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EFFECT OF PARAXON (AN ORGANOPHOSPHATE) ON BIOCHEMICAL COMPOSITION OF PAROTOID GLAND SECRETION AND ITS EXTRACT OF *BUFO MELANOSTICTUS* (SCHNEIDER)

Venkaiah Yanamala[1*], Raju Neerati[1]

[*1]Department of Zoology, Kakatiya University, Warangal-506009

ABSTRACT

The present study was aimed to investigate the effect of paraxon on biochemical composition of parotoid gland secretion and its extract of common Indian Toad (Bufo melanostictus). The parotoid glands were exposed to the toxicant organophosphate compound (OP) like paraxon, and the variations were observed on proteins, carbohydrates and ninhydrine positive substances at different time intervals i.e., 4, 8 and 12hrs in parotoid gland secretion and its extract and were estimated quantitatively. The results revealed that the components of proteins, carbohydrates and ninhydrine positive substances were found to decrease significantly in 4, 8 and 12hrs treatment with paraxon in parotoid gland secretion compared to gland extract. The maximum decrease was observed in 4hrs and 12hrs compared to 8hrs and control.

Key words: *Bufo melanostictus, Carbohydrates, Ninhydrine positive substances, Paraxon, Proteins.*

Correspondence to Author

Venkaiah Yanamala

Department of Zoology, Kakatiya University, Warangal-506009

Email: venkaiahyanamala07@gmail.com

INRODUCTION

Amphibians are treated as bio-indicators of aquatic and terrestrial ecosystems by means of their sensitivity towards environmental changes [1-3]. Amphibian skin is characterized by the presence of cutaneous glands spread over the body. Basically toads have two types of alveolar glands in the epidermal layer of their skin i.e. (i) mucous glands and (ii) granular glands [4, 5]. Mucous glands are secreting mucus, functioning as a lubricant in the water to keep skin moist and necessary for cutaneous respiration [6] and protect the skin from mechanical damages and prevent microbial settlement on the skin; these glands secrete glycoprotein rich material which plays an important role in defense mechanism. This mucous contains a variety of glycoproteins such as mucin, musinigen, and carbohydrate residues such as galactose, fukosa, and sialaic acid. Granular glands are also called poison glands, secrete serous that provides protection from predators such as birds, mammals, snakes, crocodiles [7]. These glands are generally found clustered like parotoid gland. In toads these glandular glands located between eyes and tympanum [5,8] and their secretions contain biogenic amines, steroids, peptide, proteins [9] and toxic defense molecules possessing potent therapeutic activities against microbial infection,

diabetes, cardiovascular disorders and cancer [10]. The venomous secretions of the parotoid glands of the *Bufo* species are known to contain several bioactive compounds [11] and were used by Chinese, Indian traditional medicinal practioners and Japanese physicians for centuries as folk medicines like "Kyushin" and "Chan Su" [12,13]. The granular secretions are known to be secreting a variety of compounds which are species specific .

Most of the countries have diverse amphibian population but it is surprising that much attention has not been paid to the effects of environmental pollutants upon these animals. The largest single group of potential chemical pollutants that frogs and toads might encounter is various pesticides employed in agriculture and pest management [16] which enters into the organisms through food web and contact with water [17-19] .

So far, there are few reports on the effect of paraxon an organophosphate (OP) on biochemical constituents on parotoid gland secretion and its extract. The present investigation has been undertaken for the study of effect of paraxon (OP) on biochemical composition of parotoid gland secretion and its extract of *B. melanostictus* in order to understand their possible role in potentiating/ detoxifying the venomous secretion.

MATERIALS AND METHODS

Animal materials used for the study

The toads (7cm to 10 cm in length, weighed about 50 to 70 grams.) were collected from vicinity of university hostel buildings.

Extraction and Collection of Samples

The parotoid glands were gently pressed with the help of sterile forceps to release the secretions and were collected into ice-jacketed containers [20]. After collecting secretions, the gland was dissected out, blotted to free from blood clots and other adherent tissues and weighed to the nearest milligram and processed for further analysis.

Experimental procedure for Biochemical estimations

The parotoid gland secretion and gland extract were homogenized (10%) in 10% Tri chloro Acetic Acid (TCA) to sediment of protein. The protein sediment was dissolved in 1N NaOH and protein content was determined through the Lowry's reagent[21] described by Schacterle and Pollack [22].The TCA supernatant was used to estimate TCA soluble peptides (Lowry's reagent), Ninhydrine positive substances[23] and Carbohydrates [24].

