Research Article

Morpho-ultrastructural alterations in the salivary glands of semi-engorged *Riphicephalus sanguineus* s.l. females subjected to permethrin

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Abstract

Ticks are arthropods of great medical-veterinary importance, and several methods have been developed to control them. The synthetic acaricide permethrin is one of the most widely used substances to control this pest. The present study brings cytochemical and ultrastructural data on the alterations in the salivary gland cells of *Rhipicephalus sanguineus* sensu lato semi-engorged females subjected to 206, 1031, and 2062 ppm of permethrin, showing that this chemical acts on the glandular acini, affecting the morpho-physiology of their cells, making them irregular, causing intense cytoplasmic vacuolation, altering the mitochondria and causing the emergence of lysosomes, myelin figures and spherocrystals. In addition, the results demonstrated that the chemical affected the nuclei and nucleoli of the cells, causing alterations in size, disorganizing and fragmenting them. Such alterations were mainly observed when processes of apoptotic death occurred, and indicated the occurrence of precocious degeneration of the glandular tissue caused by the exposure to the product, even in lower concentrations than the ones recommended by the manufacturer. Under normal conditions, this process would only occur in the end of the engorgement period. Therefore, these findings confirmed and demonstrated that, even at low concentrations, this acaricide would be able to accelerate the process of glandular tissue degeneration through atypical cell death, where the cell death by apoptosis (fragmentation) and autophagy (vacuolation) occur simultaneously.

Keywords nucleolus, permethrin, *Rhipicephalus sanguineus* s. l, salivary glands

Introduction

The medical-veterinary importance of ticks is determined by their capability of transmitting pathogenic agents and causing injuries to the hosts [1]. The salivary glands are vital for the success of these ectoparasites, once these organs produce substances for the fixation on the host, ensuring the feeding process [2-4].

Several methods to control these ectoparasites have been put into practice [5-6]; however, the most efficient one is based on synthetic acaricides, which have been widely used despite the high cost of the products, facilities and qualified manpower for the application of the substances.

In addition, these chemicals are harmful to the environment and public health due to potential contamination with chemical residues [7].

The pyrethroid permethrin, synthesized for the first time in 1973, with toxicological classification III (slightly toxic) [8] and neurotoxic action [9-10], is the active ingredient of the most widely used acaricides to control ticks, especially the dog tick *R. sanguineus* s. l.

Studies on tick salivary glands degeneration [11-13], mainly addressing the influence of synthetic acaricides on the morphophysiology [14-18] and ultrastructure, are scarce in the literature. Considering this, the present study had the objective to cytochemically analyze the alterations in the nucleolar structure and in the ultrastructure of the salivary gland cells of *R. sanguineus* s. l. semi-engorged females subjected to different concentrations of permethrin (206, 1031 and 2062 ppm). Studies by Nodari et al. [16-18] have demonstrated that this pyrethroid would accelerate the degeneration of the glandular tissue in this species.

Methodology

Rhipicephalus sanguineus s. l. ticks

A total of 60 semi-engorged females of *R. sanguineus* s. l., weighing 27 mg in average, supplied by the colony maintained at the Brazilian Central of Studies on Ticks Morphology (BCSTM) of the São Paulo State University-UNESP, Biosciences Institute of Rio Claro, SP, Brazil, were used in this the experiment. The ticks were kept under controlled conditions (28 ± 1ºC, 80% humidity and 12 h photoperiod) in an Eletrolab EL 202 BOD (Biological Oxygen Demand) incubator and fed (5 days) on New Zealand White rabbits (Protocol nº 5442, approved by Ethics Committee on Animal Use, UNESP, Rio Claro/ CEUA-IB-UNESP) according to the protocol described by Bechara et al. [19].

Permethrin dilution assays (CAS nº: 52645-53-1)

Permethrin (3-phenoxybenzyl (1RS, 3RS, 1RS, 3SR)-3-(2.2-dichlorovinyl)-2.2-dimethylcyclopropanecarboxylate) was purchased from Fersol Indústria e Comércio S/A (Mairinque, SP, Brazil). The concentrations were based on LC_{50} of 2062 ppm, according to Roma et al. [20]. The doses corresponded to 10% of the LC_{50} (206 ppm), 50% of the LC_{50} (1031 ppm) and the normal LC_{50} (2062 ppm). Control group was exposed to placebo (distilled water).

The ticks were washed under running tap water and dried with soft absorbent paper. Then, 45 individuals were divided into three groups (15 each group) and immersed for 5 minutes in Petri dishes containing the different concentrations of permethrin.

The ticks belonging to the control group were immersed in distilled water for the same period. After, the individuals were dried with absorbent paper and kept in BOD incubator (28 ± 1°C, 80% humidity, 12h photoperiod) for 7 days. This period was established to allow the acaricide to act on the physiology of the specimens.

Transmission Electron Microscopy (TEM)

The ticks were anesthetized by thermal shock and dissected in phosphate buffered saline (PBS) solution (NaCl 7.5 g/L, Na2HPO4 2.38 g/L and KH2PO4 2.72 g/L). The salivary glands were removed and fixed in 2.5% glutaraldehyde for 2 h, postfixed in 1% OsO4 for 2 h, contrasted in 2% uranyl acetate and dehydrated in graded acetone series (50%, 70%, 90%, and twice in 100%). The material was immersed in a mixture of acetone and resin (1:1) for 12 h, embedded in Epon Araldite for 12 h and posteriorly in pure Epon resin. Finally, it was polymerized at 60º C for 72 h, sectioned in ultrathin sections and contrasted with uranyl acetate and lead citrate during 25 and 10 min, respectively. The samples were examined and photographed using a PHILLIPS 100 Transmission Electron Microscope at the Institute of Biosciences, São Paulo State University, Rio Claro-SP, Brazil.

