IJPSR (2018), Volume 9, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 October, 2017; received in revised form, 10 June, 2018; accepted, 12 June, 2018; published 01 July, 2018

EFFECT OF METHYL PARATHION (AN ORGANOPHOSPHATE) ON BIOCHEMICAL CONTENTS OF FRESH WATER CAT FISH *HETEROPNEUSTES FOSSILIS* (BLOCH)

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Keywords:

H. fossilis,
Proteins, Carbohydrates,
Ninhydrine positive substances,
Methyl parathion, Organophosphate

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ABSTRACT: The present work was planned to study the effect of methyl parathion an organophosphate compound (OP) on biochemical contents of fresh water cat fish *Heteropneustis fossilis*. The exposure of fish to the sub-lethal concentrations of the toxicant methyl parathion was investigated and the variations were observed in biochemical contents in different tissues of the fish *i.e.* gill, liver, intestine, muscle and brain. The quantitative variations were observed in proteins, carbohydrates and ninhydrine positive substances at different time intervals *i.e.* 24, 48, 72 and 96 h. The results revealed that the components of proteins, carbohydrates and ninhydrine positive substances were found to be decreased significantly at different time intervals of methyl parathion exposure to different tissues of fish compared to control. The maximum decrease in proteins followed by ninhydrine positive substances (free amino acids) and carbohydrates was observed at 72 h and 96 h compared to 24 h and 48 h in different tissues of the fish *H. fossils*. Thus, our present investigation reports that the changes observed were depending on period of exposure of fish to the concentration of methyl parathion.

INTRODUCTION: Pesticides are used worldwide in aquaculture and agriculture to control the insects, pests and other vectors [1], which ultimately find their way into aquatic habitats like rivers, lakes and ponds. The environmental quality is determined by the assessing to toxicity of different chemicals to fish and other aquatic organisms. They ultimately enter the organisms through food webs and also through contact in water [2, 3]. Most of the chemicals used as pesticides are acutely toxic to many non-target organisms such as invertebrates, birds, mammals and fishes especially those inhibiting the marine environment [4, 5, 6].

QUICK RESPONSE CODE	DOI: 10.13040/IJPSR.0975-8232.9(7).2869-74
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(7).2869-74	

Some of the pesticides have been reported to persists the environment and tend to bio-accumulation in organisms [7]. It has been reported that pesticides can be actively toxic to fish [8, 9, 10, 11, 12, 13, 14]. Pesticide toxicity to fish has been investigated in several studies [15, 16]. Usage of pesticides in the ecosystem leads to development of various types of morphological, physiological, biochemical and behavioral changes in individual [17].

Hence it is necessary to study the immediate effect of pesticides on fish which forms a part of human diet. Among these pesticides, organophosphate compounds (OP's) are commonly used insecticides, which maintain less toxicity, persistence and also rapid biodegradability in the environment [18]. Methyl Parathion (0, 0 -di methyl-0-4 nitro phenyl phosphoro thioate)-Bayer (Germany) is a synthetic Organophosporous pesticide, known to be toxic to fish [19] and insects [20] and applied abundantly in agriculture [21].

Fishes are very sensitive to a variety of toxicants in water, which uptake and accumulate many toxicants such as pesticides [22, 23]. However pesticide exposure causes severe alternations in the tissue biochemistry of fishes. In general the toxic effects will be more when two (or) more toxicants act together in a synergistic manner [24, 25, 26, 27, 28]. The present study has been carried out to investigate the effect of methyl parathion (an Organophosphate) on biochemical contents of fresh water cat fish *Heteropneustes fossilis* (Bloch).

MATERIALS AND METHODS: In the present study, fresh water fish *H. fossilis* was exposed to different concentrations of an organophosphate pesticide methyl parathion and the variations in biochemical constituents of the fish were studied. The technical grade pesticide methyl parathion 50% E.C was taken for this present study.

The fresh water fish *H. fossilis* (ranging in weight 70 to 100 gm and in length 25 cm to 35 cm) were brought from local fresh water tanks located within the radius of 15 km from the Kakatiya University, Warangal, Telangana State, India to the laboratory in well aerated polythene bag and acclimatized to the ambient room temperature (28 ± 2 ºC) in large plastic containers. During the period of acclimatization they were fed with oil cake mixed with rice flour. The period of acclimatization lasted for 15 days.

The healthy fishes were grouped into five batches containing six and each were exposed to different concentrations of pesticide methyl parathion to calculate the medium lethal concentration LC_{50} value using Probit analysis method [29]. The methyl parathion was dissolved in ethanol and diluted with water to the required concentrations. The fishes (five groups) were exposed to the sub lethal concentration (0.1g - 0.5g) of methyl parathion for 24, 48, 72 and 96 h respectively. Another group was maintained as control without pesticide.

