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Pathological Study of Experimental Glyphosate Toxicity in Mosquito Fish *Gambusia affinis*

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Abstract. The present study deals with the effect of herbicide glyphosate on mosquito fish *Gambusia affinis*. Fish were exposed to different concentrations of the mentioned pesticide, behavioral and histological effects of various concentrations of glyphosate on mosquito fish *Gambusia affinis* in the acute exposure (96) hours were investigated. There were behavioral changes on the exposed fishes represented by nervous symptoms, loss of equilibrium, increase in respiration rate, opening of mouth and histological changes were determined in gills after acute exposure such as hyperplasia, hemorrhage, aneurysms, separation of epithelial layer, clubbing with sickle lamellae, hypertrophy, destroyed and fusion of secondary lamellae. The liver tissues were showed that hypertrophy of nucleus and necrosis and degeneration and swelling of cells, increasing of size and bleeding and congestion of blood vessels and bilirubin pigment diffusing around bile duct, in kidney tissues there were necrosis and degeneration of epithelial cells of tubules, increasing of size of Bowman's capsule, congestion and atrophy and disappear of glomerulus, separation of tubules, increasing of size and congestion of blood vessels, bleeding between tubules and bilirubin pigment diffusing around blood vessels.

Key words : mosquit fish, glyphosate, gills, liver, kidney, histology.

1. Introduction

Chemical pesticides have been widely developed in their use for the eradication of bush and pests or to increase crop productivity, This development has been accompanied by the emergence of the pollution problem in general and the pollution and rejuvenation of the aquatic environment in particular [1]. The pollution of these pesticides is due to misuse in the field in addition to the role of rainwater in the shelf residues of these pesticides from the soil and agricultural crops to ponds, rivers and swamps [2,3]. Many fishermen have also resorted to the use of these chemicals in illegal fishing as a result of the toxicity of these chemicals and their use as an easy means of fishing for their access to local markets on the one hand and the lack of effort required for her hunting on the other hand [4].

The issue of water pollution with herbicides has become very important in recent years because of the enormous quantities of these chemicals used worldwide to eliminate reed and papyrus that block waterways and thus affect the fish stocks and food chains needed to feed them [5].

Of the herbicides used in many countries, including Iraq, the glyphosate is highly important based on some of the properties that it possesses, as it is a wide spectrum of water-soluble pesticides and is used to eliminate the grass and grass broad sheets, especially unwanted and that compete with crops



which has grown all over the world. The glyphosate herbicide is used to control reed and papyrus and a large number of long-lasting bushes in the canals, fish ponds and horticultural channels [6].

The glyphosate herbicide is known by several trade names including Ground up, Round up and Lancer. Its chemical formula N (Phosphonomethyl) glycine, and its trade formula C₃H₈NO₅P. It is a organophosphate pesticides (OP), its partial weight (169.08), a colorless crystalline material, (200) C° melting, its solubility in water at (25) C° equivalent to (12) g / L. It does not dissolve in common organic solvents, its products are analyzed by high pressure liquid chromatography HPLC and by gas chromatography and thin plate chromatography [7].

The effect of herbicides in fish may be directly in the case of treatment and at rates that may exceed the (LC₅₀) concentrations of invertebrates and organisms that make up fish food or have an indirect effect. The treatment with the herbicides leads to the depletion of dissolved oxygen in the water after the decomposition of the bushes and dead plants. Therefore, the presence of pesticides in the water and the result of entry through gills and mediated blood up to the tissues of the body and various parts [8].

The current study aims at determining the toxicity of the organophosphate pesticides (glyphosate) on the mosquito fish *Gambusia affinis* by the knowledge of behavioral changes and determine the histopathological effects on gills, liver and kidney of mosquito fish acutely exposed to glyphosate.

