Effects of sub lethal doses of endosulfan on histopathological and biochemical parameters of common carp *(Cyprinus carpio)*

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Abstract

The study aim to determine the LD_{50} of endosulfan in fishes. Sixty (60) fishes of common carp 140-160 gm. body weight were utilized. The fishes were adapted and acclimated to the laboratory conditions in the college of Veterinary Medicine, AL-Qassim Green University for 2 days before starting of the experiment, then were exposed to (10 mg/L, 5mg/L, 3mg/L, 2mg/L, 1mg/L) of endosulfan for 24 hours. Results were demonstrated that the LD_{50} of endosulfan in fish was (2.28mg/L). The ALT and AST Liver enzymes were show significant elevation compare with control. Kidney sections show inflammatory cells infiltration particularly macrophage and neutrophils around the tubules with necrotic area in addition to vacuolar degeneration of epithelial lining cells of renal tubules. Histological sections of liver show cellular hypertrophy, swelling, and apoptosis of hepatic cells, and at the same time hepatocytes lost their normal polygonal structure in addition to necrosis of hepatocytes.
Key words: Endosulfan, histopathological changes, biochemical, *cyprinus carpio*.

تأثير الجرعة تحت القاتلة لمبيد الاندوسلفان على بعض الصفات المرضية النسيجية والكيموحيوية لأسماك الكارب الاعتيادي *(cyprinus carpio)*

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الخلاصة

هدفت الدراسة الى تحديد الجرعة نصف القاتلة لمبيد الاندوسلفان في الاسماك. استخدمت في التجربة 60 سمكة من اسماك الكارب الاعتيادي (*Cyprinus carpio*) تراوحت اوزانها ما بين 140- 160 غرام. أجريت التجربة في مختبر كلية الطب البيطري / جامعة القاسم الخضراء. حيث تم تكيف الأسماك وتأقلمها على ظروف المختبر لمدة يومين قبل إجراء التجربة بعدها تعرضت الأسماك إلى تراكيز مختلفة من الاندوسلفان لمدة 24 ساعة (10mg/L, 5mg/L, 3mg/L, 2mg/L, 1mg/L) اظهرت النتائج ان الجرعة نصف القاتلة كانت (2.28mg/L) ، ومن جانب أخر أظهرت فحوصات أنزيمات الكبد زيادة معنوية مقارنة بمجموعة السيطرة ، وقد أظهرت المقاطع النسيجية للكلية ارتشاح حول النبيبات الكلوية مع مناطق تنخريه بالإضافة إلى وجود الفجوات في بطانة النبيبات الكلوية وارتشاح خلايا البلعمة والعدلة حول المناطق التنخرية والنبيبات. أظهرت المقاطع النسيجية للكبد وجود تضخم في خلايا الكبد وكذلك تورم الخلايا الكبدية ، تفمم الخلايا الكبدية وفقدان شكلها الطبيعي بالإضافة إلى زيادة الخلايا التنخرية .
الكلمات المفتاحية: اندوسلفان ، التغيرات المرضية ، التغيرات الكيموحيوية ، اسماك الكارب.

Introduction

Cultural evolution has led to the use of pesticides for the control of pests and in turn brought the pollution of aquatic systems. One among such pollutants is the endosulfan which is a persistent and hazardous agent. Common carp fish (Cyprinus carpio) are sensitive to various types of pesticide such as endosulfan which is selected in this study due to its high toxicity and availability in markets (1, 2). Endosulfan has equal toxicity to insects, birds, mammals, fish and aquatic life (3,4,5). Fish can be exposed to the insecticide when dissolved in water by absorption through the gills, skin and by contaminated food. Fish gills are the first organ that adsorbs the toxicants and contaminants

which are then distributed via blood system into other organs. Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure. Intentional misuse of endosulfan for killing fish (6). The bioaccumulation of endosulfan on these animals would affect the homeostasis and result in the biomagnification. This could affect the economic growth of our country, as well as scarcity of food resources and outbreak of different type of ailments in human population and animals (7). The present work aims to determine the median lethal dose (LD_{50}) values for 24h. Study the effect on the biochemical parameters (ALT, AST, Creatinine) and on the histology of organ tissues like liver and Kidney of the fish (*Cyprinus carpio*).

