

## **Study the Changes in the Speed of Locomotors Activity and Histological Alteration in the Gills, Liver, and Ovary of Freshwater Fish *Liza Abu* (Haeckel) Exposed to Chlorthiamid Herbicide**

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**Abstract** :Acute and sub-lethal concentrations of herbicides pesticide chlorthiamid (1.5,2.5,3.5 and 4mg/l), the acute effect measuring the operculum of gills movement within the period 24-96hours ,where a significant difference was observed in the movement .The highest value was recorded ( 174.02mov/minute ) by the ( 5mg/l)through( 48hours)and the lowest recorded value of (22.03move/minute)and ( 5mg/l)and the period ( 96hours ), compared with control (72.16 ). Sub – lethal concentrations focused the tissues alternations in the gills , liver and ovaries within the concentrations of (0.12 &0.25mg/l ) for a period 10 days of exposure .These changes included in gill tissues (Blood bleeding ,Vaculues , Arches damage , Necrosis ,degeneration , splitting ,Damage of epithelial tissues ) and ovary tissues (Oocyte cells necrosis, Blood bleeding, fatty Vaculues, Thick wall of Oocytes and degeneration) and sever hazard in the liver (Glycogen, Blood Bleeding. Lipid granules, Aggregation cells, Hypertrophy of hepatic cells) . These differences depending on concentrations compared to control. Tissues a good biomarker indicator about toxic effect to fresh water fish like *Liza Abu*. This study confirms the sensevity of these fish to the hazard in the aquatic environment through the presence of these pollutants.

**Keywords:** Histology, movement operculum, *Liza Abu*, chlorthiamid

### **Introduction**

There are many damages resulting from the use of pesticide, which in turn differ from each other in their chemical composition and the degree of toxicity effectiveness. They have been used to combat agricultural pests and insects. to increase agricultural crops <sup>1</sup>as fertile material are used<sup>2</sup>. Pesticide are considered too dangerous materials to all organisms, Whether plant or animals and human It may also cause damage to aquatic organisms they leakage into rivers ,at it cause severe effects to fish ,which are source of food for human .So the knows hydro toxicity is those effect that polluted water causes to the fish and other organisms in which it live .refers Chlorthiamid Herbicide $C_7H_5Cl_2NS$ , it is an organic chlorine pesticide an specialized non-volatile herbicide and has toxic effect on plant and animals, as it has the Permeability property in to agricultural soils and facilitates its easy access to reach of river ,which effects on fish wealth and also harm effect on human health<sup>3</sup> . The researchers also emphasized the unprogrammed use and excessive uses for pesticide. In turn, it led the accumulation on the leaves of tress, such as fruits and toxic effect, through their transmutation to human during the food chain. Studies also indicated the negative effects on fresh rivers.<sup>4</sup> Where agricultural pesticides moved to rivers during discharge of polluted filed water into fresh rivers, and here the effect on fish wealth beings.<sup>5</sup> . Pesticides are ability to bind to fats Lipophilic, which leads to tissue accumulation. In addition to its effects on the

respiratory areas of fish and histological changes,<sup>6</sup>it also affects blood parameters<sup>7</sup>biochemical changes, and metabolic activates. Therefore, the study required clarification of the effects of these pesticides on the activity of gills operculum movement, which is indirect contact with its water surrounding. Pesticides are used to maintain human health by combating disease-transmitting insects, but misuse has reflect effects on human health.Also, one of the characteristics of pesticides is that they remain in the aquatic environment, which causesHarmful to some physiological organs of fish like liver and ovary, which causes changes in structure of these tissue lead to damage in physiological composition. <sup>8</sup>

## Material and Methods

### Test Animals

Specimen fish were obtained from fish farming station -Marine center of Basra , they weighted ( $9.18\pm 0.33$  gm.) and their length ( $7.52\pm 0.54$  cm).Fish were washed ( 0.5%KMno4 ) solutions for five minutes to remove the external infections .Samples were acclimated under laboratory conditions in glass aquarium (180 L) filed with dechlorinated water for 15 days period at 24°C, then transferred to the experimental aquaria ,where samples were distributed in the form 5 replicates with 5 fish for each replicate with two periods of exposure of acute and chronic

