I J R B A T, Issue (VIII), Vol. I, Jan 2020: 08-14
A Double Blind Peer Reviewed Journal
e-ISSN 2347 – 517X
Original Article

INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY

 www.ijrbat.in

KINETIC STUDY OF HEPATIC ACID PHOSPHATASE EXTRACTED FROM CATFISH, *CLARIAS GARIEPINUS*

S.B.Patharde* and A.M.Chilke**
*Sardar Patel College, Chandrapur-442402 (M.S.)
** Division of Toxicology and Biomonitaring, Department of Zoology,
Shri Shivaji Arts, Commerce and Science College, Rajura-442905 (M.S.)

Communicated : 14.11.19 | Revision : 18.12.19 & 30.12.19 Accepted : 16.01.2020 | Published: 30.01.2020

ABSTRACT:

The aim of present study was to investigate the kinetic properties of acid phosphatase extracted from liver of *Clarias gariepinus*. The activity of acid phosphatase was determined biochemically by using *p*-Notrophenol as standard and p-nitrophenyl phosphate as substrate. The effect of pH, temperature, time and substrate concentration was studied by exposing the enzyme to the range of pH from 3.37 to 6.5, 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. The enzyme was found to have maximum velocity at temperature 40^{0}C and pH 5.0, however its activity increased with increase in time and enzyme concentration. The increased in substrate concentration increases enzyme activity and found to stabilize at certain extent and it was considered to be Vmax. The Vmax and Km values were found to be 131.57 µg/g wt. and 8.64 mM of kidney from resulted graph.

Key words: - *Clarias gariepinus, Liver, Acid phosphatase, kinetics, catfish.*

INTRODUCTION:

The African Catfish *Claries gariepinus* belonging to family Clariidae 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). The lysosomes are the membrane-limited subcellular organelles that contain acid hydrolases (de Duve et al., 1966, Bainton, 1981). These organelles contain about 70 different types of acid hydrolases (Dean et al, 1976, Barett et al., 1977). The cellular acid phosphatase activity is concentrated in this sub-cellular organelle (de Duve et al., 1966, Bainton, 1981). Acid phosphatase (EC 3.1.3.2) is a lysosomal enzyme that catalyze the hydrolysis of various phosphomonoesters in an acidic pH to release an inorganic phosphate (Vincent et al., 1992; Miteva et al., 2010). These are ubiquitous enzyme and present in fungi (Leitao et al., 2010), protozoa (Amlabu et al., 2009), plants (Demir et al., 2004, Tabaldi et al., 2008, Kaida et al., 2008) and animals (Sazmand et al., 2011, Siddiqua et al., 2008, Stubberud et al., 2000). The inorganic phosphate, release in hydrolysis of phosphate monoesters, is very important in plants and animal tissues which is linked to many biological processes such as cellular signal transduction pathway and energy metabolism (Shan, 2002). Multiple forms of acid phosphatase are frequently occur (Fujimoto et al., 1984) and can be differentiated according to structural, catalytic, tissue distribution and localization (Suter et al., 2001). The acid phosphatase from *Labio rohita* (Siddiqua et al., 2008) and *Aspergillus niger* (Jain et al., 2013) has been purified and characterized from Locust (Naqvi et al., 1967).

Up to now there are no references reporting kinetic study in acid phosphatase in *Clarias,* therefore in present study, we focus on some physic-chemical and kinetic properties of acid phosphatase in liver extract from *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 was fed with standard feed containing protein once in day *ad libitum*. A substrate, p- nitrophenyl phosphate, was used, along with sodium acetate, glacial acetic acid, sodium chloride, sodium hydroxide were purchased from HiMedia, Pvt. Ltd. Mumbai.

The fish was decapitated and liver dissected out and washed in distilled water to remove traces of blood. The liver 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 3.37 to 6.5, 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 respectively, 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 0.01M acetate buffer pH 5.0. To study temperature, time and enzyme concentration, 1mM (0.001M) substrate prepared in acetate buffer (0.01M, pH 5.0) was used. However, substrate of the same molar concentration prepared in 0.01M acetate buffer with varying range of pH from 3.37 to 6.5 was used for studying the effect of pH on hydrolytic activity.

