



STUDIES ON BIOCHEMICAL VARIATIONS OF SKIN SECRETION AND ITS EXTRACT OF *BUFO MELANOSTICTUS* EXPOSED TO CHLORPYRIPHOS AN ORGANOPHOSPHATE

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ABSTRACT

The present study was under taken to analyze the biochemical variations in skin secretion and its extract of *Bufo melanostictus* exposed to chlorpyrifos an organophosphate (OP compound). The skin of *B. melanostictus* was exposed to the toxicant and the variations were observed on proteins, carbohydrates and ninhydrine positive substances at different time intervals i.e. 4, 8 and 12 hrs and variations in skin secretion and its extract. The results revealed that the components of proteins, carbohydrates and ninhydrine positive substances were found to be decreased significantly at 4, 8 and 12 hrs in skin secretion and its extract. The maximum decrease was observed at 4 hrs and 12 hrs compared to 8 hrs and control.

KEY WORDS

Skin secretion, Skin extraction, Total proteins, Ninhydrine positive substances (Free Amino Acids), Chlorpyrifos, Organophosphate.

INTRODUCTION

Amphibians show sensitivity towards changes that occur in the environment and they are used as bio-indicators of aquatic and terrestrial ecosystems [1, 2]. They lead an obligatory aquatic life and have permeable skin, which made these animals susceptible to water borne environmental chemical contaminants [3], that directly influence these organisms [2, 4]. Thus, many pesticides used in agriculture ultimately reach the food chain of the aquatic as well as terrestrial ecosystems. Amphibians actually constitute the largest group of vertebrate biomasses in some ecosystems, making them as important source of food for the higher vertebrates like fish, birds, reptiles, mammals as well as important herbivores (tadpole) and carnivores in these ecosystems [5]. But usage of pesticides is receiving an increased attention in potential causes of Amphibian decline. These chemicals unintentionally influence the life of other non-target organisms found in the

surroundings. Toads have two types of skin glands i.e. mucous and the glandular (or) alveolar glands [6, 7]. Skin glands produce mucous, peptide, biogenic amines, steroids and large no of biologically active compounds [8]. Venom is a secretion synthesized in a specific part of the body and it is a modified saliva containing different polypeptides used for defense from prey instead of defense organs [9-11]. Utilization of wide range of pesticides, insecticides, herbicides in agriculture is polluting the soil as well as aquatic ecosystem. Some of the pesticides, herbicides and nematocides show an endocrine disrupting effect [12], however in natural communities; their effect is direct or indirect on the toad species thus also becoming a cause for amphibian population decline [13-16].

In view of the above, present investigation has been undertaken to study the toxic effect of organophosphate compound Chlorpyrifos on some

biochemical components of the various secretions and extracts of the Indian common toad *B. melanostictus*.

MATERIAL AND METHODS

Collection of toad and toad skin extract (TSE) preparation

Adult live toads (40-50 gm) *Bufo melanostictus* (Schneider) were collected from the surroundings of University hostels buildings, Kakatiya University and maintained in well ventilated glass box, some insects given as feeding. Animals were pithed and their skin was separated from the body except parotid gland. The skin was kept in methanol at room temperature (RT) for 30 days. The supernatant was centrifuged and was pooled. It was evaporated to dryness by rotary evaporator and the extract was kept at RT (28°C) in a desiccator. Then Toad Skin Extract (TSE) were dissolved at definite concentration in normal saline (0.9%) for experiments [17].

Collection of toad skin secretion

The skin secretions were obtained from the frogs by gentle electrical stimulation (4-ms pulse width, 50Hz, 5V) using platinum electrodes rubbed over the moistened dorsal skin surface for 10s. Secretions were washed off into a glass beaker, using deionised water. The resultant secretions were freeze dried in a freeze dryer. Approximately 50 mg, dry weight of skin secretion was obtained. This procedure was carried out in accordance with the UK Animals Scientific Procedures (Act 1986). It is a non-invasive technique causing no distress to the frog [18]. All the qualitative and quantitative chemicals were supplied by Himeda Laboratories Pvt. Ltd. Mumbai.

The tissues were homogenized (10%) in 10% Tri Chloro Acetic Acid (TCA) centrifuged at 2000 rpm for 15 minutes and clear supernatant and sediment was used for the analysis of total proteins, carbohydrates and ninhydrine positive substances (FAA). The protein sediment and supernatant (TCA precipitated and soluble proteins) was dissolved in 1N NaOH and protein content was determined through the Lowry's reagent [19] described by Schacterle and Pollack (1973) [20]. Ninhydrine positive substances were estimated by the method of Lee and Takahashi (1966) [21] and the total carbohydrate content in the tissues were estimated by the method of (Anthrone) Carroll *et al*, 1956 [22].

