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In vitro activity of 3β-*O*-tigloylmelianol from *Guarea kunthiana* A. Juss (Meliaceae) on oogenesis and ecdysis of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) (Acari: Ixodidae)

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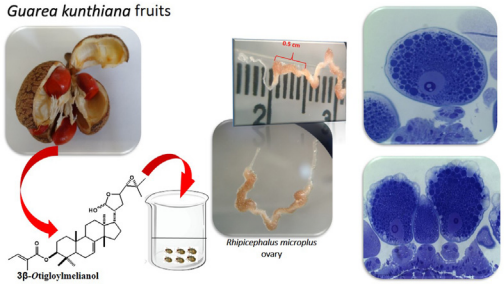
HIGHLIGHTS

- The tick *Rhipicephalus* (*Boophilus*) *microplus* is an endemic pest of cattle.
- We investigated the effects of 3β-*O*-tigloylmelianol from *Guarea kunthiana* on cattle tick.
- The new protolimonoid 3β-*O*-tigloylmelianol acts on oogenesis and ecdysis of the tick.
- This compound is an alternative to environmentally hazardous synthetic acaricides.

GRAPHICAL ABSTRACT



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ABSTRACT

We evaluated the effects of 3β-*O*-tigloylmelianol from *Guarea kunthiana* A. Juss (Meliaceae) on oogenesis, as a larvicide and on ecdysis of the larvae and the nymphs of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) (Acari: Ixodidae). On the oogenesis' test, 48 engorged females were divided into three groups, evaluated at 24, 48 and 72 h post-treatment. Half of the females were treated with 0.01% 3β-*O*-tigloylmelianol diluted in distilled water and 5% dimethyl sulfoxide (DMSO), while the other half (controls) were exposed to distilled water and 5% DMSO. After treatment, the ovaries were weighed in order to measure the gonadosomatic index (GSI) and were also subjected to standard histological technical tests. On the larvicide and ecdysis' tests, 3β-*O*-tigloylmelianol was tested at concentrations of 0.01, 0.005, 0.0025 and 0.00125%. Compared with the controls, there was a reduction of GSI of approximately 50% on the treated group, which started at 48 h post treatment. Overall, the protolimonoid 3β-*O*-tigloylmelianol has caused a significant reduction in the number of oocytes. It has also caused alteration of the cytoplasmic and germinal vesicle diameters. Morphological changes, such as vacuolization, chorion irregularity which has modified the oocytes' morphology as well as alterations on

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the yolk's granules were also observed. The compound was not larvicide, however, interfered in the ecdysis of the larvae and the nymphs. This study shows that the protolimonoid 3β-*O*-tigloylmelianol from *G. kunthiana* acts on oogenesis and ecdysis of *R.* (*B.*) *microplus*, but not as larvicide, indicating that it acts on the endocrine system of the tick.

1. Introduction

Parasites have a significant economic impact on Brazilian cattle-rearing, with economic losses of approximately 14 billion dollars annually, which 3.24 billion dollars are due to the tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) (Acari: Ixodidae) (Grisi et al., 2014), an endemic pest of cattle. Apart from Brazil, this tick often occurs in large populations in other tropical and subtropical regions of the world. The pest causes reductions on the cattle weight gain, yolk production and milk production, and it also transmits babesiosis and anaplasmosis (e.g., Jonsson, 2006). The increasing evolution of resistance of *R.* (*B.*) *microplus* to synthetic acaricides (Fao, 2004; Fernandez-Salas et al., 2012; Reck et al., 2014; Rodríguez-Vivas et al., 2014) has led the scientific community to recognize the need for further research on alternative forms of control. In this way, several tests on *R.* (*B.*) *microplus* have shown that several plant species are promising candidates, due to the substances with acaricide potential that they contain (Borges et al., 2003; Ribeiro et al., 2007, 2010; Sousa et al., 2008; Borges et al., 2011; Chagas et al., 2012; Lage et al., 2015).

