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ORIGINAL ARTICLE

Acute and chronic toxicity of Spinosad in common Carp (*Cyprinus carpio*): Implications for health and environmental safety

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ABSTRACT

Aim: The study aims to analyzes the acute and chronic effects of the Spinosad insecticide on common carp (*Cyprinus carpio Linnaeus, 1758*). Aquatic ecosystems face increasing threats from agricultural runoff, especially pesticides.

Materials and Methods: Acute toxicity tests were conducted over 96 hours with six concentrations of Spinosad (2.5, 5.0, 6.25, 7.5, 8.75, and 10.0 mg/L) to determine the median lethal concentration (LC50) and observe behavior. For chronic exposure, carp were exposed to three sublethal concentrations (1.25, 3.75, and 5.0 mg/L) for 20 days. Key physiological indicators, including blood cell counts, hemoglobin levels, liver enzyme activities (AST and ALT), and liver histology, were assessed for any histopathological changes.

Results: Acute toxicity tests show an LC50 of 7.41 mg/L for Spinosad in common carp, with behavioral changes more noticeable at higher concentrations. Chronic exposure caused significant alterations in hematological parameters (RBC, WBC, PCV, and Hb), increased liver enzymes (AST and ALT) with longer exposure times ($P \leq 0.001$ for AST; $P \leq 0.0001$ for ALT). Histological analysis revealed liver damage, including vacuolation, swelling, and necrosis, with severity rising with concentration and exposure duration.

Conclusions: This study shows that Spinosad, labeled as a "reduced-risk" pesticide, causes significant short-term and long-term toxicity in common carp, affecting behavior, blood parameters, liver enzyme activity, and tissue health. These results emphasize the environmental dangers of Spinosad runoff from agricultural fields and underline the importance of careful use and monitoring to protect aquatic ecosystems. More research is needed to explore the long-term impacts on non-target aquatic species.

KEY WORDS: Spinosad, common carp, water pollution, acute exposure, chronic exposure, hematological parameters, and histological examinations

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INTRODUCTION

The application of pesticides can significantly impact fish populations, the health of aquatic ecosystems, and the accumulation of harmful substances in humans. Consequently, this raises considerable concern since fish are particularly vulnerable to the effects of insecticides. Insecticide contamination in water is a common issue, as these chemicals can enter surface water through runoff from agricultural fields and treated soil or be sprayed directly onto the water's surface, as seen in mosquito control efforts. Such contamination can harm aquatic plants, reduce dissolved oxygen levels in the water, lead to toxic algal blooms, and negatively affect fish physiology and behavior. The overuse of fertilizers has also been linked to declines in various fish populations [1].

While using biological products is desirable and highly specific to targeted pests, creating suitable formulations poses challenges. Specifically, the formulated product must maintain the functionality of the biological agent during storage and application while also possessing good physical properties and ease of use. One natural product, Spinosad, has been authorized by various national and international certifying agencies for use in organic agriculture [2]. It is produced through the fermentation of bacteria. According to the U.S. Environmental Protection Agency (U.S. EPA, undated), Spinosad is classified as a "reduced-risk" substance and carries the lowest human hazard signal word, "Caution," underscoring its status as a naturally occurring, low-impact insecticide.

Spinosad is a pesticide within the spinosyn class, derived from the bacterium *Saccharopolyspora spinosa*, and consists of spinosyns A and D. Typically, "Spinosad" refers to a combination of spinosyn A and D, with spinosyn A accounting for between 85% and 90% of the mixture. Spinosyns can affect insects through direct contact, toxicity from body surface exposure, or ingestion via the food chain. The biochemical interaction of the bioinsecticide is associated with the disruption of nicotinic acetylcholine receptors and GABA-gated ion channels in the nervous systems of insects. Notably, Spinosad specifically stimulates nicotinic acetylcholine receptors in fleas, while it does not activate other nicotinic receptors or GABA receptors [2].

Pesticides are recognized as one of the most detrimental agricultural pollutants, contributing to both mortality and physiological changes in aquatic life. Among aquatic life, Fish eggs, larvae, yearlings, and fingerlings are particularly susceptible to contamination from pesticides and heavy metals, as these aquatic species are not equipped to cope with such pollutants. Injury to the essential organs like liver, kidneys and gills will disrupt important physiological processes, such as survival, buoyancy, osmoregulation and reproduction. These interferences could eventually lead to the loss of population and recruitment challenges to the stock [3].

A perfect example of exceeding adaptability that ensures a lot of success in various environmental conditions is the *Cyprinus carpio*, a very renowned representative of the largest freshwater fish family, *Cyprinidae,* namely, the common carp. Fisheries, whether produced in aquaculture or caught in the wild ecosystem, are a lifeblood of fishing communities and constitute a very substantial portion of the world's food supply [4].

Therefore, since *Cyprinus carpio* (common carp) is a one of the main food sources, the physiological effects of Spinosad exposure could pose risks to humans who eat contaminated fish. By examining these biomarkers, this study provides a critical link between environmental toxicology and public health, emphasizing the need for sustainable farming practices that protect both aquatic life and the human food supply.

The ability of a substance to be harmful, whether through acute or cumulative chronic action is its toxicity. The conditions that affect the toxicity include chemical composition of the substance, its concentration and duration of exposure, and the nature of the organism that is exposed, i.e. species, age, sex, and nutritional status. The exposure route, i.e., oral, inhalation, or dermal, also comes into play since some chemicals also have the potential of causing the skin and the eyes to be irritated [5]. Among the toxic compounds, pesticides are the most toxic chemicals to fish and other creatures in the food chain.

The ecotoxicity testing common to vertebrates is still acute fish toxicity testing. Mortality is normally measured using them: standard carp is normally subjected in a test material in 96 hours, and mortality is monitored after 24, 48, 72, and 96 hours. Based on such observations, the LC50 value is calculated and this is the concentration of the substance that causes 50 percent mortality of test group in the short exposure period.

Besides, the evaluation of long-term effects and regulatory data requirements should be conducted with the help of chronic toxicity tests. Biochemical and hematological indicators are widely used in fish toxicology since they are a simple and cheap mechanism of testing physiological alterations. Among them, the main predictors of immune reactions are differential leukocyte count (DLC) and white blood cell (WBC), whereas the great importance is paid to red blood cells (RBCs) or erythrocytes in providing oxygen. Such indicators are usually determined after exposure to different chemicals [6].

Biomarkers are also useful in biomarkers research, in understanding the impacts of environmental changes to living organisms and the ecosystems. Biomarkers in liver functioning, such as enzymes alanine transaminase (ALT) and aspartate transaminase (AST), are useful as sources of data on the health of organisms and in response to [7]. Histological biomarkers are of particular concern in the process of locating the damage inflicted by toxic substances and carcinogens, since they provide an opportunity to study specific organs required in respiration, excretion, and xenobiotic metabolism. The gills, kidneys and liver histopathological analysis are important data in the monitoring of the environment where the biological impact of a toxic exposure is to be determined [8].

AIM

The study aims to analyze the acute and chronic effects of the *Spinosad* insecticide on common carp (*Cyprinus carpio Linnaeus,* 1758). Aquatic ecosystems face increasing threats from agricultural runoff, especially pesticides. This study assessed the acute and chronic toxic effects of Spinosad, a low-risk, naturally derived bioinsecticide, on common carp (*Cyprinus carpio Linnaeus*, 1758), a bioindicator for water pollution.