The ACP 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 liver extract of *C. gariepinus* was added to the mixture and homogenate the reaction mixture and then 1.5 ml acetate buffer (0.01 M, pH-5.0) 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 acetate buffer (0.01 M, pH-5.0). Km and Vmax was calculated from Lineweaver-Burk Plot (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

The enzyme activity is affected by some physico-chemical parameters such as pH, Temperature, Time, Enzyme and substrate concentration.

Effect of pH:

The hydrolysis of substrate (1mM p-Nitrophenyl phosphate) was carried out at pH ranging from 3.37-6.5 at 38^{0}C for 45 minutes. The hepatic acid phosphatase activity was observed to be increases with increase in pH of 0.01M acetate buffer up to 5.0 (15.64±0.37 µg/gm wt.) and then decreases up to pH 6.5 continuously. It indicates that the optimum pH for hepatic ACP is 5.0.(Fig.1).

Effect of Temperature

After exposing the hepatic acid phosphatase reaction mixture to varying range of temperature from 30-60^{0}C, acid phosphatase showed remarkable changes in its hydrolytic activity. The reaction velocity increases from 30^{0}Cand found to be maximum at 40^{0}C (16.57±0.13µg/gm. wt.) and thereafter significantly decreases upto 60^{0}C.(Fig.2)

Effect of Time

The hepatic acid phosphatase reaction velocity increases gradually with increase in period of incubation from 10 to 40 minutes and shows slightly increase further upto 80 minutes. It indicates the enzymatic reaction velocity increases with corresponding increase in time of incubation of enzymatic reaction.(Fig.3)

Effect of enzyme concentration

The rate of enzymatic reaction was observed to be influenced by the corresponding concentration of enzyme. The release of phenol group as a product in acid phosphatase reaction increases with gradual increase in enzyme concentration from 20µl to 120µl.(Fig.4)

Effect of substrate concentration

The effect of substrate concentration on acid phosphatase activity was studied to observe the inhibitory action of substrate concentration on the rate of reaction. The rate of reaction increases with increase in substrate concentration from 0.8mM to 4.0mM up on 45 minutes of incubation at 38^{0}C and pH 5.0. The minimum velocity was observed at 0.8mM and maximum at 4.0mM substrate concentration.(Fig.5)

From the regression equation obtained from Lineweaver-Burk double reciprocal plot by

considering the inverse values of substrate concentration against reaction velocity, the Vmax and Km were calculated. It was observed that the straight line intercepts the Y-axis and gave the value 0.0076µg/gm and X-intercept gave the value -0.11mM^{-1}. However, the corresponding Vmax and Km were calculated to be 131.57 µg/gm and 8.64 mM respectively.(Fig.6)

In present study the some physicochemical parameters and kinetics of acid phosphatase was studied. In invertebrate, on the basis of molecular weight, three types of acid phosphatase reported (Saeed et al., 1990; Naz et al., 2001). The kinetics of acid phosphatase have studied in yeast (Vasileva-Tonkova et al., 1993), in insect (Naqvi et al., 1968), in fish (Siddiqua et al., 2012), in bacteria and plants (Tazisong et al., 2015). The acid phosphatase exhibits differences in molecular weight, such as high molecular weight ACP (80-200 kDa) in the lysosome and intermediate molecular weight ACP (30-50 kDa) in mitochondria of some mammalian tissues such as kidney while low molecular weight ACP (10-30 kDa) in cytosol fraction of cell (Naz et al., 2006). These differences in molecular weight lead to isoforms that hydrolyses different phosphomonoesters and thus can displayed variations in kinetic properties.

