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Protective Properties of Milk Thistle in Aquaculture: A Study on its Role in Mitigating Supracide-Induced Stress in Fish

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Abstract

This investigation sought to comprehensively evaluate the protective properties of Milk Thistle seeds against Supracide-induced oxidative stress in the Marbled Spinefoot fish. Liquid chromatography–mass spectrometry (LC–MS) was employed to identify metabolites present in *Silybum marianum* seeds. The LC_{50} value of organophosphorous insecticide was determined as 7.5 µg L^{-1} through a 24-hour bath exposure. The experimental setup involved a 14-day dietary supplementation of *S. marianum* seeds followed by exposure to the determined Supracide LC_{50}. Various physiological parameters, including lipid degradation, Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), and Total cholesterol (T.ch) levels, were measured to assess fish health and stress. Additionally, histopathological examinations were conducted on the hepatopancreas tissues of both normal and stressed fish. The obtained results indicated significant increases ($P<0.05$) in serum ALT and LDH activities in the group of fish exposed to the toxin. Moreover, the levels of lipid peroxidation products in hepatopancreas homogenates were significantly higher ($P<0.05$) in the exposed fish group, except for total cholesterol, which exhibited a significant decrease. Histological analysis further revealed notable tissue modifications in the examined organs, while the control group of fish exhibited normal appearance. Taken together, these findings suggest that Milk thistle seeds may possess protective effects against Supracide-induced oxidative stress in challenged *S. rivulatus* fish. As a result, this study provides valuable insights into potential alternative ameliorating agents for addressing aquaculture contamination caused by organophosphate insecticides.

Keywords: Malonaldehyde; Supracide; *Silybum marianum*; Oxidative stress; *Siganus rivulatus.*

1. Introduction

Aquaculture production is a crucial contributor in meeting the global demand for fish food, providing more than 46.8% of the total supply (FAO, 2020). Aquaculture production is susceptible to contamination, resulting in significant losses. However, contaminants originating from industrial chemicals that kill insects, rodents, pests, fungi, and their byproducts raise concerns about the potential health risks for aquatic organisms (Tudi et al., 2021). In particular, insecticides are highly impactful environmental contaminants that have been increasingly released into the environment in recent decades (Rad et al., 2022). Synthetic insecticides, has raised concerns about their impact on non-target organisms, particularly aquatic life. Among the synthetic insecticides, organophosphates (OPs) have gained attention due to their high toxicity and potential threat to the environment (Mali et al., 2023).The inhibition of acetylcholinesterase by OPs is well-established and explains their acute neurotoxic effects. However, recent studies have revealed that OPs can also generate reactive oxygen species (ROS), leading to oxidative stress. This occurs through various mechanisms, including the disruption of cellular redox balance, inhibition of antioxidant enzymes, and induction of lipid peroxidation (Sule et al., 2022).

An important factor influencing the development of pathological conditions in farm animals is oxidative stress, which results from an imbalance between the productions of reactive oxygen species (ROS) and antioxidant defenses. This condition can significantly affect animal production and overall well-being. It is crucial to comprehend the connection between oxidative stress and its consequences for farm animals to implement effective management strategies and enhance animal welfare (Song et al., 2023). Stressors, which can be internal or external in nature, trigger the activation of neurohormonal regulatory mechanisms aimed at maintaining homeostasis. These factors, combined with the body's ability to adapt, determine the alterations in animal maturation, productivity, growth, and all-around healthiness quality (Wahsha et al., 2023). Disruptions in homeostasis result in the overproduction of molecular species known as free radicals, overwhelming the organs' detoxification capacity (Trachootham et al., 2008). Excess free radicals can cause oxidative deterioration to lipid membranes and different organelles within cells (Martemucci et al., 2022). Animals have evolved mechanisms to counteract oxidative stress by enhancing their cellular defenses, primarily through the

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activation of antioxidant enzymes (Halliwell and Gutteridge, 2015). Moreover, certain dietary antioxidants have been shown to help mitigate the damaging consequences of ROS and promote overall health (Sies and Jones, 2020). As a result, medical plants with antioxidant properties have been considered as potential candidates for animal diets, as they may offer a protective role against various diseases (Mahima et al., 2012).

The use of plant extracts in aquaculture has recently gained attention due to their beneficial properties, which can contribute to fish health management control (Reverter et al., 2014). Medicinal plants have immense potential in the management of fish health by modulating the immune system. Through their immunomodulatory activity, these plants have the capability to enhance fish immune responses, bolster disease resistance, and improve stress tolerance, thereby serving as valuable assets in aquaculture (Nipa et al., 2021). Furthermore, these plants synthesize secondary metabolites, such as terpenoids and flavonoids, which exhibit diverse chemical structures and biological activities, playing a vital role in their mechanism of action (Yeshi et al., 2022). Among the Asteraceae family, milk thistle (*Silybum marianum* (L.) Gaertn) is indeed an important medicinal plant species. Milk thistle is native to the Mediterranean region but is now cultivated worldwide due to its therapeutic properties. The plant has a long history of traditional use and has been extensively studied for its potential health benefits (Bhattacharya, 2020; Csupor et al., 2016; Surai, 2015). In addition to its liver-protective effects, milk thistle has also shown potential in other areas of health. Studies have suggested its anti-inflammatory properties may be beneficial for conditions such as diabetes, cancer, and cardiovascular diseases (Wahsha and Al-Jassabi, 2009). Moreover, milk thistle's antioxidant activity has been linked to its protective effects against oxidative stress and certain types of cellular damage (Deep et al., 2006). Aquaculture is an important industry that often relies on the use of veterinary drugs to prevent and treat diseases in fish and other farmed organisms. However, the use of these drugs in aquaculture can have potential negative effects on the environment and human health. To address these concerns and seek more sustainable alternatives, researchers have turned to natural products derived from plants and other flora sources for use in aquaculture practices (Tadese et al., 2022). One approach is the incorporation of natural products, such as those derived from flora origins, in aquaculture approaches (Mohamad Nek Rahimi et al., 2022). The present investigation was conducted to assess the hepatopancreatic protective properties of Milk thistle (*Silybum marianum*) seed extract against the cytotoxic effects induced by Supracide insecticide, known for its oxidative properties, in the fish species *Siganus rivulatus* (Forsskål & Niebuhr, 1775). The evaluation of Supracide-induced oxidative stress involved quantifying malondialdehyde (MDA) levels as a marker of lipid peroxidation, performing serum biochemical assays, and examining histopathological alterations in the hepatopancreas.

2. Materials and methods:

2.1. Fish samples

During the summer of 2018, a study was conducted at the Marine Science Station in Aqaba (located between the coordinates 29°27'20.9"N and 34°58'26.3"E). The study focused on male fish species. According to Khalaf and Disi's (1997) classification, the fish species known as Rivulated rabbitfish was identified as *Siganus rivulatus* (Forsskål & Niebuhr, 1775). Carefully selected fish samples were chosen from the aquaculture facility and transferred to the laboratory, where the temperature was maintained at 25 ± 3 °C. The fish were given a week to acclimate to these conditions. All the selected fish samples were of the same age, approximately six months old, with an average body weight of 70 ± 15 grams and a length of approximately 10 centimetres.

