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THERAPEUTIC POTENTIAL OF ALOE VERA EXTRACT ON HAEMATOLOGY, HEPATOXICITY, AND NEPHROTOXICITY INDUCED BY COPPER IN LABEO ROHITA

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ABSTRACT

An experiment was conducted to find the therapeutic potential of Aloe vera extract on haematology, hepatotoxicity and nephrotoxicity induced by copper in Labeo rohita. Four treatments were made. T_0 served as the controlled treatment. Juveniles in the T_1 were exposed to 15 mg/L $CuCl_2$ while those in T_2 were exposed to 20 mg/L $CuCl_2$. Juveniles in the T_3 were exposed to 25 mg/L $CuCl_2$ for 21 days. After this period all groups of fish were supplemented with 20g/kg Aloe vera extract for one week. At the end of the trial, both haematological parameters and the histopathology of the liver and kidney were analyzed. The results showed a substantial decrease in RBCs, haemoglobin (Hb) and haematocrit (HCT) values across all metal-treated groups T_1, T_2, and T_3 as compared to the control group. The control group T_0 (2.05±0.05) had the greatest RBC count after receiving 0 mg/L of copper chloride. The lowest RBC count was seen in group T_3 (0.63±0.175). The control group T_0 had the greatest haemoglobin level (2.99±0.027) while the lowest amount was found in group T_3 (0.70±0.53). T_3 group had the greatest white blood cells count (18.6±0.36) while the T_0 control group had the lowest (11.64±0.050). The control group T_0 had the highest platelet count (23.5±0.9) whereas group T_3 had the lowest (9.71±0.7). The haematological parameters examined included haematocrit (Hct), haemoglobin (Hb), platelets, red blood cells (RBCs), white blood cells (WBCs), mean corpuscular volume (MCV). Copper chloride-induced hepatotoxicity appeared as hepatocyte inflammation, necrosis, clumped chromatin and cytoplasmic vacuolization. For histopathological analysis kidney were stained and examined under a compound microscope to identify any structural changes or damage. Data collected from these analyses were statistically analyzed using one-way ANOVA to determine the significance of differences between the control and treated groups.

INTRODUCTION

Aquaculture and fishing contribute significantly to global development employing 41 million people in developing nations. The growing human population has increased the demand for aquatic food products, but fisheries productivity has declined. This has led to increased demand for seafood aquaculture, accounting for 46% of global fish production. Pakistan with vast natural

water resources offers valuable resources for aquaculture but only uses 1% of available resources. Careful planning is needed to maximize productivity in underutilized sites (Anderson et al., 2017).

Rohu a thin, elongated, flattened fish has a silver-gray tint on its sides and can grow up to 45 kilograms. Its streamlined body, striking colors and adapted fins make it a global ecological and culturally relevant species, adapting well to freshwater habitats (Mahamood et al., 2021). Labeo rohita also known as the Indian big carp is a freshwater fish native to Indian subcontinent rivers and lakes. It primarily eats algae and macrophytes, but also depends on rotifers, cladocerans, and protozoans (Khan et al., 2017).

Copper chloride a mixture of copper and chlorine is used in various applications such as dyeing, disinfecting agents, wood preservation and as an organic reaction catalyst. Its white form absorbs moisture and oxidizes to blue-green(Anant et al., 2018). Copper toxicity in living organisms is crucial. Maintaining low copper levels in water bodies is essential with clean water containing only 0.5-1 µg/L. Copper's toxicity in aquatic environments is influenced by its speciation. As a transition element copper is essential in marine ecosystems (Malhotra et al., 2020).

Hematological parameters are crucial for assessing fish health after exposure to toxins and diverse environments (Xu et al., 2021). Histopathological investigations help identify malformations and illnesses (Witeska et al., 2023). Pollutant exposure can cause structural damage, strain and metabolic alterations. Measures of hemoglobin, RBC and WBC counts were used to evaluate the hematologic profile of Labeo rohita (Thangam et al., 2014). Chronic exposure to sublethal metal concentrations significantly increases leukocyte count primarily due to lymphocyte count (Köprücü et al., 2006). However, copper exposure significantly impacts leukocyte count in rainbow trout blood (Kavitha et al., 2012).