The optimum pH value for enzyme may be different in different tissues of biological system due to differences in molecular weight and consequently amino acid sequence in protein. Siddiqua et al., (2012) have reported pH 5.0 in liver of Rohu fish, Naqvi et al., (1968) recorded optimum pH 4.4 in digestive system of Locust while Vasileva-Tonkova et al., (1993) reported maximum activity at pH 5.8 in yeast ACP. The maximal hydrolysis of substrate was reported at pH 5.0 by wheat germ acid phosphatase and pH 6.0 and pH 5.0 by sweet potato and potato acid phosphatase respectively (Tazisong et al., 2015). In present study the maximal hydrolysis of substrate by hepatic ACP of *Clarias gariepinus* was 5.0.

Enzymes are proteins, whose three dimensional structure are stabilized by weak forces, and hence they are disrupted at high temperatures (Saidu *et al.*, 2005). The optimum temperature for enzyme activity was recorded to be 40^{0}C for hepatic ACP in the present investigation. This result have in agreement with the findings of Siddiqua et al., (2012) in Rohu fish liver and Naqvi et al., (1968) in desert Locust, whereas Tazisong et al., (2015) reported maximum activity of ACP at 50^{0}C from wheat germ, 60^{0}C from potato and 70^{0}C from sweet potato, however, Vasileva-Tonkova et al., (1993) reported temperature optima 55^{0}C in yeast. The variation in optimum temperature of ACP in different biological tissues might be due to differences in activation energy that outcome of structural variation owing to differences in molecular weight.

The rate enzymatic hydrolysis is influenced by the period of incubation of enzyme-substrate mixture. In the present investigation, the rate of hepatic ACP reaction was observed to be increases with gradual increase in time of incubation at constant pH, temperature, and enzyme and substrate concentration. The increased enzymatic activity with corresponding increase in exposure time might be due to increase in an interaction between the substrate and enzyme (Chilke, 2018).

The gradual increase in enzyme activity with corresponding increase in enzyme concentration was recorded in the present investigation. 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 rate of hydrolysis, catalytic efficiency and optimal pH of ACP and ALP may depend on enzyme sources and stereochemical or stereoisomeric structure of the substrates and these enzymes do exhibit a broad range of substrate hydrolysis (Tazisong et al., 2015). This enzyme shows variation in reaction kinetics (Vmax and Km) values with different substrates and enzyme source. It was observed that the enzymatic reaction velocity increases with corresponding increase in substrate concentration. The Vmax was found to be 131.57 µg/g wt. The Km for p-nitrophenyl phosphate was calculated to be 8.64 mM, however, Siddiqua et al., (2012) reported 0.25 mM in Rohu fish liver indicating that ACP from Rohu fish liver have higher affinity for p-nitrophenyl phosphate than *Clarias gariepinus* liver. This is due to nature and source of enzyme.

REFERENCES:-

Amlabu, E., A.J. Nok and A.B. Sallau, (2009). Purification and biochemical characterization of lysosomal acid phosphatase (EC 3.1.3.2) from blood serum forms, *Trypanosoma brucei brucei*. Parasitol. Int., 58:238-242.

Bainton D.F., The discovery of lysosomes. J. Cell Biol., 91(1976) 66.

Barett A.J. and Heath M.F. Lysosomal enzymes in Lysosome: A Laboratory Handbook, edited by J.T. Dingle (North-Holland Publishers, Amsterdam) (1977), 19.

Bok AH, Jongloloed BK (1984). Growth and production of Sharptooth Catfish, Clarias gariepinus in originally fertilized small ponds in the province. South Afr. Aquacult. 36:141-142

Chilke AM, (2018). Hepatic-ALP kinetics in Cichilid, *Oreochromis mossambicus* (Peter). Inter. J. Resear. Appl. Sci. and Engin. Tech. Vol. 6 Issue 1.

De Duve C and Wattiaux R. Functions of lysosomes. Annu Rev. Physol., 28 (1966) 435

Dean R. T. and Barett A.J. Lysosomes. Essays Biochem. 12 (1976) I.