The Brazilian Cerrado, which is the second largest of Brazil's major biomes (after Amazonia) (e.g., Furley, 1999; Myers et al., 2000; Klink and Machado, 2005; Marris, 2005), and the Pantanal, the world's largest wetland area (150,355 km^2) comprise a vast range of terrestrial biodiversity (e.g., Junk et al., 2006, 2014). Meliaceae plants, including *Guarea kunthiana* A. Juss, are widely distributed in some Brazilian ecosystems, such as Cerrado and Pantanal. In a previous study (Barbosa et al., 2013) we evaluated 73 ethanol extracts from 24 families of plants from Brazilian Cerrado and Pantanal for their effects on the reproductive cycle of engorged females of *R.* (*B.*) *microplus*. Of the crude extracts evaluated, the one obtained from the fruits of *G. kunthiana* was the most effective, with 99% efficacy at a 0.2% concentration (Barbosa et al., 2013). Based on these findings, a chemical investigation of this extract guided by its effect on the reproductive cycle of engorged females of *R.* (*B.*) *microplus* led to the isolation of 3β-*O*-tigloylmelianol (Fig. 1), a new protolimonoid, as the active constituent (Miguita et al., 2015). This compound strongly inhibited egg-laying and hatchability (99.2% effectiveness at a 0.01% concentration) thus revealing its potential usefulness against the cattle tick (Miguita et al., 2015). Here we investigated the effect of 3β-*O*-tigloylmelianol on oogenesis, as a larvicide and on ecdysis of the larvae and the nymphs of the cattle tick *R.* (*B.*) *microplus*.

2. Material and methods

2.1. Ethics statement

The whole research was conducted with the approval of the Animal Experimentation Ethics Committee of the Universidade Federal de Goiás (UFG, Permit Number 221/2009).

2.2. Cattle ticks colony

Engorged *R.* (*B.*) *microplus* females were collected from Holstein cattle belonging to the school farm of the UFG (Goiânia, Brazil). The cattle had not been exposed to acaricide products for at least 60 days prior to the experiment and were naturally infested. The ticks were sanitized, dried using paper towels and selected under a stereomicroscope using a 10x eyepiece lens, according to external morphological conditions. The females were weighed and those weighing 170–190 mg were selected (Bennett, 1974).

Some engorged females were incubated in order to obtain larvae, which were used to infest rabbits *Oryctolagus cuniculus* L. (Leporidae) and the engorged larvae and nymphs were used for testing. Feeding chambers made of ethyl vinyl acetate (EVA; 5 cm × 7 cm) with a central opening of 4 × 6 cm were fixed on the back of four adult rabbits, using Brascoplast adhesive. Approximately 400 larvae were placed in each chamber, which was then closed using cotton fabric. On the 6th day after the infestation, engorged larvae were collected. Engorged nymphs were collected on the 12th day. During the tests, the colonies were kept in climate-controlled chambers at 27 ± 1 °C, 90 ± 5% RH and in scotophase.

2.3. In vitro tests

The protolimonoid 3β-*O*-tigloylmelianol was previously obtained from *G. kunthiana* fruits, as described by Barbosa et al. (2013) and Miguita et al. (2015). The material was standardized for the tests and stored in a freezer at −18 °C. For the histological evaluations on oogenesis the compound was diluted to 0.01% in distilled water and 5% dimethyl sulfoxide (DMSO). This concentration was used from Miguita et al. (2015) who had the greatest action on egg-laying of *R.* (*B.*) *microplus*. Lethal and sublethal (e.g., LC50, LC90) tests were not performed because there was no mortality record in our previous experiments. To evaluate the mortality of larvae and ecdysis of engorged larvae and nymphs, tests were carried out in triplicate at concentrations of 0.01, 0.005, 0.0025 and 0.00125%. The control groups were treated with 5% DMSO in distilled H_2O.

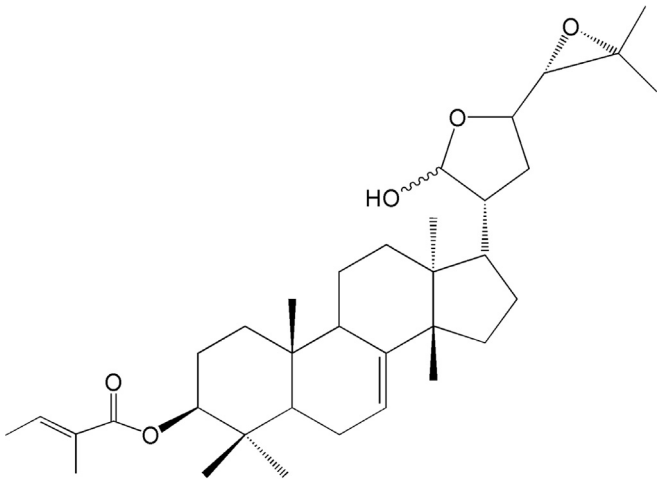

Fig. 1. Chemical structure of 3β-O-tigloylmelianol.