MATERIALS AND METHODS

EXPERIMENTAL FISH

The study used common carp (*C. carpio Linnaeus*, 1758), specifically juvenile *Cyprinus carpio* weighing 30 ± 3 grams, measured with a precise scale. These fish samples were collected from the Babylonian reservoirs and transported to

Table 1. Result of physical and chemical properties of aquariums

Physical and Chemical Properties	Range
Temperature (°C)	21- 25
Hydrogen Ion Concentration(pH)	6.9-7.6
Dissolved Oxygen (D.O) (mg/l)	5.5-7.9
Electrical Conductivity (µs/cm)	850- 1220

Table 2. Amount of pesticide added (mL) per basin volume (liters) and effective concentration [mg/L]

Amount of pesticide added [ml]	Basin volume [litres]	Effective concentration [mg/L]
0.10	40	2.5
0.20	40	5.0
0.25	40	6.25
0.30	40	7.5
0.35	40	8.75
0.40	40	10.0

Source: Compiled by the authors of this study

Table 3. Sublethal Concentrations of Spinosad for *C. carpio*

Groups	LC50 value\UI	Spinosad (mg/l)
1	1\50	0.1482
2	1\20	0.3705
3	1\10	0.741
4	control	control

Source: Compiled by the authors of this study

Table 4. Spinosad concentration (mg/L) and mortality percentage in common Carp at 24, 48, 72, and 96 hours

Concentration [mg/L]	Mortality after 24 hours [%]	Mortality after 48 hours [%]	Mortality after 72 hours [%]	Mortality after 96 hours [%]
2.5	0	0	0	0
5.0	0	0	12.5	25
6.25	12.5	25	37.5	50
7.5	25	37.5	62.5	75
8.75	37.5	50	75	87.5
10.0	50	75	87.5	100

Source: Compiled by the authors of this study

the laboratory in plastic bags. Approximately 165 fish were sampled to determine their acute exposure. Any weak or diseased fish were excluded to ensure that only healthy fish were included in the experiment (US Environmental Protection Agency, 1996). To promote acclimatization, the fish were kept under laboratory conditions for 10–14 days before starting the experiments. The control group was kept in experimental water without adding the Spinosad pesticide, keeping all other conditions constant.

GLASS AQUARIUMS

The fish tanks used for acclimatization and experiments measured 40 × 50 × 30 cm and held 40 liters of water. Oxygen was supplied to the tanks *via* a central pump that distributed it through rubber tubing to each tank. During the acclimatization and experimental periods, these tanks received a continuous flow of oxygen around the clock. Dechlorinated water was used; to achieve this, tap water was left for 72 hours to dissipate the chlorine before being added. In both acute and chronic exposure experiments, tank water was replaced every 48 hours with 40 liters, with constant aeration, maintaining a pH of 6.9 to 7.6 and a temperature of 21-25°C (Table 1). The same procedure was followed during the acclimatization and experimental phases [9].

THE CONCENTRATIONS NEEDED FOR THE ACUTE TOXICITY EXPERIMENT

The concentrations utilized in the experiments were calculated using the equation $C1 \times V1 = C2 \times V2$, expressed in mg/L. The test chemical, Spinosad, was administered to common carp over a 96-hour period, during which mortality rates were recorded at 24, 48, 72, and 96 hours. The LC50, defined as the concentration that leads to the death of 50% of the fish, will be determined when applicable. Each experimental group consisted of at least eight fish across various concentrations of Spinosad, in addition to a control group. Furthermore, the threshold concentration for this test was set at 100 mg/L, with observations conducted at specified intervals [10].

In the acute toxicity test, six different concentrations of Spinosad were examined: 2.5, 5.0, 6.25, 7.5, 8.75, and 10.0 mg/L, to ascertain the LC50 values (Table 2). Data tables presented

Table 5. The logarithm of the concentration and the probit unit

Log Concentration	Probit Value
0.796	4.33
0.875	5.00
0.942	5.67
1.0	6.28

Source: Compiled by the authors of this study

Table 6. The behavioral changes in common carp exposed to Spinosad

Concentration	Behavioral changes
Control	The fish's behavioral and swimming patterns remained unchanged, with no mortality observed throughout the entire testing period.
2.5 mg/l	No mortality was recorded, and normal fish movement was documented.
5.0 mg/l	After exposure, sudden jumping and darting were observed. All fish settled to the bottom after more than 24 hours.
6.25 mg/l	Fish exhibited increased abnormal swimming behavior, and hyperventilation was observed.
7.2 mg/l	Vertical and downward swimming patterns were observed, and jumping frequency increased. Four fish died within 24 hours.
8.75 mg/l	Swimming problems, loss of balance, and sudden jumping were observed. Increased gill movement was observed, and the fish attempted to breathe air from the surface. Six fish died within the first hours, and over time, all fish died after 24 hours.
10.0 mg/l	The fish exhibited increased gill movement and attempted to breathe air from the surface. They experienced difficulty swimming, loss of balance, and sudden jumps. After less than eight hours, all the fish died.

Source: Compiled by the authors of this study

Table 7. The primary hematological parameters in fish

Parameters	Control	0.1482 [mg/L]	0.3705 [mg/L]	0.741 [mg/L]	LSD ($P<0.05$)
RBC [10^{12}/L]	3.79 a	2.97 b	2.09 c	1.94 c	0.42
HGB [g/dL]	12.80 a	11.80 b	11.30 b	9.80 c	1.15
HCT [%]	42.20 a	36.10 b	38.70 b	30.90 c	3.8
LYM% [%]	32.04 c	27.02 b	9.07 c	27.06 b	4.95
MON% [%]	4.70 a	6.08 c	27.16 a	18.54 b	2.88
NEU% [%]	57.90a	60.30 a	57.36 a	44.45 b	6.2

Note: a = the group with the highest concentration, and it differs significantly from the groups with the other letters;
b = the group that differs significantly from the groups with the letters a and c;
c = The group with the lowest concentration, and it does not differ from any group with the same letter
Source: Compiled by the authors of this study

the logarithm of the concentrations and the probit units for the 24, 48, 72, and 96-hour periods. At elevated concentrations, Spinosad induced notable behavioral changes in the fish. Throughout the 96 hours, the behavior and condition of the fish were meticulously documented every 24 hours. A control group of fish was concurrently acclimatized and monitored, showing no mortality after 24, 48, 72, or 96 hours.

In contrast, the fish exposed to Spinosad exhibited behaviors such as sudden twitches, loss of balance, disrupted swimming patterns, difficulty breathing, surfacing, and darting movements. It is also important to highlight that many insecticides inflict harm by disrupting biological systems [11]

CHRONIC TOXICITY FOR COMMON CARP

Three groups of common carp, each comprising eight fish per aquarium, were subjected to exposure to the biocide Spinosad (1/10, 1/20, 1/50) for a duration of 20 days, alongside a control group (Table 3). To effectively eliminate waste, the water in the aquariums was refreshed every 48 hours. The fish received daily feedings, and samples were collected after 20 days.

HEMATOLOGICAL PARAMETERS

Hematological indices serve as important indicators of changes in metabolism and physiology, as blood is involved in numerous physiological processes throughout the body.

Table 8. Comparison between different groups in ALT and AST

Group	Means ±SE	
	ALT (IU/L)	AST (IU/L)
Control	30.33 ±1.45 c	32.33 ±2.34 c
Pesticide 0.1482 mg/L	34.00 ±1.15 c	36.00 ±3.05 c
Pesticide 0.3705 mg/L	56.67 ±3.75 b	51.67 ±2.03 b
Pesticide 0.741 mg/L	70.00 ±2.31 a	64.33 ±3.92 a
L.S.D.	7.708 **	9.554 **
P-value	0.0001	0.0002
Means that the different letters in the same column differed significantly. ** (P≤0.01).		

Source: Compiled by the authors of this study

Table 9. Histological changes in the liver of C. carpio cod after chronic exposure to concentrations of 0.1482, 0.3705, and 0.741 mg/L of Spinosad

Concentration	Exposure period (20 days)
Control	Normal at all times.
0.1482 mg/l	Vacuolar degeneration of the cytoplasm in hepatocytes (see Figures 6, 7, 8, and 9).
0.3705 mg/l	Hepatocyte hypertrophy and cytoplasmic vacuolation are observed, along with moderate degeneration and atrophy of the pancreatic acini (see Figures 10 and 11). There is significant thickening of the intestinal villi, which is associated with hyperplasia and aggregation of white blood cells (leukocytes). This indicates inflammation and impaired immune function (refer to Figures 12 and 13).
0.741 mg/l	Severe and widespread histological changes include cytoplasmic vacuolation, hepatocyte hypertrophy, blood congestion in the sinusoids, and necrosis of hepatocytes (Figures 14 and 15). Necrosis indicates significant toxic damage.

Note: a = the group with the highest concentration, and it differs significantly from the groups with the other letters;
b = the group that differs significantly from the groups with the letters a and c;
c = the group with the lowest concentration, and it does not differ from any group with the same letter
Source: Compiled by the authors of this study

Collecting a blood sample from live fish is generally a quick, simple, and minimally invasive procedure. To analyze the blood, researchers utilize a hemocytometer (Neubauer's counting chamber) in conjunction with a cover slip. Hematological examinations play a crucial role in assessing the physiological health of fish. Indeed, the methods employed for blood analysis are widely considered more reliable than those used for other biological or cellular samples [11, 12].

