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ORIGINAL ARTICLE

EFFECT OF WATER-BORNE SUBLETHAL DOSES OF ENDOSULFAN ON HISTOCHEMICAL PARAMETERS IN THE LIVER OF TELEOST FISH CYPRINUS CARPIO

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Abstract:

With a view to appraise the histochemical effect of sublethal doses of waterborne endosulfan (EDS) on hepatopancreatic cells of Cyprinus carpio, we estimated protein, carbohydrate and acid mucopolysaccharide content following treatment with 0.0015 and 0.002 ppm EDS concentration for periods varying from 10 to 20 days and compared them to the control one. Our results indicate concomitant loss of protein, carbohydrate, and acid mucopolysaccharide with dose and time along with a good recuperation in carbohydrate content in some hepatocytes following 0.002ppm EDS intoxication for twenty days showing carbohydrate metabolism is less affected in comparison to the metabolism of protein and acid mucopolysaccharide. The dropping off in chemical constituents combined with the pesticide contamination will certainly affect the fish health, reduce its dietetic value and affect the health and economy of mankind adversely. Another important thing that we have noted is that endosulfan also depletes dissolved oxygen (DO) level within twenty four hours that may causes fish death.

KEYWORDS:

Endosulfan, hepatopancreas, dissolved oxygen, waterborne, dietetic value.

INTRODUCTION:

The liver plays a cardinal processing and distributing role in metabolism and gets first entrée to the ingested food via the portal vein David (Nelson and Cox, Lehninger Principles of Biochemistry 5th Edi.). Endosulfan, which is a cream-to-brown colored solid (ATSDR 2000) non-systemic organochlorine insecticide (Kidd & James 1991) and belongs to the cyclodiene group, has been found to affect many vital organs including liver, intestine, and brain. EDS has not only been detected in freshwater fish in several countries including Benin and Kenya (Pazou et al 2006a, 2006b), India (Kole et al 2001; Singh & Singh 2007), Nigeria (GEF SSA 2002), Tanzania (Henry & Kishimba 2006), USA (GEF NA 2002, Hinck et al 2008, Ackerman et al 2008), Uganda (Kasozi et al 2006) , Zambia (Syakalima et al 2006) and in estuarine fish in Argentina (Lanfranchi et al 2006) but also has been found to cause monolithic fish death after a leak into rivers in Benin, Germany, India, Sudan and the USA (EJF Journal 2009) and even human death as a result of consumption of EDS contaminated fish in East Africa (GFEA-U 2007) has been reported. In Alabama (1995) endosulfan exterminated more than 24,000 fish in spite of having been applied in compliant with the instructions (IPEN 2009). Considering its toxicity sixty-two countries (EFJ Journal 2009) has banned this awful pesticide but in numerous countries including India endosulfan is still being

used indiscriminately on various crops. In India, EDS has been reported from goat and chicken (Singh et al 2008a), mustard oil and vegetable oil (GEF IO 2002), cauliflower, brinjal and okra (Shahi et al 2005), ber, grapes and guava (Kumari et al 2006), mangoes (Singh et al 2008b), farmed prawns (Amaraneni 2006), daily eatables (Kumari & Kathpal 2009), breast milk (Sanghi et al, 2003), groundnuts, and chilli powder; and in canned pineapple, chillies and onion in Sri Lanka (GEF IO 2002) and in shrimps and oysters in Jamaica (GEF CAC 2002); in honey in Turkey (Erdogrul 2007). This shows its widespread use in India and other countries. With the technical composition of α— and β— isomer (Hays, 1993), endosulfan is very toxic to almost all kinds of organisms (GFEA-U 2007) but extremely toxic to fish (Naqvi and Vaishnavi, 1993, USEPA, 2002), especially to juveniles (Dutta & Arends 2003).

