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Melaleuca alternifolia anthelmintic activity in gerbils experimentally infected by *Haemonchus contortus*

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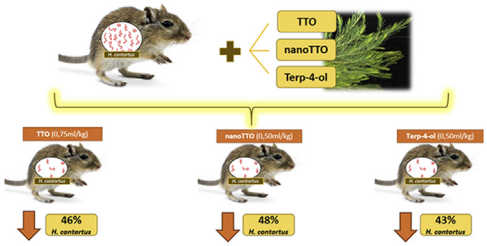
HIGHLIGHTS

- The treatments used were effective on reducing infection by *H. contortus* in gerbils.
- The nanocarriers used with essential oil of *M. alternifolia* (nanoTTO) showed highest efficiency.
- Nanotechnology can increase efficacy and bioavailability of therapeutic substances.
- Terpinen-4-ol may protect against liver damage caused by infectious agents.

GRAPHICAL ABSTRACT



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ABSTRACT

Gastrointestinal parasites are one of the biggest health problems faced in sheep, mainly due to their pathogenicity and resistance to drugs used to control these parasites. Thus, the following study aimed to assess the anthelmintic efficacy of *Melaleuca alternifolia* against *Haemonchus contortus* in gerbils (*Meriones unguiculatus*) experimentally infected. Three treatments were tested: *M. alternifolia* essential oil, popularly known as tea tree oil (TTO), a solid lipid nanocarrier made with essential oil of *Melaleuca* (nanoTTO), and terpinen-4-ol (terp-4-ol). *In vivo* studies were performed by determining the mean worm burden of *H. contortus* in gerbils TTO (0.75 mL/kg); nanoTTO (0.5 mL/kg) and terp-4-ol (0.5 mL l/kg) were able to reduce 46.36%; 48.64%, and 43.18% worm burden, respectively. *H. contortus* increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, as demonstrated by liver injury. It was found that the TTO, nanoTTO, and terp-4-ol were not toxic to liver and kidneys since hepatic and renal functions were not affected. Moreover, terp-4-ol was able to prevent increased levels of seric AST and ALT in infected animals, indicating a hepatoprotective effect. Thus, our results indicate that TTO, nanoTTO, and terp-4-ol are safe and efficient against *H. contortus* infection in gerbils, and possibly the terp-4-ol may be considered the compound present in the *Melaleuca alternifolia* responsible for parasitic action against *H. contortus*.

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1. Introduction

Infections caused by gastrointestinal nematodes are responsible for significant negative impact on sheep and goat production (Acharya et al., 2014), leading to major economic losses due to subclinical diseases (Molento et al., 2011). *Haemonchus contortus* is considered the main nematode involved in gastrointestinal infections in the tropics and subtropics with high prevalence and pathogenicity (Krecek and Waller, 2006).

The control of gastrointestinal nematodes is essential for successful production of small ruminants. However, there are many reports on parasitic resistant to anthelmintic drugs, evidencing the need of new strategies for disease control (Fortes and Molento, 2013). Thus, the use of medicinal plants with anthelmintic effect arises as a possibility (Nogueira et al., 2006). In addition, extracts from plants could be used in association to anthelmintic drugs, increasing its efficiency on parasitic control. Also, recent studies have been demonstrated the effect of nanotechnology against parasite infections, such as the use of solid lipid nanocarriers (Prabhu et al., 2012; Jain et al., 2014). Solid lipid nanocarriers is considered a novel class of nanocarriers with many advantages, such as low toxicity, better stability and high biocompatibility, that are able to improve the therapeutic efficacy and safety of many chemotherapies, including antiparasitic the drugs (Wong et al., 2004).

Tea tree oil (TTO) is an essential oil obtained from the leaves of the Australian native plant *Melaleuca alternifolia* (Mulla and Su, 1999), and its main compound is terpinen-4-ol. There are reports of many properties of this plant against pathogenic microorganisms, including parasitic activities against *Trichomonas* sp., *Leishmania* sp., *Trypanosoma* spp., *Otodectes* sp., *Lucilia* sp., *Bovicola* sp., and *Rhipicephalus* sp. (Viollon et al., 1996; Mikus et al., 2000; Baldissera et al., 2014; Neves et al., 2012; Callander and James, 2012; James and Callander, 2012; Pazinato et al., 2014). *In vitro* TTO activity against *H. contortus* was proved by our research group (Grando et al., 2016) and nanotechnology is little used in the control of this parasite, being an alternative that has some advantages, such as slow, and controlled release, increased bioavailability, and reduced side effects (Roco, 2001; Verma, and Garg, 2001). Therefore, the aim of this study was to assess the anthelmintic activities of *M. alternifolia* as essential oil (TTO), as an essential oil added to a solid lipid nanocarrier (nanoTTO), and its main compound, terpinen-4-ol in gerbils (*Meriones unguiculatus*) experimentally infected by *H. contortus*.

