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Esters from castor oil: interfering with the salivary gland secretory cycle of *Rhipicephalus sanguineus* ticks (Acari: Ixodidae)

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Abstract

The medical-veterinary importance of ticks has long been recognized and studies on the internal morphology and histology of the organs and systems in these ectoparasites have been considered of great relevance. The tick *Riphicephalus sanguineus sensu* lato (s.l.) is particularly important, considering its proximity to the human being. The present study investigated that how ricinoleic acid esters from castor oil interfere with the secretory cycle of the salivary glands in *R. sanguineus* s.l. females fed for a period of 1 to 6 days. To accomplish the objectives, females were fed on both, the rabbits fed with the food enriched in esters and on the rabbits fed with regular rabbit commercial food. The results showed that the salivary glands had changes in its secretory cycle, varying according to the considered acinus, leading to a precocious degeneration of the gland caused by the exposure of the hosts to the esters. Another important data obtained was that the esters did not interfere in the metabolism of the hosts (rabbits) and thus, the results signalize the perspective of a new strategy to effectively control these ectoparasites.

Keywords alternative control, castor oil, esters, salivary glands, ticks

Introduction

Ticks have been considered as one of the most important animals in the transmission of pathogens to the hosts, including viruses, bacteria, helminths and protozoa [1]. They are cosmopolitan, mainly spread in the tropical and subtropical regions of the planet [2, 3].

Although domestic dog is the main host of the species *Rhipicephalus sanguineus* sensu lato (s. l.) (Latreile, 1806), these ticks can parasitize humans [4] due to the proximity of these ectoparasites to the urban and domestic environment [5]. Currently, synthetic acaricides have been used to control these ectoparasites; however, due to the damage caused to non-target organisms and considering that the resistant ticks populations has been selected, the search for alternative control methods and the use of natural chemical products has been increasingly intensified [6, 7].

Studies carried out by researchers from the BCSTM (Brazilian Centre of Studies on Ticks Morphology da UNESP de Rio Claro, SP, Brazil) have demonstrated the efficacy of ricinoleic acid esters from castor oil against ticks infestations. These esters act inhibiting the vitellogenesis (oocyte growth process) and also induce the precocious degeneration of *R. sanguineus* s. l. salivary glands [8- 12], both being important organs that ensure the biological success of these ectoparasites.

Thus, considering the need of new, efficient and sustainable strategies to control this important tick species, objectives of the present study were to establish the dynamics for the action of ricinoleic

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acid esters from castor oil on the morphology of salivary gland secretory cells from *Rhipicephalus sanguineus* s.l. during the feeding process.

Methodology

Bioassay

For this study, six New Zealand White host rabbits from Central Animal Facility of UNESP Botucatu (SP, Brazil) were used. In addition, 360 *R. sanguineus* s.l. adult ticks (180 males and 180 females) were obtained from the colony maintained by the researchers from the BCSTM (Brazilian Central of Studies on Ticks Morphology), UNESP Rio Claro (SP, Brazil). The ticks were maintained under controlled conditions (28 °C, 80% humidity and 12h photoperiod) in BOD incubator.

In order to perform the bioassays, the host rabbits were divided into two groups, Control Group (CG) and Treatment Group (TG)

Control Group (CG): Three rabbits were fed with regular commercial rabbit food previously added with NaCl (5g NaCl/kg of food) and water was provided "*ad libitum*".

Treatment Group (TG): Three rabbits were fed with commercial rabbit food enriched with ricinoleic acid esters form castor oil at a concentration of 5g of esters/kg of food and was offered water "ad libitum". Each individual of both the host groups was infested with 30 couples of *R. sanguineus*.

This study was approved by the Ethics Committee on Research and Scientific Merit - UNIARARAS (protocol number 006/2009).

Histology

After the required feeding stages (1 to 6 days) were completed, the *R. sanguineus* s.l. were removed from the hosts and anesthetized by thermal shock in freezer. Further, the salivary glands were removed in saline solution (7.5 g of NaCl + 2.38 g of Na2HPO4 + 2.72 g of KH2PO4 + 1000 mL of distilled water), and fixed in paraphormaldehyde 4% for 24 hours and washed in phosphate buffer pH 7.4 for 24 hours.

The material was then dehydrated in crescent series of ethanol (70%, 80%, 90% and 95%) for 15 minutes each, and transferred to embedding resin, included and sectioned. Embedding and inclusion were performed in Leica resin. The blocks were sectioned into 3 µm-thick sections using microtome Leica RM2255 and placed in glass slides. Posteriorly, the sections were stained by hematoxylin and aqueous eosin [13]. The slides were mounted in Canada balsam and then analyzed and documented using photomicroscope, Leica DM4000.

