Kinetic study of hepatic Alkaline Phosphatase of catfish, *Clarias gariepinus*

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Abstract: Liver is metabolic center of the body and containing several important enzymes that carried out diverse metabolic functions. Alkaline Phosphatase is widely studied enzyme and it is tested for diagnosis of malfunctioning of tissues and organs. The present study is aimed to find the kinetic properties of ALP in the liver of *Clarias gariepinus* as negligible work has been carried out kinetics of enzymes.

The spectrophotometric kinetic study of liver ALP exhibited maximum activity at temperature 45^0C andpH 10.5 and these are considered to be the optimum pH and temperature respectively. The hydrolytic activity of liver ALP was observed consistentincrease with increase in time and enzyme concentration. Similarly, increase in the substrate concentration increases the reaction velocity parabolically attaining stability that results in plateau formation and it is considered as Vmax. The reaction velocity maxima (Vmax) and Michaelis-Menten constant (Km) were recorded using Lineweaver-Burk plot that was found to be 47.85 µg/g wet wt. of tissue and 2.05mM, respectively.

Keywords: Alkaline Phosphatase, enzyme kinetics, catfish, *Clarias gariepinus*

I Introduction: Fish plays crucial role not only in human diets but also in livestock nutrition. African catfish, *Clariasgariepinus* ofClariidae family is resistant to disease and survive in less oxygen condition in water and desiccation (Bok and Jongbloed, 1984).Alkaline phosphatase (EC 3.1.3.1) also referred to as orthophosphoric monoester phosphohydrolase, is a catalytic protein that hydrolyses phosphate esters in alkaline medium (Zhang *et al*., 2004; Saini *et al*., 2005) from many types of molecules, such as nucleotides, proteins, and alkaloids. This process of removing the phosphate group is dephosphorylation. The zinc atom is present near the active site of this enzyme whichis responsible for its catalytic activity (Dean, 2002; Du *et al*., 2001). The divalent cations such as magnesium (Mg^{2+}) (Kim and Wyckoff, 1990;

Du *et al*., 2001; Dean, 2002) and cobalt (Co^{2+}) (Arise et al., 2008) facilitate the activation of ALP. In almost all animal cells, the ALP enzyme is found in intrinsic plasma membrane (Mazorra et al., 2002)that involve in various metabolic processes, such as permeability, protein synthesis, cell differentiation and growth, and absorption and transport of nutrients as well as gonadal maturation (Ram and Sathyanesan,1985).

The ALP isoforms distinguished on the basis of variable and characteristic kinetic properties of alkaline phosphatase extract from different tissues and also from various biological systems (Dean, 2002). Therefore, to explore kinetic properties of alkaline phosphatase in liver of *C. gariepinus*, the present study was carried out.

II Materials and Method: The male *C. gariepinus* weighs 232 gm. was purchased from local market in Chandrapur, India. The fish was kept in aquarium and acclimatized to laboratory condition for 15 days. The aquarium tank was covered with mosquito net and fed with protein food once in a day *ad libitum*. The liver was dissected out by decapitating the fish and wash in distilled water to remove blood traces. In ice cold 0.01M PBS (pH 7.45) liver was macerated and homogenized to prepare 10% (w/v) homogenate, centrifuge it for 20 min at 5000 rpm and the supernatant was used for ALP assay.

The alkaline phosphatase activity in liver of *C. gariepinus* was studied by using the substrate p-nitrophenyl phosphate.Along with substrate,NaCl, NaOH, glycine and p-nitrophenol that was purchased from HiMedia, Pvt. Ltd. Mumbai.

The biochemical assay of alkaline phosphatase was carried out by the method described by Glogowski*et al*., (2002), but with minor modification. The physicochemical parameters of liver ALP such as pH, temperature, time, enzyme and substrate concentration was studied. The effect of pH, temperature, time and substrate concentration on enzyme activity was studied by exposing the enzyme extract to the ranges of pH 8.0-11.5, temperature 30^0C-60^0C, time 10min-80min and substrate concentration 0.8mM-4.0mM respectively. The enzyme concentration ranging from 20µl to 120µl was used to study the effect of enzyme concentration on reaction velocity.

The substrate, 1mM (0.001M) p-nitrophenyl phosphate, prepared in alkaline glycine buffer (0.1M, pH 10.5) was used to study the effect of time, temperature and enzyme concentration. However, the effect of pH on ALP activity was studied by preparing 1mM substrate in 0.1M alkaline glycine with range of pH 8.0 to 11.5. The reaction mixture of all parameters was incubated for 45 min except for time parameter and at 45^0C except for temperature parameter. The enzymatic reaction was terminated by 0.085N NaOH. The amount of p-nitrophenol formed during enzymatic reaction was measured by spectrophotometer at

wavelength 405 nm. The concentration of p-nitrophenol in µg/gm. wet wt. of tissue was estimated by interpolating from standard graph. The Km and Vmax was calculated from Lineweaver- Burk double reciprocal plot.

