Journal of
Clinical and Experimental Pharmacology

Tesfaye, et al.,, Clin Exp Pharmacol 2015, 5:2
DOI: 10.4172/2161-1459.1000171

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Invivo Anti-Trypanosomal Activity of the Leaf Extracts of *Albizia Schimperiana* (Fabaceae) Against *Trypanosoma Congolense* Infection in Mice

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Received date: January 16, 2015, **Accepted date:** March 15, 2015, **Published date:** March 20, 2015

Abstract

Objectives: The objective of this study was to evaluate effect of dichloromethane (DCM) and methanol (MeOH) extracts of the leaf of *Albizia schimperiana* against *Trypanosoma congolense* using *in vivo* mice models.

Methods: The leaf of the plant was extracted by maceration technique using DCM and absolute MeOH to obtain the corresponding crude extracts. The extracts were screened for secondary metabolites and anti-trypanosomal activity of the crude extracts was evaluated at doses of 50, 100, 200 and 400 mg/kg in Swiss albino mice infected with T. *congolense* isolated from natural infection of cattle. The animals were monitored for test parameters including parasitemia, packed cell volume, rectal temperatures, body weight and survival.

Results: The acute toxicity test showed that both solvent extracts were safe at doses of up to 2 g/kg. The methanol extract at 100, 200 and 400 mg/kg showed a statistically significant ($p<0.05$) trypanosuppresive effect, but was unable to completely clear *trypanosomes*. Significantly ($p<0.05$) higher packed cell volume (PCV), weight and survival time were observed in groups treated with higher doses of methanol extract, however, the DCM extract treated mice has not showed statistically significant ($p>0.05$) reduction in parasitemia except 400 mg/kg dose.

Conclusion: It can conclude that MeOH extract has promising activity against *T. congolense* in mice by reducing the levels of parasitemia and the activities may be due to presence of alkaloid, flavonoid and saponins which are responsible for anti-trypanosomal activity.

Keywords *Albizia schimperiana; Trypanosoma congolense;* antitrypanosomal activity; Mice

Abbreviations

DCM: Dichloromethane; MeOH: Methanol; DA: Diminazene Aceturate; PCV: Packed Cell Volume; T. *congolense*: *Trypanosoma congolense*.

Introduction

Trypanosomiasis is a parasitic disease caused by flagellated protozoan belonging to the Genus *Trypanosoma*. African animal trypanosomiasis (AAT) is transmitted cyclically by tsetse flies (Glossina species) and mechanically by biting flies. Of the tsetse transmitted trypanosomes, three species namely *T. congolense*, *T. vivax* and *T. brucei* comprise the major disease agents that affect livestock [1]. *T. vivax* and *T. evansi* can also be transmitted non-cyclically by biting flies and hence their distribution is much wider (extending to Asia and Latin America) than for the cyclically transmitted trypanosomes [2]. The human infective parasites *T. brucei gambiense* and *T. brucei rhodesiense* are the causative agents of human African trypanosomiasis (HAT), commonly known as sleeping sickness. They are transmitted by tsetse flies. *T. b. gambiense* is found in west and central Africa while *T. b. rhodesiense* is found in eastern and southern Africa [3]. A third group of trypanosome, *T. cruzi,* which is found mainly in Latin America, is a zoonotic infection which spread by blood-feeding triatomins [4].

African animal trypanosomiasis coupled with sleeping sickness has been a major obstacle to sub-Saharan African rural development and a stumbling block to agricultural production. If it were possible to eradicate trypanosomiasis from Africa it is predicted that the benefit to the overall agricultural production would gradually rise to 4.5 billion US dollar per year, and 55,000 deaths per year from sleeping sickness could be avoided [5].

The most significant clinical symptoms of trypanosomiasis are intermittent fever, anemia, enlargement of superficial lymph nodes, abortion, infertility, reduced milk yield, reduced weight gain and lowered work output and high mortality occurring in some animals during the acute phase of the disease if left untreated [6,7].

