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Lycopene's Immune-protecting, Antioxidant, and Relative Gene Expression Effects Combat Toxicity of Endosulfan in Nile Tilapia

Muna Omer Alamoudi

Biology Department, Faculty of Science, University of Hail, Hail City, Kingdom of Saudi Arabia.

Abstract

ENDOSULFAN, an organochlorine pesticide, has been widely used to control insect pets for a long time. However, it has been shown to cause harm to aquatic habitats. The present study set out to evaluate the toxic impacts of pesticide endosulfan on freshwater fish Nile tilapia as well as the lycopene protective benefit against endosulfan poisoning. Duplicated of 4 fish from each four treated groups- a control group fed a basic diet, a group fed a basic diet complement with lycopene, an endosulfan exposure group, and a group fed the basic diet complemented with both lycopene and endosulfan- were studied. The research lasted for four weeks. Endosulfan negatively impacted liver enzymes, plasma protein, and albumin. In addition to affecting immunological indicators and causing a decrease in TNF-α, IL1B, IL-8, INF-γ, and Casps 3 mRNA transcript levels, endosulfan also caused a drop in fish blood cell levels. By increasing lipid peroxide malondialdehyde (MDA) levels and lowering the antioxidant enzyme concentration, endosulfan causes oxidative stress. In contrast, adding lycopene to the endosulfan group somewhat recovered the aforementioned characteristics. The findings confirmed the benefits of adding lycopene to fish meals as a neutral antioxidant to reduce hazardous effects brought on by endosulfan.

Keywords: Organochlorine, endosulfan, lycopene, Nile Tilapia, Oxidative stress.

Introduction

Aquaculture has gradually emerged as an exciting activity for seafood sustainability and food safety. Additionally, aquiculture is involved in the financial industry, primarily in nations with low income [1]. The tilapia is probably the most significant fish of the twenty-first century [2]. Worldwide, the Nile tilapia is a highly valued commercial fish. Its ability to reproduce readily, use low protein feed effectively, and withstand a variety of environmental conditions, infectious, and stresses which give it excellent culture properties [3]. They will produce desirable flesh even when exposed to low oxygen levels, extreme crowding, and highly saline water [4]. Conversely, high concentrations of xenobiotics and toxicants negatively impacted the growth and biochemical processes involved in the digestion of aquatic species [5]. Consumers' health and reproductive of aquaculture could be affected by environmental contamination and low hygiene [6].

Pesticides and insecticide derivatives discarded from agriculture can contaminate drainage water used in raising aquatic animals. Bio-magnification of residual derivatives in the fish body usually has harmful effect on growth and overall health [7]. Organochlorines (OCs) are among the most harmful chemical pesticides in terms of environmental pollution due to their ability to bio-magnify in the food chain, persistence, and lack of biodegradability. Endosulfan is an organochlorine pesticide used worldwide to increase agricultural production and control pests. It is commonly used in the field of crop seeds such as canola, cotton, soybean, and sunflower among others [8]. Recently, its use has increased as a result of other insecticides like endrin and DDT are being used less [9].

Endosulfan is widely used and easily transported this is why the contamination of the atmosphere, soil, sediment, water, and food with this pesticide can be at great distances from its use sites [10]. It is extremely poisonous to fish and other aquatic animals [11]. The results are that fish induce a variety of responses in order to combat the negative effects, and these responses modify the metabolism of fish in a variety of ways, including growth, immunity, reproduction, and survival skills [12]. Some nations have banned chemical treatments and urged farmers to use substitutes that are less hostile to the environment [13].

