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Effect of Varying Ambiences on Glutathione Concentration in Tissues of *Labeo rohita* & *Clarias batrachus*

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Abstract: ***In these research studies, Effect of varying ambiences on glutathione concentration in tissues of Labeo rohita & Clarias batrachus under a contaminated aquatic environment were evaluated. Present study was conducted on Bisalpur reservoir. Four areas related to Bisalpur reservoir selected were Bisalpur, Nasirda, Thadoli and Negadiya for collection of fishes as well as water samples from all seasons that is moderate, extreme cold, extreme hot and moist warm ambiences. The mean values of glutathione in all the tissues were significantly lower in Thadoli area, followed by Negdiya and Nasirda. The highest values were obtained in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. Lower concentration of glutathione in fishes of Thadoli area indicated the presence of oxidative stress. In each area, the glutathione activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the activity of glutathione was highest in liver for both the fishes.***

Keywords: Glutathione, *Labeo rohita* , *Clarias batrachus*

1. Introduction

The natural aquatic bodies were extensively contaminated with heavy metals released from domestic, industrial, and other man-made activities. This may have serious effects on the ecological balance of the recipient environment. The organisms present in the aquatic environment may accumulate the toxic metals, which ultimately affect not only the productivity and reproductive capabilities of the organisms, but also the health of the human beings that depend on the organisms as a major source of protein.

Hamre *et al.* (2010) gave the hypothesis that Atlantic salmon (Salmo salar) would respond to large variations in supplementation of dietary pro- and antioxidants, and marine lipid, with adjustment of the endogenously synthesized antioxidants, glutathione (GSH) and ubiquinone (UQ). Glutathione (GSH) is an endogenous antioxidant which protect cells from reactive oxygen species such as free radicals and peroxides (Pompella *et al.,* 2003). Glutathione spares ascorbate and improves antioxidant capacity of blood (Gropper *et al.,* 2004), without it dehydroxyascorbate could not convert back to ascorbate. The ratio of reduced glutathione to oxidized glutathione within cells is used as a measure of cellular toxicity (Pastore *et al.,* 2003).

In these research studies, Effect of varying ambiences on glutathione concentration in tissues of *Labeo rohita* & *Clarias batrachus* under a contaminated aquatic environment were evaluated.

2. Material & Method

Present study was conducted on Bisalpur reservoir. Four areas related to Bisalpur reservoir selected were Bisalpur, Nasirda, Thadoli and Negadiya for collection of fishes as well as water samples from all seasons that is moderate, extreme cold, extreme hot and moist warm ambiences. Total 80 fishes collected during extreme cold condition when water bodies were having peak environmental contamination in reservoir due to fall in water level and feeble current. From each area 20 fishes were collected which constituted *Clarias batrachus* (10) and *Labeo rohita* (10). The fishes were sacrificed by decapitation, dissected and the liver, gills, kidney and heart were removed. Each organ was then homogenised in 4volumes of homogenising buffer using a tissue homogenizer. The resulting homogenate was centrifuged at 4000 rpm for 20 minutes in a centrifuge machine at 4°C to obtain the post mitochondrial supernatant fraction (Farombi et al., 2008). The supernatant fractions were used for the determination of analytes by the procedures. Glutathione was determined by the rapid colorimetric micro method as described by Maan (2010).

Procedure

A 0.2 ml supernatant was taken into a tube containing 2.5ml of 0.05 M phosphate buffer (pH7.1), 0.8ml of EDTA reagent and 0.03 ml of DTNB. The tube was shaken for 2 minutes and then kept in a water bath at 37^{o}C for 10 minutes. Then 0.1 ml enzyme solution and 0.1 ml of $NADPH_2$ solution were added. The tube was shaken again, and the optical density was recorded at 412 mμ against a phosphate buffer blank every 1st and 5th minute. The change was noted from the first to the fifth minute and used as a corrected optical density. From this optical density the concentration of glutathione was measured directly from the standard curve.
The standards were processed in the way like that of samples. Toobtain the sample values, a standard curve was prepared by taking 2, 4, 6, 8,10 and 12 µmol L^{-1} concentrations with 0.01, 0.03, 0.05, 0.07, 0.09 and 0.10 optical densities, respectively. Then the values were converted into n mol per mg of protein as described earlier.

