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RESEARCH ARTICLE

Oleic acid from Marine seaweed *Caulerpa scalpelliformis* induced toxicity in *Oreochromis niloticus*

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Abstract

Background/Objective: Oleic acid is an unsaturated fatty acid, abundant in plants and animals. It accounts for 49% to 83% of total fatty acids, and altered oleic acid leads to diabetes, liver damage, and atherosclerosis. Alteration in oleic acid consumption leads to hepatotoxicity in humans. Macro algae named *Caulerpa scalpelliformis* is well-known for its bioactivity properties like antimicrobial and anti-cancer activity. *Oreochromis niloticus* (Nile tilapia) is a freshwater breed considered a protein-rich fish also it has a fast-growing capacity, easy farm management, and pleasant flavor with fewer bones, used as a study tool in scientific research. This study aims to determine the toxic effect in *O. niloticus* by inducing oleic acid from seaweed *C. scalpelliformis*. **Methods**: In the present study, oleic acid was extracted from *C. scalpelliformis* by column chromatography and analyzed using Thin Layer Chromatography (TLC). Toxicity analysis in *O. niloticus* was performed by exposing the *C.scalpelliformis* methanol extract in a freshwater fish tank in five different concentrations. **Findings:** The mortality rate was measured after 24 hours of exposure. Fish died at 250μg/ml and 100μg/ml. In histopathology analysis, hemorrhages in the inner lamellar space, moderate lamellar epithelial cell necrosis, and sloughing of the lamellar epithelium were observed in the gill. Cerebral congestion, multifocal vacuolar degeneration, and infiltration of mononuclear cells were observed in the brain. Periportal necrosis and hypertrophy of hepatocytes were observed in the liver. Renal hemorrhage, multifocal degeneration of tubular epithelium, and sinusoid expansion were observed in the kidney. Sloughing of epithelial villi, villous atrophy, cellular infiltration, and focal necrosis of epithelial cells was observed in the kidney. **Novelty:** This is the first report from this particular study site. Even though Oleic acid has some impact on the fish, the optimum level of oleic acid from this particular seaweed can be used as a natural medicine for human society.

Keywords: Oleic Acid; Caulerpa Scalpelliformis; Oreochromis Niloticus; Hepatotoxicity; Histopathology Analysis

1 Introduction

The marine environment has enormous biological diversity in algae. Seaweeds are plant-like organisms also known as macrophytes or algae[1]. Macroalgae, also referred to as seaweeds, are highly characteristic and dominant organisms in sea ecosystems. Chlorophyta, or green seaweeds, have more than 7,000 species worldwide in colorful territories (marine, brackish, and terrestrial ecosystems). In the local community, seaweeds are frequently eaten in salads and as vegetables. Because they contain a lot of functionally useful compounds like phenolic composites, alkaloids, sterols, omega-6 adipose acids, antioxidants, carotenoids, and phenolic compounds, some species of macroalgae are eaten as a major component of the diet. A promising strategy that helps in the identification and application of specific fish species for meeting the nutrition demand of the global population is the recent development of nutrition-sensitive aquaculture for the production of different varieties of ocean food for supporting the food conditions of the general public, providing acceptable quantities of recommended salutary rudiments, and being nutritionally and culturally safe for acceptance in the global population[2]. *C. scalpelliformis* is an Indo-Pacific species that is widely distributed in the Indian Ocean. It is also found on warm-temperate Atlantic coasts and in the Pacific Ocean. According to reports, one of the plentiful fatty acids present in several kinds of seaweed is oleic acid.

The Nile tilapia (*O. niloticus*) used in this study as an experimental species has been regarded as one of the most popular freshwater-breed fish species worldwide because of its quick growth, resilience, omnivorous diet, resistance to low oxygen concentrations, ease of farm management, excellent flavor, and fewer bones. The main species among all aquatic creatures living in the Nile River is the Nile tilapia, *Oreochromis niloticus*. This species is used as a study tool in scientific research[3]. Due to its high protein content and low cost of production, tilapia can provide for the daily nutritional needs of a vast population, including the underprivileged and malnourished. Tilapia is regarded as an important nutritional fish since it serves as a main source of protein for a large population in several developing and poor nations. Information on this species' nutritional potential and aquaculture productivity is essential given its significance as a dietary and commercial fish around the world. Particularly for the assessment of toxicity, this species is utilized as an exploratory creature[4,5].

