ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Impact of an Organophosphorus Nuvan on Some Aspects of Protein Metabolism in the Fresh Water Teleost *Labeo rohita* (Hamilton)

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Manuscript Info

Manuscript History:

Received: 22 May 2015
Final Accepted: 18 June 2015
Published Online: July 2015

Key words:

Nuvan, Sublethal, *Labeo rohita*, Structural proteins, Total proteins, Protease activity and Free amino acids.

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Abstract

Impact of sublethal concentration of Nuvan (0.011mg/ml) was studied on structural proteins, total proteins, protease activity and free amino acids of the fish *Labeo rohita*. The levels of structural proteins and total proteins were declined relative to controls in all organs of fish at 1st day exposure and continued its declination up to 15th day exposure periods. From 15th day onwards their levels gradually elevated and came nearer to control at 30th day exposure period. In contrast to this the levels of protease activity and free amino acids followed an opposite trend at all exposure periods.

INTRODUCTION

Pesticides are the substances designed to control, repel, mitigate, kill or regulate the growth of undesirable biological organisms. These pests compete with humans for food, but also transmit diseases and destroy property. The uses of pesticides are necessary in agriculture to control pests and increase yielding in order to produce sufficient food for the growing global population. Now pesticides usage became an indispensible and integral part of world agriculture. Modern agriculture practices even though contributed to enhance crop production but also widely polluted aquatic environment [2]. Agriculture practices along with pest control programmers, the surface runoff and aerial spraying form the major source for translocation pesticides into aquatic ecosystems [3-5]. Pesticide pollution affects both aquatic and soil ecosystems. The contamination of water by pesticides may effect on non - target organisms like fish [6-8]. The fish is a good indicator and highly sensitive in such ecosystem where the water gets contaminated with toxic chemicals. So an attempt was made on sub lethal effect of nuvan on some aspects of protein metabolism in the fish *Labeo rohita*.

MATERIALS AND METHODS

Test Chemical:

The pesticide selected for the present investigation was an organophosphorus Nuvan. The active ingredient in Nuvan was Dichlorvos. It was widely used on diverse agricultural crops to control pests of crops, flies and mosquitoes. It has been widely used because of its degradability, non–persistent nature and low mammalian toxicity. Its commercial name was DDVP. Commercial grade was used and its effective concentration was 76%.

Experimental design:

Fresh water fish *Labeo rohita*, weighing 10±2 gm were procured from local fisheries department and stored in spacious aquaria. The water in aquaria was aerated twice day, the fish were fed daily with groundnut cake and rice bran. The physic-chemical properties of water used for experiments had pH 7.4 ± 0.2, dissolved oxygen 6-7 ml /lt, hardness 160 ppm and temperature 28±1 ˚C. Before experimentation has been executed, the fish were acclimated to the laboratory conditions for a period of 10 days. Later groups of 10 fish were exposed to different concentration of Nuvan ranging from 0.7 mg/ml to 1.4 mg/ml. The mortality was observed during 96 hrs exposure period. The LC50 / 96 hrs was determined from the percent and probit mortality versus log concentration curves (Finney, 1964) and were subsequently verified by Dragstedt and Behrens method as given by Carpenter (1975). After determination of LC 50/96 hrs (0.11mg/ml), the fish were exposed to sublethal concentration of Nuvan (1/10th of LC50/96hrs i.e. 0.011 mg/ml) for five exposure periods i.e 1, 7, 15 and 30 day.

Methods

In the present investigation the levels of total proteins, protease activity and free amino acids were estimated in the brain, liver and muscle of fish. Each experiment was carried out in the organs of six individuals and the mean of six values were taken in to consideration. The structural proteins and total proteins were estimated by Folin phenol reagent method as described by Lowry et *al* [11], protease activity were estimated using Ninhydrin method described by Davis and Smith [12] and free amino acids were estimated by the Ninhydrin method as described by Moore and Stein [13].

RESULTS

In the present investigation the levels of structural proteins, total proteins, protease activity and free amino acids were estimated in the brain, liver, gill, kidney and muscle of fish, on 1, 7, 15 and 30 days of exposure to sublethal concentration of Nuvan besides control levels were presented in tables 1,2, 3 and 4. The levels of structural proteins and total proteins declined relative to controls in all organs of fish at first day exposure and continued its declination up to 15 day exposure periods.

