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Toxicological and histopathological studies of *Boxus chinensis* oil and precocene II on larvae of the red palm weevil *Rynchophorus ferrugineus* (Oliver) (Coleoptera : Curculionidae)

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ABSTRACT

Two natural biopesticides, (*Boxus chinensis* oil and precoceneII) were tested against 10 days-old larvae of the red palm weevil, *Rhynchophorus ferrugineus*. The two biopesticidse had toxicological and pathological effects on *Rh. ferrugineus* larvae. The toxicity effect of *B. chinensis* was more than precoceneII against *Rh. ferrugineus.* In order of toxicity the LC_{50} of *B. chinensis* was 0.68% while the LC_{50} of precoceneII was 1.36%. On the other hand the mid-gut tissues of the 10days old larvae were exhibited different histopathological lesions due to effect of *B. chinensis*. The alterations included analyze and destroyed of epithelial cells, vaculation of cytoplasm and dissolve of nuclei of the epithelial cells.

Keywords: *Boxus chinensis* oil, precoceneII, histopathology, biopesticide effect , *Rhynchophorus ferrugineus* .

INTRODUCTION

The red palm weevil *Rhynchophorus ferrugineus* is a serious pest of coconut causing damage and often killing the palm trees in its prime of life. The hatched grubs burrow into the trunk and feed on tissue of the stem. The larvae and adult emergence within the same stem allow successive generations. *R. ferrugineus* (oliver) is a devastating insect pest of date palm in the Arabian Gulf region. It was reported on date palm, for the first time, from the United Arab Emirates in the mid-1980s, then its reported distributed expanded its range westwards until it reached Egypt in 1992 (Saleh, 1992; Cox, 1993).

The histopathological changes induced by different plant extracts on different insects were documented by many authors (Lusis, 1963; Abo El-Ghar *et al.,* 1994; Ramanthan *et al.,* 1997; Magd El-Din, 1999; Abdel-Ghaffar, 2004; & Wanderley-Teixeir *et al.,* 2006). However, little work has been got on the insecticidal effect against the insects larval mid-gut.

The conventional pesticides are costly and result in problems of residues, resistance, pollution and health hazards. Therefore, the biologist diverted their attention to investigate the feasibility of new generation more safety of biopesticides like natural oils, pathogenic bacteria, IGR,s pheromones, nematode and marine toxins. Many investigations have been conducted on the antifeedant effects, growth inhibition and abnormal development in various insects caused by using natural insecticides including *Boxus chinensis* oil and precoceneII (Schmutterer, 1984; Nassar1995 and Bream *et al,* 2001). *B. chinensi* oil and precoceneII as well as natural extracts are powerful insect antifeedant and repellents (Butterworth and Morgan, 1968; Zanno *et al.,* 1975). They may also disrupt growth, inhibit moulting (Koul, 1984; Garcia and Rembold, 1984; Dorn *et al.,* 1986) and oogenesis (Steets, 1976; Rembold; Sieber, 1981 and Senthilnathan and Sehoon, K. (2005)).

The objective of the present work was mainly to determine the efficacy, in the laboratory, of *Boxus chinensis* oils and precoceneII for disrupting growth and development of *Rh. Ferrugineus* larvae. Also to study the histopathlogical effects of *Boxus chinensis* oils on the mid-gut larvae.

MATERIALS AND METHODS

The experimental insect.

In the present study the larvae, pupae and adults were collected from large cavities of infested date trees in Al-Dawaser Valley region of Saudi Arabia, these trees received no chemicals such as insecticides. Using sugarcane as food, laboratory culture of *Rh. ferrugineus* was established according to methods of Nassar and Abdullah (2001).

Bioassay and administration of chemicals:

Boxus chinensis and precoceneII were bioassayed against *Rh. ferrugineus*. The purified compounds of *Boxus chinensis* oils(dissolved in water) and precoceneII (dissolved in acetone) were obtained from "Sigma Chemical Company". Five concentrations were prepared from *Boxus chinensis* oil and PrecoceneII: 0.25, 0.5, 0.75, 1.0 and 1.25% after preliminary tests against of *Rh. Ferrugineus* larvae. Three replicates contain 15 larvae (10 days old) each was topically treated with the different concentrations of each compound. Parallel to treated replicates the control replicates were prepared without any treatment. All treated and control insects were kept at 37 ± 2 C° and 50 ± 60% RH.

