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ORIGINAL ARTICLE

Impact of Aqueous Extract of *Euphorbia tirucalli* Plant Latex on Serum Protein of *Channa punctatus* Followed by Recovery

Anand Pratap Singh, Sonal Singh and Keshav Singh
Department of Zoology, Agra College, Agra
Email: sonalzoo.singh@Gmail.com

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ABSTRACT

The aim of this study was to assess the impact of aqueous extract of Euphoria tirucalli plant latex on the serum protein of snake headed Murral, Channa punctatus. 10-10 Fishes were exposed to two different sub lethal concentrations (20% and 40% of 30h-LC_{50}) of freshly prepared aqueous extract of Euphorbia tirucalli for 30 hours and 60 hours exposure time as well as in control group. In both experimental groups (20% and 40% of 30h-LC_{50} sub lethal concentrations) serum protein level was observed decreased with increase the sub-lethal concentration as well as the exposure time of aqueous extract of Euphoria tirucalli plant latex. The changes in serum protein were both dose and time dependent. For recovery, experimental fishes were transferred to normal water and kept for 7 days. After 7 days of withdrawal period, serum protein level of fishes was improved. This study reflects the changes in serum protein level of Channa punctatus due to aqueous extract of Euphorbia tirucalli plant latex and recovery results showed that its effect was reversible.

***Key words**: Serum protein, Aqueous extract, Euphorbia tirucalli, Channa punctatus*

INTRODUCTION

A major objective of today's environmental research is to improve the quality of our environment and to prevent its pollution caused by indiscriminate use of pesticides which have been proved to be highly toxic, not only fishes, but also to other organisms which form food of fishes, and also exert adverse effect on associated organs and can cause immunological disorder of freshwater animals (Richard *et al*., 1991).

In recent years, there is preference for safe and eco-friendly piscicides of plant origin than synthetic piscicides for catching fish. This is because these piscicides are less expensive, biodegradable, readily available, easy to handle and save for mankind and the environment (Singh *et al.*, 2010). The plant extract used as piscicides in fisheries are considered advantages when viewed against the backdrop of using persistent chemicals (Emmanuel *et al.*, 2019). The deliberate introduction of these plant extract in the aquatic ecosystem could eventually lead to physiological stress in aquatic productivity (Olufayo, 2009).

Fish are commonly used as bioindicators of aquatic pollution due to their sensitivity to surrounding environment (Srivastava and Kaushik, 2001). Fish blood is a pathophysiological indicator of the whole-body function and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant (Agrahari *et al*. 2006).

Euphorbia tirucalli, is a common medicinal plant of India. It is commonly known as pencil tree, milk bush, finger tree, Barki-thohar etc. It is an Indian tree spurge and indoor ornamental tree. It grows with single or multiple trunks which support a tangle of light green, thick, succulent branches with little sign of a leaf. It is a shrub that grows in semi-arid tropical climates. All parts of it ooze a milky sap when damaged or cut. The aqueous solution of the latex from the stem is used to control aphids, mosquitoes, red spiders, mites, termites, fungi and insects in general (GAIA Movement Booklet 25- Biopesticides, 2003).

The stress indicator *Channa punctatus* (Bloch) is used on account of their easy availability and quick acclimatization in the laboratory condition. It is an air breathing, predatory and carnivorous fish and widely distributed throughout plains of India up to an altitude of 600 meters. This study would form the baseline data for the assessment of Serum protein of *Channa punctatus* exposed to aqueous extract of *Euphorbia tirucalli* plant latex under laboratory condition.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF EXPERIMENTAL ANIMAL

The snake headed murral, *Channa punctatus* (Bloch) with 17.5± 1.50 cm in length and 44.0± 2.5 gm weight were collected from the different localities of Aligarh district (U.P.), India. Prior to experiment, fish were stored in glass aquaria containing 50L of dechlorinated fresh water treated with potassium permanganate solution for 1-2 minutes to remove any dermal adherent and allowed to acclimatize under laboratory conditions for a week. The aquaria water was changed every 24 hours and food was provided. Feeding of fish prior to experiment was stopped. Physio-chemical conditions of water like- atmospheric temperature, water temperature, pH, dissolved oxygen, free carbon dioxide and alkalinity were estimated prior to the experiment. These were estimated by the method of APHA/AWWA/ WPCF(1981).

