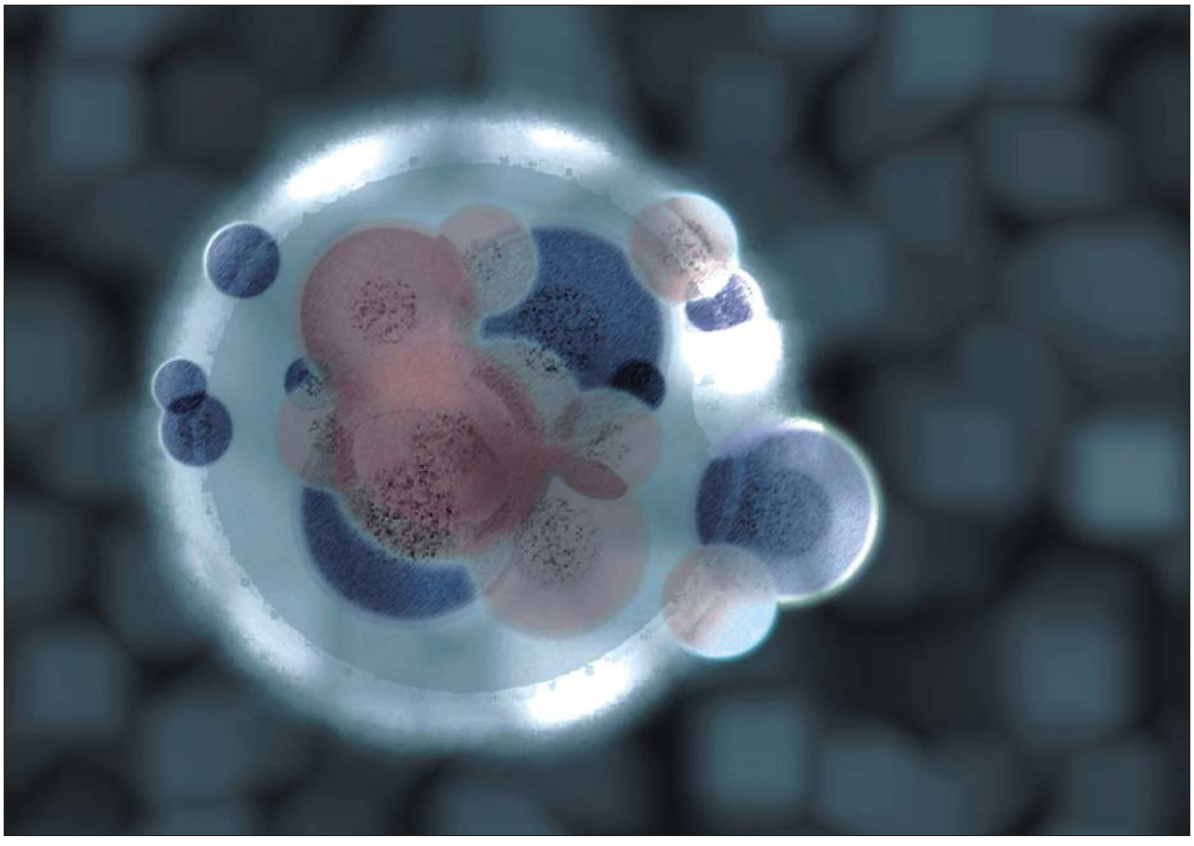

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 7 (1): 95-101, 2007
ISSN 1727-3048

Histological Effects of *Cestrum parqui* Saponins on *Schistocerca gregaria* and Spodoptera *Littoralis*

[1]Chaieb Ikbal, [2]Trabelsi Mounir, [1]Ben Halima-Kamel Mounia and [1]Ben Hamouda Mohamed Habib
[1]Entomological Laboratory, High School of Horticulture, 4042 Chott Mariem, Tunisia
[2]Histological Laboratory, Ibn Aljazzar medicine Faculty, 4000 Sousse, Tunisia