Demir, Y., A. Alayli, S. Yildirim and N. Demir, (2004). Purification and characterization of acid phosphatase from Hypericium (*Hypericum perforatum* L.). Int. J. Ahricc. Biol., 6:1089-1092

Fujimoto, S., Y. Urata, T. Nakagawa and A. Ohara, (1984). Characterization of intermediate molecular weight acid phosphatase from bovine kidney cortex. J. Biochem., 96: 1079-1088

Jain R., Garg V., Dangwal K. and Kaushish M.L. (2013). Purification and characterization of acid phosphatase from monocrotophos (MCP) hydrolyzing Aspergillus niger ITCC 7782.10 Isolated from Local Agricultural Field. Turk. J. Biochem., 38 (4): 396-402

Kaida, R., T. Hayashi and T.S.Kaneko, (2008). Purple acid phosphatase in the wall of tobacco cells. Phytochemistry, 69:2546-2551

Leitao, V.O., R.C.de Melo Lima, M.H. Vainstein and C.J. Ulhoa, (2010). Purification and characterization of an acid phosphatase from *Trichoderma harzianum*. Biotechnol. Lett., 32:1083-1088

Miteva, R., D.Zpreanova, I.V. Fasulkov, S. Yotov and T. Mircheva, (2010). Investigations on acid phosphatase activity in the seminal plasma of humans and animals. Trakia J. Sci., 8:20-23

Naqvi S.N.H., Ashrafi S.H. and Qadrt M.A.H. (1968). Acid phosphatase activity in the digestive system of the desert Locust *Schistocerca gregaria* (Forskal) (1967). Aust. J. Biol. Sci. 21:1047-1052

Naz R., N. Ikram, A. Saeed, Z.A. Khan and A.R. Khan (2001). Acid phosphatase in skeletal muscles of Uromastrix. Int. J. Agric. Biol., 3:371-344

Naz R., N., Saeed Asma and Saeed A. (2006). An 18 kDa acid phosphatase from chicken heart possessing phosphotyrosine phosphatase activity. The Protein J.., 25:135-146

Omeregie E. Thomas G, Ofogekwu PEO (1994). Chronic effects of formalin on erythrocytes count and plasma glucose of Nile Tilapia, Oreocromis niloticus. J.Asian Fish Sci. 7:1-6

Saeed A., E. Temori, G. Manao, G. Camici, G. Cappugi, and Ramponi (1990). Bovine brain low molecular weight acid phosphatase: Purification and properties. Physiol. Chem. Phys. Med. NMR, 22: 71-94

Saidu Y, Alawiyya GA, Bilbis LS, Abbas AY (2005). Kinetic studies of Rhodanese in some tissues of Sokoto Red Goat. Bulletin of Science Association of Nigeria, 26: 505-510.

Sazmand, A., A. Rasooli, M. Nouri, H. Hamidinejat and S. Hekmatimoghaddam, (2011). Serobiochemical alterations in suclinically affected dromedary camels with *Trypanosoma evansi* in Iran. Pakistan Vet. J., 31:223-226

Shan, J., (2002). Dissertation on: Trasncriptional regulation of the Human Prostatic Acid Phosphatase gene, p:15. Research Centre for

Molecular Endocrinology, Faculty of Medicine, University of Oulu, Oulu, Finland

Siddiqua, A., M. Rehmat, Asma Saeed, S. Amin, R. Naz, M. Sherazi, G.M. Khan and A. Saeed, (2008). Acid phosphatase from the liver of *Labio rohita*: Purification and characterization. Biol. Pharm. Bull., 31:802-808

Siddiqua, A., Asma Saeed, R. Naz, M. Sherazi, S. Abbas and Ahmad Saeed, (2012). Purification and biochemical properties of acid phosphatase from rohu fish liver. Int. J. Agri. Biol., Biol.,14(2):223-228

Stubberud, H.E., T.G.Honsi and J. Stenersen, (2000). Purification and partial characterization of tentatively classified acid phosphatase from earthworm *Eisenia veneta*. Comp. Biochem. Physiol., 126B:487-494

Suter,A., V. Everts, A. Boyde and S.J. Jones, (2001). Overlaping Functions of lysosomal acid phosphatase (LAP) and tartrate-resistant acid phosphatase (Acp5) revealed by doubly deficient mice. Development, 128:4899-4910

