area. The experimental trails aimed at tracing trypanocidal drugs resistance for Diminazene and Isomethamidium chloride. These drugs were temporarily clear *T. congolense* isolates from experimental mice on second day's treatment by Diminazene aceturate and third days post-treatment by Isomethamidium chloride of both doses given, and relapse occurs in tenth days in two mice treated with 3.5mg/kg BW of Diminazene aceturate and fourteen days post-treatment with Isomethamidium given at a dose rate of 0.5mg/kg BW.

Similar study results were reported on experimental sensitivity conducted on the *T.congolense* isolates from Ghibe, Arba-minch, Bedele and Sodo by [25], indicating the failure of trypanocidal drugs to clear the parasites from the experimental mice using bovine doses. In the research conducted by Afewerk *et al.* (2000) the *T. congolense* isolates from Benishangul Gumuz was not respond to trypanocidal drugs given at a dose a rate of 28mg/kg BW Diminazene aceturate and 4mg/kg BW Isomethamidium chloride in experimental mice. Similarly, the results of many studies in Ethiopia and in others African countries indicate the development of multidrug resistance: In Ethiopia [25] and Burkina Faso [26] indicate resistance to sanative pair of trypanocidal drugs for *T.congolense* isolates.

However, there is an experimental trial study that reveals the effectiveness of trypanocidal drugs conducted in mice. For instances, a study conducted in Zambia as reported by [27] indicates even using discriminatory doses at 0.1mg/kg BW. Isomethamidium chloride, and 20mg/kg BW of Diminazene aceturate, from *T.congolense* isolates 53.5% were sensitive to both drugs used in the experimental trial. The clone of identity IL 3000 from Burkina Faso, also resistance to 1mg/kg BW Isomethamidium chloride was successfully treated with 5mg/kg BW dose of Isomethamidium chloride in mice [28]. As reports from Nigeria, by [29] indicated, *T.congolense* isolates were cleared by Diminazene aceturate when given at a dose rate of 10.5mg/kg from the experimental rats.

In Ethiopia field observation conducted indicate that the prophylactic coverage of Isomethamidium chloride can extend up to four weeks of post-treatment, in opposites to manufacturer 6 to 16 weeks prophylactic coverage of these drugs. From the study results of three villages of Kindo Koysha, southern Ethiopia (Ademe & Abebe, 2000) documented that the prophylactic efficacy of Isomethamidium chloride was less than thirty days. Similarly, in Woinma field observation on *T. congolense* isolates from the area revealed four weeks for the prophylactic activities of Isomethamidium chloride on the isolates. On the other hand, researches conducted in different parts of Africa reveals the effectiveness of trypanocidal drugs in the field observations. As the reports from Kenya, by [30] Samorin and Veridium, which is Isomethamidium based products were effective in prophylactic activities for 70 days when administered at a dose rate of 0.5mg/kg BW.

At the start of the present experimental trial, the initial and the peak parasitaemia level were demonstrated on 15 days and 20 days of respectively, post inoculation of the parasites in the mice used for isolation and amplification. In the experimental mice, parasitaemia was demonstrated 7 – 11 days of post inoculum of the parasite to the mice. A similar observation by [31] detects parasitaemia 5 – 7 days of post-infection using primary isolates of *T.brucei.* The mice treated during peak parasitaemia 11th days post infection. [32], from Nigeria, reports that the procedure for the treatment of experimental mice have to be after 14 days of infection at peak parasitaemia was followed in a more recent experimental trial conducted.

The outcome of the present trypanocidal drugs resistance test in mice clearly shows the presence of trypanosome isolates have developed drugs phenotype to the currently available trypanocidal drugs. The reports by (Peregrine, 1994), indicate that the in the areas where multiple trypanocidal drugs resistance is expressed at the level of the individual parasite, chemotherapy becomes increasingly ineffective and intervention at the level of the control of vector is far most important.