III Observation and results: The enzyme activity was affected by some physicochemical parameters such as pH, temperature, time, and enzyme and substrate concentration.

Effect of pH

The hydrolysis of substrate was carried out at pH (0.01M alkaline glycine buffer) ranging from 8.0 to 11.5 at 38^0C for 45 min. The hepatic alkaline phosphatase activity was observed to be increases from pH 8.0 to 10.5 and then it decreases from pH 10.5 onwards, indicating that the optimum pH for ALP is 10.5. (Fig. 1)

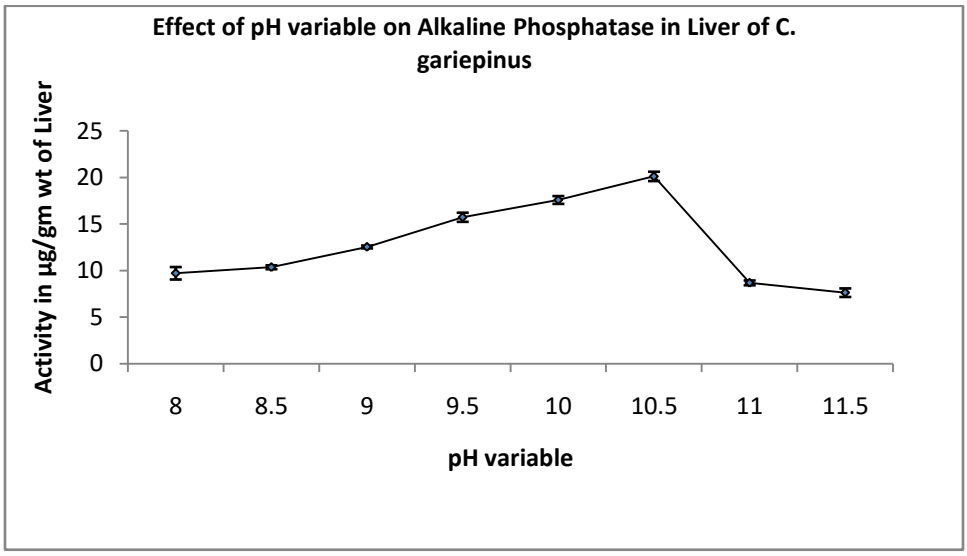

Fig. 1- Effect of pH variable on ALP activity in Liver

Effect of Temperature

The temperature affects the activity of enzyme as decrease in temperature inactivates and increase in temperature denatured the enzyme. In present assay the enzymatic reaction was carried out at temperature ranging from 30^0C to 60^0C. The exposure of ALP to this range of temperature showed remarkable changes in its hydrolytic activity. The enzymatic reaction velocity increases from 30^0C onwards and maximum velocity (26.54±0.40 µg/gm. wt.) was observed at 45^0C. The reaction velocity gradually decreases beyond 45^0C. (Fig. 2)

