Title Page

1. Name and address of reporting Institution: **Department of Zoology**
Shree Shivaji Arts, Commerce & Science College, Rajura-442 905.

2. Name of the Principal Investigator: **Dr. Arun M. Chilke**

3. Title of research project:

"Biochemical Study of β-Glucuronidase in some organs during the Reproductive Cycle of Male *Labeo rohita* (Hamilton)"

4. Project Reference No. : **F.47-206/07(WRO)- dt.-14th March 2008.**

5. Scheduled life of project : **Two years**

6. Date of research period covered by report : **24th May 2008 – 24th May 2010**

7. Objectives of the project:

a. **To study the kinetics of β-glucuronidase in the liver and kidney of *Labeo rohita"***

b. **To study the seasonal variation of β-glucuronidase in the tissues such as liver, kidney and testes of male *Labeo rohita*.**

c. **To establish the correlation between the reproductive cycle and β-glucuronidase expression in the tissues.**

8. Publications:

A. **Chilke, A.M. (2009).** In situ kinetic study of renal β- glucuronidase in teleosts, Labeo rohita (Hamilton). *Fish Physiol. Biochem.* **(Published online).**

B. **Chilke, A.M. (2010).** Kinetic study of hepatic β-glucuronidase in Indian Major Carp, Labeo rohita (Hamilton). Fish Physiol. Biochem. **(Published online).**

SUMMARY AND CONCLUSION

In the present study, the attempt was made to study the kinetics of renal and hepatic β-glucuronidase in *Labeo rohita*. The maximum enzyme activity was recorded at pH-5 and temperature 38°C in both the kidney and liver. However, reaction velocity in them was enhanced at 52°C and pH-4.5, and then the activity declined up to 70°C.

It has been observed that at 52°C, this hydrolytic activity of the enzyme shoots up to its maximum level for 1hr incubation. Below and above this temperature optima, rate of hydrolysis decreases. However, very little activity was observed even at 70°C. It is concluded that the enzyme is heat stable and native form of it changes above 52°C and completely deforms beyond 70°C. It should be noted that, the teleostean species belong to poikilothermic communities that made it able to change their body temperature with their surrounding, and therefore the adaptation may have developed in the enzyme to give response to high temperature.

Increase in the substrate and enzyme concentration resulted in simultaneous increase in reaction velocity to asymptomic value. Hepatic β-glucuronidase exhibited the reaction velocity maximum (Vmax) as 18.182 µg/hour and Michaelis-Menten constant (Km) as 2.907 mM/hour; however in the renal β-glucuronidase, the former was observed as 8.403 µg/hour and the later was 1.453 mM. It is concluded that the enzyme of both hepatic and renal origin may have same structure and functional sites and hence did not show the remarkable reaction variations in response to kinetic parameters. But the differences in the Vmax and Km indicated that the enzyme expression in the hepatic tissues is maximum than in the renal, which might be due to its functional importance. This finding indicated that the liver is a metabolic centre for enzyme β-glucuronidase.

Actually the kinetic study was carried out to standardize the method of estimation and to fix the parameters such as temperature, pH, substrate concentration, and time and enzyme concentration for studying entire reproductive cycle pertaining to β-glucuronidase activity. In *Labeo rohita*, the reproductive cycle was classified on the

basis of gonado-somatic index and seasonal variation in the testes (morphological and histological variation).

It was observed that the β-glucuronidase activity increased in the kidney and liver from preparatory to spawning phase and was lowest in the post-spawning. In testes, the lowest activity was recorded in the posts-pawning and the highest in the prespawning and early period of spawning phase. It has been concluded that the sex regulating hormones may be playing role in the modulation of this enzyme activity in the studied organs during its reproductive cycle. Further, this enzyme may not have any role in the degeneration of testes during post-spawning phase and therefore the activity in the testes decreases during post-spawning as compared to spawning phase.