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Tebuconazole-Induced Alterations in Hematological and Biochemical Responses of *Cyprinus Carpio* (L.1758): Protective Role of Propolis

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ABSTRACT

The effects of sublethal exposure to the systemic fungicide tebuconazole (TBZ) on *Cyprinus carpio*'s hematological and biochemical parameters were investigated. Fish were subjected to TBZ at concentrations of 6.47 µl/l and 8.09 µl/l for 30 days. Hematological changes included a biphasic reaction in red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Ht), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration. White blood cell (WBC) and eosinophil levels rose considerably over the exposure period. Severe erythrocyte deformations, anisocytosis, nuclear abnormalities, and Heinz bodies were detected, particularly at higher TBZ levels.Biochemical alterations included a decrease in total protein (-30.20%) and lipid levels (-56.92%), but total free amino acids increased (+76.32%) by day 30. During the 60-day propolis recuperation phase, significant gains were observed, with RBC count (+229%), Ht (+78.02%), and biochemical indicators such as lipid (+113.16%) and protein (+46.89%) exhibiting marked restoration. The findings show that TBZ causes hematotoxicity and metabolic abnormalities in fish, highlighting the preventive efficacy of propolis in mitigating pesticide toxicity in aquatic habitats.

INTRODUCTION

The global population is growing rapidly, leading to an increasing demand for agricultural products, particularly food. To meet this demand, crop production has expanded significantly. However, this rise in agricultural production is accompanied by a parallel increase in crop pests (Yeltekin, Oğuz, Kankaya, Özok, and Güneş, 2020). Consequently, the use of pesticides has escalated to safeguard crops from these pests. Pesticides are widely applied in intensive agriculture and fish farming to control various pests, including insects, weeds, molluscs, harmful bacteria, and viruses. However, pesticide contamination in the environment and food remains a major challenge in global agriculture (Li *et al.,* 2022). These chemicals can enter natural water bodies through soil runoff or direct application, posing risks to non-target organisms such as fish and prawns, which hold significant economic value(Oruç and physiology, 2010; Saravanan, Kumar, Ramesh, and physiology, 2011), Given their persistence and toxicity, pesticides contribute to long-term environmental and ecological disturbances. Among different classes of pesticides, fungicides play a crucial role in modern agriculture.

Azole fungicides, particularly triazoles and imidazoles, are widely used due to their broad-spectrum antifungal activity and high efficiency(Lass-Flörl, 2011; Peyton, Gallagher, and Hashemzadeh, 2015).These compounds function by inhibiting the ergosterol synthesis pathway, which is essential for fungal cell membrane integrity(Yeltekin *et al.,* 2020). Triazoles account for approximately 20% of the global systemic fungicide market, with India being a significant producer and consumer(Lari *et al.,* 2014).TBZ, a widely used triazole fungicide, is employed in both agricultural and public health programs(Toda, Beer, Kuivila, Chiller, and Jackson, 2021). It exerts its antifungal activity by strongly binding to cytochrome P450 (CYP450) enzymes and inhibiting the sterol 14α-demethylation process in CYP51, which is critical for fungal growth.

However, due to its persistence in the environment, TBZ can accumulate in aquatic ecosystems and exert toxic effects on non-target organisms(Becher, Wirsel, and biotechnology, 2012; Trösken *et al.,* 2006). Several studies have reported the presence of fungicides in different water sources, with TBZ frequently detected at high concentrations in surface water, groundwater, and wastewater(Elfikrie, Ho, Zaidon, Juahir, and Tan, 2020; Herrero-Hernández *et al.,* 2013; Kahle *et al.,* 2008). Given its widespread occurrence in aquatic environments, understanding its impact on aquatic organisms, particularly fish, is essential.Fish serve as reliable bioindicators of aquatic pollution due to their high sensitivity to environmental contaminants(López-López and Sedeño-Díaz, 2015). As fish are a major component of the human diet, pesticide residues accumulated in their tissues can easily enter the food chain and pose potential health

risks(Bansal, 2021). Therefore, monitoring physiological responses in fish exposed to pesticides is crucial for assessing ecological and human health risks. Hematological and biochemical parameters are widely used as sensitive indicators of toxicity in fish. These parameters provide insights into physiological and pathological changes, making fish blood an important tool in toxicological research and environmental monitoring(Saravanan *et al.,* 2011; Xu *et al.,* 2021). Hematological indicators such as hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) and white blood cell (WBC) counts, along with differential leucocyte indices like eosinophils, neutrophils, and basophils, are frequently assessed in toxicity studies(Saravanan *et al.,* 2011). Similarly, biochemical parameters, including plasma glucose, protein levels, total amino acids, total lipids, and glycogen content in vital organs, help evaluate the physiological stress induced by environmental contaminants (Sopinka, Donaldson, O'Connor, Suski, and Cooke, 2016).Although some studies have investigated the toxic effects of TBZ on aquatic organisms and mammals, research on its impact on the hematological and biochemical parameters of freshwater fish in India remains limited. To address this gap, the present study aims to evaluate the effects of sublethal concentrations of TBZ on selected hematological and biochemical parameters in *Cyprinus carpio*, a key species in carp polyculture systems in India. Given its economic and ecological significance, understanding the physiological responses of *C. carpio* to TBZ exposure will contribute to better environmental risk assessments and sustainable aquaculture practices.

MATERIALS AND METHODS

Fungicide

Commercial-grade TBZ (25.9% EC) was employed in the present investigation to assess toxicity. The appropriate concentration of the pesticide was diluted in distilled water, which was then utilized for chronic toxicity testing. Other analytical-grade chemicals (Merck, India) were sourced from reputable scientific suppliers.

Extraction of propolis

The effectiveness of propolis may vary depending on the extraction method, as different solvents extract different bioactive compounds. Ethanolandmethanol, are commonly used for biological assays (Mani*et al.,* 2006). Its chemical composition is complex and influenced by local flora. In this study, propolis was sourced from Kocaavsar village, Balikesir, Turkey, and prepared as a 30% ethanol extract (30 g propolis in 100 mL of 70% ethanol), stored at 4°C after filtration and drying. Research on rainbow trout demonstrated the protective effects of 10 ppm propolis on hematological and biochemical parameters (Talas*et al.,* 2009).

