Histological Effect of Lead Chloride on The Brain of Gambusia Affinis

Amal A. Alkshaib1*, Ameer M. Taha2
1Lecturer Biology Department, Education College for Pure Sciences, Mosul University, Mosul, Iraq
2Assistant Professor, Biology Department, Education College for Pure Sciences, Mosul University, Mosul, Iraq
*Correspondence: Lecturer Biology Department, Education College for Pure Sciences, Mosul University, Mosul, Iraq

Email: amal.biology@uomosul.edu.iq

Abstract. Heavy material pollution is one of the most critical threats to fish stocks around the world. Lead is one of the essential heavy elements that directly or indirectly affect fish. The purpose of this study is to investigate the effects of sublethal concentration of lead chloride PbCl2 on the brain of Gambusia affinis. For this purpose, 90 fish were used, which were divided into nine groups, each group containing ten fish. They divided into a control group, the acute treatment groups included four groups exposed to concentrations of 20 and 25 mg/L of PbCl2 for 24 and 96 hours, respectively. At the same time, the chronic treatment included four groups exposed to concentrations of 5 and 10 mg/L of PbCl2 for 15 and 30 days, respectively. The results showed that there were histological lesions that appeared in all the treated fish compared to the control group. These lesions appeared in different degrees of concentration and duration of use. Among the most prominent of these tissue changes that were repeated in the treatments were degeneration, vacuolar degeneration, necrosis, hemorrhage, congestion and oedema as well as disintegration between different brain tissues. The study concluded that lead chloride has a very harmful effect on brain tissues and that chronic exposure leads to irreversible histological lesions.

Keywords: Lead chloride, Gambusia affinis, Brain, Histopathology.

1. Introduction

Heavy metals are considered potent biological toxins because they are stable and toxic. They tend to accumulate in animals and plants' tissues and thus lead to biomagnification (Dinodia et al., 2002). Water pollution is a significant and dangerous problem for life inside the water (Alinnor and Obiji, 2010). This pollution increases because of humankind and industrial, agricultural, and commercial activities (Odoemelam, 2005). Many environmental mineral pollutants are toxic to nerve cells and cause tissue lesions due to their detoxification role. In fish, toxic agents affect the brain's nerves, while the gills are the first body organs to pick up these substances during breathing (Jordao and Pereira, 2002).
Lead is a non-biological toxic heavy element, as it has no known physiological role in the body of animals or even humans. It seriously affects the central nervous system and causes significant health problems to the animal's body (Davis, 2018). Lead gets into the water through sewage and agricultural waste, as well as runoff streams. Increased lead levels in water cause reproductive damage in some aquatic organisms and cause changes in blood and nerves in animals, fish, and others (Kalay and Canli, 1999).

Fish is one of the vertebral animals and widespread globally, as it is an essential source of animal protein for humans and animals. It is one of the foods that maintain human health, as it surpasses other animals in terms of the efficiency of food conversion. It also does not compete with humans for the environment and food as in animals. The other, in addition to not consuming water, but fertilizing it (EHS, 2004).

The Mosquito fish *G. affinis* plays an important and primary role in an ecosystem, as it is a secondary representative of consumption in aquatic ecosystems (Annabi et al., 2011). *G. affinis* is widespread in Iraq, especially the Tigris River. It is one of the fish introduced into the Iraqi environment to eliminate the mosquitoes that transmit malaria. It is also considered one of the laboratory models for scientific and research studies regarding fish wealth and the factors affecting it due to its ease of breeding and its small size. Therefore, the current analysis came to know the effect of lead chloride on the brain's tissue structure in mosquitofish *G. affinis*.

2. Material and methods

Fish obtained

90 male and female of Mosquito fish, *Gambusia affinis* (Order: Cyprinodontiformes, Family: Poeciliidae), were brought from Riverbanks of Tigris on the left side of Mosul/Iraq in Sept. 2019. The fish transferred to the laboratory of histology and physiology in the Biology department college of Education for pure sciences/ university of Mosul. It remained for two weeks before experimentation in flowing dechlorinated water. The temperature, oxygen content water monitored daily. The fish were fed approximately 2% body weight per day with commercial fish bait.

3. Experimental design

After a two-week acclimation period in holding tanks (maintained at 21 °C, 12:8 h light: dark), the fishes were randomly transferred to nine glass aquarium exposure tanks that operated with static systems with continuous aeration (eight experimental groups). (25, 20) mg/L PbCl₂ for 24 and 96 hours as present as acute exposure, While the concentrations (10 and 5) mg/L PbCl₂ for (15 and 30) days for chronic exposure. Before the experiments, each aquarium was continuously mixed by a motor.

