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Therapeutic potential of *Senna alata and Moringa oleifera* in reversing paracetamol-induced hepatic stress in Nile tilapia

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Abstract

Pharmaceuticals are widely used in disease management and may contaminate the environment through excretion, improper disposal, and industrial and hospital effluents. Paracetamol, a commonly used pharmaceutical, induces liver damage in *Nile tilapia* by reducing antioxidant enzyme activity. Superoxide dismutase (SOD) and catalase (CAT) are key antioxidant enzymes that protect cells against free radicals and alleviate oxidative stress. Treatment with *Moringa oleifera* and *Senna alata* leaf extracts restored SOD and CAT levels, indicating hepatoprotective effects, likely mediated by their antioxidant properties. These findings suggest that the extracts have potential in mitigating pharmaceutical-induced toxicity in fish. The results obtained were further confirmed by the histological studies of the liver tissue from all the experimental groups.

Key words: *Senna alata, Moringa oleifera*, Paracetamol

Introduction

Paracetamol, widely used and highly bioavailable, is an emerging contaminant in wastewater (Alchin *et al.,* 2022). Its overdose can cause hepatocyte toxicity, disrupting liver metabolic functions.

Superoxide Dismutase (SOD) and catalase (CAT) are key biochemical markers of liver function in fish, whose reduced levels indicate hepatic damage. Medicinal plants like *Moringa oleifera* and *Senna alata*, rich in therapeutic compounds, are widely used in traditional medicine to treat various toxicities (Stohs and Hartman, 2015).

Materials and methods

Plant Collection and Preparation of feed

Moringa oleifera and *Senna alata* leaves were collected from the local areas of Ernakulam district, Kerala, India. The collected samples were washed in clean water and dried under shade for 7-10 days. Using a mechanical homogenizer the leaves were powdered. Equal weight of powdered commercial fish feed and powdered leaves were mixed well and made into dough. It was then squeezed through a string hopper maker and dried in shade. The dried pellets thus made are stored in a clean and dry container at room temperature. Paracetamol exposed fishes were fed with this feed at a rate of 0.25g/day/kg of fish for 21 days.

Collection of Fishes and Setting up of Experiment

Male *Oreochromis niloticus* (15 ± 1 cm, ~100 g) were collected from a fish farm in Maliankara, Kerala, and acclimatized for a week. Fish were then exposed to 1/5th of the 96h LC_{50} of paracetamol (fishes were exposed to 0.330 mL/L of liquid acetaminophen for 96 hours. The 96-hr LC_{50} was determined graphically) for 4 days, and later divided into three groups (10 each), They were fed for 21 days with: commercial feed (0.50 g/day/kg of

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fish -Treatment 1), fed with *Senna* leaf powder mixed food (commercial fish feed 0.25g + 0.25g senna leaf powder/day/kg of fish -Treatment 2), and fed with *Moringa* leaf powder mixed food (commercial fish feed 0.25g + 0.25g moringa leaf powder/day/kg of fish - Treatment 3). A control group, unexposed to paracetamol, received only commercial feed.

Preparation of Extract for Biochemical Analysis

After the prescribed period of experiment, the liver was carefully dissected out from the fish, washed and wiped carefully with blotting paper to remove adhering blood and other body fluids. 1 g of tissue was homogenized in 3.0 mL potassium phosphate buffer and centrifuged at 358g for 10 minutes; the supernatant was used for the assay.

Analysis of Biochemical Parameters

Assay of Superoxide Dismutase (SOD)

SOD activity was measured following Kakkar *et al.* (1984). The reaction mixture included sodium pyrophosphate buffer (1.2 mL), PMS (0.1 mL), NBT (0.3 mL), and enzyme extract (0.2 mL). After centrifugation at 2000 rpm for 10 min, the supernatant was adjusted to 2.8 mL with distilled water. The reaction was initiated with 0.2 mL NADH, incubated at 30°C for 90 sec, and stopped with 1.0 mL glacial acetic acid. After adding 4.0 mL n-butanol, the mixture was shaken, left to stand for 10 min, and centrifuged. The butanol layer was collected, and absorbance was read at 560 nm. A control (without enzyme) was run in parallel. One unit of SOD activity equals 50% inhibition of NBT reduction per minute and is expressed as U/g/min.