Abstract: In this research try to explore the effect of injection and forced ingestion of Cestrum CSE on two insect species: *Schistocerca gregaria* and *Spodoptera littorais*. A fat body necrosis (for *Spodoptera* larvae) and a digestive tract softness (for *Schistcerca* larvae) are observed, this let us to make an histological study of these organs. Theses studies reveals a cytotoxic effect of CSE on the fat body of *Spodoptera* larvae, the cells of this tissue decreases in size and becomes more colored probably by loss of their cytoplasmic cotenant. More over we have noted a cell destruction of the foregut and of the gastric caeca of *Schistocerca* treated with CSE forced ingestion. Our hypothesis is that saponins interact with membrane cholesterol, this causes a membrane destabilization and that provokes cell death.

Key words: *Cestrum parqui*, saponins, *Schistrocerca gregaria*, *Spodoptera* littoralis, cytotoxicity, histology

INTRODUCTION

Cestrum parqui is a schurb used in Tunisia as ornamental plant. Ammar *et al.* (1995) observed for the first time the toxicity of the leaves of this plant for desert locust (*Schistocerca gregaria*) larvae. Moreover the powder of this plant is also toxic to cotton leave noctuid (*Spodoptera littoralis*) when added to its artificial diet (Chaieb *et al*., 2001). Barbouche *et al.* (2001) demonstrated that the CSE (the Crude Saponic Extract) is the active fraction in the plant, they showed that mortality of desert locust larvae was obtained by under cuticular injection of CSE solution.

Saponins are heterosidic substances synthesized by the plant in order to defend itself against some plant pest (Oleszek *et al*., 1999), many authors have shown the importance of these substances in the resistance of the plants against phytophagous insects (Agrell *et al*., 2003; Appelbaum *et al*., 1969; Harmatha, 2000). Some authors supposes that saponins interact with dietary cholesterol, witch became inassimilable by the digestive system, this provokes its undisponibility for the production of the molting hormone (Ecdysone) and this is the reason for saponin toxicity (Arnault and Mauhamps, 1984).

The hypothesis of interaction with dietary saponins is not verifiable in our case, because of the rapid action of saponins (some hours: insufficient to have an effect on ecdysone synthesis) and because of the toxicity by under cuticular injection which have not relation with dietary cholesterol.

Saponins are, in contrast, well known for their cytotoxic activity, this was demonstrated on many types of cells like erythrocytes (Bauman *et al*., 2000), cancerous cells (Croce, 2001; Hanausek *et al*., 2001; Mujoo *et al*., 2001; Shibata, 2001) and fungi (Morrissey and Osbourn, 1999; Papadopoulou *et al*., 1999). This cytotoxic activity comes from the interaction of saponins with membrane cholesterol which causes membrane structure perturbation and cell death. Encouraged by the apparition of necrotic symptoms in treated insects we try, in this work, to demonstrate a similar mode of action on insect cells. For this e will explore histological effect of CSE on two insects *Schistrocerca gregaria* and *S. littoralis* using injection and forced ingestion experiments.

MATERIALS AND METHODS

Extraction of saponins: The saponin extraction is made like is described by Barbouche *et al*. (2001). The leaves of *C. parqui* was obtained from the garden of the National Tunisian Agronomic Institute (INAT) in May 2004, dried in a steam room at 40°C during 4 days, the dried leaves are finely grounded. Hundred gram of the powder washed with petrol ether, then extracted three times with 300 mL methanol. After filtration the methanol is evaporated with rotary evaporator at 40°C. We obtained a dry residual weighing 6 g, the dissolution of 1 g of this residual in 100 mL methanol then the addition of 100 mL of ethylic ether permits to get 0.06 g of a brown precipitate symbolized CSE (Crude Saponic Extract).

Corresponding Author: Chaieb Ikbal, Entomological Laboratory, High School of Horticulture, 4042 Chott Mariem, Tunisia

Insect provenance

***Schistocerca gregaria*:** The insects eggs are brought from a breeding of desert locust *Schistocerca gregaria* at the gregarious state maintained in the laboratory of insects physiology and physiopathology of INAT, this breeding is maintained since the last great invasion of the locust in 1988-1989. The adults or the larvae undergoing of the experiments are maintained in laboratory conditions. All animal experiments where realized in 2005.