Kidney is crucial for maintaining body fluid, substance and acid-base balance and can be a marker of environmental stress in fish ecosystems. Excessive copper levels in aquatic environments can cause kidney damage, causing oxidative stress and direct cellular toxicity. This can lead to decreased activity, erratic swimming and physical signs like swelling or edema (Hozayen et al., 2011). High copper buildup can lead to renal cell loss in various parts of the nephron. This damage affects tubules, glomeruli, blood arteries and can cause proteinuria, reduced kidney filtering, glutamate aciduria and phosphaturia potentially causing renal failure (Rahman et al., 2019).

Liver is essential to an organism's health performing processes such as excretion, detoxification, metabolism, and harmful chemical elimination. Monitoring histological changes in fish liver is a highly sensitive and accurate tool for evaluating the impact of xenobiotic substances in both field and laboratory research (Alkobaby and Abd El-Wahed, 2017). Heavy metal copper significantly impacts Labeo rohita's hematology reducing parameters and causing apoptosis, cell degeneration and inflammation while Aloe Vera acts as a hepatoprotective agent (Padrilah et al., 2017).

Aloe vera a bioactive herb with over 70 active substances is crucial in aquaculture for improving fish health and well-being (Kushwaha, 2013). Its antioxidant, anti-inflammatory and antibacterial properties increase disease resistance, reduce stress and speed up wound healing. Aloe vera extract improves water quality creating a healthier environment for fish and leading to sustainable businesses (Syed et al., 2022).It also offers numerous health benefits, including anticancer, antioxidant, antiallergenic, antiviral and anti-inflammatory properties. Aloe vera extract can aid in the recovery from infectious parasite diseases improve kidney function in Labeo rohita fish and reduce the harmful effects of copper on marine species (Danish et al., 2020).

MATERIALS AND METHODS

Heavy metal pollution particularly copper contamination has adversely affected freshwater resources. This study investigates the impact of copper on the hematological, liver and kidney histopathological parameters of Labeo rohita a significant freshwater species rich in protein, calcium and vitamin A. The experiment involved exposing fish to copper chloride and subsequently supplementing their diet with Aloe vera extract.

Experimental Design

Forty fingerlings, each weighing 10-15g and measuring 6.0-8.0cm, were procured from the Punjab Fish Hatchery, Faisalabad. They were acclimated in a 70-liter glass aquarium filled with aerated dechlorinated tap water for seven days. The fish were divided into four groups (T_0, T_1, T_2, T_3) of ten fish each and fed twice daily at 5% of their body weight. The aquarium water was regularly changed and fecal matter was removed. The treatments were as follows.

o Treatment 0 : Control group o Treatment 1 : 15mg/L Copper Chloride o Treatment 2 : 20mg/L Copper Chloride o Treatment 3 : 25mg/L Copper Chloride

Specific amounts of copper chloride were dissolved in 70-liter water to achieve the desired concentrations. In the last week, the fish received 20g/kg Aloe vera extract as a supplement.

Preparation of Aloe vera Extract

Aloe vera leaves were collected, washed, dried and ground into a powder. The extract was mixed with commercial fish feed at a ratio of 20g/kg. The feed composition included corn, rice polish,

rapeseed meal, canola gluten, fish meal, poultry meal, DCP, lysine, methionine, minerals, vitamins, molasses and synthetic amino acids.

Soxhlet Extraction Method

Bioactive compounds from Aloe vera were extracted using ethanol in a Soxhlet apparatus. The process lasted 4-8 hours until the solvent became clear. The extract was then filtered, evaporated and stored under refrigeration.

Blood Sample Collection

After the 21-day exposure three fish from each treatment group were sampled. The fish were anesthetized using clove oil and blood samples were collected from the caudal vein using sterile plastic syringes containing EDTA as an anticoagulant. The samples were carefully handled to avoid contamination and were used for hematological analysis.

Hematological Analysis

Red Blood Cell Count (RBC): RBCs were counted using a hemocytometer. Blood was diluted and placed in the hemocytometer chamber for counting under 400x magnification.

White Blood Cell Count (WBC): WBCs were counted using the Battement method. Blood was diluted with Turk's solution and counted in a hemocytometer.

Platelets Count: Platelets were counted using a hemocytometer after diluting the blood with ReesEcker Fluid.

Hemoglobin Concentration (Hb): Hemoglobin was measured using the cyanmethemoglobin method, with absorbance compared to a standard solution.