3. Result & Discussion

Mean ±SEM values of glutathione in tissues of *Clarias batrachus* and *Labeo rohita* arepresented in table 1 and depicted in figures 1 and 2, respectively.

The mean values of glutathione in both the types of fish tissues obtained from different areas were compared with the control values of earlier researchers (Farombi *et al.*,2008). The mean values weresimilar in fishes from Bisalpur and Nasirda areas. The mean values were very lower in Thadoli and Negdiya areas suggesting oxidative stress in these two areas.

The mean values of glutathione in all the tissues were significantly lower in Thadoli area, followed by Negdiya and Nasirda. The highest values were obtained in *Bisalpur* area. In Thdoli area concentration of dissolved oxygen was highest. Lower concentration of glutathione in fishes of Thadoli area indicated the presence of oxidative stress.

In each area, the glutathione activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the activity of glutathione was highest in liver for both the fishes. Activity was lowest in the gills of both the fishes collected from all four areas. Similar pattern of glutathione concentration was reported by Farombi *et al. (*2008).In each area, in each tissue, the glutathione activity was significantly higher in *Clarias batrachus* than in *Labeo rohita.*

Depletion of glutathione was correlated to oxidative stress by various workers (Bernabucci *et al*, 2005 and Kataria *et al.*,2010). Dehghan*et al.* (2010) unequivocally suggested that glutathione levels changed during different environmental conditions in rams. This showed that antioxidant defense system was changed to adapt and prevent oxidative stress effects because it protects cells from oxidative damages. Farombi *et al.* (2008) observed decreased glutathione levels in the kidneys, gills and heart of fishes when exposed to the butachlor.

Based on glutathione concentration in the tissues, it was concluded that in the fishes from Thadoli and Negdiya areas, glutathione levels were significantly lower when compared to Bisalpur and Nasirda areas in present study and earlier available literature. The findings clearly reflected the presence of oxidative stress in fishes of Thadoli and Negdiya areas.

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Table 1: Effect of varying ambiences on Glutathione concentration in tissues of fishes collected from different areas /villages of Bisalpur reservoir (n=10)

Glutathione, nmol/ mg protein	Areas							
	Bisalpur		Nasirda		Thadoli		Negdiya	
	C *b*	L *r*	C *b*	L *r*	C *b*	L *r*	C *b*	L *r*
Heart	6.2 ^{d} ± 0. 09	6.1 ^{d}±0. 08	6.0 ^{d}±0. 09	5.9 ^{d}±0. 08	3.9 ^{d}±0. 07	3.8^{d}±0. 08	4.3^{d}±0. 09	4.0 ^{d}±0. 08
Kidney	5.8^{d} ± 0. 09	5.7^{d}±0. 08	5.6 ^{d}±0. 09	5.5 ^{d}±0. 08	3.6^{d}±0. 07	3.2^{d}±0. 08	3.8^{d}±0. 09	3.7^{d}±0. 08
Liver	8.0^{d} ± 0. 09	7.5^{d}±0. 08	7.3 ^{d}±0. 09	7.2 ^{d}±0. 08	4.4^{d}±0. 07	4.0^{d}±0. 08	4.7^{d}±0. 09	4.3^{d}±0. 08
Gills	5.0^{d} ± 0. 09	4.8^{d}±0. 08	4.8 ^{d}± 0. 09	4.7 ^{d}±0. 08	2.4^{d}±0. 07	2.3^{d}±0. 08	2.9^{d}±0. 09	2.7^{d}±0. 08

n= Number of fishes
All the means values of a parameter super scribed by same letter denotes significant (p≤0.05) differences among different areas.
C *b* = *Clarias batrachus*
L *r* =*Labeo rohita*