The Variant of the Critical Electrolyte Concentration (CEC)

The salivary glands were removed in saline solution (NaCl 7.5 g/L, Na2HPO4 2.38 g/L and KH2PO4 2.72 g/L, pH 7.2), and fixed in ethanol and acetic acid (3:1) at room temperature for 12 minutes. The material

was then dehydrated in crescent series of ethanol (70%, 80%, 90% and 95%), embedded and included in Leica resin, sectioned at 3-μm thickness and placed on glass slides.

For the variant of the CEC [21], slides were stained with 0.025% Toluidine Blue in McIlvane's buffer (pH 4.0) for 20 minutes. After, the material was immersed in aqueous solution of 0.05M MgCl for 2, 5, 7, and 10 minutes to detect the ideal time at which metachromasy is abolished, with the removal of Toluidine Blue molecules bound to the chromatin by $MgCl_2$. At this point, only the RNA metachromasy was maintained, staining the nucleolus and demonstrating the presence of cytoplasmic RNA (violet color). The slides were rinsed with distilled water and mounted in Entellan (Merck Millipore) for further observation under light microscope.

Results and Discussion

Transmission Electron Microscopy
Group I (control)

The salivary glands of the females belonging to the control group were composed by acini I, II and III, containing acinar cells with rounded morphology and nuclei, dispersed heterochromatin and very evident nucleoli (Fig. 1A-J).

The acini type I (agranular) presented cells with invaginations in the basal plasma membrane, characterizing the basal labyrinth, where several mitochondria and lipid inclusions were found. Smaller mitochondria could also be observed throughout the cytoplasm (Fig. 1A-B).

The acini type II and III (granular acini), contained cells with well-developed rough endoplasmic reticulum (Fig. 1J). The content of the round secretion granules varied in electron density (more electron dense / more electron lucid) depending on the properties of the substances present in them (Fig. 1C-I). Fewer mitochondria, showing intact cristae and unaltered shape (Fig. 1I), were observed in the cells of these acini. The plasma membranes of the acinar cells were also intact, as well as the basal membrane covering the acini (Fig.1 E, G-H).

Group II (subjected to 206 ppm of permethrin)

In this permethrin concentration, the acinar cells started to present the first signs of damage: cytoplasm vacuolation and alterations in shape (emergence of irregular acini) (Fig. 2A-J).

In the acini I, the nucleus of the central cell was rounded, with dispersed chromatin and evident nucleoli (Fig. 2A and C). The nuclei of the acini II and III cells were irregular, with dispersed chromatin, and the beginning of chromatin marginalization could be observed (Fig. 2G and J). The mitochondria were swollen, located in the periphery of the cell, and displayed irregular shape (Fig. 2H).

The plasma membrane was retracted, apparently separating from the basal lamina and leaving a vacuole (Fig. 2H). The rough endoplasmic reticulum was well developed and intact (Fig. 2I). Myelin figures were observed (Fig. 2A).

Group III (subjected to 1031 ppm of permethrin)

The salivary glands of the individuals subjected to 1031ppm of permethrin presented alterations in the shape of the acini, from rounded to irregular (Fig. 3A). In many cases, it was not possible to classify these granular acini; thus, these acini will herein be called indeterminate acini. The presence of several vacuoles was observed in the remaining cytoplasmic mass (Fig. 3C, E-J).