Alleviation of poverty can only start with reduction of hunger and this will be achieved through the development of sustainable agricultural systems, in which livestock plays a key role [8]. African animal trypanosomiasis is prevalent in two main regions of Ethiopia that is the northwest and the southwest regions [1]. The disease is

excluding some 180,000–200,000 km^2 of agriculturally suitable land in the west and southwest of the country; 14 million head of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at the risk of contracting trypanosomiasis at any one time [9]. The use of the limited trypanocidal drugs available in the market [10] is beset by numerous limitations, including toxicity of the drugs, development of resistance by the parasites and limited availability [11,12].

Albizia schimperiana Oliv. (Known as 'Hambabeesa' in Afaan Oromo and 'Sesa' in Amharic) is an evergreen tree that grows at an altitude of 1600 m-2600 m [13]. *Albizia spp*. is used in traditional treatment of various infections: *trypanosomiasis*, bacterial infections and stomach pain [14]. The leaf of this particular plant has shown significant antimicrobial activity on different bacterial species and potential anti-helmintic activity [15]. The report by Nibret and Wink [16] revealed *Albizia schimperiana* has promising *in vitro* trypanocidal activity on drug sensitive *Trypanosoma brucei brucei* cultured in Baltz medium with IC50 value of less than 50 µg/mL and contains spermine alkaloids as one of its active principles. The aim of the current work was to investigate the *in vivo* activity of the leaf extracts of *Albizia schimperiana* against *T. congolense* in mice model. This study reports the phytochemical constituents and *in vivo* antitrypanosomal activity of *Albizia schimperiana* against *T. congolense* infected mice.

Materials and Methods

Drugs and chemicals

The following drugs and chemicals were used in the experiment: acetic anhydride (Techno Pharm chem., India), dichloromethane (BDH Laboratory, England), diminazene aceturate (Fazrvet Laboratories BV, England), ethyl acetate (ACS, Merck), ether, ferric chloride (FISHER Scientific Company, New Jersey), ferric sulfate (ACS, Merck), Giemsa (BDH Ltd, England), 40% glucose (Pharmacure, Ethiopia), hydrochloric acid (BDH Ltd, England), lead acetate (ACS, Merck), methanol (Carlo ebra reagents, Italy), microscopic oil, PBS tablet, tween-80 (BDH Laboratorysupplies Ltd, England), potassium ferrocyanide (ACS, Merck), sulfuric acid (Carrloerbareagents, Italy), sterile water (EPHARM, Ethiopia).

Preparation of plant material

The leaves of *Albizia schimperiana* was collected from Jimma Zone, Goma Woreda from the Goma coffee farm, some 412 km south west of Addis Ababa in October 2012. Taxonomic identification was done and a voucher specimen (AT/001) was deposited at the National Herbarium, College of Natural Sciences, Addis Ababa University.

MeOH extract was prepared by maceration of 100 g powdered leaf in 1000 ml absolute MeOH in a conical flask with a rubber cork. DCM extract was prepared similarly by maceration of 100 g powdered leaf in 1000 ml of dichloromethane. The macerates were kept for three days with intermittent shaking and then filtered through sterile filter paper (Whatman No.1). The filtrates were pooled and concentrated under reduced pressure at 40°C using a rotary evaporator. The concentrates were placed in an oven at the same temperature for total removal of the extracting solvent. The dried extracts obtained were then weighed, and stored in refrigerator at 4°C as described by Peter et al. [16] until required for use.

Experimental animals

Albino mice of both sexes, aged 8-12 weeks and weighing 25-30 g were purchased from the breeding colony of the Ethiopian Health and Nutrition Research Institute. They were housed separately in polypropylene cages (6–10 mice per cage), maintained under standard condition of 12 h light/dark cycle at room temperature and fed on mouse pellets and given water ad libitum throughout the study. The animals were acclimatized for the period of 7 days before conducting any experimental procedure. The experiment was conducted in compliance with the internationally accepted principles for laboratory animal use and care [17] and this protocol was approved by research and ethics committee of Department of Pharmacology and Clinical Pharmacy.

