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KINETIC STUDY OF RENAL ALKALINE PHOSPHATASE OF CATFISH, *CLARIAS GARIEPINUS*

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ABSTRACT:

Kidney is an excretory center of the vertebrate body and it carries out excretory function. Alkaline phosphatase is widely studied enzyme and tested for diagnosing the malfunctioning of tissues or organs. The effect of pH, temperature, time and substrate concentration was studied by exposing the enzyme to the range of pH from 8.0 to 11.5, temperature ranging from 30^{0}C to 60^{0}C, time ranging from 10 to 80 min, and substrate ranging from 0.8 mM to 4.0 mM, however the range of enzyme concentration from 20 µl to 120 µl was used for studying the effect of enzyme concentration on reaction velocity. As negligible work has been done on the kinetics of enzymes in fishes in general and therefore, we aimed to study to find the kinetic properties of this enzyme in the kidney of *Clarias gariepinus*. Renal alkaline phosphatase exhibited maximum activity at temperature 40°C and pH 9.5 and these are considered to be the optima for this enzyme. However, the enzyme activity was observed to increase consistently with increase in time and enzyme concentrations. Similarly, increase in the concentration of substrate to certain extent found to stabilizes the enzyme activity and it was considered as Vmax. The Vmax and Km values were found to be 196.07 µg/gm wt. of kidney and 5.15 mM from resulted graph.

Key words: - *Alkaline Phosphatase, enzyme kinetics, catfish, Clarias gariepinus*

INTRODUCTION:

Fish plays a vital role in lives of human as it enriches with protein. African catfish, *Clarias gariepinus* of the family Clariidae, commonly called Thi-Magur is resistant to muddy water and disease and can survive in less aquatic oxygen condition and desiccation (Bok and Jongbloed, 1984) as well as it is raised in high densities (Omoergie *et al.*, 1994). Alkaline phosphatase (EC 3.1.3.1), also referred as orthophosphoric monoester phosphohydrolase, is a generic term that describes a group of catalytic proteins sharing the capacity to hydrolyze phosphate esters in alkaline medium (Zhang et al., 2004; Saini et al., 2005). Organic and inorganic phosphates are essential component of living organism; therefore it is a valuable reagent for the removal of terminal monoesterified phosphatae from ribooligonucleotide, deoxyribo-oligonucleotides, alkaloids and proteins etc. (Reid and Wilson, 1971; Fermley, 1971). It is homodimeric metalloenzyme which hydrolyzes the phosphomonoester into inorganic phosphate and corresponding alcohol (Robert et al., 2003; Trowsdale et al., 1990). Alkaline phosphatases are differing from each other in sequence of amino acids and nature and extent of glycosylation due to a number of isozymes and isoforms of this enzyme. The wide distribution of ALP in biological systems coupled with its broad specificity makes the enzyme a readily available parameter for diagnostic and research studies (Garba and Gregory, 2005). The enzyme contains a zinc atom (Zn^{2+}) near the active site that is viewed to be responsible for its catalytic activity (Le Du *et al.*, 2001; Dean, 2002). The activation of ALP activity is facilitated by divalent cation such as magnesium (Mg^{2+}) (Kim and Wyckoff, 1990; Le Du *et al.*, 2001; Dean, 2002) and cobalt (Co^{2+}) (Arise *et al.*, 2008). In almost all animal cells, this enzyme found as an intrinsic plasma membrane enzyme (Mazorra et al., 2002), that involved in metabolic processes such as protein synthesis, growth and cell differentiation, absorption and transport of nutrients, permeability and gonadal maturity (Ram and Sathayanesan, 1985). Thus the metabolism of fishes can be affected by changes in alkaline phosphatase activity. Several report showed that ALP exhibits variable but characteristic kinetic properties that serve as basis for distinguishing ALP isoforms from different tissue/organ and various biological

systems (Mayer-Sabellek, 1988; Dean, 2002). This work was aimed at evaluating some kinetic parameters of alkaline phosphatase in kidney extract of *Clarias gariepinus*.

MATERIAL AND METHODS :-

Healthy and matured male fish with a weight of 232 gm. was purchased from main fish market in Bengali Camp, Chandrapur. The fish was acclimatized to laboratory conditions in the aquarium tank for fifteen days and then use for further experiment. The aquarium tank was covered with mosquito net to prevent the fish from jumping out. The water in the tank was replaced every 48 hr. in order to maintained a healthy environment to fish. The fish were fed with standard feed containing protein once in day *ad libitum*. A substrate, p- nitrophenyl phosphate, was used, along with glycine, sodium chloride, sodium hydroxide were purchased from HiMedia, Pvt. Ltd. Mumbai.

