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Biochemical Effects to Toxicity of CCl_4 on Rosy Barbs (*Puntius conchonius*)

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Abstract
Biochemical changes in the liver, kidneys and gills of rosy barbs due to toxicity of CCl_4 were measured after 96 hour exposure. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and creatinin (CRN), levels were measured. Significant increase in ALP, ALT, LDH and BUN activities were observed in the liver in the treated groups compared to controls ($P<0.05$). AST level was significantly higher in the kidneys. This study indicates that the enzymatic activity was comparatively higher in the liver than kidneys or gills, suggesting that the liver is the target organ of CCL_4 toxicity to rosy barbs.

Keywords: Toxicity, Rosy Barb, CCl_4

Introduction

Carbon tetrachloride is a manufactured chemical that does not occur naturally. Most of the carbon tetrachloride produced is used in the production of chlorofluorocarbons (CFCs) and other chlorinated hydrocarbons.

Various substances are known to cause liver and kidney damage, and one of them is carbon tetrachloride (CCl_4), which is a well-known hepato- and nephrotoxin (Cassilas and Ames 1986; Kotsanis and Metcalfe 1991; Thrall *et al.* 2000; Ogeturk *et al.* 2005). Within the body, CCl_4 breaks down to highly toxic trichloromethyl (CCl_3) and trichloromethyl peroxyl (CCl_3O_2) free radicals by cytochrome P450 enzyme and *causes damage to hepatocytes (Abraham. et al.* 1999; Ohta et al. 2000). Short and long-term exposure to CCl_4 also causes damage to the skin, brain and blood and in some cases results in death. Besides mammals (Sundari *et al.* 1997; Abraham *et al*. 1999; Ogeturk et al. 2005), CCl_4-induced damages have been documented in the non-mammalian vertebrates such as bird (Fernandez *et al.* 1984) and teleosts including rainbow trout (Kotsanis and Metc alfe 1991) and tilapia (Chen *et al.* 2004).

The rosy barb, *Puntius conchonius*, a member of family Cyprinidae has a short life span and produces great numbers of big, transparent eggs that are fertilized externally, and has become an emerging model fish for biological and biotechnological research (Amanze and Iyengar 1990; Kiran kumar *et al.* 2003; Bhattacharya *et al.,* 2005; 2006). In fact, it has been widely used in ecotoxicological study in recent years (Gill *et al.* 1990; Kirankumar and Pandian 2003; Xu *et al.* 2005). However, little is known about the biochemical effects of CCl4 to fish (Casillas and Ames 1996). The aim of this study was to investigate the biochemical parameters of toxicity of CCl_4 to evaluate the extent of tissue damage in rosy barbs.

Materials and Methods

Chemical

Carbon tetrachloride used in the experiments was purchased from Guangcheng Chemicals LTD, Tianjin, China.

Animals and treatments

Adult rosy barbs *P. conchonius* (body weights 2.1-2.85 gram/per fish) purchased from a local fish dealer were maintained in dechlorinated water at 26±1ºC. They were fed on live bloodworms and fish flakes (Tetramin, Germany) twice a day, and acclimatized for two weeks before experiments.

A total of 5 rosy barbs per group were exposed to three concentrations (0, 5, 7.5 and 10 mg/L) of CCl_4 in dechlorinated water in 20 litre glass tanks for 96 hours. CCl_4 was always freshly dissolved in appropriate volume of absolute alcohol as a stock solution, and added immediately into the test solutions (dechlorinated water). Soon after addition of CCl_4, the glass tanks were sealed with double layers of cling plastic wrap to prevent minimum volatile loss of CCl_4 (Le Blanc, 1980). Note the volumes of ethanol were all equal in both treatment and controls.Test solutions were renewed to maintain the concentrations of both CCl_4 and dissolved O_2.

Chemical Analysis

Selected parameters for CCl_4 toxicity to rosy barbs were alanin aminotrasferase (ALT), aspartate aminotrasferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities, and blood urea nitrogen (BUN) and creatinin (CRN). Fishes were sacrificed after 96 hours of treatment and liver, kidneys and gills were dissected out and homogenised in NaCl. The homogenates were then centrifuged at 12000 RCF for 15minutes at 4 C. Supernatants were stored at -20 C until tested. The levels for total proteins, ALT, AST, ALP, LDH, BUN and CRN were measured using an AEROSET automatic biochemical analyser (Abbot, USA).

Statistical Analysis

Data obtained were analysed using Student's t test (ANOVA), the difference at $P<0.05$ was considered significant.

Results

There was significant increase in ALT activity in the liver and kidney of treated fish exposed to the highest concentration on CCl_4. The ALT activity increased significantly in the gills of fish exposed to 5 mg/L but decreased in fish exposed to 7.5 and 10 mg/L although remaining higher in comparison to controls (Fig. 1).

AST level significantly increased in the kidney of fish exposed to the highest concentration of CCl_4 (more than double). On the other hand it significantly decreased in the liver and gills of treated fish (Fig. 2).

A significant increase in ALP activity was seen in the liver of fish exposed to 7.5 mg/L whereas it dropped significantly in the liver and kidneys at the highest CCl_4 concentration (Fig. 3).

LDH level was significantly higher in liver of treated fish exposed to CCl_4 but did not show any drastic change in other organs (Fig. 4).

BUN level was found to be significantly higher in the liver of fish treated with 5 and 7.5 mg/L CCl_4 (Fig. 5). However, no significant difference was noticed in CRN levels in any of the tested organs (Fig. 6).