2.2. Plant samples:

Silybum marianum is a plant from the Asteraceae family and is also known as "ripe milk thistle". This plant is native to the Mediterranean and Middle Eastern regions. The ripe milk thistle (*Silybum marianum*) seeds were meticulously collected from locally cultivated plants in North Jordan, all of which were in the same growth stage and displayed robust physical characteristics. Per the classification by Webb et al. (1988), Milk thistle is identified as *Silybum marianum* (L.) Gaertn. The harvesting procedure adhered to the prescribed techniques documented by Curioni et al., (2002). To ensure optimal preservation and retain their freshness, the seed samples were packed in dark plastic bags and transported to the laboratory. Following transportation, the seeds underwent a 24-hour drying period at a temperature of 50˚C. Once completely dried, the samples were finely ground into a powder with particle sizes smaller than 5µm.

2.3. Metabolite determination:

Metabolite extraction, following De Vos et al. (2007) protocol, involved homogenizing and grinding dried seeds with a Retsch grinder (MM 400, Haan, Germany). Three replicated samples and three blank samples ensured precision and contamination prevention. Phenyl-$^{13}C_6$ salicylic acid served as an internal standard (IS). Seeds (50 mg ± 0.5) were weighed, extracted for 30 min in an ultrasonic bath (1.5 ml methanol/water, 75:25, v:v), and centrifuged for 20 min at 14,000 rpm. The supernatant, filtered through PTFE syringe filters (Ø 25 mm, 0.2 µm), underwent LC-MS analysis. Raw chromatographic data, aligned and corrected using MetAlign 3.0 (Lommen & Kools, 2012), and MSClust (Tikunov et al., 2012) for clustered mass signals, were processed using XcaliburTM software for molecular formulas. Metabolite identification utilized online libraries (Metlin, HMDB, Dictionary of Natural Products, and LIPID MAPS Structure Database) and literature, considering mono isotopic mass and fragmentation pattern.

2.4. Model of toxicity

In this study, Supracide (organophosphorous ≥ 95.0 %) was utilized as a representative oxidative agent. The Supracide insecticide used in the research was procured from Sigma.

2.5. Animals and ethics

In this research, the test fish were handled in strict accordance with the Global Guidelines for Animal Experimentation (1986). Every experimental protocol was thoroughly reviewed and approved by the animal welfare and ethics committees at the Oceanographic Research Centre, The University of Jordan, and Yarmouk University, Jordan. This ensured the appropriate care and welfare of the fish (session No. (4)/2015/2016, held on 7/28/2016).

2.6. LC_{50} determination

This study employed the up-and-down toxicity test method, initially introduced by Sunderam et al. (2004), to assess toxicity levels. This approach involves systematically adjusting the concentration of the toxic substance, either upward or downward in increments, until a clear pattern of responses is observed in the test organisms. The study spanned a single day, during which a functional solution of Supracide (40.03% w/v) was prepared to create specified toxin doses. Distinct quantities of the toxic substance (2.85, 1.12, 0.49, 0.2, 0.09, 0.04, 0.0175, 0.0075, and 0.0033 mg L^{-1}) were allocated to separate aquariums for the experiment, each with a volume of 60 liters. In each aquarium, ten fish, with an average weight of 70 grams and an average standard length of 10 centimeters, were housed. Throughout the 24-hour duration of the experiment, a meticulous record of the mortality rate and indicators of toxicity was consistently maintained and documented.

2.7. Fish bioassay and toxicity challenge

Forty fish were brought to the laboratory, where the water temperature was kept at 25 ± 3 °C. The fish were divided into four groups following the information in Table 1. The trials were conducted in glass aquariums containing 150 L of the specific test solution. The fish were allowed to acclimatize to the laboratory environment for seven days before the experiments began.

Table 1. Experimental design and conditions for the toxicity challenge experiment

Group	Description	Number of sacrificed fishes/time course
C	The control group did not receive any supplementation of crude extract or treatment with toxins.	Ten fish were sacrificed after 24 hrs (C24).
TC	The toxin control group was treated with Supracide (specific toxin) at a dose of 7.5 µg L^{-1}, based on the LC_{50} value.	Ten fish were sacrificed after 24 hrs (TC24).
SC	The control group for Milk thistle received a daily addition of 2.5g of the same per kg of aquatic creature mass, with this routine continued for a two-week period.	Ten fish were sacrificed after 24 hrs (SC).
ST	The fish were supplemented daily with 2.5g of Milk thistle per kilogram of fish body weight for 14 days, and after that, they were treated with Supracide at a dose of 7.5 µg L^{-1}.	Ten fish were sacrificed after 24 hrs (ST24).

Each fish was subjected to cardiac puncture to obtain blood samples using vials without anticoagulant. The collected blood was then centrifuged at 3000 X g for 30 minutes to separate the serum, which was later used for the enzyme assay. The tubes containing the serum were promptly sealed and stored at 4ºC for future use. Moreover, the hepatopancreas was immediately isolated and washed with phosphate-buffered saline at a pH of 7.2. A section of the isolated hepatopancreas was stored at -20ºC for biochemical tests, while another section was fixed in buffered formalin for histopathological analysis.

2.8. Biochemical assays

The level of malondialdehyde (MDA) in the hepatopancreas was determined following the protocol outlined by Wahsha et al. (2017). Furthermore, Cormay diagnostic kits were employed to measure the levels of lactate dehydrogenase (LDH), serum alanine transaminase (ALT), and total cholesterol (T.Ch).

2.9. Histopathological examination

To conduct the histopathological analysis, small 0.3 cm thick fragments of hepatopancreas tissues were fixed in 10% buffered formalin for 24 hours, following the method described by Al-Haj (2010). The fixed tissues were then gently washed under running tap water and dehydrated through increasing concentrations of alcohol. Subsequently, they were infiltrated with xylene and embedded in paraffin wax. Sections with a thickness of 6µm cut using a rotatory microtome, mounted with DPX and stained with Ehrlich Hematoxylin and Eosin (H&E), and the stained sections were examined using light microscopy.

2.10. Data processing and Statistical analysis.

In order to derive meaningful data from the raw chromatographic results, we employed a couple of specialized software tools. The task of spectral alignment and baseline correction was managed by MetAlign 3.0 whereas MSClust was used for the purpose of securing clustered mass signals, effectively representing reconstructed metabolites (Rizzato et al., 2017). XcaliburTM software (Thermo Scientific Inc.) was used to ascertain the molecular formulas. Further, we adhered to a set procedure for identifying metabolites, which involved predicting the most likely molecular formula according to the fragmentation pattern. We matched the mono-isotopic mass and mass spectra with published data and several online databases such as HMDB, Metlin, LIPID MAPS Structure Database, and the Dictionary of Natural Products. Sumner et al.'s (2007) method was adopted to designate the identification level. For the biochemical experiments, the statistical assessment was carried out using Sigma Stat Software version 3.5, and a significance threshold of $p < 0.05$ was set.

3. Results

3.1. Analysis of plant metabolites:

From the LC-MS chromatogram, a total of 126 secondary metabolites were detected. Out of these, 37 compounds were categorized as level 2 identifications, while the remaining compounds were classified as level 3 identifications based on Sumner et al.'s (2007) classification system. The primary constituents of the Milk thistle seed extract were found to be flavonoids and terpenoids, each accounting for approximately 22% of the total relative abundance. Additionally, glycosides comprised 14% of the identified compounds; fatty acids constituted 9%, and a diverse array of other compounds made up the remaining 33%. Notably, Table 2 showcases a selection of the most remarkable compounds among the 126 identified ones.