In fish, maximum quantity of EDS was detected in the liver followed by the intestines, gills, brain and skeletal muscles (Heath 1992, Isam et al. 2005). As the liver is the chief organ for EDS metabolism (Peterson and Bately 1993) and supplier of nutrients to all other organs and tissues, any alteration in the composition of hepatocytes will affect piscine health and dietetic value that in turn affect the piscivores. Effect of doses of EDS on different aspects such as biochemical changes, ultrastructural changes etc. on different fish organs has been studied extensively by several scientists (Hundet et al. 2012, Braunbeck, et al. 1999, Ballateros, 2009 et al., Shastry et al.1983, and many more), but little data is available on histochemical effect. Therefore, the present work is undertaken to evaluate its histochemical effect in the hepatocytes of the liver.

3. MATERIAL AND METHOD

3.1) Chemicals:

Technical grade 35% EC EDS (Acaricides; mixture of two stereoisomers -a— and ß— EDS, in a ratio of 4:1, manufactured by Excel Industries Ltd. Bombay) was procured from the local market and its solution was made using acetone having negligible toxicity for fish (12000 ppm).

3.2) Preconditioning and Treatment of Experimental Animals:

Cyprinus carpio fish was collected from the "Chandemari Fish Farm" and treated with 0.1% potassium permanganate solution for 15 minutes to get rid of dermal infections. All the fishes were kept for acclimatization in polymer fibre aquaria for 7 days. The lethal dose of EDS for

C. carpio is 0. 0057ppm but fish died even at 0.00001ppm EDS as EDS reacts with water and depletes dissolved oxygen. So the fishes were introduced after 24 hours of the solution preparation to avoid killing of fishes. Oxygen is supplied continuously throughout the experimental period with the help of aerator to maintain O2 level between 6 to 9 ppm.

Healthy fishes, measuring 12 to 14 cm and weighing approximately 50-60 gms, were selected for experimentation. A set of 10 fishes were transferred into three differently maintained aquaria with 200 L water quantity, out of which one contained dechlorinated water and the other two contained 0.0015 and 0.002 ppm EDS concentration (Lethal dose of EDS for C. carpio is 0.0057ppm). Five fishes were sacrificed after 10 days and the rest of the fishes were sacrificed after 20 days. The fishes were fed daily (with standard fish food) during the entire experimentation period. The liver of the sacrificed fishes was dissected, cut into small pieces and washed in normal saline for 5 to 10 minute.

3.3) Histochemical Tests:

The pieces of liver were fixed in 10% formalin and 8 to 10 micrometer thin sections were cut for histochemical studies of protein, carbohydrate and acid mucopolysaccharides to assess the qualitative impact of EDS. Bromphenol blue, PAS and alcian blue tests were carried out according to Homasan's Histochemical Technique book.

3.4) Water Quality Parameter:

The water quality parameters like temperature, pH, dissolved oxygen (DO), free carbon dioxide (CO2), total alkalinity, temporary hardness, permanent hardness and chloride were checked on 1st, 5th, 10th, 15th, and 20th day by standard methods and found to be within the recommended range for carp rearing.

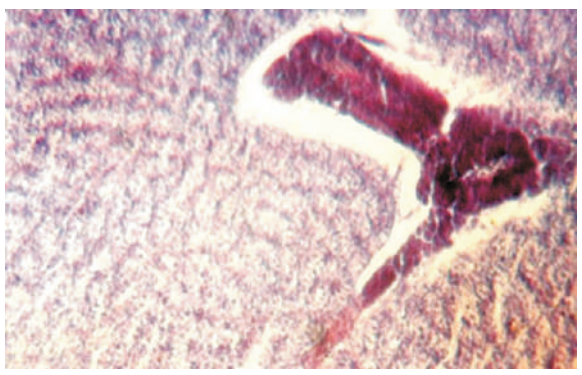
4. RESULT:

C. carpio exposed to sublethal concentrations of EDS did not exhibit any changes in movements and feeding habit likewise, growth was not retarded following introduction to all concentrations of endosulfan, and no external pathological signs were obvious.