### Preparing Solutions:

The concentrations of the Chlorthiamid pesticide prepared according to the active substance, and by dilution equation  $c_1Xv_1=c_2xv_2$  concentrations ( 0,1.5,2.5,3.5and 5mg/l )were prepared for acute period of effect as follows ( 24,48, 72and 96hours) and for chronic period ( 10 days) the sub lethal concentration are (0.12and 0.25mg/l ) respectivelyaccording<sup>9</sup>, and calculated lethal of exposure, the movement of the operculum is measured by stopwatch and a movement is calculated for every minute. Each opening and closing of the operculum count as one movement per minute (move/minute)

### Histological Examination

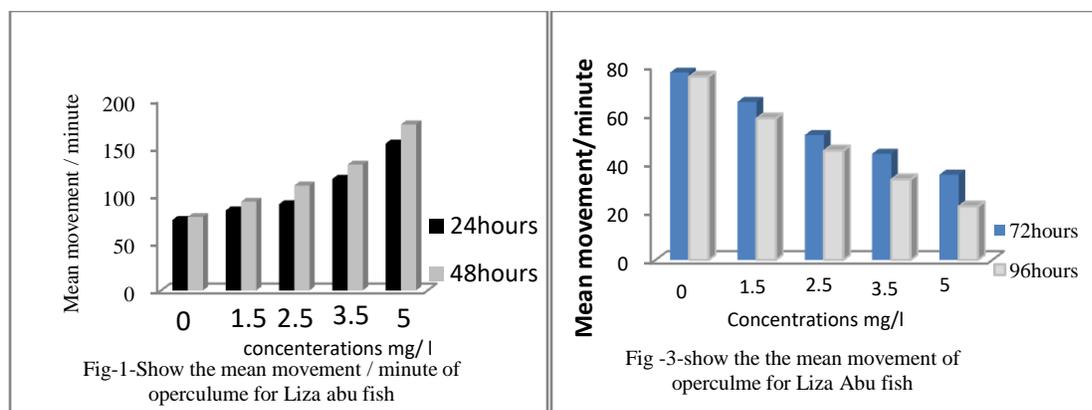
After exposure period, all of the experimental and control fishes were killed for Histopathological examination at the end of 10day according by<sup>10</sup>. Gills ,Ovary, and Liver tissues were fixed in 10% neutral formalin for 24 h. Fixed tissues were washed in running tap water for 24 and dehydrated properly through ascending series of ethanol. Then the tissues were cleared with xylene and embedded in paraffin wax. In order to specify the thickness of serial section for histological purpose, generally sections were cut at 4-5 m thickness and stained with hematoxylin. Eosin and methylene blue for light microscopic examination, and sections were examined for investigation of Histopathological lesions. Finally, the sections were observed under microscope and taken photograph at **magnifications (100 x400)**

**Statically analysis:** - was performed to compare the treated groups with the control using the Anova one –way statistical program for the purpose of comparison between locomotors activity and concentrations, correlation coefficient was used as recorded under the probability level of (0.05)

