Histopathological Changes of Common Carp (Cyprinus carpio) Vital Organs Exposed to Virkon at Different Periods

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The current study was conducted to investigate the histopathological toxic effect of Virkon at a concentration of 4 mg/L (therapeutic concentration) on the vital organs of common carp (Cyprinus carpio) fish. 120 common carp, with a mean weight of 230±10g, were distributed into eight glass aquariums (80cm x 40cm x 40cm) with their equipment (oxygen motor, water heater fixed at 25±1°C, thermometer). There were 15 fish in each aquarium, and they were randomly divided into four groups, with each group having duplicates. Group A (the control) was left untreated. Group B was continuously exposed to Virkon for 7 days. Group C was exposed to Virkon for 7 days, followed by a 3-day interval, and then a 7-day retreat with Virkon. Group D was exposed to Virkon for 14 days continuously. Tissue samples of the gill, liver, and kidney were collected on days 7, 14, and 21 of the experiment. The study revealed considerable effects of Virkon on the tissues, including hyperplasia of the primary lamella and the secondary lamella of the gill, along with hypertrophy of chloride cells. There was vacuolar degeneration of hepatocytes with hemorrhage in hepatic tissue after 7 days of exposure, and the lesions became more intensive in group D with a longer exposure period. The kidney lesions showed vacuolar degeneration of epithelial cells lining the renal tubules with hyaline casts in the renal tubules. The histopathological lesions became more exhaustive with a longer exposure period in all the studied organs, even with a short interval period.

Keywords: Virkon, Common carp, Histopathology, Disinfectant.

Introduction

Effective biosecurity reduces the fishes’ exposure to infectious agents and their susceptibility to them, as well as the economic losses associated with mortality rates so disinfectants are used in the aquaculture industry as a disease pound management process for disease eradication (stamping out) procedures or as a regular practice in biosecurity [1]. Virkon is a powdered substance with a triple salt of potassium monoperoxysulphate as its major component (49.4%). The oxidizing action is induced by potassium hydrogen sulfate and potassium sulfate. Additionally, it contains 10% malic acid, 5% sulphamic acid, 15% sodium dodecylbenzenesulphonate (as a surfactant), and 18% sodium hexanophosphate [2]. In 1986, Antec International (Antec International Limited, Sudbury, Suffolk, UK) introduced Virkon-S as a tool for agriculture and animal production. It is thought to be one of the most innovative farm disinfectants [3].

In aquaculture, Virkon is also a multipurpose disinfectant intended to eliminate bacteria, viruses, and one fungus on boots, nets, and other equipment.
Furthermore, the latest research has produced evidence that supports the effectiveness of Virkon as a tool for eliminating invasive mollusks and other organisms in farm and aquaculture settings [4].

Fish have been proven to be reliable markers of water contamination in aquatic systems as they inhabit various trophic levels and are more sensitive to many toxicants [5,6]. Fish species are frequently employed in monitoring to determine the level of toxicant accumulation and how it affects their health. Because they have more advanced osmoregulatory, endocrine, neurological, and immunological systems than invertebrates, fish are suggested in toxicological studies [7]. The gills, liver, kidney and muscles of teleost fish are the tissues that are most commonly used in ecological, toxicological, and pathological investigations [8,9]. Due to the fact that they are metabolically active tissues and have a tendency to accumulate toxicants at greater concentrations. [10-12],

Additionally, [11] believe that toxicants mostly enter fish organisms through gills, where they pass through blood to parenchymal organs where they persist for a longer period of time. Furthermore, according to [13] the concentrations of toxicants, especially in the gills, reflect the levels of toxicants in the water in which the fish exist, but the concentrations in other organs, especially the liver and kidney, reflect the storage of toxicants.

Histopathologic examination has shown to be a crucial aspect in biomarker approaches to assess the potential harm that exposure to both natural and anthropogenic pollutants pose to aquatic life [14].

Histological changes in the specific target organs are effective biomarkers indicating xenobiotic effects because they develop earlier and offer a better assessment of the impacts of water pollution than any other biochemical indicator [15-19].

Histopathologic examination of fish tissues can be used to determine the harmful effects of various toxicants and track aquatic pollution. A basic understanding of the severity of the relevant chemicals can be gained from changes in various fish tissues caused by toxicants. Damage to these vital organs has a negative impact on a fish's ability to grow, survive, develop, reproduce, and maintain a healthy immune system [20]. Therefore, this study was conducted to investigate the histopathological toxic effects of Virkon on the liver, kidney, and gills tissues of common carp fish.