In recent years, medicinal plants have been utilized for treating a broad range of xenobiotic, pharmacological, and chemical toxicity [14]. Phytochemicals are a class of molecules that include carotenoid pigments such as lycopene. Lycopene is a red, lipophilic substance commonly found in various types of natural products and vegetables, with tomatoes being the most potent source of bioavailable lycopene [15]. It is a powerful antioxidant and is 47 times more powerful than vitamin E. It can neutralize the single oxygen (IO_2), which can ability to oxidize unsaturated fatty acid, amino acid, or nucleic acid. It is also

*Corresponding authors: Muna Omer Alamoudi, E-mail: alamoudimuna7@gmail.com & m.alamodi@uoh.edu.sa.
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a powerful anticancer, neuroprotective, anti-proliferative, cognitive enhancer, anti-inflammatory, and hypocholesterolaemia agent [16]. Lycopene has lately become a focus of attention due to its extremely effective scavenging activities against free radicals and single-oxygen. Lycopene is a potent carotenoid antioxidant that reduces toxicity, oxidative damage, and diseases [4]. This research was planned to study the capability of lycopene to mitigate the destructive impacts of endosulfan on fish, in particular tilapia fish, where lycopene is one of the most effective antioxidant agents.

Material and Methods

Chemical materials

Lycopene was brought from the supplier (NOW FOOD Co. USA). Biomed Diagnostic Medical Company in Egypt provided the kits used to evaluate biochemical parameters. Unless specified otherwise, B-endosulfan and other chemicals were provided by Sigma Aldrich (St. Louis, MO, USA).

Experimental design and procedure

A local fish farm in Hail, Saudi Arabia, supplied fish utilized in this experiment. The fish were then acclimatized to the experimental circumstances and fed until full for 14 days before the experiment began. Afterward, eight appropriately prepared glass aquariums measuring (150 x 40 x 55 cm) were randomly assigned to contain four fish of comparable size (157.3 ± 21.4 gm). Aerators, thermometers, and heaters regulate the water temperature and O_2 in each tank. Periodically, the basins are cleaned, aerated, and replenished with dechlorinated tap water. The average parameters of dissolved oxygen (6.5 tons, 7.5 mg/L), pH (7.5 + 0.2), and water temperature (24 + 2 ^{0}C) were maintained as well as twelve hours of daylight and twelve hours of darkness. Throughout the trial, fish were given 2% of their body weight commercially available dry pellets twice.

Animals were divided into four groups of equal size, with four fish in each group in each aquarium. As a control, group (1) was maintained and was given a bassline diet. According to Girao et al. [17], group (2) was given the same diet along with 600 mg/kg of lycopene. Group (3) was exposed to endosulfan at a concentration of 12.795 µg/ L, 1/20 of the maximum lethal dose (LC50), as reported by Kumar et al.[8]. Group (4) had a standard meal supplemented with 600 mg/kg of lycopene and was exposed to 1/20th of the lethal dose (12.795µg/l) of endosulfan. The experiment was carried out for a duration of 4 weeks.

Sampling

Following the treatment period, feeding was halted for 24 hours before blood was sampled. Blood samples were taken from the caudal vein of control and treated animals, and placed in a tiny plastic tube containing an anticoagulant of 3% EDTA for haematological assessment. Other blood samples were taken with non-heparinized syringes then directly emptied into the tube and left for one hour at room temperature. Later on, blood samples were centrifuged for 30 minutes at a speed of 3000 rpm. Subsequently, the resulting serum was frozen at a temperature of -20 ^{o}C for biochemical analysis.

The liver of all groups of fish was carefully dissected and divided into portions. The first portion (1g) was homogenized and then centrifuged (3000 rpm for 15 minutes). The supernatant was collected and utilized for measuring antioxidant activities. The other portion (30 mg) was kept in a 2mL screw cap tube and 600 µL of RTL buffer was added and used for molecular assessment.

Haematological analysis

A haematological analyzer (HeCO Vet C; SEA, Italy) was used to analyze haematological parameters from blood samples including haemoglobin (Hb), red blood cells (RBCs), and total white blood cells (WBCs), monocytes, and lymphocytes.