## Results and Discussion

Mean speed Activity of operculum

When calculation the rate of movement after a period of 24 and 48 hours exposure to the chlorthiamid pesticide of concentration (1.5, 2.5, 3.5 and 5 mg/l) speed was (84.6, 90, 117, 32 and 145.02 move / minute) at 24 hours respectively compared with control (74 move / minute), as the 48 hours recorded a high in their rates, reaching (93.17, 110.02, 132 and 174.13 and move / minute). The rise appeared to be more than period 24 hours, where the gradient in rising was increased, compared in control (77 move / The results of the current showed from figures 1 and 2 the effect of chlorthiamid herbicide on the locomotors activity speed of operculum movement of freshwater fish *Liza Abu*. the changes in the height and reduction values of the operculum movement due to the increased need for dissolved oxygen in the water<sup>11,12</sup>. accompanied by increase in the period of exposure, It was observed, and caused as significant increase in the correlation coefficient, which is the presences of a direct relationship between the increase speed of movement in concentration and the time period, while the correlation values indicated an inverse relationship at the time of 72 and 96 hours of exposure. The means of movement speed for a 72 hours period in fig -2- founded decreases in the operculum movement speed, respectively (65.02, 51.32, 43.72, 35.14 move / minute) it was noted a significant decline between means of speed factor compared to control (72.16 move / minute), as well as the 96-hour period of exposure. There is also a clear differences in the values with means of what are (58.33, 45.04, 33.15, 22.03) move / minute) during 96 hours compared with control (72.2 move / minute) and confirmed inverse correlation coefficient values with concentrations and exposure time. These fish may suffer from a state stress by taking toxins, which leads to the inefficacy of the ability to breath and points operculum as a result of tissues alteration<sup>14</sup> And studies have confirmed that prolonging the exposure time leads to increase in a damage to the gillfillaments and respiratory platelets that work to reduce the area available degassing surfaces for the gas exchange process. Moreover the permanent contact with water to obtain dissolved oxygen, this toxic substance stand in the way of gills obtaining dissolved oxygen and the pathological changes as a result of exposure to toxic dissolved in water. therefore, due to the effective tissue damage caused by toxic pesticide and also can make the fish in a dying state because they are unable to perform respiratory movement<sup>13</sup>, in addition the lack of blood flowing to the gills to carry dissolved oxygen for this reason the axis is shattered, weak and the appearance of gaps. The results of the relative velocity during the period of acute exposure to *Liza Abu* fish it differs in some periods and concentrations, thus the relative speed values in the 24 and 48-hour exposure periods appeared to increase gradually with increasing concentration, While decreased in the 72 and 96 hour periods, it decreased with increasing concentration,<sup>14</sup> and this is what the correlation coefficient values indicated., a positive increase was recorded in the 24- and 48-hour periods, reaching 0.922r, while the lowest value of the negative correlation coefficient was recorded -0.79r, as show in table -2-.<sup>15</sup> reported the activity of the SAH enzymes in the brain and liver, which in turn leads to a state of reduce oxygenated, during that fishes (Rainbow trout) exposed to pesticide (cypermytherin minute). concentrations, correlation coefficient was recorded



After calculation the mean of the locomotors speed activity of each concentration was calculated.

$$\text{Relative speed of operculum} = \frac{\text{--speed of concentration}}{\text{Speed of control}} \times 100$$

Table -2-Show the relative speed values of operculum locomotors activity for Liza Abu fish under influence of a cute concentrations of Chlorthiamidherbicide for sort time period of exposure

Time Period	Contro l	Concentrations mg/l					R
		1.5	2.5	3.5	5		
24	100	106.7	117.9	122.0	128.8	0.922	
48	100	115.4	125.0	152.4	158.8	0.937	
72	100	87.28	69.70	59.79	48.74	-0,84	
96	100	70.96	60.03	54.19	37.44	-0.79	

### Histopathological changes in gills section

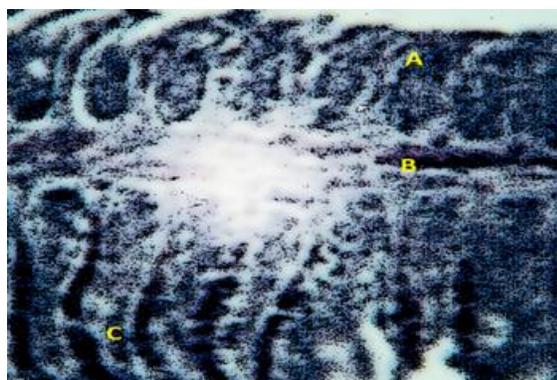


Fig-3-A-- Microscopic Examination showed no changes in control section There are no tissues changes in the untreated form, where the A – Primary lamella ,B- secondary lamella structure were in a good health,

Magnification ( 100X (H&E)

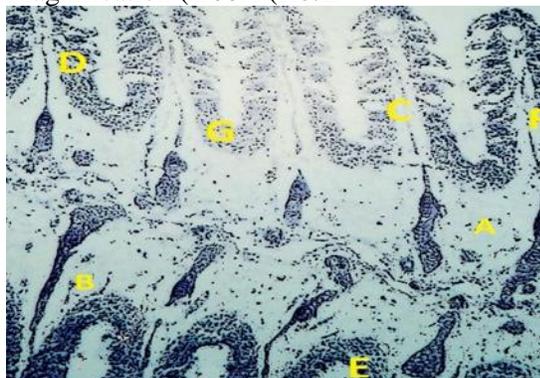
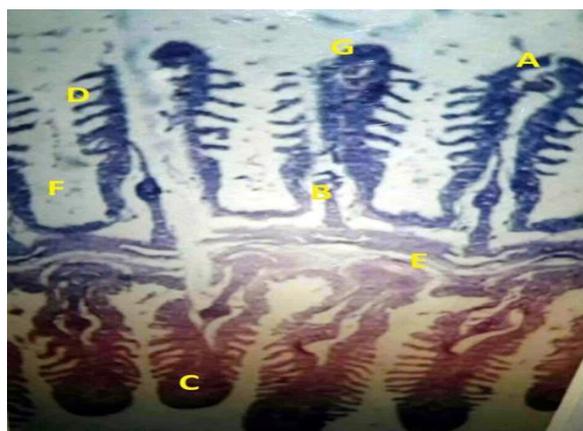