Results

The results refer to acini II and III only, because acini I do not have secretory function

Females fed for 1 day

Acini type II

In the animals from the CG, cells type a, c1, c2 and c3 (Fig. A1-A2) were observed, and their integrity was maintained. In the TG cells type a, c2, c3, c4 and c5 (Fig. G1-G2) were observed, and a cells were more active in comparison with those from CG. The acini II from TG showed fewer c3 cells in comparison with those from CG.

Acini type III

Cells d, e and f were found in both the CG (Fig. M) and TG (Fig. S). These cells were intact and their cytoplasm was full of secretion. Cells e and f were more active in the TG.

Females fed for 2 days

Acini type II

Intact cells a, c2, c3 and c4 were found in the acini II of the CG (Fig. B1-B2) and TG (Fig. H1-H2). The TG group showed cytoplasmic disorganization in some cells in the beginning (Fig. H1-H2), while others remained intact and with similar morphology to those from the CG.

Acini type III

The acini III of the CG (Fig. N) and TG (Fig. T) showed intact d, e and f cells; and these cells had secretion in the cytoplasm. The cells f of the TG group were less active than those found in the CG, and showed signs of disorganization.

Females fed for 3 days

Acini type II

The cells a, c2, c3 and c4 of the CG were intact (Fig. C). The same cell types were found in the TG .

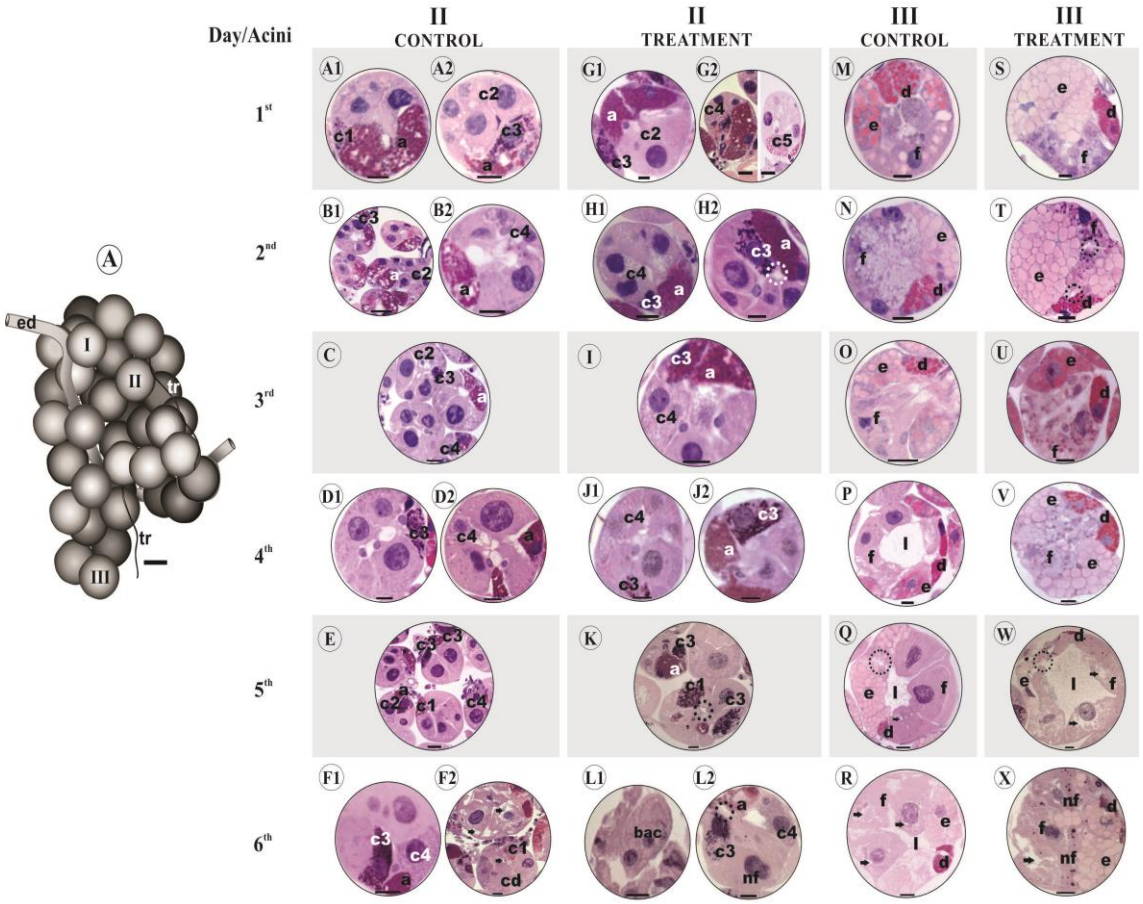

Figure captions

Figure A: Schematic draw of the salivary glands of *Rhipicephalus sanguineus* sl female ticks showing the acini types I, II and III, in addition to the common excretory duct (ed), and the tracheoles (tr) **Bar** = 100 µm.