The type I and indeterminate acini presented significant alterations in the mitochondria, which were swollen and vacuolated, with cristae forming honeycomb-like structures due to the disorganization of the internal membranes (Fig. 3A-C). Autophagic vacuoles were observed (Fig. 3B-C, I-J). The acini I cells showed a decrease in the number of lipid inclusions, mainly in the basal labyrinth membrane. The cytoplasm of the indeterminate acini showed intense vacuolation (Fig. 3 D-J), a decrease in the number of lysosomes (Fig. 3H) and autophagic vacuoles with heterogeneous content (Fig. F-G).
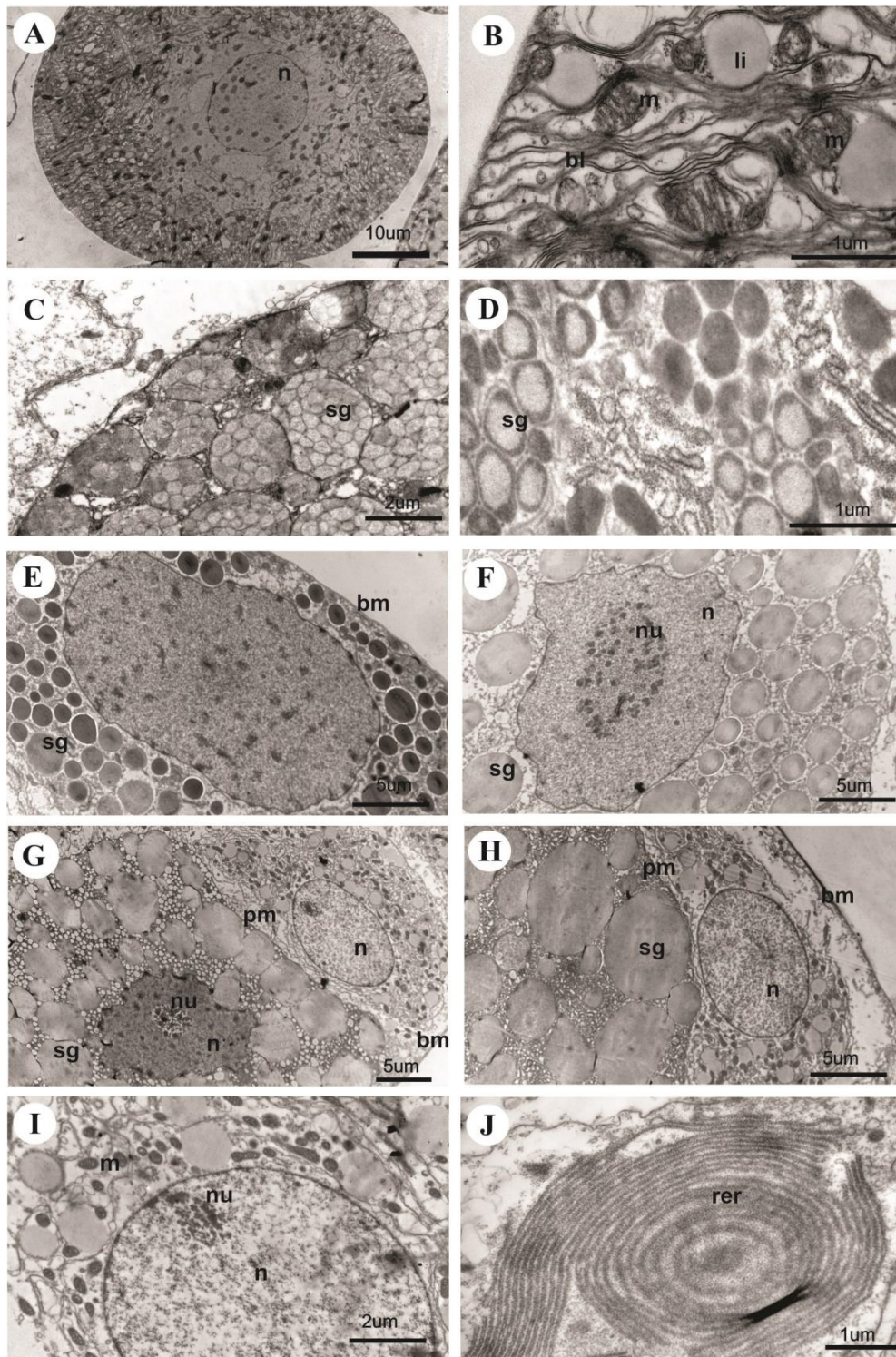
Figure 1. Transmission Electron Microscopy (TEM) of the salivary glands of *Rhipicephalus sanguineus* s. l. semi-engorged females from the control group. (A) Acini type **I** with rounded central cell nuclei (**n**) and dispersed chromatin. **(B)** Detail of the acini **I** periphery, where the basal labyrinth of the adjacent cells is observed (**bl**) the presence of lipid inclusions (**li**) and mitochondria (**m**) among the membranes. **(C-I)** Acinar cells with secretory granules (**sg**) with the content varying in electron density, presence of nuclei (**n**) with very evident nucleolus (**nu**), plasma membrane (**pm**) and basal membrane (**bm**) are intact. **(J)** Detail of the rough endoplasmic reticulum (**rer**).
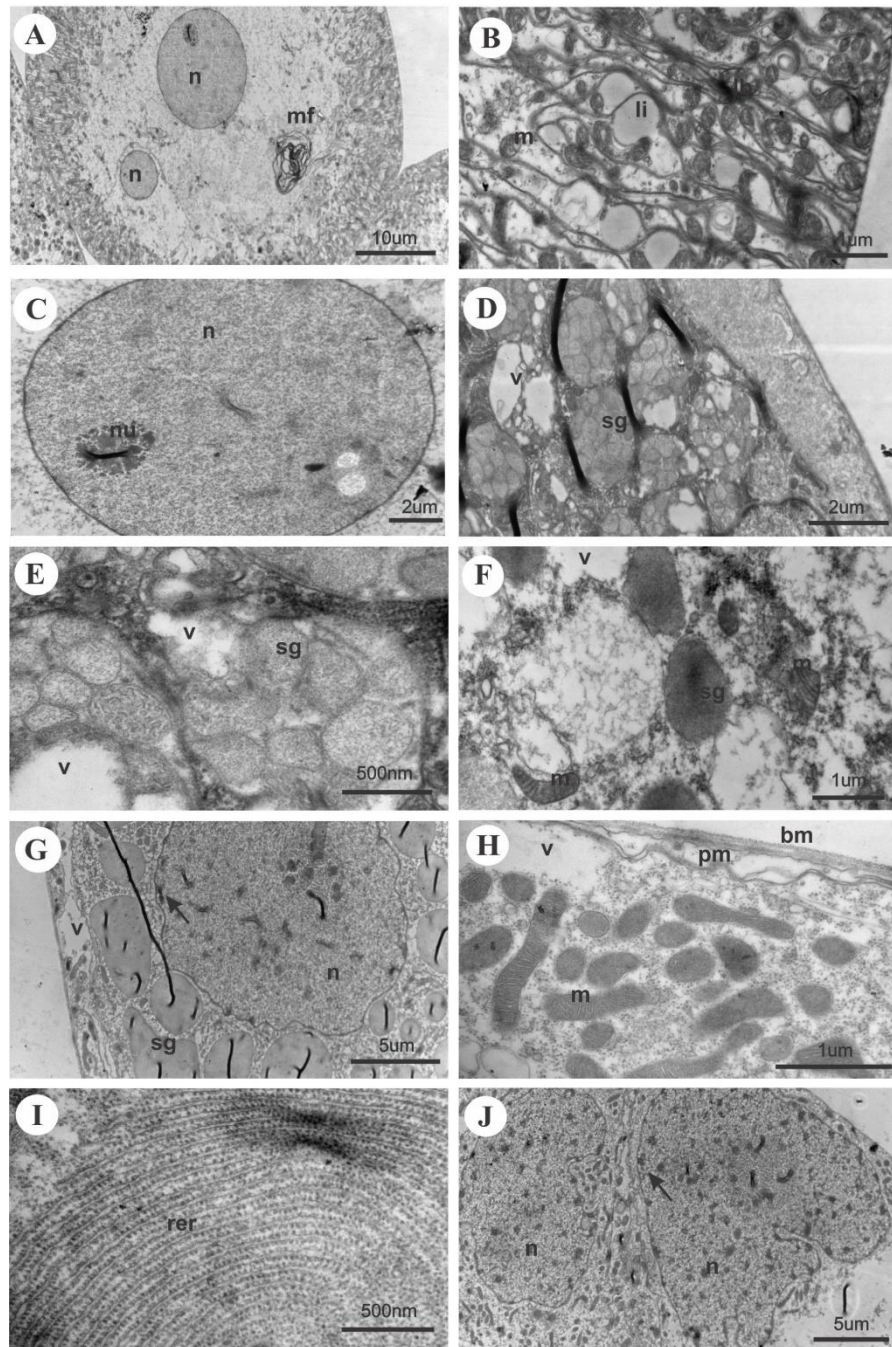
Figure 2. Transmission Electron Microscopy (TEM) of the salivary glands of *R. sanguineus* s. l. semi-engorged females subjected to 206 ppm of permethrin. (A) Acini **I** with rounded central and peripheral cell nuclei (**n**), and the presence of myelin figure (**mf**) in the cytoplasm. **(B)** Detail of the basal membrane labyrinth (**bl**) with the presence of mitochondria (**m**) and lipid inclusions (**li**). **(C)** Detail of the nucleus (**n**) showing the central cell of the acini **I** with very evident nucleolus (**nu**). **(D-F)** Granular acinar cells, with secretory granules (**sg**) presenting varied electron densities, and the presence of cytoplasmic vacuoles (**v**). **(G)** Detail of the glandular acinar cell, with secretory granules (**sg**), cytoplasmic vacuoles (**v**), and nucleus (**n**) presenting altered shape and the beginning of chromatin marginalization (→). **(H)** Detail of the acinar cell, showing swollen mitochondria (**m**) with altered shape. A retraction of the plasma membrane is observed (**pm**), where the peripheral cell separates from the basal membrane (**bm**), leaving a vacuole (**v**). **(I)** Detail of the rough endoplasmic reticulum (**rer**), well developed and intact. **(J)** Detail of the nucleus (**n**) with chromatin marginalization (→).