Table 2 Certain compounds of significance have been discovered in the seed extract of *Silybum marianum*

Formula	Compound	Biological/Pharmacological Activities	Reference
$C_{25}H_{22}O_{10}$	Silibinin (silymarin)	Antioxidant, Anti-inflammatory, Antiviral, Antitumor and Hepatoprotective.	Csupor et al., 2016.
$C_{15}H_{12}O_5$	Naringenin	Hepatoprotective, Anti-inflammatory, Anticancer, Antimutagenic, and Antimicrobial agent	Karim et al., 2018.
$C_{15}H_{12}O_6$	Eriodictyol	Antioxidant and Anti-inflammatory effects	Narvaez-Mastache et al., 2008.
$C_{15}H_{10}O_6$	Luteolin	Antioxidant, Antitumor, Anti-inflammatory, and Antiapoptotic efficacy	Zhang et al., 2011.
$C_{18}H_{18}O_6$	4'-Hydroxy-5,6,7-trimethoxyflavanone	Antimycobacterial activity	Suksamrarn et al., 2004.
$C_{25}H_{20}O_{10}$	2,3- Dehydrosilybin	Antioxidants	Reina and Martínez, 2015.
$C_{27}H_{48}O_{14}$	Naringin	Antioxidant, Lipid-lowering, Antimicrobial, Anti-inflammatory and Anticancer	Jeon et al., 2004.
$C_{28}H_{26}O_{16}$	Taxillusin	Antimicrobial activities	Fukunaga et al., 1989.
$C_{14}H_{18}O_9$	Vanillic acid 4-O-β-d-glucopyranoside	Antioxidants	Chemam et al., 2017.
$C_{16}H_{18}O_9$	Chlorogenic acid	Antioxidant and Anti-inflammatory activities	Farah et al., 2008.
$C_{17}H_{26}O_{10}$	Login	Cognitive enhancing and Free radical scavenging capacity	Lee et al. 2009.
$C_{18}H_{26}O_{11}$	Oleoside dimethyl ester	Antioxidant activity	Wang et al., 2010.
$C_{21}H_{32}O_{12}$	Darendoside B	Antioxidant activity	Pan et al., 2003.
$C_{17}H_{26}O_5$	Hymenolide	Phagostimulant activity	Juárez et al., 2014.
$C_{16}H_{32}O_2$	Palmitic acid	Antimicrobial activity	Huang et al., 2011.
$C_{18}H_{30}O_2$	Alpha-Linolenic acid	Neuroprotective and Anti-inflammatory	Nicolas et al., 2015.
$C_{18}H_{32}O_2$	Linoleic acid	Antibacterial activity	Zheng et al., 2005.
$C_{18}H_{34}O_3$	Ricinoleic acid	Antibacterial activity, Anti-inflammatory	Abdul et al., 2018.

3.2. LC_{50} determination

The fish were subjected to various concentrations of Supracide, including 2.85, 1.12, 0.49, 0.2, 0.09, 0.04, 0.0175, 0.0075, and 0.0033 mg L^{-1}, for duration of 24 hours. All groups, except for the control group, showed significant changes in behavior over the entire 24-hour test period, with the control group exhibiting no behavioral modifications and maintaining a 100% survival rate for all fish. These observed behavioral changes included loss of equilibrium, rapid and circular swimming, and dullness, surface swimmining, and increased opercular movement. The results indicated varying mortality rates among the fish, ranging from 0% to 100%. It was evident that the mortality rate was dependent on both the dosage and the duration of exposure. The LC_{50}-24h (lethal concentration at which 50% of the fish died after 24 hours) was estimated to be 7.5 μg L^{-1}.

3.3. Biochemical assays

The study aimed to understand the effects of Supracide on biochemical biomarker levels in *S. rivulatus* fish, and how Milk thistle seed extract might influence these effects. MDA levels were mainly analyzed in the fish's hepatopancreas (a digestive gland in some aquatic animals), and other blood serum biochemical markers were also measured. The data indicates significant differences in biomarker levels between the various groups, highlighting the potential impact of Supracide and the influence of Milk thistle seed extract on these biochemical parameters, as demonstrated in Table 3. Our results suggest that the organophosphorous insecticide has considerable effects on the fish's biochemical profile and the Milk thistle extract may have some mitigating effects.

Table 3. The results of the study evaluated the impact of Supracide on biochemical biomarker levels in *S. rivulatus* fish when subjected to bath administration of LC_{50} (7.5 µg L^{-1}), both with and without Milk thistle seed extract.

Group	C24	TC24	SC24	ST24
MDA+ (µmol/g)	4.2 ± 0.2	16.4 ± 0.8***	5.1 ± 0.2	5.7 ± 0.2
ALT++ (IU/ml)	11 ± 2	316 ± 2***	36 ± 1***	23 ± 3
LDH++ (IU/ml)	338 ± 9	12643 ± 45***	936 ± 17***	1455 ± 18***
T.Ch++ (IU/ml)	625 ± 12	30 ± 5***	726 ± 21***	253 ± 9***

The experimental groups were categorized as follows: C - Control group without extract or Supracide treatment, TC - Toxin Control group treated, SC - Milk thistle control group supplemented daily with 2.5g *S. marianum*/kg fish body weight for 14 days, and ST - Fish supplemented daily with 2.5 g Milk thistle/kg fish body wt for 14 days, followed by treatment with LC_{50} 7.5 µg (toxin) L^{-1}. The reported values represent the means of 3 replicates with standard deviation (SD).

+: The impact of Supracide (LC_{50} 7.5 µg L^{-1}) on MDA levels in fish hepatopancreas and++:The levels of blood serum biochemical markers. The results are expressed as means ± SD, with T representing time in hours. *** denotes statistical significance at $P < 0.05$, determined by an ANOVA test.Oxidative degradation of lipids (MDA levels)

The MDA levels in the fish hepatopancreas were significantly different among the groups ($p < 0.001$). The Toxin Control group (TC24) showed a substantially higher MDA level compared to the Control group (C24) and the Milk Thistle Control group (SC24). This indicates that exposure to the toxin (Supracide) led to increased oxidative stress and lipid peroxidation in the hepatopancreas of the fish. The Milk Thistle-supplemented group followed by toxin treatment (ST24) did not show a significant increase in MDA levels compared to the Control group, suggesting a potential protective effect of Milk Thistle extract.

3.3.1. Alanine transaminase (ALT)

ALT levels, an indicator of liver function, exhibited significant differences between the groups ($p < 0.001$). The Toxin Control group (TC24) had markedly elevated ALT levels compared to the other groups, indicating liver damage due to toxin exposure. The Milk Thistle Control group (SC24) and the Milk Thistle Toxin group (ST24) had ALT levels closer to the Control group (C24), implying that Milk Thistle supplementation might have a mitigating effect on toxin-induced liver damage.

3.3.2. Lactate dehydrogenase (LDH)

LDH levels, which reflect tissue damage, were significantly different among the groups ($p < 0.001$). The Toxin Control group (TC24) exhibited a substantial increase in LDH levels compared to the other groups, indicating tissue injury caused by the toxin. Both Milk Thistle-supplemented groups (SC24 and ST24) showed LDH levels closer to the Control group (C24), suggesting a potential protective role of Milk Thistle against toxin-induced tissue damage.

3.3.3. Total Cholesterol (T.Ch)

Total cholesterol levels also displayed significant differences between the groups ($p < 0.001$). The Toxin Control group (TC24) had remarkably reduced cholesterol levels compared to the other groups. This reduction could be due to the disruptive effects of the toxin on lipid metabolism. The Milk Thistle Control group (SC24) and the Milk Thistle Toxin group (ST24) had cholesterol levels closer to the Control group (C24), hinting at a possible protective influence of Milk Thistle on lipid homeostasis.

