PROTEIN PROFILE IN TISSUES OF ZEBRAFISH EXPOSED TO DELTAMETHRIN

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ABSTRACT

Alterations of different proteins in five different tissues namely liver, brain, kidney, ovary and testis in adult zebrafish were identified after exposure to 5µg/L of deltamethrin for 6days. Total proteins were estimated and resolved by SDS-PAGE. The protein content increased in both male and female brain, kidney and ovary, decreased in male and female liver tissue and testis. Protein bands were more prominent in brain, kidney, ovary and testis in deltamethrin treated groups compared to their control. Up/down regulated protein bands were identified as betaine-homocysteine methyltransferase, enolase and transferring A, carbonic anhydrage and NADP (H) dehydrogenase quinine1; triose phosphate isomerase in liver, aldehyde dehydrogenase 9 family and dihydropyrimidase; tubulin alpha and keratin proteins; glutathione S-transferase and cytochrome-c reductase in brain and heatshock proteins in testis.

***Key words*:** Deltamethrin, Pyrethroid, SDS-PAGE, Protein, Zebrafish.

No: of Tables : 1 **No:of Figures:2** **No:of References:24**

INTRODUCTION

Pyrethroids are structural derivatives of pyrethrins that have greater potency and environmental stability (Casida 1980; Elliott and Janes, 1978). These are considered as effective insecticides due to their high insecticidal toxicity with low mammalian toxicity (Elliott et al., 1974). Both natural pyrethrins and synthetic pyrethroids are neurotoxicants, which act directly on excitable membranes and there by interfere with membrane ionic conductance in target organisms (Wouters and Van den Bercken, 1978). Pyrethroids are lipophilic therefore biological membranes and tissues absorb them easily. Pyrethroids have been reported to be extremely toxic to fish and some beneficial aquatic arthropods, for example, lobster and shrimp (Bradbury and Coats, 1989); Srivastav et al., 1977). Residues of pyrethroids were found in many biological samples around the world. Permethrin, cyfluthrin, deltamethrin were detected in breast milk samples collected in Switzerland in 1998/99 (Zehringer and Herrmann, 2001). Bowman et al., 2006 reported mean concentrations (by wet weight) of 14.51mg/L permethrin, 41.74mg/L cyfluthrin, 4.24mg/L cypermethrin, 8.39mg/L deltamethrin in breast milk of mothers from several villages in South Africa. Table grapes were also shown to contain high concentrations of pyrethroids (Poulsen et al., 2007). The residues of deltamethrin in vegetables like head lettuce and Broccoli were found higher than the tolerance at the PHI of 6-10 days, when applied to a sandy clay loam soil at 17.5g/ha (Chen et al., 2014).

Among the synthetic pyrethroids, deltamethrin is one of the most effective and widely used (DeMicco et al., 2010). Deltamethrin, a synthetic pyrethroid pesticide with a technical name (S)-a-cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromovinyl)-2, 2dimethylcyclopropane carboxylate contaminating aquatic ecosystems as a toxic pollutant. It is a pyrethroid pesticide and considered as an endocrine disruptor by the USEPA interfering with the reproductive system (Tramujas et al., 2006). Deltamethrin affects insects by disrupting their nervous system upon physical and digestive contact. According to a review article published by Mathur and D'cruz (2011), deltamethrin has been found to affect male reproductive system by disrupting spermatogenesis, steroidogenesis of both Leydig and Sertoli cells leading to interrupted reproductive cycle. Precisely deltamethrin leads to the production of reactive oxygen species, which in turn, causes the apoptosis of nursing sertolicells.

In this study we demonstrate the protein pattern in the five tissues of zebrafish namely liver, brain, kidney, ovary and testis in response to deltamethrin toxicity. The zebrafish, *Danio rerio* (Hamilton), is one of the most important vertebrate model organism and multi-faceted research tool in chemical Biology, genetics, developmental biology, neurophysiology and biomedicine Grunwald and Eisen, 2002; Rubinstein, 2003).

Materials and Methods

Preparation of Stock Solution: Technical grade Deltamethrin (99%) (S)-a-cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromovinyl)-2, 2dimethylcyclopropane carboxylate) CAS-No: 52918-63-5 was obtained from Sigma Aldrich (USA). Stock Solution of deltamethrin was prepared by dissolving 10mg of deltamethrin in 2ml of acetone. This was stored at 4°C and from this daily requirements are taken.