The nuclei of the acinar cells were completely irregular and presented lumped and marginalized chromatin (Fig. 3D).
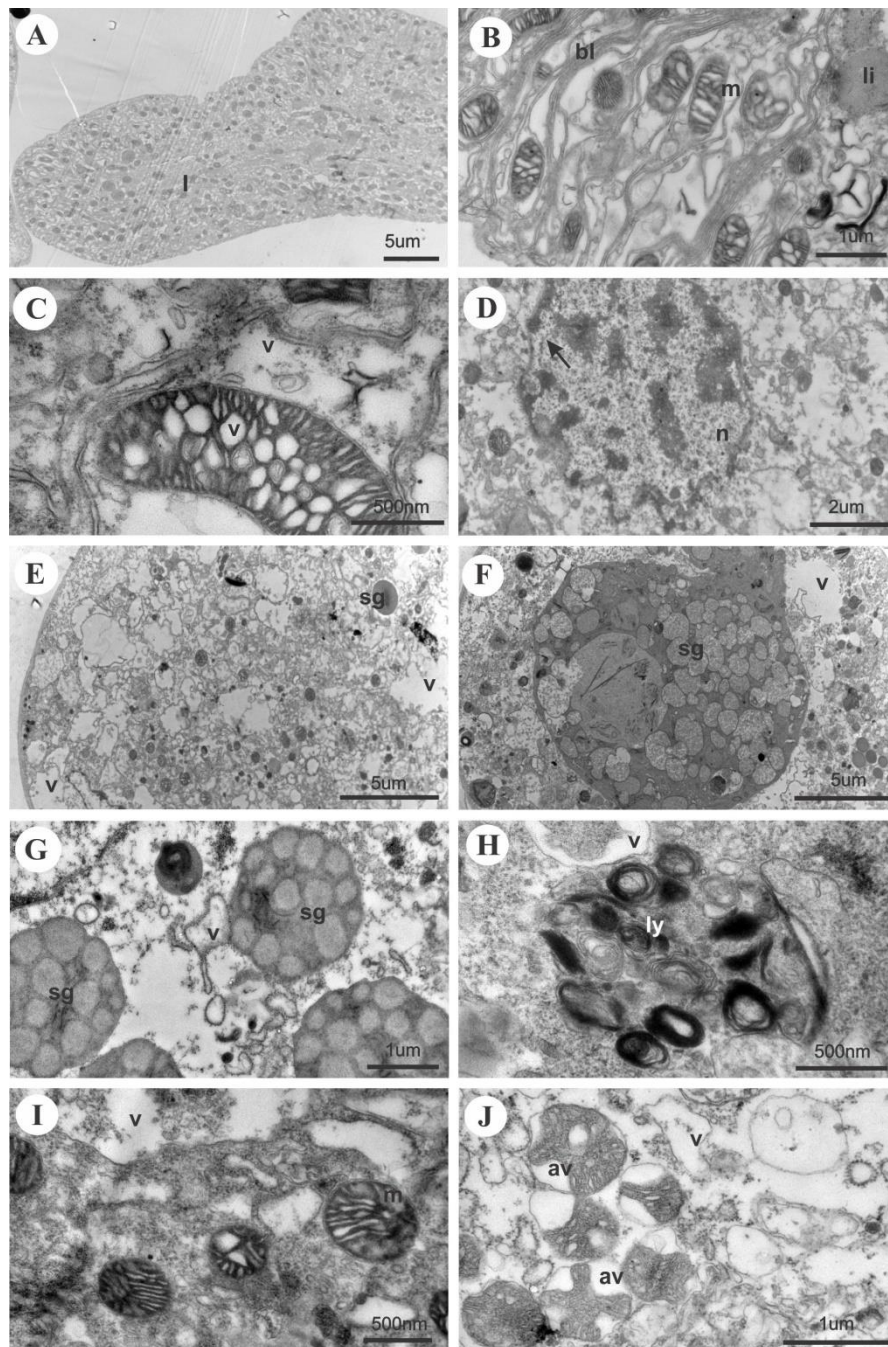