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**Material and Methods**

**Fish:** A total of 120 common carp (Cyprinus carpio) fish were procured from a private fish pond in the local fish market. The mean weight of the fish was 230±10g. The fish were allowed to adjust in the laboratory in tap water for 10 days under a natural photoperiod and ambient temperature in a glass aquarium. Before the experimental time, they were provided with an air pump (oxygen motor) in the laboratory of veterinary medicine at Duhok University. The water was changed daily, and the water temperature was maintained at 25±2 °C with a pH of 7.8±1.

**VIRKON S**

Manufactured for: Lanxess Corporation .111 RIDC Park West Drive Pittsburgh, PA 15275

Net Contents: 10LB (4.53kg)

RSL_31/VirkS/10Ib/USA/19.03.17/G

**Experimental Design**

The fish have been distributed into eight glass aquariums (80cm40cm40cm) with their equipment (oxygen motor, water heater fixed at 25±1°C, thermometer). There are 15 fish in each aquarium. Then, these aquariums are randomly divided into four groups, with each group having duplicates.

- The first group, Group A (control), is kept without any treatment.
- The second group, Group B, is exposed to virkon 4mg/L, [3] continuously for 7 days.
- The third group, Group C, is exposed to virkon 4mg/L continuously for 7 days, followed by a 3-day interval, and then retreated with virkon 4mg/L for another 7 days.
- The fourth group, Group D, is exposed to virkon 4mg/L continuously for 14 days.

**Tissues samples collection**

The spinal cord destruction technique was used to anesthetize fish prior to performing the autopsy, which involved making a longitudinal incision from the anus to the gill cover followed by a transverse incision in the direction of the lateral line, [21]. Each liver, kidney and set of gills had tissue samples taken from them. Cut the outer gill arch with associated gill filaments at the ventral and dorsal connections with scissors. The tissues were then kept in Neutral formalin 10% until the tissue slides were prepared for histopathological investigation [22].
Histopathological technique

The gill, liver, and kidney tissues, which were collected for histological examination, were prepared and stained by eosin and hematoxylin according to the procedure applied by the Luna method [23].

Results

The results showed that the histopathological lesions recorded at the gills are the normal architecture of the gill filaments in the control group, which persist as a normal one epithelial layer of the secondary lamella and normal cells of the primary lamella (Fig.1). However, after 7 days of the experiment, the gill of group B (exposed to virkon 4mg/L for 7 days) showed hyperplasia of the epithelial cells of the primary lamella and some of the secondary lamella, which looked like a drumstick appearance (Fig.2). This is the same lesion seen at the same period in group C (exposed to virkon 4mg/L for 7 days continuously, then 3 days interval, and then retreatment with virkon 4mg/L for 7 days continuously) and group D (exposed to virkon 4mg/L for 14 days continuously). After 14 days of the experiment, the gill filaments in group B exhibited the same lesions that were observed on day 7 of the experiment. However, in group C, the gill filaments showed hyperplasia of the primary lamella and the secondary lamella, along with hypertrophy of chloride cells (Fig.3). In group D, there was hyperplasia of the primary lamella with subsequent destruction of them (Fig.4). By day 21 of the experiment, the gill filaments in group B were recovered with hyperplasia of the primary lamella and hyperplasia of the second lamella and necrosis of epithelial cells of the secondary lamella (Fig.5). In both group C and group D, at 21 days, the filaments exhibited necrosis of the epithelial cells and connective tissue in the primary lamella, along with hypertrophy of chloride cells and pillar cells, and necrosis of epithelial cells in the secondary lamella, accompanied by hemorrhage.

The liver histopathology of the group B at day of experiment compared with the control group (Fig.7) show vaculation of hepatocytes with hemorrhage in hepatic tissue (Fig.8) these lesions were observed in the group C and group D at the same period. After 14 days the histopathological lesions which were seen in the group B were also vaculative degeneration but in the group C and group D there were vaculative degeneration of hepatic cells with necrosis of hepatic tissue also and hemorrhage in the pancreatic tissue (Fig.10). At 21 days of the experiment there were no pathological lesions seen in the liver of group B, but in the group C and group D showed necrosis of the hepatic tissue with infiltration of mononuclear inflammatory cells (Fig.11).

Kidneys histopathological lesions of group B at 7day of experiment compared with the control group (Fig.11) were vaculation of the epithelial cells lying the renal tables and desquamation of these cell from the basement membrane and the vaculation of the epithelial cells glomeruli (Fig.12) and these lesions were seen also in the group C and group D. At 14 day of the experiment the lesions in the kidney of the group B were necrosis of the epithelial cells lying the renal tubules hemorrhage around the renal tubules and in the interstitial renal tissue (Fig.13) but in the group C and group D there were necrosis of the epithelial cells lying the renal tubules appearance of hyaline cast in the necrotized tubules (Fig.14). After 21 day of the experiment the histopathological lesions in the kidney of the group B were different stages of necrosis of epithelial cell lining the renal tubules in some tubules and other tubules necrotized with hemorrhage in renal interstitial tissue (Fig.15). In the group C and D, the lesions were shown to be hyaline cast in the necrotized tubules in tubules there are stages of epithelial cell necrosis (Fig.16).