LIVER ENZYMES

SERUM ALANINE AMINOTRANSFERASE (ALT)

The liver functions as the primary metabolic center for most xenobiotics, making it one of the organs most susceptible to harmful chemical exposure. In line with this, [13] the liver is essential for detoxification and biotransformation processes.

The colorimetric determination of alanine aminotransferase (ALT) is based on the following reaction:

L-Alanine + ALT → Pyruvate + L-Glutamate

The pyruvate produced reacts with two molecules of 4-dinitrophenylhydrazine, yielding a colored hydrazone that can be measured at a wavelength of 546 nm (within the range of 530 to 550 nm). For precise results, it is crucial to separate serum from hemolysis from blood cells as soon as possible after collection.

SERUM ASPARTATE AMINOTRANSFERASE (AST)

AST enzymes are essential for developing resistance and enhancing defense mechanisms against pesticides [14]. Given this role, the colorimetric determination of aspartate aminotransferase (AST) involves the following reaction:

L-Aspartate + +oxoglutarate → Oxaloacetate + L-Glutamate

The resulting oxaloacetate reacts with two molecules of 4-dinitrophenylhydrazine, producing a colored hydrazone that can be measured using a spectrophotometer at a wavelength of 546 nm (within the range of 530 to 550 nm). It is crucial to separate serum that is free of hemolysis from blood cells as quickly as possible after collection.

These histopathological changes in common carp biomarkers refer to the signs of major cellular and tissue-levels changes that occur in organisms that are exposed to poisonous chemicals. This kind of information is highly valuable to scientists and health professionals, as they can be used to assess the possibility of a risk that comes with the environmental and industrial exposures [15-18] Fish are also sensitive to environmental changes, such as xenobiotics, which significantly threaten ecosystem health and non-target species.

Examples of such magnifications through the environment include the concentrations of pesticides, which pose a threat to human health through the food chain. Such chemicals are bioaccumulated in aquatic life and biomagnified as they pass up the food chain to eventually get to the human being through consumption of fish containing the chemicals [19, 20]. Besides the accumulation, the chemical contaminants may lead to colossal amounts of lesions in fish organs, including the gills, liver, and kidneys. The histopathology of such organs is important in offering critical data regarding how much and how much the environment is polluted [21].

THE HISTOPATHOLOGICAL ALTERATION IN THE LIVER OF COMMON CARP

The liver is an important organ in fish toxicology because it plays an important role in the uptake, detoxification and elimination of xenobiotics. It is a highly important organ because morphological changes of the liver to toxic exposure tend to give reproducible patterns, hence serve as an excellent organ to do toxicological studies. The anatomical and physiological properties of the fish liver can also be helpful in determining the potential of metabolism in the liver and the microscopic changes of the liver as a result of the exposure to the toxin. Data of the hepatic reaction to particular toxicants is therefore needed. Some comprehensive reviews have fully addressed these aspects [22-24].

STATISTICAL ANALYSIS

The Statistical Package of Social Sciences was used to analyze data. To determine differences between groups, pair and multiple mean comparisons were done using the Least Significant difference (LSD) test and the Multiple Range Test of Duncan respectively.

RESULTS

The aquarium laboratory has physical and chemical characteristics

The physicochemical properties of the rearing environment and the formulation of the administered pesticide directly affect the organism's behavior and distribution, such as temperature, pH, dissolved oxygen, and electrical conductivity.

Acute exposure test

MEDIAN LETHAL CONCENTRATION (LC_{50}) OF SPINOSAD

This study and others considered the ecological (physical and chemical characteristics) along with experimental factors that significantly affect pesticide toxicity to fish when measuring the LC50 values (Table 4). Accordingly, in the acute toxicity test, six different Spinosad concentrations - 2.5, 5.0, 6.25, 7.5, 8.75, and 10.0 mg/L - were used to determine the LC50 values. The results of this study showed that Spinosad exhibits varying degrees of acute toxicity on common carp, with an LC50 value of 7.41 mg/L (Fig. 1). The logarithm of the concentration and the probit unit (Table 5).

BEHAVIORAL CHANGES

The behavior of fish in test and control tanks was monitored every 24 hours for up to 96 hours. Exposure to different concentrations of Spinosad resulted in significant changes, including anxious swimming and increased movement, particularly at higher concentrations. Fish showed increased sluggishness and agitation, producing excess mucus. Some carp displayed heightened arousal, leading to unpredictable behaviors (Table 6) [25].

CHRONIC EXPOSURE TEST

BLOOD PARAMETERS

Hematological parameters serve as essential indicators of physiological changes in fish, often arising from factors such as stress, pollution, and pesticide exposure. Consequently, analyzing blood samples yields valuable insights into the impact of pesticides on fish in their environment. Notably, exposure to Spinosad has been recognized as a key factor leading to significant reductions in hemoglobin, hematocrit, and red blood cell count, critical indicators of anemia. This decline in hemoglobin levels may result from damage caused by free radicals, impaired gas exchange, and the oxidation of methemoglobin (Table 7).

RED BLOOD CELL (RBC)

The observed hematological condition of the treated fish with Spinosad pulse or continuous was erythropenia (decrease in the number of red corpuscles or anemia), thrombocytopenia (decrease in the number of platelets), and leucocytosis (increase in the number of white corpuscles). The previous studies in fish treated with pesticides exhibited the same kinds of anemia.

HEMOGLOBIN (HB)

Hemoglobin (Hb), the principal protein in red blood cells (erythrocytes), is crucial for transporting oxygen from the

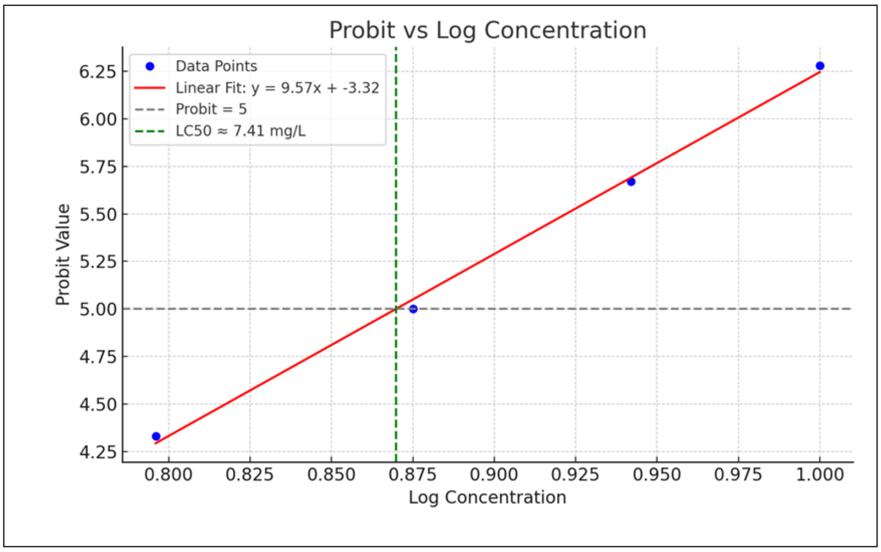

Fig. 1. The logarithm of the concentration and the probit
Source: Own materials

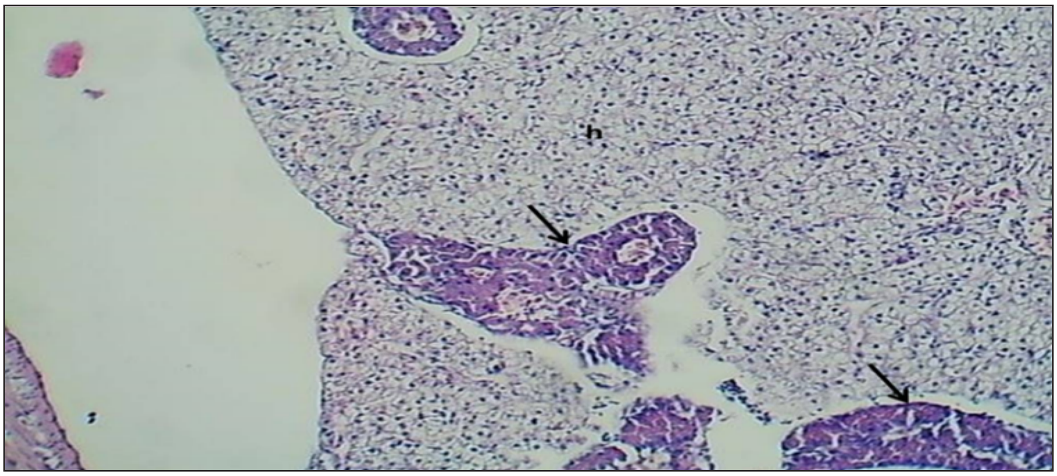

Fig. 2. Section of liver & pancreas (Control) shows normal appearance of pancreatic acini (arrows), normal hepatocytes (h). H&E.100x
Source: Own materials