Figure 3. Transmission Electron Microscopy (TEM) of the salivary glands of *R. sanguineus* s. l. semi-engorged females subjected to 1031 ppm of permethrin. (A) Acini **I** presenting irregular morphology and vacuolated basal labyrinth (**bl**). **(B)** Detail of the acini **I**, presenting vacuolated basal labyrinth (**bl**) with swollen mitochondria (**m**) presenting altered shape, and the presence of lipid inclusions (**li**). **(C)** Detail of the swollen and vacuolated mitochondria (**m**). Cytoplasm presenting vacuoles (**v**). **(D)** Detail of the nucleus of the granular acinar cell, with the beginning of chromatin marginalization (→). **(E-G)** acinar cells with secretory granules (**sg**) with altered shape and the presence of cytoplasmic vacuoles (**v**). **(H)** Detail of the lysosome (**ly**) and cytoplasmic vacuole (**v**). **(I)** Detail of the region with altered mitochondria (**m**) and cytoplasmic vacuoles (**v**). **(J)** Detail of the autophagic vacuoles (**av**) including the mitochondria.

Group IV (subjected to 2062 ppm of permethrin)

In the salivary glands of the females subjected to 2062 ppm of permethrin, as in the previous group (1031 ppm), the acini were irregular and difficult to be identified due to the action of the product. Only acini I could be identified (the other acini were herein called indeterminate). The acinar cells showed intense cytoplasmic vacuolation (Fig. 4 A-K).

A decrease in the number of lipid inclusions was observed in the basal membrane labyrinth of the acini I cells (Fig. 4A-B). The mitochondria were disorganized, presenting vacuolation and altered shape (swollen). However, the morphological alterations observed here were less intense in comparison with the ones occurred in the acini of the previous group (Fig. 4A-C, I-J).

Irregular secretory granules with heterogeneous content and varying in electron density (Fig. 4D-H, J-K) were observed in the cytoplasm of the granular cells. The emergence of spherocrystals was also be observed (Fig.4J).

The nuclei of the acinar cells were irregular, with lumped and marginalized chromatin (Fig. 4B, D-G e I).

The Variant of the Critical Electrolyte Concentration (CEC)

Considering that the Critical Electrolyte Concentration (CEC) point, (when the metachromasy abolition occurs) is always lower in the RNA than in the DNA, a variable of the CEC method is used, where the treatment with Mg^{+2} is posterior to the staining with Toluidine blue pH 2.4. Thus, the cation Mg^{+2} extracts the toluidine from the DNA ligation sites. Under these conditions, while the DNA metachromasy abolition occurs (green staining), the RNA remains metachromatic (blue or violet) [21].

The CEC critical point is stablished when the DNA of the whole nucleus has the toluidine molecules replaced by Mg^{+2} ions. In this case, the nucleus presents ortochromasy, and is stained in green. However, the RNAs, both in the nucleus (nucleolus) and the cytoplasm, remain bound to the toluidine blue molecules, and are colored blue.

This technique allows the detection of heterochromatin in the cell nucleus and of the nucleolar activity as well. The nucleolar activity is measured through the analysis of the nucleolus morphology (fibrillar and granular regions) and of the amount of RNA in the cytoplasm of the cell [21].

The results obtained with the application of CEC technique demonstrated that the metachromasy was abolished (chromatin stained in green and nucleolus in violet) after 7 minutes (Fig. 5).

Group I (control)

The nucleoli of the acinar cells from the control group were intact (Fig. 5A1); however, it was possible to observe that some of them presented signs of disorganization (irregular morphology and heterogeneous content) (Fig. 5A2).

Group II (treated with 206 ppm of permethrin)

The acinar cells of the salivary gland presented the first signs of nucleolar alterations, such as enlarged and disorganized nucleoli, and some nucleoli started to show the first signs of fragmentation (Fig. 5B1-B4).

Group III (treated with 1031 ppm of permethrin) and group IV (treated with 2062 ppm of permethrin)