Materials and methods

The study was conducted at the college of Veterinary Medicine of AL-Qassim green University, Ichthyology laboratory. A total of 60 fish of common carp (*Cyprinus carpio*) ranging between16-18 cm in total length and 140-160 gm. in body weight, with no visible signs of disease or morbidity. Fishes were obtained from Al-Forat hatchery and acclimated to the laboratory conditions for 2 day before the commencement of the experiment. The fishes acclimatized in aquarium measuring 80x40x40 cm, supplied with 90 L tap water and oxygen. The temperature were fixed between 20-23C˚, and the pH between 7- 7.5. Fishes were fed twice daily with commercial pellets with ratio of 3% of initial body weight per day, having 30% crude protein. Using the Endosulfan insecticide. Compound of Endosulfan - Active material (Endosulfan) 35% weight/volume. Determination of median lethal dose of endosulfan A pilot study was carried out to determine the median lethal dose (LD_{50}) of endosulfan (8). To determine the endosulfan LD_{50}, 6 groups of 10 fishes each were transferred to 90 L of water aquaria and maintained for 2 day period of acclimation in the same conditions as mentioned above except for the feeding that was suspended 24 hours before the beginning of the experiment. One control group of fish were also established, both with water only. Five different concentrations of treatment of endosulfan were used; each concentration was added 3 times to each group. The concentration at which 50% mortality of fishes occurred after 24h was selected as the medium lethal dose (LD_{50}). The LD_{50} concentration for 24h was calculated by the probit analysis method. In this study, LD_0 and LD_{100} were determined. The concentrations that using in this experiment were (10mg/L, 5mg/L, 3mg/L, 2mg/L, 1mg/L). Survival animal at dose 1mg/kg was let for 7th day for biochemical and histological study Biochemical study was design for estimation of serum ALT and AST level (9). Histopathological changes were studied in fish that exposed to endosulfan 7th day 1mg/kg f. After dissection, at least sampled pieces of liver, kidney or were collected per fish and fixed in 10% formalin and stained with Hematoxylin and Eosin stain (H&E) (10).

Statistical analysis

Results are expressed as M± SE. Statistical analysis of data was performed on the basis of one- way analysis of variance (ANOVA I) for experiment Group differences were determined using least significant difference (LSD) (Snedecor and Cochran, 1973).

Results

The LD50 of endosulfan estimated by (Probit method) in (*Cyprinus carpio*) was shown in Table (1). In the acute toxicity test, approximately 1 hours after exposure to the various lethal endosulfan concentrations, the fishes showed behavioral abnormalities such as: increase jerking, frequent jumping, erratic swimming, spiraling, and convulsion, escape attempts from the aquarium, loss of equilibrium, molting, color changes and paralysis (Fig.1). The results showed no mortality of fishes in the control treatment. The acute toxicity of endosulfan concentrations at different exposure period and the mortality percentages are shown in diagram (1). The mortality of the fish indicated that the toxicity of endosulfan was entire concentration dependent. Mortality was found after 24 h of exposure, while at higher concentrations (10mg/L), mortality

occurred after 4 hours of exposure. However, the mortality of (*Cyprinus carpio*) exposed to either low or high concentrations of endosulfan remained constant after exposure for 24 h. The present study determined the LD_{50} of endosulfan (2.28mg/L), the LD_0 (1mg/L), and the LD_{100} (10mg/L, 5mg/L). Also it reveals the significant increase in aspartate amino transferase (AST) and alanine amino transferase (ALT) in blood and liver, table (2), and it showed an increase in the creatinine level as in the diagram. Histopathological section in the kidney of fish at one week post-treatment shows inflammatory cells infiltration around the tubules with necrotic area (Fig. 2), also there was inflammatory cells infiltration particularly macrophage and neutrophils around necrotic area and tubules (Fig. 3). On the other hand the section of liver in control fish at 7^{th} day show hepatic tissue (hepatocyte and sinusoids) that present in normal proportion while hypertrophy and cellular swelling of hepatocyte and appearance of apoptotic hepatocytes (Fig.4). Also show focal necrosis of liver tissue surrounded by MNCS infiltration (Fig. 5).

Table 1: Show LD_{50} of Endosulfan on (*Cyprinus carpio*) by Probity method.