Packed Cell Volume (PCV): PCV was determined using the micro hematocrit method where blood was centrifuged and the hematocrit value calculated.

Mean Corpuscular Volume (MCV): MCV was calculated to determine the average mass of red blood cells.

Histopathological analysis of liver

Fish liver tissues were removed, formalin-fixed overnight, and tiny slices were embedded in paraffin and stained with hematoxylin and eosin for microscopic examination. Tissues were submerged in 10% formalin for 11 to 15 days. After fixation, 0.1 cm^2 slices were rinsed for 6-7 hours, then dehydrated with paraffin, ethanol, and xylene. This procedure prepared the samples for histopathological analysis.

Histopathological Evaluation of Kidney

Histopathological analysis involved tissue preparation including fixation, dehydration, embedding, sectioning, mounting and staining.

Fixation: Kidney tissues were fixed in 10% formalin solution for 3-5 days to prevent cell disintegration.

Post-Fixation Treatment: Tissues were dehydrated using ethanol and xylene of increasing concentrations.
Embedding: Dehydrated tissues were embedded in paraffin to form blocks.
Sectioning: Thin tissue sections were prepared using a microtome and mounted on slides.
Mounting: Sections were affixed to slides with Meyer's egg white and dried.
Staining: Slides were stained with Hematoxylin and Eosin to differentiate tissue structures.
Statistical Analysis
Data were analyzed statistically and represented as mean ± SD. ANOVA was used to compare means of normally distributed data.

RESULTS
The current trial was conducted to determine therapeutic potential of aloe Vera extract on hematological parameters, hepatotoxicity and nephrotoxicity induced by copper in Labeo rohita. For acclimatization purpose the fishes were fed with basal diet and kept under laboratory conditions for about one week and then transferred to fish tanks after sampling.
In this four week trial least RBCs, Platelets, Heamoglobin and hematocrit were observed in the treatment T3 (25mg/L of $CuCl_3$) followed by Treatment T2 (20mg/L $CuCl_3$) , treatment T1 (15mg/L $CuCl_3$) and the normal control group. The copper chloride treatment increased the WBCs and MCV in the T3 (25mg/L of $CuCl_3$) followed by T2 (20mg/L $CuCl_3$), T1 (15mg/L $CuCl_3$) and control group.
In this trial after treatment with Aloe vera extract it was found that Aloe vera didn't showed any significant results on fishes treated with $CuCl_2$. The extract didn't reversed or enhanced the RBCS, platelets, haemologbin and hematocrit in T3. It aslo didn't significantly lowered the WBCs and MCV in T3.
The following table shows the mean values for the fishes treated with different concentration of copper chloride and table no 2 shows the mean values for fishes treated with 2 % of Aloe vera extract.
Table 1

Treatments	RBCs	WBCs	Platelets	Heamoglobin	Hematocrit	MCV
Treatment (Control)	2.05	11.6	23.5	2.99	15.67	65.93
Treatment 1	1.84	14.7	16.25	1.96	14.037	68.57
Treatment 2	1.267	16.52	12.9	1.43	12.417	70.70
Treatment 3	0.63	18.6	9.82	0.70	10.407	72.71

Table 1: illustrates the mean values of RBCs, WBCs, Platelets, Haemoglobin, Hematocrit and MCV in the Labeo rohita treated with copper chloride. The lowest RBCs were recorded in the T3

as 0.63 while the control, T1 and T2 had 2.05, 1.84 and 1.267 respectively. Likewise the lowest platelets, haemoglobin and hematocrit were observed in the T3 as 9.82, 0.70 and 10.407 when compared to the control group. Table shows that the highest WBCs were recorded in the T3 as 18.6 while control, T1 and T2 had values of 11.6, 14.7 and 16.52. in the same way highest MCV was observed in the T3 as 72.71 whereas control, T1 and T2 had the MCV as 65.93, 68.57 and 70.70 after treatment with copper chloride.

