

Histopathological Change and Gene Expression of Heat Shock proteins (Hsp90 & Hsp70) in Snail *pomaceacaniculata* (Lamarch, 1822) Exposed to Stress by the Pesticide Lannate

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The current study aimed to investigate the snail's pathological changes and their relationship with the amount of gene expression of heat shock proteins HSP90 & HSP70 under the influence of lannate pesticide. *P. canaliculata* were collected during the period between 5th-10th May 2018. The laboratory generation snails were divided into 2 groups, the first was exposed to a concentration of 10 µg/L of the pesticide, as the lethal concentration of the pesticide was 12.6µg/L, and the second was left as a control group. After that, three snails from each group were dissected by the end of the following intervals (10mins, 1hr, 8hrs, 24hrs, 3days, 7days, and 15day). The gene expression was studied using qPCR method. Results of the genetic analysis for the abundance of the HSP70-HSP90 showed a clear effect of the pesticide on the treated snails during the above interval.

On the other hand, the results of the histological study of the gills showed that the pathological changes varied in their intensity during the intervals, while there was no clear effect to variation of concentrations. These changes involve loss of cilia in the epithelial cells, infiltration of granulocytes in the cilia loss regions, hyperplasia, degradation and necrosis of cells as well as the accumulation of non-living brown and grey materials within the epithelial cells. Moreover, it was noticed that the mucous materials secreted from cystic cells has increased.

Keywords: *Pomacea canaliculata*, Heat shock proteins, HSP70, HSP90, Lannate.

Introduction

As a matter of fact, *Mollusca* has a wide variety of species, including *Gastropoda*.⁽¹⁾ *P.canaliculata* feeds on aquatic plants and can destroy rice and other aquatic or semi-aquatic plants as in Southeast Asia.⁽²⁾

Subsequently, this snail has spread steadily through its leakage through irrigation and drainage canals until it became an important part of the ecosystem in Taiwan. Hence, its rapid growth in Southeast Asia has turned it into a serious pest that threatened rice fields and inflicted heavy economic losses.⁽³⁾ In 2000, *P.canaliculata* was considered one of the 100 worst pests that invaded the world. This was in terms of the International Union for Conservation of Nature and Natural Resources (IUCN) classification. Such abundance may be attributed to the lack of its enemies in the environments in which it lives and, in most cases, to its exceptional ability to adapt to

the environments with high temperatures drought, lack of food sources and water pollution.⁽⁴⁾

As a result of these features, the snail was capable of conquering several countries. It was recently registered in the Iraqi environment for the first time by Al- Abbad in 2015.⁽⁵⁾ It is a pest of multiple botanical species and is viewed as one of the most serious pests on rice in Iraq if it spreads in planting zones.⁽⁶⁾

In 1962, so-called stress proteins or Heat Shock Proteins (HSPs) were discovered. These are one of the stress-affected proteins.⁽⁷⁾ Afterwards, they have been known as Molecular chaperon. They play an active role in preserving the functions of proteins as well as stimulating immune responses against pathogens. In addition, they take part in preserving cellular functions by folding the newly formed amino acid chains into their correct protein forms. They also prevent wrong protein

folding and help the cell get rid of damaged proteins. Moreover, they protect proteins and accompany them on their way to combine with their escorts. They also fulfill a very important function to the immune system, as they act as a presentation antigen to show antigens of the immune system cells in order to weaken or eliminate.⁽⁸⁾ HSPs are normally expressed and their expression increases at stress conditions such as high temperatures, exposure to chemical toxic compounds, heavy metals, infections, and diseases.⁽⁹⁾ They have been classified into several species based on their molecular weight, including HSP100, HSP90, HSP70, HSP60, and HSP40.⁽⁹⁾ This study tried to investigate the snail's pathological changes and their relationship with the amount of gene expression of heat shock proteins HSP90 & HSP70 under the influence of lannate pesticide