Oleic acid (18:1 n-9) is a type of unsaturated fatty acid found in both plants and animals. In the bodies of healthy individuals, it is the most prevalent and plentiful fatty acid. Cell membranes, human plasma, and adipose tissue all contain it. A change in the levels of circulating fatty acids is linked to diseases like cancer, diabetes mellitus, coronary heart disease, obesity, and atherosclerosis. Consuming olive oil, which contains oleic acid in amounts ranging from 49% to 83% of the total fatty acids, has been linked to increased pancreatic and liver secretory activity as well as a decreased risk of gastric and duodenal ulcers. Endothelial toxicity caused by oleic acid injection results in endothelial necrosis, epithelial damage, and neutrophil infiltration[6]. A diet rich in oleic acid may have health problems for humans, including increasing the risk of coronary heart disease and other related diseases.

Histopathology is the study of disease symptoms using a microscopic examination of a biopsy or surgical specimen that is processed and fixed onto glass slides. The sections are stained with one or more stains to visualize different tissue components under a microscope[7].

Despite having many advantages, oleic acid has certain negative consequences for both people and marine life. Consuming too much of this omega-9 fatty acid might result in the development of certain deficits, even though a good level of oleic acid improves heart health. A diet high in omega-9 fatty acids may eventually result in heart failure. Hence, careful assessment is demanded before anything is brought into remedial value. For the toxicity of *C. scalpelliformis*' oleic acid in *O. niloticus*, a relatively small number of studies have been conducted. In view of this, this work assesses the histopathological investigation and the acute toxicity studies of *C. scalpelliformis*, an active chemical. Furthermore, no previous research has been conducted at the current study site. Overall, this research showed that oleic acid ingestion from macroalgae might have an impact on marine life. The findings in this study pave the way for the upcoming research to gain more information about the toxicity study of oleic acid and other bioactive chemicals from seaweeds in *O. niloticus*.

2 Methodology

2.1 Collection of algae

A species of seaweed belonging to the Caulerpaceae family is called *Caulerpa scalpelliformis*. Marine macroalgae *C. scalpelliformis* were collected from the Periyathalai, Tuticorin district (Latitude-8°20'12.81"N, 77°58'20.77"E). The algae samples collected from the seawater were put into a plastic container for further analysis. The algae were rinsed with the water to remove any inorganic matter.

2.2 Extraction Process by Soxhlet Extractor

Soxhlet extraction is highly helpful for preparative purposes in which the analyte is concentrated from the matrix as a whole or separated from specific interfering components. The test sample (SJ-11 M) can be fresh or dried. The Sample was crushed. The test sample should be enough to fill the cellulose thimble. It should take the entire process four hours. Once the extraction procedure has been set up, it is not advised to leave the equipment alone because it is near moving water and an electrical device. The apparatus should be turned off after informing a technician or other lab user of the situation.

2.3 Isolation of Oleic acid from *Caulerpa scalpelliformis*

Isolation of a compound, using silica gel for column chromatography, the SJ 11M (*C. scalpelliformis*) extract was analyzed (mesh size-60-120). Wet packing with methanol was used to prepare the silica column, and 100 ml of a separate solvent system was used to wash the column. The SJ11M extract was then combined with silica gel to create a fine powder for sample distribution. The pre-packed silica column was topped with the powdered sample mass. Methanol was then used to begin the elution process. Each fractional volume of 10 ml was collected in a vial for further thin-layer chromatography analysis.