3.4. Histopathological changes in hepatopancreas

The control group's fish exhibited a typical histological structure in their hepatopancreas. The hepatocytes displayed central nuclei with a spherical shape, along with a significant presence of blood sinusoids (Figure 1). Melanomacrophage centers (MMC) were found in the hepatic parenchyma, usually near the hepatic arteries or bile ducts. In contrast, the liver sections of fish exposed to the LC_{50} concentration of Supracide for 24 hours displayed a range of histopathological changes. These changes included more severe damage characterized by dilation of intercellular spaces, blood congestion, and necrosis accompanied by cytoplasmic vacuolization (glycogen and lipid). Furthermore, there were some observed hepatic morphological alterations, albeit to a lesser extent, in the fish treated with the toxin and supplemented with Milk thistle (Figure 1).

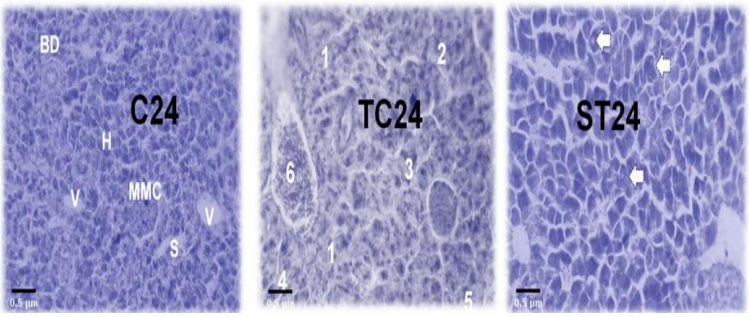

Figure1. Illustrative photomicrograph of hepatopancreas tissue slices derived from the following groups of fish: Control (C24), Toxic control (TC24), and toxin/Milk thistle pre-exposed (ST24) groups.C24: (H) Hepatocellular cells; (V) Hepatic vein; (BD) Bile duct; (MMC) Melanomacrophage clusters; (S) Sinusoidal cavity. TC24: (1) Localized necrosis and absence of nuclie; (2) Pyknotic nuclei; (3) Nuclier hypertrophy; (4) Cytoplasmic vacuolization; (5) Fatty Change; (6) Vascular congestion. ST24: Fish pre-exposed to toxin/Milk thistle (arrow) showcasing Cytoplasmic vacuolization. Stain used: H & E; Magnification at 40X.

4. Discussion

In the past few years, there has been a significant increase in the number of studies presenting compelling evidence on the connection between organophosphate pesticides, particularly Supracide, and oxidative stress induction in aquatic life (Sule et al., 2022; Özkan-Yılmaz et al., 2015). Despite a wealth of scientific data underscoring the harmful effects of organophosphates, their influence on Aquaculture has been given minimal consideration by researchers globally. Supracide (S-[(5-Methoxy-2-oxo-1,3,4-thiadiazol-3(2H)-yl)methyl] O,O-dimethyl phosphorodithioate), a potent organophosphate pesticide, sees broad application. Our study determined the Supracide concentration that caused mortality in 50% of the test fish (LC_{50}) to be 7.5 µg L^{-1} by employing an adjusted up and down toxicity test (Sunderam et al., 2004). It is noteworthy that this value diverges from previous findings on aquatic organisms such as Sheepshead minnow, rainbow trout, bluegill, and goldfish (Flaherty et al., 2009). Differences in LC_{50} values across various animal models can be traced back to elements such as species, age, and sex of the organisms involved (Ghosh and Saha, 2022).

In the present investigation, we documented signs of cellular harm following Supracide exposure, evaluated through particular biomarkers and histopathological specimen analysis. The main toxicity mechanism related to organophosphate pesticides is the suppression of acetylcholinesterase, a critical enzyme for neural operation (Fernandes et al., 2015; Lu et al., 2018; Sandoval-Herrera et al., 2019; Tsai and Lein, 2021). Latest studies imply that oxidative stress plays a pivotal role in organophosphates' toxic effects by boosting the generation of reactive oxygen species (ROS) and unsettling the equilibrium between ROS and antioxidant defense mechanisms (Martinez-Alvarez et al., 2005; Cortes-Iza and Rodriguez, 2018; and Sandoval-Herrera et al., 2019). In addition, research has suggested that lipid peroxidation (LPO) is a molecular mechanism involved in organophosphate toxicity and liver injury (Oruc, 2011, and Karami-Mohajeri et al., 2017).

Our study discovered a shift in the MDA (malondialdehyde) level in the liver and pancreas tissue of the fish used in the experiment following exposure to Supracide at the LC_{50} value. These outcomes corroborate prior research carried out by Modesto and Martinez, (2010) and Abhijith et al., (2016) and propose that reactive oxygen species (ROS) play a part in the onset of Supracide-induced damage. Several studies have reported that organophosphate pesticides could disturb antioxidant defense enzymes, leading to a surge in lipid peroxidation products by directly interacting with organic molecules in the cell membrane (Wu et al., 2011; Abhijith et al., 2016, and Yang et al., 2020). MDA, a lipid peroxidation byproduct, has been used as a biomarker for oxidative stress and as an indicator of the degree of lipid peroxidation (Tsikas, 2017, and Ito et al., 2019). In our investigation, we noticed an increase in MDA levels in the liver and pancreas tissue of *S. rivulatus* fish treated with the LC_{50} value of Supracide, suggesting that MDA levels could serve as an indicator of Supracide-induced damage.

Animal cells have various methods to neutralize oxidative stress's detrimental effects, either by repairing the inflicted damage or by inhibiting and neutralizing reactive oxygen species (ROS) via enzymatic and non-enzymatic antioxidant defense systems (Ponnampalam et al., 2022). Polyphenols, including flavonoids, are part of the non-enzymatic defense mechanism (Panche et al., 2016) and have demonstrated potential as therapeutic agents against a broad spectrum of ROS (Kumar and Pandey, 2013; Ullah et al., 2020; and Khan et al., 2021). Our results indicate that Supracide poisoning may incite the production of highly unstable free radicals capable of attacking lipids containing carbon-carbon double bonds, especially polyunsaturated fatty acids (PUFA). Past studies in our lab have underscored the impacts of milk thistle seed extracts on lipid peroxidation (Wahsha and Al-Jassabi, 2009) and antioxidant enzymes (Wahsha et al., 2012). Wilson Magdy et al (2016) showed that treatment with alpha-tocopherol (vitamin E) and ascorbic acid (vitamin C) post-Supracide administration could mitigate liver damage in rats. Karami-Mohajeri et al (2017) reported that sublethal Supracide doses could compromise the antioxidant enzymatic defense system and escalate lipid peroxide levels in the liver and pancreas of fish.

