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LIVER, KIDNEY AND GILL BIOCHEMICAL CHANGES IN *CARASSIUS AURATUS GIBELIO* DURING LINDANE ACUTE INTOXICATION

Cristina Munteanu, Andreea Cristina Staicu, Eugen Simionica, Lotus Elena Mester, Elena Ionica, Marieta Costache, Anca Dinischiotu*

University of Bucharest, Department of Biochemistry, Molecular Biology Center , 91-95 Spl. Independentei, 050095 Bucharest, Romania
adin@bio.bio.unibuc.ro

ABSTRACT. This study aims to investigate the effects of the insecticide lindane on liver, kidney and gill antioxidant enzyme activities in *Carassius auratus gibelio*. Fish were exposed to 0.05 mg/l for 96h. Activities of catalase (EC 1.11.1.6) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) were increased by the pesticide exposures. Glutathione reductase (EC 1.6.4.2) and glutathione-S-transferase (EC 2.5.1.18) activities decreased significantly in all analyzed intoxicated organs compared to control. Furthermore, glutathione peroxidase (EC 1.11.1.9) activity decreased in the liver exposed to lindane and increased in kidney and gill. These results indicate that *Carassius auratus gibelio* could not counteract the oxidative stress caused by lindane acute intoxication by antioxidant mechanisms.

KEY WORDS. lindane, catalase, glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione -S- transferase, glutathione peroxidase

INTRODUCTION

The organochlorine γ-hexachlorocyclohexane commercially available as lindane, is used as a treatment insecticide in agriculture for crop protection or in both human and veterinary medicine against ectoparasites (1). Lindane is poorly hydrolysed and biodegrades slowly in the environment (2). It is persistent in food chains and is readily accumulated by animals in the adipose tissue due to its lipophilic nature (3). Fish absorb this pesticide directly from water or by ingesting contaminated food. Lindane toxicity commonly involves increased central nervous system excitability as muscle spasms and convulsions (4). Petrescu et al. (5) showed that lindane exposure led to increased gestation length in rats. Criswell and Loch-

Caruso (6) had demonstrated contractility of rat uterine strips exposed to lindane in vitro. Studies made by Samanta et al. (7) have shown impairment of testicular functions in adult age as a consequence of some permanent lesions induced by hexachlorocyclohexane during critical stages of sexual maturation. γ - Hexachlorocyclohexane-induced hepatotoxicity is associated with oxidative stress (8).

Normal cellular function depends on a balance between reactive oxygen species produced and antioxidant defence mechanisms available to the cell. Reactive oxygen species, including the superoxide radical, hydrogen peroxide, and the hydroxyl radical, arise as by products of normal cellular metabolism or may be the consequence of exposure to certain toxicants (9).

Oxidative stress occurs when enzymatic and non enzymatic cellular antioxidants are unable to counteract the reactive oxygen species.

Our study was done in order to evaluate the oxidative stress induced by lindane 0.05 mg/l in liver, kidney and gill of *Carassius auratus gibelio*.

MATERIAL AND METHODS

Animals and treatments

Freshwater goldfish Carassius auratus gibelio *of 20±2cm in length and 200±30.0g in weight, were obtained from The Nucet Fishery Research Station and housed in a 60 l glass aquarium at 25ºC. Prior to exposure, fish were held for 15 days for acclimatization and evaluation of overall fish health under laboratory conditions. During the experiments, the individuals were exposed for 96h to lindane (0.05 mg/l) and were not fed. The toxicant was added only in the first day of experiment. After 96h, tissues (liver, kidney, and gills) of 10 animals were collected and frozen at -80ºC. The enzymatic studies were done in the next two weeks.*

Methods

The frozen tissues were homogenized in ten volumes of 0.1M Tris-EDTA buffer, pH=7.4 and centrifuged at 8000xg for 30min at 0-4°C. Aliquots of the supernatant were utilized for enzymatic assays.

Catalase (CAT) activity was assayed by the method of Aebi (10). Change in absorbance was recorded at 240nm. Catalase activity was calculated in terms of k/min/mg protein, where k is first order rate constant.

Glucose-6-phosphate dehydrogenase (G6PD) activity was assayed by the method of Löhr and Waller (11). The rate of NADPH formation is a measure of the enzyme activity and it can be followed by means of the increase in extinction at 340nm.

Glutathione reductase (GR) activity was recorded by Goldberg and Spooner method (12). The enzyme activity was calculated as μmol NADPH oxidized/min/mg protein.

