Research in Veterinary Science 100 (2015) 226–231

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

In vivo antitrypanosomal activity of *Garcinia hombroniana* aqueous extract

H.O. Dyary [a,b], A.K. Arifah [a,*], R.S.K. Sharma [a], A. Rasedee [a], M.S. Mohd Aspollah [c], Z.A. Zakaria [d], A. Zuraini [d], M.N. Somchit [d]

[a] Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[b] College of Veterinary Medicine, University of Sulaimani, Iraq
[c] Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[d] Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 22 August 2014
Accepted 1 March 2015

Keywords:
Garcinia hombroniana
Trypanosoma evansi
Trypanosomiasis
Surra

ABSTRACT

The anti-*Trypanosoma evansi* activity of *Garcinia hombroniana* (seashore mangosteen) leaves aqueous extract was tested on experimentally infected Sprague–Dawley rats. Treatment of infected rats with *G. hombroniana* extract resulted in a significantly extended post-infection longevity ($p < 0.05$), compared to the untreated control group. The possible mode of antitrypanosomal effect of the plant extract was also investigated on cultured *T. evansi* in HMI-9 medium with the addition of 25 μg/ml *G. hombroniana* aqueous extract. It was observed that the addition of *G. hombroniana* extract resulted in the inhibition of trypanosomal kinetoplast division, with no significant inhibitory effect on nuclear division. It is concluded from the current study that the aqueous extract of *G. hombroniana* has a potential antitrypanosomal activity through the inhibition of kinetoplast division, as one of the possible mechanisms of its antitrypanosomal effect. This plant could serve as a possible source of new antitrypanosomal compounds.

1. Introduction

Trypanosoma evansi is a hemoflagellate parasite causing often a chronic disease known as "surra" (Hoare, 1972). The disease infects many species of animals such as camels, horses, cattle, buffaloes, dogs, cats, sheep, goats, as well as a variety of wildlife animals (Luckins, 1988).

Surra has a wide geographical distribution, including Asia, Africa, Central and South America. Animals are infected through mechanical transmission via blood-sucking flies, especially those belonging to the genera *Tabanus* and *Stomoxys* (Stevens and Brisse, 2004). In South America, vampire bats are also involved in the transmission of this disease (Hoare, 1972).

Although it has been discovered more than a century ago (Luckins, 1988), the treatment of surra is limited to a few drugs that have been in use for a long time (Steverding, 2010). These drugs are toxic and sometimes ineffective due to the emergence of resistant strains of *T. evansi* to the currently available therapies (El Rayah et al., 1999; Zhou et al., 2004) and this makes the treatment of surra ineffective.

Plants continue to provide lead compounds for the treatment of various diseases, even with the advent of modern technology (Balunas and Kinghorn, 2005). In 2008 only, 48% of 225 compounds undergone preclinical and clinical trials were originated from plants (Harvey, 2008). Many researchers studied the antitrypanosomal potential of plants in the recent years and some plant extracts exhibited promising antitrypanosomal potentials (Bawm et al., 2010; Gressler et al., 2012; Habila et al., 2011; Talakal et al., 1995). Any new drug could be used in combination with the currently available ones for better efficacy, to improve their potency and/or pharmacological activities, as well to reduce the burden of use of the old drugs.

Garcinia hombroniana (Clusiaceae) is an evergreen small tree commonly known as "seashore mangosteen". Extracts prepared from the plant parts were reported to have antioxidant and platelet aggregation-inhibitory effects (Jantan et al., 2011). The plant species belonging to the genus *Garcinia* have been known to contain a number of xanthone compounds. Xanthones isolated from different *Garcinia* species were reported to exhibit antioxidant (Minami et al., 1994), antiplasmodial (Elfita et al., 2009; Mbwambo et al., 2006) and antitumor (Aisha et al., 2012; Chitchumroonchokchai et al., 2012) activities. The aqueous extract of the plant's leaves exerts *in vitro* antitrypanosomal activity with a considerably high selectivity index (Dyary et al., 2014).

This study investigates the antitrypanosomal effect of *G. hombroniana* aqueous extract on Sprague–Dawley rats experimentally infected with *T. evansi* and the possible mode of antitrypanosomal activity of the extract *in vitro*.

* Corresponding author: Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel.: +60 3 8609 3403; fax: +60 3 8947 1971.
E-mail address: arifah@upm.edu.my (A.K. Arifah).

http://dx.doi.org/10.1016/j.rvsc.2015.03.007
0034-5288/

2. Materials and methods

2.1. Preparation of plant extract

Fresh leaves of *G. hombroniana* were collected from the Agricultural Conservatory Park at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM). The plant species was identified in the Biodiversity Unit in IBS, UPM.

The leaves were cleaned from debris, dried at 40–45 °C in a hot-air oven and ground to powder. A measured amount of the plant powder was soaked in ten times the amount (wt/vol) of sterilized water in a tightly sealed container and the container was put under continuous shaking for 4 hours in a rotary incubator shaker (Dyary et al., 2014). The mixture was then filtered using filter paper (Whatman no. 4) and the solvent was separated using freeze dryer. The extract powder was stored in a tightly-sealed container at –28 °C until used.