The acinar cells subjected to higher concentrations of permethrin (1031 ppm and 2062 ppm), presented the most significant nucleolar alterations (increase in size, disorganization and fragmentation) (Fig. 5C1-C4; D1-D4).
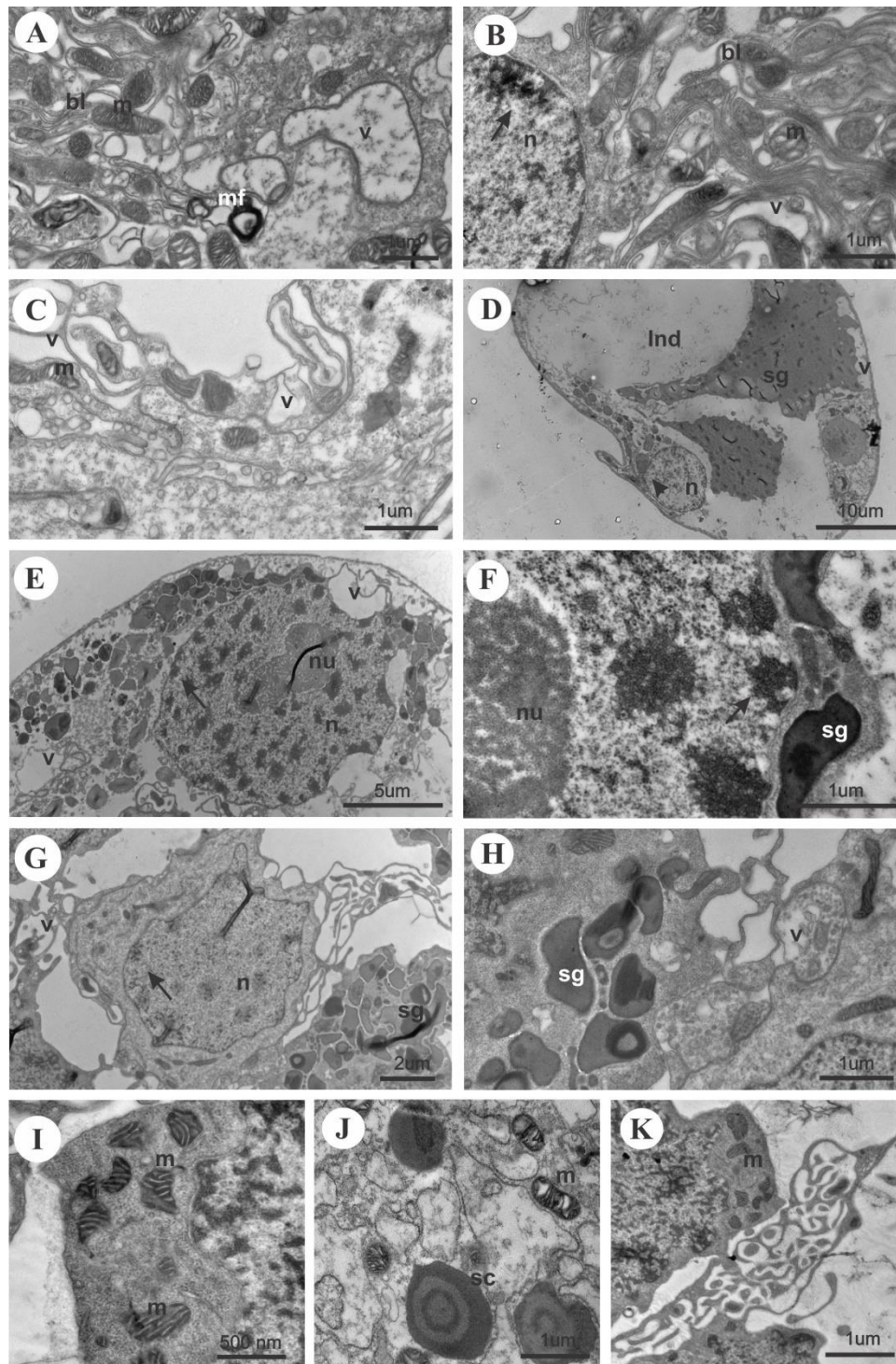
Figure 4. Transmission Electron Microscopy (TEM) of the salivary glands of *R. sanguineus* s. l. semi-engorged females subjected to 2062 ppm of permethrin. (A-B) Detail of the basal labyrinth (**bl**) of the acini **I** cells, with the presence of altered mitochondria (**m**), myelin figure (**mf**), cytoplasmic vacuoles (**v**), disorganized membranes and nucleus (**n**) with marginalization of the chromatin (**→**). **(C)** Detail of the acinar cells with altered mitochondria (**m**) and cytoplasmic vacuoles (**v**). **(D)** Indeterminate acinus with secretory granules (**sg**), cytoplasmic vacuoles (**v**) and nucleus (**n**) with chromatin marginalization (**→**). **(E-H)** Details of the acinar cells with cytoplasmic vacuolation (**v**) and the nuclei (**n**) presenting marginalization of the chromatin (**→**) and secretion granules (**sg**) with altered shape. **(I-K)** Detail of the acinar cells with mitochondria (**m**) presenting alterations in shape, swollen and vacuolated. In **(J)** the presence of spherocrystals can be observed (**sc**).
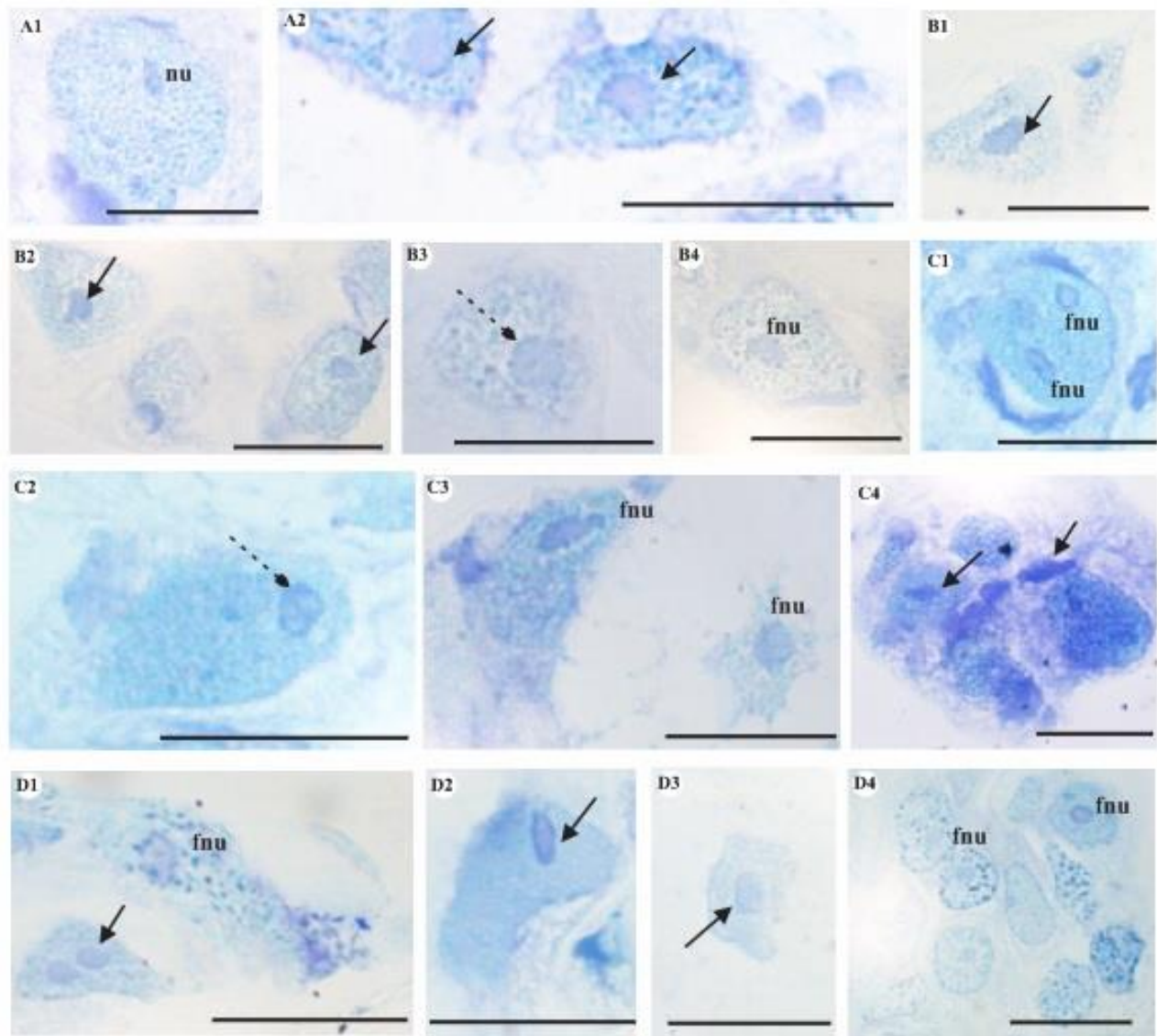
Figure 5. Histological sections of the cells salivary glands of *R. sanguineus* s. l. semi-engorged females, subjected to Critical Electrolyte Concentration (CEC). (A1-A2) Salivary glands of the females from the control group presenting intact nucleoli and the first signs of disorganization. **(B1-B4)** Salivary glands of the individuals subjected to 206 ppm of permethrin presenting disorganized and enlarged nucleoli, with the first signs of fragmentation. **(C1-C4)** Salivary glands of semi-engorged females exposed to 1031 ppm of permethrin presenting enlarged and disorganized nucleoli and the first signs of fragmentation. **(D1-D4)** Salivary glands of semi-engorged females subjected to 2062 ppm of permethrin with significant alterations, such as fragmentation and disorganization of the nucleoli.
nu= intact nucleolus **fnu**= nucleolus with the first signs of fragmentation **arrow**= disorganized nucleolus **dotted arrow** = enlarged and disorganized nucleolus
Bars: A1-D4= 20 μm