Preparation of Paraxon (OP) concentration for induction

To estimate the biochemical constituents variation after exposure to paraxon (0, 0-di-ethyl-4-nitrophynyl phosphate ($2X10^{-3}M$) concentrations and normal saline were induced sub- cutaneously into parotoid gland contra laterally. The *in vivo* effects of Paraxon (OP) on biochemical constituents of parotoid gland secretion and its extract were studied according to the procedures[21-24] at different time intervals i.e., 4H, 8H and 12H hours were observed.

Statistical analysis

The results were expressed as means + SE. Results were analyzed by one-way ANOVA using Dunnett's multiple comparison tests using Graph pad Prism 5 software of the results between the tissue components. A probability level of 0.001 or less was accepted as significant.

RESULTS

The values obtained from the quantitative estimates on effect of paraxon (OP) on biochemical composition of parotoid gland secretion and its extract of *B. melanostictus* are presented in table-1, 2 & 3 and figure-1, 2 & 3 respectively.

In this experiment when parotoid glands of toad were injected with the desired concentrations of the test chemical paraxon at different time intervals, a drastic reduction was observed in total biochemical constituents in paratoid gland secretion and its extract compared to control.

The results presented in table 1 and figure 1 shows that the protein content was decreased in

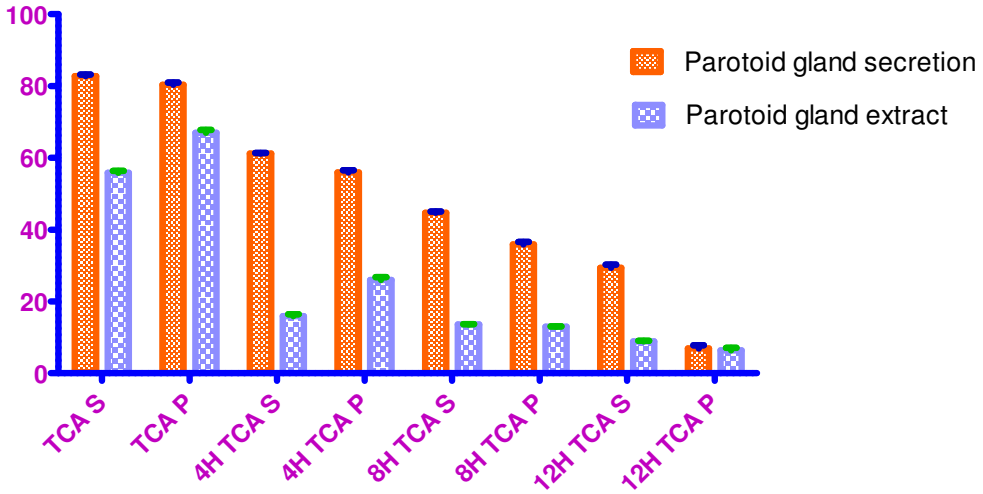
supernatant in paraxon treated samples after 4, 8 hrs inductions and the reduction in soluble protein content (supernatant) and the structural protein content (sediment) was found to be $p < 0.001$ in parotoid gland secretion and its extract.

Table-1 Biochemical constituents variations of proteins after induction of Paraxon into the Parotoid gland secretion and its extract of *Bufo melanostictus*

Period of Exposure	Parameter	Variation of protein content after induction of Paraxon	
		Parotoid gland secretion	Parotoid gland extract
Control	TCA soluble proteins	82.75 ± 0.46	55.83 ± 0.58
Control	TCA precipitated proteins	80.35 ± 0.62	67 ± 0.81
4 hrs	TCA soluble proteins	61.25 ± 0.13***	15.9 ± 0.55***
4 hrs	TCA precipitated proteins	55.9 ± 0.65***	26 ± 0.82***
8 hrs	TCA soluble proteins	44.66 ± 0.38***	13.6 ± 0.12***
8 hrs	TCA precipitated proteins	35.83 ± 0.71***	13 ± 0.13***
12 hrs	TCA soluble proteins	29.41 ± 0.81***	8.91 ± 0.13***
12 hrs	TCA precipitated proteins	6.91 ± 0.97***	6.33 ± 0.75***

Values are expressed as mean ± SE; n = 6,
Statistically significant value to respective control value.* P<0.05,** P<0.01, *** P<0.001.

