

Effect of Acute Toxicity of Dimethoate on Some Organs of Mosquito Fish, *Gambusia Affinis Hollobrokii*

Original
Article

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ABSTRACT

Background: Dimethoate (DM) is one of a category of insecticides referred to as organophosphates. DM is an insecticide used to kill mites and insects systemically and on contact. DM is middling toxic by ingestion, inhalation and skin absorption. One of the major advantages of using histopathological (HP) biomarkers in environmental oversight is that this group of biomarkers allows examining particular organs, including gills, kidney and liver that are responsible for necessary functions, such as respiration, excretion and accumulation in the fish.

Aim of Work: This study aimed to calculate the LC₅₀ of DM in case of *G. affinis hollobrokii* as a result of exposure to different concentrations of DM. The exposure was continued to 96h. with concentrations of 30, 60, 90 mg/L and control group. Also, clarify HP deformation on some vital organ as gill, liver and kidney of *G. affinis hollobrokii* as biomarker indicator for destructed effect of DM pesticide.

Materials and Methods: A total of 40 specimens of mosquito fish, *Gambusia affinis hollobrokii* with a good condition were obtained in large plastic bag containing approximately 20 L of water and enough oxygen, fish were acclimatized for one week in well aerated large glass tank (100x 50x 50 cm) and fed daily on a commercial fish diet, before the starting of the experiment. A graduated algorithmic concentration of DM was added to the treated tanks. The exposure was continued to 96h. with concentrations of 30, 60, 90 mg/L and control group. During the monitoring, the dead fish were extracted immediately. Also HP effect of DM on some organs of *Gambusia affinis hollobrokii* (gills, liver and kidney), normal and treated fish with maximum concentrations (90 mg/L) were processed for paraffin blocks and stained with H&E, then photographed and described.

Results: The results showed that the calculated LC₅₀ of DM in case of *G. affinis hollobrokii* equal to 63.33 mg/L. The mortality increases with the increasing of concentrations. From investigate the HP variation induced in some organs (gills, liver and Kidney) of *Gambusia affinis hollobrokii* as a result of exposure to 90 mg/L of DM and control group for 96h. The microscopic examination of gill fabric of the fish reveal to 90 mg/L of DM pesticide showed hyperplasia and hypertrophy of the epithelial cells (EC) in secondary gill lamellae (SGL) with partial oedema, lifting up epithelial layer (EL), atrophy of the SGL and congested blood vessels. Liver tissue of the same group of fish showed that, the liver architecture was destroyed, congestion of blood vessels, fatty degeneration in hepatocytes and appearance of some necrotic areas. Kidney tissue showed degenerated renal tubules, severe congestion of the blood vessels, fluid stagnation in renal tubules, oedema, necrotic of renal tubular cells and macrophage leucocytes were also detected.

Conclusion: The random use of pesticides, especially DM and their accumulation cause severe damage to the water streams, including fish, which ultimately result in severe harm to humans.

Received: 17 March 2019, **Accepted:** 23 April 2019

Key Words: Dimethoate; *Gambusia affinis*; histopathology; LC₅₀.

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ISSN: 1110-0559, Vol. 43, No.4

INTRODUCTION

Heavy dependence of newfangled agriculture on agrochemicals such as fertilizers and pesticides is emerging as a menace to the ecological equilibrium of aquatic ecosystems. Artificial pesticides used for monitoring pests in agriculture are one of the considerable causes of aquatic contamination. Sometimes pesticides are immediately used in water bodies for halting pests and vectors, but their vestige mostly reach into aquatic ecosystems through surface water run away and impact the health of non- goal organisms

including fish. Among pesticides organophosphates (OPH) are widely used in agriculture and hygiene programs due to their high efficacy as insecticide but fewer steadinesses in the environment. They are favored over organochlorines (OCL) which have long persistence and consequently readily bioaccumulate in food chain. The shift from (OCL) to (OPH) has resulted into excess occurrence of (OPH) into water bodies causing acute and chronic toxicity to fish fauna^[1-4].

The pollution of surface waters by pesticides used in agriculture is a trouble of wide-ranging importance^[5].