2. Materials and Methods

Fish samples:

Samples of mosquito fish *Gambusia affinis* used in this study, which ranged in length from (3-5)cm and (0.8-2.35)g in weights, were collected from fish shops in Babil Governorate. The collected fish were placed in plastic tubs with a capacity of (50) liters containing pre-stored tap water at least one week for the removal of chlorine, so that it can be adapted under appropriate laboratory conditions of air temperature (2±25) C°, water (2±20) C°, PH (0.3±7.4) and salinity (0.2±1.3) g/L with appropriate lighting and ventilation using an electric air pump where dissolved oxygen (0.4±8.1) mg/L. and 28% protein diet was used to feed the fish during the 10-day period, with the water being replaced every 2 days for disposal.

Pesticide:

The herbicide glyphosate was used in the form of its commercial product, which contains (48%) glyphosate isopropyl amine salt and a biologic substance (17%), liter capacity provided by the Veterinary and Agricultural Pharmaceutical Industries Company (VAPCO).

Experimental design:

The mosquito fish were subjected to concentrations (LC₁₀=1.08, LC₂₅=1.54, LC₅₀=1.59) ppm of the pesticide for (96) hours for the study of the acute effect using a glass aquaria with a capacity of (8) liters with artificial ventilation by electric pumps and by (4) aquarium including control treatments and one aquarium containing (8) fish. During the period of exposure, fish behavior was observed inside the aquarium and observed changes in breathing. The number of respiratory movements was calculated per minute for groups exposed to the pesticide and compared with the control group. After the exposure period, the fish were killed and dissected. Samples of gills, liver and kidney tissues were collected for all the experimental groups (acute and control) in Bouin's solution for at least (20) hours and then followed the Humason's method [9] in the preparation of the tissue slides, and all were examined under the light microscope to observe differences between the tissues of the fish exposed and not exposed to the pesticide.

3. Results

3.1. Behavioral changes:

There were changes in the behavior of the mosquito fish, after (30) minutes of the start experiment, were the emergence of neurological signs such as sudden short emotional movements in aquarium as the fish suddenly jump to the top of the aquarium and then descend calmly and settle still moments in the bottom of the aquarium and then jump back and continue for many times and then return to Stabilization in the aquarium floor is in a state of inactivity and loss of balance and does not respond when a wall is clicked or when the torsion is pinned.

It was also observed on the mosquito fish speed of the movement of the Operculum, as it was observed increasing the number of respiratory movements gradually proportional to the increased concentration used, the highest respiratory rate was recorded (106.2movement/minute) at the highest concentration used ($LC_{50}=1.59$)ppm when compared with the control group(87.1 movement/minute)(**Table1**).

Table(1): Effect of glyphosate pesticide on mosquito fish respiration rate after (96) hours from exposure time.

glyphosate concentration(ppm)	Respiration rate(movement/minute)
0(control)	5.01±87.1
$LC_{10}=1.08$	3.37±97.1
$LC_{25}=1.54$	19.02±99.8
$LC_{50}=1.59$	4.76±106.2