Fig 1 Biochemical constituents variations of proteins after induction of Paraxon into the Parotoid gland secretion and its extract of *Bufo melanostictus*



TCA S-TCA soluble proteins,
TCA P- TCA precipitated proteins.

The results presented in table 2 and figure 2 revealed that carbohydrate content was decreased in a drastic reduction in total carbohydrate content in paratoid gland secretion and its extract when compared to control. In our observations paraxon treated samples with 4 & 8 hrs treatment, the p value of carbohydrate content was found to be non significant, but at 12

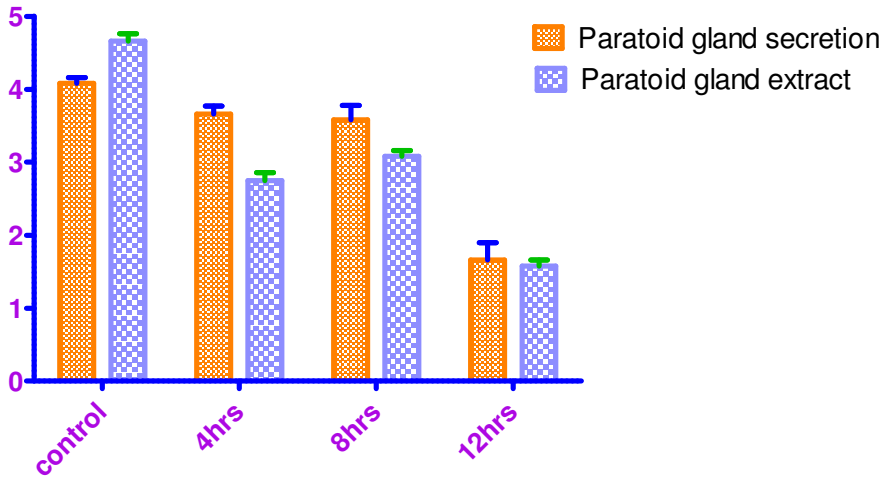
hrs treatment we noticed that the p value of carbohydrate content was found to be p <0.001 in parotoid gland secretion and its extract. Hence, it can be concluded that there is a significant variation between the parotoid gland secretion and its extract.

Table-2 Biochemical constituents variations of Carbohydrates after induction of Paraxon into the Parotoid gland secretion and its extract of *Bufo melanostictus*

Period of Exposure	Variation of carbohydrate values after induction of Paraxon	
	Parotoid gland secretion	Parotoid gland extract
Control	4.08 ± 0.08	4.66 ± 0.10
4 hrs	3.66 ± 0.11^{ns}	2.75 ± 0.11***
8 hrs s	3.58 ± 0.20^{ns}	3.08 ± 0.08***
12 hrs	1.66 ± 0.24***	1.58 ± 0.08***

Values are expressed as mean ± SE; n = 6,
Statistically significant value to respective control value.* P<0.05,** P<0.01, *** P<0.001. ns- Non significant

Fig-2 Biochemical constituents variations of carbohydrates after induction of Paraxon into the Parotoid gland secretion and its extract of *Bufo melanostictus*



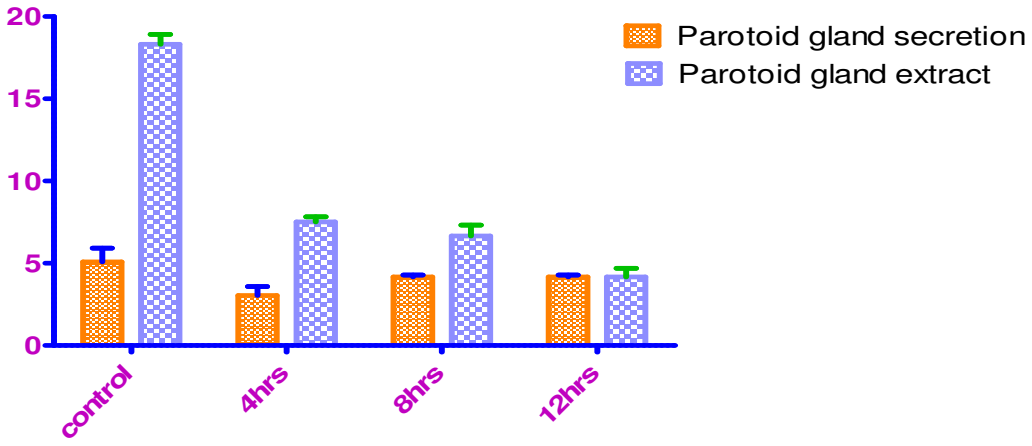
The results presented in table 3 and figure 3 revealed that the Ninhydrine positive substances (free amino acids) were decreased in a drastic reduction in free amino acid content in paratoid gland secretion and its extract compared to control with p<0.05 at 4 hrs, while non significant at 8&12hrs intervals in gland secretion where as the gland extract also showed decreased free amino acid content with a significant value of p<0.001.

