In vitro and in vivo antitrypanosomal activities of Echinops kebericho root extract

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Research note

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Abstract

Objective: Microbial resistance to the few conventional antitrypanosomal drugs, increasing resistance of vectors to insecticides, lack of effective vaccines and adverse effects of the existing antitrypanosomal drugs justifies the urgent need for effective, tolerable and affordable drugs. We assessed antitrypanosomal effect of hydromethanolic extract of *Echinops kebericho* Mesfin roots against *Trypanosoma congolense* field isolate using *in vitro* and *in vivo* techniques. Parasite load, packed cell volume (PCV), body weight and rectal temperature in Swiss albino mice were assessed. This finding is part of the outcomes of drug discovery research for neglected tropical diseases. Result: The extract ceased motility of the trypanosomes within 40 min at 4 and 2 mg/ml concentration whereas in the untreated control motility continued for more than 160 min. The extract also reduced parasitemia, prevented drop in PCV and body weight significantly ($p < 0.05$), as compared to control. Phytochemical analysis showed the presence of flavonoids, triterpines, steroids, saponins, glycosides, tannins and alkaloids. It is observed that this extract has activity against the parasite. Isolation and purification of specific compounds are required to identify hit compounds responsible for the antitrypanosomal activity of the studied medicinal plant.

Introduction

Current trypanosomiasis control relies on trypanocidal drugs, use of trypanotolerant cattle breeds and control of the tsetse fly vector. The major strategy to control trypanosomiasis relied on the use of trypanocidal drugs, which is challenged by an increasing problem of resistance [1]. The search for new chemical entities that are effective against trypanosomes, safe and affordable for disease-endemic countries is rational to fight against the trypanosomiasis [2]. To this effect, exploring natural products and synthetic sources are required to feed the pipeline of drug developments for trypanosomiasis control and elimination.

Plants are potential sources of new drugs due to the presence of countless number of secondary molecules that have pharmacological effects [3]. Exploring traditionally claimed medicinal plant for the biological activity gave humankind a number of antiprotozoal medications.

Validation of medicinal plant for their antitrypanomal activity will guide the society for the best approach to employ their indigenous knowledge and at the same time provide hit compounds to feed future pipeline for antitrypanomal drug development. *Echinops kebericho* (Mesfin), [Amharic vernacular name: kebericho], belongs to family Asteraceae/Compositae, endemic to Ethiopia, is erect perennial herb or shrub [4]. Its varied medicinal applications are documented in the ancient medico-religious pharmacopoeia, and are well-recognized by modern-day traditional professionals/specialists [5].

Echinops kebericho root is used for the treatment of animal trypanosomiasis in Ethiopia [6]. However, there is no laboratory-based evidence for the effectiveness and safety of this plant. The objective of this study was, therefore, to assess the *in vitro* and *in vivo* antitrypanosomal effects of hydromethanolic

extract of *E. kebericho* roots using field isolate of *T. congolense*, which is the most important cause of domestic animal trypanosomiasis [7]. Experimental mice infection model has been selected given the provision of this model new insight in both human and animal trypanosomiasis [8].

Method

Plant collection and authentication

Roots of *E. kebericho* were collected in November 2015 in Jimma Arjo Woreda of Eastern Wollega, Ethiopia. Leaves with flower specimen of the plant were identified and authenticated at Aklilu Lema Institute of Pathobiology (ALIPB), and the vouchers were deposited at the National Herbarium of Addis Ababa University with number DA 01.

Preparation of plant extracts

Air dried powdered plant material was macerated in an Erlenmeyer flask with 80% methanol at room temperature for a period of 72 hours. It was then filtered with gauze followed by Whatman filter paper (No.1). The residue was re-macerated once again to increase the yield. The filtrate was concentrated using rotary evaporator to remove methanol. Then the concentrated filtrate was lyophilized to remove water.

Test organism and experimental animals

Trypanosoma congolense was obtained from Department of Veterinary Parasitology, Addis Ababa University by infecting Swiss albino mice via intraperitoneal inoculation.

Swiss albino mice of either sex, weighing 30-35 g (age 10-12 weeks) were purchased from Ambo University (Ethiopia). They were fed with standard pellet and provided water *ad libitum*; maintained at room temperature of 23-25°C with relative humidity of 60-65%. The care and handling of animals were in accordance with internationally accepted guidelines for use of animals [9].

Experimental procedures

Evaluation of *in vitro* antitrypanosomal activity

In vitro test was performed in triplicates to detect any motile trypanosomes in a 96 well microplate. Twenty microliter of blood containing about 16-32 organisms per field were mixed with 5 µL of the test substance at concentrations of 2.5, 5, 10, 20mg/mL to produce test concentrations of 0.5, 1, 2, 4.0 mg/mL, respectively.