COLLECTION AND PREPARATION OF AQUEOUS EXTRACT OF PLANT LATEX

The plant *Euphorbia tirucalli* (family- Euphorbiaceae) was collected from botanical garden of D.S. College, Aligarh (UP), India.

Euphorbia tirucalli latex was obtained by incisions or cut the twigs and collected in the pre-weighted test tubes containing 3ml distilled water. After collecting the exudates, the test tube was weighted again. The accurate weight of latex was calculated by subtracting the initial weight of test tube containing 3 ml of distilled water from the final weight of test tube. Now for preparation of aqueous extract, 1000 ml of distilled water was added into the latex. Thus, the aqueous extract of known concentration was obtained from which required dose was calculated. This aqueous extract was stored in the refrigerator and used within a period of 48 hours (Jurberg *et al*., 1985).

COLLECTING BLOOD AND SEPARATION OF SERUM FROM FISH

The blood samples were obtained from the caudal region of the living fishes. The blood was collected using 2 ml sterile disposable syringe. The blood samples were transferred carefully into the sterilized centrifuge tubes without anticoagulant. Each tube was left undisturbed in the slanting position for about 2 hours at room temperature. When the blood clot starts retracting, it was centrifuged at 2500 rpm for 30 minutes, and the supernatant serum was separated from the sedimented cell debris by a fine pipette and transfer to airtight plain sterilized vials and finally stored in the freezer below 0^{o}C until used.

EXPERIMENTAL DESIGN

For the experiment glass aquaria containing 20L dechlorinated tap water were used. Each aquarium containing 10 fish. A set of 10 fish were also simultaneously maintained in fresh water as the control each time the test was repeated. During experiment, water conditions were; atmospheric temperature 29±2°C, water temperature 28±2°C, pH 7.2-7.4, dissolved oxygen 5.4-7.2 mg/L, free carbon dioxide 4.3-6.2 mg/L, alkalinity 104-106 mg/L (APHA,1998). Fish were exposed to two different sub- lethal concentrations, Dose A (9.3 mg/L =20% of 30h-LC_{50}) and Dose B (18.6 mg/L = 40% of 30h-LC_{50}) of aqueous extract of *Euphorbia tirucalli* plant latex for 30 and 60 hours respectively (Table-1).

Table 1: Physio-chemical parameters of water used in Experiments

S. No.	Parameters	Control	Latex
1.	Atmospheric temperature (^{o} C)	28 ±2	29 ± 2
2.	Water temperature	26 ± 2	28 ± 2
3.	pH	7.0 - 7.2	7.2 - 7.4
4.	Dissolved oxygen (DO) (mg/L)	6.9 - 7.5	5.4 - 7.2
5.	Free carbon dioxide (mg/L)	7.2 - 8.0	4.3 - 6.2
6.	Alkalinity (mg/L)	106.8 - 107.6	104 - 106

RESULT AND DISCUSSION

The physio-chemical parameters of water with latex were showed partial increase in atmospheric temperature, water temperature pH and showed decrease in dissolved oxygen, free carbon dioxide and alkalinity at in both sub- lethal concentrations of aqueous extract of *E. tirucalli* plant latex. This study agrees within the report of warren C.E., (1977) who documented that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration which will in turn impair respiration thereby leading to asphyxiation. The reduction in the dissolved oxygen level across the treatments in the findings of Ayoola and Idowu (2008) on exposure of *Clarias gariepinus* to aqueous extract of *Ipomea aquatica* leaf. The pH range recorded in this study falls within the physiological range of 7-7.4 which is ideal for biological productivity of fishes as reported by Abowei and Ekubo (2011).