Experimental design: Mature and healthy adult zebrafish were randomly chosen from our aquarium stock and were placed in each of the three glass aquaria of 20L capacity. One tank served as control, second tank served as solvent control and the third tank was used for toxicant. All aquaria were fitted with aerators for constant supply of dissolve oxygen. Fish were fed daily with artemia and dry flakes in the morning and evening respectively at a regular time. Room and aquaria temperature were maintained at 27°C ± 1°C. They were maintained for one week and every day eggs were collected to confirm that fish were reproductively active. After one week 5µg/L of deltamethrin was added every day for 6 days. After every 24h, water from each aquarium was exchanged with fresh dechlorinated water. The exposure was conducted for 6 days. After the stipulated period fish were scarified, liver, brain, kidney, ovary and testis tissues were removed carefully and immediately kept in liquid nitrogen to overcome autolysis.

Sample preparation and estimation: 50mg of liver, brain, kidney, ovary and testis tissues were homogenized using Teflon Pestle in sodium phosphate buffer (0.1M $Na_2HPO_4.H_2O$, 0.1M NaH_2PO_4 anhydrous). Homogenized samples were centrifuged at 12000rpm for 15 minutes at 4°C and supernatant was collected for further analysis. Total proteins in the tissues were estimated by Bradford method (1976).

Sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE): This is carried out according to the procedure of Laemmli, (1970). The glass plates of the size 10cm X 10cm and spacers with 1.5mm thickness were used to cast 12% gel. The protein samples were diluted 1:2 with SDS–PAGE sample buffer [50mM Tris-Cl (pH 6.8), 100mM dithiotheriotol, 2% (w/v) SDS, 0.1% bromophenol blue, 10% (v/v) glycerol]. The sample was then centrifuged at 12000rpm for 10min using a table centrifuge (C 1205 Eppendorf, U.S.A.). From this 20µg protein was loaded into the wells along with 10µl protein molecular weight marker in the first well. Electrophoresis was carried out in a cold room initially at 50V. Voltage was increased to 100V after the sample has entered the resolving gel. Once the tracking dye has reached the end, the run was stopped and the gels were carefully removed from glass plates. Gels were then placed in 100ml of coomassie blue R-250 solution for staining. After 4hr the gels were transferred to destaining solution to remove the extra stain. Gel images were captured by Image Scanner.

Statistical Analysis: Standard deviation and level of significance was calculated for data obtained from three independent

experiments. One-way ANOVA was carried out by Duncan's test using SPSS software version 16.0 and P value.

Results and discussion

Results: Estimation of protein content: The total protein content in five different tissues of zebrafish in control and experimental animals are presented in Table-1 and Figure-1. From this it was observed that the total protein content decreased in the liver tissue of male and female treated fish as well as in the testis. In contrast, six days of exposure caused an increase of the total protein content in brain and kidney of both male and female fish along with ovary.

Protein analysis by SDS-PAGE: Protein pattern as revealed by SDS-PAGE in control and deltamethrin treated fish are depicted in Figure-2 (A-D). Homogenates of five tissues of both control and experimental zebrafish were resolved on 12% gels with molecular weight marker in the range of 3 to 205kDa. In the liver tissue of both male and female fish nine prominent bands were observed with one band each at 205KDa region, 97kDa region and 3kDa region, three bands between 66-43kDa, one band between 43-29kDa and two bands between 29-20.1kDa. The bands were more prominent in the control liver than in the experimental liver. Among the controls the bands were more prominent in females than males.

In brain tissue seven bands were observed in both male and female fish (Figure-2 B) with three prominent bands between 66-43kDa, one band between 43-29kDa, two bands between 29-21kDa and one band at 3kDa. In male fish bands were more prominent in experimental than control. With regard to the kidney sample (Figure-2 C) five prominent bands were observed in both control and experimental fish. Two bands each between 97-66kDa and 66-43kDa and one band at 3kDa was observed. In female and male fish the bands were more prominent in experimental compared with its controls.

Protein profile of ovary and testis of deltamethrin exposed fish along with their control are presented in Figure-2 D. Seven prominent bands were observed in both the tissues which are noticed near to 205kDa marker, other at 97kDa, another band in between 97-66kDa, two bands between 63-43kDa, one band each at 29kDa and 3kDa. The bands were more prominent in ovary than in testis. All seven bands were more prominent in experimental ovary/testis compared to their control.

Discussion: Proteins are among the most abundant biological molecules present in living tissue and have varied functions in various biochemical and physiological processes(Wilson et al., 1975). Since proteins play a key role in major events in cell assessment of its content and understanding its profile in different tissues of mature adult zebrafish is important when the fish is under deltamethrin stress.