Commercially available moisture-free TLC plates were taken. With a pencil, 0.5 cm of a thin mark was made at the bottom of the plate to apply the sample spots. Using a micropipette, each solvent extract was loaded 0.5 cm from the base of the TLC plate after being diluted with the appropriate solvent. The samples were then left to dry. As the mobile phase, various solvent systems were employed, including a 5:4:0.2 ratio of toluene, ethyl acetate, and formic acid. The mobile phase was kept inside a closed chamber where the TLC plate was allowed to develop before being thoroughly dried. The plates were examined using a UV lamp (254 nm and 366 nm wavelengths) and white light. Followed by TLC, HPLC was done for confirmation of Oleic acid in *C. scalpelliformis* extract.

The formula for calculating the Rf value is given below

The Rf value (retardation factor) was calculated by using the below formula

Rf value= Distance travelled by the compound/Distance traveled by the solvent front

2.4 Toxicity analysis with Oleic acid in fish models

From a fish farm, Nile tilapia (*O. niloticus*) was obtained. In 6-liter tanks, fish were kept in a lab environment. Commercial dry fish pellets were used twice daily to feed the fish. A marine macroalgae sample, *C. scalpelliformis*, was exposed to methanol extract to perform a cytotoxicity test on freshwater fishes. In a fish tank with 3 liters of water, 6 groups (36) of Nile tilapia fish were kept in separate tanks. The stock was made by mixing 0.05g of the sample with 1ml of DMSO (dimethyl sulfoxide). Five different concentrations of the extract was made: 5000 ppm(300μl), 1000ppm(60μl), 500ppm(30μl), 100ppm(6μl), 50 ppm(3μl). Five different concentrations and one control were used to test Nile tilapia fish.

2.5 Histopathology

Fish that died after the bioassay test was completed in 24 hours were set aside for histopathological examination.The gill, brain, liver, kidney, and intestine of the fish were removed from both the control and experimental groups. To prevent autolysis, these organs were fixed in Bouin's fluid. The wooden block was mounted with embedded tissues. After 2 minutes of being restrained in 1% Eosin, sections were rinsed. The sections that were dehydrated were cleaned in xylene. The sections that had been cleared were mounted in DPX (Diethyl Butyl Phthalate) and left to dry. Various microscope objectives were used to view the mounted sections, and the results were recorded.

2.6 Statistical analysis

One-way ANOVA was used to analyze the data from the toxicity study of oleic acid in fish using Graph Pad Prism software. P-values are found to be higher than 0.05, as a result, the current study's hypothesis is accepted.

3 Results and Discussion

The GC-MS results revealed that many active compounds were present in the methanol extract of *C. scalpelliformis*. Among the active compounds, oleic acid was found to be the major constituent in the extract. Then the active compounds were separated through the column chromatographic technique. The eluted fractions were spotted on TLC and run with the suitable solvent system to isolate the oleic acid compound.

Totally 16 fractions were collected from the silica gel column chromatography. The Rf value of the isolated compound through TLC analysis was found to be 0.79 cm. The isolated compound was subjected to a UV lamp for the observation of the visible band.

3.1 Toxicity analysis in *O. niloticus*

Oleic acid from *C. scalpelliformis* extract was induced in *O. niloticus*. After 24 hours of exposure, the results were noted. The percentage of mortality in *O. niloticus* is depicted in Table 1 . The percentage of viability was noted in Table 2 . Figure 1 shows the graphical representation of the mortality rate of the fish, *O. niloticus*. The dead fish was found in 250μg/ml and 100μg/ml. Figure 2 shows the graphical representation of the viability. This figure depicts that *O. niloticus* survived in different concentrations and controls except at 250μg/ml and 100μg/ml. Overall; the survival rate was high in *O. niloticus* treated with oleic acid of extract. The LC 50 values were found to be 187.6μg/ml and 70.76 μg/ml.

