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ABSTRACT

The purpose of this study was to examine whether or whether the serum acid and alkaline phosphate levels of the snake-headed Murral, Channa punctatus, would change after being exposed to an aqueous extract of the latex that is produced by the Euphoria tirucalli plant. Fishes were subjected to two sub-lethal concentrations (20 percent and 40 percent of 30h-LC50) of freshly made aqueous extract of Euphorbia tirucalli for 30 hours and 60 hours, as well as in a control group. This was done in addition to the standard protocol. Serum levels of acid and alkaline phosphate were found to have increased with increasing sub-lethal concentrations and the amount of time the animals were exposed to an aqueous extract of Euphoria tirucalli plant latex. This was observed in both of the experimental groups (20 percent and 40 percent of 30h-LC50 sub lethal concentrations). Both the dose and the amount of time passed had an effect on the alterations in acid and alkaline phosphate. For the purpose of recuperation, experimental fishes were moved to their natural environment and maintained there for one week. The levels of acid and alkaline phosphate in fishes showed signs of recovery after a withdrawal period of seven days, going from extremely high values to low values. According to the findings of this study, the unusually high values of serum acid and alkaline phosphate in Channa punctatus are caused by the latex of the Euphorbia tirucalli plant.

Keywords: *Aqueous extract, serum acid phosphate, serum alkaline phosphate, Euphorbia tirucalli, and C hanna punctatus etc.*

INTRODUCTION

It has been determined that the application of plant extracts as piscicides in fisheries can be advantageous as an alternative to the use of persistent pesticides. It is possible that the deliberate introduction of these phytoconstituents into the aquatic ecosystem will result in a stress response in the productivity of the aquatic environment. 2

Euphorbia tirucalli, a shrub that is utilised for medicinal purposes, is quite popular in India. This shrub thrives in tropical regions that are semi-arid in nature. Latex can be found in every section of this plant (a milky sap). Fungi and insects in general can be kept under control with an aqueous solution of the latex that is extracted from the stem. The fish are rendered immobile by the terpene 4-deoxyphorbol ester, which is the active component of the latex produced by the Euphorbia tirucalli plant.

Because of its ready availability and their capacity to quickly adapt to the conditions of the laboratory, the stress indicator Channa punctatus (Bloch) is frequently utilised. It is a fish that consumes other animals and breathes air; it is a predator and a carnivore; and it is widespread across the plains of India. In this study, an aqueous extract of plant latex was taken from the Euphorbia tirucalli plant. The serum acid and alkaline phosphatase levels discovered in the blood of the Channa punctatus cichlid fish were measured to identify the effect that the extract had.

The Components and Procedures

Experimental animal

The snake-headed murral, Channa punctatus, was 17.5 1.50 cm in length and weighed 44.0 2.5 g. It was obtained from the Aligarh district in the state of Uttar Pradesh in India. Before the experiment, the fish were kept in aquaria that contained 50 litres of fresh water that had been dechlorinated and treated with a potassium permanganate solution for two minutes. This was done to remove any skin that might have been adhered to the fish. The fish were then given a week to acclimate to the conditions of the laboratory. A fresh supply of water was delivered every day, and there was also food that was manufactured. The fish were not fed in the time leading up to the experiment.

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Before beginning the experiment, the physiochemical conditions of the water, such as the temperature of the air, the alkalinity of the water, the amount of dissolved oxygen in the water, the pH, and the amount of free carbon dioxide, were all estimated. The approach proposed by the APHA/AWWA/WPCF (19815) was utilised in order to arrive at these figures.

Experimental plant

In the botanical garden of D.S. College in Aligarh (UP), India, the latex from the Euphorbia tirucalli (Euphorbiaceae) plant was extracted by making incisions or chopping twigs, and it was then placed in pre-weighted test tubes with 3 ml of distilled water. The garden was located in the state of Uttar Pradesh. After collecting the latex, the test tube was reweighed in order to ascertain the exact quantity of latex that was extracted (the exact quantity of latex = the final weight of the test tube minus the weight of the test tube at the commencement of the experiment). To make an aqueous extract, all you need to do is combine one litre of water that has been distilled with one litre of latex. The latex of the plant was therefore extracted into water, and after that, the required concentration was figured out. After being extracted into the refrigerator, this aqueous extract of plant latex was supposed to be used no more than forty-eight hours later.