***Spodoptera littoralis*:** L5 larvae were obtained from a rearing maintained at the Entomological Laboratory of the high school of horticulture. The caterpillars kept individually in Petri disches and fed on simplified artificial substrate according to the formula of Poitout and Bues (1974). Larvae are reared in culture rooms under a temperature of 25°C, with a relative humidity of 70% and 8 h photoperiod of illumination.

Injections experiments: The needle of the syringe is introduced usually ventrally between the tergits of the 2nd and the 3rd abdominal segments. The end of the needle is introduced parallel with the cuticule to not touch the internal bodies. The needle is introduced with a depth from 3 to 5 mm into the direction of the Thorax, the injection is done on the level of the first abdominal segment. for these experiments we use a 25 μL micro syringe Hamilton. In all the cases in this kind of experiments one should not exceed 20 μL of volume injected.

The injections can also be carried out on larvae of *S. littoralis*, generally, we use the caterpillars of the 5th stage.

Forced ingestion experiments: These experiments consist in the introduction of the solution to be tested artificially in the digestive system of the insect. For that a syringe with insulin provided with a point on which one fixed a 10 cm length catheter and 0.5 mm of diameter.

The catheter is introduced between mandibles of the insect until reaching the esophagus, thus the substance is injected. One can also inject relatively significant quantities (50 μL). The same experiments are carried out on L_5 larvae of *S. littorlis*.

Histological methods: After shunt anesthetize by freezing, midgut, fat bodies and gastric caeca where dissected in physiological liquid using surgical material employed in ophthalmological surgery and placed in the appropriate fixative.

For the haematoxylin, phloxin, orange G (HPO) procedure, the tissues where fixed in trichloroacetic Bouin's fluid for 32 h and this was followed by paraffin embedding and sectioning. The sections, 7.5 thick where sticked on slides and coloured by HPO.

RESULTS

Symptomatic effects of CSE: Injections in the thoracic cavity of the locusts provoked animal mortality accompanied by a blackening and a necrosis of tissues in the injection zone (Fig. 1C). A dissection of the animal shows a total or partial blackening of the fat body in the zone of injection. We think that it is a necrosis due to a cytotoxic action of saponins. These lasts are known for their interference with membrane cholesterol, which probably creates pores thus disturbing the permeability of the membrane and causing cellular death. This assumption hypothesis deserves to be checked.

The injection of a saponic solution under the cuticle of the larvae of *Spodoptera* results in their death with blackening and liquefaction of the insect body. The dissection shows a blackening of all the bodies.

Caterpillars bound between the 3rd and 4th abdominal segment undertake an injection on the level of the former or posterior part of the body. After 24 h, the same type of necroses and liquefaction is noted only in the treated part of the insect, the other part remains alive (Fig. 1A and B).

Histological effect of the CSE: When we started our investigations on the toxicity of the ESB, we began by making experiments of injection and forced ingestion, two principal symptoms had then challenged us:

- Necrosis on the level of the zone of injection accompanied by the change of the color of the fat body particularly for *Spodoptera*;
- The digestive tract of the insects having undergone a forced ingestion became flabby particularly for *Schistocerca*.

For this reason we will try in this work to study the effect of CSE on *Spodoptera* fat body and *Schistocerca* digestive system. The purpose of this work is to check the hypothesis of cytotoxic effect of *Cestrum* saponins on the two species of insects. *Saponins* are known for their cytotoxic effect, especially, noted on fungi and cancerous cells.