The fish was decapitated and kidney dissected out and washed in distilled water to remove traces of blood. The kidney was macerated and homogenized using ice cold 0.01M PBS, pH 7.45 to obtain 10% (w/v) homogenate. The homogenate was centrifuged for 20 min. at 5000 rpm and the supernatant was used for enzymatic assay.

To study the effect of pH, temperature, time and substrate concentration, the enzyme was exposed to the range of pH from 8.0 to 11.5, temperature ranging from 30^{0}C to 60^{0}C, time ranging from 10 to 80 min, and substrate ranging from 0.8 mM to 4.0 mM, however the range of enzyme concentration from 20 µl to 120 µl was used for effect of enzyme concentration on reaction velocity. The enzyme activity was determined in a final volume of 1.7 ml, containing 0.1M alkaline glycine buffer pH 10.5. To study temperature, time and enzyme concentration, 1mM (0.001M) substrate prepared in alkaline glycine buffer (0.1M, pH10.5) was used. However, substrate of the same molar concentration prepared in 0.1M alkaline glycine with varying range of pH from 8.0 to 11.5 was used for studying the effect of pH on hydrolytic activity.

The ALP activity was determined by taking 0.1 ml substrate (1 mM p-nitrophenyl-phosphate) solution in test tube followed by incubation for 5 min at 38^{0}C. Later 0.1 ml kidney extract of *C. gariepinus* was added to the mixture and homogenate the reaction mixture and then 1.5 ml alkaline glycine buffer (0.1 M, pH-10.5) was added to the reaction mixture and incubation was carried out for 45 minutes, except for studying the effect of temperature. At the end of incubation, the reaction was terminated by adding 8.3 ml 0.085 N NaOH. The amount of p-nitrophnol released, in terms of intensity of color developed, was measured by spectrophotometer at wavelength 405 nm. The concentration of p-NP was estimated by interpolating from standard curve.

For substrate kinetic analysis, the concentration of substrate was varied over the range of 0.8 mM to 4.0 mM prepared in alkaline glycine buffer (0.1 M, pH-10.5). Km and Vmax was calculated from Lineweaver-Burk double reciprocal plot by using the inverse values of substrate concentration and respective reaction velocity. The effect of enzyme concentration was studied by using subsequent increasing concentration of 10% stock tissue extract.

RESULTS AND DISCUSSION

Effect of pH:-

The renal alkaline phosphatase activity was found to be increases from pH 8.0 to 9.5 and beyond that decrease up to pH 11.5 upon exposing the enzyme to pH ranging from 8.0 to 11.5. The maximum reaction velocity was observed to be 31.10±0.68 at pH 9.5 indicates that optimum pH value for renal alkaline phosphatase is 9.5. (Fig.1)

Effect of Temperature:-

The renal alkaline phosphatase activity was observed to be maximum at temperature 40^{0}C when the enzyme was exposed to the temperature ranging from 30^0 to 60^{0}C. The reaction velocity gradually increases from 30^{0}C to 40^{0}C and above 40^{0}C it decrease up to 60^{0}C at which very low reaction velocity was observed. (Fig.2)

Effect of Time:-

The renal alkaline phosphatase activity increases gradually with corresponding increase in period of incubation from 10 to 80 minutes. (Fig.3)

Effect of Enzyme concentration:- The enzyme concentration influences the reaction velocity was observed. The reaction velocity was found to be gradually increases with increase in alkaline phosphatase concentration upon incubating the reaction mixture with pH 10.5 for 45 minutes at 38^{0}C. (Fig.4)

Effect of Substrate concentration

The renal enzymatic activity increases with corresponding increase in substrate concentration with pH 10.5 for 45 minutes at 38^{0}C. (Fig.5).

It was observed from the regression equation obtained from Lineweaver-Burk double reciprocal plot by considering the inverse values of substrate concentration against reaction velocity that straight line intercepts Y-axis at 0.0051µg/gm and X-axis giving the value -0.19mM. The Km and Vmax values for renal alkaline phosphatase calculated to be 5.15 mM and 196.07 µg/gm wt. of tissues. (Fig.6).