In the present investigation serum protein of *Channa punctatus* were examined at the end of 30-hrs., 60 hrs. exposure to two different sub-lethal concentrations (20% and 40% of 30 h-LC_{50}) and after 7 days of withdrawal (recovery period) of sub-lethal concentrations of aqueous extract of *Euphorbia tirucalli* plant latex. The mean value of serum protein in *Channa punctatus* was 7.40 g/100ml in the control group. The serum protein was decreased with increase the sub-lethal concentration as well as exposure time of aqueous extract of *E. tirucalli* plant latex. It was decreased by 14.05% and 25.95% at Dose A; and by 38.51% and 50.54% at Dose B after 30 hours and 60 hours respectively. The serum protein level showed significant recovery at both doses after 7 days of withdrawal. It was decreased by 5.05 at Dose A and 5.81% at Dose B after recovery period (Table-2).

Table 2: Changes in serum protein (g/100ml) of *Channa punctatus* in control and in different sub-lethal concentrations of aqueous extract of *Euphorbia tirucalli* plant latex

S.No.	Concentrations (mg)		Exposure Time		
			30-Hours Range Mean ±S.E.	60-Hours Range Mean ±S.E.	Recovery Range Mean ±S.E.
1.	Control		5.41 – 9.14 7.40 ± 0.62	5.41 – 9.14 7.40 ± 0.62	5.41 – 9.14 7.40 ± 0.62
2.	Dose A (20% of 30 h-LC_{50})	% Alteration	4.43 – 7.06 6.36 ± 0.44 (-14.05)	3.84 – 6.19* 5.48 ± 0.38 (-25.95)	6.25 – 7.57 7.63 ± 0.20 (-5.0)
3.	Dose B (40% of 30 h-LC_{50})	% Alteration	2.72 – 5.81# 4.55 ± 0.49 (-38.51)	2.37 – 4.21# 3.66 ± 0.29 (-50.54)	6.60 – 7.58 6.97 ± 0.15 (-5.81)

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 $p < 0.01$
\- = Parvbar me koi indicates decrease % alteration

The mean value of total serum protein content in *Channa punctatus* was 7.40 g/ml in the control group which was higher than those reported by Singh *et al*. (2010) in the same species, *Channa punctatus*, Zaki *et al*. (2011) in *Clarias lazera*; Prasad and Priyanka (2011) in *Pangasiano hypophthalmus*, Gaafer *et al*. (2010) in *Oreochromis niloticus* and lower that those reported by Yaji *et al*. (2011) in *Oreochromis niloticus*, Adewoye (2010) in *Clarias gariepinus*,

In the experimental groups, the total serum protein content decreases with increase in the exposure time and sub-lethal doses of aqueous extract of *E. tirucalli* plant latex. Similar decrease in serum protein were also reported by Hamid and El- Sayed (2019) in *Oreochromis niloticus* exposed to *Moringa oleifera* leaf extract, Prasad and Priyanka (2011) in *Pangasiano hypophthalmus* due to effect of *Garcinia gummigutta*, Adewoye (2010) in *Clarias gariepinus* exposed to biopesticide *Tephrosia vogelii* and by Hassanain and Okail (2008) in *Ctenopharyngodon idella* exposed to biopesticide *Azadirachta indica*, Gaafar (2010) in *Oreochromis niloticus* exposed to edifenphos, However, increase in the total serum protein content has been reported by Yaji *et al*. (2011) in *Oreochromis niloticus* exposed to cypermethrin, Adewoye (2010) in *Clarias gariepinus* exposed to

Tephrosia vogelii at higher doses. Mostafa *et al*. (2009) and Metwally (2009) in *Oreochromis niloticus* exposed to fenugreek seeds and *Allium sativum* respectively.

CONCLUSION

From the present study, the serum protein content was decreased with increase the sub-lethal concentrations as well as exposure time of plant latex. The decrease in serum protein may stimulate the degradative processes like proteolysis and utilization of degraded products for enhanced energy metabolism. Which reveals that the different sub lethal concentrations of aqueous extract of *Euphorbia tirucalli* plant latex showed deleterious consequences on the entire physiology of fish, *Channa punctatus,* but after 7 days of recovery impact of aqueous extract of *Euphorbia tirucalli* plant latex on serum protein were recovered.

Therefore, we concluded that the impact of aqueous extract of plant latex were reversible and used as biopesticide but some preventive measures must be adopted to use of *Euphorbia tirucalli* plant latex into the water bodies.

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