In the present study decreased total protein content observed in deltamethrin exposed liver and testis could be due to breakdown of proteins under stress condition. In support of this decreased protein content under pyrethroid toxicity

was reported earlier in *Cyprinus carpio* (David et al., 2004). The decreased trend of protein content may be due to metabolic utilization of keto acids in the synthesis of glucose or for the osmatic and ionic regulation. Deltamethrin caused increase in total protein content in brain and ovary tissues which could be due to stepping up of protein synthesis under stress. Similar trend was reported earlier under pyrethroid toxicity in *Cyprinus Carpio* (Philip and Rajasree, 1996).

Protein expression profile is a good approach to visualize the pattern of proteins expressed under deltamethrin stress. SDS-PAGE examination of zebrafish tissues is carried out in control and after exposure of mature zebrafish for 6 days to deltamethrin. Due to deltamethrin energy demands were shown to be different in different tissues. For this reason we noticed up-regulation of proteins in ovary, brain and kidney tissues and down regulation in liver and testis tissues.

Not much work has been done with regard to identification of specific proteins in different tissues under stress. For this reason it can't be said clearly which is/are the protein/s that are showing up in the gels. In liver tissue the bands observed in between 66-43kDa marker could be betaine-homocysteine methyltransferase (BHMT), enolase3 and transferrin A. These proteins are involved in aminoacid metabolism and also BHMT is involved in the production of reduced GSH as shown by Kling et al., 2008) when treated with BFR mixture. In between 43-29kDa marker the proteins could be anti-oxidant defense enzymes like carbonic anhydrase and NADP (H) dehydrogenase quinine1. Earlier these proteins were also reported in zebrafish liver(Kling et al., 2008; Jury et al., 2008). The band between 29-21kDa region can be identified as triosephosphate isomerase. It is involved in interconversion of glyceraldehydes 3-phosphate to dihydroxy acetone phosphate. The isomerization of these 3-carbon phosphorylated sugars is important, without which generation of ATP will be lost. The decreased level of this protein band could be causing depletion of energy source under deltamethrin stress.

In brain two out of three bands present between 66-43kDa region may be identified as aldehyde dehydrogenase 9 family and dihydropyrimidase. Aldehyde dehydrogenase plays an important role in ethanol metabolism where in it converts acetaldehyde to acetate. Whereas dihydropyrimidase is involved in hydrolase activity. Expression of these two proteins in brain tissue of zebrafish treated with mixture of methyl parathion and cadmium have been reported earlier Ling et al., 2012). Cytoskeletal proteins like tubulin a and keratin which play a role in structural molecular activity with molecular weight of 55.6 and 55.5kDa respectively have been shown to increase in female fish exposed to deltamethrin. The band between 29-20.1kDa was identified as glutathione S-transferase which is known to have a molecular weight of 26.5. This enzyme which plays a crucial role in detoxification was observed to be increased in experimental fish probably playing a important role in detoxification of deltamethrin. Through one band is

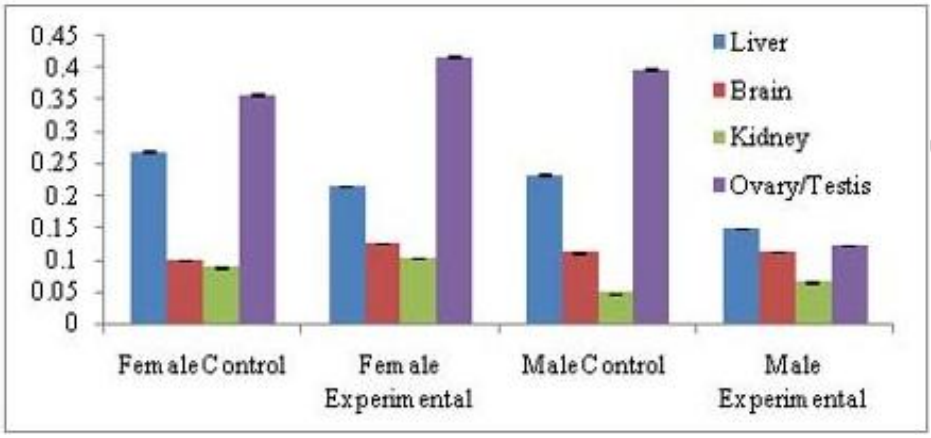
prominent at 3kDa region in both liver and brain tissues, it can't be conclusively said which protein it is. Further studies are required to identify this protein.