Collection of fish and maintenance

Healthy specimens of *C. carpio* (length 20 ± 2 cm, weight 180–250 g) were procured from the Fish Seed and Breeding Farm, Deoli, District Bilaspur, Himachal Pradesh. The fish were transported to the laboratory in aerated, water-filled polythene bags to minimize stress. Upon arrival, they were immersed in a 0.2% potassium permanganate solution for 2–4 minutes to prevent infections. Fish were acclimatized for 15 days in glass aquaria (80 L capacity) under laboratory conditions. The water was treated with an anti-chlorine solution and aerated for 24 hours before use. Aerators and filtration systems were installed, and 40% of the water was replaced daily to maintain quality. Fish were fed commercial supplementary feed twice daily (10:00 AM and 5:00 PM), and uneaten food and waste were removed periodically. The following physicochemical parameters of the water were maintained: temperature 22 ± 1.4°C, pH 7.5 ± 0.2, dissolved oxygen 8 ± 1 mg/L, total dissolved solids 155 ± 5 ppm, alkalinity 165 ± 8mg/L, and hardness 120 ± 4 mg/L.

Sublethal Studies

Fish were divided into three groups: one control (Group 1) and two experimental groups (Group 2 and Group 3). Group-2 fish were exposed to 6.47 µl/l of TBZ (lower sublethal concentration) and group-3 fish were exposed to 8.09 µl/l of TBZ (higher sublethal concentration) for 30 days. The toxicant was renewed daily to maintain a constant concentration after removing the same volume of water. Fish were randomly selected from the control and experimental groups at 10, 20, and 30-day intervals for analysis. No mortality was observed during the experimental period.

Blood Collection

Blood collection is a critical preanalytical step in haematological analysis. After the experiment, blood samples were collected in Eppendorf tubes using 0.5M EDTA as an anticoagulant. Fish were first anaesthetized using MS-222 or clove oil, with dosage adjusted based on species and size. Once the fish reached a sedated state with regular gill movement, the caudal vein was accessed for blood collection. The puncture site, located just posterior to the anal fin, was cleaned with sterile saline and ethanol.

A needle was inserted at a 30-45° angle, and blood was drawn slowly to minimize haemolysis and clotting. Depending on the fish's size, 0.5-2 mL of blood was collected. For larger fish, alternative collection sites such as the dorsal aorta or cardiac puncture were considered if a higher volume was required.

Hematological and biochemical analysis

The Neubauer's hemacytometer kit was used to determine the RBC and WBC counts. The Sahil's hemoglobinometer was used to test hemoglobin concentrations, and the microhematocrit method was used to quantify hematocrit (Hct) levels. Furthermore, using established methods, erythrocyte indicators such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were computed. By creating blood smears, the percentage of various leukocytes—mononuclear (lymphocytes and monocytes) and polymorphonuclear (neutrophils and eosinophils)—was determined using the Fatayer (2006) method.
Formulas used to calculate;

Total RBC count ($\times 10^3$ mm^3) = N × 10,000 mm^{-3} (N is the number of RBC).

Total WBC count ($\times 10^3$ mm^3) = N × 50 mm^{-3} (N is the number of WBC).

DLC=(Number of mononucler/ polymorphonuclear cells)/(Total number of cells)x 100

Hct = %Hb X 3

$$\textbf{MCH} = \frac{\%\text{Hb}}{\text{RBC}} \times 100$$

$$\textbf{MCV} = \frac{\%\text{Hct}}{\text{RBC}} \times 100$$

$$\textbf{MCHC} = \frac{\%\text{Hb}}{\%\text{Hct}} \times 100$$

Erythrocytic Nuclear and Cellular Abnormalities

The assessment of erythrocytic nuclear and cellular abnormalities followed previously described techniques (Al-Emran*et al.*, 2022; Sadiqul *et al.*, 2016; Shahjahan *et al.*, 2019). Blood samples were put onto glass slides and allowed to air dry for 10 minutes. The smears were then fixed in methanol for 10 minutes before staining with 5% Giemsa solution. After cleaning with distilled water, the slides were air-dried overnight and DPX-mounted for analysis. The observations were conducted using an electronic microscope under 100× magnification. Three slides were made for each fish, with each slide containing 2000 cells with intact nuclear and cellular membranes. The erythrocytic nuclear abnormalities (NA) and erythrocytic cellular abnormalities (ECA) were observed under criteria provided by Shahjahan et al. (2019) and Sadiqul et al. (2016)

Collection of Spleen

The fish were dissected after blotting dry with absorbent paper. The spleen was carefully removed and stored in respective plastic vials for biochemical analysis.

Estimation of biochemical alterations in spleen tissues

Biochemical parameters like total protein were measured by the method of(Bradford, 1976), total amino acidsby Moore and Stein (1954) and total lipid contentby the method of Folch et al. (1957).

Statistical analysis

The results of the studies were expressed as mean ± SE. The significance of sample means between control and TBZ-treated fish was tested according to the two-way ANOVA followed by Tukey's test ($p < 0.05$), error bars represent standard errors (SE) and capital letters indicate significant differences in mortality over different days, while lowercase letters above the bars show significant differences between concentrations.

RESULTS

Hematological observation

Figure 1a–k illustrates the hematological parameters of *Cyprinus carpio*exposed to sublethal concentrations of TBZ. A biphasic response was observed in hematological profiles, including hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and neutrophils (Figure 1. a,b,g and j). Throughout the study period, there was a gradual increase in eosinophil level with a maximum increase of 76.19% observed on the 20th day of exposure to 8.09 µl/l of TBZ, whereas lymphocytes, monocytes, and erythrocyte indices such as hematocrit (Ht), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) exhibited a continuous decline (Figure 1. c,d,e,f,h,i and k).At the end of the 30-day exposure period, the maximum percentage

increase in WBC count (84.16%) and eosinophils (68.10%) was recorded (Figure 1g and i). Conversely, the highest percentage decrease was observed in hematocrit (-48.74%) and MCH (-45.11%) at the same time point (Figure 1. c and d) compared to the control group.

No statistically significant differences were noted in hemetological parameters between the control group and the group treated with propolis only.During the recovery phase with propolis treatment, a significant restoration in hematological parameters was noted (Figure 1 a-k). The RBC count showed a maximum recovery of 229%, MCH of 102%, Ht of 78.02%, and lymphocyte percentage increased by 65.15% on the 60th day compared to the group exposed to 8.09 µl/l of TBZ.