Phosphate buffer saline (pH 7.2) and standard trypanocidal drug, diminazene aceturate (DA) were used to serve as untreated control and treated controls, respectively. The mixtures were incubated at 37°C for up to 3 h. During the period, motility of the parasites was checked in 20 min interval under microscopy

(X40 objective lens). Briefly, about 2 μ L of test mixtures was placed microscope slide and covered with cover slips and the parasites observed for reduced motility or complete cessation of motility.

Evaluation of *in vivo* antitrypanosomal activity

Thirty mice of either sex were randomly grouped into five (I- V) groups of 6 animals per group. They were intraperitoneally infected with 0.2 mL of *T. congolense* (5×10^5 parasites/mL) suspension. Groups I and II were administered 0.3 mL distilled water per orally and DA (3.35mg/kg) per orally respectively to serve as untreated and treated controls, while groups III, IV, and V were administered with the extract at daily doses of 100, 200 and 400mg/kg body weight respectively for 7 consecutive days per orally from 10th days of parasite inoculation. Parasitemia and packed cell volume (PCV) were observed every 4 days for 21 days while body weight and rectal temperature was monitored every 2 days [10].

Determination of parasitemia

On the tenth day post infection and every four days, the parasitemia level of mice was checked. Parasitemia was monitored by examining blood drawn from the tail of mice under microscopy at $\times 400$ magnifications using the “Rapid Matching” method of Herbert and Lumsden [11]. Monitoring of parasitemia was performed every four days until the 21th day post-treatment initiation [12,13].

Determination of packed cell volume

PCV was determined using microhematocrit centrifuge and microhematocrit tube reader. PCV was monitored on day of treatment initiation and every 4 days until 21th day post treatment initiation [14,15].

Determination of body weight

Body weight of experimental animals were recorded on the day of parasite challenge, day of treatment initiation and every other day for 21days [16].

Determination of rectal temperature

Rectal temperature was measured using digital rectal thermometer (Mettler Toledo, Switzerland) on the day of parasite inoculation, day of treatment commencement and every other day thereafter for 21days [14].

Phytochemical screening for secondary metabolites

Standard screening tests of the extract was carried out for secondary metabolites according to the methods described in the literature [17–22].

Statistical analysis

Data were presented as mean $\pm$ SEM and analyzed using Statistical Package for Social Science version 20. Analysis of variance was employed to test statistical difference within all groups followed by Tukey test for significance test between two groups. P values less than 0.05 were considered statistically significant.

Results

Experimental animals follow up

Parasite load, body temperature and weight of animals were recorded. When the parasite load increased and confirmed that experimental animals could not survive due to infection, inhalation anesthetic in a transparent euthanasia chamber was used for humane euthanasia of experimental animals. Data set collected from solvent control and low dose extract treated groups were only for few days (Figure 1 and 2) due to rapid increment of parasite load.

In vitro antitrypanosomal activity

Hydromethanol extract of *E. kebericho* roots ceased motility of the trypanosomes within 40 min at 4 and 2 mg/ml concentration. At 0.5 mg/ml of hydromethanol extract of *E. kebericho* root, the motility was maintained for 80 min after which motility of the parasite is completely ceased. The motility of parasites ceased at 60 minutes for *E. kebericho* roots extract at dose of 1mg/ml (Table 1).

Table 1: *In vitro* activity of hydromethanolic extract of *E. Kebericho* roots

Duration (min)	<i>E. kebericho</i> extract (Motility)				DA 3.35mg/ml (Motility)	Control (Motility)
	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml		
0	+	+	+	+	+	+
20	+	+	+	+	-	+
40	+	+	-	-	-	+
60	+	+	-	-	-	+
80	+	-	-	-	-	+
100	-	-	-	-	-	+
120	-	-	-	-	-	+
140	-	-	-	-	-	+
160	-	-	-	-	-	+
180	-	-	-	-	-	-
200	-	-	-	-	-	-

Effect on parasitemia of *T. congolense* infected mice

Parasitemia level was assessed at day 4 post-treatment in all thirty experimental animals randomly assigned to different groups (30/30). All doses of the extract including the standard drug suppressed

parasitemia at day 4 post-treatment ($p < 0.05$). Treatment with extract at 200 mg/kg and 400 mg/kg and DA3.35mg/kg showed statistically significant ($p < 0.05$) reduction in parasitemia on day 8 to day 12 post-treatments compared to 100mg/kg body weight (Figure 1-A).