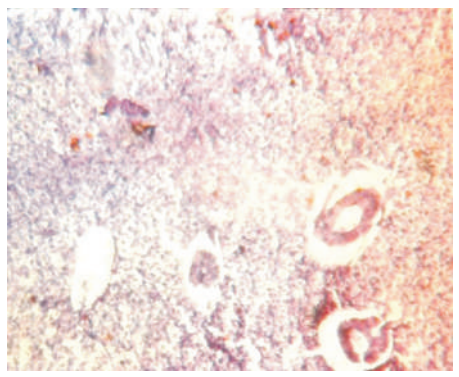
Protein: Bromophenol Test

Bromophenol test of the control fish liver showed nuclei of the hepatic cells and exocrine pancreatic mass strongly positive, blood vessels, blood cells and endocrine pancreatic mass positive, cytoplasm of hepatic cells and its reserve material negative and the interhepatic spaces strongly positive. (fig.1)

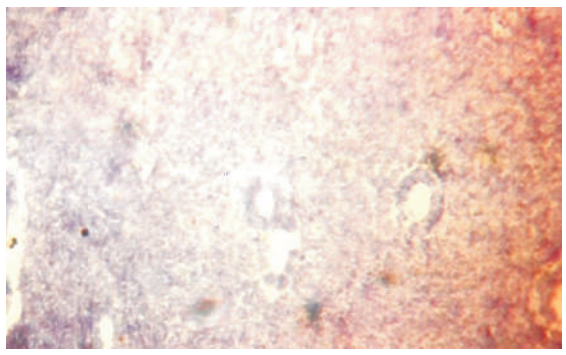
Endosulfan treated liver showed increase of protein content in the hepatic cells and decrease in the exocrine pancreatic tissue in comparison to control liver following 0.0015-ppm EDS intoxication for 10 days while after 20 days intoxication further decrease in protein content was noticed in hepatic as well as pancreatic tissues (fig. 2&3). 0.002-ppm endosulfan intoxication for 10 days showed less protein in the pancreatic and hepatic cells while 20 days exposure showed maximum decrease in protein content in the whole of the liver mass (fig 3 & 4).



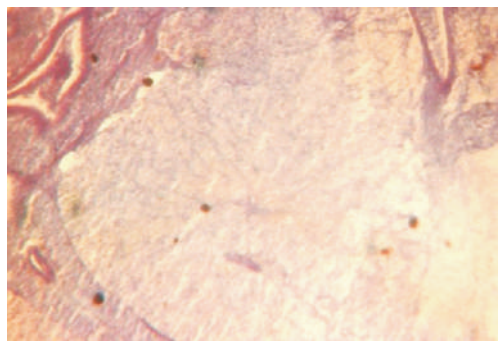
(Fig. 1. Photomicrograph of T.S. of the liver of untreated C. carpio
Bromophenol Blue Test, x-100.)



(Fig. 2. Photomicrograph of T.S. of the liver after 10 days intoxication to 0.0015ppm EDS, x-100.)



(Fig. 2. Photomicrograph of T.S. of the liver after 20 0.0015ppm EDS exposure X-100)

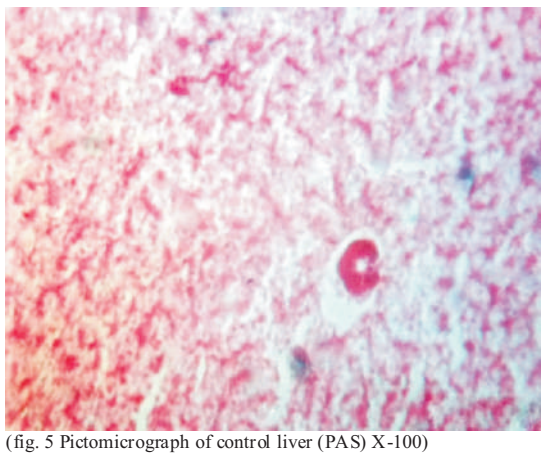


(Fig. 4. Pictmicrograph after 10 days 0.002 PPM EDS 0.002ppm EDS exposure X-100)