Discussion

CCl_4 toxicity is reported to depend on mixed-function oxidase (MFO) activity to form free radical intermediates (Recknagel et al., 1977) and liver is known to contain the highest MFO specific activity among fish tissues (Stegeman, 1981). Due to these reasons increased ALP, ALT, LDH and BUN activities in the liver were observed in

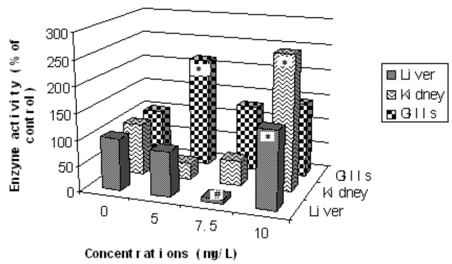

Figure 1. ALT levels in liver, kidneys and gills of rosy barbs exposed to CCl4 for 96 hours. * Significantly increased in comparison to controls. # Significantly decreased in comparison to controls. (P<0.05).

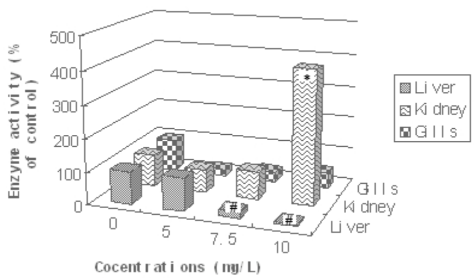

Figure 2. AST levels in liver, kidneys and gills of rosy barbs exposed to CCl4 for 96 hours. * Significantly increased in comparison to controls. # Significantly decreased in comparison to controls. (P<0.05).

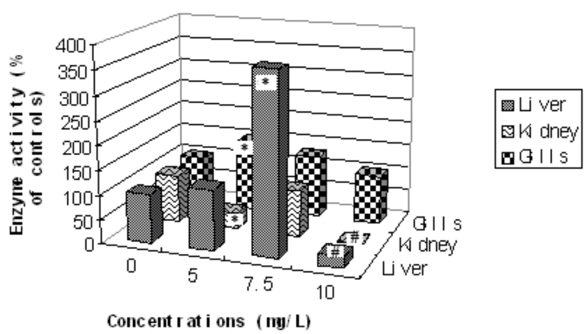

Figure 3. ALP levels in liver, kidneys and gills of rosy barbs exposed to CCl4 for 96 hours. * Significantly increased in comparison to controls. # Significantly decreased in comparison to controls. (P<0.05).

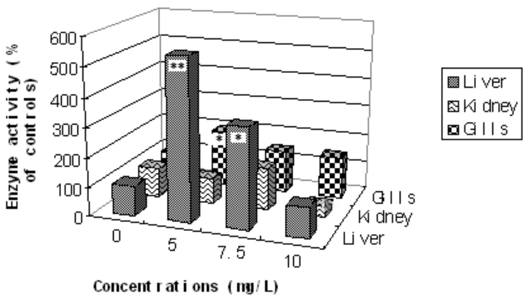

Figure 4. LDH levels in liver, kidneys and gills of rosy barbs exposed to CCl4 for 96 hours. * Significantly increased in comparison to controls. (P<0.05).

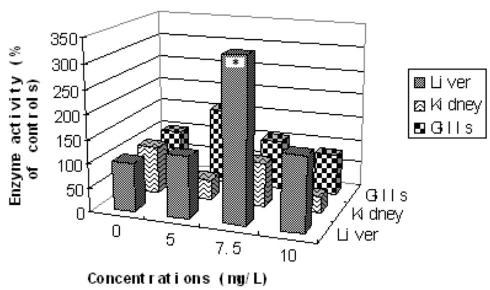

Figure 5. BUN levels in liver, kidneys and gills of rosy barbs exposed to CCl4 for 96 hours. * Significantly increased in comparison to controls. (P<0.05).

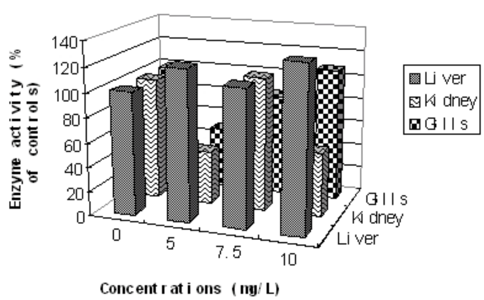

Fig. 6.

Figure 6. CRN levels in liver, kidneys and gills of rosy barbs exposed to CCl4 for 96 hours.

this study. Casillas and Ames (1986) reported that the ALP level in English sole exposed to CCL4 was highest in the kidneys. However, our data showed that ALP, LDH and BUN were more active in the liver than in other tissues.

AST level was significantly higher in the kidneys indicating an increase in glutamate transaminase activity, a mitochondrial enzyme. This could be due to kidney injury caused by CCl_4, which may stimulate tissue repair through protein turnover and increased respiration.

Surprisingly it was found that the CRN level did not change significantly in liver, kidney or gills of rosy barbs exposed to CCl_4. Unlike the BUN, the creatinine level is not affected by hepatic protein metabolism. Only renal dysfunction changes the results. The serum creatinine level does not rise until at least half of the kidney's nephrons are destroyed or damaged.

It is of interest to note that significant rise in enzyme parameters were noticed at 5 and 7.5 mg/L and a drastic fall at 10 mg/L of CCl_4 exposure. This indicates that exposure of rosy barbs at lower sub-acute concentrations of CCl_4 causes tissue damage which initiates tissue repair. However, at higher concentration of CCl_4, tissues are damage beyond repair and hence enzymatic activity decreases. In addition, enzyme levels were comparatively higher in the liver than kidneys or gills, suggesting that the target organ of CCL4 toxicity to rosy barb is the liver.

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