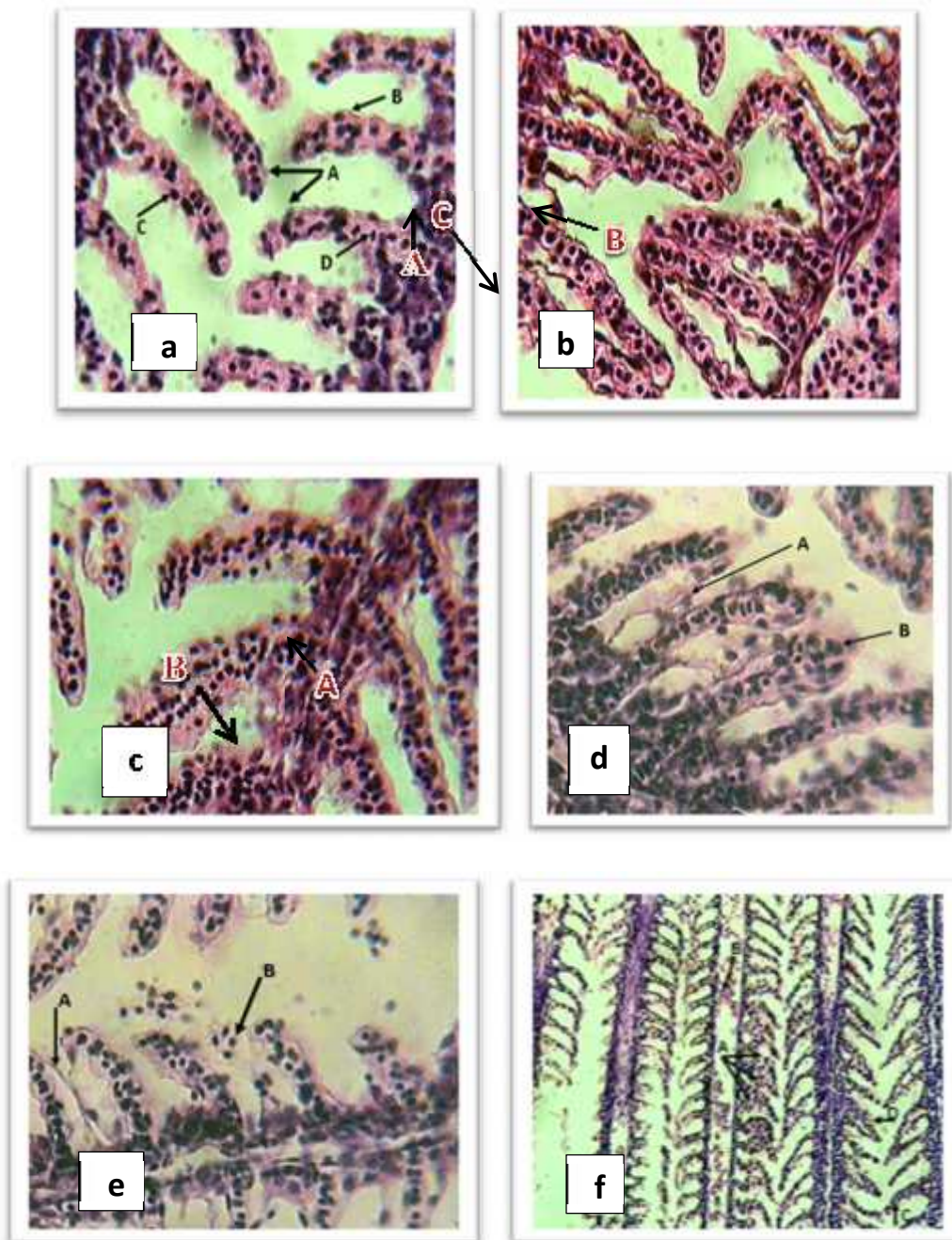
Values are expressed as (mean ± S.D.)

3.2. Histopathological Changes

3.2.1. Gills:

gills of the control fish were composed from primary filament or primary lamella which are formed by secondary lamella located vertically on the upper and lower edges of each primary lamella, usually clad in epithelial tissue characterized by two layers of epithelial cells separated by supporting cells Pillar cells, as well as the red blood cells present in the spaces between the supporting cells, and that each of the above layers consists of squamous cells called respiratory cells based on a thin basal membrane and a thin layer of connective tissue (**Fig.1a**)

Acute exposure to different concentrations of glyphosate ($LC_{10}=1.08$, $LC_{25}=1.54$ and $LC_{50}=1.59$) ppm resulted in histopathological changes in mosquito fish gills, with concentration at ($LC_{10}=1.08$) ppm noticed fusion of secondary lamella and separation of epithelial layer (**Fig.1b**), aneurysms, hemorrhagic and hyperplasia, as observed at concentration ($LC_{25}=1.54$) ppm Hypertrophy of secondary lamellae and formation of clubbing forms, in addition to previous symptoms (**Fig.1c,d**), ($LC_{50}=1.59$) ppm resulted in a destroyed in some secondary lamella and the formation of Sickel forms in addition to the aforementioned symptoms at the concentrations ($LC_{10}=1.08$ and $LC_{25}=1.54$) ppm (**Fig.1e,f**).