Dimethoate (DM) is one of a category of insecticides referred to as organophosphates. DM is an insecticide used to kill mites and insects systemically and on contact. DM is middling toxic by ingestion, inhalation and skin absorption. As with all (OPH), DM is easily absorbed through the skin. Pesticides accumulate in the tissues and lead to many physiological and biochemical alteration thereby affecting on the activities of many enzymes and metabolites and finally causes disturbance in the entire metabolic process^[6].

Histopathological (HP) studies are one of the effective tools for ecotoxicology and risk assessments^[4,7]. Particular lesions occurring in organs of fish exposed to toxic material under laboratory circumstances are helpful as biomarkers of exposure. As a result (HP) examination is increasingly being known as a worthy tool for assessing the influence of environmental pollutants on fish^[8,9]. Fish, among the group of non-target aquatic organisms, represent the most diverse group of vertebrates. A number of features make them stellar experimental models for the toxicological research, essentially for the contaminants which are probably to extend their impact on aquatic systems^[10,11].

One of the major advantages of using (HP) biomarkers in environmental oversight is that this group of biomarkers allows examining particular organs, including gills, kidney and liver, that are responsible for necessary functions, such as respiration, excretion and accumulation and bio-transformation of xenobiotics in the fish^[12] and serve as warning marks of deterioration and damage to animal health^[13].

The principal thematic of the present work is to scrutinize the pathological effects of squeaky toxicity of DM on structure of gills, liver and kidney of mosquito fish, *Gambusia affinis holbrokii*

MATERIAL AND METHODS

I- Experimental fish

A total of 40 specimens of mosquito fish, *Gambusia affinis holbrokii* with a good condition were obtained from El-Saidah Eisha at Cairo Governorate. Fish were transported to the fish laboratory at Animal House of Zoology Department, Faculty of Science, Al-Azhar University; in large plastic bag containing approximately 20 L of water and a lot of enough oxygen for fish life. In the laboratory, fish were acclimatized for one week in well aerated large glass tank (100x 50x 50 cm) and fed daily on a commercial fish diet ad libitum, before the starting of the experiment.

II-Determination of 96h.LC₅₀

The 96h.LC₅₀ of Dimethoate (DM) of *Gambusia affinis holbrokii* was conducted according to the method of^[14]. Four groups of 9 fish were isolated in plastic tank 10L, well aerated and filled with de-chlorinated water (PH= 7.2 ± 0.50). A graduated algorithmic concentration of DM was added to the treated tanks. The exposure was continued to 96h. with concentrations of 30, 60, 90 mg/L and control group (0.00 mg/L). Daily surveillance for the treated and control groups

were done to examine the dead and life fish. During the monitoring, the dead fish were extracted immediately. Sum of the recorded dead fish after 96h. were used to calculate the value of LC₅₀ according to^[14] by the following formula:

$$LC_{50} = MC - \Sigma (z * d) / m$$

Where

MC = the maximum concentration used.

z = the number of dead fish of two consecutive concentrations divided by two.

d = the difference between two consecutive concentrations.

m = the number of fish in each group.

III-Histological and histopathological studies

To investigate the effect of Dimethoate (DM) on some organs of *Gambusia affinis holbrokii* (gills, liver and kidney), normal and treated fish with maximum concentrations (90 mg/L) were fixed "in toto" in Bouin's fluid at room temperature for 48 h. Then, the specimen were transferred to 70% ethyl alcohol after fixation and decalcification in Decal solution, then dehydrated in ascending concentrations of ethyl alcohol, purified in xylene and deeply surrounding in paraplast wax (M.P.: 58°C). Transverse sections were cut by the microtome at the thickness of 4-6 microns and stained with Harris's haematoxylin and eosin solutions^[15]. Finally, the staining slides were observed by light microscope (XSZ-N107T) at different magnifications, then photographed using Digital Camera (Toup Cam, Ver. 3.7) and described.

RESULTS

Determination of LC₅₀

Data in (Table 1 and Figure 1) showed the changes in percentage of mortality for *Gambusia affinis holbrokii* exposed to the different concentrations of Dimethoate (DM) for 96 h. The results showed that the calculated LC₅₀ of DM in case of *G. affinis holbrokii* was equal to 63.33 mg/L.

However, according to the regression relationship obtained by plotting the different concentrations against the mortality percentage of the fish, the relation between mortality and the different concentrations was found to be significant according to the equation:

% mortality = - 0.07 + (0.077 x concentration), with R² = 0.86.

