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Toxicity impact of *Cestrum nocturnum* on respiratory metabolism of Fish *Clarias batrachus*

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ABSTRACT

The effect of alcoholic extract of *C. nocturnum* was observed on *Clarias batrachus* and LC_{50} value was noted. The animal was exposed to ⅓ LC_{50} value, (0.933ppm) for 48 hrs. Oxygen consumption was decreased in the treated fish as compared to the control fish.Reduced SDH and elevated LDH levels were observed in liver, gills and muscles upon exposure to piscicidal extract. This indicates that the piscicidal extract of *C. nocturnum*leaves impairs the respiratory enzyme activity in *Clarious batrachus* and hence has a lethal effect.

KEY WORDS: *Cestrum nocturnum*, piscicide, LDH, SDH, *Clarias batrachus*, Oxygen consumption.

INTRODUCTION

There are over 300 species of *Cestrum* and most of the species are native of warm subtropical and tropical areas, now widely distributed throughout the tropics and grown in warm countries for the attractive and fragrant flowers (Amin and Parle, 2016) among them *Cestrum nocturnum* and *Cestrum diurnum* are found in India and cultivated as an ornamental plant.These species have been a subject of interest to some investigators from the phytochemical point of view. Particularly they are steroid alkaloids and saponin (Silva et al., 1962)

Chatterjee et al., (1964) made pharmacological studies with the saponin extracted from the leaves of *Cestrum sp.* for its passiblecardio tonic property on the isolated heart of guinea pig and frog and concluded that the saponin from *C. diurnum* produce systolic contraction of frog heart. *C.nocturnum* and *C.diurnum*has been known for their exhibit cardiotonic and cardiotoxic property (Ray et al., 1986).Recently*C. nocturnum* has beenreported to havepiscicidal (Jawale et al., 2012), larvicidal (Jawale et al., 2010), and insecticidal (Jawale and Dama 2010)effect. *C.nocturnum* still has lots of potential to be evaluatedas safe piscicidal agent in aquaculture. In the present study an attempt has been made to evaluate its effect on respiratory metabolismof predatory fish *Clarias batrachus*, when exposed to sublethal dose of alcoholic extract of *C. nocturnum* leaves.

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MATERIAL AND METHODS

Fishes, *Clarias batrachus* of standard length (20-25 cms) and weight (75-100gms) were procured and maintained in the laboratory in well-aerated water for a period of 3 weeks. They were fed with fish feed. Feeding was stopped a day prior to experimentation. The alcoholic extract was prepared from the powder of dried leaves of *C. nocturnum* using percolation method (Ikan 1977). A stock solution of dried alcoholic extract was prepared in distilled water with the concentration of 10 mg total extract per liter equivalent to 10 ppm and different dilution were prepared by adding required amount of distilled tap water.

A batch of ten animals in triplicate (total 30 fishes) was exposed to each concentration and mortality was noted at 48 hrs (Finney, 1971). The experiments were repeated thrice to get concordant results. For estimation of Oxygen consumption pattern and Respiratory enzyme activty in control and experimental conditions, similar sets were exposed to sublethal concentration of alcoholic extract of *C. nocturnum* (1/3 of LC_{50} value for 48 hr.0.933mg/lit).

Oxygen consumption: A respiratory chamber was prepared by using glass aquarium, containing 15 lit.Tap water. Acclimatized fishes were transferred to the respiratory chamber. The desired concentration of alcoholic extract of leaves of *C.nocturnum* was added to each respiratory chamber and a layer of liquid paraffin was added to avoid interference with atmospheric oxygen. After one hour, the fishes were removed from respiratory chamber and 250 ml of water was taken out in a reagent bottle for estimation of oxygen content. The oxygen consumption of fish was determined by Winklers method (Welsh, 1953). The results were calculated in ml. of oxygen consumed per hr per gm body wt. per lit of water at NTP. The't' test was applied to calculate the level of significance (Dowdeswell, 1957).

Respiratory enzyme estimation: The succinate dehydrogenase activity in Gill, liver and muscles tissue of the fish was estimated by the method described by Marvin et al., (1960). The succinate dehydrogenase activity was expressed in µg of INT per mg of protein per hr. The lactate dehydrogenase activity in different tissues of the fish was estimated by the Kornberg's method (Plummer, 1989). The lactate dehydrogenase activity was expressed in µmol/mg of protein per hr. The results are calculated by applying 't' test of significance and presented in table No.1.

Table No. 1: Respiratory enzyme activity and oxygen consumption pattern in *C. batrachus* exposed to sub lethal concentration of alcoholic extract of *C. noturnum.*

Parameter	Tissue	control	Experimental
Oxyegen consumption rate	--	0.2493± 0.013	0.2024±0.0119
Succinate dehydrogenase	Gills	0.057 ± 0.021	0.035 ±0.023

(SDH)	Liver	0.301 ± 0.021	0.281 ±0.023
	Muscles	0.221 ± 0.014	0.205 ±0.007
Lactate dehydrogenase (LDH)	Gills	0.128 ± 0.012	0.175 ± 0.015
	Liver	0.150 ± 0.011	0.162 ± 0.017
	Muscles	0.710 ± 0.031	0.721 ± 0.003

1/3 LC_{50} = 0.933ppm for 48 hrs.