Assay of Catalase (CAT)

The method of Luck (1974) was followed for determining the catalase activity. H_2O_2-Phosphate buffer (3.0 mL) was taken in experimental cuvette, followed by the rapid addition of 40 micro liter of enzyme extract. The mixture was centrifuged at 2000 rpm for 10 minutes, and the supernatant was used for the assay. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm. The enzyme solution containing H_2O_2 free phosphate buffer served as a control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance 240 nm by 0.05 units. The activity is expressed as U/ mg/ minutes.

Histopathology studies

The tissue was fixed in Bouin's fluid for 24 hours and processed into a wax block. Thin sections (5 µm) were cut using a microtome and stained with Haematoxylin and Eosin. The stained sections were observed under a phase-contrast microscope (Nikon Eclipse 80i) at 400× magnification.

Statistical Analysis

To assess the statistical significance of the results, one-way analysis of variance (ANOVA) was performed using R statistical software (version 4.4.1).

Results and Discussion

Paracetamol overdose is a leading cause of acute liver failure, driven by oxidative stress and inflammation. Biochemical parameters of liver showed significant changes after 96 hours of exposure.

Fish fed with plant materials for 21 days, showed improved SOD and catalase levels. Compared to the Control, SOD levels increased in Treatments 2 and 3 but decreased in Treatment 1, indicating that both plant extracts helped restore SOD levels towards normal (Table I and II: Figure1). Similar to our findings, *Moringa oleifera* caused increased SOD levels in tilmicosin-induced rats, suggesting its strong antioxidant properties (Abarikwu *et*

al., 2017). SOD has been described as the first line of defense against free radical chemicals (Ighodaro and Akinloye, 2018). The endogenous antioxidant systems, which consist of the antioxidant enzymes (e.g., SOD and CAT) and non-enzymatic antioxidant components (e.g., GSH), are known to play an important role in cellular defense against oxidative damage (Otto and Moon, 1996).

Our study showed complete loss of catalase activity in Treatment 1, with slight recovery in fish fed with Senna and Moringa leaves, though levels remained much lower than Control (Table I and II: Figure 1). The experimental exposure of paracetamol causing toxicity in liver and kidney, remarkably decreasing SOD and CAT activities has been reported by Uysal *et al.* (2016) and Karaali *et al.* (2018). Significant increases in SOD, CAT, and GSH were observed in *Senna alata*–treated rats versus untreated diabetic controls, indicating its potential antioxidant effects against alloxan-induced oxidative stress (Attama *et al.,* 2023). Abamectin exposure in tilapia reduced biochemical and immune functions, while a 1% *Moringa oleifera* extract in the diet improved antioxidant status, immune response, and protected against hepato-renal damage (Reda *et al.,* 2023).

The alterations observed in antioxidant enzyme activities were correlated with the histopathological changes in the liver. In the present study, *Nile tilapia* exposed to paracetamol exhibited marked hepatic damage characterized by enlargement of hepatocytes, increased cellular density in the hepatic parenchyma, cytoplasmic degeneration, extended sinusoids and peripheral vacuolization in several regions (Figure 3, Figure 2-control) These histological alterations are consistent with findings reported in previous studies. Vliegenthart *et al.* (2014) examined hepatocyte necrosis in zebrafish exposed (bath exposure) to very high concentrations of (20-40 mM; about 3000-6000 mg/L) acetaminophen which showed severe hepatotoxicity. The observed decrease in antioxidant enzyme levels indicates oxidative stress–induced liver damage caused by paracetamol exposure, thereby supporting the relationship between impaired antioxidant defense and hepatic tissue injury.

Summary

Paracetamol reduced Superoxide dismutase and catalase levels, indicating toxicity, while *Senna alata* and *Moringa oleifera* effectively restored these parameters, showing protective potential.