Table-1. Measured total protein concentration [µg/50mg] in control and deltamethrin exposed zebrafish, *Danio rerio* at 6days.

		5µg/L concentration of Deltamethrin					
S. No	**Samples**	**Female Control**	**Female Treated**	**% change**	**Male control**	**Male Treated**	**% change**
1	Liver	0.269±0.001	0.215±0.001	-20.07	0.233±0.001	0.150±0.000	-35.6
2	Brain	0.100±0.000	0.127±0.001	27	0.112±0.001	0.113±0.001	0.892
3	Kidney	0.088±0.001	0.102±0.001	15.9	0.048±0.001	0.066±0.001	37.5
4	Ovary/ Testis	0.359±0.001	0.417±0.001	16.15	0.398±0.001	0.123±0.001	-69.09

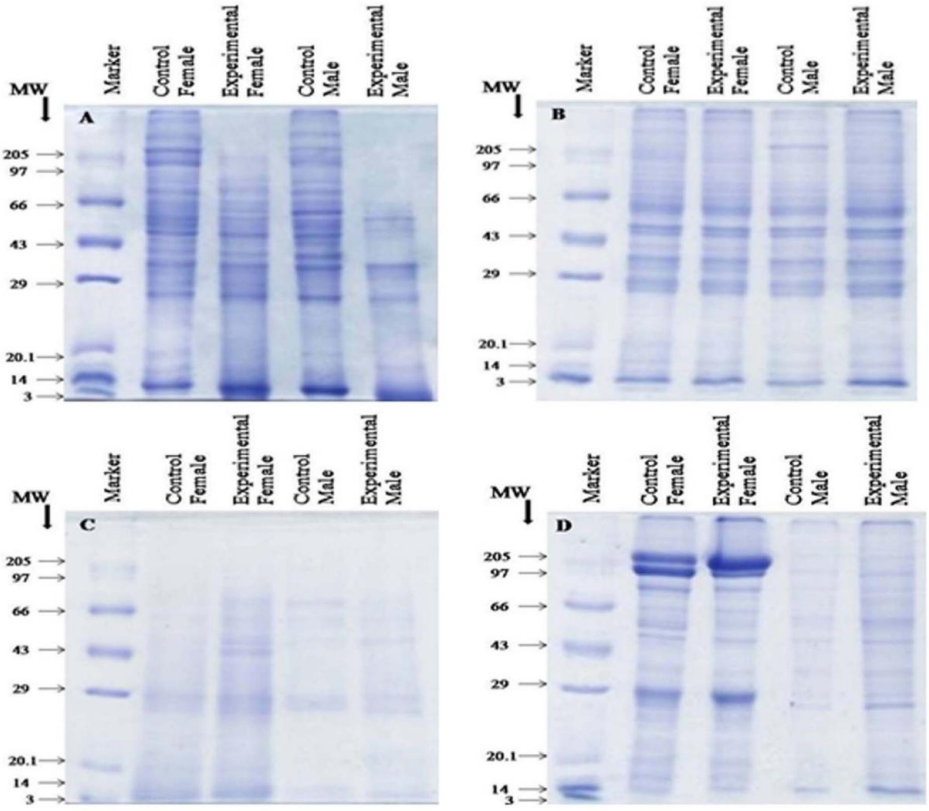
Data are expressed as mean±SD. Values of each group shown were significantly different (P<0.05).

Fig. 1 Graphical representation of protein concentration in zebarafish exposed to 5µg/L of deltamethrin.



Data are expressed as mean±SD. Each group shown were significantly different (P<0.05).

Fig. 2 Changes in the proteins band pattern of zebrafish exposed to 5µg/L concentration of deltamethrin.



A: Liver; **B:** Brain; **C:** Kidney; **D:** Ovary/Testis

In ovary and testis out of the seven prominent bands observed, only one band between 96-66kDa could be identified which belong to heat shock protein family with molecular weight of 71.4kDa and 74.2kDa. This has been reported to be present in testis of zebrafish exposed to microcystin-RR (Zhao et al., 2012). Other special proteins like calmegin identified by this group could not be identified in our study. From these studies it can be said that alteration in protein content under deltamethrin treatment is contributed by differently up and down regulated proteins in different tissues of adult zebrafish.

Conclusion

From these studies it can be said that protein expression pattern in brain, liver, kidney and ovary/testis are different. Protein bands which are prominent in one tissue are not noticed in the other tissue. The prominent proteins expressed were cytoskeletal proteins, enzymes involved in detoxification, stress proteins like HSPs.

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