At the end of each exposure period, fishes were scarified and the tissues such as gill, liver, intestine, muscle and brain were dissected out and stored on ice-jacketed container for biochemical studies. The tissues were weighed to the nearest milligram and processed for further analysis.

The tissues were homogenized (10%) in 10% Tri Chloro Acetic Acid (TCA) centrifuged at 2000 rpm for 15 min 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 method [30] described by Schacterle and Pollack (1973) [31]. Ninhydrine positive substances were estimated by the method of Lee and Takahashi (1966) [32] and the total carbohydrate content in the tissues were estimated by the method of Carroll *et al.*, (1956) [33].

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 effect of Methyl parathion on biochemical contents of various tissues of fresh water cat fish *H. fossilis* are presented in **Table 1, 2, 3, 4** and **Fig. 1, 2, 3, 4** respectively. In this study when the fish tissues *i.e.* gill, liver, intestine, muscle and brain after treatment with different concentrations of the test chemical methyl parathion at different time intervals a drastic reduction was observed total biochemical content of different tissues of *H. fossilis* compared to control. The results presented shows that the protein content was significantly decreased in the gill of fish exposed to methyl parathion (**Table 1, 2** and **Fig. 1, 2**).

TABLE 1: TCA SOLUBLE PROTEINS IN VARIOUS TISSUES OF *H. FOSSILIS* EXPOSED TO VARYING PERIODS OF SUB-LETHAL CONCENTRATION OF METHYL PARATHION

Dose / Tissue	Control	24 h	48 h	72 h	96 h
Gill	4.99 ± 0.22	4.368 ± 0.26**	3.10 ± 0.26***	1.83 ± 0.48**	0.88 ± 0.50
Liver	6.10 ± 0.22	4.22 ± 0.45	3.55 ± 0.45	2.05 ± 0.50**	1.05 ± 0.45
Intestine	3.42 ± 0.17	1.65 ± 0.33	1.33 ± 0.34	0.83 ± 0.82**	0.66 ± 0.38
Muscle	4.49 ± 0.11	4.05 ± 0.28	3.50 ± 0.28	1.44 ± 0.54**	0.88 ± 0.41
Brain	2.49 ± 0.21	1.94 ± 0.33	1.21 ± 0.33	0.71 ± 0.24	0.33 ± 0.15**

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

TABLE 2: TCA PRECIPITATED PROTEINS IN VARIOUS TISSUES OF *H. FOSSILIS* EXPOSED TO VARYING PERIODS OF SUB LETHAL CONCENTRATION OF METHYL PARATHION

Dose / Tissue	Control	24 h	48 h	72 h	96 h
Gill	16.11 ± 0.25	13.38 ± 0.98**	9.77 ± 0.98**	7.72 ± 0.55**	4.05 ± 0.31*
Liver	14.38 ± 0.31	11.05 ± 0.28*	7.82 ± 0.28*	6.05 ± 0.85	3.32 ± 0.90**
Intestine	11.21 ± 0.59	10.99 ± 0.61 **	8.99 ± 0.61**	7.94 ± 0.39**	6.05 ± 0.72**
Muscle	10.66 ± 0.52	9.38 ± 0.52	8.16 ± 0.52	6.16 ± 0.73**	3.94 ± 0.14*
Brain	6.49 ± 0.37	5.38 ± 0.42**	3.99 ± 0.42**	2.44 ± 0.40**	0.88 ± 0.33***

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

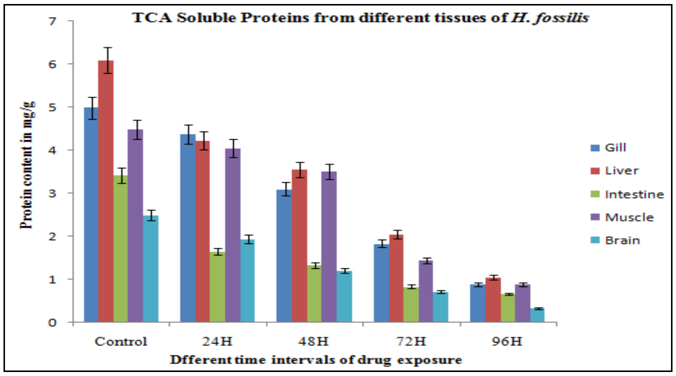

FIG. 1: TCA SOLUBLE PROTEINS IN VARIOUS TISSUES OF *H. FOSSILIS* AND THE VALUES ARE EXPRESSED AS MEAN ± SE mg/gm OF WET WEIGHT OF TISSUE