Table 1. At concentrations of 500 ppm/ml and 1000 ppm/ml, oleic acid caused toxicity

Name of the sample	5000 ppm/ml	1000 ppm/ml	500 ppm/ml	100 ppm/ml	50 ppm/ml	Control
SJ- 11M	0	16.66667	16.66667	0	0	0
	0	16.66667	16.66667	0	0	0

Table 2. 100% viability was observed in 5000 ppm/ml, 100 ppm/ml, 50 ppm/ml, and control groups

Name of the sample	5000 ppm/ml	1000 ppm/ml	500 ppm/ml	100 ppm/ml	50 ppm/ml	Control
SJ- 11M	100	83.33333	83.33333	100	100	100
	100	83.33333	83.33333	100	100	100

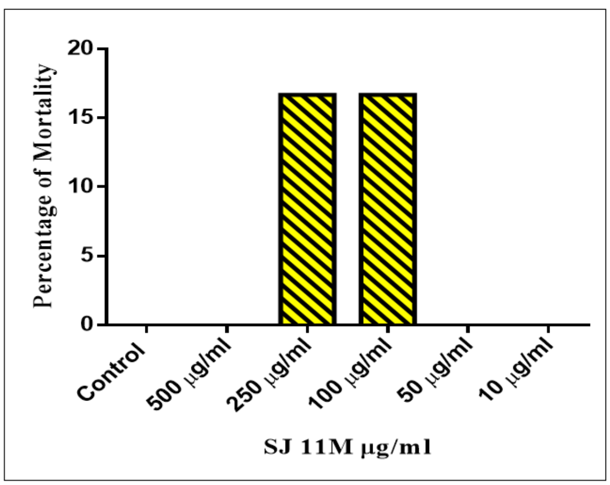

Fig 1. Mortality rate of *Oreochromis niloticus* observed in 250 μg/ml and 100 μg/ml

3.2 Histopathology study on *Oreochromis niloticus*

Oleic acid-induced *O. niloticus* showed some behavioral patterns at the particular concentrations (250μg/ml and 100μg/ml) of the extract. The histopathology microscopic images are shown in Figure 3 . The tissues of the fish were observed under a microscope. Some morphological changes and ecological stress occurred in the tissues of the fish. The juveniles showed some loss of balance, inactivity, and slow opercular movement. It might be due to the toxins produced from the oleic acid extracted

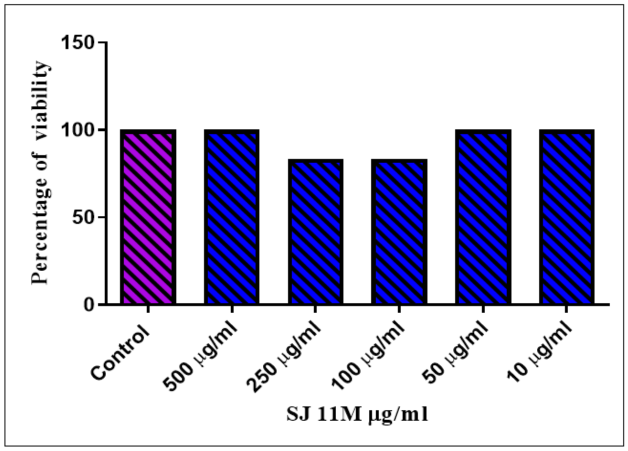

Fig 2. Percentage of viability of *Oreochromis niloticus* in control and different concentration

from *C. scalpelliformis*, less immunity of the fish, and some ecological changes. Even though fish exhibited a better survival rate, dead fish have some severe tissue damage Figure 3 .

The marine seaweeds have been known to have many bioactive compounds. It plays a vital role in Medicine, food, and industrial products. Monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and highly unsaturated fatty acids (HUFA), as well as omega-3 and -6 (-3 and -6, respectively), are particularly produced by seaweeds[8]. Oleic acid belongs to the family of monounsaturated fatty acids. Even though Marine seaweeds provide many beneficial products to society, it has some major impact on Animal and human. The present studies focused on the effect of Oleic acid from *C. scalpelliformis* in Nile tilapia (*O. niloticus*). Oleic acid from *C. scalpelliformis* extract was induced in *O. niloticus*. The results were noted after 24 hours of exposure. The survival and death rate of the fish was counted as the percentage of mortality and viability [Tables 1 and 2]. It shows some minor differences. Figure 1 shows the graphical representation, the mortality rate was found in 250μg/ml and 100μg/ml concentrations. The LC 50 values were found to be 187.6μg/ml and 70.76 μg/ml. Histopathology was also done and the tissues were observed under a microscope [Figure 3]. The brain, gill, intestine, liver, and kidney were highly affected by the oleic acid at this particular concentration. The death in particular concentration may be due to ecological stress, a weak immune system and toxins produced by the oleic acid.