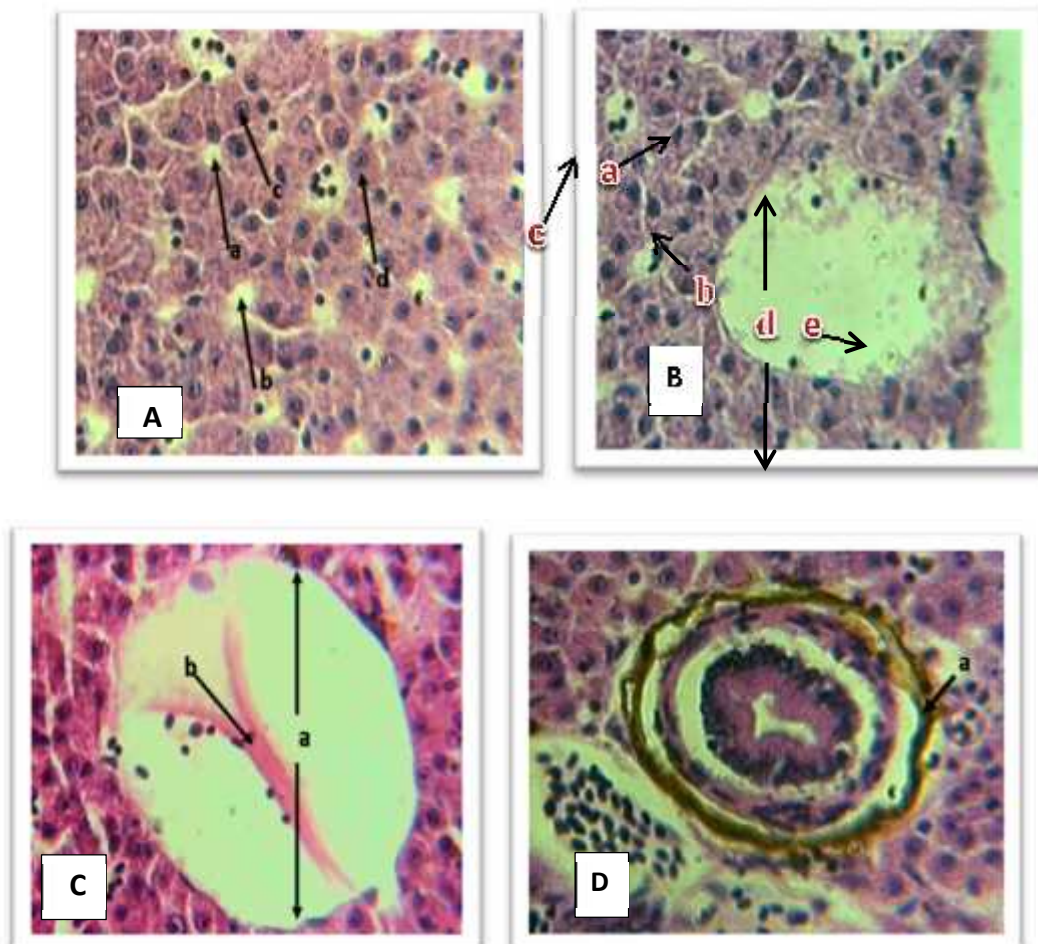


Figure(1): Gill tissue of *Gambusia affinis* (a)Control-(A)secondary gill lamella,(B) epithelial layer , (C) pillar cells , (D) red blood cells.H&E,600x ;(b) exposed to (LC₁₀=1.08) ppm glyphosate-(A) separation of epithelial layer,(B)fusion between two of adjacent secondary gill lamella,(C)fusion between more of secondary gill lamella.H&E, 600 x ;(c) exposed to (LC₂₅=1.54) ppm glyphosate-(A)separation of epithelial layer,(B) hypertrophy of secondary gill lamellae .H&E, 600x ;(d)exposed to (LC₂₅=1.54)ppm glyphosate-(A) separation of epithelial layer,(B)formation of Sickle forms.H&E, 600x ;(e)exposed to(LC₅₀=1.59) ppm glyphosate - (A)separation of epithelial layer , (B) destroyed of secondary gill lamella.H&E, 600x ; (f) exposed to (LC₅₀=1.59)ppm glyphosate-(A) Sickle forms,(B) hemorrhagic,(C) fusion between two of adjacent secondary gill lamella,(D) fusion between more of secondary gill lamella.H&E, 600x

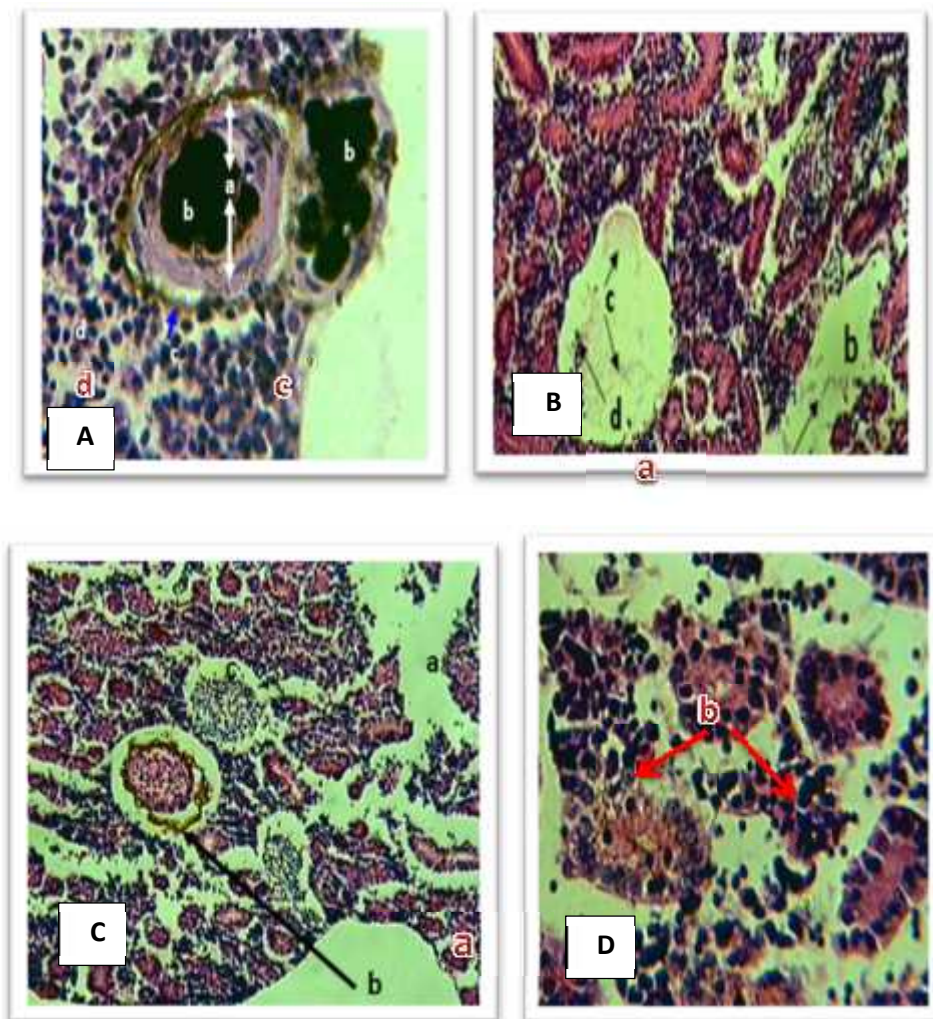
3.2.2. Liver & Kidney:

Mosquito fish exposed to different concentration of glyphosate ($LC_{10} = 1.08$, $LC_{25} = 1.54$ and $LC_{50} = 1.59$)ppm showed clear histopathological changes in liver and kidney after (96) hours of exposure compared to control fish, observed on liver of Fish exposed to pesticide degeneration, necrosis, nuclear hypertrophy and swelling of hepatocytes cause loss of radial regulation of hepatic cords as well as inability to distinguish between the trabeculae of their cells. As observed on the liver symptoms of the diffusion of yellow pigment to the brown around the bile duct represent the bilirubin pigment (**Fig. 2**).

The kidney was also characterized by the symptoms of congestion of blood vessels and hemorrhagic bleeding between the tubules and bleeding and atrophy and the decay of many glomeruli, also noted on the kidney the diffusion of the bilirubin pigment around blood vessels, also observed symptoms of necrosis and degeneration of epithelial cells which accompanies increasing of size of the Bowman's capsule as well as separation of tubules (**Fig.3**).



Figure(2): Liver tissue of *Gambusia affinis*.(A)exposed to ($LC_{10}=1.08$)ppm glyphosate-(a) degeneration of hepatocytes(b) necrosis of hepatocytes(c)nuclear hypertrophy(d)swelling of hepatocytes.H&E , 600x ;(B)exposed to ($LC_{10}=1.08$)ppm glyphosate-(a)degeneration of hepatocytes (b) nuclear hypertrophy(c)swelling of hepatocytes(d)increasing of size of blood vessels(e) bleeding of blood vessels.H&E, 600x ; (C) exposed to ($LC_{50}=1.59$)ppm glyphosate-(a)increasing of size of blood vessels(b)congestion of blood vessels.H&E,600x;(D)exposed to ($LC_{25}=1.54$)ppm glyphosate-(a)diffusion of bilirubin pigment around bile duct.H&E, 600x.



Figure(3):Kidney tissue of *Gambusia affinis* . (A) exposed to ($LC_{50}=1.95$)ppm glyphosate- (a) increasing of size of blood vessels (b) congestion of blood vessels (c) diffusion of bilirubin pigment around blood vessels (d) degeneration of epithelial cells of tubules .H&E,x600;(B) exposed to ($LC_{25}=1.54$) ppm glyphosate - (a) bleeding between the tubules (b)separation of tubules (c) bleeding of glomerulus (d) atrophy of glomerulus.H&E,x600;(C) exposed to ($LC_{10}=1.08$)ppm glyphosate-(a) separation of tubules (b)diffusion of bilirubin pigment around blood vessels(c) increasing of size of Bowman's capsule. H&E,x600; (D)exposed to($LC_{10}=1.08$) ppm glyphosate- (a)separation of the tubules (b)degeneration of the epithelial cells of tubules.H&E,x600.

4. Discussion

4.1. Behavioral changes:

The results of this study showed the emergence of changes in the behavior of fish after (30) minutes of the start of the experiment represented by the emergence of neurological signs, such as sudden short emotional movements within the aquarium as the fish suddenly jump to the top of the aquarium and then descend calmly and sit still moments in the bottom of the aquarium and then jump back and continue for many times, This is due to the neurotoxic effect of fish, by inhibiting the activity of an enzyme Acetyl Choline Esterase (AChE) phosphorous organic pesticides (glyphosate) on the central nervous system is the important enzyme in the nervous system as it enters the Serine phosphorylation

of the closed metabolites of the active sites in the neurotransmitter transport, resulting in non-polarized Depolarized neural tube membranes, causing a malfunction in the neurotransmitter process, Neurosurgery [10].