Table-3 Biochemical constituents variations of Ninhydrine positive substances (Free amino acids) after induction of Paraxon into the Parotoid gland secretion and its extract of *Bufo melanostictus*

Period of Exposure	Variation of Free amino acid values after induction the Paraxon	
	Parotoid gland secretion	Parotoid gland extract
Control	5.08 ± 0.83	18.3 ± 0.62
4 hrs Dose	3.04 ± 0.54*	7.5 ± 0.32***
8 hrs Dose	4.16 ± 0.11^{ns}	6.66 ± 0.66***
12 hrs Dose	4.16 ± 0.11^{ns}	4.16 ± 0.54***

Values are expressed as mean ± SE; n = 6,
Statistically significant value to respective control value.* P<0.05,** P<0.01, *** P<0.001. ns- Non significant

Fig-3 Biochemical constituents variations of Ninhydrine positive substances (Free amino acids) after induction of Paraxon into the Parotoid gland secretion and its extract of *Bufo melanostictus*



DISCUSSION

The organophosphates are compounds widely used as insecticides and extremely toxic in some cases, these materials are generally short lived in the environment compared to halogenated organics and related compounds[25]. 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 *Bufo periglenes* (Bufonidae)[26].The predominant mechanism of organophosphate toxicity is inhibition of acetylcholinesterase in the nervous system causing accumulation of acetylcholine[27],which causes hyper excitability and multiple postsynaptic impulses generated by single presynaptic stimuli. Minimal work has been conducted on effects of organophosphorus compounds on disease susceptibility.

The parotoid gland secretions are abundant proteinaceous material having much content of esterases and were sensitive to OP compounds like Paraxon and Physostigmine[28,29]. The present work has been carried out by the injection of paraxon (OP) into parotoid gland of *B. melanostictus*. In our present investigation on the effect of paraxon on biochemical composition of parotoid gland secretion and its extract of *B. melanostictus,* showed a considerable variation between the parotoid gland secretion and its extract compared to control.

Generally biochemical constituents of parotoid gland secretion and its extract are rich in protein content. Whereas, when treated with paraxon, the decrease in total protein content of both parotoid gland 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 proteins and pesticides disturb the protein synthesis. In the present study the total protein content in both parotoid gland secretion and its extract in Indian toad decreased after induction of Paraxon.

Carbohydrates are less sensitive as compared to proteins towards OP compounds. Carbohydrates and Ninhydrine positive substances (free amino acids) are very low in control. The biochemical variations were observed in three intervals (4hrs, 8hrs and 12hrs) after induction of OP compounds like paraxon. The results revealed that the great decrease in three intervals in both parotoid gland secretion and its extract compare to control but the p value was not significant in gland secretion compared to gland extract.

The reduction in total protein contents after pesticide application in different insects was reported by many workers. It has also been reported by many authors that a significant reduction was noticed in total protein content in many insects, fishes and reptiles when exposed to pesticides[30-33] and was also found in our present investigation. The protein reduction in the liver and

kidney of reptiles was also reported as the present investigations also appear to be in line with the earlier findings.

CONCLUSION

The present studies showed that the parotoid gland secretion and its extract were rich in protein content compared to carbohydrate and free amino acids. Hence, it can be concluded that the bio active molecules were more sensitive to OP compound effecting on their physiological activities and xenobiotic metabolism which are species specific. This needs further investigation.

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