Additionally, WBC count showed a mixed trend with a substantial decrease of 8.42% recorded on the 30th day in the treated group, highlighting the immunotoxic effects of TBZ on fish The alteration in blood cell morphology observed through smears were presented in Figure 2.TBzexposure (6.47 µl/l and 8.09 µl/l) resulted in severe hematological changes in *C. carpio*, including erythrocyte deformation, nuclear abnormalities, and enhanced anisocytosis, which worsened over time(Figure 2a-k).

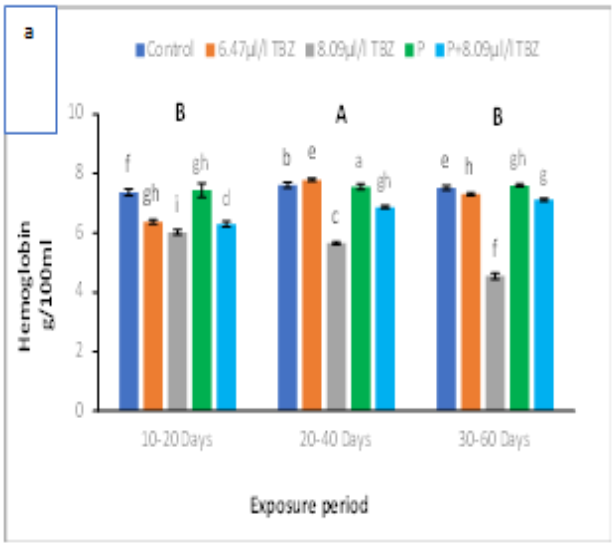
The higher dose (8.09 µl/l) caused significant cellular damage, including binuclear development, Heinz bodies, and nuclear degeneration(Figure 2. f-h). However, co-treatment with propolis for 60 days resulted in significant hematological recovery, with reduced erythrocyte abnormalities, improved cell shape, and normalized leukocyte profiles, suggesting the preventive efficacy of propolis against TBZ-induced toxicity(Figure 2. i-k).

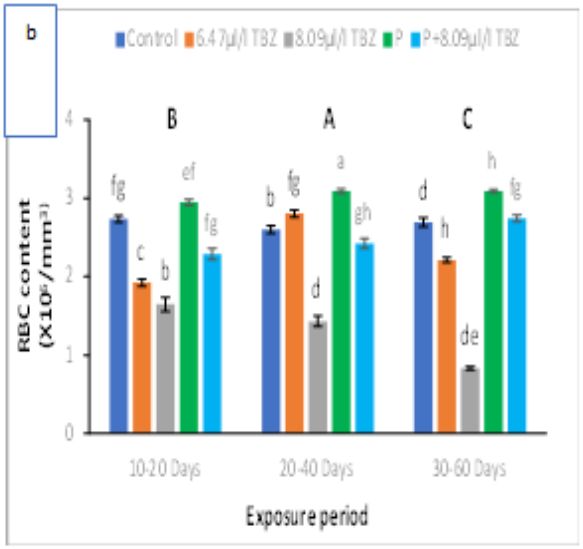
Biochemical observation

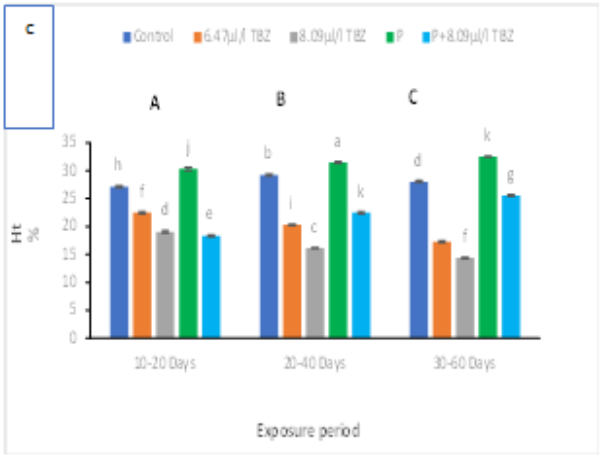
Figure 3. a–c, presented the effects of sublethal toxicity of TBZ on the biochemical parameters of the spleen in *Cyprinus carpio*. A gradual decline in total protein and total lipid levels was observed in TBZ-treated fish throughout the experimental period, with a maximum percentage decrease of -30.20% and -56.92%, respectively, by the end of the 30th day (Figure 3. A and c).

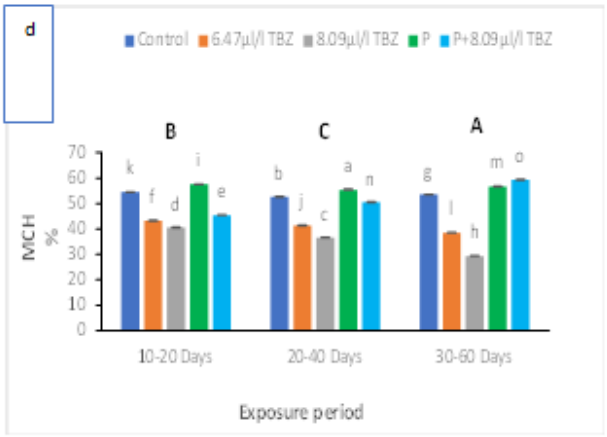
In contrast, the total free amino acid levels increased progressively throughout the exposure period, reaching a maximum percentage increase of 76.32% on the 30th day compared to the control group (Figure 3b).During the recovery phase with propolis treatment, significant restoration in biochemical parameters was observed.