Fig 3B-Shows - Microscopic Examination showed section in the Histopathological alteration in gills after exposure to sub lethal concentration 0.12 mg /gm. of chlorthiamid pesticide at 10 days of exposure. A - Blood bleeding B- Necrosis C- Arches damage D- secondary epithelial damage Necrosis E- degeneration F- splitting G-Damage of epithelial tissues Magnification ( 400X (H&E)

found damage to the axis and hyperplasia, necrosis these findings were similar to the results found by the others <sup>16</sup>. However . when exposing *Liza Abu* fish to sub lethal concentrations, the period 10 days was notice, changes in the tissue at 0.12mg/l were tissue necrosis .separation of epithet cells . The histological alterations of the gill tissues appear as a result of the toxic effects of the pesticide, which in turn led to the dislocation and weakening of the tissue and appearance of pathological symptoms on it , as the continuous water contaminated by use pesticide as toxic substance can make the fish in case of death due to lack of breathing because the hazard of tissues breakage of the weakness ).The experimental study of the *Liza Abu* fish under sub lethal concentrations ( 0.12 ) mg/l were observed changes include 2b fig .These alternations have made uncertainly in the performance of its respiration function , where to obstructs the work of the primary and secondary lamella , many studies indicated to effect and dysfunction <sup>17</sup>the finding in the present study showed hyperplasia , degeneration ,vacuolation of gill epithelium were similar observed by <sup>18,19</sup>.Gilllifting and necrosis might sever a defensive mechanisms leading to a decrease in the respiratory surface in the toxicant blood diffusion distance <sup>20</sup>.



Fi-3c – Microscopic Examination changes A-epithelial hyperplasia B- glubing of secondary lamella C-degeneration D- lifting curling E- necrosis of epithelial vaculeouse F- Vaculeouse , Magnification ( 400X (H&E).

In the shape 3c , I found sever histological changes in this concentration 0.25mg/l in clouding hyperplasia and increase breakdown of epithelial cells and also noticed that the secretion of the mucus layer increase on the gills , which leads to the difficulty of gas exchange for the decline in oxygenation , thus reflected in the reduction the level of blood coming in to the gills and the absence of energy during blood loaded with dissolved oxygen , as it is negatively effect on the physiological blood , and biochemical process ,this found were found by<sup>21</sup> , In this group has shown changes in the epithelial cells and the disintegration of the tissues , excessive hematoma , necrosis of the secondary epithelial lamella , the appearance vacuolation , narrowing of the axis and its weakness, and this is what indicated by <sup>22</sup><sup>23</sup>