Glutathione-S-transferase (GST) activity was determined by the method of Habig et al. (13). The change in absorbance was recorded at 340nm and the enzyme

activity calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6x 10^3 M^{-1} cm^{-1}.

Glutathione peroxidase (GPx) activity was assayed according to the method described by Beutler (14). Oxidation of NADPH was recorded spectrophotometrically at 340nm at 25ºC. The enzyme activity was calculated as nmoles NADPH oxidized/min/mg of protein, using a molar extinction coefficient of 6.22 x 10^3 M^{-1} cm^{-1}.

The protein estimation was done by the method of Lowry et al. (15) using bovine serum albumin (BSA) as standard.

All the spectrophotometric analysis were done with a Perkin-Elmer Lamda 25 Double Beam Spectrophotometer at 25ºC.

The Student test analysis was used to determine the statistic significance of enzymatic activity changes.

RESULTS

The specific activity of CAT after 96h exposure was higher than the control lot (Figure 1) in liver, kidney and gill of *Carassius auratus gibelio.*

The specific activity of G6PD increased following exposure to the pesticide for 96h in liver in a very significant manner ($P<0.001$). In kidney, the same activity was increased, but not significant from the statistical point of view (Figure 2).

The specific activity of GR after 96h exposure of fish decreased very significantly in liver and distinct significantly in kidney and gill (Figure 3). The same pattern is present for the specific activity of GST following acute intoxication with lindane (Figure 4).

Individuals treated with pesticide showed a significant decrease in liver and a significant increase in kidney and gill for the GPx specific activity (Figure 5).

DISCUSSION

Catalase removes H_2O_2 and provides the first defence against reactive oxygen species toxicity. In our study we noticed an increase which is not significant in the intoxicated liver and gill and statistically significant in kidney. Our previous studies reported that catalase activities increased in liver and intestine and decreased in kidney and gill at the effect of manganese (II) treatment on *Carassius auratus gibelio* (16,17). Other observations concerning the effects of 2,4 D (2,4-dichlorophenoxy acetic acid dimethyl amine salt) and azinphosmethyl on antioxidant enzymes in liver of Oreochromis niloticus revealed that this activity is not affected by the both pesticides and also the combined treatment (18).

The increased levels of catalase in liver, kidney and gill of fish treated with 0.05mg/l lindane suggest a compensatory response to oxidative stress induced by this xenobiotic.

The increased levels of glucose-6-phosphate dehydrogenase in liver (very significant from the statistical point of view) and kidney could appear due to the increased necessity for NADPH, used by glutathione reductase to produce GSH in order to maintain the protein thiols in reduced state during the oxidative stress or to

react directly with the pesticide. Taking in account that the liver has an important role in degradation and bioactivation of pesticides, this enzyme activity increase could be expected. Other studies (18, 19) demonstrated increased glucose-6-phosphate dehydrogenase activity in response to pesticide toxicity.

The decreasing (very significant in liver and distinct significant in kidney and gill) of the specific activity of glutathione reductase may be due to the increase of NADPH concentration in the respective tissues. The inactivation of glutathione reductase was found to depend on NADPH concentration (20). Also it is possible that glutathione reductase could be a target for lindane whose action could inactivate it.

Because, probably the GSH concentration is lower in intoxicated fish, the glutathione S transferase specific activity in all the studied tissues is also significantly decreased. Glutathione transferases play an important role in the detoxification and excretion of xenobiotics. This process involves conjugation of electrophilic metabolites with GSH. The decrease of this activity suggests a lower capacity of detoxification in the analysed organs.

Glutathione peroxidase specific activity in intoxicated fish liver is significantly lower compared to control, possibly because of the decreased GSH content. The decreased activity may be the result of superoxide production (21) or a direct action of pesticides on the synthesis of the enzyme (22). In kidney and gills this activity is significantly increased, probably due to the higher level of lipid peroxidation**.**

CONCLUSION

The lower specific activities of glutathione reductase and glutathione-S-transferase of all analyzed organs in intoxicated individuals compared to the control indicate that fish can't counteract efficiently the effect of the xenobiotic. The lower activity of glutathione peroxidase in the intoxicated liver compared to control support the previous assumption.

Taking in account these data we concluded that *Carassius auratus gibelio* exposed to lindane intoxication didn't resist properly by antioxidant mechanisms to the oxidative stress.