4. Histological Analysis

The histological analysis steps were performed according to the method of (Al-Hajj 2010). The histopathological observations were made from the brain of the control and experimental groups that were exposed to PbCl₂. Fish killed, and the brain tissue was fixed in 10% neutral buffered formalin. Fixed tissues were dehydrated in increasing gradient of alcohol (70, 80, 90, and 100%) for 30 min each and were eventually dried in acetone and cleared in xylene for 2 min. The tissues were infiltrated by embedding in molten wax and sectioned at 6µ. The paraffin sections were then mounted on a slide, stained with haematoxylin and eosin then examined by light microscope for studying the histological changes.

5. Result

The normal structure of the brain

The brain consists of *G. affinis* fish is made up of several parts. The cerebrum composed of the nuclei of neurons, glial cells and myelinated axonal processes, which divided into two parts: the gray matter and the white matter. The Valvula cerebella have two layers. The first is the granular layer, which consists of small cells and Purkinje cells that randomly distributed and surrounded by astroglia. The second layer is the molecular layer, which
includes the axodendrites of the granular layer. There is also an intermediate layer that provides for ganglion cells and Purkinje cells (Figures 1a,b; Table1).

**Figure (1) (1a,b)** Light Micrograph Section brain of Control group of mosquito fish showing the normal structure of the brain in G. affinis ,100x. Abbreviations: GL= granular layer; ML= Molecular layer.

6. **Acute treatment**

The fish treated at a concentration of 20 mg/L of PbCl₂ for 24 hours showed many histopathological changes that appeared in the brain, including degenerations, vacuolar degeneration, edema, necrosis, and hemorrhage (Figures 2a,b; Table1).

**Figure (2) (2 a,b)** Fish treated at a concentration that appeared in the brain, including degenerations, vacuolar degeneration, edema, necrosis, and hemorrhage

When treatment with a concentration of 20 mg/L of PbCl₂ for 96 hours, the changes were more severe and included a separation of the granular layer from the molecular layer, focal necrosis, vacuolation in the granular and molecular layers, degeneration of the granular layer, as well as the emergence of edema in multiple regions of the nervous tissue. There was also bleeding between the components of this tissue (Figures 3a,b; Table1). Whereas, the treatment at a concentration of 25 mg/L of PbCl₂ for 24 hours showed disintegration and separation between the components of the brain, especially in the cerebellum in its different regions, as well as vacuolation and vacuolar degeneration in the granular layer.
Figure 3 (a,b): histopathological effects in the brain in *G. affinis* treated with 20 mg/L of PbCl$_2$ for 96 hours. 100X and 400X respectively.

There was also a clumping of glial cells, and edema appeared widely among the brain's components, as well as hemorrhage and congestion in the blood vessels and degeneration of Purkinje cells (Figures 4a,b; Table 1). While at a concentration of 25 mg/L of PbCl$_2$ for 96 hours, dissociation of the granular and molecular layers and edema and vacuolar necrosis of the molecular layer, were also shown. As the nuclei of the granular layer clustered separated by disjunctions. Hemorrhage between the molecular layer's fibers and degeneration of Purkinje cells was also demonstrated (Figures 5a,b; Table 1).

Figure 4(a,b) histopathological effects in the brain in *G. affinis* treated with 25 mg/L of PbCl$_2$ for 24 hours 100X and 400X respectively.

Figure 5 (a,b) histopathological effects in the brain in *G. affinis* treated with 25 mg/L of PbCl$_2$ for 96 hours 100X and 400X respectively. Abbreviations: S= Separation; C= Congestion; D= Degeneration; ED= Edema; VD= Vacuolar Degeneration; N= Necrosis; H= Hemorrhage; P= Pyknosis; VN= Vacuolar Necrosis; V= Vacuolation.
7. Chronic treatment

As for the chronic treatment, a concentration of 5 mg/L of PbCl₂ for 15 days showed the disintegration and separation of brain components and the appearance of edema between members and degeneration and necrosis in different brain regions. Also, vacuolar degeneration appeared and the migration of some cell nuclei from the granular layer to the molecular layer. Also, Purkinje cells necrosis as well as hemorrhage (Figures 6a,b; Table1). As for the treatment at a concentration of 5 mg/L of PbCl₂ for 30 days, the granular layer appeared relatively thick, and that was at the expense of the molecular layer, congestion appeared in most blood vessels in the brain, as well as the disintegration and separation of the components of the two layers of the cerebrum, vacuole degeneration, necrosis, edema, and hemorrhage also appeared (Figures 7a,b; Table1).