Effects on *Spodoptera* fat body: We noticed that the fat body which is normally yellow for control individuals, bleaches then blackens in treated individual. Thus we chose to carry out this work on *Spodoptera* either by

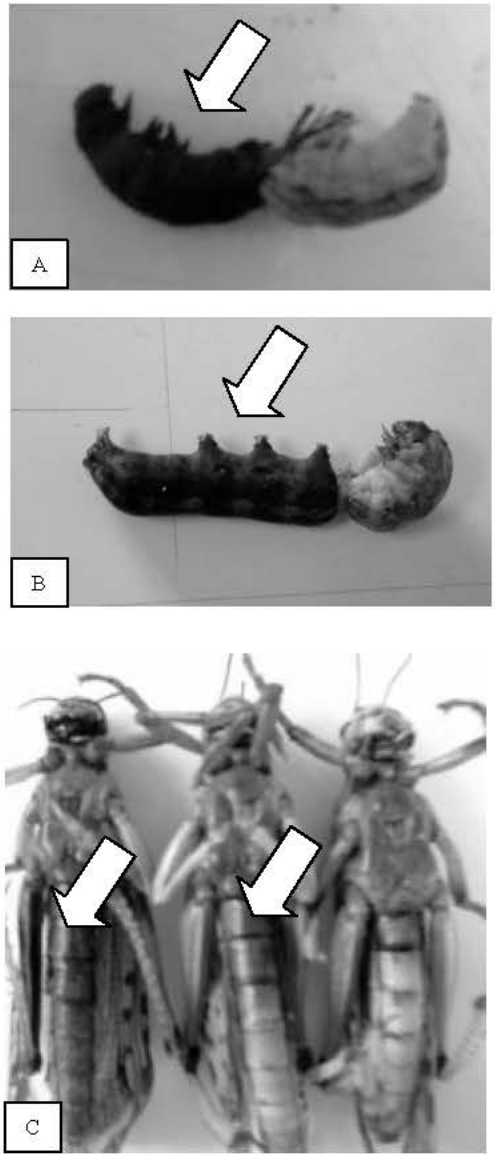


Fig. 1: Effect of CSE injection in the posterior (A) or anterior part (B) part of bounded *Spodoptera*; (C): under cuticular injection of CSE on *Schistocerca* adults

directly injecting saponins with an alive animal, or by dissecting portions of caterpillars and by making them marinate in the ESB dissolved in a physiological liquid.

Histological cuts made for untreated animals show a fat body made up of cellular clusters which often take the form of cellular cords. The cells appear rectangular with a core and a cytoplasm filled with many not colored

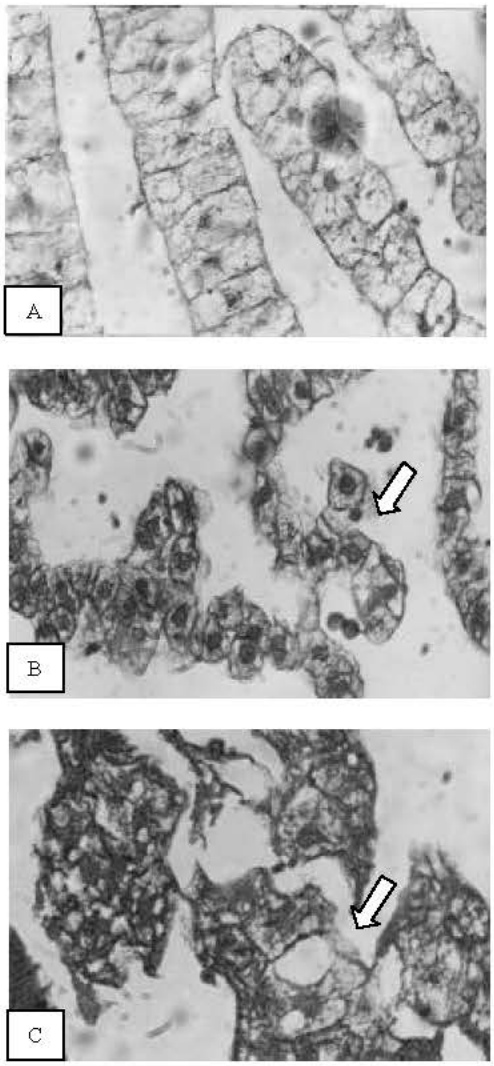
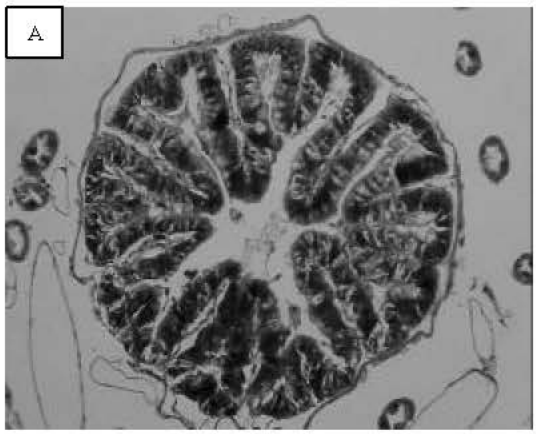