Materials and Method

One hundred 100 *Pomacea canaliculata* snails (Lamarck, 1822) were collected manually from the eastern bank of the Shatt al-Arab in the Salihiya area in the Governorate of Basra in southern Iraq between 5th-10th of May 2018. The snails were then transported to the breeding laboratory in the animal house of the Department of Biology. They were left in the breeding basins to obtain a laboratory generation. After hatching, newly-hatched snails were left under laboratory conditions. Three months later, the laboratory snails were divided into two groups of equal weights of approximately 21g. Next, histological changes were studied especially in the snails subjected to concentration 10 µg/L for the intervals (24hs, 7days, and 15days). 6 snails were taken from each interval, 3 of which were treated and 3 were untreated. Accordingly, histological sections were prepared according to the Humason method (1972).⁽¹⁰⁾ Thus, 3 snails from each group were dissected after the end of the following intervals (10mins, 1hr, 8hrs, 24hrs, 3days, 7days, and 15days). Then, 20µg of the soft parts of the body (gastrointestinal gland and kidney) were taken for the extraction of RNA according

to the method mentioned in the extraction tools SV Total RNA Isolation System of Promega Company. The concentration of RNA was determined with the use of Nanodrop Technologies. After that, the cDNA was formed using the transformation tools GoTaq® 2-Step RT-qPCR System from Promega company. Moreover, the starters for the HSP90 & HSP70 genes were used.⁽¹¹⁾

Afterwards, SPSS program was adopted in the statistical analysis of results. Finally, the significance of the differences between gene expression for genes and periods were tested using the T test at a significant level of $p \leq 0.05$

Results

The results of the microscopic examination ME of the tissue sections of the gills of *P. canaliculata* treated with lannate showed the presence of various histopathological effects on most of the components of the gill plates but basically are centered on the epithelial cells lining them. It was also noted that there was no effect of the pesticide concentration on the type and severity of these pathological effects, as the pathological changes in each of the two concentrations µg/L (10 and 5) were similar.

After 24 hours of the experiment: The results showed the presence of histopathological changes in the gills of treated *P. canaliculata* compared to the control group samples (Figure 1). These changes were represented by loss of cilia from the epithelial cells lining the plates. The specific study of the successive histological sections showed that the losses were in small areas distributed on different locations of the single placenta plate. An accumulation of granulocytes was also observed in these small areas. That accumulation followed a pattern, as the microscopic examination showed that granular cells are abundant in the base of the epithelial cells lining the gill plate that lost their cilia or are burst into the epithelial cells, but it was not noticed in the epithelial cells lining the gill plates that still maintain their cilia (Figure 1).