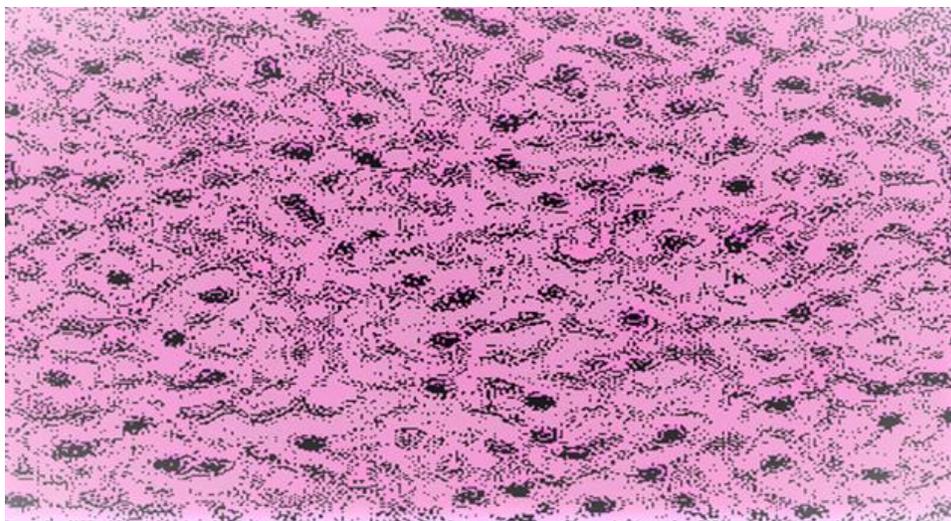


Fig-4-A Microscopic Examination no changes in the liver control . ( Magnification 100X) (H&E)

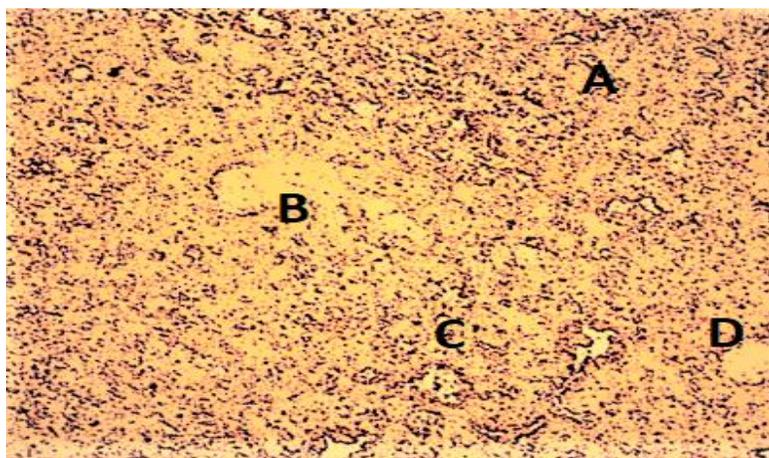


Fig 4b -Microscopic Examination changes the alteration Histological in the liver tissues exposure to 0.12 mg/l of pesticide for period 10 days present A- Magnification ( 100X (H&E)A- Hemorrhage ,B-Hypertrophy C- Fatty vaculeouse ,D- Necrosis

The liver is one of the important organ in its vital function, which in turn constitutes and antioxidant, as it stores glycogen for energy .In addition to its immune function it is considered one of the lymphoid

organs of the immune system, also the liver to regulate the carbohydrate and glucose. The pathological alterations were evident at the concentration of 0.12 mg/l as it was in the form of necrosis of hepatocytes and hemorrhaged, this was also found by many studies<sup>24</sup>. Also from the present study the presence of fatty vacuoles and reduction in the size of hepatocytes, this is consistent with study<sup>25,26</sup>

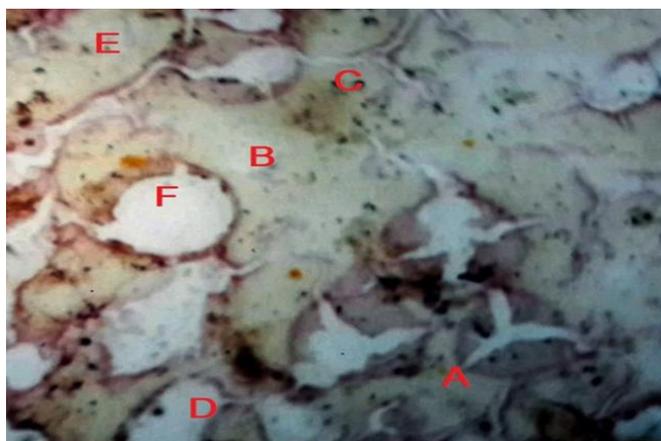


Fig 4c- Microscopic Examination changes the alteration histological in the liver tissues for histological in the liver tissues exposure to 0.25 mg/l chlorthiamid of pesticide for period 10 days present A -Abnormal shape of hepatic cells, B. Fatty insinoid degeneration C. Lipid granules D- Hypertrophy of hepatic cell, E- Necrosis, F-Glycogen. (Magnification 400X) (H&E)