II- Histological studies

1- Gills

Normally each gill arch endures a double row of gill filaments (non-respiratory or primary filaments) that carry two rows of gill lamellae (respiratory or secondary gill lamellae (SGL)). The SGL are detached by distinct inter-lamellar spaces. The primary filament is composed of multilayered epithelium cells (primary epithelium). Many and scattered mucous cells in the inter-lamellar epithelium

were apparent in between the SGL. Each SGL be composed of a double gaunte sheet of epithelial layer (EL) (secondary epithelium), detached by the centrally existing pillar cell system that supports the EL and limit blood lacunae (Figure 2 A).

The microscopic study of gill sections of the treated fish, *G. affinis holobrokii* with third concentration of Dimethoate (DM) (90 mg/L) showed somewhat extensive hyperplasia of the epithelial cell (EC) that caused complete fusion of some SGL and partial fusibility of most of them. Vacuolated spaces between the pillar system and epithelial lining of the SGL were noticed. Also massive hyperplasia of the secondary lamellar epithelium resulted in a complete obliteration of inter-lamellar space between SGL. Proliferation of the mucous cells at the tips of the gill filaments and edema were also observed resulting in separation or lifting of the respiratory epithelium from the pillar system. Bending or curling of some SGL and degeneration of lamellar lining epithelium were also observed (Figure 2 B-D).

2- Liver

The liver tissue of *G. affinis holobrokii* consists of hepatocytes that aggregate in masses separated by blood sinusoids and arranged in anastomosing laminae and in rings around a central vein. Each hepatocyte is polygonal or spherical in shape with well-defined boundaries and contains a large rounded nucleus. The granular eosinophilic cytoplasm of the hepatocytes has small vacuoles of various sizes that are formed of lipid droplets. The blood sinusoids are slit-like structure filled with nucleated red blood cells. These sinusoids are lined by a layer of flat EC (endothelial cells) with flat elongated nucleus. Branches of hepatic portal vein and bile duct are seen in the liver tissue. The liver compartments were separated by scarce connective tissue (Figure 3 A).

The histological examination of treated fish liver with 90 mg/L of DM revealed some alterations compared to the normal structure. Degeneration of some region of the central

vein epithelial lining was present. The liver architecture was destroyed, where necrotic areas and highly vacuolated hepatocytes as well as crammed blood vessels and cytoplasmic vacuolization of the hepatocytes were observed. Also, the liver exhibited signs of fatty degeneration where the extensive deposits of hyaline intracellular materials forming large vacuoles or oil droplets and occupy the cytoplasm led to displacement of the cell nucleus to marginal position and thickening of the cell walls was seen (Figure 3 B).

3- Kidney

Histological observations of the control kidney of *G. affinis holobrokii* reveals that kidney is mainly consist of renal tubules and renal corpuscles. The renal tubules are lined with tall simple columnar epithelial (SCE) cells, whereas the renal corpuscle is composed of glomerulus within Bowman's capsule which is formed of a double-walled from EC and has a crescent-shaped lumen recognized as the capsular space. The renal tissues also have numerous blood supplies and hematopoietic tissue. The renal tubules are composed of proximal tubules, distal tubules and collecting ducts. The proximal tubules are lined by tall SCE cells with basal nuclei, whereas distal tubules were lined with huge relatively clear SCE cells with central nuclei. The collecting duct is bigger in diameter than the far segment and containing SCE cells with basal nuclei (Figure 3 C).

Histological examination of the kidney of fish treated with high concentration (90 mg/L) of DM showed marked histopathological (HP) alterations to the ordinary architecture pattern of the renal tissue. Congested blood vessels, oedema and fluid stagnation in the renal tubules and vaculation in the renal tubular cells were noticed. Microscopic examination showed predominating renal lesions. Most of the renal tissue had severe reduction of hemopoietic tissue. Severe lymphocytes infiltration, hemorrhage and blood hemolysis were also observed. Swelling of the renal tubular epithelium with hydropic degeneration and marked reduction of peritubular lymphoid tissue leaving large necrotic renal tubular cells were noticed (Figure 3 D).