2. Materials and methods

2.1. Preparation of test solutions

Essential oil of *M. alternifolia* (TTO) was purchased from *Importadora Química Delaware Ltda*® (Porto Alegre, Brazil). Lipid nanocarriers of TTO (nanoTTO) were obtained from *Inventiva*® company (Porto Alegre, Brazil), and were prepared with 7.5% TTO using a method based on a high-pressure homogenization. The terpinen-4-ol (terp-4-ol) was purchased from Sigma-Aldrich®. NanoTTO present a final concentration of 7.5%.

2.2. TTO characterization

Oil composition and yield was analyzed by gas chromatography (GC) using an Agilent Technologies 6890N GC-FID system, equipped with DB-5 capillary column connected to a flame ionization detector (FID). Injector and detector temperatures were set at 250 °C. The carrier gas was helium, at a flow rate of 1.3 mL/min. The thermal programmer was 100–280 °C on a rate of 10 °C/min. Two replicates of samples were processed in the same way. Component relative concentrations were calculated based on GC peak areas without using correction factors. GC-Mass Spectroscopy (GC-MS) analyses were performed on an Agilent Technologies AutoSystem XL GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250 °C). The transfer line temperature was 280 °C. Helium was used as carrier gas (1.5 mL/min) and the capillary columns used were an HP 5MS (30 m × 0.25 mm x 2.5 μm film thickness) and an HP Innowax (30 m × 0.32 mm i.e., film thickness 0.50 mm). The temperature programed was the same as that used for the GC analyses. Essential oil injected volume was 1 μL.

Identification of TTO components was performed on the basis of retention index (RI), determined with reference of the homologous series of n-alkanes, C7–C30, under identical experimental conditions, comparing with the mass spectra library search (NIST and Wiley), and with the mass spectra literature date (Adams, 1995). The relative amounts of each component was calculated based on the CG peak area (FID response).

2.3. In vivo experiment

2.3.1. Haemonchus contortus

The infective third larval stage (L3) of *H. contortus* used in this study was obtained from a sheep previously infected by a monospecific culture of *H. contortus* (Almeida et al., 2010). The L3 was obtained by fecal culture, following the technique of Roberts and O'Sullivan modified by Ueno and Gonçalves (1994). The experimental protocol was approved by Ethics Committee on Animal Use (CEUA) of the Universidade Federal de Santa Maria, under protocol number 043/2014.

2.3.2. Animals and infection

Forty-seven outbred 60-day old Mongolian gerbils (*Meriones unguiculatus*) of both sexes, weighing an average of 35–40 g were used as an experimental model. The gerbils are considered good models for *H. contortus* because parasites establish in the gerbil at a site anatomically similar to their predilection site in the abomasum of sheep (Conder et al., 1992).

The animals had free access to water and food and were maintained in a room under controlled humidity and temperature (22 ± 2 °C) with 12-h of light-dark cycle. All animals were immunosuppressed with 100 μL dexamethasone (Azium®-Coopers-Saúde Animal, Brazil) intramuscularly for five consecutive days according to Jesús-Gabuíno et al. (2010). On the third day of immunosuppression, 35 animals (35 out of 47) were inoculated with 2000 infective sheathed larvae (L3) of *H. contortus* via oral gavage. The remaining animals were left uninfected.

2.3.3. Experimental design

Gerbils were randomly divided into seven infected groups of five animals each and uninfected animals were divided into four groups of three uninfected animals each. The uninfected group of animals were used to assess whether the treatment would not cause any damage or toxic biochemical changes. All animals were treated according to their group during three days, i.e., on days five, seven and nine after inoculation with *H. contortus*.

Infected groups:

Group 1 - infected control - treated with 0.75 mL/kg of distilled water.
Group 2 - TTO (0.50) - treated with 0.50 mL/kg of TTO
Group 3 - TTO (0.75) - treated with 0.75 mL/kg of TTO
Group 4 - nanoTTO (0.20) - treated with 0.20 mL/kg of nanoTTO
Group 5 - nanoTTO (0.50) - treated with 0.50 mL/kg of nanoTTO