Preparation OP compound

To estimate the biochemical variations in *B. melanostictus* after exposure to Chlorpyrifos (Alkyl Sulphonate and Poly Oxy ethyl Ether) 20%E.C; different concentrations of insecticide and normal saline were injected sub-cutaneously into skin. The *in vivo* effects were observed by following procedures at different time intervals i.e. 4h, 8h, 12hours.

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance ANOVA to compare the results between the tissue components.

RESULTS

The results obtained from the quantitative estimates on the toxic effect of Chlorpyrifos on biochemical constituents of various tissues of *B. melanostictus* are presented in table 1,2,3, and fig.1, 2, 3 respectively with dose exposure at different time intervals of 4,8,12 hours.

In this experiment when the skin of toad was injected with the desired concentrations of test chemical Chlorpyrifos at different time intervals, a drastic reduction was observed on total biochemical constituents in skin secretion and its extract compared to control.

The results presented in table and figure1 shows that the protein content was decreased in supernatant in Chlorpyrifos treated sample after 4, 8 and 12 hrs inductions and the reduction in soluble protein content (supernatant) and the structural protein content (sediment) was found to be $P < 0.001$ in skin secretion and its extract.

The results presented in table 2 and figure 2 revealed that the carbohydrate content was decreased in a drastic reduction in total carbohydrate content in skin secretion and its extracts when compared with control. In our observations chlorpyrifos treated samples with 4 hrs, 8 hrs and 'p' value of carbohydrate content was found to be non-significant but at 12 hrs treatment we noticed that the 'p' value was found to be $P < 0.001$ in skin secretion and its extract. Hence it can be concluded that there is a significant variation between the skin secretion and its extract.

The results presented in table 3 and figure 3 revealed that the ninhydrine positive substances (FAA) were decreased in a drastic reduction in FAA content in skin secretion and its extract, compared to control with

P<0.05 at 4 hrs, while non-significant at 8 hrs and 12 hrs intervals, and showed decreased FAA content with significant value of P<0.001.

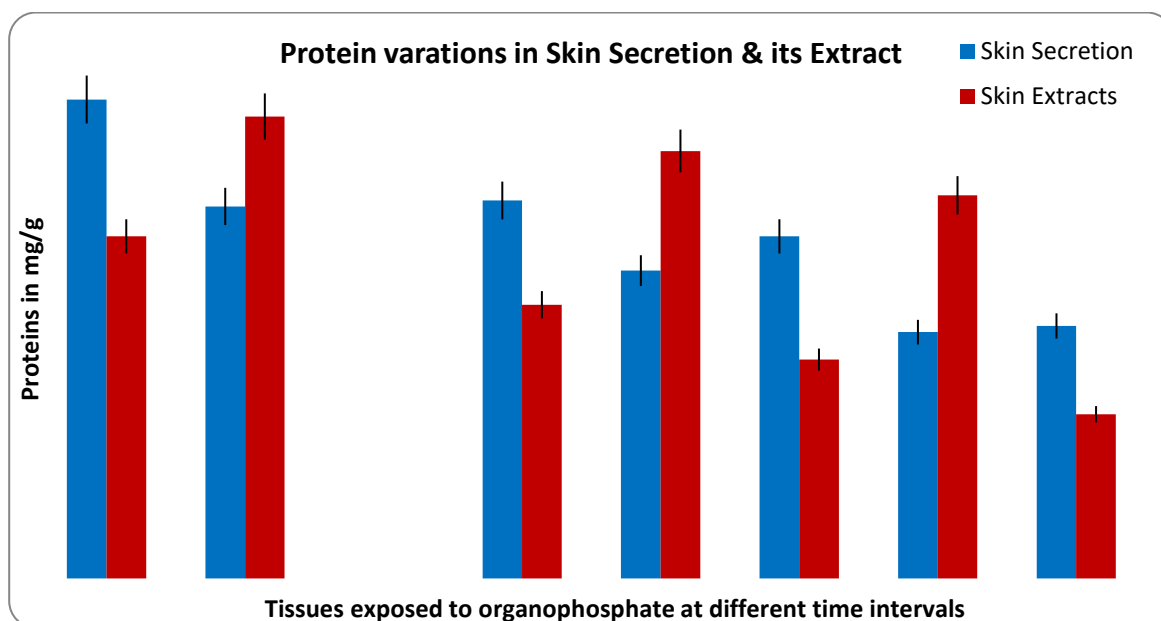
Tab: 1. Effect of Chlorpyrifos on Protein variation in skin secretion and its extract of *B. melanostictus*.