The present study was carried out for kinetic parameter of alkaline phosphatase in kidney of *Clarias gariepinus*. The kinetics of ALP have studied in bacteria (Yu-Hao Chu *et al.*, 2018, Zappa S. *et al.*, 2001, Garen A. *et al.*, 1960, Qader A.U.S. *et al.*, 2009), in pisces (Chilke, 2018), in reptile (Abubakar MK *et al.*,2013) and in Mammal (Njoku *et al.*, 2011). Coburn et al., (1998) and Zhang *et al.*, (2004) have reported the occurrence and diversity of alkaline phosphatase in biological system. All the alkaline phosphatase exhibit differences in amino acid sequence and their three dimensional structures, though they catalyzes the hydrolysis of any phosphomonoester. The alkaline phosphatase displays variable affinity for wide range of phosphorylated substrates depending on the molecular nature of the substrate, isoforms and source of enzyme.

Chilke, (2018) reported the increased enzymatic reaction velocity with corresponding increase in time period of incubation might be due to increase in interaction between the enzyme and substrate. In present investigation, it was recorded that the rate of ALP reaction increases gradually with increase in exposure time of incubation at constant pH, enzyme and substrate concentration.

The optimum pH values may differs in different tissues and organs differing with organism due to differences in amino acid sequence in protein and consequently the conformation of protein. Aberomand *et al.*, (2008) have reported the optimum pH of 10.5 for ALP from hydatidiform while Yu-Hao Chu *et al.*, (2018) recorded maximum ALP activity at pH 8.5 in lactic acid bacteria. In present study, optimum pH for ALP from kidney of *C. gariepinus* was recorded 9.5 at temperature 38^{0}C for 45 minutes of incubation. The optimum ALP activity have been reported at pH 9.9 in liver of *Agama agama* (Abubakar *et al.*,2013) however, Chilke, (2018) reported 8.5 optimum pH value for liver ALP of *O. mossambicus*. The increase in pH value beyond the optimum pH decreases the enzyme activity due to internal electrostatic repulsion of the charges on the side chains of amino acids which leads to change in native conformation of protein (Ali ul Qader S *et al.*, 2008). The increase in enzyme activity was recorded with corresponding increase in enzyme concentration at pH 10.5 at 38^{0}C for 45 minutes incubation. The increase in enzyme activity is the result of increase binding competition of enzyme with substrate. The similar result was noticed for substrate concentration.

The renal alkaline phosphatase activity was found to be increases with increase in substrate concentration by gradually decreasing slope in fig.5. The Km value for p-nitrophenyl phosphate for renal ALP was calculated by Lineweaver-Burk double reciprocal plot (Fig.6). The Vmax and Km were calculated to be 196.07µg/gm wt. and 5.15mM respectively. The ALP kidney extract of present investigation exhibited lower affinity for p-NPP (Km = 5.15 mM) than enzyme extracts from fish (*O. mossambicus*) liver (Km = 0.216 mM) (Chilke, 2018) and rabbit (Lepus townsendii) liver (Km = 0.5±0.25 mM) (Njoku, *et al.*, 2011); however, it showed higher affinity than enzyme extracts from rat (*Rattus novergicus*) kidney (Km = 6.41 mM) (Arise *et al.*, 2008) and human placental isoenzyme (Km = 5.55 mM) (Saini et al., 2005). These findings give the conformation to the occurrence of isoforms of ALP.

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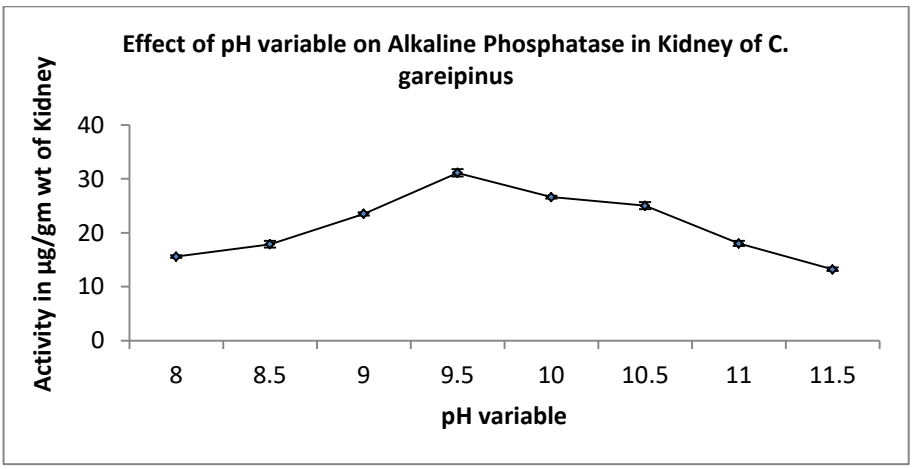