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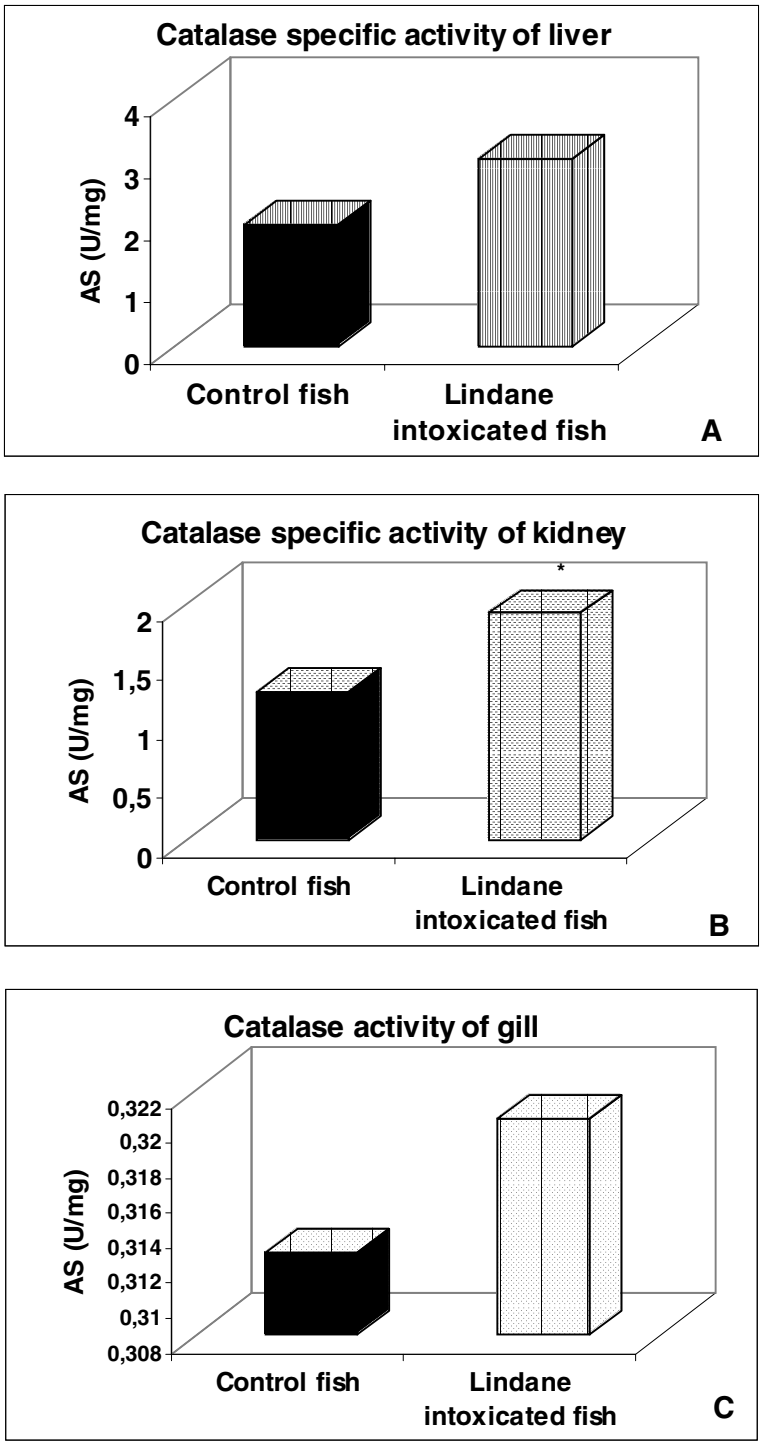

Figure 1. *Effects of 0.05 mg/l lindane on catalase specific activity (U/mg protein) in the liver (panel A), kidney (panel B) and gill (panel C) of* Carassius auratus gibelio. ** P<0.05*