Figures A1-X: Histological sections of acini type II (**A1-L2**) and III (**M-X**) of the salivary glands of *Rhipicephalus sanguineus* female ticks stained by hematoxylin-eosin (HE)

A1-F2: Acini II of the Control Group; **M-R:** acini III of the Control Group. **Figures G1-L2:** Acini II of the Treatment Group; **S-X:** acini III of the Treatment Group.

Bars = 20 µm.

II = acinus II; **III** = acinus III; **a-f**= cell types (**a; c1**; **c2**; **c3**; **c4**; **c5**; **d**; **e**; **f**) **va**= vacuole; **ed**=common excretory duct; **id**= intermediary duct, **dotted circle** = loss of cell contact, * = cell fragmentation, **nf**= nuclear fragmentation; **bac**= ruptured acinus; **ab**= apoptotic bodies; **tr**=tracheoles.

(Fig. I). The cells c4 were more active in the TG than in the CG

Acini type III

In the CG, these acini showed intact d, e and f cells (Fig. O), and the later did not present secretory activity. The same cell types were found in the TG; however, the cells f still contained secretion in the cytoplasm (Fig. U).

Females fed for 4 days

Acini type II

In the CG (Fig. D1-D2) and TG (Fig. J1-J2) the cells a, c3 and c4 were intact, and in the TG these cells presented reduced size.

Acini type III

In these acini, the cells d, e and f were intact, both in the CG (Fig. P) and in the TG (Fig. V). Specifically in the CG, the acinar lumen was enlarged. Few acini III were observed in the TG group, and the cells f still contained cytoplasmic secretion.

Females fed for 5 days

Acini type II

In the acini II, the cells a, c1, c2, c3 and c4 were intact in the CG (Fig. E). In the TG (Fig. K), the cells presented reduced size and lower secretion activity and showed signs of degeneration.

Acini type III

The acini III of the groups CG and TG showed enlarged lumen and cells type d, e and f (Fig. Q and W) were in degeneration process, evidenced by the cytoplasmic vacuolation and loss of cell.

Females fed for 6 days

Acini type II

The cells type a, c1, c3 and c4 observed in the acini III of individuals from the both groups (CG and TG) (Fig. F1-F2 and L1-L2, respectively), showed signs of degeneration. In the TG, the degenerative characteristics, such as cell (Fig. L1) and nuclear (Fig. L2) fragmentation, cytoplasmic vacuolation (Fig. L2), and rupture of the acini (Fig. L1) were less intense.

Acini type III

The acini III of the CG (Fig. R) and TG (Fig. X) showed reduced lumen, and the cells type d, e and f presented characteristics of degeneration. In the TG, the cells showed more intense degeneration signs in comparison with the CG: nuclear fragmentation, cytoplasmic vacuolation loss of cell contact and rupture of the acini (Fig. X).

Discussion

This study investigated the action of ricinoleic acid esters from castor oil (*R. communis*) as acaricide agent to control the dog tick *R. sanguineus* s.l., obtaining results that demonstrated the potential of the esters to interfere with the secretory cycle of the salivary glands of these ectoparasites and morpho-physiologically altering these organs in specific periods of the secretory cycle.

Previous studies, conducted by Messetti et al. [14], have demonstrated that the castor oil esters would be widely used to control microorganisms due to their hydrolytic activity on polysaccharides and derived compounds, such as glycoproteins and proteoglycans, conferring them a wide range of actions, including bactericide and fungicide. Although several studies have been developed and these esters were already known to act as acaricide agents, the literature lacked information on how these substances would act on the ectoparasite cells and tissues. The data to understand the control mechanisms is of great importance.

Moreover, few studies have been aimed to demonstrate the acaricide action of esters by adding them to the food offered to the rabbits and then artificially infested them with *R. sanguineus* s. l. females in the stage of complete engorgement [8-12]. These studies have demonstrated that in addition to the indirect interference of the esters on the oogenesis process and on the secretory cycle of the salivary glands, this substance acted directly on the hydrolysis of the yolk composition material (mainly polysaccharides and glycoproteins) and also on the composition of the molecules secreted by the salivary glands.