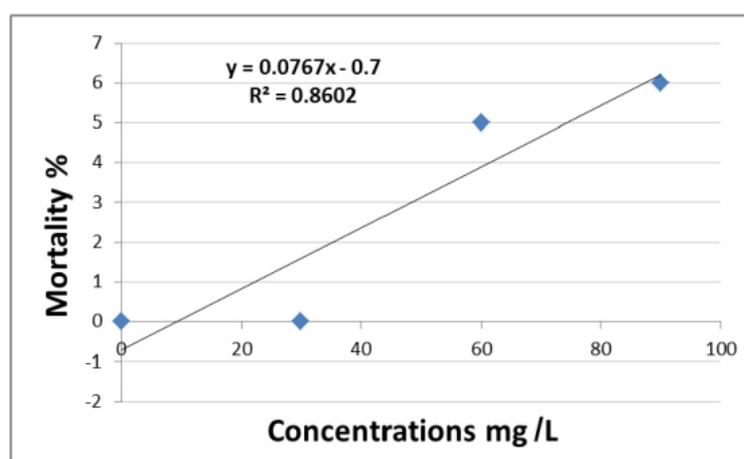


Fig. 1: The relationship between percentage of mortality and concentrations of Dimethoate for *G. affinis holobrokii*

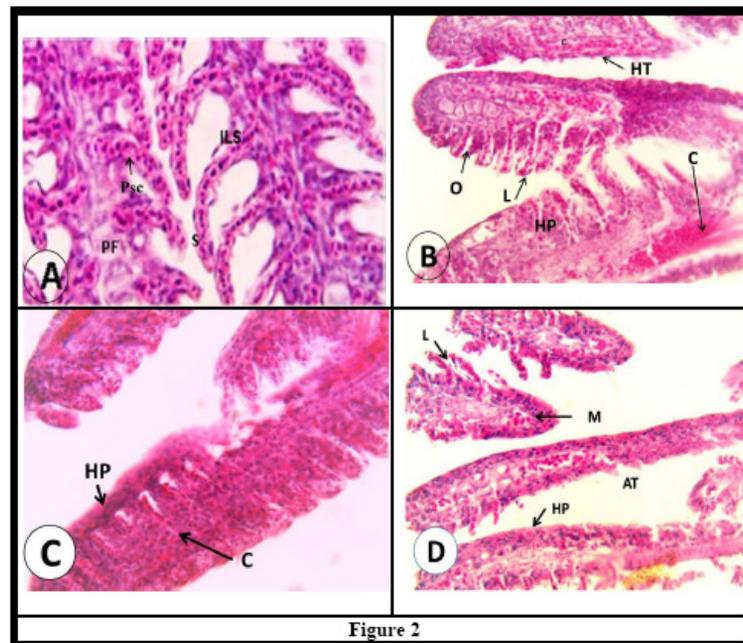


Fig. 2: A. Photomicrograph of T.S. in gills of the control *Gambusia affinis* showing: primary gill filaments (PF) bear double rows of the secondary lamellae (S) with interlamellar space (ILS) and pillar system cells (PSC) (H & E x 400).

B. Photomicrograph of T.S. in gills of the treated *G. affinis* showing: abnormal gill structure, hyperplasia (HP) and hypertrophy (HT) of the secondary gill lamellae with partial oedema (O), lifting up epithelial layer of the secondary lamellae (L) and congested blood vessels (C) (H&E x100).

C. Photomicrograph of T.S. in gills of the treated *G. affinis* showing: hyperplasia of the secondary gill lamellae (HP) and congested blood vessels (C) (H&E x400).

D. Photomicrograph of T.S. in gills of the treated *G. affinis* showing: hyperplasia (HP), atrophy (AT), lifting up epithelial layer of the secondary lamella (L) and macrophage leucocytes (M) (H&E, X100).