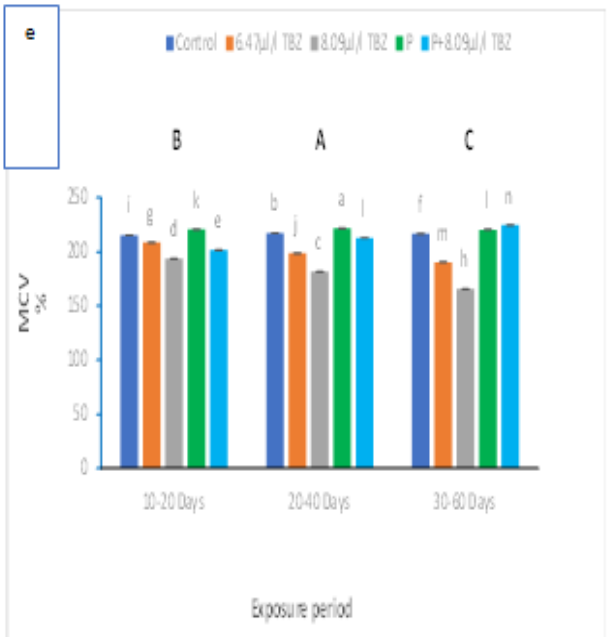
By the 60th day, lipid content exhibited a maximum recovery of 113.16%, while total free amino acids and total protein content showed recoveries of -32.84% and 46.89%, respectively, in comparison to fish exposed to 8.09 µl/l of TBZ. These findings suggest the potential of propolis in mitigating the biochemical alterations induced by TBZ toxicity

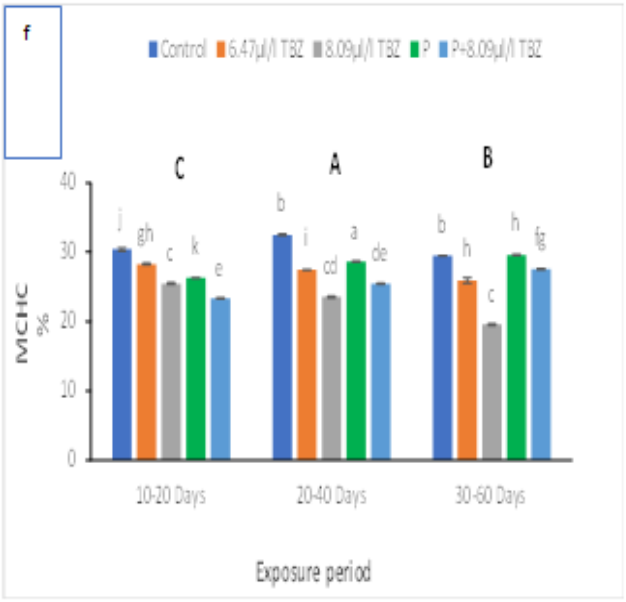


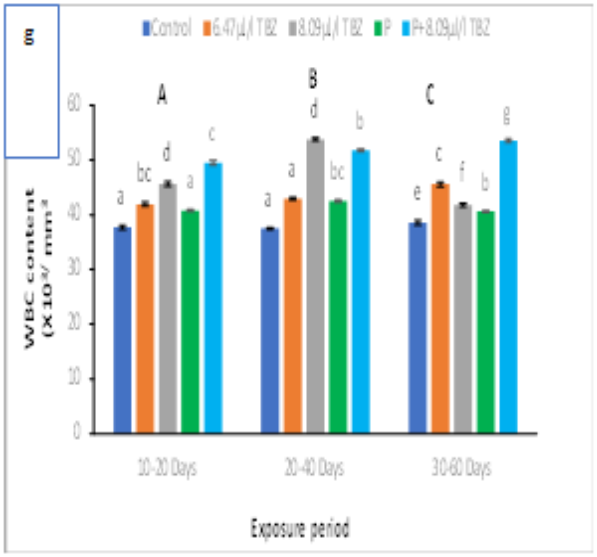


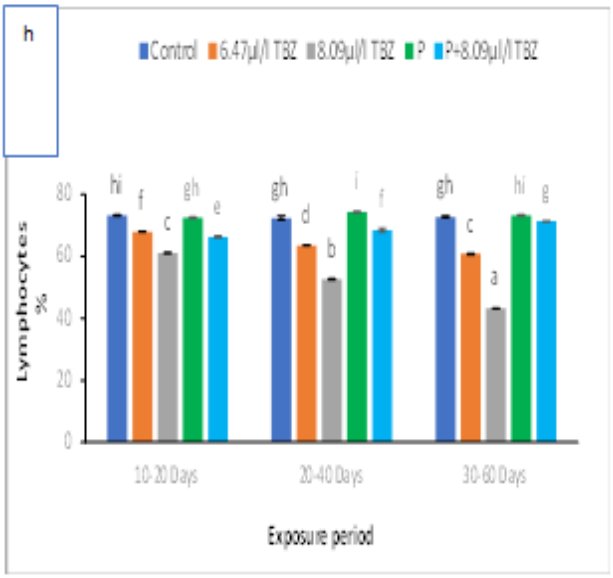

c
Control
6.47µl/l TBZ
8.09µl/l TBZ
P
P+8.09µl/l TBZ
A
B
C
Ht %
10-20 Days
20-40 Days
30-60 Days
Exposure period


d
Control
6.47µl/l TBZ
8.09µl/l TBZ
P
P+8.09µl/l TBZ
B
C
A
MCH %
10-20 Days
20-40 Days
30-60 Days
Exposure period


e
Control
6.47µl/l TBZ
8.09µl/l TBZ
P
P+8.09µl/l TBZ
B
A
C
MCV %
10-20 Days
20-40 Days
30-60 Days
Exposure period


f
Control
6.47µl/l TBZ
8.09µl/l TBZ
P
P+8.09µl/l TBZ
C
A
B
MCHC %
10-20 Days
20-40 Days
30-60 Days
Exposure period


g
Control
6.47µl/l TBZ
8.09µl/l TBZ
P
P+8.09µl/l TBZ
A
B
C
WBC content (X10³/ mm³
10-20 Days
20-40 Days
30-60 Days
Exposure period


h
Control
6.47µl/l TBZ
8.09µl/l TBZ
P
P+8.09µl/l TBZ
Lymphocytes %
10-20 Days
20-40 Days
30-60 Days
Exposure period