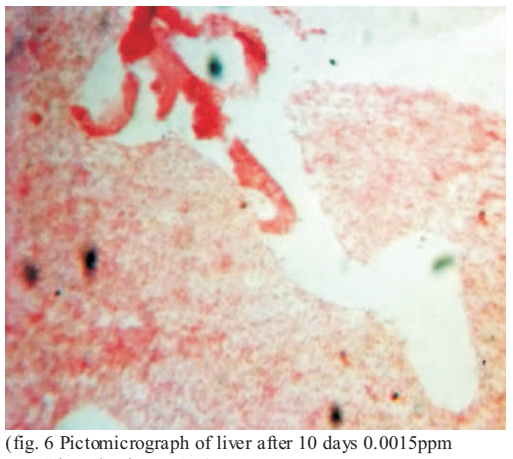
CARBOHYDRATE: PICRIC ACID SCHIFF'S TEST

In the normal liver, carbohydrate was detected in the pancreatic tissue, blood vessels, and blood cells. Little carbohydrate was detected in the nuclei of the hepatic cells and very little in the cytoplasm of hepatic cells (fig. 5). Following 0.0015-ppm endosulfan intoxication for 10 days, carbohydrate content decreased and subsequent to 20 days exposure heavy loss of carbohydrate was observed (fig. 6). Likewise diminution was also recorded following vulnerability to 0.002-ppm endosulfan for 10 days but after 20

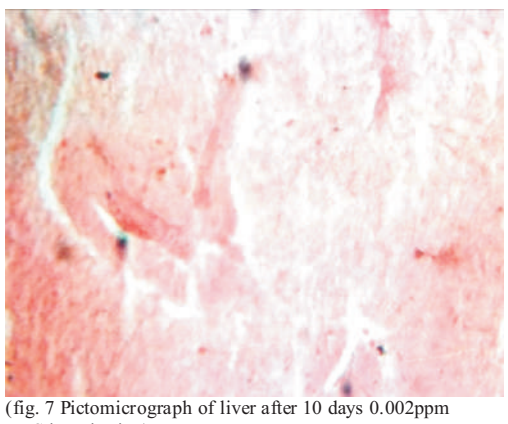
days some cells showed good amount of carbohydrate (fig. 7 & 8).



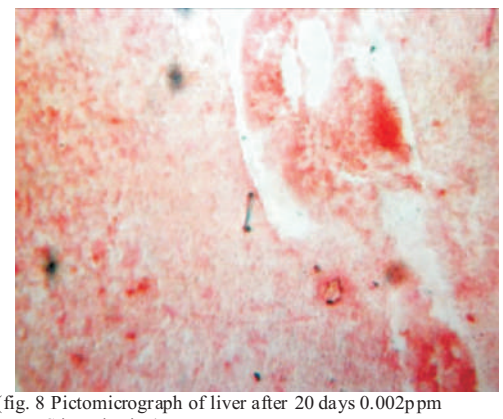
(fig. 5 Pictomicrograph of control liver (PAS) X-100)



(fig. 6 Pictomicrograph of liver after 10 days 0.0015ppm EDS intoxication X-100)



(fig. 7 Pictomicrograph of liver after 10 days 0.002ppm EDS intoxication)

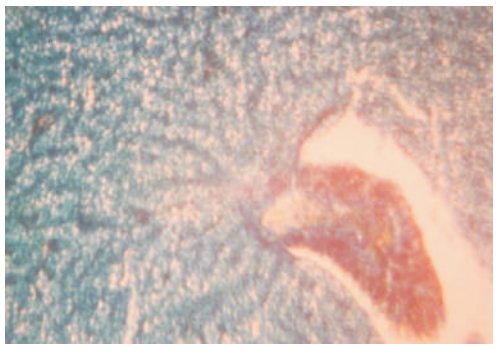


(fig. 8 Pictomicrograph of liver after 20 days 0.002ppm EDS intoxication)