- **Oleic acid**

According to earlier research, monounsaturated fatty acids (OA, oleic acid) are less toxic than saturated fatty acids (PA, palmitic acid). This might be the reason why the death rate of *O. niloticus* was lower in the recent study. Oleic acid and linoleic acid are plant-derived fatty acids that come from *Nigella sativa L*. It is characterized by an abnormally high level of liver fat deposition (>5% in hepatocytes), which has a direct association with obesity, insulin resistance, and cardiovascular illnesses[9,10]. Oleic acid from the seaweed *C. scalpelliformis* was used in the current study, and it had an effect on the tissues of *O. niloticus*. As a result, the consumption of fatty acids (FA) has a strong correlation with its development.

- **Histopathology**

Treatment with OA harms *O. niloticus* in the present study. The gill and liver are the primary sensitive target organs of xenobiotic toxicity and injury for fish. In the present study, gill and liver were impacted by oleic acid [Figure 3 (a) and (c)]. Gills were impacted by Hemorrhages in the inner lamellar space,moderate lamellar epithelial cell necrosis and sloughing of the lamellar epithelium were found to be detrimental to gills in the current investigation [Figure 3 (a)]. Liver was impacted by Periportal necrosis and Hypertrophy of hepatocytes in the current study [Figure 3 (c)]. Heavy metal treatment of *O. niloticus* resulted in histological changes in the kidney and liver. Hepatocytes were affected by inflammation, edema, central vein congestion, degeneration, and necrosis of hepatocytes. Cytoplasmic disorganization, cytoplasmic vacuolation, dilation and sinusoid, necrosis of hepatic cells, blood cell congestion, and oedema were the abnormalities seen in the liver[11]. In the present study, the kidney was affected by renal hemorrhages, multifocal degeneration of tubular epithelium, and Expansion of sinusoids [Figure 3 (d)]. Experimental *O. niloticus* demonstrated that kidney tissue was affected by necrosis in renal tubular cells, tubular hemorrhage, oedema, damaged glomerulus, collecting duct damage, and blood congestion, as well as kidney affected by degeneration of the glomerulus tissue, necrosis, and occlusion in tubular lumen[12].

Oxidative pressure is one of the primary causes of death fish in the current investigation. Deltamethrin exposure caused oxidative damage to the liver, kidney, and gills of freshwater Nile tilapia (*O. niloticus*), and its toxic effects were also examined. Previous research has documented additional liver changes caused by exposure to various toxic chemicals in *O. niloticus* and other fish species. The oxidative stress brought on by atrazine (ATZ) exposure is likely responsible for the damaged intestinal characteristics and the inflammation[13]. Due to the deleterious effects of ATZ-induced inflammation on the structure and function of tissue cells, which results in irregular release of enzymes and lipid droplets, Nile tilapia's liver and kidney function were disrupted.[14]. In this present study, gill, liver, brain, kidney and intestine of *O. niloticus* may be affected due to oxidative stress [Figure 3].