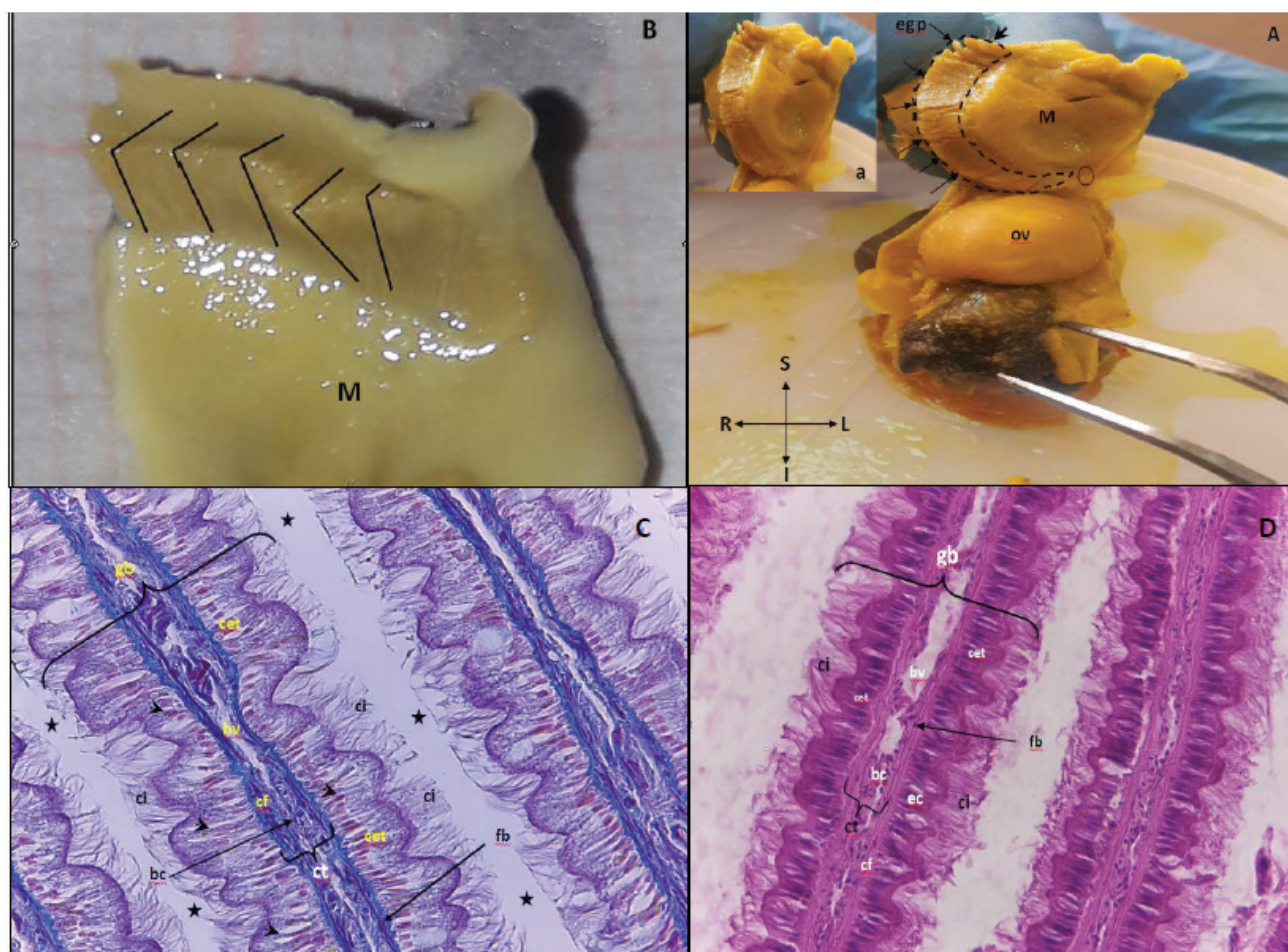


Figure (1): Anatomical image of P.canaliculata snail

After 7 days of the experiment: The results showed the persistence of conjugation of the immune granulocyte cells in the areas that suffer the loss of cilia in the gill plate. In addition, the accumulation of non-living brown color substances was observed in the epithelial cells. These substances usually accumulate as an oval or spherical mass in the cytoplasm directly above the oval nucleus (Figure 2).

The examination also revealed the presence of hyperplasia in the vertical epithelial tissue lining the gill plates and the appearance of epithelial tissue similar to the papilla, as the cells at the top of the hyperplasia lose their polarity. On the other hand, the original cells still maintain their polarity and have vertical shape. Furthermore, their basal surface resides on the basement membrane. In addition, hyperplasia cells are seen as smaller than the mother cells and their large nuclei.

Hyperplasia also presses on the tops of adjacent cells, causing their decline (Figure 2). The degeneration of ciliated epithelial cells has been observed too.

After 15 days of the experiment: Results showed the extension of the area of cilia loss accompanied by the loss of the immune granulocyte infiltration. It was also noticed that in some areas of the granulocyte infiltration, these cells form a cyst-like structure as a result of their aggregation in one area under the lining tissue of the gill epithelium, which leads to the rush of the epithelial tissue outward towards the gill fissure, and sometimes eruption of the epithelial tissue leads to pressure on the adjacent gill epithelial cells and, consequently, changes their vertical shape. It was also noticed that there was an accumulation of non-living brown substances in the cytoplasm of the epithelial cells of the gill plate (Figure 3).