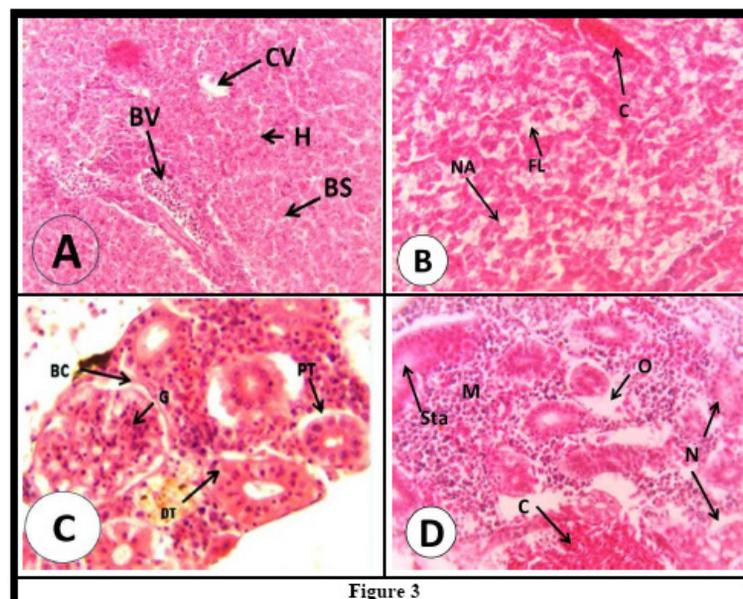


Fig. 3: A. Photomicrograph in liver section of the control *Gambusia affinis* showing: the hepatic polygonal cells (H) with blood vessels (BV) and blood sinusoids (BS) around central vein (CV) (H & E x 100).

B. Photomicrograph in liver section of the treated *G. affinis* showing: moderately pathogens effect such as congested blood vessels (C), fatty degeneration of hepatocytes (FL) and appearance of some necrotic areas (NA) (H&E x100).

C. Photomicrograph of T.S. in kidney of the control *G.affinis* showing normal Bowman's capsule (BC), glomeruli (G), proximal (PT) and distal tubules (DT) (H&E x100).

D. Photomicrograph of T.S. in kidney of the treated *G. affinis* showing abnormal renal tubules; severe congested blood vessels (C), fluid stagnation (Sta) in the renal tubules, oedema (O), necrotic in renal tubular cells (N) and macrophage leucocytes (M) (H&E x100).

DISCUSSION

The present study showed that the calculated LC50 of Dimethoate (DM) in case of *G. affinis holbrokii* is equal to 63.33 mg/L. This result is differ in comparison with LC50 of DM in case of *Heteropneustes fossilis* which equal to 2.98 mg/L^[16] and *Labeo rohita* (24.55 µg/L)^[17]. The differences in LC50 of DM between the different fishes may be due to differences in habitats, behavior, resistant and target organs in the different fish species.

Histological examinations of organs and tissues of fish are of extreme importance in cases of fish poisoning. Histological findings can play significant role in diagnostics of fish injuries or mortalities^[18]. The gills are the most vulnerable structures of the teleost fish because of their external site and intimate contact with water. Gill is vital organ for respiration and has direct connect with water, which permit the pesticides to enter through it and cumulative in the fish body. So, they are liable to damage any irritant material whether dissolved or suspended in the water^[19,20].

In the current study, the gill of mosquito fish, *G. affinis holbrokii* after 96 h of expose to 90 mg/L of DM pesticide showed different histopathological (HP) deformations; hyperplasia and hypertrophy of epithelial cells (EC) in the secondary gill lamellae (SGL) with partial oedema, lifting up epithelial layer (EL), atrophy of secondary lamellae (SL) and congestion of blood cells. The damage of gill epithelium due to pesticide had been notified by^[21-25]. The increase in mucus deposition on the gills and damage caused to gill lamellae by the toxicant would reduce gases exchange^[19,20,26].

Hyperplasia may in some times represent an acclimation by the organism to conserve underlying tissues from any irritation or agitation. However, increased thickness of the EL including mucous cell hyperplasia and fusion of adjacent SL as a result of hyperplasia would not only decrease the surface area available for oxygen extraction but also would increase oxygen diffusion distance between water and blood^[18]. Thus, while hyperplasia may indeed be having a protective function, it may also be hindering the respiratory, secretory and excretory functions of the gills. In addition,^[27] suggested that the gill hyperplasia may increase epithelial thickness so as to retard or prevent the entry of toxic ions into the blood stream.