Biochemical markers are vital in the diagnosis of diseases in fish and for the detection of tissue impairment instigated by environmental toxins, functioning as sensitive detectors of metabolic perturbations in the organism (Bharti and Rasool, 2021). Enzymes such as ALT and LDH are often deployed in the identification of fish diseases, with their serum levels reflective of physiological changes or stress responses. These enzymes are valuable indicators of minor cellular disruptions (Ahmet Deveci et al., 2022).Our research demonstrated that the group of *S. rivulatus* fish exposed to Supracide exhibited a significant elevation in serum ALT and LDH activities compared to the control group. This observation indicates that severe stress conditions in fish lead to an augmented requirement for energy, driving the need for carbohydrates and other key compounds to uphold the glycolytic pathway and tricarboxylic acid cycle (Tiwari and Singh, 2004). Fish affected by toxicants adapt their metabolic processes to mitigate the stressors and sustain homeostasis (Petitjean et al., 2019). Prior research has shown that stress commonly results in enhanced aminotransferase activity, and specific studies have noted increased ALT activity, indicative of increased energy requirements due to tissue damage (Jee et al., 2005; Samanta et al., 2014). Additionally, ALT increase signifies liver tissue damage, caused by toxicant-induced harm to the liver, resulting in serum leakage of cellular enzymes. Hence, elevated enzyme activity is linked to liver damage inflicted by pesticides (Gowda et al., 2009; Vasantharaja et al., 2015; Xavier Martins et al., 2021).

LDH, a pivotal enzyme in fulfilling the energy needs of cells under varying oxygen conditions, can be used to signal the release of the enzyme from damaged cells, shifts in protein and carbohydrate metabolism, and cell leakage (Alarifi et al., 2012; Klein et al., 2020). A significant upsurge in LDH activity was recorded during Supracide exposure in our study, implying disruption in carbohydrate metabolism due to Supracide. Additionally, the notable rise in LDH activity could be associated with tissue harm inflicted by Supracide toxicity. Such alterations in LDH activity underscore the stress conditions experienced by the fish. Other organisms exposed to substances like carbamazepine, selenium, ibuprofen, clofibric acid, and

diclofenachave also demonstrated similar changes in LDH activity (Jos et al., 2003; Saravanan et al., 2011; Saravanan et al., 2012; Ramesh et al., 2014).Moreover, in our study, when fish were pre-exposed to Milk thistle seeds before Supracide exposure, an amplified serum LDH value was recorded, implying increased LDH activity. A more pronounced increase was observed in the group pre-treated with Milk thistle seeds than in the control group. These findings suggest that a 14-day pre-treatment with Milk thistle seeds offers significant recovery protection against Supracide toxicity.

In our experiment, serum T.Ch levels significantly decreased after Supracide exposure. Similar outcomes were reported in previous studies where various insecticides, such as dichlorvos, acephate, and cypermethrin, were used on different experimental animals (Ryhanen et al., 1984; Choudhari and Chakraharti, 1984; Shakoori et al., 1988). Lipids, vital components of animal cell plasma membranes, participate in numerous biological processes in the body (Muro et al., 2014).Earlier studies showed that Supracide could trigger the oxidative degradation of lipids through reactive oxygen species (ROS) action (Sule et al., 2022).Additionally, Supracide and other organophosphate insecticides might inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, an essential enzyme for cholesterol synthesis (Ryhanen et al., 1984). The reduced serum total cholesterol levels could be due to the organophosphate-stimulated activation of the low-density lipoprotein (LDL) receptor, increasing the cholesterol clearance from the bloodstream (Ibrahim and El-Gamal, 2003).

Histological findings and biochemical data contribute to a thorough understanding of the death mechanisms in organisms exposed to toxic substances (Orrenius et al., 2011). As such, they are effective tools for assessing the health status of fish. The results of our study showed increased histopathological alterations in hepatopancreas tissue after Supracide exposure. However, pre-treatment with Milk thistle seed led to a remarkable decrease in these histopathological alterations. Similar observations were reported where Supracide exposure led to a notable rise in rat liver enzyme activities and morphological liver changes in carp exposed to Supracide (Asztalos et al., 1990; Altuntas et al., 2002; Karami-Mohajeri et al., 2017).

The phytochemical analysis of milk thistle seed extract revealed a remarkable array of bioactive compounds with diverse biological and pharmacological activities. Among the compounds identified, silibinin (silymarin) stood out for its numerous benefits. Csupor et al. (2016) reported silibinin to possess antioxidant, anti-inflammatory, antiviral, antitumor, and hepatoprotective properties, making it a potential candidate for combating oxidative stress, inflammation, viral infections, and even supporting liver health. Additionally, naringenin, as studied by Karim et al. (2018), demonstrated hepatoprotective, anti-inflammatory, anticancer, antimutagenic, and antimicrobial activities, indicating its potential in protecting the liver, combating inflammation, and acting as an agent against cancer and various microorganisms.

Other compounds found in milk thistle seed extract also exhibited promising attributes. Eriodictyol, as identified by Narvaez-Mastache et al. (2008), exhibited antioxidant and anti-inflammatory effects, making it valuable in neutralizing free radicals and reducing inflammation. Luteolin (Zhang et al., 2011) showed antioxidant, antitumor, anti-inflammatory, and antiapoptotic efficacy, suggesting its potential role in cellular protection, tumor inhibition, and antiapoptotic properties. Moreover, compounds like 4'-hydroxy-5,6,7-trimethoxyflavanone, 2,3-dehydrosilybin, and chlorogenic acid (Suksamrarn et al., 2004; Reina and Martínez, 2015; Farah et al., 2008) were found to possess antioxidant properties, which could contribute to their potential health benefits in combating oxidative stress-related disorders. The presence of other compounds, as reported in the table 2, further highlights the diverse pharmacological potential of milk thistle seed extract.

In summary, the results suggest that Supracide has detrimental effects on the biochemical biomarkers in *S. rivulatus* fish, leading to oxidative stress, liver damage, tissue injury, and disruption of lipid metabolism. Milk Thistle seed extract supplementation appears to provide some level of protection against these negative impacts, as indicated by the reduced levels of these biomarkers in the Milk Thistle-treated groups. The observed hepatoprotective effects may be attributed to the rich presence of active phytoconstituents found in the Milk thistle extract, including flavonoids, terpenoids, polyphenols, glycosides, and essential fatty acids. These findings highlight the potential benefits of Milk Thistle extract in mitigating the adverse impacts of the toxin on fish health and biochemistry of the fish hepatopancreas. However, further research is necessary to better understand the mechanisms underlying these effects and to validate the potential therapeutic role of Milk Thistle in such contexts.

References

Abdul WM, Hajrah NH, Sabir SM, Al-Garni SM, Sabir MJ, Kabli SA, Saini KS and Bora RS. 2018. Therapeutic role of *Ricinus communis L.* and its bioactive compounds in disease prevention and treatment. ASIAN PAC J TROP MED, **11(3):** 177-185.

Abhijith BD, Ramesh M and Poopal RK. 2016. Responses of metabolic and antioxidant enzymatic activities in gill, liver and plasma of Catla catla during methyl parathion exposure. J Basic Appl Zool, **77**, 31–40.

Ahmet Deveci H, Nur G, Deveci A, Kaya I, Mavioglu Kaya M, Kükürt A, Gelen V, Faruk Başer O and KarapehlivanM. 2022. An Overview of the Biochemical and Histopathological Effects of Insecticides. IntechOpen. doi: 10.5772/intechopen.100401

Alarifi S, Al-Doaiss A, Alkahtani S, Al-Farraj SA, Saad A, Al-Dahmash B, Al-Yahya H and Mubarak M. 2012. Blood chemical changes and renal histological alterations induced by gentamicin in rats, Saudi J Biol Sci, **19**: 103–110.
doi: 10.1016/j.sjbs.2011.11.002.

AL-Haj H. 2010. **Optical laboratory preparations**, Massira Press, Amman, Jordan, P238.