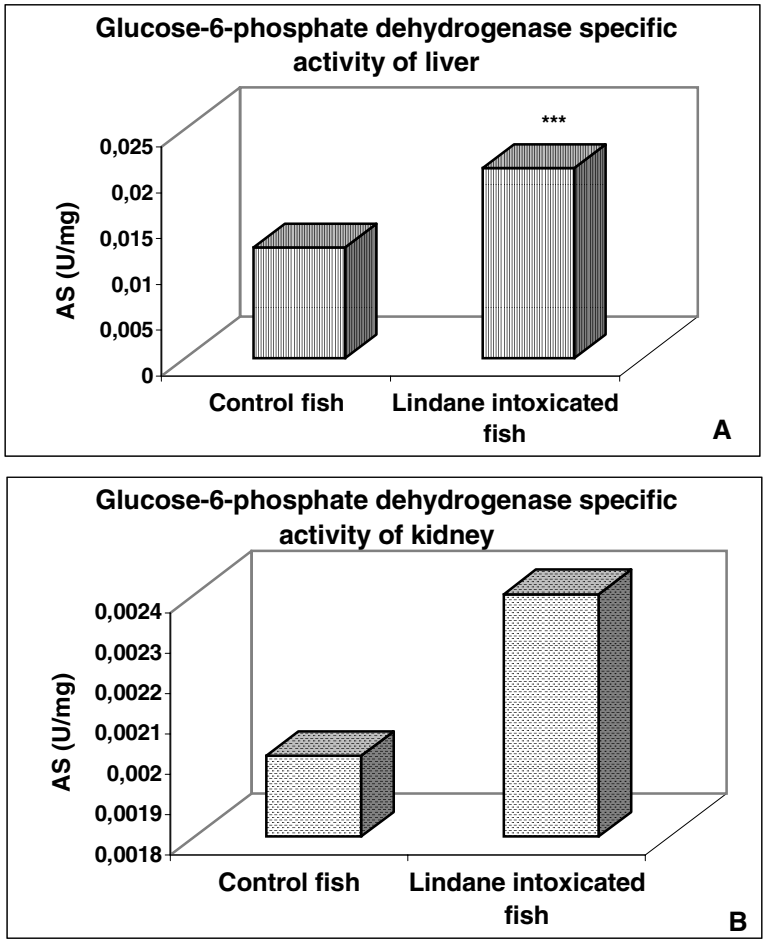

Figure 2. *Effects of 0.05 mg/l lindane on glucose-6-phosphate dehydrogenase specific activity (U/mg protein) in the liver (panel A) and kidney (panel B) of* Carassius auratus gibelio. *** *P<0.001*