Effect 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 the extract and DA 3.35mg/kg shows an increase in mean PCV values from day 0 to day 12 post-treatment while treatment. PCV measurement in blood of infected mice treated with *E. kebericho* at dose of 200mg/kg and 400mg/kg and DA 3.35mg/kg showed statistically significant ($p < 0.05$) improvement compared with those untreated control on day 4 post treatment initiation (Figure 1-B).

Effect on body weight of *T. congolense* infected mice

There is statistically significant ($p < 0.05$) body weight changes in 200mg/kg, 400mg/kg and DA 3.35mg/kg treated groups compared with 100mg/kg through day 4 to day 6 post-treatment initiation and with untreated control on day 4 post-treatment (Figure 2-A).

Effects 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 2-B).

Phytochemical screening

Phytochemical screening revealed the presence of saponins, tannins, phenol, terpenes, flavonoids, glycosides and alkaloids.

Discussion

In the present study, antitrypanosomal activities of the *E. kebericho* roots suggested that the extract could contain trypanocidal constituents that are active in the *in vitro* and *in vivo* environments. Parasites motility constitutes a relatively reliable indicator of viability of most trypanosomes [23] and a complete elimination or reduction in motility of trypanosomes when compared to the control could be taken as index of trypanocidal activity [24].

In vivo assessment of the extract revealed a marked suppression of parasite load at 200 and 400mg/kg compared to group treated with vehicle control even though the extract failed to clear the parasite. Further investigation is required to see whether the extract will have an improved effect or not when administered by injection to minimize the negative impact of limited bioavailability from the gut.

The mean PCV in the untreated control group continued to decrease until all the animals in the group died due to infection while in treated groups the value shows normal range. The decrease in PCV value for

untreated control may be due to anemia which is the most outstanding clinical and laboratory feature of African trypanosomiasis [25].

The present study showed that the *E. kebericho* extract contain secondary metabolites: saponins, tannins, phenol, terpenes, flavonoids, glycosides and alkaloids. The responsible active components were yet to be isolated. Previous studies showed that flavonoids are effective antitrypanosomal substances against different trypanosome species [26]. Phenolics and polyphenols have also been reported to have antitrypanosomal activity by inhibiting the trypanosome alternative oxidase [27]. Alkaloids affect trypanosomes by DNA intercalation in combination with the inhibition of protein synthesis [26]. The *in vitro* and *in vivo* activities of the *E. kebericho* extract in the present study might be contributed by multiple secondary metabolites.

The present study also demonstrated that the extract is safe and tolerable since no treatment-related signs of toxicity were noticed in the animals throughout the observation period. Per oral administration of the hydromethanolic extract of *E. kebericho* root extract produced neither significant toxic signs nor death during the observation period of 14 days after a single administration of 2000 mg/kg with oral median lethal dose greater than 2000 mg/kg in mice. Another study showed that the hydroethanolic extract of *E. kebericho* root (up to a dose of 5,000 mg/kg) did not produce sign of toxicity [28].

In conclusion the present study provides evidence to the antitrypanosomal activity of hydromethanolic extract of *E. kebericho* root and validates the traditional practice of Ethiopian community to control trypanosomiasis. Further *in vitro* and *in vivo* activities of the extract on other species of trypanosome are recommended. In addition, the responsible compound (s) for activity shall be characterized to identify hits and develop lead compounds.

Limitation of the study

The main limitation is that the screening was conducted using one type parasite isolates, and therefore extrapolating the result to all *Trypanosoma* species is unjustified.

List Of Abbreviations

PCV	Packed cell volume
ALIPB	Aklilu Lema Institute of Pathobiology
DA	Diminazene aceturate
OECD	Economic Co-operation and Development

Declarations

Ethics approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Ethical approval for the conduct of the research project was obtained from the Scientific and Ethics Committee of the Department of Pharmacology, School of Medicine, Addis Ababa University.

Availability of data and materials

Data will be available up on request.

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Consent for publication

Not applicable.

Competing interests

The author(s) declare that they have no competing interests.

Author's contributions

DA participated in the study design, carried out the experiments, performed the statistical analysis, and drafted the manuscript; MG, NK, GT and SMA participated in the study design and the execution of the experiments; SMA contributed to the revision of the manuscript; MG, NK, GT and SMA critically revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Effect of *E. kebericho* root extract. A) Effect on parasitemia parasitemia of *T. congolense* infected mice, B) Effect on PCV of *T. congolense* infected mice. Values are expressed as mean $\pm$ SEM, Day 0= 10th day after infected blood inoculation, DA= diminazene aceturate

Figure 2

Effect of *E. kebericho* root extract. A) Effect on body weight of *T. congolense* infected mice, B) Effect on rectal temperature of *T. congolense* infected mice. Values are expressed as mean $\pm$ SEM, n=6, Day 0= 10th day after infected blood inoculation, DA= diminazene aceturate

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