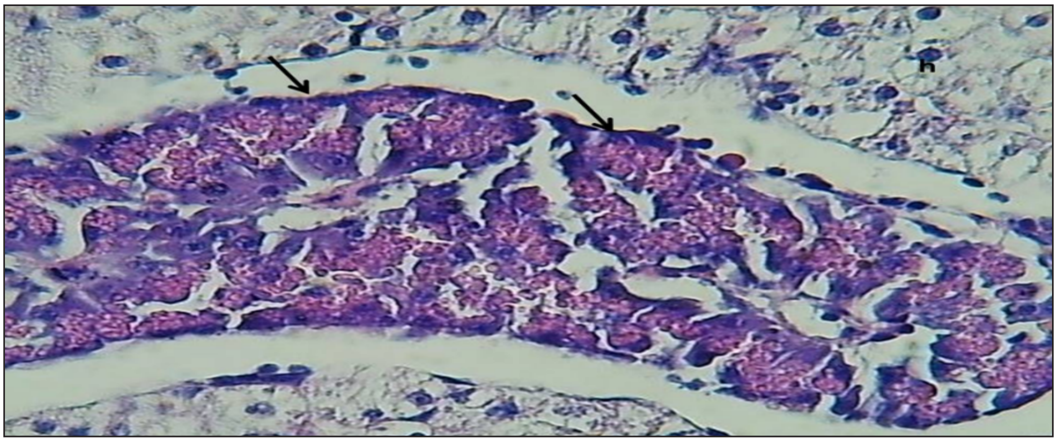

Fig. 3. Section of liver & pancreas (Control) shows normal appearance of pancreatic acini (arrows) & normal hepatocytes (h). H&E.400x
Source: Own materials

environment to body tissues. In fish, it also facilitates the rapid release of oxygen into the swim bladder and the choroid rete of the eye. This distinctive oxygen-transport system in fish possesses characteristics that are typically absent in other vertebrates, underscoring the specialized physiological role of fish hemoglobin.

PACKED CELL VOLUME (PCV)

The hematocrit (PCV) refers to the percentage of blood that is occupied by red blood cells of the total blood volume (100 milliliters). Different health problems may be represented by the abnormal values of hematocrit. In this study, the researcher tested an average packed

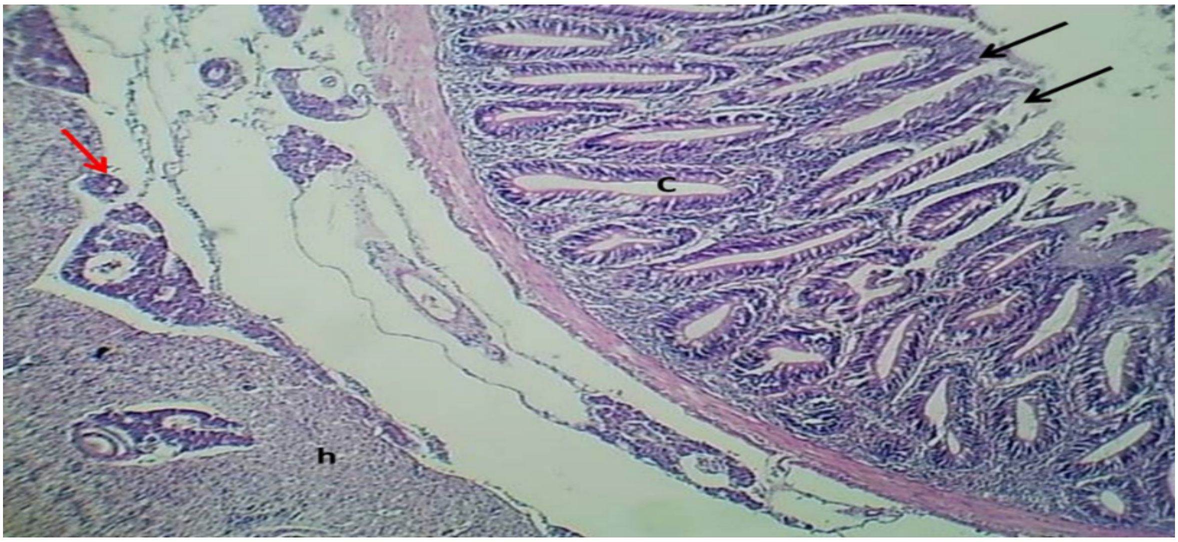

Fig. 4. section of liver with pancreas & intestine (Control) shows normal appearance of pancreatic acini (red arrows), normal hepatocytes (h) & intestinal villi (black arrow) with crypts (C). H&E.100x
Source: Own materials

Fig. 5. A section of intestine (Control) shows) show normal intestinal villi (black arrow) with crypts (C). H&E.200x
Source: Own materials

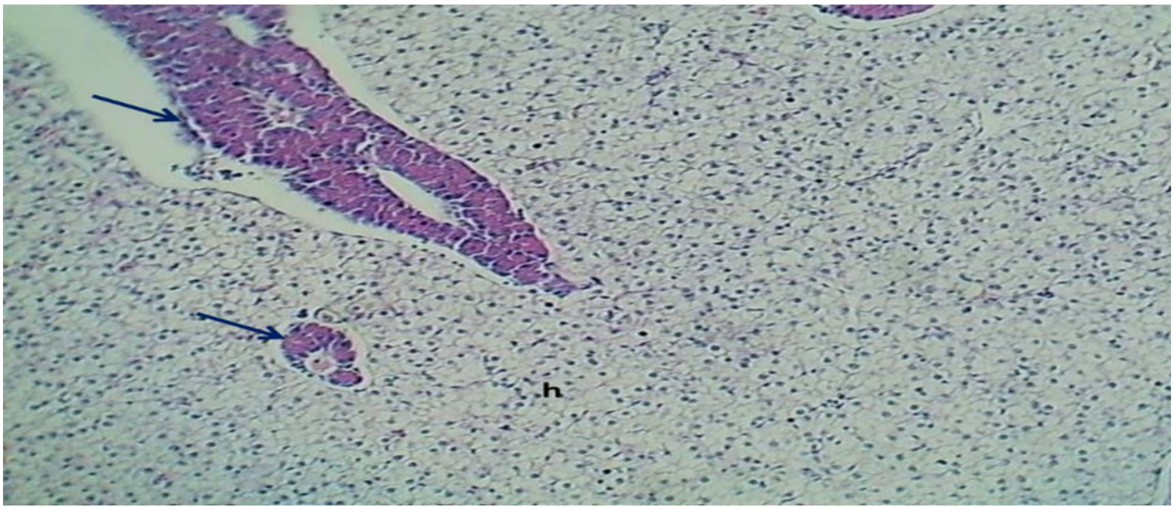

Fig. 6. Section of liver & pancreas (0.1482) shows normal appearance of pancreatic acini (Arrows), normal hepatocytes (h). H&E.100x
Source: Own materials