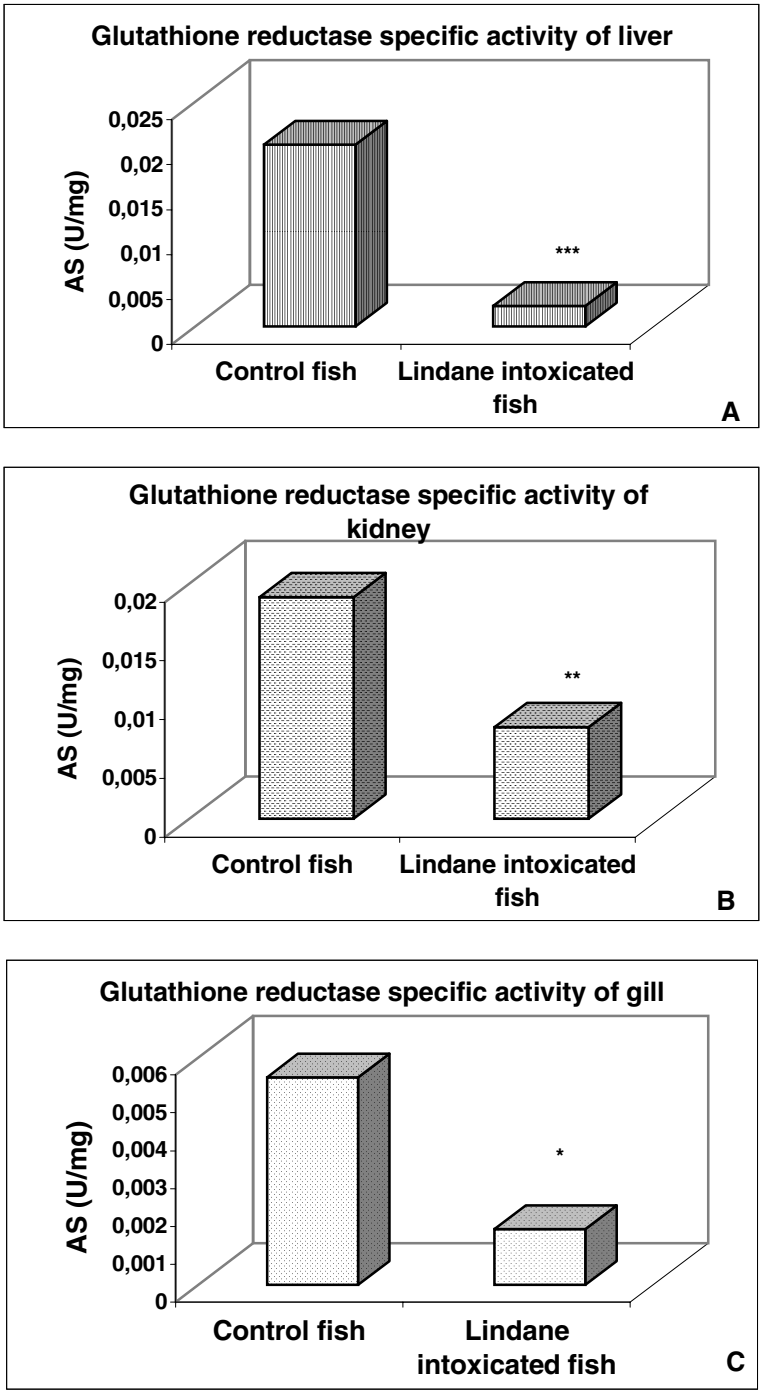

Figure 3. *Effects of 0.05 mg/l lindane on glutathione reductase specific activity (U/mg protein) in the liver (panel A), kidney (panel B) and gill (panel C) of* Carassius auratus gibelio. ** P<0.05, ** P<0.01, *** P<0.001*