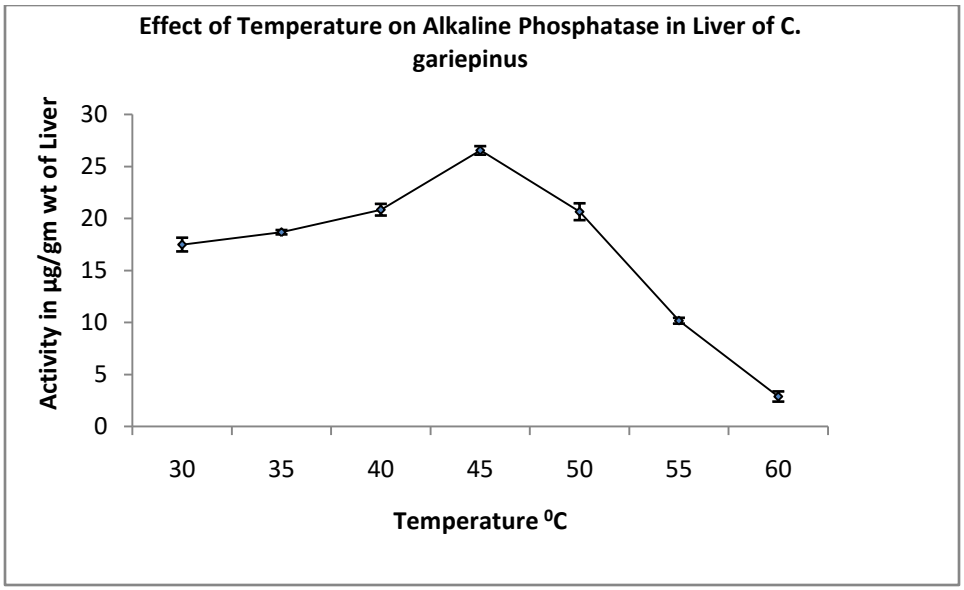

Fig.2- Effect of Temperature on ALP in Liver

Effect of Time

The reaction velocity increases with corresponding increase in time of incubation from 10 min. to 80 min. at pH 10.5. The reaction velocity increases drastically from 10 min. to 40 min. and onwards little increase in reaction velocity was observed up to 80 min. of incubation. (Fig.3)

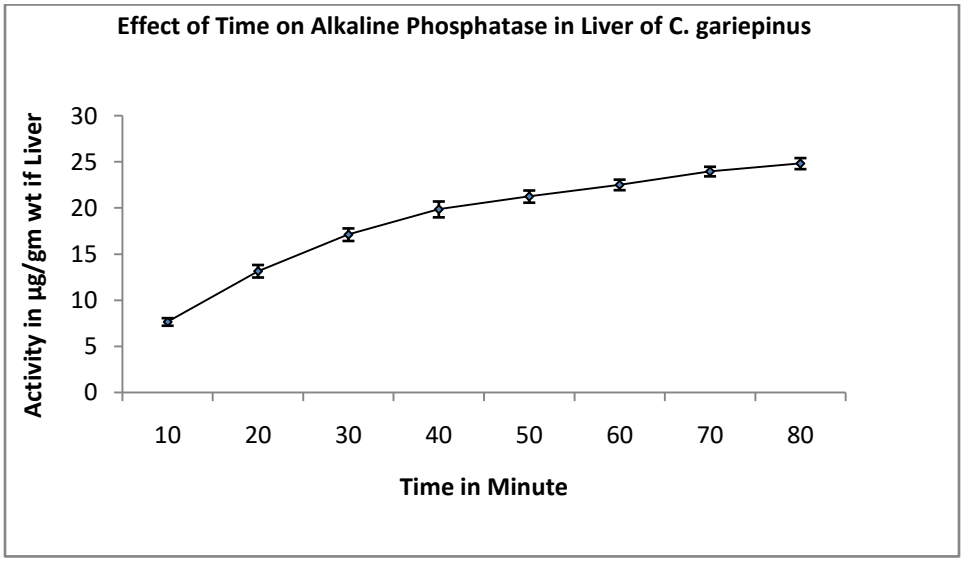

Fig. 3-Effect of Time on ALP in Liver

Effect of Enzyme concentration

The rate of enzymatic reaction was observed to be influenced by concentration of enzyme. The reaction velocity increases gradually with increase in concentration of alkaline phosphatase upon 45 min. of incubation at 38^0C at pH 10.5.(Fig. 4)