From 15 day onwards their levels gradually elevated and came nearer to control at 30 day exposure period. Where as the levels of protease activity and free amino acids followed an opposite trend on exposure periods. Mean and standard deviation are a pool of six individual measurements. The percent change in the protease activity at different periods was calculated in relation to the protease activity in the control medium. The differences between control and exposure period days were found to be statistically significant ($P < 0.01$).

Table 1: **Structural Proteins** (mg/g wet wt) in the fish *Labeo rohita* on exposure to sublethal concentration of Nuvan. Mean and standard deviation are a pool of six individual measurements. The differences between control and exposure period days were found to be statistically significant ($P < 0.001$).

Sl.No	Organs	Control	Exposure period in days			
			1 day	7 day	15 day	30 day
1	Brain SD± PC	58.13 2.12	52.61 1.72 -9.49	44.08 2.25 -24.16	39.27 1.84 -32.44	48.06 1.68 -17.32

2	Liver SD± PC	66.34 2.24	54.26 2.12 -18.20	42.35 1.80 -36.16	33.12 1.76 -50.07	58.32 2.14 -12.08
3	Muscle SD± PC	86.52 2.36	74.38 1.89 -14.03	61.75 2.52 -28.62	54.56 1.95 -36.93	78.24 2.26 -9.57

SD – Standard Deviation; **PC** – Percent change

Table 2: **Total Proteins** (mg/g wet wt) in the fish *Labeo rohita* on exposure to sublethal concentration of Nuvan. Mean and standard deviation are a pool of six individual measurements. The differences between control and exposure period days were found to be statistically significant ($P < 0.001$).

Sl.No	**Organs**	**Control**	**Exposure period in days**			
			1 day	**7 day**	**15 day**	**30 day**
1	Brain SD± PC	87.23 1.28	79.67 1.36 -8.66	66.03 1.56 -24.30	56.40 1.82 -35.34	70.36 1.76 -19.33
2	Liver SD± PC	98.62 1.36	83.92 1.84 -14.90	63.57 1.53 -35.54	44.53 1.65 -54.84	86.79 1.88 -11.99
3	Muscle SD± PC	122.54 2.52	107.24 6.36 -12.48	74.85 2.18 -38.91	50.15 1.85 -59.07	110.81 1.98 -9.57

SD – Standard Deviation; **PC** – Percent change

Table 3: **Protease Activity** (µM amino acid nitrogen/mg protein/hr) in the fish *Labeo rohita* on exposure to sublethal concentration of Nuvan. Mean and standard deviation are a pool of six individual measurements. The differences between control and exposure period days were found to be statistically significant ($P < 0.001$).

Sl.No	**Organs**	**Control**	**Exposure period in days**			
			1 day	**7 day**	**15 day**	**30 day**
1	Brain SD± PC	0.192 0.022	0.219 0.016 +14.06	0.236 0.025 +22.91	0.275 0.018 +43.22	0.253 0.024 +31.77

2	Liver SD± PC	0.220 0.018	0.278 0.032 +26.36	0.315 0.020 +43.18	0.372 0.026 +69.09	0.329 0.028 +49.54
3	Muscle SD± PC	0.138 0.006	0.166 0.018 +20.28	0.187 0.013 +35.50	0.224 0.008 +62.31	0.194 0.016 +40.57

SD – Standard Deviation; **PC** – Percent change

Table 4: **Free Amino acids** (mg amino acid nitrogen/g wet wt) in the fish *Labeo rohita* on exposure to sublethal concentration of Nuvan. Mean and standard deviation are a pool of six individual measurements. The differences between control and exposure period days were found to be statistically significant ($P < 0.001$).