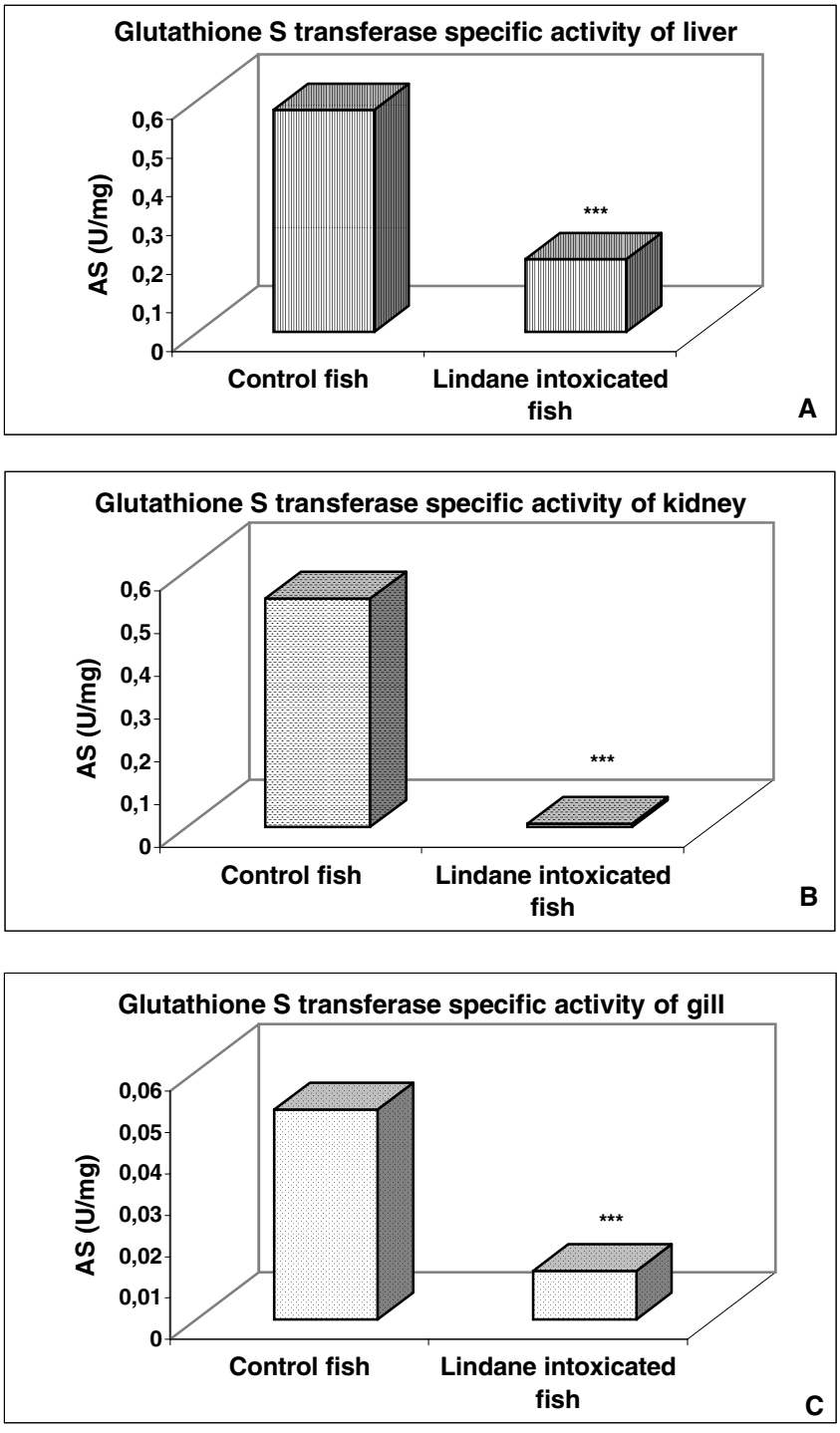

Figure 4. *Effects of 0.05 mg/l lindane on glutathione-S-transferase specific activity (U/mg protein) in the liver (panel A), kidney (panel B) and gill (panel C) of* Carassius auratus gibelio. **** P<0.001*

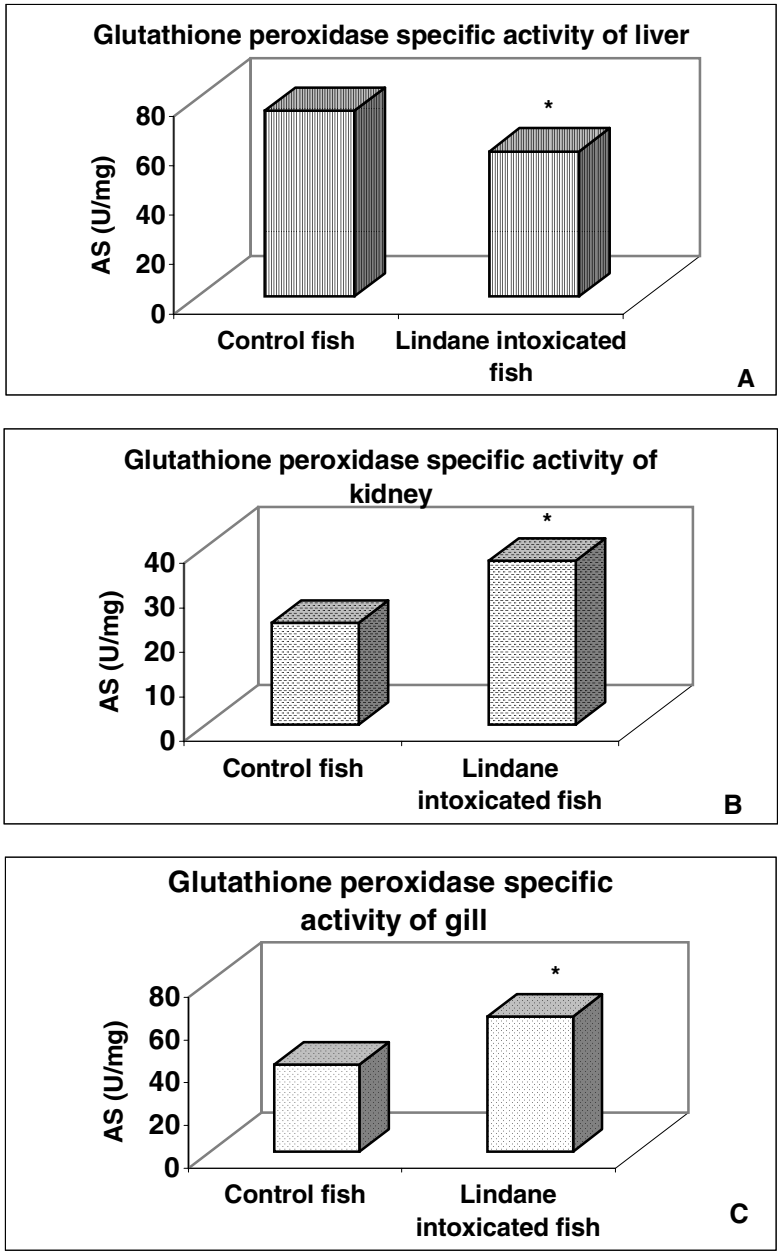

Figure 5. *Effects of 0.05 mg/l lindane on glutathione peroxidase specific activity (U/mg protein) in the liver (panel A), kidney (panel B) and gill (panel C) of* Carassius auratus gibelio. * *P<0.05*