Altuntas I, Delibas N, Demirci M, Kilinc I and Tamer N. 2002. The effects of methidathion on lipid peroxidation and some liver enzymes: role of vitamins E and C, Arch Toxicol, **76**: 470–473, Doi:10.1007/s00204-002-0359-1.

Asztalos B, Nemcsok J, Benedeczky I, Gabrie R, Szabo A and Refaie OJ. 1990. The effects of pesticides on some biochemical parameters of carp (*Cyprinus carpio L.*)., Arch Environ Contam Toxicol, **19**: 275-82, https://doi.org/10.1007/BF01056097.

Bharti S and Rasool F. 2021. Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on Channa punctatus (Bloch) fish. Toxicol Rep, **25(8),** 443-455. doi: 10.1016/j.toxrep.2021.02.018.

Bhattacharya S, Milk thistle seeds in health. In: Preedy VR, Watson RR. (Eds.). **Nuts and Seeds in Health and Disease Prevention.** 2nd Ed., London, San Diego: Academic Press (an imprint of Elsevier Inc.); 2020, p. 429-438.
DOI: 10.1016/B978-0-12-818553-7.00030-9.

Chemam Y, Benayache S, Marchioni E, Zhao M, Mosset P and Benayache F. 2017. On-line screening, isolation and identification of antioxidant compounds of *Helianthemum ruficomum*. Molecules, **22**: 239.

Choudhari PD and Chakraharti CH. 1984. Effect of acephate (Orthene), an organophosphorus insecticide, on lipid metabolism in albino rats. Indian J Exp Biol, **22**: 45-49.

Cortés-Iza SC, Rodríguez AI. 2018. Oxidative Stress and Pesticide Disease: A Challenge for Toxicology. Rev. Fac. Med, https://doi.org/10.15446/revfacmed.v66n2.60783.

Csupor D, Csorba A and Hohmann J. 2016. Recent advances in the analysis of flavonolignans of *Silybum marianum*. J Pharm Biomed Anal. **130**: 301–317.

Curioni A, Garcia M, Alfonso W and Arizio O. 2002. Milk thistle harvest prediction through the external characteristics of the heads. Acta Hortic, **569**, 257–261.

Deep G, Agarwal R, Kroll DJ and Agarwal C. 2006. Silymarin and silibinin cause G1 and G2-M cell cycle arrest via distinct circuitries in human prostate cancer PC3 cells: a comparison of flavanone silibinin with flavanolignan mixture silymarin. Oncogene. 16;**25(7):**1053-69. doi: 10.1038/sj.onc.1209146.

De Vos RCH, Moco S, Lommen A, Keurentjes JJB, Bino RJ and Hall RD, 2007. Untargeted large-scale plant metabolomics using liquidchromatography coupled to mass spectrometry. Nat Protoc. **2(4),** 778–791.

Farah A, Monteiro M, Donangelo CM and Lafay S. 2008. Chlorogenic acids from green coffee extract are highly bioavailable in humans. J Nutr, 138(12): 2309-2315.

Fernandes LS, Emerick GL, Santos NA, de Paula ES, Barbosa FBF, Jr and Santos AC. 2015. In vitro study of the neuropathic potential of the organophosphorus compounds trichlorfon and acephate. Toxicol In Vitro, **29**, 522–528. 10.1016/j.tiv.2015.01.001

Flaherty C, Moore K and Hetrick J. 2009. Risks of Methidathion Use to Federally Threatened California Red-legged Frog - Pesticide Effects Determination. Environmental Fate and Effects Division Office of Pesticide Programs Washington, D.C. 20460.

FAO. 2020. The State of World Fisheries and Aquaculture 2020.Sustainability in action. Rome.
https://doi.org/10.4060/ca9229en.

Fukunaga T, Kajikawa I, Nishiya K, Takeya K and Itokawa H. 1989. Studies on the constituents of the Japanese mistletoe, *Viscum album* L. var. coloratum Ohwi grown on different host trees. Chem Pharm Bull. **37(5):** 1300–1303.

Ghosh D, and Saha SK. 2022. Determination of the Lethal Concentration 50% (LC_{50}) of Hexavalent Chromium in Nile Tilapia (Oreochromis niloticus). Advances in Zoology and Botany. **10(4),** 123-131.

Halliwell B and Gutteridge JM. 2015. **Free Radicals in Biology and Medicine**. Oxford University Press.

Huang CB, Alimova Y, Myers TM and Ebersole JL. 2011. Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. Arch Oral Biol. **56(7):** 650–654.

Lommen, A., and Kools, HJ, (2012). MetAlign 3.0: Performance enhancement by efficient use of advances in computer hardware. Metabolomics : Metabolomics. **8(4),** 719–726.

Ibrahim NA and El-Gamal BA. 2003. Effect of Diazinon, an Organophosphate Insecticide, on Plasma Lipid Constituents in Experimental Animals. J. Biochem. Mol. Biol**. 36(5),** 499-504.

International Guiding Principles for Biomedical Research Involving Animals issued by CIOMS. 1986. Veterinary Quarterly, **8**:4 350-352, DOI:10.1080/01652176.1986.9694068.

Ito F, Sono Y and Ito T. 2019. Measurement and Clinical Significance of Lipid Peroxidation as a Biomarker of Oxidative Stress: Oxidative Stress in Diabetes, Atherosclerosis, and Chronic Inflammation. Antioxidants. **8(3):** 72.

Jee JH, Masroor F and Kang JC. 2005. Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, Sebastes schlegeli (Hilgendorf). Aquac Res. **36(9):**898–905.

Gowda S, Desai PB, Hull VV, Math AA, Vernekar SN and Kulkarni SS. 2009. A review on laboratory liver function tests. Pan Afr Med J, 22;**3**:17.

Jeon SM, Park YB and Choi MS. 2004. Antihypercholesterolaemic property of naringin alters plasma and tissue lipids, cholesterol-regulating enzymes, fecal sterol and tissue morphology in rabbits.Clin Nutr, **23**: 1025-1034.

Jos A, Repetto D, Rios JC, Hazen MJ, Molero ML, Peso A, Salguero M, Ferna´ndez-Freire P, Pe´rez-Martı´n JM and Camea´n A. 2003. Ecotoxicological evaluation of carbamazepine using six different model systems with eighteen endpoints. Toxicol In Vitro, **17**, 525–532.

Juárez ZN, Fortuna AM, Sánchez-Arreola E, López-Olguín JF, Bach H and Hernández LR. 2014. Antifeedant and phagostimulant activity of extracts and pure compounds from *Hymenoxys robusta* on *Spodoptera exigua* (Lepidoptera: Noctuidae) Larvae. Nat Prod Commun, **9(7):** 895-8.

Karami-Mohajeri S, Ahmadipour A, Rahimi H and Abdollahi M. 2017. Adverse effects of organophosphorus pesticides on the liver: a brief summary of four decades of research. Arh Hig Rada Toksikol, **68**,261-275.

Karim N, Jia Z, Zheng X, Cui S and Chen W. 2018. A recent review of citrus flavanone naringenin on metabolic diseases and its potential sources for high yield-production. Trends Food Sci Technol, **79**: 35–54.

Khan J, Deb PK, Priya S, Medina KD, Devi R, Walode SG, and Rudrapal M. 2021. Dietary Flavonoids: Cardioprotective Potential with Antioxidant Effects and Their Pharmacokinetic, Toxicological and Therapeutic Concerns. Molecules. **26**, 4021. 10.3390/molecules26134021

Klein R, Nagy O, Tóthová C and Chovanová F. 2020. Clinical and Diagnostic Significance of Lactate Dehydrogenase and Its Isoenzymes in Animals. Vet Med Int. ID 5346483, https://doi.org/10.1155/2020/5346483

Kumar S and Pandey AK. 2013. Chemistry and biological activities of flavonoids: An overview. Scientific World Journal, 1, 16. doi: 10.1155/2013/162750.