Biochemical analysis

Albumin concentration and serum total protein were determined colorimetrically. The kinetic enzyme activities of liver, namely, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assessed utilizing a micro lab 200 Vital Scientific-Netherlands' spectrophotometer. The activities of liver supernatant biomarkers of oxidative stress including glutathione peroxidase (GPx) catalase (CAT), and superoxide dismutase (SOD) malondialdehyde (MDA) were determined according to the methodology described in Hamed and El-Sayed [18]. The lysosome commotion was evaluated using turbidity assay, and immune-globin M (IgM) concentration in serum samples were calculated using Elisa Kit for fish (Cusabio Biotic Co., Ltd), both according to the instruction provided by the manufacturers.

Extraction of RNA, synthesis of cDNA and Real-time PCR

According to the manufacturer's instructions, total RNA was extracted utilizing Trizol and TRI reagent (NtRON Biotechnology, InC., Korea). SensiFASTTM cDNA synthesis kit (Bioline, UK) was used to synthesize complementary DNA (cDNA). The primers sequences utilized in this investigation comprise the following: interferon (INF-γ), interleukin 1 beta (IL- β), tumor necrotic factor alpha (TNF-α) interleukin 8 (IL8), and cellular apoptosis caspase 3 (Casp 3) and β-actin as housekeeping gene. All those genes with β–actin were listed in (table 1). The mRNA relative expression level of targeted genes in liver was determined using Real-Time PCR with β-actin and SYBR green internal reference [19].

Statistical estimation:

The statistical analysis of the data was done using the student's test and two- factors of variance (ANOVA). The significance of the findings was established at $p < 0.05$.

Results

Effects of lycopene and endosulfan on the haematological parameters:

The Hb with (p<0.003), RBCs with (p-value = 0.000), and WBCs lymphocyte and monocytes with (p-value =0,000) showed a significant reduction in endosulfan exposed group versus control (table 2). Administration of lycopene significantly improves the decline in parameters compared to endosulfan group and becomes near to normal control group for RBCs, Hb and monocyte parameters. There was no observed disparity noticed between lycopene's treated group and control group.

The data is shown as Mean ± SE (n=8). Significant differences were shown in values with subscript letters (p<0.05).

Effects of lycopene and endosulfan on the liver functions:

Nile tilapia subjected to endosulfan showed a notable rise in the level of serum liver enzymes (ALT. ALP, AST) with p=0.000 and a reduction in albumin and total protein versus control and lycopene-treated groups. The treatment with lycopene showed marked mitigation to endosulfan harmful effects compared to endosulfan group with p-value < 0.001 for albumin and AST and p-value <0.000 for the other parameters compared to endosulfan group (table 3). There was no discernible difference between the lycopene-treated group and control group.

The data is shown as Mean ± SE (n=8). Significant differences were shown in values with subscript letters (p<0.05).

Effects of lycopene and endosulfan on oxidative stress and antioxidant biomarkers:

Comparing the endosulfan-treated group to control group, a significant decrease was observed in the level of SOD (p-value = 0.000), CAT (p-value < 0.002), GPx (p-value = 0.000) as well as a notable increase in the concentration of MDA (p-value = 0.000). In comparison to the endosulfan group, the endosulfan group treated with lycopene showed a significant increase in SOD (p-value= 0.000), CAT (p-value <0.02), and GPx (p-value = 0.000) as well as a decrease in MDA (p-value <0.002) (table 4). There were no notable differences in the antioxidant enzyme and lipid peroxidation between control and lycopene-treated group.

The data is shown as Mean ± SE (n=8). Significant differences were shown in values with subscript letters (p<0.05).

Endosulfan and lycopene effects in immunological parameters

Endosulfan alone therapy resulted in a significant decrease in lysozyme (p=0.000) and IgM (p=0.000) compared to control (Fig. 1). Endosulfan and lycopene combined treatment resulted in a substantial rise in IgM and Lysozyme compared to endosulfan alone treatment (p=0.000). The differences between the lycopene-only treatment and control group did not reach statistical significance for these measures.