Test organisms

The test organism *T. congolense*was isolated from naturally infected cattle in Bedele area, 480 km south west of Addis Ababa. The detection of *T. congolense* from blood samples collected from the animals was based on the type of motility in the microscopic field and confirmation of *T. congolense* species by morphological characteristics was done by examination of Giemsa stained slides under a microscope [18,19]. Then the infected blood collected from the cattle was inoculated to mice and transported to laboratory at Aklilu Lemma Institute of Pathobiology, Addis Ababa University where the organisms were maintained by serial passages in mice until required.

Preliminary phytochemical screening

Standard screening tests of the crude extracts were carried out for secondary metabolites such as phenolic compounds, tannins, saponins, flavonoids, cardiac glycosides, and anthraquinones according to the methods discussed in the literatures [20,21]

Acute toxicity study

Acute toxicity test was done on the limit test recommendations of OECD 425 Guideline. The extracts were reconstituted in 2% Tween-80 in distilled water and administered orally by gavage. Two female Swiss albino mice were fasted for 3-4 h and DCM and MeOH extracts were given for each animal at a dose of 2000 mg/kg. Based on the result from the first two mice, eight mice, four for each extracts were given a single dose of 2000 mg/kg of each extracts. The animals were observed for 14 days for gross behavioral changes such as, hair erection, lacrimation, tremors, convulsions, salivation, diarrhea, mortality and other signs of toxicity manifestations [22].

Experimental designs and *In Vivo* activity of the extracts

The mice were divided into eleven groups (MeOH50, MeOH100, MeOH200, MeOH400, DCM 50, DCM100, DCM200, DCM400, diminazene aceturate (DA), Tween 80 2% and un-infected un-treated (UIUT)) each comprising of 6 mice. All experimental groups of mice except un-infected un-treated were then infected intraperitoneally with 0.2 ml of the inoculums (infected blood diluted in PBS) containing approximately about 104 trypanosomes/ml collected from donor mice by cardiac puncture. The mice were monitored for development of infection by microscopic examination of blood film. The treatment of each groups were begun on 13 days post-infection at the average parasitemia of approximately antilog (7.46) parasites/ml. The plant extracts were freshly reconstituted using 2% Tween 80 and

administered intraperitoneally at doses of 50, 100, 200 and 400 mg/kg, daily at 9 a.m for seven days. The doses were selected based on the acute toxicity test and pilot studies. Diminazene aceturate (28 mg/kg) and 2% Tween 80 were used as positive and negative controls respectively. Mice were checked daily after the first treatment to estimate the number of trypanosomes in their blood using a wet blood film for 21 days and twice weekly for next 21 days by two independent laboratory technicians. For the assessment of anti-trypanosomal effect of the extracts, the level of parasitemia (expressed as log of absolute number of parasites per milliliter of blood) in the treated was compared to that of the control animals. Animals that survived to the end of the experiment with no parasite in their blood sample were considered as cured.

Determination of parasitemia

A wet film of the blood of the infected mouse was made under a 7 × 22-mm cover glass. Evenly distributed field was chosen and examined under 400X magnification. The absolute number of parasites per milliliter of blood was taken as a log using the rapid matching method for estimating the host's parasitemia. At higher levels of parasites, this was achieved by matching microscopic fields of wet blood film against charts and, when fewer parasites were present, by counting the number of trypanosomes in 5, 10 or 20 microscopic fields as described by Herbert and Lumsden [23].

Determination of PCV

PCV was measured to predict the effectiveness of the test extracts in preventing haemolysis resulting from increasing parasitemia associated with trypanosomiasis. It was monitored on days 0, 7, 14 and 21. For this purpose, blood was collected from tail of each mouse in heparinized microhaematocrit capillary tubes filled up to 3/4th of their length. The tubes were sealed immediately and placed in a microhematocrit centrifuge. The capillary tubes with the blood were then centrifuged in a micro-centrifuge for 5 min at 10000 rpm. After centrifugation, the height of the red blood cell column was measured by use of haematocrit reader and compared to the total height of the column of the whole blood.