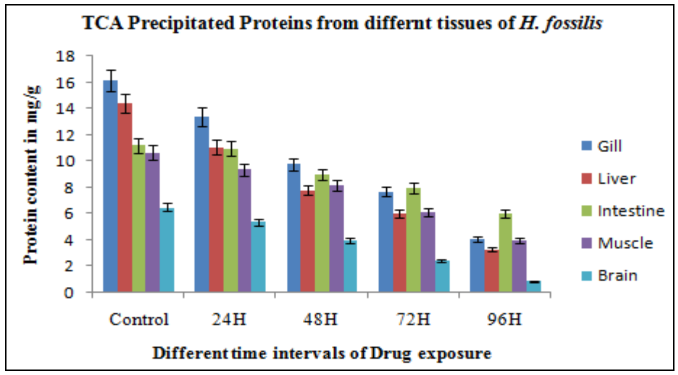

FIG. 2: TCA PRECIPITATED PROTEINS IN VARIOUS TISSUES OF *H. FOSSILIS* AND THE VALUES ARE EXPRESSED AS MEAN ± SE mg/gm OF WET WEIGHT OF TISSUE

It was observed that the TCA soluble proteins were decreased and is more pronounced in the gill at 48, 72, 96 h exposure gill, liver, intestine, muscle and brain estimated greater reduction in TCA soluble proteins (**Table 1** and **Fig. 1**). Whereas in TCA precipitated protein contents were significantly decreased at 24, 48, 72 and 96 h of exposure of methyl parathion (**Table 2** and **Fig. 2**).

While the Carbohydrates were more in brain compared to other tissues in control and also highly decreased in brain and liver at 96 h (**Table 3** and **Fig. 3**). The Free amino acids were found be highest in control muscle and gradually decreased in brain (**Table 4** and **Fig. 4**) from 24 h, 48 h, 72 h, 96 h.

TABLE 3: CARBOHYDRATES CONTENT IN VARIOUS TISSUES OF *H. FOSSILIS* EXPOSED TO VARYING PERIODS OF SUB LETHAL CONCENTRATION OF METHYL PARATHION

Dose / Tissue	Control	24 h	48 h	72 h	96 h
Gill	3.60 ± 0.39	3.47 ± 0.45**	1.94 ± 0.54 **	2.08 ± 0.25**	1.24 ± 0.30
Liver	6.38 ± 0.59	3.88 ± 0.58***	1.24 ± 0.581	1.38 ± 0.13	1.10 ± 0.27
Intestine	4.85 ± 0.41	3.46 ± 0.56	2.63 ± 0.561	1.52 ± 0.45	0.96 ± 0.37
Muscle	2.36 ± 0.55	1.80 ± 0.28	1.38 ± 0.29	0.96 ± 0.35	0.83 ± 0.13
Brain	7.22 ± 0.99	4.30 ± 0.87**	1.52 ± 0.87**	1.10 ± 0.46	0.83 ± 0.17

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

TABLE 4: FREE AMINO ACIDS/NINHYDRINE POSITIVE SUBSTANCES IN VARIOUS TISSUES OF *H. FOSSILIS* EXPOSED TO VARYING PERIODS OF SUB LETHAL CONCENTRATION OF METHYL PARATHION

Tissue / Dose	Control	24 h	48 h	72 h	96 h
Gill	3.09 ± 0.39	2.66 ± 0.35	1.83 ± 0.36	1.27 ± 0.35	0.60 ± 0.22**
Liver	2.88 ± 0.44	2.49 ± 0.18**	1.94 ± 0.187**	1.38 ± 0.26	0.83 ± 0.18**
Intestine	2.66 ± 0.35	2.71 ± 0.34	1.99 ± 0.35	1.38 ± 0.38	0.71 ± 0.25
Muscle	3.83 ± 0.39	3.83 ± 0.50	3.05 ± 0.50	1.86 ± 0.52**	0.44 ± 0.33***
Brain	2.49 ± 0.16	2.16 ± 0.17	1.83 ± 0.17	1.66 ± 0.32	0.38 ± 0.26***