The importance of liver has a marker for pathological variation reflects the major role of teleost hepatic tissue in nourishment, lipid and carbohydrate storage, synthesis of protein and enzymes, fatty acid metabolism and bio-transformation and elimination of xenobiotics^[28]. The current work showed that the liver of mosquito fish, *G. affinis holbrokii* after 96 h of expose to 90 mg/L of DM pesticide was moderately affected and showed pathogens effect such as: destroyed, congested blood vessels, fatty degeneration of hepatocytes and appearance of some necrotic areas. Fatty degeneration of hepatocytes and cumulation of large vacuoles occupying the cytoplasm of hepatocytes may be due to the reduction of lipoprotein production, releasing and cumulation of triglycerides in liver cells. These results were in agreement

with analogous observations with pesticides in different fish such as *Brachydanio rerio*^[29]; a neotropical fish^[30]; *Tilapia zillii* & *Solea vulgaris*^[31]; *Oreochromis mossambicus*^[7]; *Cyprinus carpio*^[32]; *Heteropneustes fossilis*^[33] and *Clarias batrachus*^[26,32] mentioned that the changes in structure of liver such as cytoplasmic vacuolization appeared as manifestation of stress on fish.

The kidney is an important organ of body and the appropriate kidney function is to maintain the homeostasis. It is not only involved in eliminate of wastes from blood, but it is also accountable for sensible reabsorption, which helps in preserving volume and pH of blood and body fluids and erythropoieses^[34]. Kidney serves as a vital route of elimination of metabolites of xenobiotics, and receives the highest proportion of post branchial blood and therefore it is more likely to undergo HP alterations under pesticide squeeze^[35].

The current study revealed that, kidney of mosquito fish, *G. affinis holbrokii* after 96 h of expose to 90 mg/L of DM was highly affected and sensitive organ in fish. Kidney tissue showed numerous changes such as: degenerated renal tubules, severe congestion of the blood vessels, fluid stagnation in the renal tubules, oedema, necrotic renal tubular cells and aggregated macrophage leucocytes. In accordance with the current results; the degenerative process leads to tissue necrosis. The necrosis of the renal tubular cells has high influence on the metabolic activities and elevates metabolic abnormalities in fish^[36]. The current results are in compatible with those detected in *Cyprinus carpio*^[37]; *Prochilodus lineatus*^[38]; *Lates calcarifer*^[39]; *Oreochromis mossambicus*^[7]; *Cyprinus carpio*^[32] and *Clarias batrachus*^[26].

CONCLUSION

Accumulation of Dimethoate (DM) pesticide in the water body essentially affects the non-goal organism especially fish and become deposited. These fish through food chain have elevated influence on humans and causes deleterious effects. Hence, the usage of the DM pesticide should be restricted to protect human healthy and conserve ecology equilibrium.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربي

تأثير السمية الحادة للدايمثوايت على بعض الأعضاء لسمة جامبوزيا أفينيس هولوبروكي

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الخلفية: الدايمثوايت هي واحدة من فئة المبيدات الحشرية المشار إليها باسم الفوسفات العضوي. كما يستخدم كمبيد حشري لقتل العث والحشرات بشكل منهجي وعند التلامس. الدايمثوايت مادة سامة يمكن أن تنتقل عن طريق الأبتلاع والاستنشاق وامتصاص الجلد. تتمثل إحدى المزايا الرئيسية لإستخدام المؤشرات الحيوية النسيجية المرضية في الرقابة البيئية في أن هذه المجموعة من المؤشرات الحيوية تسمح بفحص عدد من الأعضاء، بما في ذلك الخياشيم والكلية والكبد والتي بدورها مسؤولة عن معظم الوظائف الضرورية في الأسماك، مثل التنفس والإفراز والتراكم، كما تعتبر التشوهات النسيجية الواضحة في الأعضاء دلالة واضحة علي مدي تأثير المبيد المستخدم من عدمه.

الهدف من العمل: تهدف هذه الدراسة إلى تحديد الجرعة المميته لنصف عدد أسماك الجامبوزيا بعد التعرض لسمية الدايمثوايت لمدة ٩٦ ساعة عند تركيزات ٣٠، ٦٠، ٩٠ ملجم/ لتر. كما تهدف هذه الدراسة إلى تقييم الأضرار النسيجية التي لحقت بعدد من الأعضاء الهامة مثل الكبد والكلية وخياشيم أسماك الجامبوزيا أفينيس هولوبروكي بعد التعرض للسمية الحادة للدايمثوايت ٩٠ ملجم/ لتر لمدة ٩٦ ساعة.