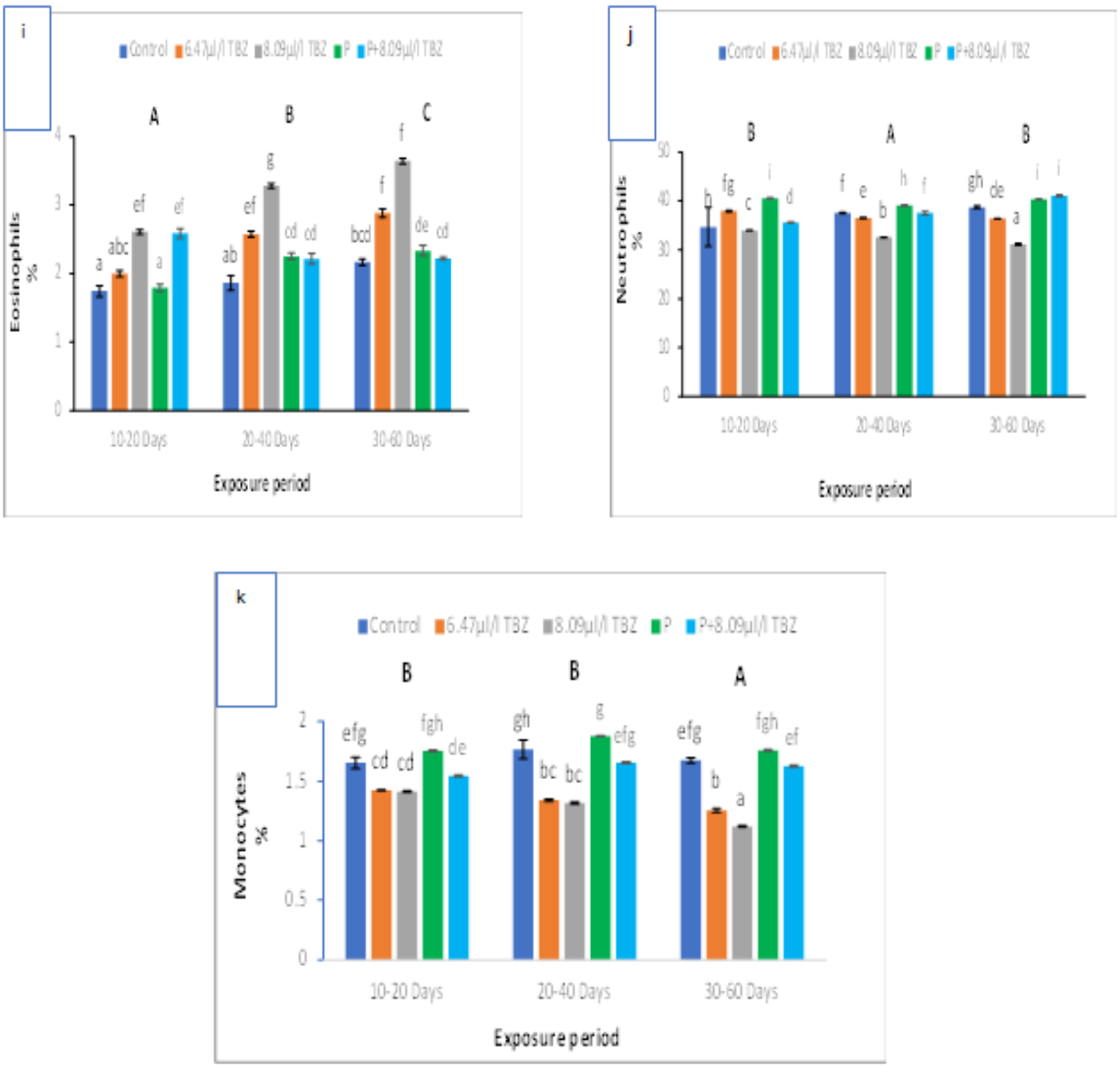
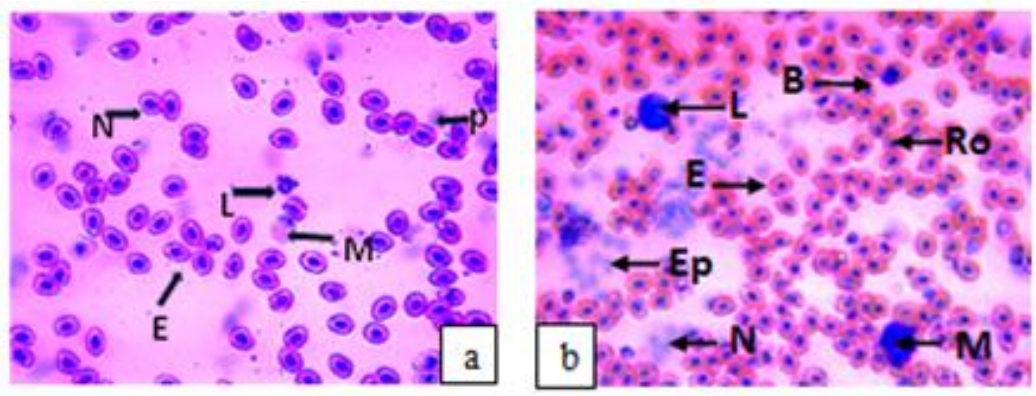
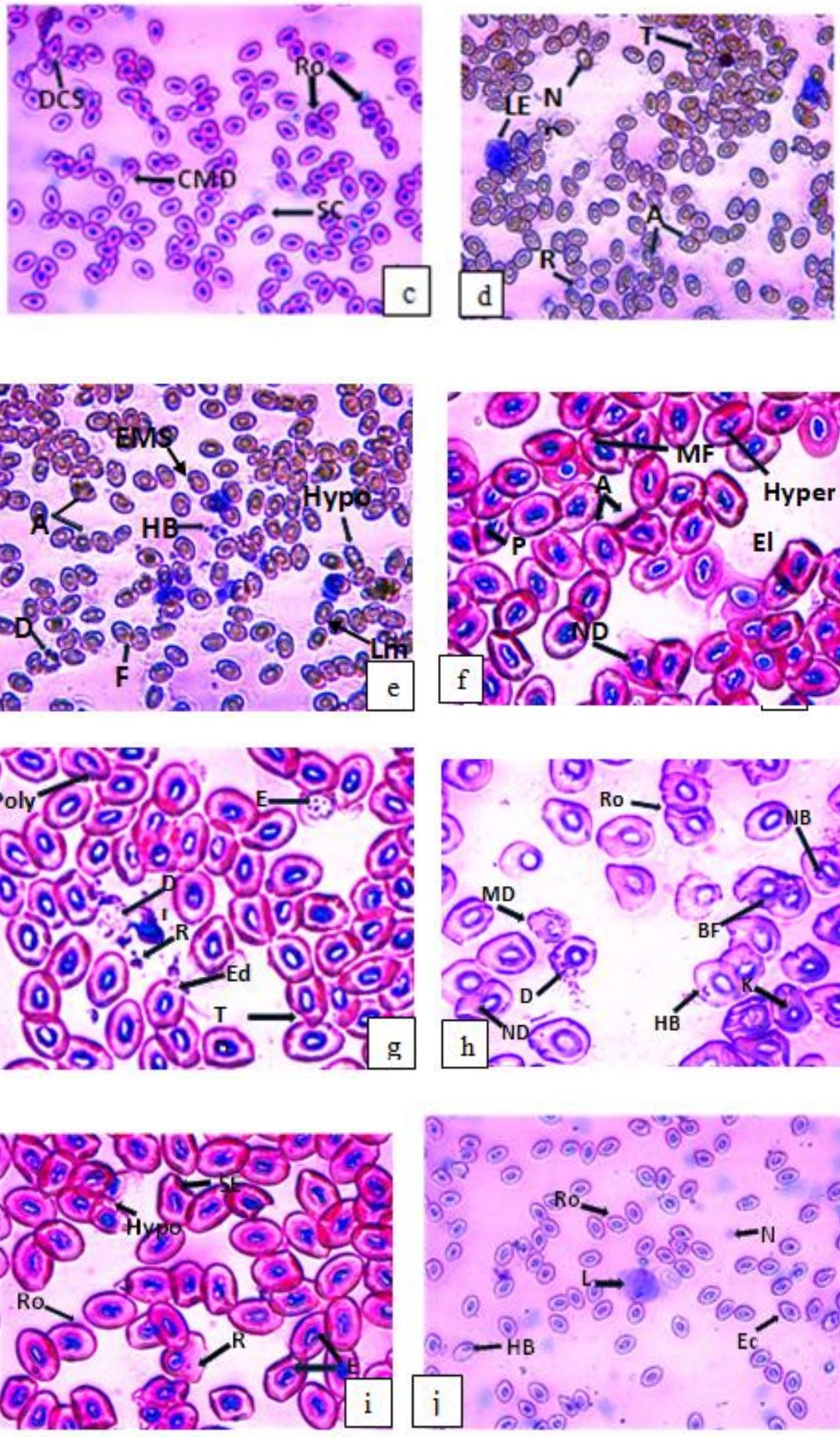