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

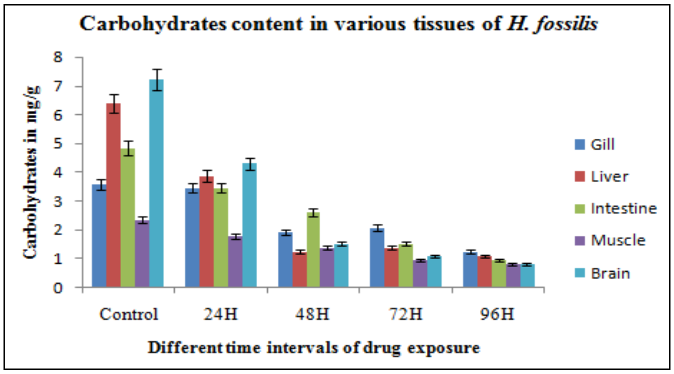

FIG. 3: CARBOHYDRATE CONTENT IN VARIOUS TISSUES OF *H. FOSSILIS* AND THE VALUES ARE EXPRESSED AS MEAN ± SE mg/gm OF WET WEIGHT OF TISSUE

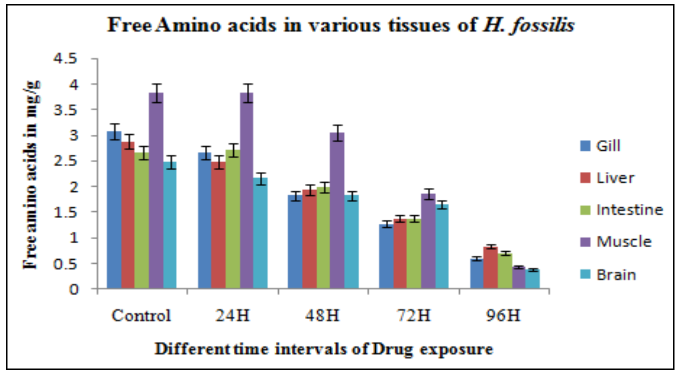

FIG. 4: NINHYDRINE POSITIVE SUBSTANCES IN VARIOUS TISSUES OF *H. FOSSILIS* AND THE VALUES ARE EXPRESSED AS MEAN ± SE mg/gm OF WET WEIGHT OF TISSUE

DISCUSSION: Organophosphates are non-persistent in nature and their degradation is rapid [34]. These are short lived and highly toxic when compared to a number of other pesticides for example organophosphate pesticide is found to be more toxic to fish than organochlorine [35, 36, 37]. Therefore, organophosphates can affect the organisms exposed even for a very short period.

The degree of toxicity produced by the poisonous substances is dose dependent upon environmental conditions such as temperature, pH, O_2 concentration and presence of residual molecules [38]. Due to Methyl parathion in toxification, the normal functioning of cells with the resultant alterations in the fundamental biochemical mechanisms in test fish was noticed. Similar results were also noticed in freshwater Labeo rohita upon chronic exposure to the pesticide methyl parathion [39]. In our present investigation, the effect of methyl parathion on biochemical constituents of fresh water fish *H. fossilis* showed a considerable variation in different tissues of fish *H. fossilis* [40]. Findings were correlated on pesticide treated mollusks.

In the present study methyl parathion caused reduction in the total structural and soluble protein content in various tissues of *H. fossilis i.e.,* gill, liver, intestine, and muscle brain were found to be declined during the exposure period of 24, 48, 72 and 96 h. The studies on Methyl parathion toxicity cause metabolic dysfunction in fish [41, 42, 43]. Carbohydrates are less sensitive as compared to proteins towards the OP compounds. The results of the present findings showed a significant decrease in carbohydrate content in all the tissues.

The decrease in carbohydrates and ninhydrine positive substances (Free amino acids) was observed in various tissues of *H. fossilis* compared to control. The decrease in carbohydrate content is significant and may result in impairment of carbohydrate metabolism due to toxic effects [44, 45, 46].

CONCLUSION: From the present study it is concluded that the Methyl parathion exposure has a strong potential to alter the biochemical constituents in various tissues of *H. fossilis*. Further research has to be focused to evaluate the effect of alternative pesticides and related chemicals to reduce the adverse effects on physiological and biochemical aspects of fish. Therefore, the use of pesticides in the field may be a threat to a human, fauna and flora of the environment.

ACKNOWLEDGEMENT: The author expresses their deep gratitude to the Head of the Department of Zoology, Kakatiya University for providing logistic Support.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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How to cite this article:
Rao TB, Thirupathi K and Venkaiah Y: Effect of methyl parathion (an organophosphate) on biochemical contents of fresh water cat fish *Heteropneustes fossilis* (bloch). Int J Pharm Sci & Res 2018; 9(7): 2869-74. doi: 10.13040/IJPSR.0975-8232.9(7).2869-74.