Sl.No	**Organs**	**Control**	**Exposure period in days**			
			1 day	**7 day**	**15 day**	**30 day**
1	Brain SD± PC	20.53 1.76	23.05 1.66 +12.27	26.85 2.08 +30.78	29.52 1.56 +43.78	26.24 2.02 +27.81
2	Liver SD± PC	13.54 1.24	16.12 1.86 +19.05	19.18 1.42 +41.65	23.16 1.53 +71.04	20.56 1.95 +51.84
3	Muscle SD± PC	10.26 0.96	1.76 0.89 +14.61	13.52 1.21 +31.77	15.85 1.15 +54.48	13.12 1.34 +27.87

SD – Standard Deviation; **PC** – Percent change

DISCUSSION

Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism. Proteins can be expected to be involved in the compensatory mechanism of stressed organisms. The survival ability of animals exposed to stress majorly depends on their protein synthetic potentials. The proteins are the major source of energy during chronic conditions besides carbohydrates [15]. Young [16] reported protein budget of a cell can be taken as an important diagnostic tool in the evaluation of its physiological standards. Pesticides are known to interfere in protein synthesis and degradation there by altering the dynamic equilibrium [17-31].

In the present study relative to controls the levels of total proteins declined on first day exposure and continued its depletion up to 15 day exposure period. Whereas the levels of protease activity and free amino acids initially elevated on 1 day exposure period and continued its elevation up to 15 day exposure period. The decline in total protein levels followed by elevation in the levels of protease activity and free amino acids at initial exposure periods may indicates the high energy demand associated with imposed nuvan stress. To overcome this animal tends to mobilize the proteins by stimulating the protease activity.

Seshagiri Rao *et al.,* [32] observed an increase in free amino acid level in the organs of the fish *Sarotherodon mossambicus* which could be due to degradation of proteins by proteolysis or due to decreased protein synthetic potentials in the pesticide induced pathological condition also supports the present trend in protein metabolism.

Some of the observations were also supports the present trend in the decline of total proteins and elevation in free amino acid are as follows. Bhavan and Geraldine [33] reported the decline in soluble proteins in tissues of prawn *M. malcomsonii* on exposure to sublethal concentration of carbaryl. Magar and Shaik [34] observed decline in protein content and elevation of free amino acids in tissues of fish *Channa punctatus* on exposure to sublethal concentration of malathion. Pratap and Singh [35] reported significant decrease in total proteins and elevation in free amino acid levels in *Chnanna punctatus* on exposure to sublethal doses of apigenin. Ram Yadav and Ajay Singh [36] reported decline in protein content and elevation in free amino acids in snail *Lymnea acuminata* exposed to plant pesticide. Furthermore Singh and Singh [37] reported decline in total proteins in the fish *Trichogaster fasciatus* on exposure to pesticide dipterex. Fahmy [38] reported significant decrease in total proteins in various tissues of *Oreochromis niloticus* on exposure to malathion. Arun Kumar and Jawahar Ali [39] observed decrease in protein content in the tissues of shrimp *Streptocephalus dichotomus* on exposure to sublethal concentration of malathion and glyphosate. Vidya and Nair [40] observed protein content decreased in tissues of *Etroplus suratensis* on exposure to sublethal concentration of λ- cyhalothrin. Binakumari and Vasanthi [41] reported decline in protein content in the tissues of fresh water fish *Labeo rohita* on exposure to pesticide dimethoate. Nagaraju and Venkataratnamma [42] observed protein depletion in tissues of fresh water fish *Labeo rohita* exposed to sublethal concentration of profenofos. Shivanagouda *et al* [43] observed decline in protein content in tissues of marine fish *Mugilcephalus* on exposure to sublethal concentration of carbaryl. Suneel Kumar [44] reported significant decrease in total proteins in *Channa punctatus* on exposure to lethal concentration of nuvan. Neeraja and Giridhar [45] observed Significant decrease in total proteins and an increase in protease activity and free amino acids in the fish *Labeo rohita* on exposure to deltamethrin. All these studies correlate with the decline in total proteins and elevation in levels of protease activity and free amino acids.

In later half of exposure the total proteins gradually elevated and came nearer to control at 30 day exposure period. Whereas the levels of protease activity and free amino acid levels goes on decreasing and came nearer to control on 30 day exposure period. Pratibha *et al* [46] reported decrease in soluble and insoluble protein in tissues of fish *Channa punctatus* at initial exposure period and elevation in protein content in later exposure periods also coincides with the present study. Dixon and Sprague [47], Kito *et al* [48], and Pampatwar *et al* [49] reported an increase in protein content may also help to fortify the organs for developing resistance to the imposed toxic stress and synthesis of enzymes necessary for detoxification. In the present study the shifts in protein metabolism might to compensate with situation shown by the animal for its survival.

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