المواد وطرق البحث: تم الحصول على ما مجموعه ٤٠ عينة من أسماك الجامبوزيا أفينيس هولوبروكي بحالة جيدة في كيس بلاستيكي كبير يحتوي على ما يقرب من ٢٠ لترًا من الماء والأكسجين الكافي، ثم تم البدء في عملية الأقامة للأسماك لمدة أسبوع واحد في خزان زجاجي كبير جيد التهوية (١٠٠ × ٥٠ × ٥٠ سم) وتتغذى يوميًا على العلف التجاري الخاص بها قبل البدء التجربة. كما تمت إضافة تركيزات متدرجه من الدايمثوايت إلى الخزانات المعالجة حيث إستمر التعرض حتى ٩٦ ساعة بتركيزات ٣٠، ٦٠، ٩٠ ملجم/ لتر ومجموعة الكنترول، وأثناء المراقبة تم إستخراج الأسماك النافقة على الفور وإحتساب أعدادها. كما تم تقييم الأضرار النسيجية الناتجة عن تأثير الدايمثوايت والمعالجة بتركيزات قصوى تصل إلي (٩٠ ملجم/ لتر) على بعض أعضاء أسماك الجامبوزيا أفينيس هولوبروكي (الخياشيم والكبد والكلية) ومقارنتها بالأسماك الطبيعية، حيث تمت معالجة أنسجة الأعضاء محل الدراسة بتركيزات مختلفة من الكحول الأيثيلي ومن ثم تنقيتها بالزيلين لإعداد بلوكات التقطيع بواسطة الغمس في شمع البارافين النقي ثم يتم صباغتها بالهيماتوكسيلين والإيوسين، حتي يتم تصويرها ووصفها.

النتائج: أوضحت النتائج أن الجرعة المميته لنصف عدد أسماك الجامبوزيا تساوي ٣٣ و٦٣ ملجم/ لتر. كما أوضحت العلاقة بين الوفيات ومختلف التركيزات أنه كلما زاد التركيز زاد عدد الوفيات وقد أظهر الفحص الميكروسكوبي بعض التغيرات النسيجية المرضية التي حدثت عند تعرض خياشيم الأسماك لتركيز ٩٠ ملليجرام من الدايمثوايت لكل لتر لمدة ٩٦ ساعة، ظهور زيادة فائقة في عدد وحجم الخلايا الطلائية مما أدى إلى إتحاد كامل للصفائح الخيشومية الثانوية وإغلاق الفراغات بين تلك الصفائح، انفصال الطبقة الطلائية عن الشعيرات لدموية (خلايا بيلار)، حدوث إنتفاخ جزئي وضمور في الصفائح الخيشومية الثانوية وإحتقان بالأوعية الدموية. كما أوضحت نتائج الدراسة أنه عند تعرض كبد الأسماك لتركيز ٩٠ ملليجرام من الدايمثوايت لكل لتر لمدة ٩٦ ساعة، شوهد تكسير لخلايا أنسجة الكبد وإحتقان بالأوعية الدموية وتكون دهون في الخلايا الكبدية وظهور بعض مناطق النخر الخلوي في أنسجة الكبد. كما بينت نتائج الدراسة أنه عند تعرض كلى الأسماك لتركيز ٩٠ ملليجرام من الدايمثوايت لكل لتر لمدة ٩٦ ساعة، لوحظ تقطيت الأنبيبات الكلوية وإحتقان بالأوعية الدموية مع بداية ظهور ركود للسوائل في تجويف الأنابيب الكلوية وكذلك حدوث إنتفاخ ونخر خلوي لبعض أنسجة الكلية وظهور بعض التجمعات من الخلايا الليمفية الأكلة.

الإستنتاج: إستخدام الدايمثوايت في الزراعة يؤدي إلى تراكمه في الماء وبالتالي في الأسماك ويصل إلى الإنسان من خلال السلسلة الغذائية ويضر بصحته. وبالتالي يجب تحديد إستخدام الدايمثوايت من أجل الحفاظ على التوازن البيئي والصحة العامة للإنسان.