2.2. Antitrypanosomal activity of G. hombroniana aqueous extract

The antitrypanosomal activity of *G. hombroniana* aqueous extract was tested *in vivo* using Sprague–Dawley rats of both sexes. The experiment was conducted with permission of Animal Care and Use Committee (ACUC animal utilization protocol number 11R127), Faculty of Veterinary Medicine, Universiti Putra Malaysia.

2.3. Trypanosomes passage in the laboratory rats

Trypanosoma evansi strain Te7, originally isolated from a buffalo (*Bubalus bubalis*) in Selangor, Malaysia, was used for the experiment. The trypanosomes were passaged twice in Sprague–Dawley rats before being used in the experiment. Blood was collected from the infected rat during parasitemia and diluted using phosphate buffered saline–glucose (PBS–glucose) before being injected into the experimental animals.

2.4. Animal groups

Thirty male and female rats, aged 8–10 weeks and weighing 179.50 ± 20.96 grams were used in the experiment. After 2 weeks adaptation to the laboratory environment, all the animals were infected by intraperitoneal injection of 1×10^5 trypanosomes and were divided randomly into six groups of five rats each. The first group, the untreated control, was orally drenched with sterile water (20 ml/kg of body weight), for 3 days, starting from day 4 post-infection. The second group, the treated control, was treated with a single intramuscular injection of 3.5 mg/kg diminazene aceturate (Berenil®) at day 4 post-infection. Groups T1–T3 were orally treated with 300, 600 and 1200 mg/kg of *G. hombroniana* extract (dissolved in water) for three consecutive days, starting from day 4 post-infection. The extract volume was adjusted to 20 ml/kg for each rat. Group T4 was treated with 300 mg/kg of *G. hombroniana* extract for 3 days, starting from the same day of parasite inoculation.

2.5. Measurement of parasitemia and packed cell volume

The level of parasitemia was monitored daily by taking blood from the tail vein. The concentration of trypanosomes in the blood was determined using a Neubauer hemocytometer. The trypanosomes were counted in the four large squares used for leukocyte count under 200× magnifications. In case of extreme parasitemia, the blood was first diluted using PBS–glucose to facilitate the parasite count. The parasitemia level was calculated according to the following formula:

$$\text{Trypanosome concentration (number/ml blood)} = \frac{N}{4} \times 10^4 \times DF$$

where N is the total number of trypanosomes in the four large squares and DF is the dilution factor.

The hematocrit or packed cell volume was measured immediately before the rats were infected and every 3 days thereafter. This was done by filling approximately two-thirds of a heparinized capillary tube with blood taken from the tail and centrifugation at 10^4 RPM for 5 minutes.

2.6. Mode of antitrypanosomal effect of G. hombroniana aqueous extract

The effect of *G. hombroniana* on the kinetoplast division of *T. evansi* was studied by culturing trypanosomes in HMI-9 medium with the addition of *G. hombroniana* aqueous extract (25 µg/ml). Trypanosomes were cultured in 25 cm^2 tissue culture flasks at 37 °C, 5% CO_2 and 85% relative humidity for 24 hours. Negative control flasks and flasks containing 15 ng/ml diminazene aceturate as positive control were also included. Three flasks were prepared for each of the *G. hombroniana* extract-containing, negative and positive control cultures.

After 2, 6, 12 and 24 hours of incubation, 1–1.5 ml of the trypanosome culture was taken from the culture flask and put in a 1.5 ml capacity centrifuge tube. The tube was centrifuged for 5 minutes at 2500 *g* and 10 °C in a refrigerated microcentrifuge. The supernatant was discarded and the trypanosome pellet was resuspended in 0.5 ml of 4% paraformaldehyde. The content of the tube was then poured into a 1.5 cm diameter well on a glass slide that was made using nail varnish. The glass slide was kept in a humid chamber for 30 minutes to allow the trypanosome cells to settle on the slide. The slides were then dipped in 1% triton X100 for 5 minutes, and were put in phosphate buffered saline pH 7.3 to wash off the triton. Finally, the slides were dipped in Hoechst (bisbenzimide H33258 from Sigma-Aldrich, Germany) stain solution (100 parts per billion in PBS pH 7.3) for 5–15 minutes. The slides were covered with a cover slip and stored in the refrigerator overnight until examined in the following day.

The slides were examined using 400× magnifications under a fluorescent microscope (Nikon Eclipse 80i, Japan) to quantify the number of kinetoplasts and nuclei in each trypanosome cell which fluoresce under ultraviolet light. From each slide, 200–400 trypanosomes were counted and classified according to the number of nuclei and kinetoplasts in each cell as cells containing (a) one nucleus and one kinetoplast (1N1K), (b) one nucleus and two kinetoplasts (1N2K), (c) two nuclei and two kinetoplasts (2N2K), (d) one nucleus and no kinetoplasts (1N0K), (e) two nuclei and one kinetoplast (2N1K) and (f) two nuclei and no kinetoplasts (2N0K). The percentage of each cell type was extrapolated and the change in their percentage at 2, 6, 12, and 24 hours of incubation was compared statistically between the control and treatment groups.

2.7. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by post hoc test (Duncan). A probability value less than 0.05 was considered statistically significant.

3. Results

3.1. Antitrypanosomal activity of G. hombroniana aqueous extract

3.1.1. Measurement of the level of parasitemia and packed cell volume

After the rats were infected with *T. evansi*, trypanosomes appeared in the blood of the control, T1, T2 and T3 group rats as early