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Table I- SOD (U/g/ min) and CAT activity (U/mg/ min) in the liver of *Oreochromis niloticus* - Control, Treatment 1, Treatment 2 and Treatment 3

Group	Mean ± SD*		p-value
	SOD	CAT	
Control	1.31 ± 0.03	0.16 ± 0.04	0.000
Treatment 1	0.11 ± 0.01	0 ± 0	
Treatment 2	1.01 ± 0.01	0.01 ± 0	
Treatment 3	1.01 ± 0.01	0.01 ± 0	

*Values are the mean of 6 different sets of experiments ± SD

Table II- Analysis of Variance of SOD and CAT activity between Control, Treatment 1, Treatment 2 and Treatment 3 in the liver of *Oreochromis niloticus*

Groups	Mean difference		Calculated value		P value	
	SOD	CAT	SOD	CAT	SOD	CAT
Control - Treatment 1	1.201	0.163	135.502	12.929	0.000	0.000
Control - Treatment 2	0.301	0.155	33.969	12.294	0.000	0.000
Control - Treatment 3	0.296	0.155	33.424	12.255	0.000	0.000
Treatment 1 - Treatment 2	-0.900	-0.008	-101.532	-0.635	0.000	0.920
Treatment 1 - Treatment 3	-0.905	-0.009	-102.077	-0.674	0.000	0.906
Treatment 2 - Treatment 3	-0.005	-0.001	-0.545	-0.040	0.947	1.000

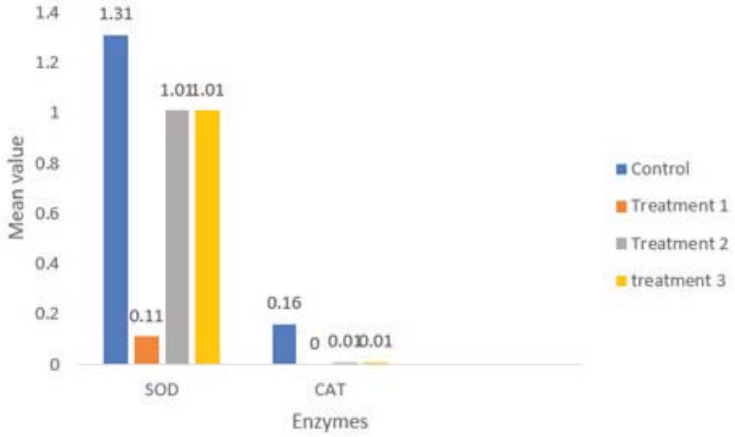

Fig.1 SOD and CAT activity bin the liver of Oreochromis niloticus - Control, Treatment 1, Treatment 2 and Treatment 3

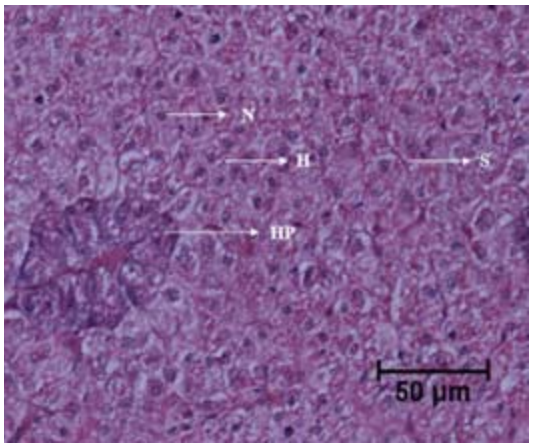

Fig. 2 Liver of Control fish (not exposed to paracetamol) showing a uniform arrangement of hepatocytes (H) with normal nucleus(N), normal sinusoids (S), and normal hepatopancreas (HP); (N);

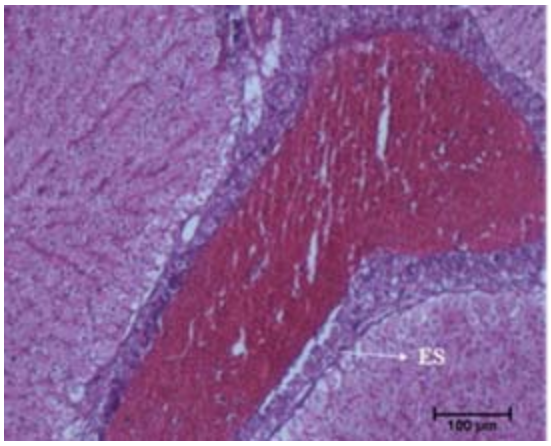

Fig. 3 Liver of fish treated with 1/5th of 96-hour LC50 of paracetamol showing extended sinusoid (ES)