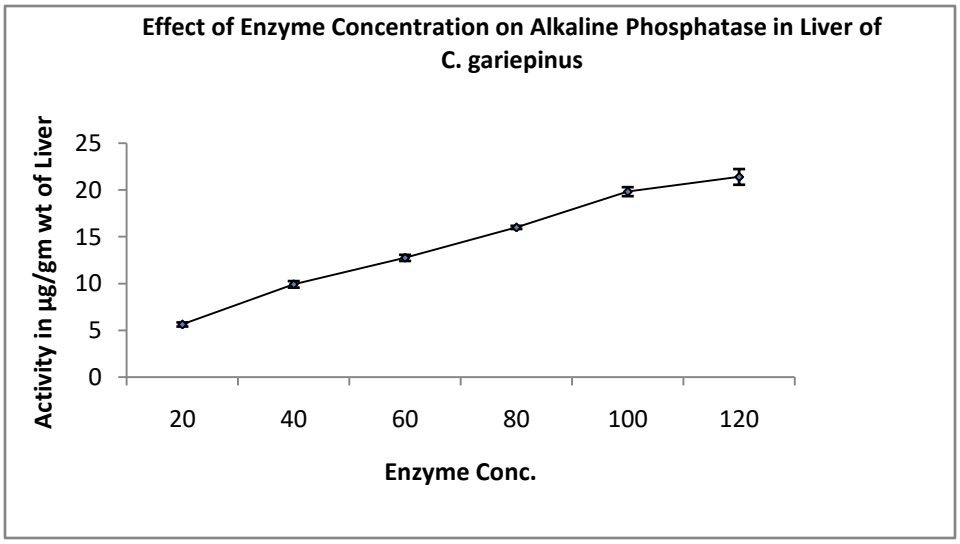

Fig. 4-Effect of Enzyme concentration on ALP in Liver

Effect of Substrate concentration

The inhibitory action of substrate concentration on the rate of enzymatic reaction was studied by the effect of substrate (p-NPP) concentration on enzyme activity. The rate of enzymatic reaction increases with increase in substrate concentration from 0.8 mMto 4.0 mMfor 45 minutes of incubation at 38^0C at pH 10.5. The minimum reaction velocity was observed at 0.8 mM and maximum at 4.0 mM substrate concentration. (Fig. 5)

The Km and Vmax were calculated from regression equation obtained from Lineweaver-Burk plot by considering inverse values of substrate concentration against the reaction velocity. It was observed that the straight line intercepts the y-axis and gave 0.0209 µg/g wet wt. of tissue and x-intercept gave the value -0.48 mM^{-1}. However, the corresponding Vmax and Km were calculated to be 47.85 µg/g wet wt. of tissue and 2.05 mM respectively. (Fig. 6)

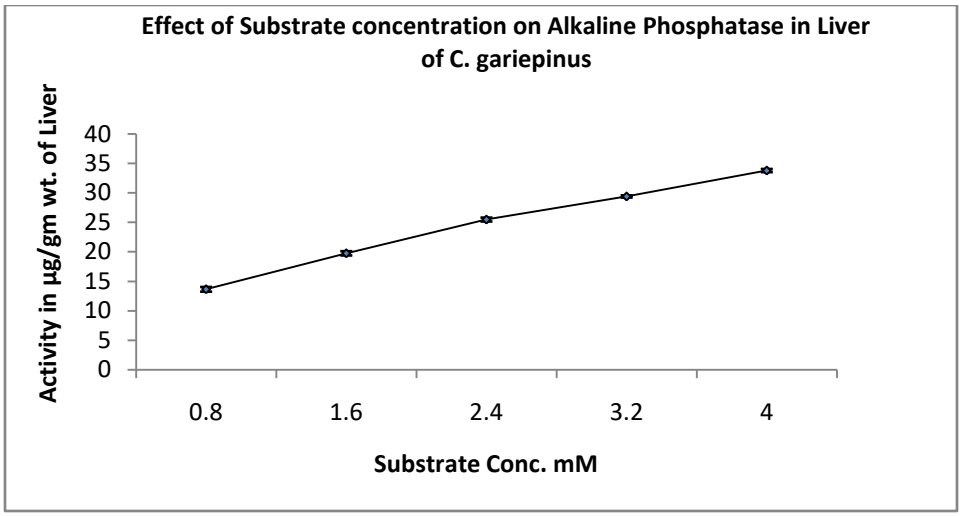

Fig. 5-Effect of Substrate concentration on ALP in Liver

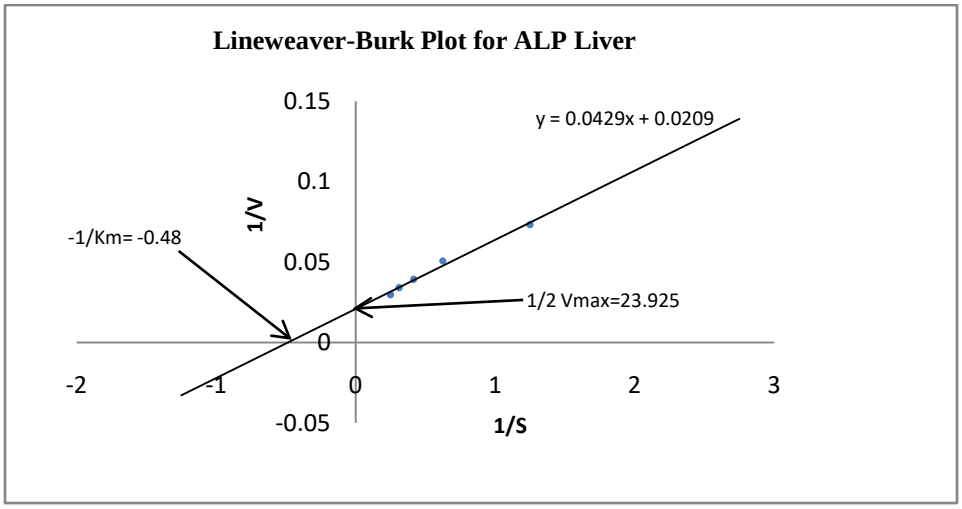

Fig.6-Lineweaver-Burk double reciprocal plot of 1/v versus 1/s showing straight line intercepting Y-axis giving the value 0.0209 µg/g wt. and the X-intercept -0.48 $mM^{-1}$with Vmax 47.85 µg/g wt. and Km 2.05 mM at pH 10.5 and temperature 38^0 C.