Lee S, Najiah M, Wendy W and Nadirah M. 2009. Chemical composition and antimicrobial activity of the essential oil of *Syzygium aromaticum* flower bud (clove) against fish systemic bacteria isolated from aquaculture sites, Front Agric China, **3**: 332–336, https://doi.org/10.1007/s11703-009-0052-8.

Lu J, Zhang M, and Lu L. 2018. Tissue Metabolism, Hematotoxicity, and Hepatotoxicity of Trichlorfon in Carassius auratus gibelio After a Single Oral Administration. Front Physiol, 23, **9**: 551. doi: 10.3389/fphys.2018.00551.

Mahima A, Rahal R, Deb SK, Latheef H, Abdulsamad R, Tiwari AK, Verma A, Kumar K and Dhama. 2012. Immunomodulatory and therapeutic potentials of herbal, traditional / indigenous and ethnoveterinary medicines, Pak J Biol Sci, **15**: 754-774, DOI: 10.3923/pjbs.2012.754.774

Mali H, Shah C, Raghunandan B, Prajapati AS, Patel DH, Trivedi U and Subramanian R. 2023. Organophosphate pesticides an emerging environmental contaminant: Pollution, toxicity, bioremediation progress, and remaining challenges. J. Environ. Sci. **127**, 234-250.

Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. 2022. Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. Oxygen. **2(2):**48-78.

Martinez-Alvarez RM, Morales AE and Sanz A. 2005. Antioxidant Defenses in Fish: Biotic and Abiotic Factors. Rev Fish Biol Fish, **15**, 75–88. doi: 10.1007/s11160-005-7846-4.

Modesto KA and Martinez CB. 2010. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish Prochilodus lineatus. Chemosphere, **78**, 294–299.

Mohamad Nek Rahimi NN, Natrah I, Loh Y, Ervin Ranzil FK, Gina M, Lim HE, Lai S and Chong M. 2022. Phytocompounds as an Alternative Antimicrobial Approach in Aquaculture. Antibiotics. 11(4).

Muro E, Ekin Atilla-Gokcumen G, Eggert US and Bement W. 2014. Lipids in cell biology: how can we understand them better?. MBoC. DOI:10.1091/mbc.E13-09-0516.

Narvaez-Mastache JM, Novillo F and Delgado G. 2008. Antioxidant arylprenylcoumarin, flavan-3-ols and flavonoids from *Eysenhardtia subcoriacea*. Phytochemistry, **69**: 451–456.

Nicolas B, Robert HL, Miled B, Mark WD, Philip BG and Ann MM. 2015. Alpha-Linolenic Acid: An omega-3 fatty acid with neuroprotective properties ready for use in the stroke clinic. Biomed Res Int. https://doi.org/10.1155/2015/519830.

Nipa G, Shikta R and Anindita C. 2021. A Review on Medicinal Plants and Immune Status of Fish. Egypt J Aquat Biol Fish. **25(2),** 897 – 912.

Orrenius S, Nicotera P and Zhivotovsky B. 2011. Cell Death Mechanisms and Their Implications in Toxicology. Toxicological Toxicol. Sci. **119(1),** 3–19.

Oruc E. 2011. Effects of diazinon on antioxidant defense system and lipid peroxidation in the liver of *Cyprinus carpio* (L.). Environ. Toxicol, **26(6),** 571-8. doi: 10.1002/tox.20573.

Özkan-Yilmaz F, Hunt A, Gül Gündüz S, Berkoz M, Yalin S and Sahin, N, 2015. Effects of methidathion on antioxidant system and expression of heat shock protein 70 (HSP70) gene in the liver of *Oreochromis niloticus* L. 1758. Fresenius Environ Bull. 24, 2650-2658.

Pan J, Yuan C, Lin C, Jia Z and Zheng R. 2003. Pharmacological activities and mechanisms of natural phenylpropanoid glycosides. Pharmazie. **58**: 767–775.

Panche AN, Diwan AD and Chandra SR. 2016. Flavonoids: an overview. J Nutr Sci., 5, e47. doi: 10.1017/jns.2016.41.

Petitjean Q, Jean S, Gandar A, Côte J, Laffaille P and Jacquin L. 2019. Stress responses in fish: From molecular to evolutionary processes. Sci. Total Environ. 684,371 - 380. ff10.1016/j.scitotenv.2019.05.357ff.

Ponnampalam EN, Kiani A, Santhiravel S, Holman BWB, Lauridsen C and Dunshea FR. 2022. The Importance of Dietary Antioxidants on Oxidative Stress, Meat and Milk Production, and Their Preservative Aspects in Farm Animals: Antioxidant Action, Animal Health, and Product Quality—Invited Review. Animals. **12(23),** 3279.

Rad SM, Ray AK and Barghi S. 2022. Water Pollution and Agriculture Pesticide. Clean Technol. **4(4):**1088-1102.

Ramesh M, Sankaran M, Veera-Gowtham V and Poopal RK. 2014. Hematological, biochemical and enzymological responses in an Indian major carp *Labeo rohita* induced by sublethal concentration of waterborne selenite exposure. Chem Biol Interact. **207**: 67–73, http://dx.doi.org/10.1016/j.cbi.2013.10.018.

Reina M and Martínez A. 2015. Silybin and 2,3-Dehydrosilybin Flavonolignans as Free Radical Scavengers. J Phys Chem B, **119(35):** 11597–11606.

Reverter m, Tapissier-bontemps N, Lecchini D, Banaigs B and Sasal P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture. **433**, 50–61. 10.1016/j.aquaculture.2014.05.048. OK

Rizzato G, Scalabrin E, Radaelli M, Capodaglio G and Piccolo O. 2017., A new exploration of licorice metabolome. Food chemistry, **221**: 959–968.

Ryhanen R, Herranen J, Karhonen K, Penttila I, Popvilanpi M and Puhakainen E. 1984. Relationship between serum lipids, lipoproteins and pseudocholinesterase during organophosphate poisoning in rabbits. Int J Biochem, **16**: 687-690.

Samanta P, Pal S, Mukherjee AK and Ghosh AR. 2014. Evaluation of metabolic enzymes in response to Excel Mera 71, a glyphosate-based herbicide, and recovery pattern in freshwater teleostean fishes. Biomed Res Int, 425159. doi: 10.1155/2014/425159.

Sandoval-Herrera N, Mena F, Espinoza M and Romero A. 2019. Neurotoxicity of organophosphate pesticides could reduce the ability of fish to escape predation under low doses of exposure. Sci Rep, 19, **9(1):**10530. doi: 10.1038/s41598-019-46804-6.

Saravanan M, Karthika S, Malarvizhi A and Ramesh M. 2011. Ecotoxicological impacts of clofibric acid and diclofenac in common carp (*Cyprinus carpio*) fingerlings: hematological, biochemical, ionoregulatory and enzymological responses, J Hazard Mater, **195**: 188–194, doi: 10.1016/j.jhazmat.2011.08.029.

Saravanan M, Usha Devi K, Malarvizhi A and Ramesh M. 2012. Effects of Ibuprofen on hematological, biochemical and enzymological parameters of blood in an Indian major carp, *Cirrhinus mrigala*, Environ Toxicol Pharmacol, **34**: 14–22, http://dx.doi.org/10.1016/j.etap.2012.02.005.