Discussion

The result indicated a significant pathological change of the gill in the treated groups after 7 days compared with control group and this result due to that the mostly toxic agent enters the fish body through gills, where they pass through blood to parenchymal organs where they persist for a longer period of time [11]. The hyperplasia of the epithelial cell’s primary lamella of as well as few secondary lamella which is the first response for toxicity [24]. After 14 days of the experiment the lesions become more severe in the group C and group D and severity of the lesions increase with long exposures of the fish to virkon, as that whenever an animal exposed to chemical or toxic material in concentration lower than that which can result in mortality, agree with the result of [25].

The necrosis of the primary lamella's epithelial cells and connective tissue along with hypertrophy of the chloride cells and pillar cells, as well as necrosis of the secondary lamella's epithelial cells and hemorrhaging, which Similar histological alterations in the gills of several fish species exposed to pesticides, petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals have been reported by numerous researchers, [26-29].

The liver is considered a major organ for metabolism, xenobiotic detoxification, and toxic substance excretion but the capacity to break down harmful substances in high levels of these substances may overload the liver’s regulatory system, causing...
structural damage, [30]. The results of current study on Liver histopathological showed Hepatocyte vacuolation (vaculative degeneration) which is corresponding as the primary change of cell injury by toxic [31], and these lesions could be due the oxidative stress and production of ROS induced by pesticide [32], and virkon is consider as pesticide also. After 14 days liver lesion in group B remain same to that of 7 days but in group C and D there is necrosis of hepatic tissue also and hemorrhage in the pancreatic tissue and. At day 21 of the experiment, group B's liver showed no pathological lesions, whereas groups C and D's livers showed necrosis of the hepatic tissue with infiltration of mononuclear inflammatory cells, these lesions occurred as a result of persist of toxic material in parenchymal organs which is pass through blood from the gill [11] and these lesions agrees with other studies on different toxic chemicals on different fish species [17,29,32] s study

The histopathological lesions which was seen in the kidney at group B,C and D after 7 day and includes the vaculation of the epithelial cells lying the renal tables ,desquamation of these cell from the basement membrane and the vaculation of the epithelial cells glomeruli could be due to the oxidative stress effect of virkon which is consider as pesticide [32]. The necrosis of the epithelial cells lying the renal tubules with hemorrhage around them and in the interstitial tissue and hyaline cast in the necrotized tubules, these histopathological findings is due to the accumulation of toxic material in the kidney as the kidney is a parenchymal organs,[11] and these also agree with other investigation [33-40].

In this study the most intensive histopathological lesions were seen in the liver and the kidney and increase with longer exposure period of virkon due to the fact that these organs are metabolically active tissues and have a tendency to accumulate toxicants at greater concentrations [10-12].

**Conclusion**

In conclusion, exposure to virkon causes severe histopathological lesions in the gill, liver and kidney tissues of common carps, even in therapeutic concentration and these lesions become more severe with the longer exposure periods of time. So time exposure to virkon should be minimized in fish ponds less than a day.

**Acknowledgments:**

The authors thank Duhok University and Duhok research center of the college of veterinary medicine.

**Conflicts of Interest:**

The authors of this study have revealed no conflicts of interest.

**Funding statement:**

No funding

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**Fig.1. Normal gill lamella of control group shows primary lamella (blue arrow) and the secondary lamella (red arrow)**  
100x H&E
Fig. 2. Gill filaments of the group B at 7 days of experiment show the hyperplasia of the primary lamella (Green arrow) and some of the secondary (red arrow) especially the tip of them and appear drumstick (Blue arrow) 100x H&E

Fig. 3. Gill filament of group C after 14 days of experiment show hyperplasia of the primary lamella (Blue arrow) and the secondary lamella (Red arrow) with hypertrophy of chloride cells (Green arrow) 100x H&E
Fig. 4. Gill filament of group C at 14 days show hyperplasia of the primary lamella (Blue arrow) with destruction of them (Red arrow) 40x H&E.

Fig. 5. Histopathological features of gill filament of group B at 21 day of experiment show the recovery of the filament with hyperplasia of the primary lamella (Blue arrow) and hyperplasia of the second lamella (Green arrow) with necrosis of epithelial cells of the secondary lamella (Red arrow) 100x H&E.
Fig. 6. Gill filament of the group D at 21 day of experiment show necrosis of the epithelial cells and the connective tissue of the primary lamella (Orange arrow) with hypertrophy of the chloride cells (Blue arrow) and pillar cells (Red arrow) and necrosis of epithelial cells of secondary lamella (Green arrow) and hemorrhage (Yellow arrow) 100x H&E.