Fig. I

Fig.1.Hematological values ((a) Hb; (b) RBC; (c)Ht; (d)MCH; (e) MCV; (f) MCHC; (g)WBC; (h) Lymphocytes; (i)Neutrophils; (j) Monocytes; (k) Eosinophils of C. carpio exposed to sublethal concentration of TBZ for 30 days. Bars represent SE. Lowercase letters representsignificant at P < 0.05 (based on the Tukey test).




DCS
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MF
A
Hyper
P
El
ND
f
Poly
E
D
R
Ed
T
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ND
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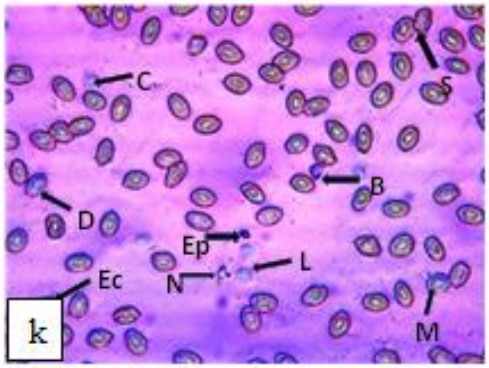

Fig.2

Fig 2. Blood smears of control group (a), and treated with only propolis (b),showing normal erythrocytes (E), neutrophils (N), lymphocytes (L), eosinophiles (Ep), monocytes (M), basophiles (B) with little signs of rouleaux formation (Ro) slides (c-e);6.47 µl/l of TBZ treated group showing elliptocytosis (El), nuclear degeneration (ND), micronuclear formation (MF), pyknosis (P), anisocytosis (A), reticulocytosis (R), hyperchromasia (Hyper), dacrocytes (TD), polychromasia (Poly), degenerating lymphocytes (DL), erythrocyte degeneration (Ed), degenerating eosinophils (E), binuclear formation (BF), Heinz bodies (HB), nuclear budding (NB), rouleaux (Ro), karyopyknosis (K).,slides (f-h);8.09 µl/l of TBZ treated groupshowing deformed cell shape (DCS), sickle cell formation (SC), cell membrane damage (CMD), lymphocyte enlargement (LE), twin formation (T), lacrymocytes (Lm), fusion (F), degeneration (D), erythrocytic membrane shrinkage (EMS). and slides (i-k);P+8.09 µl/l of TBZtreated group with reduced rouleaux (Ro), lower reticulocyte count (R), decreased hypochromia (Hypo), fewer sickled erythrocytes (SE), increased erythrocyte production (E), normalized lymphocytes (L) and neutrophils (N).