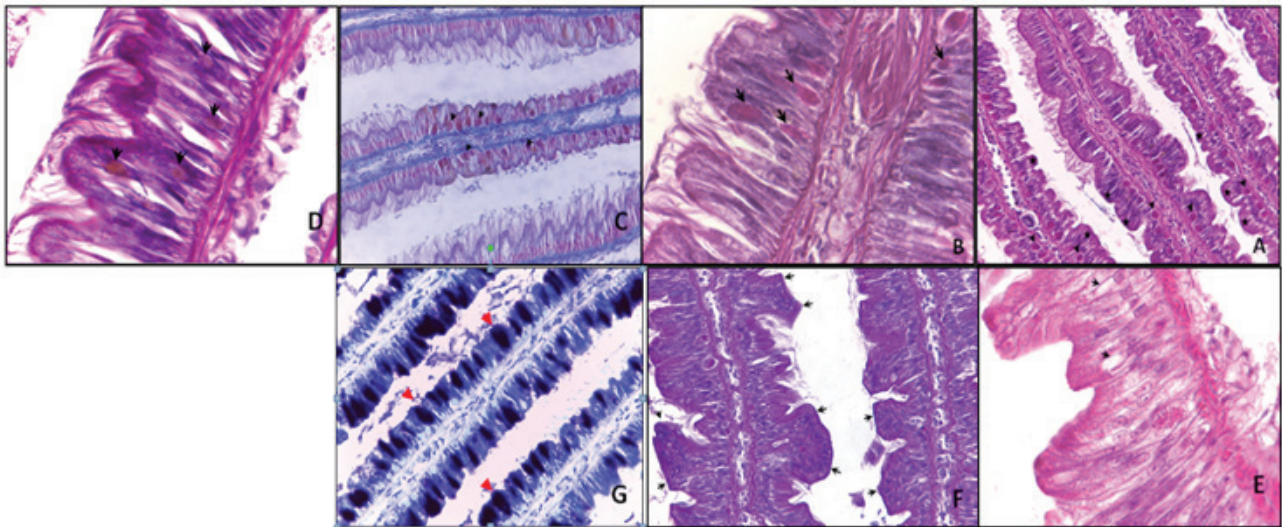


Figure (2): A- Gills plate of *P.caenaliculata*

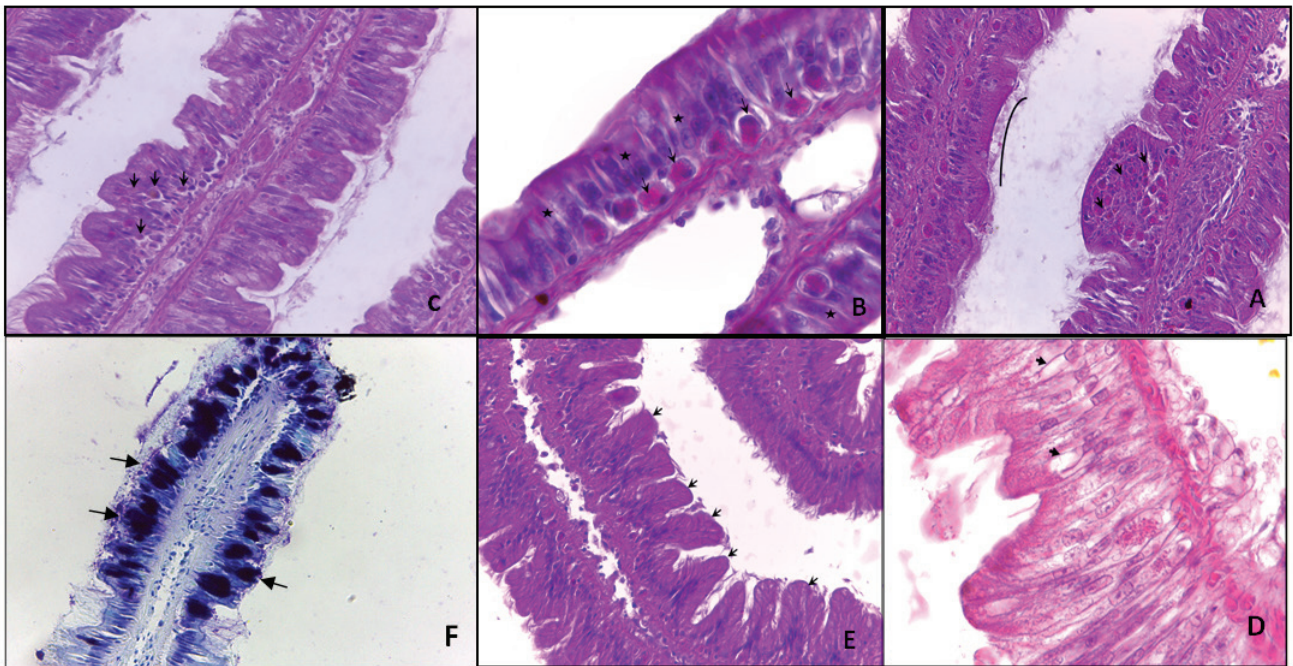


Figure (3): The gill plate

There were also instances of hyperplasia in the form of multiple papilla. In this respect, the histological examination showed the emergence of a number of vertical epithelial cells for the gill plate which suffered from cytoplasm degeneration, so it seemed faded or nonabsorbent to the pigment (Figure 3).