Fig.1- Effect of pH on alkaline phosphatase in Kidney of *C. gariepinus*

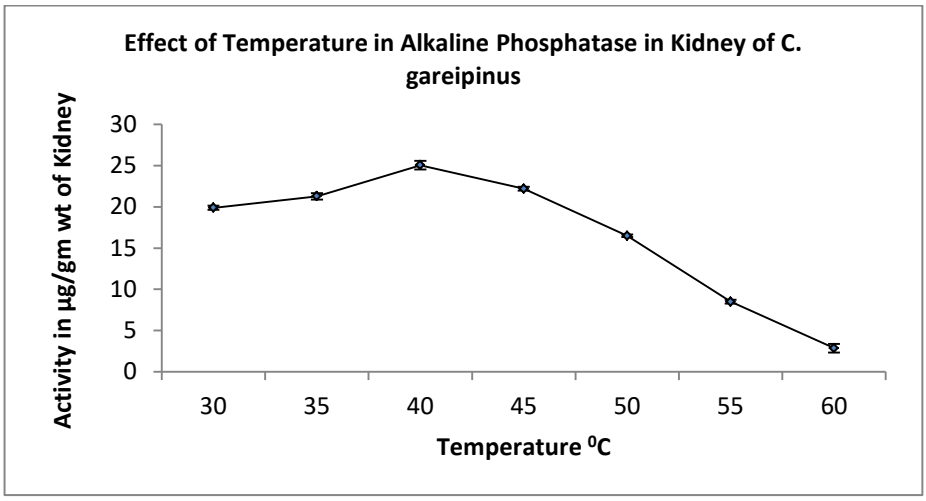

Fig.2- Effect of Temperature on alkaline phosphatase in Kidney of *C. gariepinus*