Effects of endosulfan and lycopene on gene expression profile:

The endosulfan group exhibited a statistically significant upregulation of IL-8, IL- 1β, INF-γ, TNF-α, and casp3 gene expression in comparison to control group (p= 0.000). Adding lycopene to endosulfan group significantly mitigates the effect of endosulfan in comparison to endosulfan treated group. The differences between the lycopene-only treatment and control group did not reach statistical significance for these measures (Fig. 2).

Discussion

Hematology is frequently used to identify physio-pathological changes brought on by exposure to various stressors. A lot of information can be obtained from haematological parameters, which are normally used to assess the health condition of poisoned fish [20]. In this study, there was a reduction in haematological parameters including RBCs, Hb, WBCs, lymphocytes and monocytes in endosulfan treated group. The reduction in haematological parameters in endosulfan exposed group was observed in rats [21], Crap [22], and fish [23]. A decline in RBCs indices indicated the presence of anaemia, which could be caused by an osmoregulatory, hemosynthetic, erythropoietic, or hematopoietic disorder, or an acceleration of the rate at which erythrocytes in hematopoietic organ are destroyed. Using lycopene ameliorates harmful effects of endosulfan on those parameters. Previous studies showed that exposure to pesticides leads to severe anaemia due to haemolysis of RBCs [24]. In our study, there was a decline in WBCs count, lymphocytes, and monocytes. These alterations in the leukocyte deferential count indicate that the fish have a lower level of non-specific immunity after being acutely exposed to hazardous chemicals. According to Modaresi and Jalalizand [21] endosulfan poison suppresses the body's defence mechanisms which in turn results in a decline in lymphocyte production.

Aspartate transaminase (AST) and Alanine transaminase (ALT) are enzymes that participate in metabolic process of amino acids. Changes in their level can used to detect tissue damage in organs, particularly the liver [25]. Exposure of *O. niloticus* to endosulfan resulted in a statistical increase in serum concentration of AST, ALP, and ALT, and a decline in albumin and total protein (table 3). Bharti and Rasool [26], reported that ALT, ALP, and AST adjust the physiological activities by facilitating transamination reaction which contributes to metabolism of xenobiotics and other macromolecules. As a result, changes in their behaviours enable immediate verification of damage in functions of kidney and liver and serve as indicators to demonstrate the health condition of these organs in animals. Like many toxic chemicals, endosulfan has been well known to affect metabolic enzymes profiles [27] and thus can alter the biochemical and physiological responses of aquatic organisms. The elevation of these enzymes in blood bloodstream is typically caused by hepatotoxic effects of pesticide poisoning, leading to cellular destruction and necrosis in liver tissues [28]. Apoptosis of the hepatocytes and destruction of liver tissue may be the primary crucial process causing the fall in the tilapia liver' s production of immunoglobulin, albumin and total protein [4]. Interestingly, using lycopene against endosulfan toxicity attenuated liver activates nearly to normal level, and improved the level of total protein and albumin, which demonstrates that lycopene has advantageous properties such as hepatoprotection. Our findings align with the research conducted by [29]. Who concluded that lycopene has ability to counteract liver damage caused by diazinon.

This study found that endosulfan induced a strong oxidative stress response, as evidenced by a considerable rise in hepatic MDA level as well as a substantial decrease in scavenging abilities of SOD, CAT, and GPx. Meanwhile, lycopene can withstand these significant alterations due to antioxidant qualities. Fish liver exposed to endosulfan is susceptible to oxidative damage due to an increase in the generation of oxygen free radicals [8]. Lycopene scavenges the reactive oxygen species by transferring the energy from singlet oxygen to lycopene molecules converting it to an energy-rich triplet state and thus preventing their harmful action [30]. Furthermore, Nrf2 and NF-iB transcription factors are highly expressed

by lycopene and are essential for the activation of phase II detoxifying enzymes. The body uses these enzymes to defend itself against free radicals [31].