The used herbal remedies (*E. montanum* and *H. villosa)* or *"Africa potato"* known as for its long history of medicinal use in Africa continent [33]. The extracts of these herbal remedies, when given at a dose rate of 300mg/kg and 600mg/kg did not produce any clinical signs or mortality due to its toxicity. Different extracts were used for the screening test to detect their anti-trypanosomosis activities given at 600mg/kg BW. In the South Africa primary health care community currently using *Hypoxis* as immuno-stimulant for the patients with HIV/AIDS, a daily dose of 2400 mg of raw plant purported to be therapeutically effective [34]. The results of the used herbal remedies *E. montanum* and *H. villosa* indicate that one mouse was died from the group treated with *H.villosa* during the fourth days of treatment started. The mean parasitaemia load of the infected mice with *T. congolense* those treated by extracts of *E. montanum* and *H. villosa* extracts were significant ($P < 0.05$) as compared those treated with trypanocidal drugs.

During the period of experimental activities in the laboratory the average parasitaemia level measured in each group treated with herbal remedies indicate that the average minimum parasite load counts (24.84 average wet film field count of parasite for five days) (Figure 1*)*, detected in those group treated with *H.villosa* extracted product using chloroform extracted and in *E.montanum* chloroform extract and *H.villosa* methanol extracts relatively similar parasite load detected during the study period. Similar to (Maikai *et al.*, 2008), who report that the preliminary screening of *X. americana* methanol and aqueous extract reduce motility of *T.congolense* in vitro, *H.villosa* chloroform extracted used in this experiment also indicate a reduction in the parasite loads in mice treated orally by this herbal extract.

In this study, the PCV value result observed in all groups of infected mice treated with *E. montanum* and *H. villosa* extract was reducing from time to time, which indicate that the parasite load was not cleared or reduced to the minimum level that the animals can cop up. Besides, the body weights of the experimental mice assigned to groups of the three extracts: *E.montanum* methanol extract, *E.montanum* chloroform extract, and *H.villosa* chloroform extract showed similar measurement; and *H.villosa* treated group showed there was no reduction in body weight measurement. The study results also revealed that the body weight of experimental mice treated with trypanocidal drugs and herbal remedies shows a similar pattern of reduction in weight even though there was initially clearance of the parasite from those groups treated with trypanocidal drugs

Similarly, researches were conducted by a different researcher on herbal remedies against trypanosomosis. As reviewed by [35], in 2013 *Abedo,* showed *in vitro* activity was observed on nine extracts from plant materials of *Tapinanthus globiferus* and *G. latifolium* were against *T.congolense* at various concentration. In Ethiopia, reports [36], speculate that *Dovyalis abyssinia* might be a promising candidate for phytotherapy of trypanosomosis. [37-40] also reported that methanol extract of *Artemisia abyssinica* showed appreciable *in vitro* and *in vivo* anti-trypanosomal activity against field isolates of *T.congolense*.

Conclusion

In this study, the administration of chloroform extracts of *Hypoxis villosa* indicates good results in the treatment of trypanosomosis. During the experimental period mice treated with the *Hypoxis villosa* group increased in body weight and low mortality rate compared to the other groups. The parasitaemia load in this group was highly reduced, even though no total cleared. Therefore, further study is required to determine the active chemical constituents of these herbal remedies extracts and mechanisms of actions against the parasites [41-46].

Authors' Contribution

AB: conducted laboratory activities and drafted the manuscript. FR and TB: implemented the study design, drafted and revised the manuscript and supervised the laboratory experimentation. FA and SS: edited the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors are grateful to Addis Ababa University College of Veterinary Medicine and Agriculture, all staff of Benishangul Gumuz Region Bureau of Agriculture and Rural Development, Assosa and Bambasi, Agriculture and rural development office for the Department of Animal Health and quality control stuffy member, Benishangul Gumuz Regional State Animal Health and Fisher Agency and all individual who play vital role for the accomplishment of this study.

Funding

This study was supported by Addis Ababa University thematic research project.

Competing interests

The authors declare that they have no competing interests.

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