Body weight and rectal temperature

The weights of the mice were determined starting from the day of treatment and every other day up to day 21 by a sensitive digital weighing balance. The rectal temperature was determined on day 0, 7, 14 and 21 at 9 am by digital thermometer. The tip of the thermometer was deep into 2% Tween 80 so as to minimize bleeding of the mice rectum.

Determination of mean survival time

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow up period for 42 days.

Statistical analysis

Data were analyzed using Statistical Package for Social Science (SPSS version 17. Results are presented as Means ± standard error of mean (SEM), the means were compared by analysis of variance (ANOVA) and post test analysis was done using the Tukey multiple comparison tests. Probability level at ($P<0.05$) was considered significant.

Results

Percentage yields of 6.8% and 14.1% (w/w) were obtained for the DCM and MeOH crude extracts of *Albizia schimperiana* leaf, respectively. Phytochemical screening of the extracts showed the presence of various chemical constituents. The MeOH extract was found to contain the highest number of phytochemicals such as alkaloids, saponins, flavonoids, tannins and anthraquinones. On the other hand alkaloid and steroids were found in the DCM extract.

Acute toxicity studies revealed the non-toxic nature of both solvent extracts of *Albizia schimperiana* leaf as where no death and gross behavioral changes recorded at a dose of 2000 mg/kg.

The pre-treatment mean parasite count for all groups was antilog (7.46) parasites/ml. Treatments with both solvent extracts affected the course of parasitemia. Despite the administration of varying doses of both extracts to mice infected with *T. congolense*, only the higher doses of MeOH extract (100,200 mg/kg and 400 mg/kg) showed a statistically significant ($p<0.05$) trypanosuppresive effect when compared with the negative control (Figure 1).

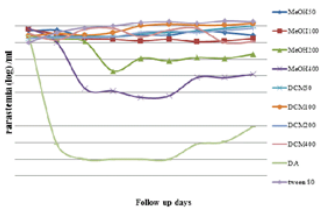

Figure 1: Comparison of effect of the DCM and MeOH crude extracts of leaf of *Albizia schimperiana* on parasitemia of *T. congolense* infected mice. Data are presented in log10 and expressed as Mean ± SEM; N=6; D0=the day treatment commenced; D: Day; MeOH: Methanol; DCM: Dichloromethane; DA: Diminazene acceturate.

Treatment with this extract (MeOH) resulted in a statistically significant ($p<0.05$) reduction in the level of parasitemia within 4 days of post-treatment (Table1).

Treatment	Follow up days								
	D0	D3	D5	D7	D9	D12	D14	D18	D21
MeOH 50	7.70 ± 0.12	7.75 ± 0.092	7.35 ± 0.16	7.35 ± 0.16	7.45 ± 0.12	7.45 ± 0.09	7.70 ± 0.06	7.60 ± 0.10	7.45 ± 0.09

MeOH 100	7.80 ± 0.03	7.50 ± 0.15	7.25 ± 0.12	7.20 ± 0.13	7.10 ± 0.06	7.20 ± 0.13	7.05ak ± 0.06	7.10 ± 0.06	7.25 ± 0.12
MeOH 200	7.40 ± 0.10	7.20 ± 0.13	7.05 ± 0.10	5.30** ± 0.07	6.05 ± 0.21	5.90 ± 0.10	6.10** ± 0.20	6.05 ± 0.18	6.30** ± 0.20
MeOH 400	7.85 ± 0.09	7.00a ± 0.06	4.25 ± 1.34	4.10* ± 1.30	3.70 ± 1.17	3.75 ± 1.20	3.82** ± 0.98	4.90 ± 0.98	5.10* ± 1.03
DA	7.40 ± 0.10	0.90** ± 0.90	0.00 ± 0.00	0.00** ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.90 ± 0.90	1.05 ± 1.05	1.95 ± 1.23
Tween 80 2%	7.00 ± 0.06	7.60k ± 0.12	7.90 ± 0.06	8.00jk ± 0.06	8.20 ± 0.10	8.25 ± 0.13	8.16 ± 0.14	8.3 ± 0.14	8.25 ± 0.15

Table 1: Effect of MeOH crude extract of the leaf of *Albizia schimperiana* on parasitemia of *T. congolense* infected mice.