Statistical analysis of data:

Percentages of the larval mortalities were observed during the larval period, and were calculated as suggested by Jimenez-Peydro et al. (1995). Data obtained were analyzed by the Students t-distribution and refined by Bessel correction (Moroney, 1956).

Histopathological studies:

Treated larvae with *Boxus chinensis* oil and the untreated larvae of the red palm weevils were dissected in Ringer's solution. The midgut was isolated and fixed in Bouin's solution then embedded in paraffin. Many sections were obtained and stained with haematoxyline and eosin according to method of Drury and Wallington, (1980).

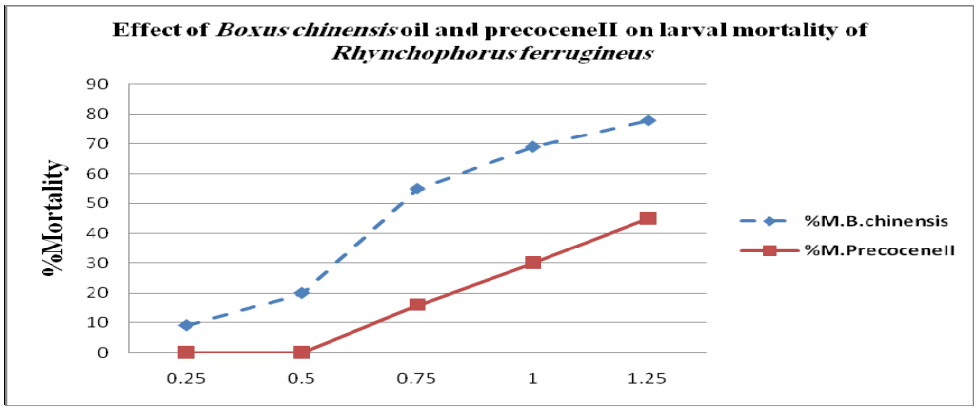
RESULTS AND DISCUSSION

Lethal effects of *Boxus chinensis* oil and precocene II:

Lethal action of *Boxus chinensis* oil and precoceneII against the 10-days larvae of *Rhynchophorus ferrugineus* was shown in Fig. (1). According to the obtained data, larval mortalities were increased in an ascending course by increasing concentration levels. Good evidence for the total mortalities, where it was 78% at the highest concentration (1.25%) of the *Boxus chinensis* oil and 8% at the lower concentration (0.25%). PrecoceneII caused 45% and 00 which exhibited the lethal action with the application of the same previous concentrations. Depending to the recorded data in the present work of *Boxus chinensis* oil and precoceneII against 10-days old larval instars of *Rhynchophorus ferrugineus*, it was found that LC_{50} of *Boxus chinensis* was 0.68% and of precocene II 1.37%. El-Sherif *et al.* (1994) reported LC_{50} *A. nobilis* was 1.05 ppm against 1st instar larvae of *Pectinophora gossypiella* and *Erias insulana* which lower than our finding. This may due to the different in species and instar ages. Lethality effect of plant oil suggested due to its active contents, which may disrupt the metabolism by increasing of malate dehydrogenase enzyme against many insect larvae

(Mostafa *et al.* 1995). On the other hand precoceneII was found less toxic (LC_{50} 2.6%) than *B. chinensis* oil. Similar results were obtained by Heyde *et al.* (1983) who reported that neem extract was effective only at the high concentration (1-50%)against the Homopteran insects. *Boxus chinensis* oil may affect the neuroendocrine system of insects which affect the production of ecdysone and consequently disturb the moulting process and finally cause insect death (Gujar and Mehrotra, 1983 and Mostafa *et al.*, 1995). In addition the higher toxicity of *Boxus chinensis* oil than precoceneII may be due to the effect of some insecticides as a gangalion poisons which inhibit cholenestrase enzyme for regulate insect metabolism (Naqvi *et al.*, 1992).