Both IgM and lysozyme are good markers for immune status in fish [4]. In this research, there was a decrease in the level of IgM and lysozyme activities in endosulfan-exposed fish which modulated by supplementation of lycopene. These results inconsistent with the previous study done by Hussein et al. [4] Dysfunction of lysozyme is virtually invariable lined to cellular malfunction, degenerative and inflammatory disorders, apoptosis, and cellular death [32]. Caspase 3, IL-β1, IL-8 INF-α and TNF-γ levels increased in our research. Endosulfan is a stressor that has been lined to negative effects on immune system by suppressing cellular immunity, which can lead to immune toxicity [33]. According to our findings, lycopene, as an antioxidant, increases fish immune.

Conclusion

In the current research, endosulfan activated several harmful toxicological sequences on serum biochemistry, haematological parameters, and oxidative indicators of Nile tilapia. In addition, endosulfan had an immune and inflammatory harm role on the animal, which possibly elevated the severity of toxicological effects. However, dietary supplementation of lycopene alleviates the toxic effects of endosulfan. We suggest adding lycopene, a natural substance, to fish diets as a way to decrease endosulfan toxicity.

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Conflicts of interest

There is no conflict of interest

Ethical of approval

Nil

TABLE 1. A list of real-time PCR analyzer primers:

Target gene	Sequences (5'----------3')	Reference
IL-1β	F**:** CAAGGATGACGACAAGCCAACC R**:** AGCGGACAGACATGAGAGTGC	(20)
IL8	F: GCACTGCCGCTGCATTAAG R: GCAGTGGGAGTTGGGAAGAA	(3)
TNFα	F: GAGGTCGGCGTGCCAAGA R: TGGTTTCCGTCCACAGCGT	(3)
IFN-γ	F: AAGAATCGCAGCTCTGCACCAT R: GTGTCGTATTGCTGTGGCTTCC	(21)
Casp3	F: GGCTCTTCGTCTGCTTCTGT R: GGGAAATCGAGGCGGTATCT	(21)
β-actin	F: CAGCAAGCAGGAGTACGATGAG R: GTGTGGTGTGTGGTTGTTTTG	(3)

TABLE 2. Blood haematological parameters of Nile tilapia after 4 weeks of treatment:

Group/ Parameters	Control	Lyco	Endo	Endo+Lyco
Hb (g/100 ml)	7.12±0.36^{a}	8.26±0.35^{a}	5.4±0.23^{b}	6.98±0.94^{a}
RBCs (×10/mm³)	1.85±0.09^{a}	2.07±0.09^{a}	0.89±0.05^{b}	1.46±0.13^{a}
WBCs (×10³/mm³)	27.44±0.13^{a}	26.58±0.34^{a}	15.79±0.01^{b}	23.68±0.33^{c}
Lymp (%)	56.80±1.28^{a}	54.23±0.81^{a}	41.85±1.15^{b}	49.13±1.15^{c}
Mon (%)	1.71±0.01^{a}	1.70±0.03^{a}	2.17±0.05^{b}	1.65±0.05^{a}

The data is shown as Mean ± SE (n=8). Significant differences were shown in values with subscript letters (p<0.05).

TABLE 3.Liver parameters of Nile tilapia after 4 weeks of treatment:

Group/ Parameters	Control	Lyco	Endo	Endo+Lyco
Total protein (g/dl)	5.85±0.16^{a}	5.47±0.18^{a}	1.89±0.199^{b}	4.04±0.08^{c}
Albumin (g/dl)	1.78±0.06^{a}	1.85±0.05^{a}	0.85±0.03^{b}	1.32±0.07^{c}
ALP (U/l)	9.05±2.83^{a}	9.1±0.31^{a}	21.39±0.67^{b}	15.99±0.56^{c}
AST (U/l)	43.17±1.01^{a}	41.12±0.61^{a}	113.01±3.64^{a}	66.30±7.55^{c}
ALT (U/l)	14.01±0.64^{a}	13.56±0.63^{a}	36±1.25^{b}	24.73±0.56^{c}

The data is shown as Mean ± SE (n=8). Significant differences were shown in values with subscript letters (p<0.05).