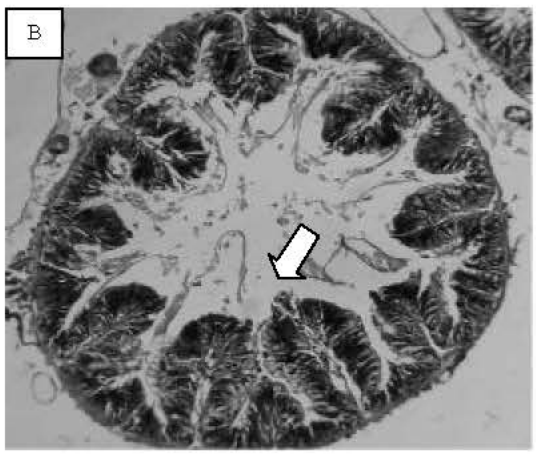
Fig. 2: Effect of CSE injection on *Spodoptera* fat body. (A): untreated control; (B): 6 hours after injection; (C): 24 h after injection. (Magnification x 400)

vacuoles (Fig. 2A). When coloring with the hematoxylin, phloxin, G orange, the non-colored vacuoles correspond to lipidic vacuoles, major component of the cells of the fat body.

The histological observations of *Spodoptera* larvae treated by saponins enabled us to observe two types of symptoms in comparison with the control (Fig. 2A):

- The fat cells treated become smaller and more colored. This structural change becomes rather visible after 6 h of treatment (Fig. 2B).





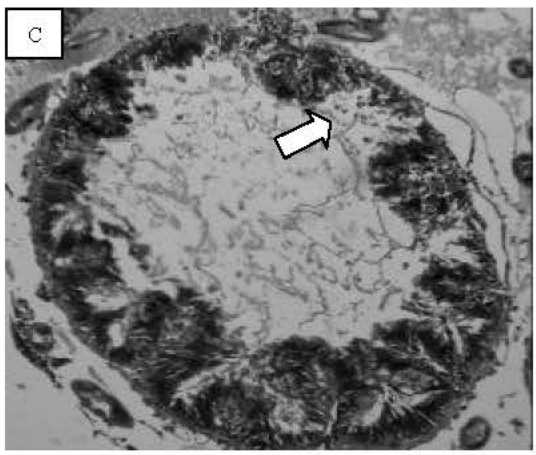
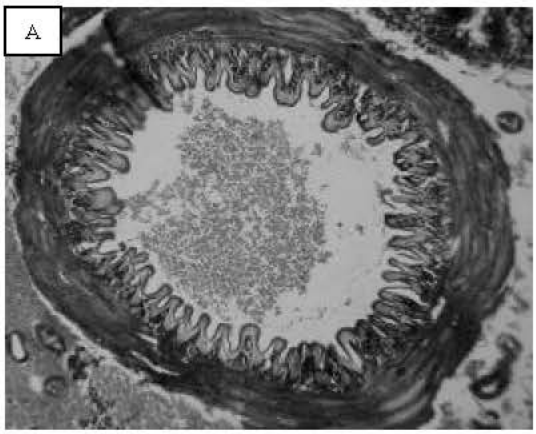
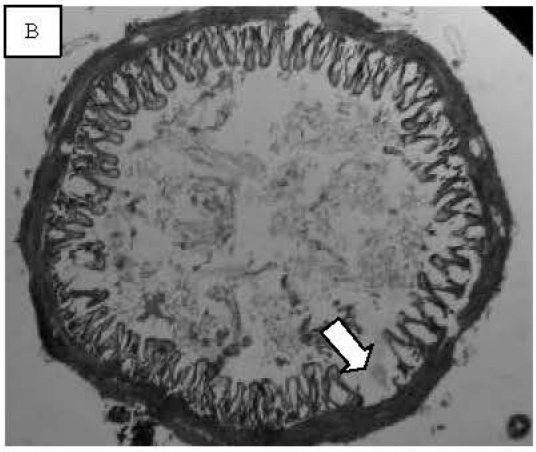