Results:

Rate of oxygen consumption is a bioindicator of stress phenomenon in aquatic organisms, when exposed to toxic media. In the present study sub lethal dose of alcoholic extract of *C. nocturnum* leaves was calculated using probit analysis (Finney 1971) and was found to be 2.799 ppm SE ±3.14. O_2 consumption was calculated for*C. batrachus* exposed to sub lethal dose of alcoholic extract of *C.nocturnum* leaves. From Table 1, it is interpreted that the toxic concentration at sublethal dose affects the oxygen consumption in fish. SDH& LDH activity in liver, muscles and gills was calculated and expressed in Table no. 1. It shows that SDH activity in liver, muscles and gillshas decreased at 48 hr.whereas the LDH activity hasincreased in gills and muscles and liver, when exposed to sublethalconcntration of alcoholic extract of *C. nocturnum* leave.

DISCUSSION

Respiration is an important parameter to gauge the stress on fishes due to toxic environment. It provides a sensitive measure of the physiological condition of the fish (Adams 1990). Respiration or transfer of oxygen from water into the cell of fish is vital and difficult job. Most fish interrupt their respiratory pumping periodically and backflush the gills to remove food particles and clear any debris, this is commonly called coughing reflex in fishes. The total amount of oxygen transfer from water to blood also depends upon the availability of surface area (Lynwood, 1999). Thus oxygen consumption is a sensitive process, as an indicator of stress in the animal exposed to toxicants (Bhagyalakshmi and Ramamurthi, 1981). In the present study oxygen consumption rate was decreased in *C.batracus* when exposed to piscicidal compound. This suggests the alterations in respiratory surface due to the toxicants. Similar results were noted by Jawale and Patil (2002) in histopatholoigcal changes in gill of *Cyprinuscarpio* exposed to alcoholic extract of *C. nocturnum*. These supportive evidences indicate that the toxicant causes thedamage to gill surface and RBC(Jawale and Dama 2010). In the current results oxygen consumption of fish was lowered in experimental group which could be the probable outcome of drop in the transport of oxygen through collapsed gill structure and rupture of RBCs.This interpretation was in accordance with the findings of McLeay (1973). The other possibility of reduced oxygen consumption may be related to unconsciousness or tissue anoxia which is a reflex mechanism for reducing mechanical activity in the fish, Hence, during this short exposure to the

toxicants may have developed lethargy or unconsciousness in the fishes which resulted in less demand of oxygen (Lynwood, 1999).

Respiratory metabolism can be used as an indicator to assess the energy utilization of an organism under stress (Yang et al., 2006; Wang et al., 2011). Change in the respiratory rate of an organism can be reflected in the form of changes in respiratory metabolic enzyme activity. The usual mode of respiration in aquatic animals is aerobic although under hypoxic stress they respire anaerobically to sustain themselves (Paschke et al., 2010; Qiu et al., 2011). As Mitochondria is the main site for respiration there are certain enzyme working as key marker which can be analyzed to reflect the respiratory metabolism. Succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) are such markers which have been used in the present study. SDH plays an important role in aerobic respiratory metabolism (Gao et al., 2016) where as LDH is a critical regulator of anaerobic glycolysis as it catalyzes the conversion of pyruvate into lactic acid (Carlsson and Gade, 1986). Therefore, the activities of SDH and LDH represent the degree of aerobic and anaerobic respiratory metabolism.

The activity of respiratory enzymes indicate the change in tissue oxygen level in fishes exposed to toxicants.So, In the present study, the activity of lactate dehydrogenase and succinate dehydrogenase enzymes in the tissues of *C. batrachus*, exposed to alcoholic extract of *C. nocturnum*leaves has been done to throw light on respiratory metabolism, which generates energy through both aerobic and anerobic pathways. In the present study, SDH activity decreased at 48hr, inliver, muscle and gills.Similar inhibition in SDH activity has been recorded by many workers in fish exposed to various toxicants (Parveen and Vasantha, 1994; Raju et al. 1994, James et al. 1992, Rajamanickam 1992).This decline in SDH levels has been explained as an impairment of oxidative metabolic cycle as the pyruvate converts into lactate leading to shortage of acetyl CoA to enter the kreb's cycle leading to the dependency of an organism on anaerobic glycolysis to meet its energy requirement under stressful conditions. (Rajamanickam 1992;James et al. 1992)

On the other hand, the LDH levels increased in the present study similar elevation of LDH activity has also been observed in fish treated with different toxicants by many authors (Parveen and Vasantha 1994; Raju et al., 1994; Sivakumari et al., 1997; Rajamannar and Manohar, 2000). Golovina (1977) has mentioned that an increase in LDH activity as an indication of disturbances in the aerobic oxidation processes. It is known that under conditions of lack of oxygen, anerobic metabolism is favored, and in this case LDH converts pyruvic acid to lactic acid.

It is also possible that under stressful conditions, the pituitary-adrenal axis may become active resulting in the release of cortisol in *C. batrachus*. These cortisols are known to increase activity of LDH in rat tissue (Bolyarska, 1977). It is therefore quite likely that cortisol released in response to stress for a short or long duration, may result in increased activity of LDH. The stimulation of LDH and inhibition of SDH activity in present investigation, indicates the inhibition of oxidation of pyruvate to carbon dioxide and water through TCA cycle in the absence of aerobic condition. Consequently there is a shift towards the reduction of pyruvate to lactic acid in liver in anerobic conditions which reflected in high LDH and lowSDH activity. The muscle also show same trend of results.

The type of metabolism compensation, together with the changes in oxygen consumption, SDH & LDH observed in present study,efficated the toxicity mechanism in *C. batrachus*. These study explain the metabolic phenomenon in fishes related with mechanism of toxicity and physiological changes occurring during intoxication by the plant extract.

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