It was also observed on the mosquito fish speed of the movement of the operculum as a significant increase in the number of respiratory movements was observed, and this increase was gradually proportional to the increased concentration used. The highest respiratory rate (106.2 movement/min) was observed at the highest user concentration ($LC_{50}=1.59$) ppm when compared with control group (87.1 movement / min), This is a sign of the respiratory effect of these pesticides in fish. It inhibits the enzymes involved in the regulation of respiratory processes. The levels of the effectiveness of the Succinate dehydrogenase (SDH) and Malate dehydrogenase (MDH) in the liver and brain are reduced and cause hypoxia in tissues. The fish will speed up the movement of the operculum exposing it to more pesticides, which makes it [11].

4.2. Histopathological changes :

The mosquito fish exposed to different concentrations of glyphosate ($LC_{10}=1.08, LC_{25}=1.45, LC_{50}=1.59$) showed clear histopathological changes in the studied organs (gills, liver, kidney) after (96) hours of exposure compared with control fish. The histopathological changes in the gills can be divided into two groups. The first group due to the toxic effect of the pesticide and the second changes resulting from the defensive response to fish [12]. Where the first group changes are swelling, as well as the breakdown and necrosis of many epithelial cells lining the secondary gill plates. As for the changes in the second group, it is usually the symptoms of hyperplasia in the epithelium and the integration of adjacent secondary filaments, as well as the formation of the bulbous forms [13]. Drewitt & Abel [14] confirmed that the fusion between the epithelial cells of the neighboring gill plates was a result of the swelling of these cells, as well as the intensity of their infiltration of the inflammatory cells, causing the convergence and adhesion of their epithelial cells together. Mallatt [15] confirmed the hyperplasia of the secondary gill plates produced by exposure to pesticides often leads to complete fusion of adjacent secondary platelets. Bindont *et al.* [16] reported that acute infections doing separate the epithelial lining of the secondary plates, and that this separation leads to a decrease in surface area of gills causing the blocking of the functions of the osmosis organization and ionian them.

It has been observed that the liver of the mosquito fish exposed to different concentration of glyphosate pesticide decomposing, degenerating and thickening the nuclei and swelling of the liver cells, causing the loss of the radial regulation of the liver cordes as well as the inability to distinguish between the boundaries of their cells. This swelling is due to the fact that most of the phosphorus pesticides, including the (glyphosate pesticide) are compounds in nature, hepatocellular membranes, affecting the work of these semi-permeable membranes, increasing their influence on water, resulting in the swelling of these cells [17]. Vader and Sputel [18] said that the loss of the radial regulation of liver cords comes as a result of the attack of toxic chemicals by the cytoskeleton, causing blurring of contiguous cells. The expansion, bleeding, and congestion of blood vessels can be seen as an expected response due to the increased concentration of the pesticide in the blood of exposed fish in an attempt to keep the liver cells away from damage by reducing absorption while accelerating the metabolism of these toxins [19], also observed on the liver symptoms of the spread of the bilirubin pigment. This is consistent with the findings of the study of Mutar [20] when exposed *Ctenopharyngodon idella* fish to the glyphosate herbicide .

As for kidney, the symptoms of vascular expansion, congestion, hemorrhagic hemorrhage, bleeding, atrophy, and the decay of many glomeruli have been observed. This is due to the work of the inhibitor in the production of renal prostoglandin, which is important in reducing the blood vessels. The fragility of the walls of these vessels can help in the process of hemorrhage through glomeruli and

renal tubules, as this may allow the explosion and destruction of renal glomerular epithelium, thus causing hemorrhage [21,22]. It was also noticed on the kidney symptoms of the spread of the bilirubin pigment around the blood vessels and this is consistent with the findings of the study of Mutar [20] when exposed *Ctenopharyngodon idella* fish to the glyphosate pesticide.

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