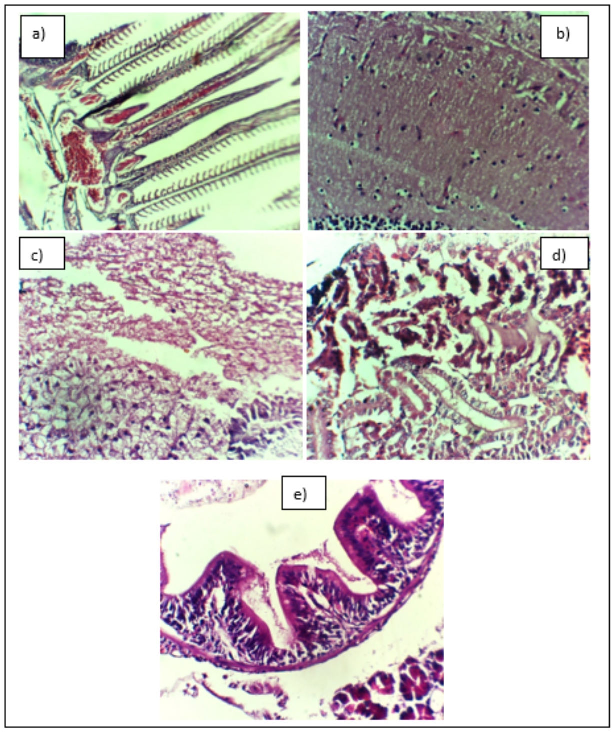

Fig 3. The histology microscopic images of treated *O. niloticus.* (a) Gill was impacted by Hemorrhages in the inner lamellar space, Moderate lamellar epithelial cell necrosis and Sloughing of lamellar epithelium. (b) Brain was impacted by Cerebral congestion, multifocal vacuolar degeneration and Infiltration of mononuclear cells. (c) Liver was impacted by Periportal necrosis and Hypertrophy of hepatocytes. (d) Kidney was impacted by Renal hemorrhages, multifocal degeneration of tubular epithelium and Expansion of sinusoids. (e) Intestine was impacted by Sloughing of epithelial villi villous atrophy, cellular infiltration, and Focal necrosis of epithelial cells

High quantities of cortisol are produced by stressed fish, which causes the liver to secrete glucose through a process called gluconeogenesis. This provides the fish body with the energy it needs to combat the effects of stress[15]. The neurological, reproductive, and developmental damage brought on by pyrethroids is greatly influenced by oxidative stress. Representative pyrethroid insecticides that produce histopathological alterations by inducing oxidative stress in organs including fish gills, livers, and muscles include lambda-cyhalothrin, cypermethrin, and deltamethrin. Fish can survive in a wide range of aquatic environments, including those with different temperatures, salinities, and concentrations of dissolved oxygen, all over the world. When external influences surpass adaption limitations, environmental pressures emerge that negatively impact fish physiology[16]. Polyvinyl chloride-induced oxidative stress and histopathological alterations were seen in *O. niloticus*[17]. According to the current investigation; external pressures are one of the reasons why fish die.

In the present study, the brain of the experimental species was affected; it has cerebral congestion, multifocal vacuolar degeneration, and Infiltration of mononuclear cells [Figure 3 (b)]. *O. niloticus* treated to Aluminium nanoparticles also exhibited neurobehavioral alterations, including a decrease in comfort behavior and an increase in aggression [18]. Another study that revealed decreased expression of genes involved in PUFA and MUFA synthesis in the frontal cortex of depressed patients provided some support for these findings. Previous research demonstrates that oleic acid contributes hyperglycemia in diabetes patients. In the current research, the product of hyperglycemic emulsion from oleic acid may be the reason for the fish mortality rate.

The gills, intestines, kidneys, and swim bladder are among fish's osmoregulatory organs. The largest surface area and closest contact with the outside world among them are provided by the gills [19]. Chlorpyrifos exposure caused congestion and telangiectasia in the gills, catarrhal enteritis followed by fatty degeneration and vacuolation of hepatocytes in addition to congestion of blood sinusoids in the liver, and catarrhal enteritis followed by degeneration and sloughing of the apical part of the intestinal villi. [20]. In the current investigation, periportal necrosis and hepatocyte hypertrophy impacted the liver [Figure 3 (c)] and Intestine was impacted by Sloughing of epithelial villi villous atrophy, cellular infiltration, and Focal necrosis of epithelial cells [Figure 3 (e)].