Data are presented as log10 and expressed as Mean ± SEM; N=6; D0=the day treatment commenced; D: Day; MeOH: Methanol; DA: Diminazine acceturate; All superscripts indicate significance at $p<0.05$ (a = against the negative control, j=against MeOH 200, k=against MeOH 400, **against all group *against all group except MeOH 200, Parasitemia were compared with each other mainly at days 3, 7, 14, 21.

The significant inter-group variations were first noticed from the group treated with DA followed by groups treated with 400 mg/kg compared to the negative control. The 400 mg/kg MeOH extract showed significant inter-group difference ($p<0.05$) on parasitemia level on 12th day of post-treatment compared to all the groups. Parasitemia measurement in blood of infected mice treated with the DCM extract hasn't showed statistically significant ($p>0.05$) reduction. However a group of mice treated with DCM 400 mg/kg body weight revealed the lowest number of parasite count compared to the negative control and the lower concentration of same extract on 21 day of post-treatment. However, the group treated with diminazene aceturate (positive control), showed a sharp reduction in parasites count, with no parasite seen in 20 microscopic field counting on day 17 of post-infection (5 days post-treatment). Relapse was recorded in positive control group, in which, parasite starts to be detected in blood of some of the mice, with antilog (0.9) parasites/ml on 14th day of post-treatment (Figure 1). However, *in vivo* anti-trypanosomal activity of DCM and MeOH leaf extracts of *Albizia schimperiana* revealed that none of the doses used completely cleared trypanosomes from the blood of infected mice.

The mean PCV pattern for the different experimental groups prior and after the initiation of the study period is shown in Table 2.

Treatment	**Dose (mg/kg)**	**PCV(Treatment/Follow up)**			
		PCV0	PCV7	PCV14	PCV21
MeOH	50	48.85 ± 0.27	48.00 ± 0.30	45.63 ± 0.68	41.60 ± 1.15
MeOH	100	48.20 ± 0.33	45.96 ± 1.18	45.03 ± 1.03	44.78 ± 1.26^{a}
MeOH	200	48.65 ± 0.51	48.83 ± 0.38^{bc}	48.73 ± 0.47	48.03 ± 0.54^{ab}
MeOH	400	49.15 ± 0.27	49.76 ± 0.37^{bc}	49.83 ± 0.27	50.01 ± 0.14^{ab}
DCM	50	49.20 ± 0.36	45.96 ± 1.25^{bce}	45.28 ± 0.89	40.01± 0.89
DCM	100	48.46 ± 0.55	45.46 ± 1.29^{bce}	40.76 ± 1.31	37.70 ± 1.35
DCM	200	49.16 ± 0.42	47.40 ± 0.58	46.25 ± 0.89	43.26 ± 1.50
DCM	400	49.53 ± 0.23	49.23 ± 0.29	48.73 ± 0.45	47.71 ± 0.41^{a}
Tween80	2%	49.31 ± 0.24	47.66 ± 0.52	46.58 ± 0.51	42.65 ± 1.57^{fhjk}
DA	28	49.31 ± 0.35	49.26 ± 0.32^{bc}	49.88 ± 0.35	50.56 ± 0.23^{ec}
UIUT	-	50.51± 0.20	48.00 ± 0.30	50.95 ± 0.23	50.83 ± 0.36^{ec}

Table 2: Effect of the DCM and MeOH crude extracts of leaf of *Albizia schimperiana* on PCV of *T. congolense* infected mice

Value are Mean ± SEM; N=6; D0=the day treatment commenced; D: Day; MeOH: Methanol; DA: Diminazene aceturate; DCM: Dichloromethane, All superscripts indicate significance at $p<0.05$ (^{a}compared to the negative control, ^{b}compared to DCM 50, ^{c}compared to DCM 100, ^{f}compared to DCM 400, ecompared to DA, ^{h}compared to MeOH 100, ^{j}compared to 200, ^{k}compared to MEOH 400).