Percent *100/D+S	Died accumulation (D)	Survived accumulation (S)	Mortality %	Mortality	Survival Fish	Fish No.	Conc. mg/L
100	10	0	100	10	0	10	mg/L10
100	20	0	100	10	0	10	mg/L5
50	25	5	50	5	5	10	mg/L3
30	28	12	30	3	7	10	mg/L2
0	28	22	0	0	10	10	mg/L1

$$\text{Proportional distance} = \frac{\text{50- mortality below 50 percent}}{\text{Mortality above 50 percent – mortality below 50 percent}} = 0.280$$

LD_{50}= 0.280 + 2(Concentration Below 50) = 2. 28

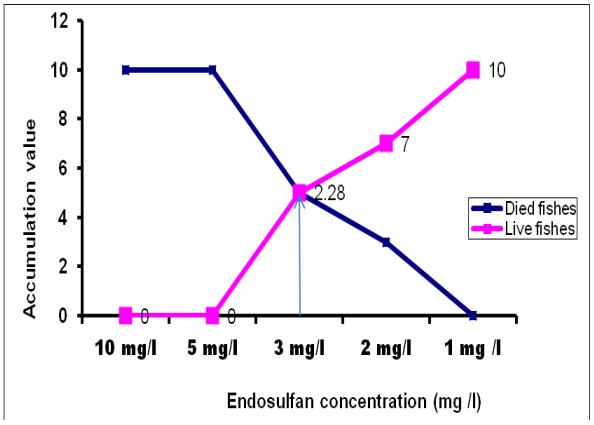

Diagram 1: Linear relationship between probity response and concentration of median lethal dose for endosulfan in 24h.

Table 2: The result show significant elevation of ALT ,AST and creatinine in treatment group when compared with control.

Parameters	Control	Treatment
ALT(GOT)	b74.60± 14.74	a226.40±29.46
AST(GPT)	b 57.98±6.76	a184.20±21.20
Creatinine	b3.76±0.28	a18.10±0.78

Fig. 1: **Show fish sinking to the bottom of the aquarium**.

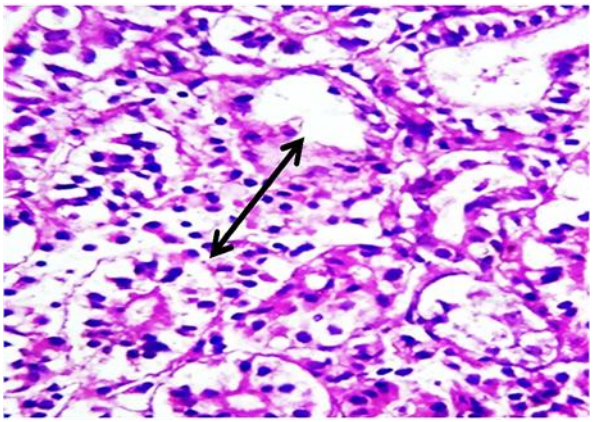

Fig. 2: Histopathological section in the kidney of fish at one week post treatment show inflammatory cells infiltration around the tubules with necrotic area (arrow) (H& E Stain 40X).

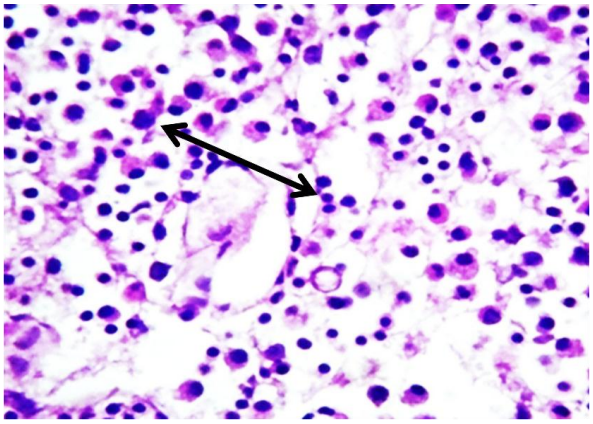

Fig. 3: Histopathological section in the kidney of fish at one week post-treatment show inflammatory cells infiltration particularly macrophage and neutrophils around necrotic area and tubules (arrow) (H& E Stain 40X).