Tabaldi, L.A., R. Ruppenthal, L.B. Pereira, D. Cargnelutti, J.F. Goncalves, V.M. Morsch and M.R.C. Schetinger, (2008). Presence of multiple acid phosphatase activity in seedlings of cucumber, raddish and rocket salad, Ciencia Rural. Santa Maria, 38:650-657

Tazisong I.A., Senwo Z.N. and He Z. (2015). Phosphatase hydrolysis of organic phosphorus compound. Adv. Enzy. Resea. 3:39-51

Vasileva-Tonkova E., Galabova D.N., Balasheva M.A. and Sotirova A.V. (1993). Purification and partial characterization of acid phosphatase from *Candida lipolytica*. J. Gener. Microbiol., 139:479-483

Vincent, J.B., M.W. Crowder and B.B. Averill, (1992). Hydrolysis of phosphate monoester: a biological problem with multiple chemical solution. Trends Biochem. Sci., 17:105-110

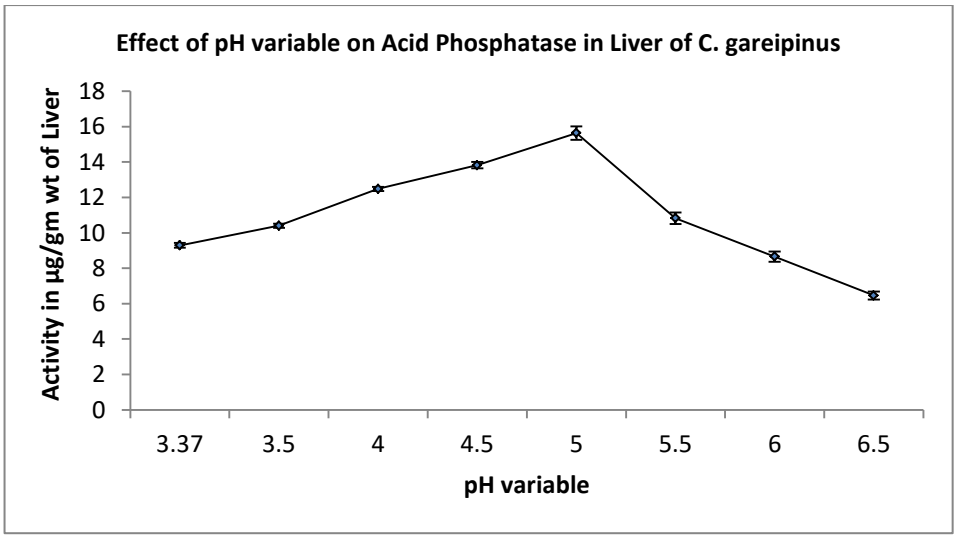

Fig.1- Effect of pH on Acid phosphatase in Liver of *C. gariepinus*

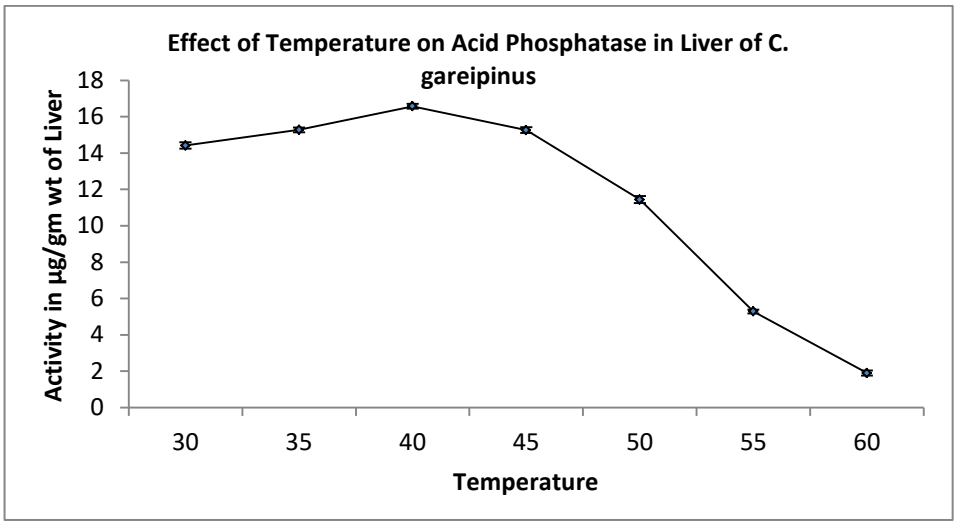

Fig.2- Effect of Temperature on Acid phosphatase in Liver of *C. gariepinus*

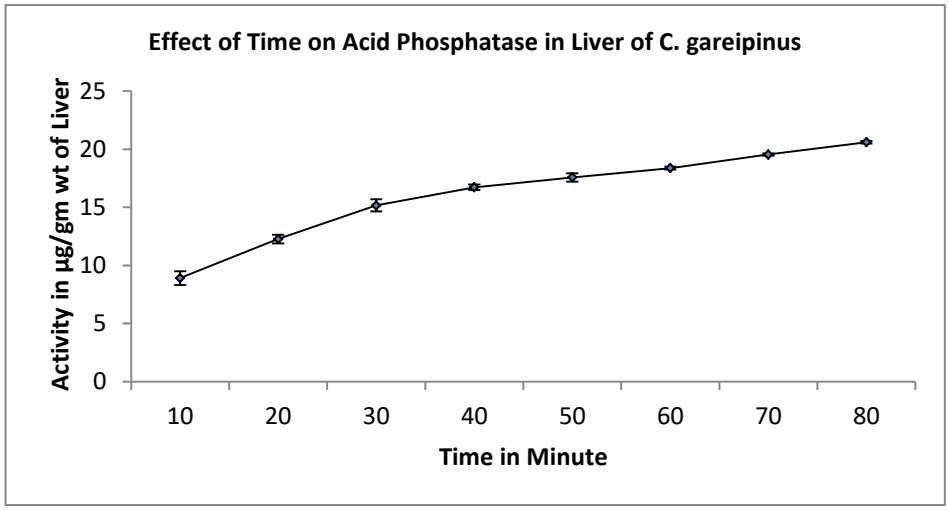

Fig.3-Effect of Time on Acid phosphatase in Liver of *C. gariepinus*

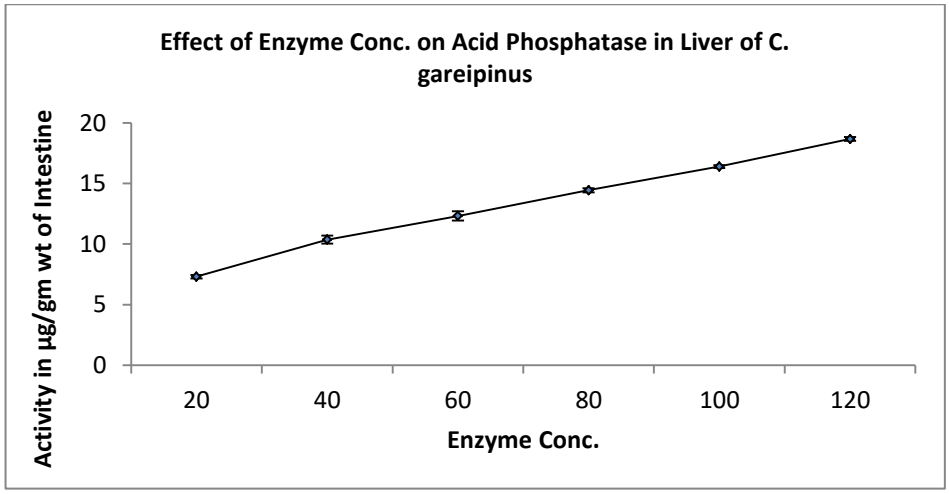

Fig.4- Effect of Enzyme concentration on Acid phosphatase in Liver of *C. gariepinus*