Fig. (1): Toxicity of *B. chinensis* oil and precoceneII on the 10-days old larval instars of the red palm weevil *Rhynchophorus ferrugineus*



%M.*B. chinensis*: % of larval mortality by effect of *Boxus chinensis* oil
%M.PrecoceneII % of larval mortality by effect of precoceneII

Histopathological study:

The obtained results were revealed that, larval mortalities by the effect of *Boxus chinensis* oil more than precoceneII. Therefore alimentary canal of the larvae treated with *B. chinensis* was dissected in order to complete the histopathological study. The midgut of the 10 days old larvae of the red palm weevil *Rhynchophorus ferrugineus* divided into three regions(Midgut-1, midgut-2, and midgut-3). Midgut-1 contains epithelial cells and regenerative cells (Fig.A). The interior surface of the epithelial cells provided with borders. The epithelial cells rest on a basement membrane. Midgut-2 the longitudinal muscle layer appears externally followed by an internal circular muscle layer. The caecal pouches are thrown into folds directed to gut lumen as illustrated in Fig. (B). Where the muscle layer appeared detached from the gut wall. Also the disappeared of cell boundaries between epithelial cells, vacuolization, shrinkage of the epithelial cells. The transverse sections of the treated midgut-2, revealed that the nuclei moved toward the distal part of the epithelial cells and the nuclear membrane disappeared. Also, the cytoplasm of the columnar epithelial cells appeared less condensed at the basal part of the columnar cells. As shown in Fig. (C), degeneration of the epithelial cells of the midgut-3 is clearly noted in the larvae treated with *B. chinensis* oil. In addition the nuclei lose the nuclear chromatin granules and the peritrophic membrane appeared wrinkled and vacuolated.

The present work showed that the *B. chinensis* oil caused histopathological changes in the midgut of the 10 days old larvae of *Rhynchophorus ferrugineus* larvae. These changes included degeneration, vaculation and shrinkage of the epithelial cells, movement of nuclei twords the apical part of the cell, vaculation of peritrophic

membrane and detachment of muscle layers. On the other hands, the midgut-1 (A2) of *Rh. ferrugineus* larvae treated with *Boxus chinensis* oil showed a hazard appeared in the elongation and deatachment of epithelial cells from the basement membrane, these distortions was either shredding or completely degenerated. Also, Fig. B2 of midgut-2 of treated larvae showed disorders as the previous characters. The obtained results revealed that midgut-2 and midgut-3 were affected more than midgut-1. Similar result was obtained by using different plant extracts against different insects as well as Abdel-Ghaffar (2004) who discussed the disorders effect of Margosan-0 combined with sesame oil against the berseem hopper *Euprepocnemis plorans* and Wanderley-Teixeir *et al*. (2006) who descriped the midgut and the pyloric valve alterations of the orthopteran, *Tropidacris collaris.* Hussein *et al.*(1994) mentioned that the effect of plant extracts on midgut of *Erias insulana* may due to digestion and absorption of plant oil. On the other hand Ahmed (1995) reported that histopathological effect oil extract of chamomile plant was produced enlargement of epithelial cells, appearance of vacuoles at the apical part of the cell and destruction of the peritrophic membrane of *Culex pipiens* larvae. Nasiruddin and Mordue (1993) studied the histological and ultrastructure changes caused by azadirachtin on the midgut of locust. His findings revealed necrosis of epithelial cells, enlargement of cytoplasmic inclusions and small sized striated borders. Various aspects of histopathology in the midgut of locust Heteracris arnulosa caused by neem seed extract were discussed by Naqvi et al (1994). These results agree with the finding of Ruscoe (1972) during his studies that antifeedants have growth disruption effects on insect organs, while Rembold *et al.* (1988) confirmed that *Azadirachtin* a good factors for tissue specific in corporation in Malpighian tubules of *Locusta migratoria.* Zudaire *et al.* (1998) cleared that, the gut of locusts disturbed and showed disrupter in the endocrine cells as affected by food nutrient content, insect age and stage. On the other hand, Abo El-Ghar *et al.* (1994) provided histological effects of abmectin on mid-gut of *Spodoptera littoralis* which had similar hazard shredding and erosion on the lining epithelium. Also, Magd El Dein (1999) has discussed the toxicological and histological effects of lemon grass; *Cymbopogen citrates* on *Agrotis ipsilon* showed the same previous histological alterations.