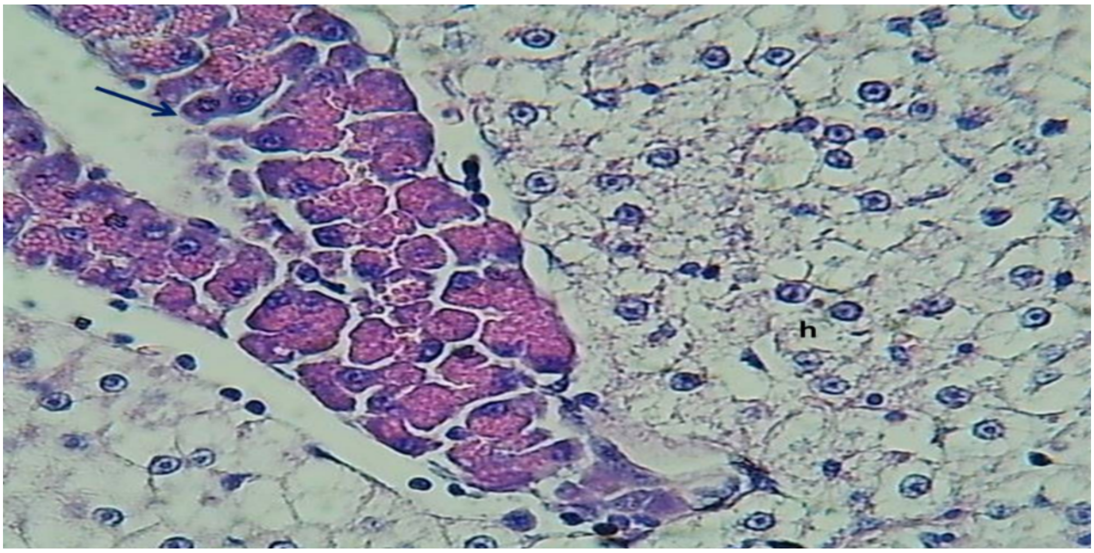

Fig. 7. Section of liver & pancreas (0.1482) shows normal appearance of pancreatic acini (Arrows) & normal hepatocytes (h). H&E.400x
Source: Own materials

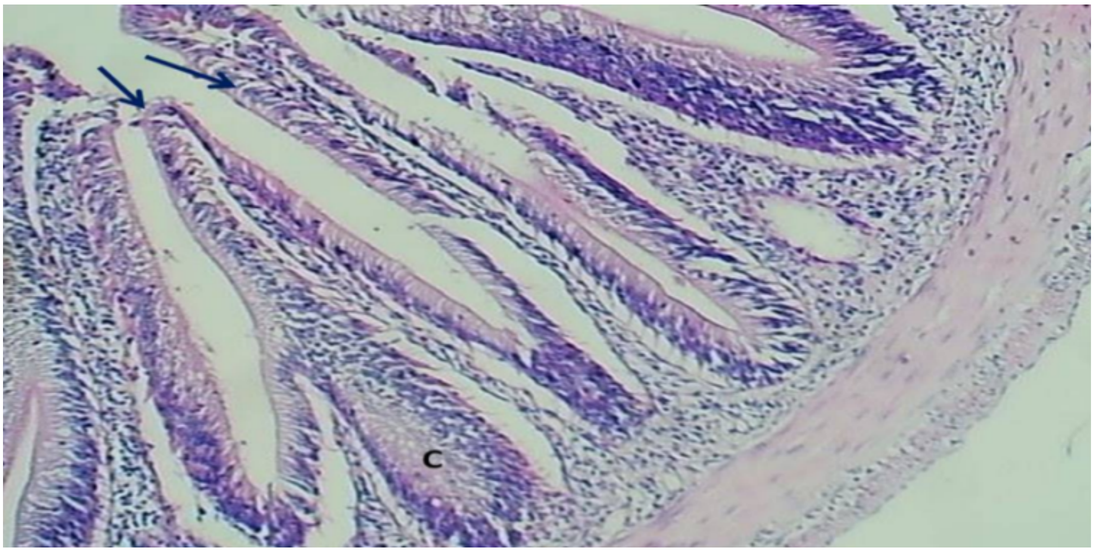

Fig. 8. A section of small intestine (0.1482) shows normal appearance of intestinal villi (black arrow) with crypts (C). H&E.100x
Source: Own materials

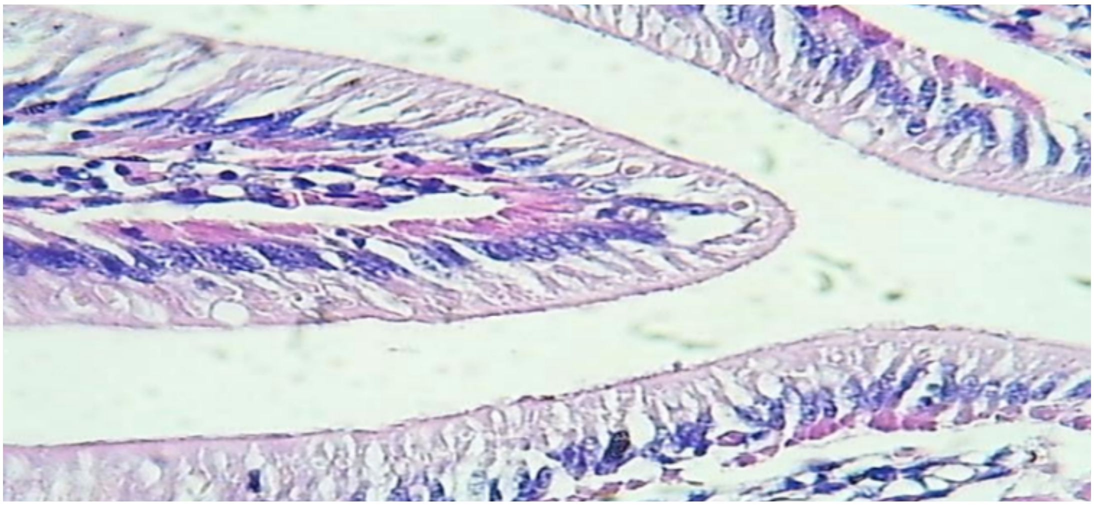

Fig. 9. A section of small intestine (0.1482) shows the normal appearance of enterocytes of the intestinal villi. H&E.400x
Source: Own materials

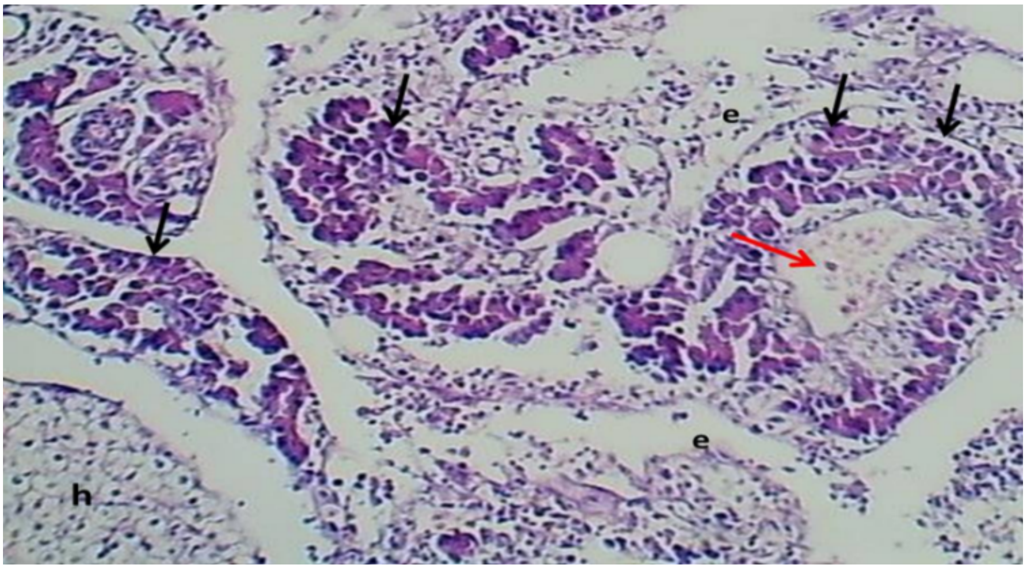

Fig. 10. Section of liver & pancreas (0.3705) shows moderate degeneration with atrophy of pancreatic acini (black arrows), congestion with dilation of central veins (red arrows), with edema (e). H&E.100x
Source: Own materials

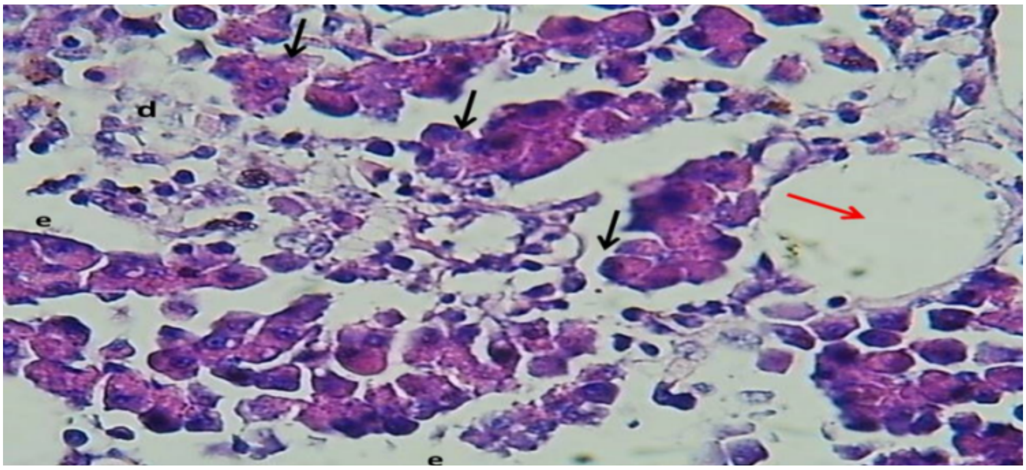

Fig. 11. A section of liver & pancreas (0.3705) shows moderate degeneration with necrosis and atrophy of pancreatic acini (black arrows), dilation of central veins (red arrows) with edema (e) & tissue depletion (d). H&E.400x
Source: Own materials