TABLE 4. Antioxidant biomarkers and oxidative stress parameters of Nile tilapia after 4 weeks of treatment:

Group/ Parameters	Control	Lyco	Endo	Endo+Lyco
SOD (U/g)	60.93±0.84[a]	60.33±0.32[a]	40.9±0.41[b]	49.26±0.24[c]
CAT(U/g)	43.93±1.48[a]	43.8±0.76[a]	26.34±0.78[b]	31.83±0.32[c]
GPx (U/g)	128.77±1.6[a]	127.03±1.29[a]	84.22±0.67[b]	116.51±1.01[c]
MDA (nM/g)	38.18±2.83[a]	39.61±2.83[a]	77.97±1.84[b]	49.41±1.44[c]

The data is shown as Mean ± SE (n=8). Significant differences were shown in values with subscript letters (p<0.05).

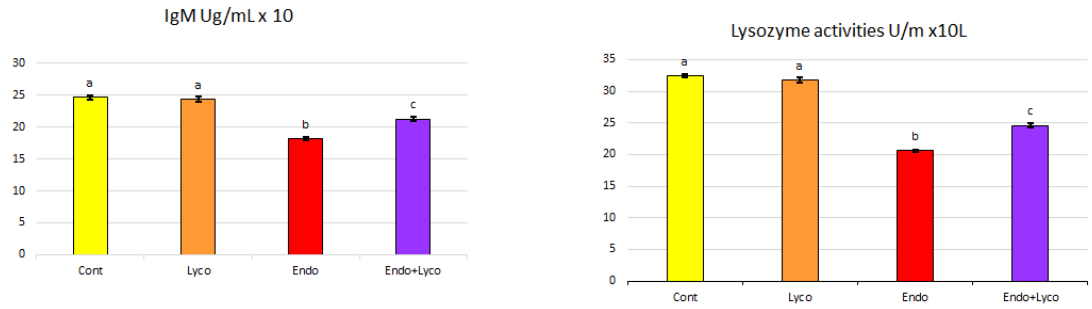

Fig. 1. Effects of endosulfan and lycopene on serum IgM (Ug/mL) and lysozyme activities (U/mL). Bars indicate Means ± SE. The bars with different subscript letters are significantly different