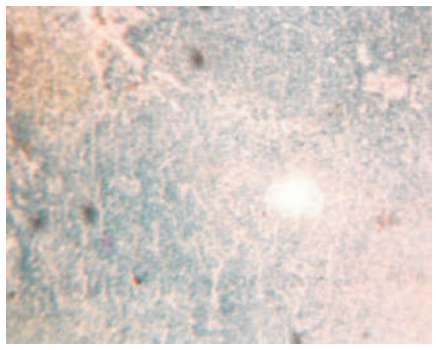
ACID MUCOPOLYSACCHARIDE: ALCIAN BLUE TEST

Acid mucopolysaccharides were detected with Alcian Blue test in the liver of control and treated fish. In the liver and pancreatic mass, acid mucopolysaccharides got extremely reduced in 0.0015-ppm endosulfan treated fishes for 10 days (fig. 10) in comparison to control fish (fig. 9) and subsequent to 20 days exposure (fig. 11) further minification was detected. Similar decrement was also observed following 0.002 ppm EDS exposure for 10 days and 20 days

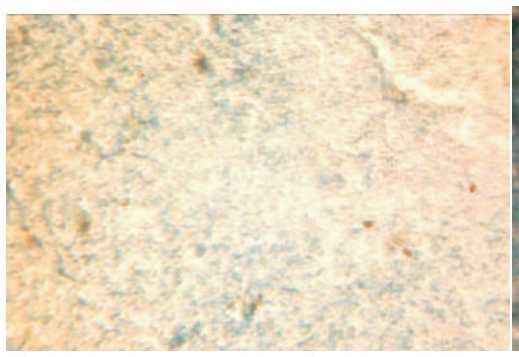
(fig. 12&13).



(fig. 9. Pictomicrograph of liver control Alcian Blue test X-100)

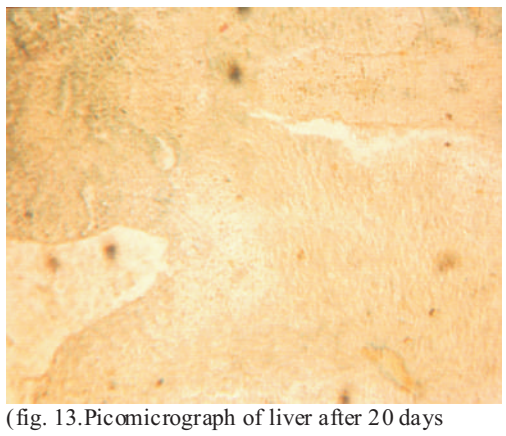


(fig. 10.Picomicrograph of liver after 10 days 0.0015ppm EDS intoxication X -100)



(fig. 11.Picomicrograph of liver after 20 days 0.0015ppm EDS intoxication X -100)

(fig. 12.Picomicrograph of liver after 10 days 0.002ppm EDS intoxication X -100)



(fig. 13.Picomicrograph of liver after 20 days 0.002ppm EDS intoxication X -100)

5. DISCUSSION

Increase in protein content in the beginning seems to be the result of enhanced protein synthesis in response to the pesticide but thereafter decline in protein content seems the result of inequity in metabolic activity as proteolytic activity increased in response to the pesticide while synthetic activity decreased as EDS induces inhibition of messenger RNA production (G. Tripathi et al. 2004). Braunbeck et al.(1999) in cytological studies of liver cells in C. carpio exposed to ultra low dose of oral EDS noticed significant increase in number and extension of RER stacks and proliferation of lysosomes. The former may be related to biotransformation process as they have indicated and the latter can be correlated with enhanced proteolysis under starvation and oxidative stress caused by reduced absorption of nutrients in intestine by increased mucous cells in response to endosulfan. All these facts support that endosulfan either directly or indirectly contribute more to protein degradation. Under starvation, selective ordered protein degradation by the process of autophagy has also been reported (Anders, 2008). EDS is shown to induce oxidative stress (Omurtag, 2008, Balleteros, 2009).