The result indicated that the mean PCV levels of all infected groups before starting treatment was 49.12 ± 0.12 compared to uninfected untreated group with mean PCV levels of 50.51 ± 0.20. The diminazene aceturate treated group sustained their PCV throughout the study period, with final mean values of 50.56 ± 0.23 on day 21 comparable to that of the uninfected untreated group. Conversely, mean PCV changes in mice treated with MeOH extracts (200 mg/kg and 400 mg/kg) and DCM extract (400 mg/kg) revealed statistically

significant difference with negative control. The MeOH 400 mg/kg treated group has showed a gradual increase in mean PCV until the end of the experimental period, with the final mean value of 50.01 ± 0.1, which is comparable to the positive control. The group treated with DA has displayed significantly higher PCV compared to the negative control group ($p<0.05$) starting from day 7 of treatment. The drop in the level of the PCV that correspond to the relapse of parasitemia, in the DA group was not recorded.

The pattern of mean body weight changes of the groups involved in the study are given in Figure 2.

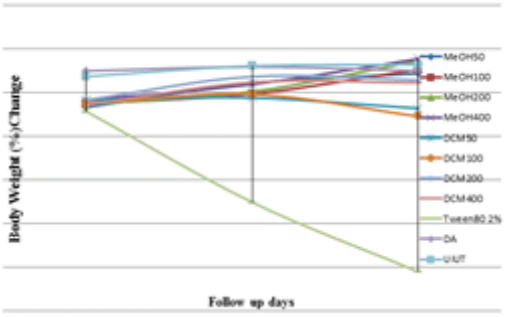

Figure 2: Effect of the methanol and dichloromethane crude extracts of leaf of *Albizia schimperiana* on body weight change (g) of T. congolense infected mice. Values are Mean ± SEM; N=6; D0=the day treatment commenced; D: Day; MeOH: Methanol; DCM: Dichloromethane; DA: Diminazine acceturate; UIUT: Uninfected Untreated

On the 13th day of post-infection (just prior to commencement of treatment) the experimental animals had a mean weight ± SEM of 27.17 ± 0.22g. In the DA treated group, the animals showed a gradual increase in weight up to 14 days of follow up which starts to drop at the end of the experiment. The mean body weight of all the groups treated with MeOH extract showed a similar pattern of loss up to day 7 of post treatment. Treatment with all doses of MeOH crude extracts prevented loss of weight starting from day 9 of post-treatment. A statistically significant drop in mean body weight was recorded in infected control group through the study period. The MeOH extract (400 mg/kg) shows statistically detectable differences in preventing weight loss ($p<0.05$) associated with parasitemia compared with the entire group on day 21 of post treatment. There was no statistically significant body weight improvement between the groups treated with DCM extract when compared with the positive control through the study period. The pre-treatments mean rectal temperature of *T. congolense* infected mice was 35.71 ± 0.6. The mean rectal temperature of uninfected untreated group was 34.9 ± 0.4. The analysis of result showed that temperatures of all the experimental groups were fluctuating when compared on day 7, day 14 and day 21 of post treatment. The DA and MeOH 400mg/kg treated group has also revealed 2.3% and 1.4% reduction in rectal temperature, respectively on day 7 of post-treatment (Figure 3)

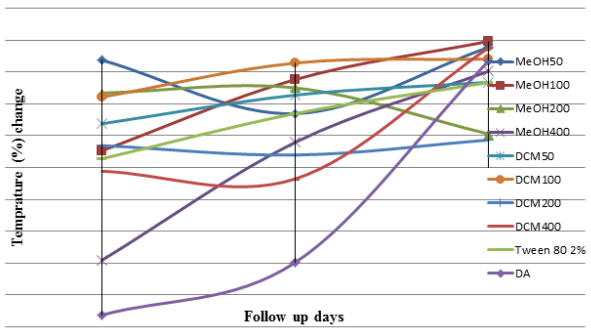

Figure 3: Percentage temperature change of T. congolense infected mice treated with DCM and MeOH crude extracts of leaf of Albizia schimperiana. Values are Mean ± SEM; N=6; D0=the day treatment commenced; D: Day, Data are calculated as percentage change.