In the concentration (0.25 mg/l) the changes were severe due to the toxicity of the pesticide. In a recent study found fatty vacuoles in the cytoplasm. Hypertrophy of hepatocytes emphasized by<sup>27</sup> found in this study fatty insinoid degeneration and dilution of hepatocytes were similar with study<sup>27</sup> during exposure of cirrhinus mrigala fish to Dichloro pesticide.<sup>28</sup> reported the toxicity of chlorpyrifos pesticide causes degeneration and hypertrophy of hepatocytes. Some of the other changes that were found through microscopic examination of liver cells, the presence of degeneration, necrosis, fatty vacuoles, hemorrhage, hypertrophy, changes in shape.<sup>29,30,31</sup> Exposure of fish to these pollutants changes the function and shape of the hepatic cell. The histopathological changes were different between the control and sub-lethal concentrations, which were (0.12 and 0.25 mg/L) for a 10-day exposure period of chlorthiamid pesticide, as there was a severe decrease in the hepatocytes and fatty vacuoles, which in turn leads to fatty generation, which is the result of fat droplets and the decline in hepatocytes is significant by the toxicity effect of the pesticide and the time of exposure period.

. Histopathological changes in the ovary section

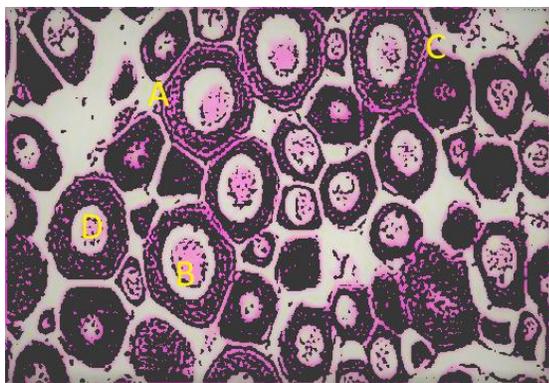


Fig-5-A- Microscopic Examination no changes in control section and normal tissues of Oocyte cells ovary A- vitelline envelop B-yolk Vacuules ,D-yolk droplets. C -Epithelialenvelop - Magnification ( 100X (H&E)

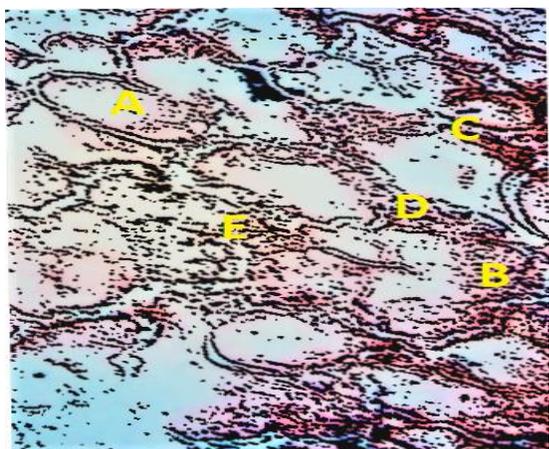


Fig 5B- Microscopic Examination changes The alteration histological in the ovary tissues exposed to 0.12 mg/l of chlorthiamid pesticide for period 10 days present - A .Fatty Vacuules B. . Droplet of yolk, C-Necrosis D – Hyperplasia epithelial , F. cytoplasmic Vacuules of Oocytes. (Magnification 400X) (H&E).

Ovary as the reproductive organs in its vital physiological function of the fish ,and contains multi oocytes , and during their exposure to damage due to pollution of the water environment in which live , changes in the ovaries tissues contain a large amount of fat that is qualified to pesticide accumulate in it after that abnormal tissue changes <sup>32</sup>.As far the sub lethal concentration 0.12mg/l for chlorthiamid herbicide , ovarian necrosis as found ,cytoplasmic vacuoles, a decrease in mature Oocytes ,hyperplasia epithelial and sticking to each other .Moreover there were pathological changes in fish ovary with 0.21mg/l concentration of chlorthiamid herbicide. Some alteration appeared, clear ticking-off the Oocyte wall, hemorrhage, necrosis , Hyperplasia epithelial tissue and degeneration fatty Vacuules. And fragment ova with abnormal shape. Several authors dealt with tissue changes of the reproductive organs, including the ovaries <sup>33</sup>Safiya and KulkarniThe present study observed the pathological changes of the ovaries , including necrosis , emergence of cytoplasmic vacuoles' and appearance degeneration , this is what was refer to <sup>34</sup>in his study on *Channa punctuates* fish during exposed to Malation pesticide showed

changes in the damage to epithelial cells ,hemorrhage. As well as showed in this work the size of the Oocyte cells and increase's the vacuoles ,and this indicates the extent of the chlorthiamid toxicity pesticide within the concentration treated with it