Discussion

This study confirmed the occurrence of alterations in the salivary glands of semi-engorged *Riphicephalus sanguineus* s. l. females subjected to different concentrations of permethrin.

The ultrastructural data regarding the salivary glands of the females from the control group corroborate light microscopy analyses by Nodari et al. [16-18] , who demonstrated that in this group the acini were intact, maintaining original shape; i.e., rounded, and presenting intact cells. The acini I cells presented a well-defined basal membrane labyrinth, as described in literature [22], with randomly distributed mitochondria throughout the cytoplasm and the presence of lipid inclusions (drops). The presence of cells showing a basal membrane labyrinth and containing a great number of mitochondria is also a characteristic of the cells that transport ions in the vertebrates. These cells need energy from the organelles to perform this function [23]; therefore, the acini type I of *R. sanguineus* s. l. female salivary glands would be responsible for the transportation of ions. Similar data were found by Needham and Teel [24] and Gaede and Knulle [25] for other species of ticks.

The acini types II and III and the salivary gland cells of the females from the control group were intact, presenting rounded secretion granules and different electron densities, as well as a developed rough endoplasmic reticulum. The presence of these granules and organelles is explained by the physiology of these acini, responsible for producing the salivary secretion, a mixture of products associated with the fixation of the tick on the host; tissue digestions [3]; inhibition of blood coagulation [3, 26-27] and the secretion of kinases that catalyze the bradykinin, a mediator of the host's pain [28].

In this group, the presence of cells with intact nuclei and nucleoli could be observed; however, some acinar cells started to show disorganized nucleoli, due to the continuous process of glandular degeneration; which, in normal conditions, occurs in the end of the engorgement process, when the salivary gland does not play a fundamental role in the survival of the tick. These data corroborate Furquim et al. [29], who observed the occurrence of this and other alterations in the nucleoli of the salivary gland cells of fully engorged females three days after engorgement. All the nucleoli were intact in unfed females.

The acinar cells of the salivary glands subjected to 206 ppm of permethrin presented few ultrastructural alterations in relation to the control group. These alterations in the shape of the cell consequently modified the shape of the acini. The cytoplasm presented vacuolation and it was possible to observe alterations in the shape and size of the mitochondria, which became irregular; however, the rough endoplasmic reticulum was still intact. Some cells presented retraction in the cytoplasmic membrane, indicating the initial stage of acinar degeneration, which would be preceded by the formation of blisters on the surface of these membranes and the emergence of myelin figures, structures typically found in cells undergoing a process of recycling or death [30].