The survival time (days) of the mice showed 27.5 ± 4.2 and 41.6 ± 0.33 days respectively for the negative and positive control. The administration of various doses of the MeOH extract of *Albizia schimperiana* showed the following survival times for the mice at the doses stated: 50 mg/kg (34.1 ± 0.28 days); 100 mg/kg (34.6 ± 2.1 days); 200 mg/kg (40.6 ± 0.88) and 400 mg/kg (41.1 ± 0.54 days). The result showed MeOH extract at 200 mg/kg and 400 mg/kg were the most effective doses in prolonging the survival time of the infected mice when compared with the negative control (Figure 4).

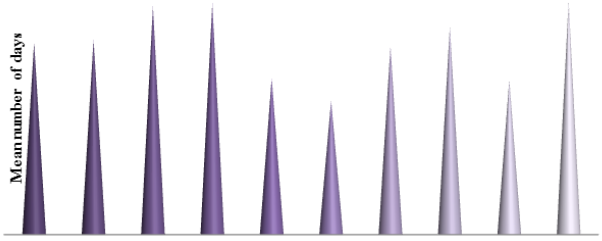

Figure 4: Effect of the DCM and MeOH crude extracts of leaf of *Albizia schimperiana* on mean survival time of *T. congolense* infected mice. Value are Mean ± SEM; N= 6; D: Day; D0= the day treatment commenced

Discussion

The present study investigated phytochemical constituents and *in vivo* antitrypanosomal activity leaf extract of *Albizia schimperiana*. The absence of death following administration of the extracts at 2000 mg/kg showed that the extracts were well tolerated. However, this result does not rule out the possible cumulative toxic effects of administering the extracts for 7 consecutive days. As arbitrary use of crude extracts in treatment without carrying out an acute toxicity test could be fatal [24], toxicity tests together with pilot study were employed in determining the possible dosages at which crude extracts administered to experimental animals.

The phytochemical analysis of the DCM and MeOH crude extracts of *Albizia schimperiana* revealed a range of compounds that could be responsible for the trypanosuppresive activity. Most plants with anti-trypanosomal properties have been found to contain metabolites such as alkaloids [25-27], flavonoids [26,28] and saponins [29].

The parasites were not completely eliminated by both solvent extracts in the in vivo test, despite the significant reduction in parasitemia recorded in group treated with MeOH extracts. The parasite counts in animals treated with methanol extract at dose of 200 and 400 showed maximum reduction at the D7 and D9 and afterwards the count began to increase due to relapse of infection, associated with loss of activity of extract may be due to pharmacokinetic reasons. This pattern was similar to DA treatment where parasitemia increased due to parasite resistance.

Different researchers [30-32] have made similar observations on reduction in parasitemia and speculated that the degradation or metabolism of the active principle or high parasite load could mask the efficacy of crude extracts. Although the extracts mechanism for observed *in vivo* trypanosuppresive effect is not known, it is obvious that extract contain phytochemicals that could interfere with survival of the parasite in vivo. The result of the phytochemical screening of both crude extracts of Albizia schimperiana leaf revealed pharmacologically active metabolites such as alkaloids, anthraquinones, flavonoids, saponins, steroidal compounds and tannins. The trypanocidal property of the extracts may probably be due to the action of one of these constituents or two or more acting in concert.

The present study did not involve detailed characterization and isolation of different compounds that could be responsible for the observed activity. However, some of the mentioned secondary metabolites were either confirmed or isolated for anti-trypanosomal activity. Rosenkranz and Wink [33] characterized alkaloids by their general property to inhibit protein biosynthesis, to intercalate DNA, to disturb membrane fluidity, to inhibit microtubule formation or induced programmed cell death in blood stream forms of *Trypanosomes*. *In vitro* and *in vivo* activities of flavonoids on the trypanosomal parasite were reported by different authors [26,34]. Flavonoids when administered *in vivo,* significantly reduced the level of parasitemia, and did not completely clear the parasites. The possible chemotherapeutic targets could be interference with glycolysis for energy production and membranes of the parasites [35].