According to earlier reports, the fish used in this study perished as a result of oxidative stress, oleic acid-induced neurobehavioural changes, and other ecological imbalances that made the fish less aggressive, lose weight, and eat less food. According to the current investigation's histopathological component. Fish exposed to oleic acid exhibit severe tissue damage despite having a higher survival rate. Oleic acid from *C. scalpelliformis* has some impact on the *O. niloticus*, according to these findings.

4 Conclusion

The current study highlights how oleic acid induced Nile tilapia (*O. niloticus*) mortality rates at specific concentrations and resulted in severe tissue damage to the dead fish. The present study draws the conclusion that oleic acid from *C. scalpelliformis* has some effect on *O. niloticus*. This is the initial report from this specific study site. The oleic acid-induced toxicity of nile tilapia fish has probably never been documented before.

Prospects and Recommendations: It is possible to consider using the oleic acid isolated from *C. scalpelliformis* to treat human diseases, but further research is strongly encouraged to elucidate the effects of oleic acid and its potential effects on using the right amount of oleic acid to treat diseases in the human population isolated from *C. scalpelliformis* extract.

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References

1) Pandey AK, Chauhan OP, Semwal AD. Seaweeds-a potential source for functional foods. *Defence Life Science Journal*. 2020;5(4):315–337. Available from: https://doi.org/10.14429/dlsj.5.15632.
2) Gephart JA, Golden CD, Asche F, Belton B, Brugere C, Froehlich HE, et al. Scenarios for Global Aquaculture and Its Role in Human Nutrition. *Reviews in Fisheries Science & Aquaculture*. 2021;29(1):122–138. Available from: https://doi.org/10.1080/23308249.2020.1782342.
3) El-Sappah AH, Seif MM, Abdel-Kader HH, Soaud SA, Elhamid MAA, Abdelghaffar AM, et al. Genotoxicity and Trace Elements Contents Analysis in Nile Tilapia (Oreochromis niloticus) Indicated the Levels of Aquatic Contamination at Three Egyptian Areas. *Frontiers in Veterinary Science*. 2022;9:818866. Available from: https://doi.org/10.3389/fvets.2022.818866.
4) FAO Fisheries & Aquaculture-Cultured Aquatic Species Information Programme- Oreochromis niloticus . 2023. Available from: https://www.fao.org/fishery/docs/CDrom/aquaculture/I1129m/file/en/en_niletilapia.htm.
5) Iqbal S, Atique U, Mahboob S, Haider MS, Iqbal HS, Al-Ghanim KA, et al. Effect of supplemental selenium in fish feed boosts growth and gut enzyme activity in juvenile tilapia (Oreochromis niloticus). *Journal of King Saud University - Science*. 2020;32(5):2610–2616. Available from: https://doi.org/10.1016/j.jksus.2020.05.001.
6) Piccinin E, Cariello M, De Santis S, Ducheix S, Sabbà C, Ntambi JM, et al. Role of Oleic Acid in the Gut-Liver Axis: From Diet to the Regulation of Its Synthesis via Stearoyl-CoA Desaturase 1 (SCD1). *Nutrients*. 2019;11(10):2283. Available from: https://doi.org/10.3390/nu11102283.
7) Chen D. Histological Staining and its Methods. *Journal of Interdisciplinary Histopathology*. 2022;10(9):1–2. Available from: https://www.ejmjih.com/ejmjih-articles/histological-staining-and-its-methods-92034.html.
8) Rocha CP, Pacheco D, Cotas J, Marques JC, Pereira L, Gonçalves AMM. Seaweeds as Valuable Sources of Essential Fatty Acids for Human Nutrition. *International Journal of Environmental Research and Public Health*. 2021;18(9):4968. Available from: https://doi.org/10.3390/ijerph18094968.
9) Arrese M, Barrera F, Triantafilo N, Arab JP. Concurrent nonalcoholic fatty liver disease and type 2 diabetes: diagnostic and therapeutic considerations. *Expert Review of Gastroenterology & Hepatology*. 2019;13(9):849–866. Available from: https://doi.org/10.1080/17474124.2019.1649981.
10) Younossi Z, Tacke F, Arrese M, Sharma BC, Mostafa I, Bugianesi E, et al. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. *Hepatology*. 2019;69(6):2672–2682. Available from: https://doi.org/10.1002/hep.30251.
11) Mahboob S, Al-Ghanim KA, Al-Balawi HF, Al-Misned F, Ahmed Z. Toxicological effects of heavy metals on histological alterations in various organs in Nile tilapia (Oreochromis niloticus) from freshwater reservoir. *Journal of King Saud University - Science*. 2020;32(1):970–973. Available from:

https://doi.org/10.1016/j.jksus.2019.07.004.
12) De Oliveira EA, Merigueti YFFB, Ferreira IB, Garcia IS, Pereira AS, Santos RDS, et al. The Role of Nile Tilapia (Oreochromis niloticus) in the Life Cycle of Toxocara spp. *Frontiers in Veterinary Science*. 2021;8:685911. Available from: https://doi.org/10.3389/fvets.2021.685911.
13) Abdel-Warith AWAW, Younis EM, Al-Asgah NA, Gewaily MS, El-Tonoby SM, Dawood MAO. Role of Fucoidan on the Growth Behavior and Blood Metabolites and Toxic Effects of Atrazine in Nile Tilapia Oreochromis niloticus (Linnaeus, 1758). *Animals*. 2021;11(5):1448. Available from: https://doi.org/10.3390/ani11051448.
14) Neamat-allah ANF, Hakim YAE, Mahmoud EA. Alleviating effects of β-glucan in<i>Oreochromis niloticus</i>on growth performance, immune reactions, antioxidant, transcriptomics disorders and resistance to<i>Aeromonas sobria</i>caused by atrazine. *Aquaculture Research*. 2020;51(5):1801–1812. Available from: https://doi.org/10.1111/are.14529.
15) Hedayatirad M, Mirvaghefi A, Nematollahi MA, Forsatkar MN, Brown C. Transgenerational disrupting impacts of atrazine in zebrafish: Beneficial effects of dietary spirulina. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2020;230:108685. Available from: https://doi.org/10.1016/j.cbpc.2019.108685.
16) Yang C, Lim W, Song G. Mediation of oxidative stress toxicity induced by pyrethroid pesticides in fish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2020;234:108758. Available from: https://doi.org/10.1016/j.cbpc.2020.108758.
17) Jawdhari A, Mihăilescu DF, Stan MS, Bălănescu MVV, Vlăsceanu RII, Staicu CA, et al. Ingestion of Polyvinylchloride Powder Particles Induces Oxidative Stress and Hepatic Histopathological Changes in Oreochromis niloticus (Nile Tilapia)—A Preliminary Study. *Sustainability*. 2023;15(8):6494–6494. Available from: https://doi.org/10.3390/su15086494.
18) Farag MR, Zheng C, Gharib HSA, El-Hady E, Mahdy EAA, Abo-Elmaaty AMA, et al. Physiological and Neurobehavioral Disturbances Induced by Al2O3 Nanoparticle Intoxication in Nile Tilapia Fish: Benefits of Dietary Chamomile Essential Oil. *Aquaculture Nutrition*. 2023;2023:1–14. Available from: https://doi.org/10.1155/2023/6700708.
19) Cui Q, Qiu L, Yang X, Shang S, Yang B, Chen M, et al. Transcriptome profiling of the low-salinity stress responses in the gills of the juvenile Pseudopleuronectes yokohamae. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*. 2019;32:100612. Available from: https://doi.org/10.1016/j.cbd.2019.100612.
20) Dawood MAO, Abdel-Razik NI, Gewaily MS, Sewilam H, Paray BA, Soliman AA, et al. β-Glucan improved the immunity, hepato-renal, and histopathology disorders induced by chlorpyrifos in Nile tilapia. *Aquaculture Reports*. 2020;18:100549. Available from: https://doi.org/10.1016/j.aqrep.2020.100549.