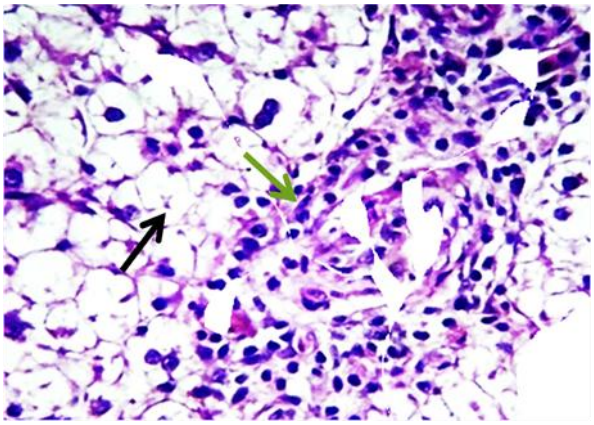

Fig. 4: Histopathology of the liver tissue of fish treated with 0.1 mg/L of endosulfan at 7th day showing hypertrophy and cellular swelling of hepatocyte and appearance of apoptotic hepatocytes (H& E Stain 40X).

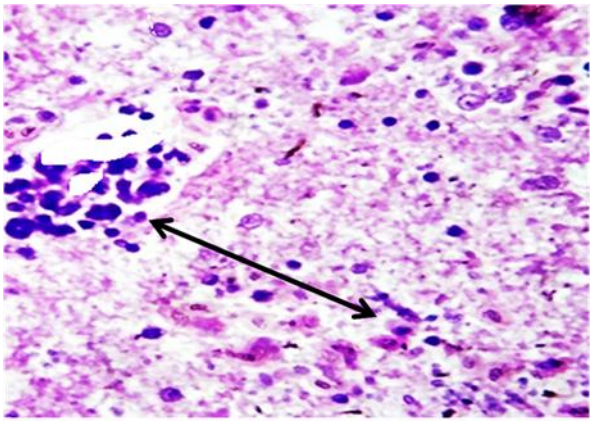

Fig. 5: Histopathology of the liver tissue of fish treated with 0.1 mg/L of endosulfan at 7th day showing focal necrosis of liver tissue surrounded by MNCS infiltration (H&E Stain 40X)

Discussion

Fish can be exposed to the insecticide when dissolved in water by absorption through the gills, skin and by contaminated food. Fish gills are the first organ that adsorbs the toxicants and contaminants which is distributed via blood system into other organs. Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure. The present study determined the acute toxicity of endosulfan on the (*Cyprinus carpio*) during 24 h. The LD_{50} of endosulfan was (2.28mg/L).On another the study, LD50 has been established in (2.86mg/L) found in (*Cyprinus carpio*) (10). The endosulfan had 96h LD50 of (0.93 mg/L) found by Macrobrachiumrosenbergii, post larvae (11). In grass shrimp where 96-h LD50 was (0.62- 1.01 mg/L), with a range of (0.35-1.43mg/L) (12,13). The U.S. Environmental Protection Agency (USEPA) has assessed the acute toxicity standard for endosulfan as (0.22 mg/L) in freshwater (14