Diminazene aceturate temporarily cleared trypanosomes from circulation of infected mice to no detectable level, but relapse occurred during the experimental period. This might be, due the fact that, trypanocidal drugs developed resistance in several African countries, including Ethiopia [36,37]. Regarding the test stock, Chaka and Abebe [38] reported *T. congolense* originating from southern Ethiopia including Bedele area, were resistant to the standard trypanosomal drug.

The measurement of anemia gives a reliable indication of the disease status and productive performance of trypanosome infected animals and also the primary cause of death [20,39]. Anemia, as indicated by PCV level, is known to worsen with increasing parasitemia. This may be due to trypanosome generated reactive oxygen species can attack red blood cells membranes, induce oxidation and subsequently hemolysis [40,41]. The slightly lower PCV observed in all the infected groups before the commencement of the treatment may be as a result of acute hemolysis due to increasing parasitemia. These findings are in agreement with a report of Ekanem et al. [20]. The results of this study showed the highest dose of MeOH extracts have comparable potential to diminazene in minimizing sudden drop in PCV values. This may be attributed to free radicals scavenging property of the flavonoids, in the MeOH extract. In untreated and lower doses of DCM extracts treated mice, the parasite count increased and the PCV decreased markedly from day to day until the death of the animals, which was also observed in a similar study [42].

The trypanosuppresive effect of the extracts against trypanosome infection can be further signified by the effect on the weight of the mice. Animals treated with the highest dose of MeOH extract maintained their body weight throughout the post treatment period while the animals in the infected untreated group showed progressive reduction in body weights. This body weight change was found to be consistent with the observation made on parasitemia. The highest body weight loss recorded may be due to trypanosomosis causing a drop in feed intake hence there is energy deficit and loss of tissue associated with catabolism of body fat, deficiencies of vitamin C, B and essential amino acids [43]. Those mice treated with highest dose of both extracts and positive control were in better physical state to eat and reduced loss of protein from tissues due to the administered extracts, possibly by depletion of proliferating parasites [20,42].

Significant prolongation in survival time (in days) was recorded in groups treated with 200 and 400 mg/kg MeOH extracts compared with the negative control. Parasitemia in initial appearance of trypanosomes in peripheral circulation are usually high, fluctuating and evident in most days. At this acute crises and sub-acute stage, death commonly occurs due to severe pancytopenia and other pathologies [43,47]. Prolongation in mean survival time may be attributed to the parasite suppressive effect of the extracts, hence overpasses the acute crisis and sub-acute stage of infection. Then, after a sufficiently balanced situation in the host-parasite relationship was established allowing the establishment of a chronic infection as reported by Takeya et al. [47-49].

Conclusion

The present study has established *in vivo* anti-trypanosomal activities of DCM and MeOH crude extracts of *Albizia schimperiana* leaf against *T. congolense*. The anti-trypanosomal activities of DCM and MeOH crude extracts of *Albizia schimperiana* leaf may be due to the active constituents present within the extracts. The MeOH crude extract have shown a better *in vivo* anti-trypanosomal activity at higher doses. This study has also provided evidence that Albizia schimperiana leaf extracts are trypanosuppresive, prevent drop in PCV level, promote weight gain and prolong survival time of mice. This work has also demonstrated lack of acute toxicity of the crude extracts of *Albizia schimperiana* leaf in mice supporting its ethnopharmacological use by the society.

Acknowledgements

The authors would like to thank staffs of the National Tsetse and Trypanosomosis Investigation and Control Center for technical assistance during isolation of the test organism; Vice President for research and Technology transfer of Addis Ababa University for partly funding the research and staffs of Aklilu Lemma Institute of Pathobiology, for their cooperation and unreserved help during the laboratory work and Jimma University for granting study leave for first author.

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