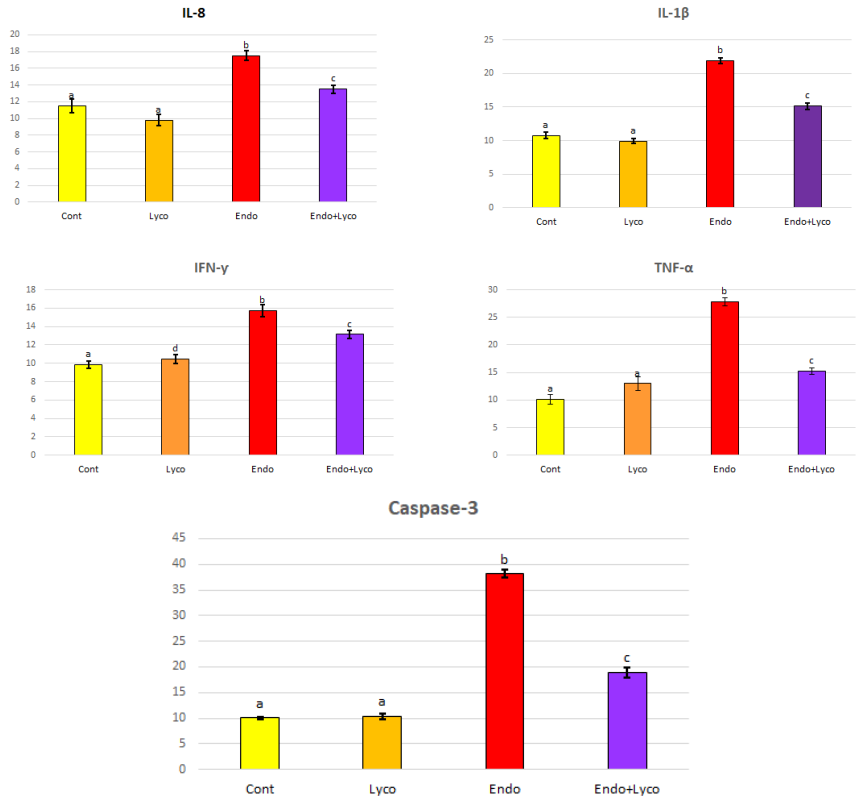

Fig. 2. Effects of endosulfan and lycopene on liver IL8, IL-1β, TNF-α, and IFN-γ. Bars indicate Means ± SE. The bars with different subscript letters are significantly different.

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تأثير الليكوبين في مكافحة سمية الإندوسلفان على الحماية المناعية ومضادات الأكسدة والتعبير الجيني النسبي في البلطي النيلي

منى عمر العمودى

قسم البيولوجى - كلية العلوم- جامعة الحائل – المملكه العربيه السعوديه.

الملخص

أستخدم الإندوسلفان، وهو أحد مبيدات الآفات الكلورية العضوية، اقتصاديًا لفترة طويلة لمكافحة الحشرات ولكن ثبت أنه يضر بالبيئة المائية. تهدف الدراسة الحالية إلى تقييم التأثيرات السامة لمبيد الإندوسلفان على أسماك المياه العذبة من نوع البلطي النيلي بالإضافة إلى تقييم التأثيرات الوقائية لمادة اللايكوبين ضد التسمم بالإندوسلفان. تمت دراسة ما مجموعه 4 أسماك مزدوجة من كل مجموعة معالجة و التي تبلغ أربع مجموعات - المجموعة الضابطة وتم تغذيتها بالغذاء الاساسي، ومجموعة الليكوبين و تم تغذيتها بالغذاء الأساسي مضاف اليه اللايكوبين، ومجموعة الإندوسلفان وتم تعريضها لمادة الإندوسلفان ، ومجموعة اللايكوبين + الإندوسلفان وتم تتغذيتها بالغذاء الأساسي مضاف اليه كلا من اللايكوبين مع تعريضها لمادة الإندوسلفان. استمر البحث لمدة أربعة أسابيع. وجد ان الإندوسلفان يؤثر سلباً على إنزيمات الكبد وبروتينات البلازما والألبومين. وبالإضافة إلى تأثيره على المؤشرات المناعية، فقد تسبب في انخفاض مستوى التعبير الجيني لكل من TNF-α وIL1B وIL-8 وINF-γ و Casps 3، كما تسبب الإندوسلفان في انخفاض مستوى خلايا الدم في الأسماك. .زاد الاندوسلفان من الاجهاد التاكسدي عن طريق زيادة مستوى بيروكسيد الدهون المالونديالدهيد (MDA) وخفض تركيز الإنزيمات المضادة للأكسدة. وفي المقابل، فإن إضافة اللايكوبين إلى مجموعة المعرضة للإندوسلفان أدى الى تحسن الاثار السمية السابقة الذكر. وأكدت النتائج فوائد إضافة اللايكوبين إلى وجبة السمك كمضاد أكسدة طبيعي لتقليل التأثيرات الخطرة الناجمة عن الإندوسلفان.

الكلمات الدالة: الكلور العضوي الاندوسلفان، اللايكوبين ، البلطي النيلي ، الاكسدة.