). it observed variations in LD50 values for the same species and that toxicant depends on size, age and condition of tested species along with the other experimental factors. These variations in 24 h LD_{50} value may be attributed to the fact that endosulfan induced changes in physiology and survival rate of aquatic organisms under stress is complicated because such changes differ from compound to compound, species to species and from one experimental condition to another. The exact causes of mortality due to endosulfan poisoning are multiple and depend mainly on period-concentration combinations. The kidney sections exposed to different concentrations revealed various degenerative changes. The renal tubular epithelial may become very swollen (Fig.1) and hydropic degeneration observed in others as shown at 0.0001and 0.0002 µg/L concentration, however most tubules showed necrosis of epithelium with sloughing and others disrupted as well as cystic distention of some tubules as in (Fig.2). Some glomeruli showed congestion of glomerular capillary (Fig.3). The specific structural lesions observing in the liver parenchyma exposed to 1mg/kg (Fig.4), with focal area of coagulative necrosis that extensive karyolytic and karyorrhexis were noticed (Fig.5), and in some regions some hepatocyte showed regenerative changes with nuclear hypertrophy. Many toxic chemicals are metabolized in the liver and these processes may cause liver injuries (15).In general fish liver showed a significant finding in different concentrations through this study and this may be related to the fact that most of endosulfan metabolites and excreted via the bile, this indicated that liver is the major site of endosulfan metabolism and excretion in fish (16). The pathological lesions may be classified into irreversible degenerative reactions and regeneration response mediating defense mechanism (17,18). The degree diffusion of this insecticide depends on lipid solubility and the removal by blood depend on the lipid content of blood and once it is absorbed toxicant transported by blood circulated to liver for transformation and/or storage. However liver of fish is more sensitive to environmental contamination because it may be tend to accumulates in the liver at high level than other organ (19).The hepatocyte damage was more sever and prominent under high level. the endosulfan toxin could cause more hepatic lesion ,sever at higher concentration which has associated with degenerative changes (vacuolation with presence of lipid vacuoles) and showing inhibit effect in protein content of liver (20).Moreover, endosulfan also produced cytological and histological effects in the endothelial and epithelial tissue of common carp liver (21). These results are consistent with the results of the present study. The present study also reveals the significant increase in aspartate amino transferase (AST) and Alanine amino transferase (ALT) in blood and liver. This study was in agreement with (22) that indicate endosulfan cause destruction of liver and increase ALTand AST enzyme. Such insecticide induced increase in AST and ALT has been reported by earlier authors (23), They have also reported an increase in enzyme levels in the three major concentrations of organophospherous insecticide (chlorpyrifos). They have also suggested that the increase in tissue AST and ALT was the indication of incorporation of amino acids by way of amino transferase activities of these enzymes into Kreb's cycle to overcome the acute stress posed by the endosulfan. This may be a reason for the increased in the enzymes levels in tissues of exposed fishes. The result showing increased hypertrophy of hepatocytes, hepatocyte cloudy swelling, and apoptosis hepatic cells, at the same time hepatocytes lost their normal polygonal structure, aggregation of mononuclear cells in addition to necrosis of hepatocytes, This study was in agreement with (22). Indicate hepatic lobules also showed some loss of it's parenchyma accompanied with mild infiltration of macrophage, lymphocyte and plasma cells that mainly surrounding the bile ducts, also hydropic swelling of hepatocyte were observed with reticulation of the cytoplasmو as well as sever congestion and dilation of central vein .The pathological lesions may be classified into irreversible degenerative reactions and regeneration response mediating defense mechanism

(17,20).The degree diffusion of this insecticide depends on lipid solubility and removal from blood depend on the lipid content of blood and once absorbed toxicant transported by blood circulated to liver for transformation and/or storage. However liver of fish is more sensitive to environmental contamination because it may be contaminated to that tend accumulated in liver in high level than other organ (5). The hepatocyte damage was more sever and prominent under high level, the endosulfan toxin could cause more hepatic lesion ,sever at higher concentration which has associated with degenerative (18). Moreover, endosulfan also produced cytological and histological effects in the endothelial and epithelium of carp liver (24), these results are consistent with the results of the present study .The endosulfan also mediated reduction in some important metabolic suggested impairment of metabolism and the protein synthesis (25). Alteration in lipoprotein synthesis which resulted in accumulation of lipid vacuole in hepatocyte which is a common findings in most concentrations and was more sever at higher concentration, this may be due to failure in transcription and protein synthesis and alteration in lipoprotein synthesis necessary for the release and transport lipid from the hepatocyte (26). In regard to liver lesions these results also reviled vacuolization, focal necrosis and hydropic swelling and this finding was resulted from excessive work required by the fish to remove the toxicant from body through detoxification by liver as the liver is the main organ for detoxification (27). Histopathological changes of kidney shows inflammatory cells infiltration around the tubules with necrotic area, in addition to vacuolar degeneration of epithelial lining cells of renal tubules, inflammatory cells infiltration particularly macrophage and neutrophils around necrotic area and tubules. these result was in agreement with (28, 29), they indicates changes were degeneration and dissolute of renal epithelial tubules and necrotic changes, this may be due to the kidney of fish which plays an important role in maintaining osmatic hemostasis and receiving large volume of blood flow and serum as a major role of excretion, so a higher effects of toxic chemical were observed. The micro vascular changes finding in most organs like kidney show a state of hypoxia (30). Macrophages are the key for dealing with foreign material and cellular debris (31, 32). The presence of renal hemosiderosis in hemopoitic tissue, where there is excessive breakdown of erythrocytes and these finding are similar to (32) with suggestion that endosulfan induced several hematological changes probability results in inhibition of erythropoiesis, hemosynthesis and increase in the mature erythrocyte destruction in kidney.

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