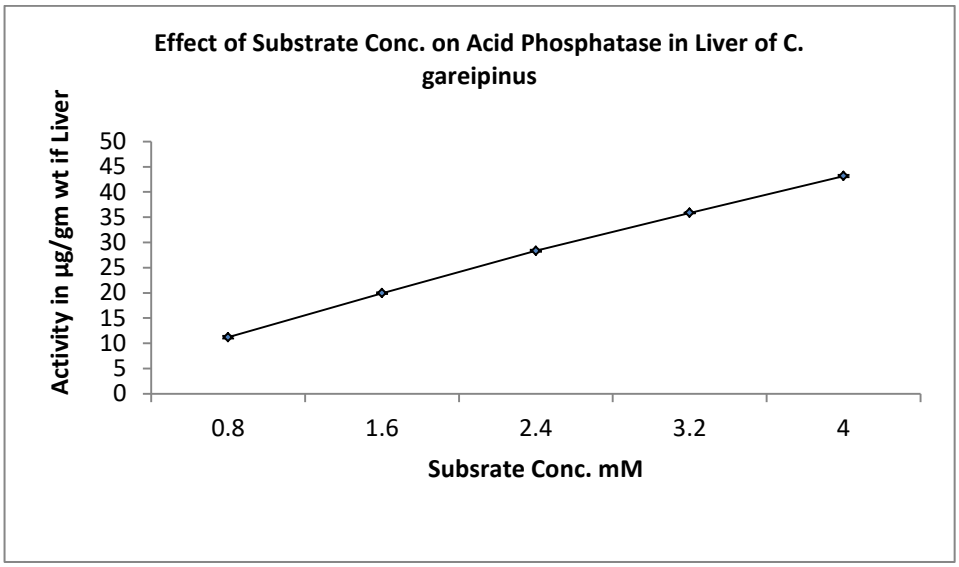

Fig.5- Effect of Substrate concentration on Acid phosphatase in Liver of *C. gariepinus*

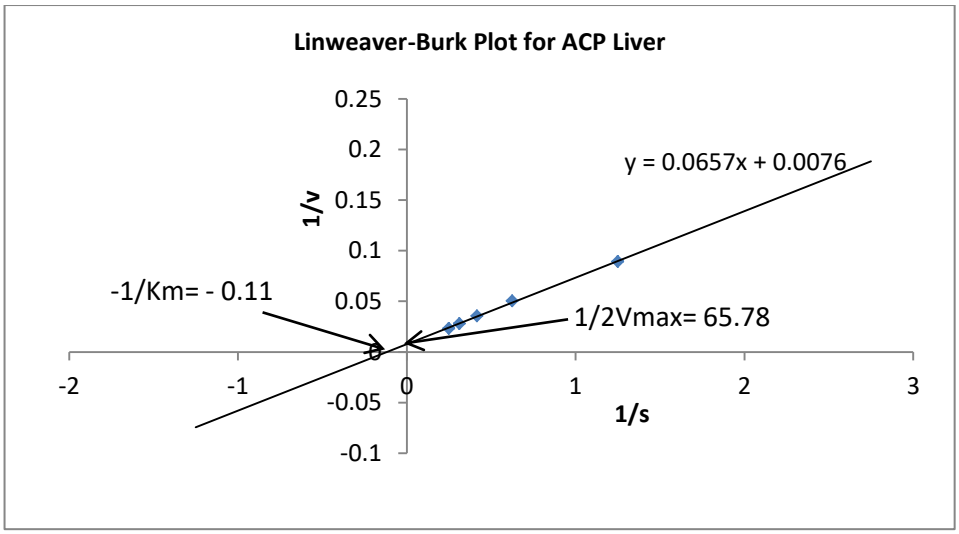

Fig.6- Lineweaver-Burk double reciprocal plot of 1/v versus 1/s showing straight line intercepting Y-axis giving the value 0.0076 µg/g wt. and the X-intercept -0.11 mM^{-1} with Vmax 131.57 µg/g wt. and Km 8.64 mM at pH 5.0 and temperature 38^{0} C.