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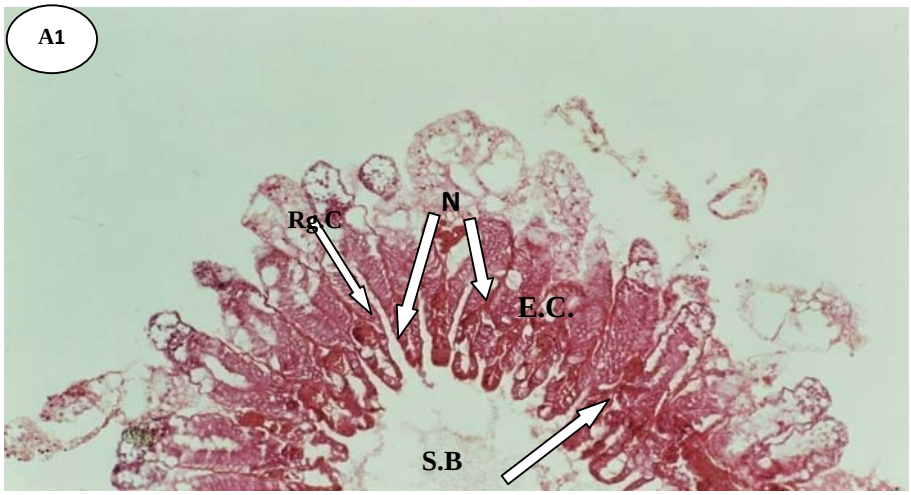

Fig. (A1): T. S. of the midgut-1 of control *Rh. ferrugineus* larva (H/E X200).

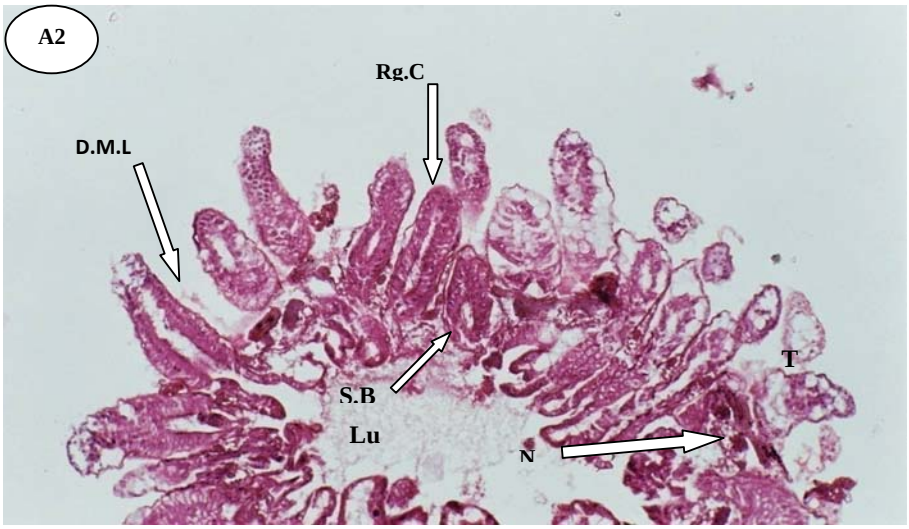

Fig. (A2): T. S. of the midgut-1 of *Rh. ferrugineus* larvae treated with Boxus chinensis oil (H/E X 200).