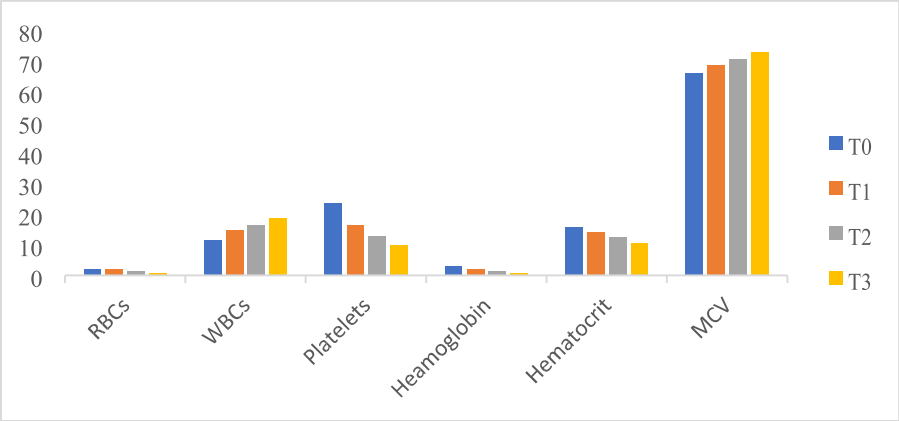
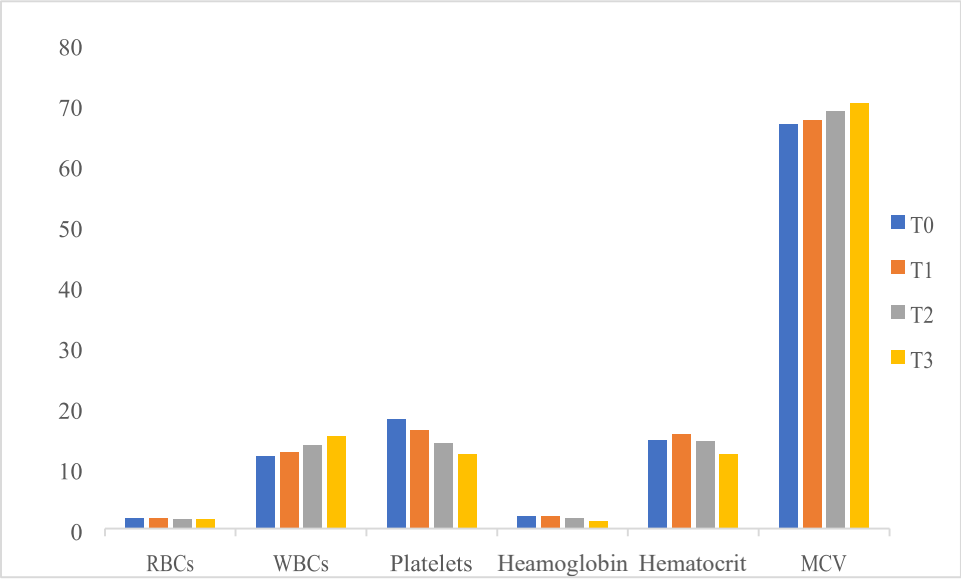


Table 2

Treatments	RBCs	WBCs	Platelets	Heamoglobin	Hematocrit	MCV
Treatment (Control)	1.7	12.02	18.0	2.06	14.6	66.7
Treatment 1	1.75	12.6	16.23	2.02	15.5	67.4
Treatment 2	1.5	13.8	14.0	1.73	14.5	68.8
Treatment 3	1.5	15.2	12.2	1.2	12.2	70.2

Table 2 shows the mean values of blood parameters after treating with 2% Aloe vera extract. The table shows that aloe vera didn't significantly mitigate the toxic effects of copper chloride as it didn't lower the WBCs and MCV. It also didn't raise significantly the RBCs, platelets, haemoglobin and hematocrit.



Effects of Copper on liver and kidney tissues of Labeo rohita

Histological changes in liver and kidney tissues were observed through the help of ligh microscope after treating with different concentrations of copper chloride. The slides showed the comparison of alterations in treatments T_0, T_1, T_2 and T_3.

