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In vitro and *in vivo* trypanocidal action of aescin and aescin liposomes against *Trypanosoma evansi* in experimental mice

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PEER REVIEW

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Comments

The present study has addressed an important aspect of trypanosomosis research by evaluating the effectiveness of a potential adjuvant to trypanosomosis treatment in animals, aescin and aescin liposomes. Undoubtedly, the rearch results will add value to the current knowledge in this field.

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ABSTRACT

Objective: To verify the trypanocidal effectiveness of aescin and aescin liposomes against *Trypanosoma evansi in vitro* and *in vivo*.

Methods: Aescin and aescin liposomes were used *in vitro* on trypomastigotes at different concentrations (0.5%, 1.0% and 2.0%) and exposure times (0, 1, 3, 6 and 9 h). *In vivo* tests were performed using mice as the experimental model. *Trypanosome evansi* infected mice were treated with aescin and aescin liposomes with doses of 60 and 100 mg/kg during 4 d.

Results: The three concentrations tested in free form and nanoencapsulated showed trypanocidal activity *in vitro*, completely eliminating the parasites in small concentration after 6 h of assay. Animals treated with aescin (100 mg/kg) and aescin liposomes (100 mg/kg) showed increase in longevity, however without curative effect.

Conclusions: Active compounds present in natural products, such as aescin, may potentiate the treatment of trypanosomosis when used in association with other trypanocidal drugs.

KEYWORDS
Surra, Nanotechnology, *Aesculus hippocastanum*, *Trypanosome*

1. Introduction

Trypanosoma evansi (*T. evansi*) is the etiologic agent of a disease known as *Mal das cadeiras* or surra in horses[1]. Surra is an important disease in a wide geographic region (Africa, Asia, and South and Central America), and infects mainly camels, cattle, horses, buffalos and some wild animals[2-4]. The parasite is transmitted by infected blood through haematophagous insects such as tabanid flies[5,6]. The disease is endemic in some regions (Mato Grosso, Pantanal in Brazil,) and in other regions it occurs in outbreaks, hindering the prophylaxis and control.

The treatment of this disease in Brazil is based on diminazene aceturate (DA), but this drug is ineffective for umpteen animals[7,8]. Most of the drugs used for the treatment of the disease do not provide total elimination of the infection and are associated with recurrence and mortality[8]. In many cases, DA treatment may not be effective, leading to recurrent parasitemia[8-10], as well as hepatotoxicity, and nephrotoxicity[1,11]. As a result, researchers have tested natural products such as oils of copaiba, andiroba, aroeira, tea tree[12-14], and propolis extract[15].

Aescin is the predominant active constituent of *Aesculus hippocastanum* seed extract, which is a mixture of triterpene, saponins, consisting of A, B, C and D aescin[16]. Research has been focused on plant secondary compounds, such as saponins,

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for the control of parasite in sheep[17,18]. According to Carrasco and Vidrio aescin has been shown to be effective in the treatment of inflammatory conditions[19]. Actually, some studies have indicated that aescin is also a potential anticancer agent[20,21].

The field of nanoparticle synthesis, assembly, and application in biology is a fast growing area of nanotechnology and nanomedicine[22]. Through the development of materials that exhibit novel optical, chemical, and electrical properties at the nanometer-sized scale, it is hoped that it will be possible improve therapies for disease[23]. In this context, it can be highlighted the liposomes. Liposomes are spherical self-closed structures, composed of curved lipid bilayers, that encapsulate both hydrophilic and lipophilic substances[24]. The simplicity of production, their biocompatibility, low toxicity, size and similar composition to cells make them a revolutionary tool in biomedical domain. Based on the needs for curative therapy of *T. evansi* and on the properties of aescin and the antiparasitic properties of saponins mentioned above, the present study analyzed for the first time the *in vitro* and *in vivo* activity of aescin and aescin liposomes against *T. evansi*.

2. Materials and methods

2.1. T. evansi isolate

This study was set up in two consecutive experiments (*in vitro* and *in vivo*). The same *T. evansi* isolate was used in both experiments[10]. Two rats (R1 and R2) were infected intraperitoneally with trypomastigotes contaminated blood kept cryopreserved in liquid nitrogen. This procedure was performed to obtain a large amount of viable parasites for *in vitro* tests (R1), and to infect the experimental groups (R2).