Fig. 7. Microscopical features of liver liver of the control group demonstrates the normal hepatic tissue the central hepatic vein (Blue arrow) and the hepatocytes (Red arrow) 100x H&E.

Fig. 8. Liver of B group at 7 day of experiment show vaculation of hepatocytes (Blue arrow) with hemorrhage in hepatic tissue (Red arrow) 100x H&E.
Fig. 9. Liver of the group C at 14 day of experiment show vaculation of hepatocytes (Blue arrow) with necrosis of hepatic tissue (Red arrow) and hemorrhage in the pancreatic tissue (Yellow arrow) 100x H&E.

Fig. 10. Liver of the group D show necrosis of the hepatic tissue (Blue arrow) with infiltration of mononuclear inflammatory cells (Red arrow) 100x H&E

Fig. 11. Normal kidney of control group show the normal renal tubules (Blue arrow) and normal interstitial renal tissue (Red arrow) 100x H&E.
Fig.12. Kidney of the group B at 7 days of experiment showing the vaculation of the epithelial cells lining the renal tables and desquamation of these cells from the basement membrane (Red arrow) and the vaculation of the glomerular epithelial cells (Blue arrow) 100x H&E.

Fig.13. Kidney of the group B at 14 day of experiment show necrosis of the epithelial cells lying the renal tubules (Blue arrow) hemorrhage around the renal tubules (Yellow arrow) and in the interstitial renal tissue (Red arrow) 100x H&E.
Fig. 14. Histopathological features of kidney of the group C at 14 day of experiment show necrosis of the epithelial cells lying the renal tubules (Blue arrow) appearance of hyaline cast in the necrotized tubules (Red arrow) 100x H&E.

Fig. 15. Kidney of the group B after 21 day of experiment show different stage of necrosis of epithelial cell lying the renal tubules in some tubules (Blue arrow) necrosis of other renal tubules (Red arrow) 100x H&E.
FIG. 16. Kidneys of the group D at 21 days of experiment show hyaline cast in the necrotized tubules (Blue arrow), in tubules there are stages of epithelial cell necrosis (Red arrow) 100x H&E.

References


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التغيرات المرضية النسجية في الأعضاء الحيوية الأسماك الكارب الشائع (Cyprinus carpio) المعضة للفيركون في فترات متبقية

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أجريت الدراسة الحالية لمعرفة التغيرات النسجية في الأعضاء الحيوية (الغلاصم والكبد والكلى) لأسماك الكاري الشائع (Cyprinus carpio) حيث تنتج من التسمم باستخدام التركيز العلاجي 4 ملغم/لتر للفيركون. استخدمت 120 سمكة كارب بمعدل وزن 230±10 غم وزعت في ثمانية أحواض زجاجية بأبعاد 40 سم × 40 سم × 80 سم مجهزة بمضخة أوكسجين وشير ذو ترموستات ومحرار ثابت توضع درجة الحرارة على 25±1 درجة مئوية في كل حوض. قسمت الاحواض عشوائيا إلى أربعة مجموعات لكل مجموعة، المجموعة A عرضت الفيركون لمدة 7 أيام، ثم تم إيقاف العرض، المجموعة B عرضت الفيركون لمدة 7 أيام فقط، المجموعة C عرضت الفيركون لمدة 7 أيام فقط، المجموعة D بشكل مستمر للفيركون لمدة 14 يوما. تم جمع عينات الأنسجة من الغلاصم والكبد والكلى في الأيام 3 و7 و14 من التجربة. خشلت الدراسة عن تأثيرات كبيرة للفيركون على الأنسجة، بما في ذلك تكاثر الظهارية للصفائح الغلصمية الأولية والثانوية الأولية إضافة إلى تضخم الخلايا الكلوية وكان هناك تنكس فجوي للخلايا الكلوية مع نزف في النسيج الكديي في اليوم 7 من التجربة كما كان هناك تنكس فجوي للخلايا الظهارية المبطنة للنباتات الكلوية وازدادت شدة الأفات المرضية بزيادة مدة العرض للفيركون حيث لوحظ وجود القوالب الزجاجية في النباتات الكلوية بعد 14 يوم من العرض واستمرت الأفات حتى بعد 21 يوم من التجربة.

أوضح الدراسة أن استخدام الفيركون لمدة 7 أيام يمكن أن يحدث أفات نسجية حتى ولو استخدام بالتركيز العلاجي

الكلمات الافتتاحية: الفيركون، الآفات النسجية، أسماك الكارب، المعقمات.