Histopathological alterations in the liver of fish treated with different concentrations of copper chloride were observed under light microscopic exams by making paraffin- embedded slides. The slides showed the comparison of morphological alterations of liver tissues in treatments T_1, T_2, T_3 and T_O (control group). The microscopic observation of the liver in T_1 which was exposed to 15mg/L copper chloride showed distinct morphological alterations like degeneration, hemolysis, necrosis and inflammation of hepatocytes. The microscopic observation of the liver in T_2 which was exposed to 20 mg/L copper chloride showed individual cell necrosis. The copper in hepatocytes was clumped. The intensity of damage noticed in T_3 was comparatively higher than that of T_1. The apoptosis of hepatocytes was prominent. Extensive inflammation in portal vein and mild cytoplasmic degeneration (CD) were recorded. The kidney tissues of control group in slide A shows normal nephrocyte pattern, normal paranchyma, nephrocyte nuclei and vesicular structure. The microscopic observation of T_1 (15 mg/L $CuCl_2$) showed different alterations in tissues like hemolysis necrosis, degeneration and inflammation of nephrocytes. The parenchyma was constricted. Kupffer cells were observed highly along the walls. In addition, few cells have glycogen deposited in them. The observation of T_2 kidney tissues (20 mg/L $CuCl_2$) showed cell necrosis. Nephrocyte chromatin was clumped. The cytoplasm become heavily vacuolated. Lymphocytic infiltration was shown between nephrocytes. The highest damaging effects were recorded in T_3 as compare to other treatments. The apoptosis of nephrocytes was prominent. Extensive inflammation in portal vein and mild cytoplasmic degeneration (CD) were recorded. The degree of histopathological changes had significantly reduced with Aloe vera supplementation in the diet in T_1. Hepatocytes of the fish liver treated with 2% Aloe vera extract on T2. The

histological appearance of the fish liver treated with 2% aloe vera extract in T3 showed less apoptosis and mild portal vein inflammation but edema was persistent in T3. This appearance indicates poor protection of hepatocytes against hepatoprotective agents. Supplementation of aloe vera showed good recovery in T_1. Nephrocytes of the fish kidney treated with 2% Aloe vera extract on T_2. The Nephrocytes showed less hemolysis, necrosis and inflammation of Nephrocytes. The necrosis was absent and regeneration of kidney cells. The histological appearance of the fish kidney treated with 2% aloe vera extract in T_3 showed less apoptosis and mild portal vein inflammation but edema was persistent in T_3. This appearance indicates poor protection of Nephrocytes against nephroprotective agents

DISCUSSION

The study investigates the therapeutic effect of aloe vera on hematology, hepatotoxicity and nephrotoxicity caused by copper chloride in Labeo rohita. Results showed that copper chloride exposure for 21 days causes toxicity in Labeo rohita. Aloe vera when supplemented in fish feed acts as hepatoprotective and nephroprotective agent and strong antioxidant.

The study found that treatment T_3 with 25mg/L copper chloride significantly reduced red blood cell count compared to treatment T_1 and T_2 compared to the control group. This could be due to increased oxidative strain inhibition and reduced cellular antioxidants. Anemia was also observed possibly due to impaired erythropoiesis and defective Fe metabolism. The results align with previous studies by Gandhewar and Zade (2010) and Chaudhary et al. (2023) which found significant reductions in red blood cells in Labeo rohita.

The study found that copper exposure significantly increased white blood cells (WBCs) in heavy metal-treated Rohu fish compared to the control group. The increased in WBCs could be attributed to the immune system's response to toxic metals. The immune system fights against intruders and produces antibodies for immunity. The increase in WBCs could be attributed to inflammatory reactions and cell degeneration. Similar results were reported by Garai et al. 2021 in Channa punctatus due to copper sulphate and copper chloride-induced toxicity. Similar results was explained by Radha and Laxmipriya (2022) Copper exposure can stimulate the immune system and lead to elevated WBC counts as a defense mechanism against toxicity.

The study found that exposure to different concentrations of copper chloride significantly decreased the platelet levels in Labeo rohita. The decrease in platelet production may be due to toxins which are destroyed in the immune-mediated system. Platelets are coated with antiplatelet antibodies and removed from circulation by the fixed phagocyte system. This leads to thrombocytopenia a condition characterized by excessive sequestration of platelets. Similar findings were found in Pant et al., (2017) and Khan et al. (2022), supporting the idea that platelets are targeted by antiplatelet antibodies and removed from circulation.

Heavy metal treatment in Labeo rohita led to a significant decrease in hemoglobin levels compared to the control group. Treatments T_3 and T_1 showed lower mean values possibly due to heavy metal accumulation in red blood cells (RBCs) that hindered hemoglobin formation. Copper metal restrains hemoglobin synthesis by inhibiting vital enzymes involved in the heme group synthesis pathway. Olaifa et al. (2004) have shown similar results in Oreochromis niloticus

indicating that copper metal can significantly affect hemoglobin levels and closest results were written by Bazari Moghaddhan et al. (2022) that copper inhibits the sysnthesis of haemoglobin by interfering with several key enzymes in the heme synthesis pathway.