2.2. Aescin and aescin liposomes

Aescin was purchased from (Sigma-Aldrich®, St. Louis, USA). Aescin liposomes were prepared with 0.5% aescin using a proprietary method from Inventiva®. Particle size and polydispersion index were evaluated by dynamic light scattering. Zeta potential was evaluated using electrophoretic mobility technique. The samples were diluted in Milli-Q water (500) and the assays were performed using Zeta Sizer Nanoseries, Malvern. The pH was assessed by direct use of Digimed potentiometer according to Da Silva[25].

2.3. In vitro tests

The culture medium for *T. evansi* was adapted from Baltz as previously published by Baldissera[13,26]. The trypomastigotes were acquired from the infection of one rat (R1) with a *T. evansi* isolate. Five days post-infection rat showed high parasitemia (7.5×10^6 trypanosomes/μL). The rat was was anesthetized with isoflurane for blood collection by cardiac puncture, and blood was stored in ethylene diamine tetraacetic acid tubes. For trypanosomes separation, each 200 μL blood was diluted in complete culture medium (200 μL), stored in microcentrifuge tubes and centrifuged for 10 min at 1 550 r/min. The supernatant was removed and resuspended in culture medium and the number of parasites was counted in a Neubauer chamber.

The culture medium with the parasites was distributed in microtiter plates (270 μL/well), followed by the addition of 25 μL of aescin (diluted in culture medium) at concentrations of 0.5%, 1.0% and 2.0%. The aescin liposomes also were used at concentrations of 0.5%, 1.0% and 2.0 %. A positive control (DA at a dilution of 0.5%) was also used, at the same volume (25 μL). The tests were performed in duplicates and the parasites were counted at 1, 3, 6 and 9 h after the onset of the experiment in Neubauer chambers. The microtiter plates were placed in a 5% CO_2 incubator at 37 °C according to Baltz[26].

2.4. In vivo test

2.4.1. Animal model

Forty-two, female, 60-day-old-mice weighing an average of (23.0±0.7) g were used as the experimental model. They were kept in cages with six females each, housed on a light/dark cycle of 12 h, in an experimental room with controlled temperature (23±1) ℃ and humidity 70%. They were fed with commercial feed, and water *ad libitum*. All animals were subjected to a period of 10 d for adaptation.

2.4.2. Experimental design and parasitemia estimation

The mice were divided into seven groups (A to G). Group A consisted of uninfected mice and untreated (negative control); Group B consisted of infected mice and untreated (positive control); Group C was composed of animals infected and treated with aescin 60 mg/kg; Group D was composed of animals infected and treated with aescin 100 mg/kg; Group E was composed of animals infected and treated with aescin liposomes 60 mg/kg; Group F was composed of animals infected and treated with aescin liposomes 100 mg/kg; Group G was composed of animals infected and treated with DA. Infected animals were inoculated intraperitoneally with 0.05 mL of blood from one rat containing 1.1×10^6 trypanosomes.

The DA was administered in a single dose of 7.0 mg/kg, intraperitoneally injection, and 1 h after infection of the animals. Aescin and aescin liposomes were administered orally for 4 d, starting the 1 h following infection.

The evolution of parasitemia and the effect of the treatment were daily monitored through blood smear. Each slide was prepared with fresh blood collected from the tail vein, stained by the panoptic method, and visualized at a magnification of 1000× according to Da Silva[27].

2.4.3. Treatment efficacy

Treatment efficacy was determined by the number of mice that did not show clinical signs of *T. evansi* infection after treatment. Prepatent period, longevity and animal mortality were also observed.

2.5. Statistical analysis

Data from *in vitro* were analyzed by analysis of variance for repeated measures and comparison of concentrations tested for aescin. Data of the prepatent period and longevity were submitted for analysis of variance according to Duncan test ($P<0.05$).

3. Results

3.1. Aescin liposomes

The prepared suspension was evaluated regarding to their physical-chemical properties. The particle size was (223±6) nm and the