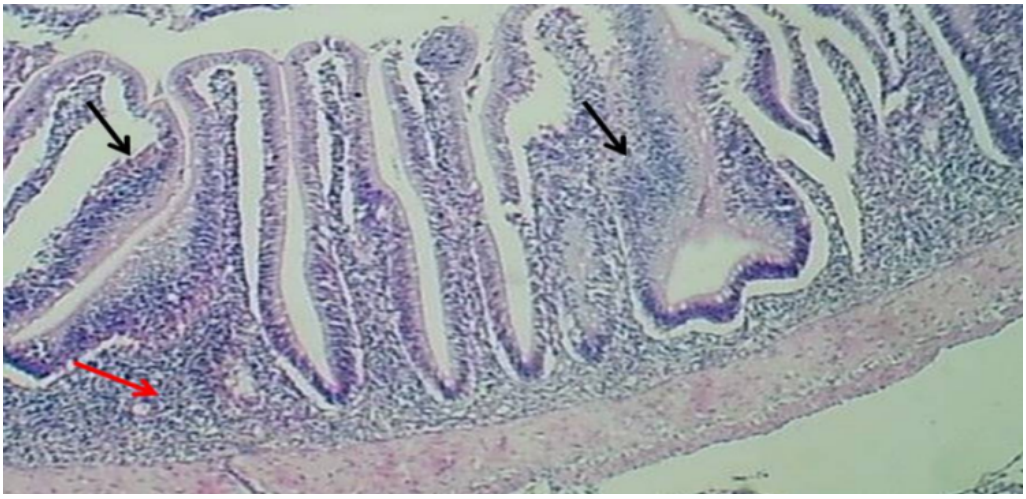

Fig. 12. A section of small intestine (0.3705) shows marked mucosal thickening of intestinal villi associated with hyperplasia (black arrow) & aggregation of leukocytes (red arrows). H&E.40x
Source: Own materials

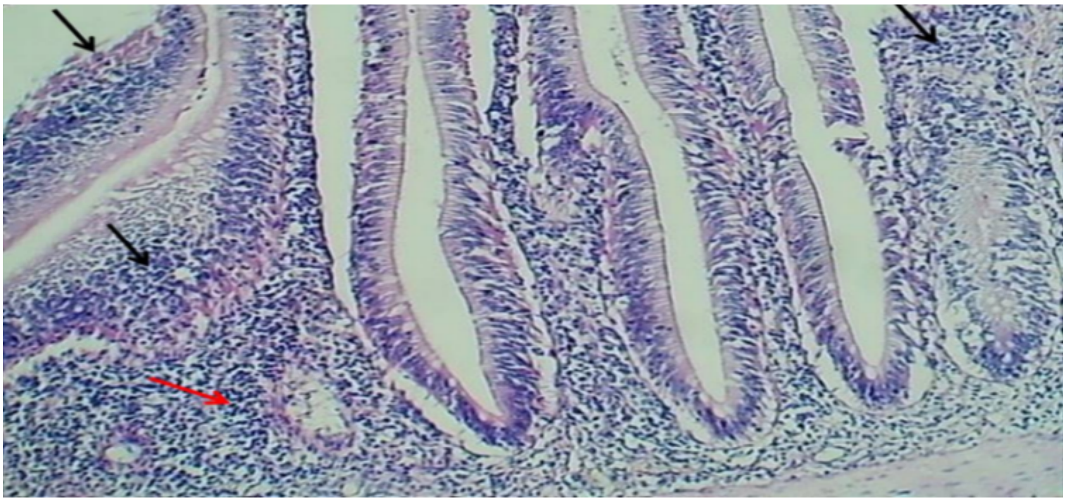

Fig. 13. A section of small intestine (0.3705) shows marked mucosal thickening of intestinal villi associated with hyperplasia (black arrow) & aggregation of leukocytes (red arrows). H&E.100x
Source: Own materials

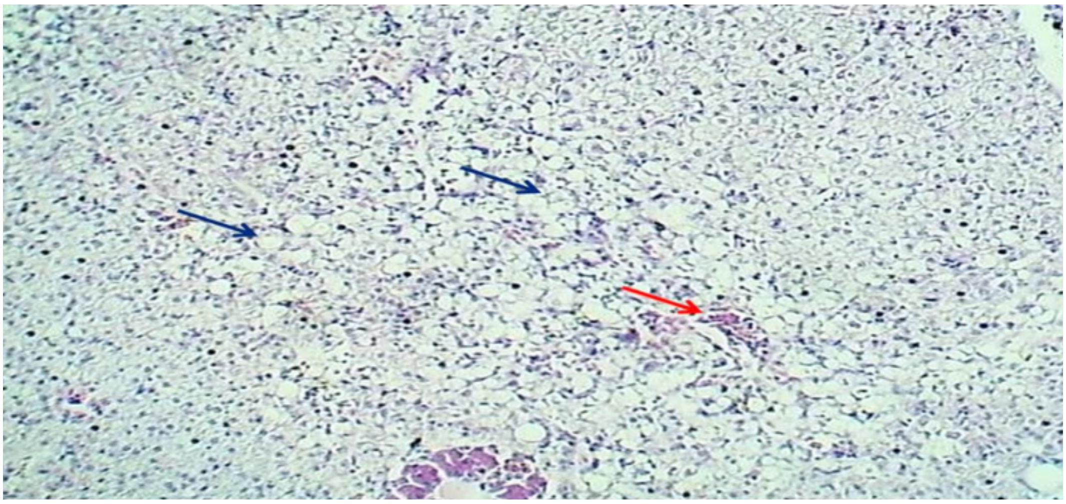

Fig. 14. A section of liver & pancreas (0.741) shows moderate vacuolar degeneration with cellular swelling of hepatocytes (black arrows) & sinusoidal congestion (red arrow). H&E.100x
Source: Own materials

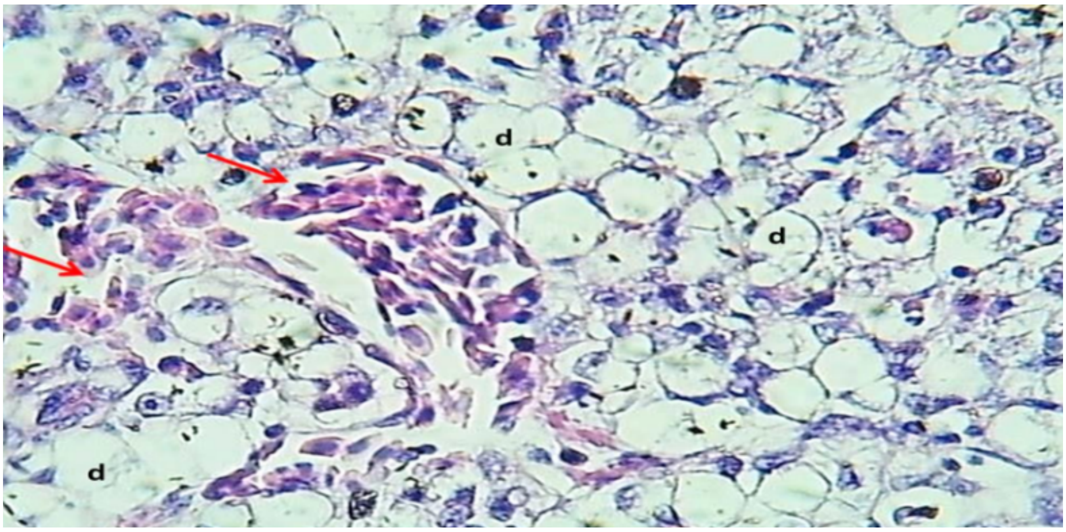

Fig. 15. A section of liver (0.741) shows moderate vacuolar degeneration with cellular swelling of hepatocytes (d) & sinusoidal congestion (red arrow). H&E.400x
Source: Own materials

cell volume (PCV) of fish that were subjected to a series of concentrations of a pesticide.