The study found that different concentrations of copper chloride significantly decreased the hematocrit percentage in heavy metal-treated Rohu fish compared to the control group. Treatments with 25mg/L copper chloride showed a lower mean value (10.40±0.72) compared to 20mg/L and 15mg/L copper chloride respectively. Chung et al. (2009) observed decline in hematocrit levels was associated with anemia indicating an insufficient supply of healthy red blood cells and a large number of white blood cells due to long-term illness infection. Mekkawy et al. (2011) reported a significant decrease in RBCs, Hb, and Het content in Oreochromis niloticus exposed to Cd for 15 and 30 days. Our findings were consistent with these observations.

The copper showed hepatotoxicity in T_1 by causing degeneration in hepatocytes. The degeneration of hepatocytes is due to oxidative stress. The oxidative stress is due to the production of reactive oxygen species (ROS) and hydroxyl radicals (OH). Hepatotoxicity of copper in T_2 showed severe inflammation. Copper chloride activates inflammatory proteins like TNF-a, nuclear factor-kappa B (NF-kB), is specifically involved in the occurrence of inflammation. Treatment (T_3) is adversely affected by copper chloride toxicity. Hepatocyte showed apoptosis. Hepatotoxicity induced by exposure to copper stimulate inflammation in hepatocytes. These results were also observed by Dini et al., (2002). Bainy et al., (1996) also showed similar results that heavy metals cause reduction of catalase enzyme and elevation of super- oxide dismutase that indicates liver oxidative stress.

Aloe vera showed non-significant effects on hematology. RBC count in treatment T3 (1.5±0.1) was non-significantly lower than T1 (1.5±0.02), T2 (1.75±0.05), and the control (1.7±0.6). WBCs in T3 (15.2±0.55) were non-significantly higher than T2 (13.8±2.4), T1 (12.6±1.33), and the control (12.02±0.84). This hematological response is due to Aloe vera's insufficient potency to counteract copper toxicity. Haghighi et al. (2014) similarly found no significant impact of Aloe vera on RBCs, WBCs, Hct, or Hb in Labeo rohita. Jin et al. (2023) also suggested Aloe vera might not mitigate copper-induced immunosuppression effectively.

Aloe vera has been found to have a significant nephroprotective effect in various tissues including kidneys. It reduces nephrocyte oxidative stress inhibits reactive oxygen species generation and enhances antioxidant activity. It also reduces inflammation in nephrocytes, reduces receptor-4-mediated inflammation and reduces cytokine production. Additionally, aloe vera contains lupeol, quercetin and kaempferol, which enhance mitochondrial antioxidant and redox status and can increase antioxidant enzymes in the kidney.

SUMMARY

The present study was conducted to investigate therapeutic potential of Aloe Vera extract on hematology, hepatotoxicity and nephrotoxicity in Labeo rohita. Labeo rohita were divided into four treatments T_0, T_1, T_2, and T_3. The fish were acclimatized for a week and then given sub-lethal concentrations of copper chloride for four weeks. After 21 days the fish were given a sub-lethal concentration of copper chloride and supplemented with Aloe Vera 20g/kg in their feed. Changes

in hematological parameters were recorded under different treatments. Histopathological studies were conducted on the liver and kidney sections. Results showed a significant decrease in hematological parameters in T_1, T_2 and T_3. Hemoglobin concentration and hematocrit levels were significantly higher in T_0 (control) and T_2 (control) respectively. White blood cells and platelet count increased significantly in heavy metal treatments while mean corpuscular hemoglobin levels were significantly higher in T_0 (control). Histopathological evaluation showed no pathological alteration in the kidney of T_0 (control), but changes in kidney histo-architecture were observed in T_1, T_2 and T_3. Histopathological examination revealed no pathology changes in the liver of T_0 (Control) Rohu. Increased blood sinusoids, broken blockages in the bile duct and portal vein, and hepatocyte destruction in T1, T2, and T3 demonstrated changes in the liver's histo-architecture. Aloe Vera showed non-significant results on hematology, but it is hepatoprotective and nephroprotective.

Conflict of Interest: The authors have no conflict of interest.
Contribution: All authors contributed equally.

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