The findings of Arnosti et al. [8, 9] have motivated the search for further information on how these ester would morpho-physiologically act at cell level by directly modifying the cells and altering the whole dynamics of the secretory cycle in the salivary glands of *R. sanguineus* s.l. females. Therefore, considering the alterations observed in the salivary glands of *R. sanguineus* s. l. females, it was clear that the esters selectively modified the

different types of acini – structures composed of different types of cells – significantly modifying the types II and III; i.e., those regarded granular and responsible for producing and releasing the saliva components. Even though the acini type I have undergone alterations in response to the esters, these acini were not analyzed here because they are classified as agranular, therefore not producing the saliva components.

The results obtained in this study showed that the alterations in the glandular secretory cycle started to occur on the first feeding day in case of the ectoparasites attached to the rabbits fed with the ester-enriched food. In the beginning of the cycle, once an increase in the secretory activity of cells a from acini II and cells d/e from acini III was observed in the individuals from the TG. These morphological alterations in cells a, e and d suggested the occurrence of modifications at physiological level as well. Moreover, these cells would be responsible for the processes of ectoparasite fixation on the host (secreting glycoproteins that compose the cement cone) [2, 15, 16]. In addition to these alterations, it was verified that only acini II of the TG presented c5 cell type in activity, which indicates the precocity of the secretory activity, possibly due to the difficulty of ectoparasites to complete the feeding process [17].

In the individuals from the TG fed for two days, cells a, c2, c3, c4 and c5 of some acini II were observed in early cytoplasmic disorganization. This indicated a precocious degenerative process in these acini, which in normal conditions is expected only during and/or after the final feeding stage and never in the initial stage [18].

From the physiological point of view, according to Wikel [17] and Fawcett et al. [19], the acini II and III are responsible for the synthesis and release of molecules, which are present in the final secretion (saliva) and responsible to manipulate the immune response of the host, acting as immunosuppressant. Thus, in the individuals from TG that were collected on the third feeding day, higher secretory activity in the cells c4 of the acini II was observed. This indicated that this super-production is a strategy of the ectoparasites to accelerate the blood ingestion process when the hosts are resisting (via the presence of esters in the system) the tick infestation. Therefore, the esters act to accelerate the degeneration of the responsible organ i.e., the salivary glands.

In the individuals from TG, although the f cells of the acini III were in the beginning of the degeneration process and showing cytoplasmic disorganization, the majority of the cells were still performing their secretory and physiological functions [2, 15, 19, 20].

In the individuals from TG collected on the fourth feeding day, although the cells type f of the acini III still had the secretory activity, the cells a, c3 and c4 of the acini II suffered significant morphological alterations, however their size were reduced in comparison with those from the CG acini, which consequently caused the physiological alteration. Specifically, regarding the alterations occurred in the cells c, they certainly had the influence of the esters and additionally, these cells are responsible for the synthesis and secretion of glycoproteins that are the target of action of these substances [21].

The results observed in acini II and III of the salivary glands of the 5th-day fed individuals from the CG showed typical signs of degeneration, which included cell disarrangement and the loss of the cell characteristics. This degeneration was expected under natural conditions, after the completion of the feeding process, when salivary glands lose their function. However, in the 5-day fed individuals from the TG, these acini (type II and III), displayed similar degeneration signs as those of the CG. For the latter, in addition to being precocious, these signs were more aggressive, including the loss of acinar contact, cytoplasmic vacuolation, decrease in size and shape alterations (the acini became completely irregular), concurring for the decrease, or even cessation of the secretory activity.

Accordingly, on the sixth feeding day, in the individuals from the TG, the esters had the intensified degenerative alterations (cell/nuclear fragmentation and cytoplasmic vacuolation). In addition, ruptured acini were observed, which prevented the identification of the cells types present in each acinus. These degeneration characteristics in natural conditions were observed only in completely engorged females or in those in post-engorgement stage [18, 22] which indicated that the exposure to ricinoleic acid esters from castor oil accelerates the degeneration process without altering the process dynamics, corroborating data found by Arnosti et al. [9].

Thus, the results obtained in our study confirmed that in the near future, the ricinoleic acid

esters from castor oil could be effectively used to control *R. sanguineus* s.l. ticks, considering their potential to modify the glandular morphophysiology and cause, via the responses of the host fed with enriched food, the emergence of difficulties for the ectoparasite to fix and feed on the host, impairing the feeding capacity and decreasing the chances of its development.

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