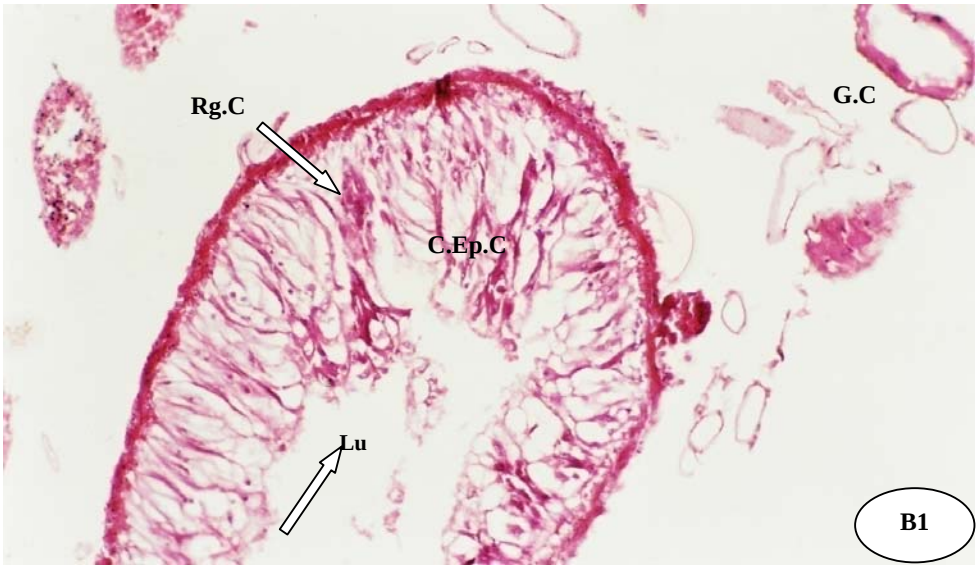

Fig. (B1): T. S. of the midgut-2 of control *Rh. ferrugineus* larva. (H/E X200).

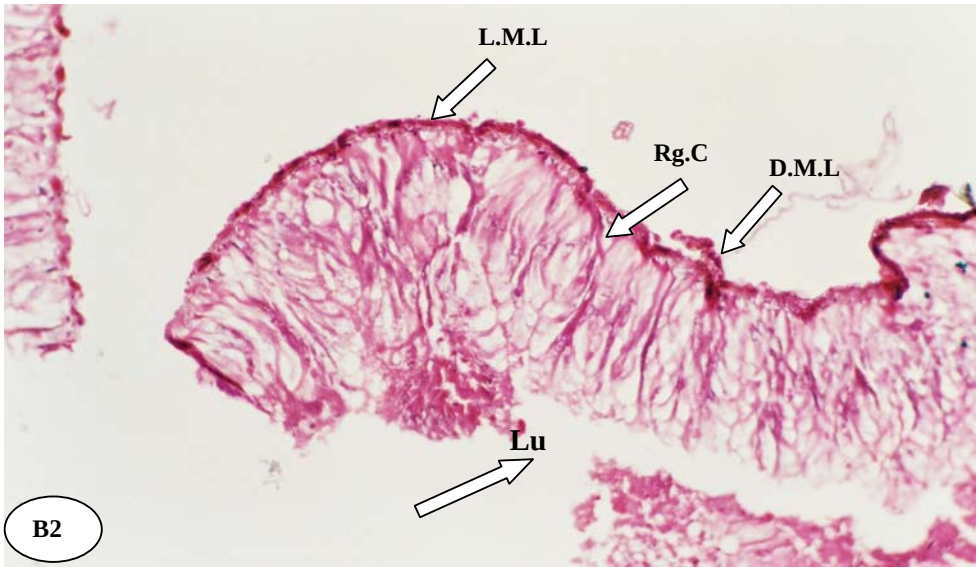

Fig. (B2): T. S. of the midgut-2 of *Rh. ferrugineus* larvae treated with Boxus chinensis oil. (H/E X200).

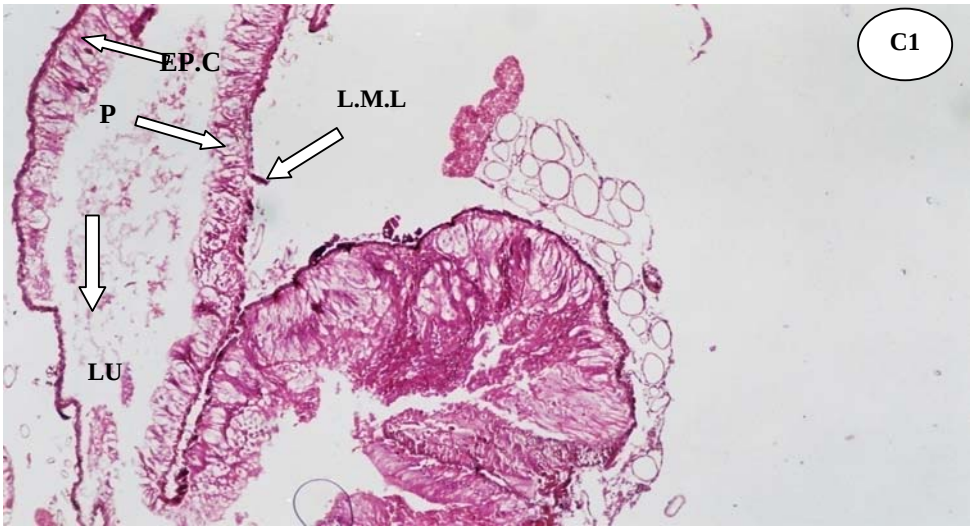

Fig. (C1): T. S. of the midgut-3 of of control *Rh. ferrugineus* larva (H/E X200).