Scalabrin E, Radaelli M, Rizzato G, Bogani P, Buiatti M, Gambaro A and Capodaglio G. 2015. Metabolomic analysis of wild and transgenic Nicotiana langsdorffii plants exposed to abiotic stresses: Unraveling metabolic responses. Anal Bioanal Chem. **407**: 6357-6368.

Shakoori AR, Ali SS and Saleem MA. 1988. Effects of six months feeding of cypermethrin on the blood and liver of albino rats. J Biochem Toxicol, **3**: 59-72.

Sies H and Jones DP. 2020. Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. Nat Rev Mol Cell Biol. **21(7),** 363-383.

Song C, Sun C, Liu B and Xu P. 2023. Oxidative Stress in Aquatic Organisms. Antioxidants. **12(6):**1223.

Suksamrarn A, Chotipong A, Suavansri T, Boongird S, Timsuksai P, Vimuttipong S and Chuaynugul A, 2004. Antimycobacterial activity and cytotoxicity of flavonoids from the flowers of Chromolaena odorata. Arch Pharm Res. **27**: 507-11.

Sule RO, Condon L and Gomes AV. 2022. A Common Feature of Pesticides: Oxidative Stress-The Role of Oxidative Stress in Pesticide-Induced Toxicity. Oxid Med Cell Longev. **19**, 2022:5563759. doi: 10.1155/2022/5563759.

Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, Fan TWM, Fiehn O, Goodacre R, Griffin JL, Hankemeier T, Hardy N, Harnly J, Higashi R, Kopka J, Lane AN, Lindon JC, Marriott P, Nicholls AW, Reily MD, ThadenJJ and Viant MR. 2007. Proposed minimum reporting standards for chemical analysis. Metabolomics, **3**: 211–221. doi:10.1007/s11306-007-0082-2.

Sunderam RM, Patra RW, Julli M and Warne J. 2004. Use of the Up-and-Down Acute Toxicity Test Procedure to Generate LC50 Data for Fish. Bull Environ Contam Toxicol, **72**, 873–880.

Surai PF. 2015. Silymarin as a Natural Antioxidant: An Overview of the Current Evidence and Perspectives. Antioxidants. **5(4),** 43.

Tadese DA, Song C, Sun C, Liu B, Liu B, Zhou Q, Xu P, Ge X, Liu M, Xu X, Tamiru M, Zhou Z, Lakew A and Kevin NT. 2022. The role of currently used medicinal plants in aquaculture and their action mechanisms: A review. Rev Aquac. **14(2),** 816-847.

Trachootham D, Lu W, Ogasawara MA, Valle NR and Huang P. 2008. Redox regulation of cell survival. Antioxid Redox Signal, 10: 1343–1374, DOI: 10.1089/ars.2007.1957.

Tikunov YM, Laptenok S, Hall RD, Bovy A and De Vos RCH, 2012. MSClust: A tool for unsupervised mass spectra extraction of chromatography mass spectrometry ion-wise aligned data. Metabolomics. **8(4),** 714–718.

Tiwari S, and Singh A. 2004. Piscicidal activity of alcoholic extract of *Nerium indicum* leaf and their biochemical stress response on fish metabolism. Afr J Tradit Complement Altern Med, 1:15-29.

Tsai YH and Lein PJ. 2021. Mechanisms of organophosphate neurotoxicity. Curr Opin Toxicol, **26**, 49-60. doi: 10.1016/j.cotox.2021.04.002.

Tsikas D. 2017. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 1, **524**:13-30.

Tudi M, Daniel Ruan H, Wang L, Lyu J, Sadler R, Connell D, Chu C and Phung DT. 2021. Agriculture Development, Pesticide Application and Its Impact on the Environment. International Int J Environ Res Public Health. **18(3):**1112.

Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH and Jaremko M. 2020. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules., **25(22),** 5243. doi: 10.3390/molecules25225243.

Vasantharaja D, Ramalingam V and Aadinaath Reddy G. 2015. Oral toxic exposure of titanium dioxide nanoparticles on serum biochemical changes in adult male Wistar rats. Nanomed J, **2(1):** 46-53.

Wahsha m, Al-Zibdah M, Al-Jawasreh R and Wahsheh H, 2023. Harnessing Garlic Extract and AI for Sustainable Disease Mitigation in Aquaculture. J Adv Zool. 44, S-5, 133-141.

Wahsha M and. Al-Jassabi S. 2009. The role of silymarin in the protection of mice liver damage dgainst microcystin- LR toxicity, Jordan J Biol Sci, **2**: 63-68.

Wahsha M, Al-Omari A, Hassan M, Abuadas FA, Ahmed ET, Mostafavi K, Moradi M and Ghotbi M. 2012. Protective action of flavonoids extracted from different Jordanian plants against oxidative stress, IJBPR, **3**: 450-456.

Wahsha M, Juhmani AS, Buosi A, Sfriso A and Sfriso A. 2017. Assess the environmental health status of macrophyte ecosystems using an oxidative stress biomarker. Case studies: The Gulf of Aqaba and the Lagoon of Venice. Energy Procedia, **125**, 19-26.

Wang HS, Gan DH, Zhang ZP and Pan YM. 2010. Antioxidant capacity of the extracts from pulp of *Osmanthus fragrans* and its components. LWT-Food Science and Technology. **43(2010),** 319–325.

Webb C,J, Sykes W,R and Garnock-Jones, P,J, 1988. **Flora of New Zealand Vol IV: Naturalised Pteridophytes, Gymnosperms, Dicotyledons. Christchurch, New Zealand: Botany Division**, Department of Scientific and Industrial Research.

Wilson Magdy B, El-sayed Mohamed F, Seleem Amin A and Sarhan Rana S. 2016. Ameliorative effect of antioxidants (vitamins C and E) against abamectin toxicity in liver, kidney and testis of male albino rats. J Basic Appl Zool. **77**, 69-82.

Wu H, Liu J, Zhang R, Zhang J, Guo Y and Ma E. 2011. Biochemical effects of acute phoxim administration on antioxidant system and acethylcholinesterase in *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae). Pestic Biochem Physiol, **100**: 23-26.

Xavier Martins R, Vieira L, Ribeiro Souza J, Flor Silva M, Silva Muniz M, Souza T, Queiroga F, Ferreira Machado M, Silva B and Farias D. 2021. Exposure to 2,4-D herbicide induces hepatotoxicity in zebrafish larvae. Comp Biochem Physiol C Toxicol Pharmacol. **248**, 109110.

Yang C, Lim W and Song G. 2020. Mediation of oxidative stress toxicity induced by pyrethroid pesticides in fish. Comp Biochem Physiol C Toxicol Pharmacol, **234**, 108758.

Yeshi K, Crayn D, Ritmejerytė E and Wangchuk P. 2022. Plant Secondary Metabolites Produced in Response to Abiotic Stresses Has Potential Application in Pharmaceutical Product Development. Molecules. 5, **27(1),** 313.

Zhang BC, Zhang CW, Wang C, Pan DF, Xu TD and Li DY. 2016. Luteolin attenuates foam cell formation and apoptosis in Ox-LDL-stimulated macrophages by enhancing autophagy. Cell Physiol Biochem, **39**: 2065–2076.

Zheng CJ, Yoo JS, Lee TJ, Cho HY, Kim YH and Kim WG. 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Letters, **579(23):** 5157–5162.