Whereas, the treatment with 10 mg/L of PbCl₂ for 15 days showed more severe histopathological effects than the previous treatment, and also included

![Image](image_url)

**Figure 6 (a,b)** histopathological effects in the brain in *G. affinis* treated with 5 mg/L of PbCl₂ for 15 days. 100X and 400X respectively.

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**Figure 7 (a,b)** histopathological effects in the brain in *G. affinis* treated with 5 mg/L of PbCl₂ for 30 days 100X and 400X respectively. Abbreviations: S= Separation; C= Congestion; D= Degeneration; ED= Edema; VD= Vacuolar Degeneration; N= Necrosis; H= Hemorrhage; P= Pyknosis; VN= Vacuolar Necrosis; V= Vacuolation; CL= Clumping of glial cells; DP= Degeneration of Purkinje cells; PN= Necrosis of Purkinje cells.
Separation between layers of the brain in different regions, the emergence of edema in the white matter and the rest of the components, and diffuse vacuolar degeneration appeared in most parts. As well as vacuolation and necrosis of nerve cells and Purkinje cells, bleeding in some areas, and congestion in blood vessels. A distinctive finding was a thrombus in the granular layer (Figures 8a,b; Table1). As for the treatment at a concentration of 10 mg/L of PbCl₂ for 30 days, the results showed that the histopathological effects are similar to what appeared in the previous concentration, with some additional effects. In addition to necrosis, cell degeneration, and edema among the brain's components, a breakdown between the parts of the brain and infiltration of inflammatory cells appeared between the components of the molecular layer and the white matter. As well as congestion in the blood vessels, bleeding in some areas, and the appearance cluster of glial cells (Figures 9a,b; Table1).

8. Discussion

Freshwater is affected by heavy metals that come from untreated material discharges from factories, agricultural operations, and some daily activities; this leads to water pollution with toxic heavy metals and this lead to negatively affects both aquatic organisms and even humans (Mahurpawar, 2015; Elarabany and Bahnasawy, 2019). Many vertebrates, including fish, have developed strategies and regulatory systems that allow them to absorb and selectively use essential minerals

![Figure 8 (8a,b)](histopathological effects in the brain in G. affinis treated with 10 mg/L of PbCl₂ for 15 days. 100X and 400X respectively)

![Figure 9 (9a,b)](histopathological effects in the brain in G. affinis treated with 10 mg/L of PbCl₂ for 30 days. 100X and 400X respectively. Abbreviations: S= Separation; C= Congestion; D= Degeneration; ED= Edema; VD= Vacuolar Degeneration; N= Necrosis; H= Hemorrhage; P= Pyknosis; VN= Vacuolar Necrosis; V= Vacuolation; CL= Clumping of glial cells; DP= Degeneration of Purkinje cells; PN= Necrosis of Purkinje cells; TH= Thrombus; IN= Infiltration of inflammatory cells.)
Table 1. Effect of PbCl$_2$ (Acute exposure and Chronic exposure) on brain histopathology. (−) absent; (+) mild; (++) moderate; (+++) severe.

| Histological lesions                  | Control | Acute Effect | Chronic Effect |
|---------------------------------------|---------|--------------|----------------|
| Hemorrhage                            | -       | ++           | +++            |
| Separation between layers             | -       | +++          | +++            |
| Edema                                 | -       | +++          | ++             |
| Necrosis                              | -       | ++++         | +              |
| Congestion                            | -       | ++           | ++             |
| Vacuolar degeneration                 | -       | +++          | ++             |
| Clumping of glial cells               | -       | +            | -              |
| Degeneration of Purkinje cells        | -       | +            | +++            |
| Thickness of granular layer           | -       | -            | +              |
| Thrombus                              | -       | -            | +              |
| Infiltration of inflammatory cells    | -       | -            | +              |

and reduce their reactive forms of non-essential elements that hitting the system (Jumawan, 2015). In the fish, the brain is the crucial organ that controls these systems. The brain's anatomical and histological structure differs in different fishes, but the number of parts of the brain is similar (Abdelnaeim and Cao, 2018). The typical brain structure in G. affinis, identical to other teleost fish (Al-Baker, 2013; Rajini et al., 2015; Abdelnaeim and Cao, 2018).