Dose	Tissue	Variation in Protein values after induction of Chlorpyrifos	
		Skin Secretion	Skin Extracts
Control	TCA Soluble proteins	80.00 ± 0.70	57.14 ± 0.54
	TCA precipitated Proteins	62.14 ± 0.52	77.16 ± 0.46
4H	TCA Soluble proteins	63.13 ± 0.25*	45.71 ± 0.22*
4H	TCA precipitated Proteins	51.42 ± 0.52***	71.40 ± 0.42*
8H	TCA Soluble proteins	57.14 ± 0.26*	36.57 ± 0.56*
8H	TCA precipitated Proteins	41.14 ± 0.30**	64.00 ± 0.41*
12H	TCA Soluble proteins	42.18 ± 0.39	27.42 ± 0.64
12H	TCA precipitated Proteins	30.85 ± 0.75	48.00 ± 0.61**

The values are expressed as mean mg/g ± SE of wet weight of tissue; n=6;

Statistically significant value to respective control value *P <0.001, **P < 0.05, ***P <0.005

Fig.1. Effect of Chlorpyrifos on Protein variation in skin secretion and its extract of *B. melanostictus*.



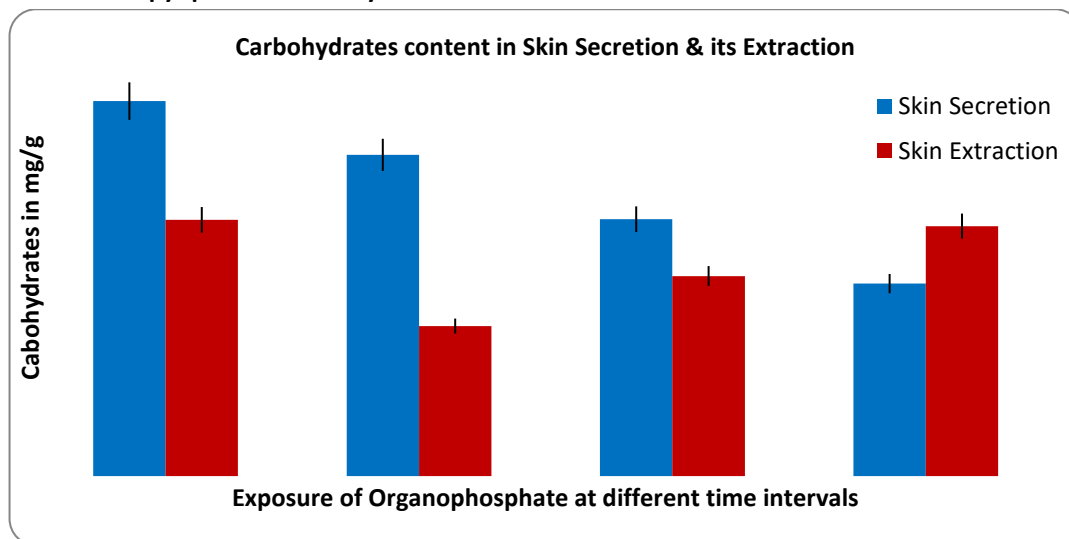
Tab.2. Effect of Chlorpyrifos on Carbohydrate variation in skin secretion and its extract of *B. melanostictus*.

Dose	Tissue	Variation of carbohydrate values after induction of Chlorpyrifos	
		Skin Secretion	Skin Extracts
Control		6.00 ± 0.49	4.10 ± 0.69
4H		5.14 ± 0.73	2.40 ± 0.27*
8H		4.11 ± 0.39*	3.20 ± 0.20*
12H		3.08 ± 0.25*	4.00 ± 3.20*

The values are expressed as mean mg/g ± SE of wet weight of tissue; n=6;

Statistically significant value to respective control value *P <0.001, **P < 0.05, ***P <0.005

Fig 2. Effect of Chlorpyrifos on Carbohydrate variation in skin secretion and its extract of *B. melanostictus*.