Fig. 3: Effect of CSE forced ingestion on *Schistocerca* gastric caeca. (A) untreated control; (B) 6 h after injection; (C) 24 h after injection. (Magnification x 40)





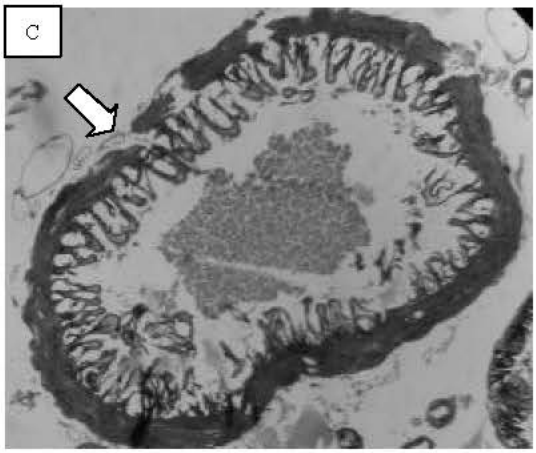


Fig. 4: Effect of CSE forced ingestion on *Schistocerca* foregut. (A): untreated control; (B) 6 h after injection; (C) 24 h after injection. (Magnification x 40)

- The fat body becomes less provided with a significant fall of the number of fat cells. This tissue loss is often accompanied by cellular exudate (Fig. 2C).

The change of the size and the color of the cells is probably due to a change of permeability causing a loss of the cellular contents, the cells of the greasy substance contain in particular reserves in lipids which are not colored in the histological cuts one supposes that the treated cells lose this clear color because of the loss of the lipids.

The destruction of the cellular structure is probably due to the membrane destabilization followed by the bursting of the membrane and the discharge of the cellular contents.

Effect on the gastric caeca: We notice that for treated adults of Schistocerca the gastric caeca presents notable histological modifications compared to the control (Fig. 3A). After 6 h of treatment (Fig. 3B), we notice a dilation of the gastric caeca showing a quite visible light accompanied by a fall height of the epithelial folds. This modification would be probably due to the disturbance of the muscular layer surrounding the caeca. Moreover Cellular perturbation and epithelial bursting located after 24 h (Fig. 3C).

We think that the modifications are due to the same phenomena of cytotoxicity described in the fat body of Spodoptera.

Effect on the foregut: The same phenomena are observed on the level of the foregut (Fig. 4A). A progressive destruction begin with a separation of the intestinal epithelium and muscular layers appear 6 h after the treatment, we observe a total disorganization of the cells after 24 h from the beginning of the treatment (Fig. 4B) these observations were noted even on the level of muscular bases (Fig. 4C).

DISCUSSION

The injection of the CSE of Cestrum causes the apparition of necrotic tissues on the level of the injection zone. In the same way a forced ingestion of the CSE provokes a softening of the consistency of the digestive tract of *Schistocerca*.

Histological studies enabled us to note that the various symptoms are due to structural modifications observed as well on the level of the fat body of *Spodoptora littoralis* as on the level of the foregut and the gastric caeca of *Schistocerca gregaria*. These modifications were interpreted as being due to a cytotoxicity caused by Cestrum saponins.