In the acute treatment, the fish that were treated at a concentration of 20 mg/L of PbCl$_2$ for 24 hours showed many histopathological changes, including degenerations, vacuolar degeneration, edema, necrosis, and hemorrhage. It founded that the lead exposure increased the production of reactive oxygen species observed in the brain of the fish Clarias batrachus this may lead to the same effects that appeared in this study (Maiti et al., 2010). Whereas, in 20 mg/L of PbCl$_2$ for 96 hours, the changes were more severe, included a separation of the granular layer from the molecular layer, focal necrosis, vacuolation in the granular and molecular layers, degeneration of the granular layer, and others changes. The same effects appeared in the Fish Catla catla (Bose et al., 2013) and fish Carassius gibelio (Berillis et al., 2014) when exposure to Heavy Metals and Toxic Cyanobacteria, respectively.

A concentration of 25 mg/L of PbCl$_2$ for 24 hours showed disintegration and separation between the brain's components, vacuolation, and vacuolar degeneration in the granular layer. These changes are similar to those recorded in Africa catfish that exposure to Glyphosate Herbicide (Erhunmwunse et al., 2014) and in cyprinus carpio that exposure to quinalphos (Chamarthi et al., 2014). For a concentration of 25 mg/L of PbCl$_2$ for 96 hours, there was the dissociation of the granular and molecular layers and edema and vacuolar necrosis of the molecular layer. In the fish Cyprinus carpio exposed to lethal concentrations of an organophosphate insecticide phorate, it caused mild damage to the fish's brain on day. Further exposure for four days it caused apparent damage (Lakshmaiah, 2017). The same effects were noted in this study.

In the Chronic treatment, a concentration of 5 mg/L of PbCl$_2$ for 15 days showed the disintegration and separation of brain, edema, degeneration, and necrosis in different brain regions. In the study of Savari et al. (2020) that treated the brain of spotted grouper Epinephelus coioides with methylmercury, the results showed hyperemia, some extent of hemorrhage, karyolysis, necrosis, nuclear dust, hyper chromatin, vacuolation, endothelium hypertrophy, cloudy swelling, hydropic degeneration, and ectopic granular accumulation. This study's results are very similar.
to our results, and this indicated that heavy substances have a similar effect on the brain. At a concentration of 5 mg/L of PbCl₂ for 30 days, the granular layer appeared relatively thick, and congestion appeared in most blood vessels. The same results occurred when the brain of *Labeo rohita* Induced by Lambda-cyhalothrin 5% EC and Marshal (Carbosulfan 25%EC) drastically affected molecular and granular regions causing necrosis, which is evidenced by the appearance of white patches, pycnotic nuclei (Dey and Kumarsaha, 2016).

The treatment with 10 mg/L of PbCl₂ for 15 days showed more severe histopathological effects than the previous treatment, and also included separation between layers of the brain in different regions. The same changes recorded in brown trout exposed Cadmium (Topal et al., 2014). Pearce (2007) found that a low level of exposure during early development may lead to long-term neurocognitive and behavioral impairment. Also, when exposed to high or chronic levels of lead (40-60 μg / dl), it can cause severe damage to the brain and kidneys, leading to death. That indicates that the effective lead concentration differs between the different classes of vertebrates. Still, it remains harmful to animals, especially the brain's tissue structure, as was found in our study.

In a concentration of 10 mg/L of PbCl₂ for 30 days, therewere histopathological effects similar to what appeared in the concentration of 10 mg/l for 15 days, with some additional results. These also occurred when the brain's tissues exposed to Cadmium as in Zebrafish (Alsawafi et al., 2017). Some studies indicate that the accumulation of heavy metals, especially lead, in the brain of some types of fish leads to decreased reproductive capacity in fish (Tulasi et al., 1989; Doaa and Hanan, 2013).

The accumulation of metals in fish may cause several pathological effects such as change in enzyme activities and damage to organ structure (Alkshab, 2017). Lead toxicity is aimed at brain memory and learning processes, and can be controlled by three processes. Lead may impede brain learning and memory by inhibiting the N- methyl aspartate receptor (NMDAR) and can block neurotransmission by inhibiting neurotransmitter release, blocking neuronal voltage calcium (Ca²⁺) channels (VGCCs) and growing neurotrophic information extracted from the brain (Nava-Ruiz et al., 2012).

9. Conclusion

The present study results point to the fact that the toxins of pollutants affect the aquatic life of the freshwater fish. The problem can be more severe in the fish culture farms as the culture ponds are invariably located near the agricultural land, which was already loaded with toxic residues of all kinds.

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