The nuclei and nucleoli of the cells in this treatment group, as well as in treatment group III and IV, presented alterations in size (enlarged), shape (irregular), and in the organization of the nucleolus, including the fragmentation of the RNA content. These alterations are similar to the ones found by Furquim et al. [29] studying fully engorged females, indicating that the permethrin would accelerate the degeneration of the glands without causing alterations in the process through which it occurs; i.e., an atypical cell death, by apoptosis (fragmentation) and autophagy (vacuolation) simultaneously.

The nuclear and nucleolar alterations observed in the treatment groups (206 ppm, 1031 ppm and 2062 ppm of permethrin) corroborate Kerr et al. [31], who reported nuclear alterations in apoptotic cells, including fragmentation and enlargement of the nucleus, chromatin marginalization and fragmentation of the nucleolus, resulting from the biochemical and morphological alterations in the nucleus during apoptosis [29,32-36]. Thus, considering the similarities between the nuclear [18] and nucleolar alterations found in this study, it can be inferred that the salivary glands of semi-engorged *R. sanguineus* s. l. females subjected to different concentrations of permethrin would also be undergoing the process described by Kerr et al. [31]; i.e., apoptotic death.

Furthermore, the RNA was strongly stained in the nuclei of the acinar cells of the salivary glands subjected to higher concentrations of permethrin (1031 and 2062 ppm), which, according to Bowen and Bowen [32] and Furquim et al. [29], would indicate high synthesis of RNA and protein, essential in the

initial stages of cell death by apoptosis. The RNA molecules would also be used in the synthesis of acid phosphatase [29], corroborating Nodari et al [18], who reported intense staining of this enzyme in the acinar cells of female *R. sanguineus* s. l. salivary glands subjected to these concentrations of permethrin. The presence of this enzyme would be directly associated with the degenerative process of the tissue [18, 29], once the increase in the level of activity of the acid hydrolases (acid phosphatase) is a characteristic of autophagic cell death [34, 35, 37-42].

Significant ultrastructural alterations were observed in the salivary glands of the females subjected to higher concentrations of permethrin (1031 ppm and 2062 ppm), such as vacuolation of the cytoplasm and mitochondria, presence of irregular nuclei with chromatin marginalization and alterations in the shape of the acini. These characteristics [32], in addition to a decrease in lipid inclusions in the acini I cells, indicate apoptotic death. Needham et al. [43] and Barker et al. [44] reported alterations in the diameter of the acini I of *Amblyomma americanum* during the engorgement process, a decrease in the number of lipid inclusion, and an increase in the autophagic activity through the emergence of myelin figures and dense lysosomal bodies. In this study, the presence of myelin figures and lysosomes (structures that should only be observed in glands in more advanced stages of engorgement) was also observed. These data corroborate Nodari et al. [16-18], who reported that permethrin would be accelerating the degeneration of the salivary glands of the *R. sanguineus* s. l. females subjected to different concentrations of the product.

According to Sauer and Hair [45], this decrease in the number of lipid inclusions in the glandular cells would occur due to the intracellular consumption of this element to be used as energy source, aiming the increase in the transportation of water from the environment to the animal, a function attributed to acini type I. However, Barker et al. [44], have suggested that the disappearance of lipid inclusion would be associated with the lysis, which would provide the lipid basis for the formation of the cement to adhere the tick's buccal apparatus to the host's skin.

The intense cytoplasmic vacuolation observed in the acinar cells of all the treatment groups (206, 1031 and 2062 ppm), was more evident when higher concentrations were applied. The vacuoles would result from the process of cell autophagy, which would be recycling the damaged components to reuse the amino acids resulting from this degradation, mainly of the membranes [46]. The acinar cells of the individuals subjected to 1031 ppm of permethrin showed cytoplasmic and autophagic vacuoles, the latter presenting mitochondria – important in the control of the apoptosis [47]. Pereira et al. [15] reported the sequestration of mitochondria to the interior of autophagic vacuoles in the salivary glands of *R. sanguineus* s. l. females subjected to different concentrations of fipronil, suggesting that the granular cells might have activated a mechanism of protection against the action of the toxic product [48].

Significant mitochondrial alterations were observed in the acinar cells of the salivary glands exposed to higher concentrations, mainly at the intermediate concentration (1031 ppm of permethrin). According to Pimentel [49], such alterations would be related with modifications in the nuclear genes, confirming the ultrastructural data obtained in this study mitochondria with honeycomb-like cristae, a consequence of the disorganization of the inner membranes.

In the highest concentration (2062 ppm) of permethrin, the formation of spherocrystals was observed in the remaining cytoplasm mass in the indeterminate acini cells. These structures are composed of an organic matrix forming calcium crystal complexes [50]. The spherocrystals (in diplopoda) are believed to be related with: a) the storage of calcium for the formation of the embryonic skeleton [50]; b) the detoxification of the organism exposed to high concentrations of minerals from the soil [51]; and c) the processes of ionic balance which may include several phenomena, such as recycling, storage and secretion of minerals [52]. Spherocrystals were also observed by Pereira et al. [15] in *R. sanguineus* s. l. salivary gland cells in degeneration when subjected to different concentrations of fipronil; however, their exact function in the salivary glands has not been elucidated. They probably play a role in the detoxification of the organism exposed to the acaricide, once these structures were not observed in the salivary gland cells obtained from the control group.

Thus, the ultrastructural and cytochemical data obtained in this study complement studies by Nodari et al. [16-18], confirming the neurotoxic action [9], of permethrin. Even in lower concentrations than the ones commercially recommended, the product would act on the morphophysiology of the salivary

glands of *R. sanguineus* s. l. semi-engorged females, accelerating the degeneration process through atypical cell death, by apoptosis (fragmentation) and autophagy (vacuolation) simultaneously.

Conflict of interest statements

The authors declare that there are no conflicts of interest.

Acknowledgments

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