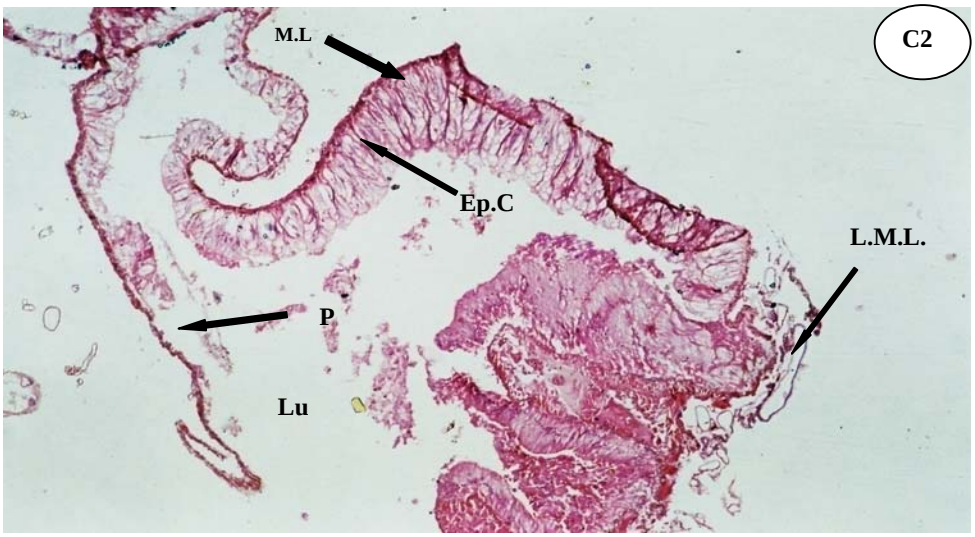

Fig. (C2): T. S. of the midgut-3 of *Rh. ferrugineus* larvae treated with *Boxus chinensis oil*. (H/E X200).

B.M, Basement membrane; Caecal pouch(C.P); Circular muscle layer(C.M.L); Columnar epithelial cells (C.Ep.C); Detached muscle layer (D.M.L); Epithelial cells (Ep. C); Fat bodies (F.B); Gastric caeca (G.C); Longitudinal muscle layer (L.M.L); Lumen (Lu); malpighian tubules (M.T); Nucleus (N); Peritrophic membrane (P); Regenerative cells (Rg.C); Ruptured epithelial border (R.Ep); Striated border (S.B); Tracheols (T); Vacuoles (V); Vacuole cells (V.C).

ARABIC SUMMARY

دراسات توكسيكولوجية وانسجة مرضية بتأثير زيت نبات البوكسس شيننسس والبريكوسين2 على يرقات سوسة النخيل الحمراء، رينكوفرس فريجينس (أوليفر) (غمدية الاجنحة : كيركليونيدى)

محمد عبد الله على
قسم علوم الحياة – كلية العلوم – جامعة جازان - السعودية

تم اختبار اثنان من المبيدات الطبيعية، زيوت نبات البوكسس شيننسس والبريكوسين2 على عمر عشرة ايام ليرقات سوسة النخيل الحمراء، رينكوفرس فريجينس. ومن الدراسة تبين ان هذين المبيدين الحيويين لهما تأثيرات توكسيكولوجية ومرضية على اليرقات. وكان زيت نبات البوكسس اكثر فاعلية من البريكوسين2 على السوسة. وبدراسة درجة السمية فقد وجد ان التركيز القاتل عند مستوى 50% لزيت البوكسس هو 0.68 % بينما كان للبريكوسين 1.36 %. ومن ناحية اخرى بالدراسة التشريحية لتأثير زيت البوكسس على اليرقات وجد ان المعى الوسطى قد حدث لة تشوهات وتهتكات. وقد اشتملت هذة التشوهات على تحلل وتدمير الانسجة الطلائية، وكذلك حدوث فجوات فى السيتوبلازم وايضا اختفاء بعض الانوية فى الخلايا الطلائية.