IV Discussion

In present study, kinetics of alkaline phosphatase was studied in liver of *C. gariepinus*.The occurrence of ALP and its diversity in biological system have reported by several authors (Coburn *et al*., 1998; Zhang *et al*., 2004). The sequence comparison between different ALP from mammalian and *E.coli* exhibits about 25 to 30% homology (Weissig*et al*., 1993). The kinetics of ALP have studied in bacteria (Yu-Hao Chu *et al*., 2019, Zappa S. *et al*., 2001, Garen A. *et al*., 1960, Qader A.U.S. *et al*., 2008), in pisces (Chilke, 2018), in reptile (Abubakar M.K.*et al*.,2013) and in Mammal (Njoku*et al*., 2011). Although all ALP exhibits differences in amino acid sequence and three dimensional structures, it catalyzes the hydrolysis

of any phosphomonoester with release of inorganic phosphate and alcohol (Njoku*et al*., 2011).Additionally, ALP displays variable affinity for wide range of phosphorylated substrates depending on source of the enzyme, isoform and molecular nature of the substrate.

The interaction of enzyme and substrate is greatly affected by period of incubation of enzymatic reaction. The rate of enzymatic reaction gradually increases with increase in time of exposure at constant pH and enzyme and substrate concentration was recorded in the present investigation.The increased exposure time of enzymatic reaction increases the enzymatic activity that might be due to increase in an interaction between the substrate and enzyme (Chilke, 2018).

The optimum pH value for enzyme activity may be precisely different in various biological systems due to variability in amino acid sequence of protein. Abubakar*et al*.,(2013) have reported 9.9 optimum pH for ALP activity in liver of *Agamaagama*, however, Chilke(2018) recorded 8.5 optimum pH for hepatic ALP of *O. mossambicus.* The optimal activity of ALP in lactic acid bacteria have reported to be at pH 8.5 (Yu-Hao Chu *et al*., 2019) while the study of ALP from humanhydatidiform by Aberomand*et al*., (2008) reported the optimum pH 10.8. In present study, the optimum pH for ALP of *C. gariepinus* was recorded 10.5 at temperature 38^0C for 45 minutes of incubation. As pH increases beyond the optimum value causes internal electrostatic repulsion of the charges on the side chains of amino acid which leads to structural changes of protein and resulting in decrease in enzyme activity (Qader*et al*., 2008).

Enzyme activity and temperature linked properties reported elsewhere (Lee *et al*., 2007; Copeland *et al*., 1985; Aberomand *et al*., 2008) defined the so called enzyme "temperature optimum" of alkaline phosphatase activity.Enzymes are proteins, whose three dimensional structure are stabilized by weak forces, and hence they are disrupted at high temperatures (Saidu*et al*., 2005). In the present investigation optimum temperature for hepatic ALP activity was recorded 45^0C. These findings are consistent with previous studies by Njoku*et al*.,(2011) in which they reported optimum temperature 45^0C for hepatic ALP of rabbit. However, Yu-Hao Chu *et al*., (2019), and Qader*et al*., (2008) was recorded 37^0C optimum temperature in bacteria and Abubakar et al.,(2013) recorded 35^0C optimum temperature for hepatic ALP of *Agama agama* lizard.

The increase in enzyme activity was recorded with corresponding increase in enzyme concentration at constant pH, temperature and substrate concentration. The gradual increase in the enzyme activity might be consequence of increase in binding competition of the enzymes with the substrate. The same result was noticed about substrate concentration.

The ALP has high substrate specificity to p-nitrophenyl phosphate (Robert *et al*., 2003) and shows variation in Km and Vmax in different organisms and also in the different tissues of same organism. It

was observed that the alkaline phosphatase activity increased with increase in substrate concentration. The Michealis constant for p-nitrophanyl phosphate at pH 10.5 was calculated by Lineweaver-Burk plot (Fig.6). It was observed that the straight line intercepts the Y-axis and gave the value0.0209 µg/g wt. and the X-intercept -0.48 mM^{-1}. The corresponding Vmax and Km were calculated to be 47.85 µg/g wt. and 2.05 mM. The Km and Vmax value for hepatic ALP of *O. mossambicus* was calculated 106.95 µg/Hh and 0.216mM/Hh (Chilke,2018).

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