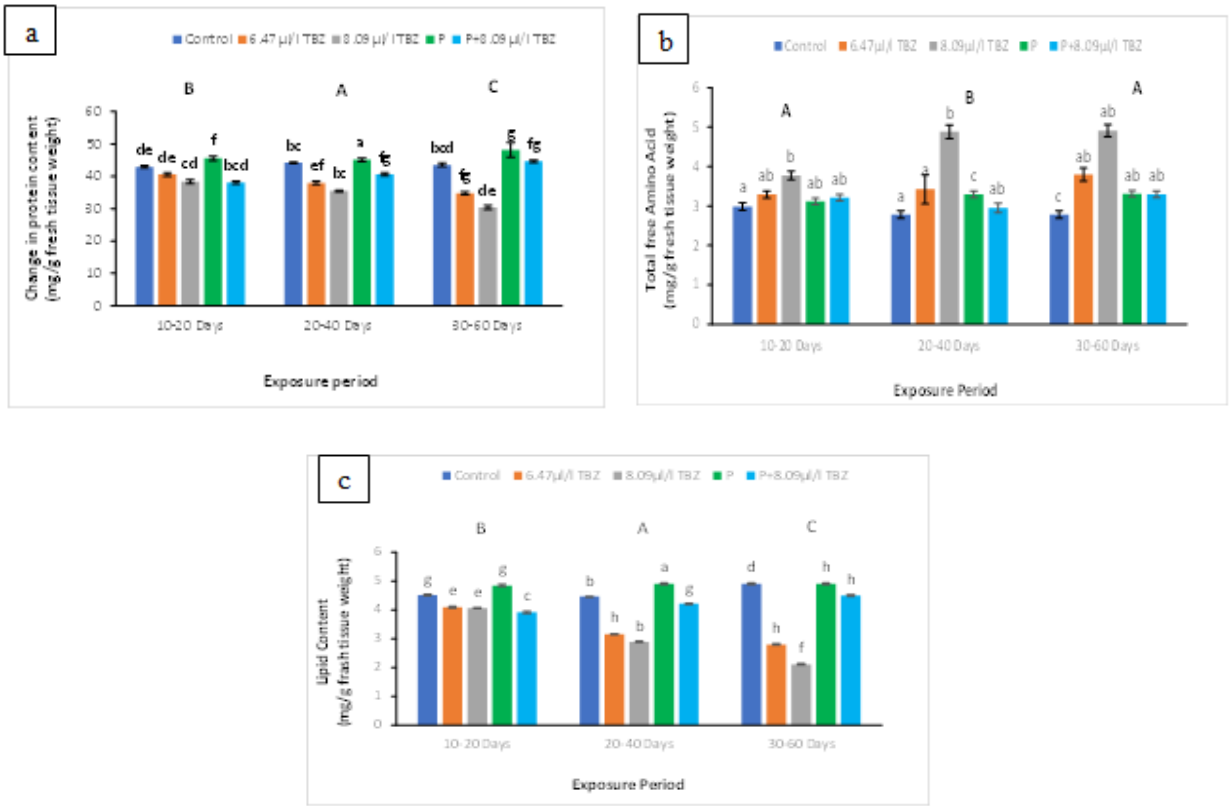

Fig.3

Fig.3 Alterations in biochemical parameters(a)total protein content; (b) total free amino acids; (c) total lipid content of spleen of *Cyprinus carpio* treated with different concentrations of TBZ for different exposure periods of 30 days and ameliorative effects of propolis within 60 days. Bars represent SE. Lowercase letters represent results significant at P<0.05 (based on Tukey's test).

DISCUSSION

Blood serves as a crucial indicator of toxic stress, and the evaluation of hematological parameters in fish is commonly utilized to assess toxic stress levels and the overall health status of the organism (Kavitha*et al.,* 2012; Yonar*et al.,* 2014). The hematological profiles of *Cyprinus carpio*subjected to sublethal amounts of TBZ showed a biphasic reaction in this investigation.In the present study, the reduced RBC count, hemoglobin (Hb), and hematocrit (Hct) levels in *Cyprinus carpio*exposed to TBZ may be attributed to hemolysis induced by the toxic effects of the fungicide. This decline suggests potential damage to erythrocytes, leading to impaired oxygen transport and hematological dysfunction.

Additionally, this reduction serves as an indicator of anemia, potentially resulting from the inhibition of erythropoiesis, impaired hemoglobin synthesis, or osmoregulatory dysfunction. It may also be linked to an increased rate of RBC destruction in hematopoietic organs (Vani *et al.,* 2011). Similar observations were noted in *Alburnus tarichi* exposed to TBZ(Yeltekin *et al.,* 2020), *Cyprinus carpio*treated with *Moringa oleifera* seed extract (Kavitha *et al.,* 2012)and those exposed to lindane (Saravanan *et al.,* 2011). Comparable decreases in these parameters were also observed in *Clarias gariepinus* exposed to plant extracts, including tobacco (*Nicotiana tabacum*) and cassava effluents(Adeyemo, 2005; Omoniyi*et al.,* 2002). The lower Hb concentration may indicate a slower rate of hemoglobin synthesis, hindering oxygen delivery to tissues and causing erythrocyte lysis due to stress (Atamanalp., 2003). In contrast, Ayotunde (2004)reported an increase in RBC count, hemoglobin, and hematocrit levels in *Oreochromis niloticus* exposed to aqueous extracts of Moringa oleifera seeds(Kavitha *et al.,* 2012).

A decline in MCV, MCH, and MCHC values in *Cyprinus carpio*exposed to TBZ suggests the development of microcytic hypochromic anaemia due to a high proportion of immature red blood cells in circulation, similar to findings by (Yonar *et al.,* 2014)in *Cyprinus carpio*treated with malathion and by (Prusty *et al.,* 2011)in *Labeorohita* treated with fenvalerate. MCHC serves as an indicator of red blood cell swelling, and its reduction during acute exposure may be attributed to the release of immature erythrocytes with lower hemoglobin content into circulation (Kavitha *et al.,* 2012). Additionally, an increase in erythrocyte volume under stress conditions could be another contributing factor. However, fish treated with propolis during the recovery phase exhibited hematological parameters similar to the control group, significantly differing from those exposed solely to TBZ. These findings indicate that propolis may mitigate the hematotoxic effects of TBZ by helping maintain optimal blood parameters. Similar observations regarding the ameliorative properties of propolis were noted by (Yonar *et al.,* 2014)in *Cyprinus carpio*exposed to malathion.

Erythrocytic nuclear abnormalities (ENAs), including micronucleus formation, binucleation, and nuclear degeneration, observed in TBZ-exposed *C. carpio*, strongly indicate genotoxicity and chromosomal instability. This aligns with findings by (Lim *et al.,* 2009), who reported that ENAs serve as critical biomarkers of genotoxic stress in fish exposed to pollutants. (Shahjahan *et al.,* 2019) further emphasized that micronucleus formation results from DNA damage due to chromosomal breakage and spindle apparatus dysfunction. Similar genotoxic responses have been reported in *Heteropneustesfossilis*(Akter *et al.,* 2020), *Cirrhinusmrigala*(Bhatnagar*et al.,* 2016) and *Danio rerio*(Shahjahan *et al.,* 2019) following exposure to various fungicides and pesticides. Furthermore, apoptosis associated with nuclear abnormalities is often mediated by caspase-activated DNase, leading to nuclear fragmentation and cytoskeletal protein cleavage (Bolognesi and Hayashi, 2011; Kroemer., 2007), further supporting the findings of the present study.

Morphological changes in erythrocytes, including teardrop and fusion-shaped cells, observed in *C. carpio* exposed to TBZ, indicate membrane instability and oxidative stress (Moss and Hathway, 1964) described similar erythrocyte deformities as a consequence of altered membrane permeability and cytoskeletal protein disruption. Comparable hematotoxic effects have been reported in *Clarias batrachus*(Narra., 2017) and *Labeorohita*(Ghaffar *et al.,* 2020) following pesticide exposure, highlighting the common occurrence of erythrocyte abnormalities in fish subjected to environmental contaminants.