Gene expression abundance analysis of Heat shock protein: The results of the genetic analysis for the abundance of heat shock genes HSP70-HSP90 of the control group snail samples showed that each of the two

genes is expressed in the natural conditions at different degrees. In this respect, the statistical analysis revealed that there are noticeable differences between the two genes as the gene expression of HSP90 was highly significant, of about 5,000 times more likely than that of HSP70 at a probability level of $P \leq 0.05$.

Discussion

High abundance of these genes means an increase in the amount of the production of HSPs, usually at the

expense of the rest of the necessary cell proteins.⁽¹²⁾ This may reveal the causes for the appearance of some histopathological changes in the gills and kidneys of the snail, such as degeneration and necrosis. Therefore, the low gene expression in the intervals (8 hours -3 days -7 days - 15 days) may be under those regulatory mechanisms, as the high intensity of HSP after ten minutes may have provided the cell with sufficient amounts of protein necessary to adapt to the stress experienced during this interval.

The gills are the most affected organs by the surrounding environment. Being in direct contact with the surroundings, it is expected that they may experience histopathological effects faster than other organza's.^(13,14) they provide a very large space for direct and continuous contact with pollutants and toxins in water.⁽¹⁵⁾

The results have also showed the high toxicity of the lannate on the organs of *P. canaliculata*. The pathological changes were observed during the snail's exposure to the pesticide in both groups exposed to a concentration of 10 µg/l and 5 µg/l of the lannate. These changes have increased with the increase of the time of exposure to the pesticide. In this respect, it is worth mentioning that the current study is in line with a previous study that showed the relationship between the intensity of changes occurring in different organisms and the length of time of exposure to toxic substances.⁽¹⁶⁾ The nature of the synthesis of the pesticide may play a role in that, or perhaps the cell receptors in the snail tissue respond with the same degree in different concentrations to urge the cells to react against the pesticide.⁽¹⁷⁾

Perhaps this explains the speed of their infiltration and accumulation in some areas of the epithelial connective gill tissue, as they have infiltrated from the first day of the snail's exposure to lannate. So, when these substances or free radicals act on the oxidation of fats in the plasma membranes, causing a change in the permeability of those membranes, a swelling for the cells may appear and liquids may accumulation inside.⁽¹⁸⁾ This was observed in the histological sections of epithelial tissue cells.

It is known that a change in the permeability of membranes or their damage can finally lead – with the continued presence of the causing factor- to greater damages that may contribute to the death of cells.⁽¹⁹⁾ This explains the large number of tissue areas in the organs which suffer from necrosis and degeneration

in the members of the studied snails in all intervals. This indicates the speed of divisions and overgrowth triggering the possibility of cancer with the continued exposure to the pesticide,⁽²⁰⁾ as previous study indicated that lannate could be carcinogenic.⁽²¹⁾ Hyperplasia in the organism is a form of reaction to exposure to pathogens. This may lead to cancer with the organism's continued exposure to these pathogens.⁽²²⁾

Increasing the reproduction of cystic cells in the epithelial tissue plate is a primary defensive means for the snail against environmental stress exerted by the pesticide in order to the increase mucous material, this may lead to lessen the area exposed to the water polluted with the lannate.⁽¹⁷⁾ Mucous material is produced for defensive action in order to protect cells from pollutants

Conclusion

The study concluded that the intensity of histopathological changes increases in the gills and kidneys of snails with an increase in the extension of the period of exposure to the pesticide more than the increase in the concentration of the pesticide itself. It became evident that there was a direct relationship between the increase in the intensity of histopathological changes and with increased genetic expression of heat shock proteins during the chronic period upon exposure For the pesticide..

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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