Our result does not corroborate the finding of Indirabai et al (2010) who found increase in protein content in hepatocytes of Labaeo rohita treated with sublethal doses of endosulfan but supports the findings of several other workers who have obtained analogous results in different fish species. Bakthavathsalam & Srinivas (1984) during acute exposure of Anabas testudneus to Lindane; Sastri et al. (1984) in Channa punctatus after endosulfan exposure; Ambrose et al. (1994) in the liver of Cyprinus carpio communis after tannery effluent exposure; Rajan (1990) in Cyprinus carpio exposed to effluent, Saravanan et al. (2010) in Labeo rohita.

Decrement in carbohydrate content in hepatocytes may be due to either oxidative stress (Omurtag et al 2008) or inhibition of insulin biosynthesis by EDS or due to inhibition of hexokinase (HK), to decreased activity of succinate dehydehydrogenase (SD), malate dehydrogenase (MD) in response to EDS (Sastry K.V. 1983) or diminution of citrate synthase (CS) activity by endosulfan (Tripathi, 2004). HK inhibit glucose phosphorylation in liver cells while decreased activity of SD, MD and CS drops off TCA cycle. Hypersecretion of Mucous in gills makes gaseous exchange more difficult (Bols et al. 2001) that

paves the way for more anaerobic respiration to meet extra energy demand resulting into the heavy depletion of glycogen. Glycogen depletion may also be attributed to damage of hepatocytes due to EDS or to hormone mediated stress phenomena (Hanke et al. 1983, Gluth & Hanke 1985), or to reduced intestinal absorption of carbohydrates (Sastry et al 1982). All the facts are suggestive of different mode of action of EDS in carbohydrate metabolism.

Our results substantiate the findings of Murty & Devi (1982), Sastry & Siddiqui (1983), Verma et al. (1983), Sharma & Maya (1987) and Sastri et al. (1987), who noticed diminution in hepatic glycogen in other fish species. Vishwaranjan et al, (1988) recorded remarkable decrease in the carbohydrate level after the higher dose exposure of tannic acid in the fish, Oreochromis mossambicus. Palanichamy et al. (1989) studied the effect of chemical effluent on Mystus vittatus and found that body constituents like protein, carbohydrate and lipid content of muscle, liver, gill and intestine decreased with increasing concentration of effluent. Jebakumar et al (1990) studied the carbohydrate level of L. thermalis exposed to sublethal levels of Cypermethrin and noticed very little difference from control upto 9 days and thereafter they observed decreasing trend. Grant and Mehrle (1970) have reported inhibition and mobilization of liver glycogen by low doses of Endrin and blockage by high doses.

Decrease in acid mucopolysaccharide content seems logical as both protein and carbohydrate content decreased heavily.

The diminution in protein, carbohydrate and acid mucopolysaccharide content in liver following endosulfan exposure indicate heavy changes in chemical composition of fish reducing its dietetic value. EDS can also affect human health through food chain and biological magnification.

6. CONCLUSION:

Despite being classified as an "extremely hazardous" pesticide (ITRC 1989), India is currently the leading producer and user of endosulfan (EJF, 2009). Seeing its extensive use in agriculture and massive killing and contamination of fish and other food items, humans seem to be the next major victims of EDS. People are using this awful organochlorine pesticide for their selfish gain ignoring its hurtful side effects. Its biochemical, cytological, histological, and histochemical effects alarms against a dreadful hidden ensuing danger. Now, time has come to see towards other ecofriendly pesticide.

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