This study highlights the protective effects of propolis in reducing erythrocytic nuclear abnormalities and oxidative stress in *C. carpio* exposed to TBZ. Propolis, known for its antioxidant and anti-inflammatory properties, stabilizes cell membranes, reduces DNA damage, and prevents chromosomal instability. Studies (Nassar *et al.,* 2020; Orun and Erdogan, 2014; Yonar *et al.,* 2014), have shown that propolis enhances antioxidant enzyme activity, neutralizing reactive oxygen species (ROS) and mitigating genotoxic effects. Additionally, it inhibits apoptotic pathways, preserving erythrocyte integrity and preventing cytoskeletal disruptions. These findings support the potential of propolis as a natural protective agent against pesticide-induced toxicity in fish.

A significant rise in WBC count, notably eosinophils, was observed, suggesting an immunological response to TBZ-induced toxicity. The greatest rise in WBC (84.16%) and eosinophils (68.10%) implies that TBZ functions as an immunostimulant, initiating lymphopoiesis and increasing leukocyte release from hematopoietic regions (Ates *et al.,* 2008; Nussey., 2002; Saravanan *et al.,* 2011). This is consistent with prior research showing increased WBC counts in

response to environmental stressors(Al-Emran *et al.*, 2022; Ates *et al.*, 2008; Begg and Pankhurst, 2004). Comparable increases in WBC counts have also been documented in *Pangasius hypophthalmus* exposed to agrochemicals(Hedayati and Tarkhani, 2014) and *Channa gachua* subjected to pesticide-contaminated water(Pala and Dey, 2016). However, some studies have reported reductions in WBC counts, such as those observed in *C. carpio* exposed to other pesticides (Kaya *et al.*, 2015). These discrepancies suggest that immune responses may vary depending on species, toxicant type, and exposure duration.Propolis has been shown to have antioxidative and immunomodulatory effects, which could aid in hematopoietic regeneration and immune system restoration. Propolis has been demonstrated to modulate lymphocyte activity in mice (Kalsum*et al.*, 2017), enhancing immune responses. Studies suggest that propolis stimulates lymphocyte proliferation, increases cytokine production, and boosts antibody responses, improving disease resistance (Orun and Erdogan, 2014). Overall, the hematological alterations observed in this study highlight the toxic effects of TBZ on *Cyprinus carpio*.

The decline in RBC-related parameters and the initial leukocytosis followed by a decrease in WBC count underscore the immunotoxic and hematotoxic nature of TBZ. However, the observed recovery with propolis treatment suggests potential therapeutic applications for counteracting pesticide-induced toxicity in fish.Protein is a crucial biochemical parameter used to assess overall health and metabolic responses under pollutant-induced stress(Saravanan *et al.*, 2011). Fish experiencing stress may utilize protein as an energy source to sustain heightened physiological activity(Martinez*et al.*, 2004). In the present study, the observed decline in total protein and total lipid levels in the spleen of *Cyprinus carpio* exposed to sublethal concentrations of TBZ suggests significant biochemical disturbances induced by the fungicide. The reduction in total protein content may be attributed to impaired protein synthesis or increased proteolysis as a stress-induced adaptive response to maintain energy demands. Comparable results were also noted by(Saravanan *et al.*, 2011)in lindane treated *Cyprinus carpio* and (Sastry and Sharma, 1981) in *Ophiocephalus punctatus* treated with diazinon.

Similarly, the depletion of lipid reserves could indicate their mobilization to compensate for the increased energy requirements under toxic stress. These findings align with previous studies demonstrating the metabolic adjustments in fish exposed to environmental toxicants(Gurushankara*et al.*, 2007). The decrease in total lipid levels and the increase in lipase activity in the spleen of fish exposed to lethal concentrations of TBZ indicate a significant accumulation of the pesticide in this organ. This disruption suggests an impaired homeostatic mechanism, likely due to enhanced lipid metabolism as a stress response. The excessive breakdown of lipids may reflect an adaptive strategy to meet energy demands under toxic stress, ultimately leading to metabolic imbalances and compromised spleen function.These findings align with the earlier study by (Ravinder*et al.*, 1988)which reported similar effects in *Clarias batrachus* exposed to decis. (Bradbury*et al.*, 1987)suggested that the reduction in protein content could be linked to cellular damage or necrosis, leading to impaired protein synthesis. Protein depletion in tissues may serve as a physiological adaptation, functioning as a compensatory mechanism under cypermethrin-induced stress by supplying intermediates for the Krebs cycle. Additionally, this decline in protein levels has been associated with increased osmolality, potentially helping to counteract osmoregulatory disturbances caused by ion leakage and the loss of essential molecules during pyrethroid toxicity (Rafat, 1986).

Conversely, in the spleen, enhanced protease activity and reduced protein levels resulted in a significant increase in free amino acid (FAA) content across all time intervals. This accumulation of FAA is likely due to protein degradation, as well as a decreased utilization of amino acids(David*et al.*, 2004). Additionally, the elevated FAA levels may play a role in maintaining osmotic and acid-base balance, as suggested by (Moorthy et al .,1984).Similar stress-induced elevations in FAA have been reported by (Awasthi*et al.*, 1984). Additionally, the activation of alternative metabolic pathways, such as aminotransferase reactions, could result from the inhibition of key oxidative enzymes, including succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, and cytochrome-c-oxidase.

A comparable metabolic alteration was previously demonstrated by (Ghosh, 1989), in *Labeorohita* under cypermethrin toxicity.During the recovery phase with propolis supplementation, a significant restoration of biochemical parameters was observed, consistent with findings from previous studies (Abdelmagid *et al.*, 2022; Orun and Erdogan, 2014; M. E. Yonar*et al.*, 2012). The substantial recovery in lipid and protein levels suggests that propolis plays a crucial role in mitigating TBZ-induced toxicity by enhancing antioxidant defence mechanisms, facilitating cellular repair, and stabilizing metabolic processes.

Furthermore, the partial normalization of free amino acid levels indicates a progressive return to homeostasis, reflecting improved physiological conditions in fish.Similar protective effects of propolis have been reported in *Cyprinus carpio* exposed to trichlorfon (M. E. Yonar*et al.*, 2015) and in pesticide-treated rainbow trout (Fuat Gulhan*et al.*, 2012), reinforcing its efficacy in counteracting toxicant-induced damage. These findings underscore the potential of propolis as a natural therapeutic agent in mitigating pesticide-induced biochemical alterations, highlighting its role in enhancing metabolic stability and promoting overall fish health.

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