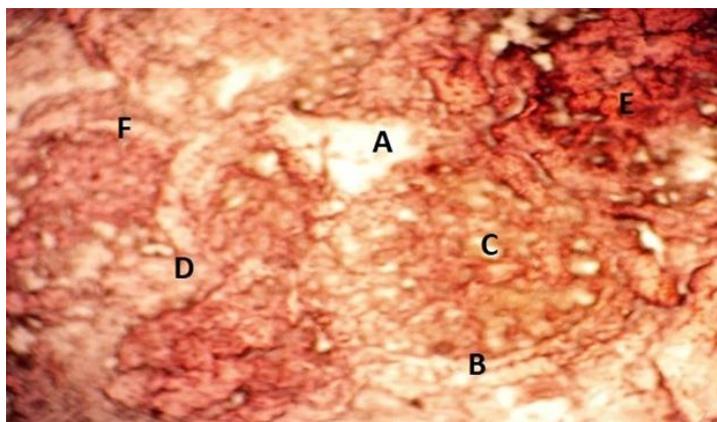


Fig-5C- Microscopic Examination showed the effect of 0.25 mg/l concentration of pesticide in the ovarian structure of the fresh water fish *Liza Abu* , has been observed A-Necrosis of Oocyte ,B- degenerated epithelium degenerated oocytes , C-yolk droplet, D- degenerated oocytes E-Hyperplasia ,F- fatty vaculeouse . (Magnification 400X) (H&E).

The results its of present study are similar with what found<sup>34</sup>their study of the changes resulting in the ovaries of fish *Mystus tengara* when exposed to pesticide chlorpyrifos and cypermethrin .It is also agrees with <sup>35</sup>reached in their study on some changes in the ovaries of a fish that were exposed to monocrotophors , as it indicated the presence of necrosis ,hemorrhage that degenerated the epithelial cells of oocytes .In addition to many authors have indicated to the environmental damage from the presence of pesticide in the environment of aquatic fish causing tissues damage and alternation to the productive organs such as necrosis , Hyperplasia , fatty vaculeouse . changes in the shape , increasing in the thickness of epithelial oocytes and the presences of yolk droplet <sup>36'37'38</sup>. The Histopathological changes were different between the control and sub lethal concentrations, which were (0.12and 0.25 mg/L) for a 10-day exposure period of chloromethidine pesticide and severe decreases in the Oocyte cells and this gathering of fatty vacuoles leads in turn to fatty generation, which is the result of fat droplets and tis decline in Oocyte cells is significant by the toxicity effect of the pesticide and the time of exposure period. These changes under laboratory conditions as an indicator of the changes that occur in the aquatic ecology unfit for the livelihood and the presence of the fish. Moreover the harm in the toxic substance of the pesticide or other pollutants is to cause damage to the environment and transfer to fish, for this reason the aquatic environment loses its vitality .

#### Behavioral Response

During this study , The fish were observed to acute and sub lethal concentrations of pesticide were behavior was seen by jumping from the jar test and increased the speed of swimming in a random movement .It was slow response only in sub lethal exposure by the slowly or move it by stick to be responded as an increase of mucus martial in all of the body .There were also irregular behavioral responses, including concentrations and exposure period ,where it was observed at acute lethal

concentration accelerated ventilation and spasm, in addition to the movement of the operculum and opening of the mouth very quickly and this is what many authors have indicated<sup>39,40</sup>. The behavior affected compared to the control samples, as increase in rotational swimming and imbalance was noticed especially in the first time of the experiment, furthermore gathering crowded around the source of ventilation, this similar study by<sup>41</sup>. After that a appearance of extreme tired, it falls to the bottom of jar experiment<sup>42,43,44</sup>. Here are all toxic pressure from pesticides and other harmful chemicals to water environment with different concentrations so that fish will be on high sensitive for these pollutants.<sup>45</sup> The report for uptake of oxygen, and also decline oxygen causes inhibition muscular work.<sup>46</sup>

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