The PCV values varied considerably, ranging from 30.90 in fish exposed to the highest concentration of 0.741 mg/L to 38.70 in those exposed to the lowest concentration of 0.1482 mg/L. The average PCV at a concentration of 0.741 mg/L was 36.10. In contrast, the control group exhibited a mean PCV of 42.20, significantly higher than the lowest recorded value.

Our analysis of variance showed significant differences ($P \leq 0.001$) among the various pesticide concentrations. Furthermore, the duration of exposure had a notable impact on the results ($P < 0.05$). A least significant difference (LSD) test, which yielded an LSD value of 3.8, confirmed the significant differences across the different exposure periods (Table 6).

WHITE BLOOD CELL (WBC)

Fish immune systems are essential for host defense and act as significant biochemical indicators in environmental assessments. However, these systems can be adversely affected by exposure to various environmental toxins. A decrease in white blood cell count may indicate tissue damage due to exposure to Spinosad, while lower-than-normal lymphocyte levels can signify a compromised immune system. It is important to note that white blood cell counts can exhibit considerable variability.

In our study, this was identified a significant reduction ($p < 0.05$) in the white blood cell count of common carp subjected to sublethal concentrations of Spinosad for 20 days. Specifically, at a concentration of 0.3705 mg/L, the percentage of lymphocytes (LYM%) dropped to 9.07%, compared to 32.04% in the control group. This difference surpassed the least significant difference (LSD) value of 4.95, indicating a suppression of cellular immunity. Conversely, the percentage of monocytes (MON%) increased to 27.16%, reflecting heightened inflammatory activity, which was statistically significant. The percentage of neutrophils (NEU%) decreased at the higher concentration but remained within normal fluctuation limits up to 0.3705 mg/L (Table 6).

Our analysis demonstrated that both the concentration of the pesticide and the duration of exposure had highly significant effects ($P \leq 0.01$) on the distribution of white blood cells (WBC). This finding was further supported by an LSD value of 1.539.

LIVER ENZYMES

SERUM ALANINE AMINOTRANSFERASE (ALT)

The liver's vital role in the body and its anatomical positioning, along with its rich blood supply, make it particularly vulnerable to waterborne pollutants

In the study, a pronounced increase in ALT enzyme activity was noted in groups treated with Spinosad doses of 0.1482 mg, 0.3705 mg, and 0.741 mg, with recorded ALT values of 34.00 ± 1.15 IU/l, 56.67 ± 3.75 IU/l, and 70.00 ± 2.31 IU/l, respectively. In contrast, the control group exhibited an ALT value of 30.33 ± 1.45 IU/l (refer to Table 7).

These results indicate that ALT levels rise with increasing pesticide concentrations and extended exposure durations compared to the control group. Elevated ALT levels may signal liver injury, as they are typically associated with damaged liver cells. This aligns with prior research indicating that exposure to xenobiotics can disrupt an organism's antioxidant defense system

LIVER ENZYMES

SERUM ALANINE AMINOTRANSFERASE (ALT)

Fish liver biomarkers associated with conjugation enzymes, carboxylesterase activity, and antioxidant defenses can indicate exposure to biopesticides [26].In the study, a significant increase in ALT activity was observed in groups treated with Spinosad at doses of 0.1482 mg/L, 0.3705 mg/L, and 0.741 mg/L, with values of 34.00 ± 1.15, 56.67 ± 3.75 IU/L, and 70.00 ± 2.31 IU/L, respectively. The control group had an ALT value of 30.33 ± 1.45 IU/L (Table 8).

These results indicate that ALT levels increase with higher pesticide concentrations and longer exposure, which may suggest liver injury. Furthermore, exposure to xenobiotics can disrupt the antioxidant defense system in organisms [27].

SERUM ASPARTATE AMINOTRANSFERASE (AST)

The liver plays an essential role in maintaining homeostasis and is the primary site for metabolizing xenobiotics. However, the AST test results showed notable variations in average values. Specifically, AST enzyme activity was significantly higher in groups exposed to pesticide concentrations of 0.1482 to 0.3705 mg/L and 0.741 mg/L, with values of 36.00 ± 3.05, 51.67 ± 2.03 IU/L, and 64.33 ± 3.92 IU/L, respectively. This differs from the control group, which exhibited a value of 32.33 ± 2.34 IU/L (Table 7). Additionally, the analysis of variance indicated highly significant effects of both pesticide concentration and duration of exposure ($P \leq 0.001$) on the results, with a least significant difference of 9.554, highlighting clear distinctions among the groups.

SERUM ASPARTATE AMINOTRANSFERASE (AST)

AST enzymes play a crucial role in developing resistance and enhancing defense mechanisms against pesticides

[28]. The results from the AST test showed significant variations in mean values. Specifically, AST enzyme activity increased notably in groups exposed to pesticide concentrations of 0.1482 to 0.3705 mg/L and 0.741 mg/L, with values of 36.00 ± 3.05 IU/L, 51.67 ± 2.03 IU/L, and 64.33 ± 3.92 IU/L, respectively. In contrast, the control group exhibited a value of 32.33 ± 2.34 IU/L (see Table 8). Furthermore, the analysis of variance revealed highly significant effects of both pesticide concentration and duration of exposure ($P \leq 0.001$) on the results, with a least significant difference of 9.554, indicating clear distinctions among the groups.

In this study, we observed an increase in mean AST values compared to the control group as both pesticide concentrations and exposure durations increased. As noted by [29], these toxic metabolites can interact with intracellular macromolecules, potentially leading to hepatocellular necrosis (Table 8).

BIOMARKERS FOR HISTOPATHOLOGICAL ANALYSIS

Histopathological studies conducted in both laboratory and natural environments are effective in identifying the potentially harmful effects of pesticides on the target organs of fish. The further use of histopathological changes as the main parameter in the environmental monitoring is gaining growing relevance, especially in the determination of specific organs.

HISTOPATHOLOGICAL OBSERVATIONS ON THE COMMON CARP LIVER

The fish liver can be also infected by toxins, as well as is an active part of the metabolism and excretion of foreign substances. Liver injury or dysfunction may result in severe changes in a number of physiological functions that cause inflammation and metabolic dysfunction. The experiments have indicated that the glycol and glutamic acid can be toxic to the fish liver, and other animals as well. The analysis of common carp has found a disturbed antioxidant defense capacity, heightened serum glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) that are two essential enzymes used to assess liver health. After 20 days of exposure to varying concentrations of Spinosad (0.1482,0.3705, and 0.741 mg/L), histological changes were observed in the liver (cytoplasmic vacuolation, hepatocyte hypertrophy, blood congestion, and cellular necrosis), while the pancreatic glands and hepatocytes in the control group retained a normal appearance (Fig. 2 and 3 and Table 8).

HISTOLOGICAL CHANGES IN THE LIVER OF COMMON CARP (C. CARPIO)

The liver is a key organ in aquatic toxicology, crucial for the uptake, excretion, and detoxification of xenobiotics. Toxicant-induced changes in the liver reflect general findings on fish liver damage, highlighting the need to study hepatotoxic effects of specific toxins [30]. In common carp exposed to Spinosad, liver cells showed light vacuoles, indicating fatty degeneration, and necrosis occurred in some areas, possibly due to the fish's detoxification efforts and inability to produce new hepatocytes [31]. Studies suggest that glycol and glutamic acid may harm liver function in fish and other animals. Research has shown that common carp exhibit reduced antioxidant defenses and elevated serum enzymes GOT and GPT, indicating liver dysfunction [32]. The liver is vital for maintaining metabolic balance [33].