The histological observations show cells of smaller size than the control and this as well on the level of the fat body of *Spodoptora* as on the level of the digestive tract of Schistocerca In addition these cells of the greasy substance appear darker probably by loss of their contents due to a modification of their membrane permeability and even with the disorganization of their molecular architecture.

Several works was interested in the disturbing effect of saponins on the biological and synthetic membranes. This work presents a model, explaining this phenomenon, as being based on the interaction property between saponins and membrane cholesterol (Fig. 5). This interaction structurally modifies the phospholipids double layer which would be at the origin of disturbances of the cellular exchanges leading to a cytotoxicity.

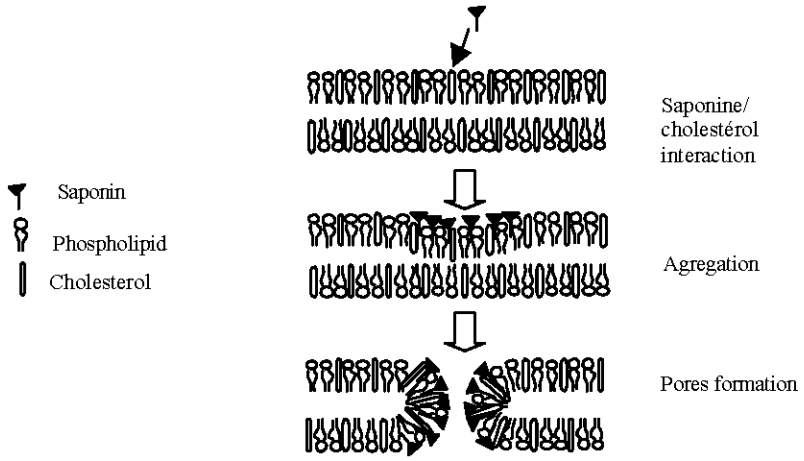


Fig. 5: Hypothesis about *Saponins* cytotoxic mode of action

Sung *et al*. (1995) showed that the use of saponins of the soybean in the treatment of the cancerous cells induced a deformation of the plasmic and nuclear membrane These same authors remarked that the saponins of *Gypsophila* induce a complete destruction of plasma membrane.

The digitonine (spirostanic saponin with 5 sugars chain) causes according to Menger and Keiper (1998) the rupture and the disintegration of synthetic giant vesicle containing cholesterol in their membrane. Whereas the vesicle deprived of cholesterol are not sensitive to this saponin. These authors in addition observed the formation of fibrous tubules on the surface of the membrane of the treated vesicle.

Commercial saponins induce lesions on the level of the membrane of the erythrocytes (Bauman *et al*., 2000). The same observations were made on cells of the intestinal epithelium and pancreatic cells, The digitonine causes deformations on the surface of membrane and the formation of hemi-tubes (Miller, 1984).

These saponins act also on the membrane structure by modifying their permeability, these results arises from a work of Hu *et al*. (1996) once proportioning dyes after their passage through membranes of liposomes permeabilized with α-choacine has and α-tomatine.

Present results are considered closer these phenomena, reason for which the cells observed in the fat body of *Spodoptora* decreases considerably by size and become denser probably by loss of their contents. These modifications of the exchanges were also observed and measured for erythrocytes permeabilized by saponins (Bauman *et al*., 2000).

Our observations on *Shistocerca* are in favour of a total loss of the cellular integrity which reaches even muscular bases on the level of the foregut and on the level of the gastric caeca. we think that at the beginning, the complexation of saponins with membrane cholesterol would have as a consequence the opening of breaches on the level of the cellular membranes (Fig. 5), which accentuates the exchanges with the extracellular medium. These breaches will end up leading to a loss of the integrity of the membrane and the mortality of the cell itself. These observations on *Schistocerca* are to be brought closer the observations carried out by Armah *et al*. (1999) by using Avenacine A1 on liposomes.

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