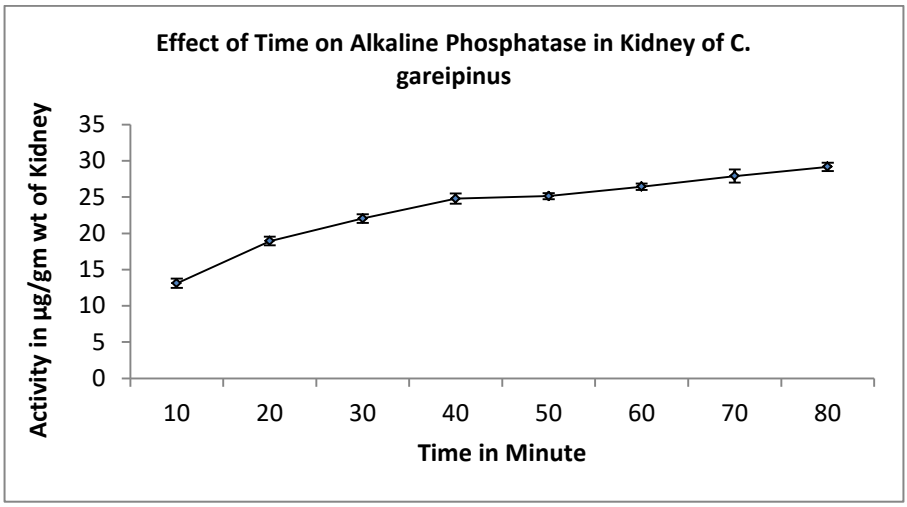

Fig.3- Effect of Time on alkaline phosphatase in Kidney of *C. gariepinus*

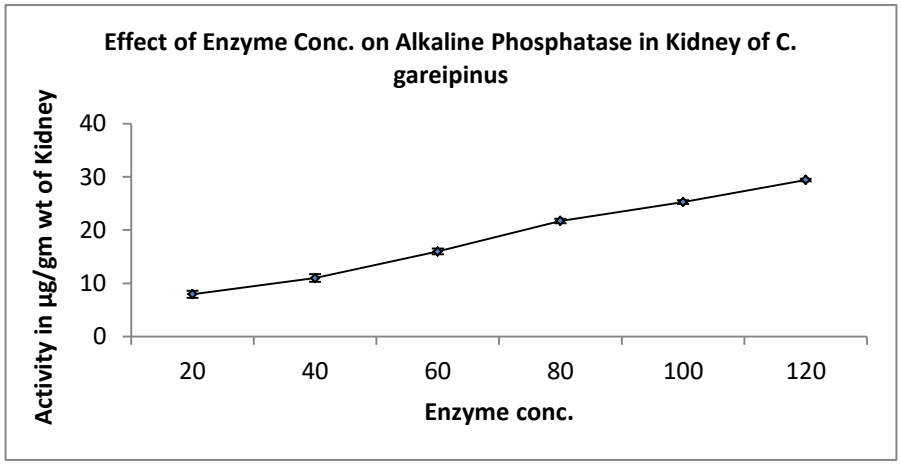

Fig.4- Effect of Enzyme concentration on alkaline phosphatase in Kidney of *C. gariepinus*

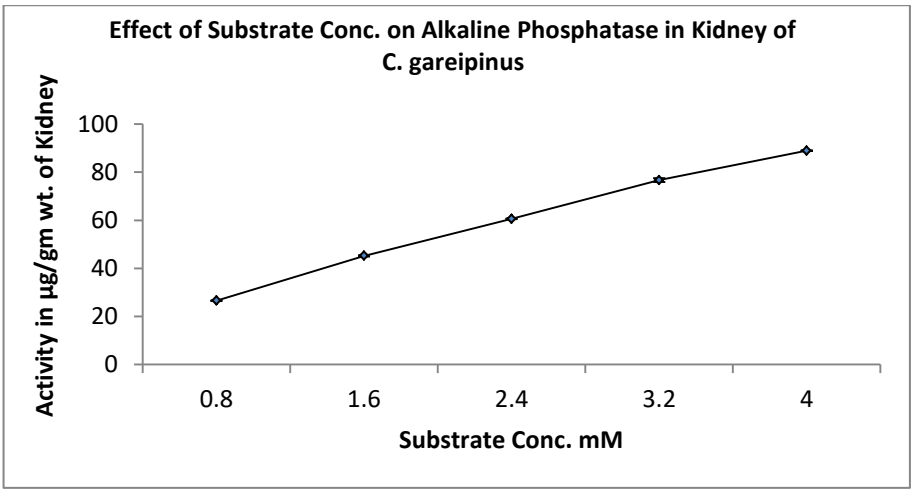

Fig.5- Effect of Substrate concentration on alkaline phosphatase in Kidney of *C. gariepinus*

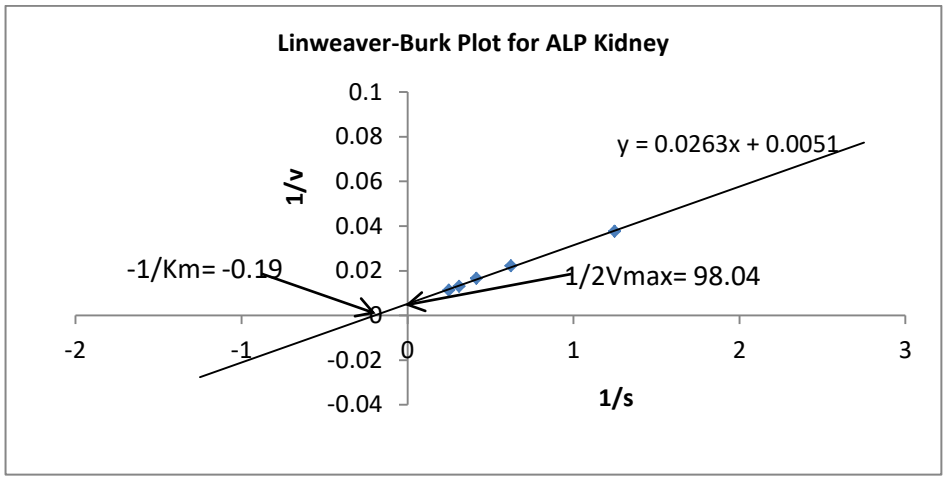

Fig.6- Line weaver-Burk double reciprocal plot of 1/v versus 1/s showing straight line intercepting Y-axis giving the value 0.0051 µg/g wt. and the X-intercept -0.19 mM^{-1} with Vmax 196.07 µg/g wt. and Km 5.15 mM at pH 10.5 and temperature 38^0 C.