Histological changes in the liver were consistently observed after 20 days of exposure to different concentrations of Spinosad (0.1482, 0.3705, and 0.741 mg/L), as shown in Table 9 and Figure 2, 3, 4 and 5, which illustrate the normal appearance of pancreatic glands and hepatocytes in the control group. Figures 6-15 show

Microscopic analysis of common carp (Cyprinus carpio) tissues exposed to the Spinosad insecticide revealed a series of concentration-dependent changes. Lowest concentration group (0.1482 mg/L) – figures. 6, 7, 8, and 9. In this group, the histological appearance remained within normal limits. Both the pancreatic acini and hepatocytes (liver cells) maintained a normal appearance. Similarly, the small intestine showed normal structures of intestinal villi, crypts, and enterocytes. Medium concentration group (0.3705 mg/L) – figures 10, 11, 12, and 13. Exposure to this dose led to distinct damage. The liver and pancreas showed moderate degeneration, necrosis, and atrophy of the pancreatic acini. Dilation of central veins, edema, and tissue depletion were also noted. In the small intestine, marked mucosal thickening of the villi was observed, associated with hyperplasia and leukocyte aggregation, indicating inflammatory activity. Highest concentration group (0.741 mg/L) – figures 14 and 15. At a concentration of 0.741 mg/L, degenerative changes intensified. Liver tissue exhibited moderate vacuolar degeneration and cellular swelling of hepatocytes. Additionally, sinusoidal congestion was observed, confirming the pesticide's toxic effects on the vascular system and organ parenchyma.

Microscopic analysis of common carp (Cyprinus carpio) tissues exposed to the Spinosad insecticide revealed a series of concentration-dependent changes. Lowest concentration group (0.1482 mg/L) (Fig. 6-9). In this group, the histological appearance remained within normal limits. Both the pancreatic acini and hepatocytes (liver cells) maintained a normal appearance. Similarly, the small intestine showed normal structures of intestinal villi, crypts, and enterocytes. Medium concentration group (0.3705 mg/L) (Fig. 10-13). Exposure to this dose led to distinct damage. The liver and pancreas showed moderate degeneration, necrosis, and atrophy of the pancreatic acini. Dilation of central veins, edema, and tissue depletion were also noted. In the small intestine, marked mucosal thickening of the villi was observed, associated with hyperplasia and leukocyte aggregation, indicating inflammatory activity. Highest concentration group (0.741 mg/L) (Fig. 14 and 15). At a concentration of 0.741 mg/L, degenerative changes intensified. Liver tissue exhibited moderate vacuolar degeneration and cellular swelling of hepatocytes. Additionally, sinusoidal congestion was observed, confirming the pesticide's toxic effects on the vascular system and organ parenchyma.

DISCUSSION

The condition and behavior of fish in various test and control tanks were assessed every 24 hours for a period of up to 96 hours. When exposed to different concentrations of Spinosad, the fish exhibited notable changes in behavior, particularly at elevated concentrations. Accelerated movement patterns and anxious swimming behaviors have been observed [34]. Significantly, these behavioral alterations may serve as indicators of water pollution resulting from pesticide exposure [35]. The excessive mucus secretion acts as a nonspecific defense against toxins, likely reducing their interaction with harmful substances. Specifically, mucus aids in scavenging toxins through the epidermis or establishes a barrier between the fish's body and the surrounding medium, thereby mitigating irritating effects.

Rao (2006) reported comparable findings when fish were exposed to RPR-V, a novel phosphorothionate insecticide (2-butenoic acid-3[diethoxy phosphinothionyl] ethyl ester). Blood analysis can effectively identify and assess stressful conditions and diseases affecting fish production performance. This comprehensive evaluation enhances our understanding of the physiological status and overall health of fish [36]. What was observed in this case is that the maximum mean red blood cell (RBC) level of 2.97 million cells per microliter was measured at a concentration of 0.1482 mg/L that was observed in 20 days of exposure. Notably, this count was significantly lower than that of the control sample, which had a mean RBC count of 3.79 million cells per microliter.

Conversely, the lowest mean RBC counts recorded were 2.09 million cells per microliter and 1.94 million cells per microliter, noted at concentrations of 0.3705 mg/L and 0.741 mg/L, respectively, after the same exposure duration (see Table 6). These results suggest that increasing pesticide concentrations, along with extended exposure times, have a significant impact on red blood cell counts in the examined common carp. Analysis of variance indicated significant differences in red blood cell counts among the various pesticide doses ($P \leq 0.001$) and exposure durations. Furthermore, the least significant difference (LSD) value of 0.42 reinforces these findings. Research has indicated that hematopoietic dysfunction in fish exposed to insecticides can result in significant reductions in hemoglobin and red blood cell levels [37].

Indeed, the findings of the current study corroborate this, reporting diminished levels of both red blood cells and hemoglobin. Among the hemoglobin level explored, the maximum mean of 11.80 g/dL was attained among those fish exposed to pesticide concentration of 0.1482 mg/L during 20 days. The average of the level of 0.3705 mg/L of exposure was 11.30 g/dL and the lowest average of 9.80 g/dL was of those exposed to the level of 0.741 mg/L of the pesticide (Table 6). All these were significantly lower than the control sample which had an average hemoglobin concentration of 12.80g/dL.

According to [38] anemia can arise from various disorders that lead to a decrease in either the quantity or size of red blood cells. These disorders may include excessive bleeding, dietary deficiencies, cell death due to a transfusion reaction, or complications with hemoglobin formation. A lower hematocrit level reflects a reduction in both the number and size of red blood cells, consequently decreasing the space they occupy. The diagnosis of anemia is reinforced by abnormal blood test results that indicate a low hematocrit level. There was a significant statistical variance difference of both doses of the pesticide and exposure time on the level of hemoglobin in fish blood (P 0.001). The lowest significant difference (LSD) calculated was 1.15 which indicated that there were significant differences in some of the measured samples especially in the 0.1482 mg/L and 0.741 mg/L dosage.

These results indicate that ALT levels increase with higher pesticide concentrations and longer exposure, which may suggest liver injury. Furthermore, exposure to xenobiotics can disrupt the antioxidant defense system in organisms [27].

In this study, the researcher noted an increase in mean AST values compared to the control group as both pesticide concentrations and exposure durations increased. These toxic metabolites can interact with intracellular macromolecules, potentially resulting in hepatocellular necrosis.

Extensive application of systemic insecticides in agriculture has led to contamination of waters and soils of the treated crops with pesticide residues. As a result, human contact with stored pesticides is achieved by consuming contaminated fish. A major concern is the bioaccumulation of Spinosad residues in fish tissues, which may lead to biomagnification in humans via the food chain. As *Cyprinus carpio* (common carp) is a major protein source, consuming contaminated fish poses public health risks and may lead to long-term physiological disruptions, as these compounds are not easily eliminated by the human body. However, the initial symptoms of pollution can manifest as altered fish behavior or physical appearance, which indicates that both the consequences of pollution and the effects of pollution can be observed at both cellular and structural levels. Spinosad can consequently react with common carp on its entry into the water system.

The results from the AST test showed significant variations in mean values. Specifically, AST enzyme activity increased notably in groups exposed to pesticide concentrations of 0.1482 to 0.3705 mg/L and 0.741 mg/L, with values of 36.00 ± 3.05 IU/L, 51.67 ± 2.03 IU/L, and 64.33 ± 3.92 IU/L, respectively. In contrast, the control group exhibited a value of 32.33 ± 2.34 IU/L (see Table 8). Furthermore, the analysis of variance revealed highly significant effects of both pesticide concentration and duration of exposure ($P \leq 0.001$) on the results, with a least significant difference of 9.554, indicating clear distinctions among the groups.

In this study, we observed an increase in mean AST values compared to the control group as both pesticide concentrations and exposure durations increased. As noted by [29], these toxic metabolites can interact with intracellular macromolecules, potentially leading to hepatocellular necrosis.

CONCLUSIONS

This experiment comprehensively evaluated the effects of Spinosad exposure on common carp. Our results revealed significant dose-dependent toxicity, manifested by severe histopathological damage to the liver, along with significant changes in hematological and biochemical parameters. Relevant observations would involve those that shed light on the role of Spinosad in the natural freshwater environment and the impacts this runoff may have should it run off farms. These dynamics are important to understand in an attempt to enhance the water management practices and reduce the environmental risks. Furthermore, given that common carp represent a vital global food source, the observed toxicological impairments raise concerns about potential risks to human consumers through the food chain. Although Spinosad is a natural genetic option in terms of natural origin, the present study emphasizes the fact that, albeit low in concentration, it may have immense negative effects on aquatic life, including common carp. This emphasizes the highest need to use the environment wisely and monitor its use in order to achieve a balance between agricultural demands and the conservation of aquatic organisms. The longitudinal effects of Spinosad toxicity and molecular machineries on non-target aquatic organisms should be highlighted in future studies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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