Blood Isolation from Fish, followed by the Collection of Serum:

The caudal regions of the living fishes were dissected in order to collect the blood samples. The blood was drawn into the sterile and disposable syringe of 2 millilitres capacity. The blood samples were properly transferred into the sterile centrifuge tubes, but no anticoagulant was used in the process. At room temperature, each tube was allowed to stand in an angle for approximately two hours without being moved or disturbed. The blood clot was centrifuged at 2500 rpm for thirty minutes, after which the supernatant serum was separated from the sedimented cell debris using a fine pipette. The serum was then transferred to airtight plain sterilised vials, and it was finally stored in the freezer at a temperature below 0 degrees Celsius until it was required.

The method developed by King and Jagatheesan in 1959 for measuring serum acid phosphate, and the method developed by Kind and King in 1954 for measuring serum alkaline phosphate.

Experimental Design

For the purpose of the experiment, glass aquaria with a capacity of 20 litres each were filled with dechlorinated tap water. Every aquarium has ten different fish in it. Concurrently, a control group consisting of ten fish that were kept in fresh water was also maintained. During the experiment, the parameters of the water were measured and recorded (atmospheric temperature of 29.2 degrees Celsius, alkalinity of 104-106 mg/L, water temperature of 28.2 degrees Celsius, dissolved oxygen of 5.4-7.2 mg/L, free carbon dioxide of 4.3-6.2 mg/L, and pH of 7.2-7.4) 7. Fish were subjected to sub-lethal concentrations of Euphorbia tirucalli plant latex at Dose A (9.3 mg/L =20 percent of 30h-LC50) and Dose B (18.6 mg/L =40 percent of 30h-LC50) for a period of 30 and 60 hours, respectively.

Results and Discussion

In the current study, the serum acid and alkaline phosphatase levels of Channa punctatus were measured at the conclusion of two different exposure times of sub-lethal concentrations (20 percent and 40 percent of 30 h-LC50) and after 7 days of withdrawal (recovery period) of aqueous extract of Euphorbia tirucalli plant latex. The exposure times ranged from 30 hours to 60 hours, and the sub-lethal concentrations were 20 percent and 40 percent, respectively The control groups of Channa punctatus had a mean value of 1.086 mg/dL for serum acid phosphatase and 1.232 mg/dL for serum alkaline phosphatase. These values were taken from their blood. Both of the serum phosphatases rose to higher levels as the sub-lethal concentration of the aqueous extract of E. tirucalli plant latex was raised as well as the amount of time the animals were exposed to it. After 30 hours and 60 hours, the serum acid phosphatase level increased by 18.35 percent and 43.12 percent at Dose A, and it increased by 27.52 percent and 52.39 percent at Dose B. The serum alkaline phosphatase level increased by 22.56 percent and 35.06 percent at Dose A, and it increased by 38.15 percent and 39.77 percent at Dose B. The levels of serum acid and alkaline phosphatase exhibited signs of recovery during the phases of recovery, going from very high values to low values. At Dose A, the blood acid phosphatase level climbed 15.50 percent,

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and at Dose B, the serum acid phosphatase level increased 16.51 percent. At Dose A, the serum alkaline phosphatase level increased 3.90 percent, and at Dose B, it increased 6.33 percent. At both the sub-lethal doses after 30 hours and the sub-lethal doses after 60 hours, the elevated levels of blood acid and alkaline phosphatase were statistically highly significant. After 7 days of withdrawal, blood acid phosphatase levels were statistically highly significant in recovery phases, while serum alkaline phosphatase levels were significant at both the sub-lethal doses. (Table-1 & Table-2).

Table 1: changes in serum acid phosphatase (mg/dL) of Channa punctatus in control, in both concentrations of aqueous extract of Euphorbia tirucalli latex and after recovery in both doses.

S. No.	Concentrations (mg/L)		Exposure Time		
			30-Hours Range Mean ± S.E.	**60-Hours Range Mean ± S.E.**	**Recovery Range Mean ± S.E.**
1.	Control		1.040–1.140 1.086 ± 0.017	1.040–1.140 1.086 ± 0.017	1.040–1.140 1.086 ± 0.017
2.	Dose A (20% of 30 h-LC_{50})	% Alteration	1.330 –1.390# 1.294 ± 0.012 (+18.35)	1.530 – 1.590# 1.56 ± 0.01 (+43.12)	1.200 – 1.280# 1.258 ± 0.013 (+15.60)
3.	Dose B (40% of 30 h-LC_{50})	% Alteration	1.360 – 1.440# 1.388 ± 0.012 (+27.52)	1.580 – 1.780# 1.664 ± 0.035 (+52.29)	1.22 – 1.28# 1.266 ± 0.011 (+16.51)

Values are Range, Mean ± S.E. of 5 individual observations

S.E.= Standard Error of mean

* = Values were significant at $p < 0.05$

\# = Values were significant at $p < 0.01$

\+ = indicates increase % alteration

Table 2: changes in serum alkaline phosphatase (mg/dL) of Channa punctatus in control, in both doses of Euphorbia tirucalli latex aqueous extract, and after recovery in both doses.