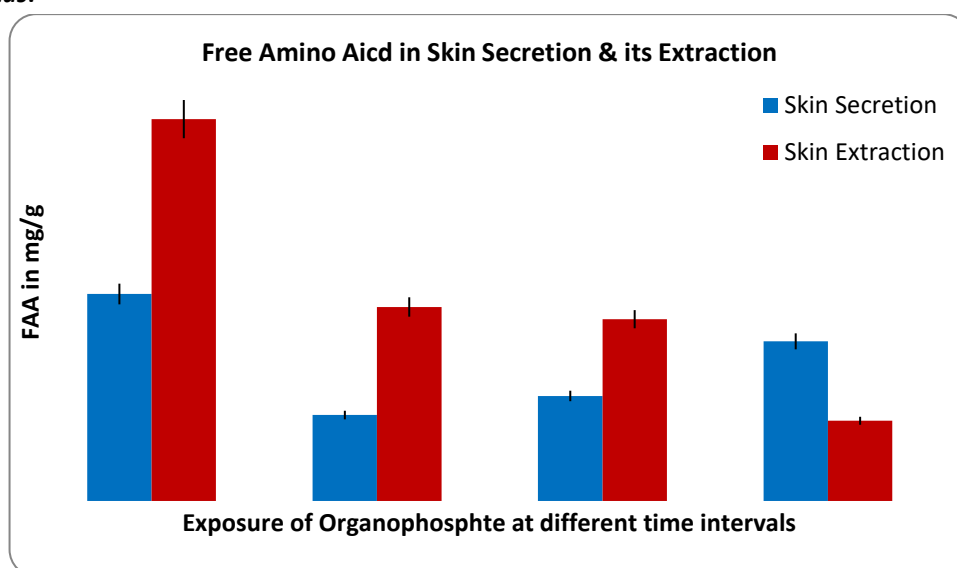


Tab. 3. Effect of Chlorpyrifos on Ninhydrine positive substances (FAA) variation in skin secretion and its extract of *B. melanostictus*.

Tissue Dose	Variations in FAA values after induction of Chlorpyrifos	
	Skin Secretion	Skin Extracts
Control	5.42 ± 0.47	10.00 ± 0.26
4H	2.25 ± 0.72*	5.08 ± 0.35***
8H	2.75 ± 0.56*	4.76 ± 0.08**
12H	4.18 ± 0.79	2.10 ± 0.58*

The values are expressed as mean mg/g ± SE of wet weight of tissue; n=6;
Statistically significant value to respective control value *P < 0.001, **P < 0.05, ***P < 0.005

Fig.3. Effect of Chlorpyrifos on Ninhydrine positive substances variation in skin secretion and its extract of *B. melanostictus*.



DISCUSSION:

Amphibian populations are declining globally at an alarming rate. Pesticides are among a number of proposed causes for these declines. Although a sizable database examining effects of pesticides on amphibian exists, the vast majority of these studies focus on toxicological effects (lethality, external malformations, etc.) at relatively high doses (PPM). Very few studies focus on effects such as endocrine disruption at low concentrations [23].

The organophosphate compounds are widely used as insecticides and are extremely toxic in some cases, generally these are short lived in the environment compared to halogenated organics and related compounds [24]. Further, most of the studies examine exposure to single chemical, but amphibians especially in agricultural areas are exposed to mixture of pesticides. Adverse effects are due to the continuous use of pesticides in agriculture over the last 50 years, which have played and will continue to play a role in amphibian declines. In particular, the effects described here are very important. Pesticide-induced declines in populations as a result of decreased prey availability and increased susceptibility to predators may be difficult to discern in the wild [23].

Several Anuran species have become extinct due to the events related to the amphibian decline before their bioactive molecules have had a chance to be discovered, such as the golden toad *B. periglenes* (Bufonidae) [26]. The predominant mechanism of organophosphate toxicity is inhibition of Acetylcholine esterase in the nervous system causing accumulation of acetylcholine. Thus, the present investigation, on effect of chlorpyrifos on variations in biochemical composition of skin secretion and its extract of *B. melanostictus*, revealed that the total protein content found to be decreased in both skin secretion and its extract after injection of chlorpyrifos. The decrease of total protein content in both skin secretion and its extract may be due to less incorporation of amino acids in the translation process i.e., a reduced incorporation into any kind of protein and the disturbance caused by the pesticides during protein synthesis [27]. Regarding pesticides, the present study demonstrates that the examination of effect of single pesticide is inadequate to assess adverse impacts on amphibian development or to address the role of pesticides in amphibian declines.

CONCLUSION:

From the present study it can be concluded that the skin secretion and its extract of *B. melanostictus* were rich in protein content compared to carbohydrates and free amino acids and that the bioactive molecules were more sensitive to OP compound effecting on their physiological activities and xenobiotic metabolism which are species specific. Further examinations are needed to characterize pesticide interactions on multiple combinations of pesticides at multiple concentrations.

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