S. No.	Concentrations (mg/L)		Exposure Time		
			30-Hours Range Mean ± S.E.	**60-Hours Range Mean ± S.E.**	**Recovery Range Mean ± S.E.**
1.	Control		1.200–1.260 1.232 ± 0.009	1.200–1.260 1.232 ± 0.009	1.200–1.260 1.232 ± 0.009
2.	Dose A (20% of 30 h-LC_{50})	% Alteration	1.460 –1.550# 1.510 ± 0.015 (+22.56)	1.570 – 1.780# 1.664 ± 0.032 (+35.06)	1.210 – 1.320* 1.280 ± 0.017 (+3.90)
3.	Dose B (40% of 30 h-LC_{50})	% Alteration	1.640 – 1.780# 1.702 ± 0.023 (+38.15)	1.780 – 1.750# 1.722 ± 0.011 (+39.77)	1.260 – 1.380* 1.310 ± 0.017 (+6.33)

Values are Range, Mean ± S.E. of 5 individual observations

S.E. = Standard Error of mean

* = Values were significant at $p < 0.05$

\# = Values were significant at $p < 0.01$

\+ = indicates increase % alteration

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The control group of Channa punctatus had a mean value of serum acid phosphatase that was 1.086 mg/dl, which was greater than the value that was reported by Gupta8 in the same species, Channa punctatus. In addition, Inyang et al.9 demonstrated that the fish had a larger value of acid phosphatase, while Clarias gariepinus and Luskova et al.10 demonstrated that Cyprinus carpio had a lower value of acid phosphatase.

Within the experimental groups, the exposure period and both concentrations of the aqueous extract of E. tirucalli plant latex resulted in an increase in the mean value of serum acid phosphatase. Both Inyang et al. 9 in Clarias gariepinus treated to a greater dose of diazinon and Gupta8 in Channa punctatus exposed with methomyl have reported observations that are comparable to our findings. On the other hand, Luskova and colleagues10 found that exposure to diazinon led to a reduction in the amount of serum acid phosphatase in Cyprinus carpio.

The control group's Channa punctatus had a mean value of 1.232 mg/dl for their blood alkaline phosphatase, which was lower than the values that Gupta8 reported for the same species of Channa punctatus. Gaafar et al.12 in Oreochromis niloticus, whereas higher than that reported by Inyang et al.9 in Clarias gariepinus, Zaki et al.11 in Clarias lazera, and Velisek et al.13 in Oncorhynchus mykiss.

In the experimental groups, the mean value of serum alkaline phosphatase increased with an increase in the exposure time and dosages of an aqueous extract of the plant latex produced by the E. tirucalli plant that did not result in death. This was the case even though the exposure did not result in death. In their studies of Clarias lazera exposed to phenol, Gaafar et al.12 and Velisek et al.13 made observations that were fairly comparable to what we saw in Oncorhynchus mykiss exposed to bifenthrin. In contrast, a lower serum alkaline phosphatase level was observed by Inyang et al.9 in the case of Clarias gariepinus that had been exposed to diazinon.

CONCLUSION

According to the findings of the current investigation, the levels of serum acid and alkaline phosphate increased as the sub-lethal concentrations of aqueous extract as well as the exposure period of E. tirucalli plant latex were raised. Verma et al. suggest that increased osteoblast activity and necrosis of the liver, both of which cause enzymes to be released into the bloodstream, may be responsible for the rise in serum phosphatase levels. 14 After receiving treatment for seven days with a latex obtained from the Euphorbia tirucalli plant that had been water-extracted, serum acid and alkaline phosphate levels reverted to normal. As a result, I came to the realisation that the Channa punctatus was negatively affected by the latex of the Euphorbia tirucalli plant, even at quantities that were not deadly. When using the latex from the Euphorbia tirucalli plant in water bodies, it is necessary to take certain precautions first.

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