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Investigating the inhibitory effects of hydro alcoholic extracts of *Aloe Vera, Anethum graveolens* and *Kelussia Odoratissma* on acid phosphatase derived from rat liver.

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ABSTRACT

Acid phosphatase called orthophosphoric-monoester phosphohydrolase (Ec: 3.1.3.2) is produced by plants and microbes and is a group of enzymes which catalyze hydrolysis of a variety of orthophosphate esters reactions in acid medium. This study aims to comparatively investigate the inhibiting effect of the three extracts Aloe Vera, Anethum graveolens, and Kelussia Odoratissma on the activity of acid phosphatase. This was an experimental in vitro study. Acid phosphatase extracted from rat liver was partially purified by successive chromatography based separating technique on the sephadex G50. This enzyme was purified and its final specific activity was 479.35 U/mg. The inhibiting effect of the three extracts on the activity of acid phosphatase was determined. All three tested plant extracts inhibited the activity of the enzyme acid phosphatase. *Aloe Vera* showed the most inhibiting value (82.57%), follwed by *Kelussia Odoratissma* (5.6%) and *Anethum graveolens* (4.07%). The results of this study show that all *Aloe Vera* can extract the acid phosphatase enzyme activity, and reduce plant extracts.

Key words: Enzyme Acid phosphatase, *Aloe Vera, Anethum graveolens, Kelussia Odoratissma*, Inhibiting.

INTRODUCTION

Enzymes are catalysts which accelerate the rate of reaction trough providing an alternative reaction pathway which requires less energy than uncatalyzed reaction ([1]). As reacting molecules bind to the catalysts surface, they are oriented in a manner which increases the likelihood of product formation. As products are formed, they leave absorption sites, which are then available for other reaction molecules ([2]).

The difference between inorganic catalysts and enzymes is directly related to their structure. In contrast to inorganic catalysts, each type of enzyme molecule contains a unique, intricately shaped binding surface called an active site. Reaction molecules called substrates, bind to the enzymes active sit, which is typically a small cleft or crevice of an otherwise large protein molecule ([3]).

The "lock–and key model" assumes that the enzyme's active site has a rigid structure that is complementary in structure to the substrate. In the "induced–fit model" the structure of protein is taken into account. In this model, substrate does not fit precisely into a rigid active site. Instead, non – covalent interaction between the enzyme and substrate cause a change in the three – dimensional structure of the active site. As a result of these interaction, the shape of the active site conforms to

the shape of the substrate ([4]4. Sigman DS. Mooser G., Clinical studied of enzyme active site, Ann. Rev. Biochem., 44: 889, 1975. ,[5]). The thousands of enzyme – catalyzed chemical reactions which occur in living cells are organized into a series of biochemical pathways.

Regulation is essential for the following: maintenance of an ordered state, conservation of energy, and responsiveness to primarily by adjustments in the concentration and activities of certain enzymes ([6]). Control is accomplished by utilizing various combination of the following mechanisms: genetic control, covalent modification, allosteric regulation, and compartmentalization ([7]). Each enzyme is classified and named according to the type of chemical reaction it catalyzes. The six major enzyme categories are Oxidoreductases, Transferases, Hydrolase, Lyases, Isomerases, and Ligases ([8]). Hydrolase enzymes catalyze the hydrolysis of their substrates by adding constituents of water across the bond they split. The substrates include ester, glycosyl, ether, peptide, acid-anhydride, C-C, halide and P-N bonds ([9]). Phosphatases are hydrolase enzyme and one of the most important animal enzymes. They act as enzyme bound to the cell membrane and its role is dephosphorylation of organic phosphate into inorganic phosphate ([10]). Phosphatase process depending on the optimum pH for enzyme activity divided into two categories of acid phosphatase and alkaline phosphatase. More than pH=7, is suitable for alkaline phosphatase and less than 7 is suitable for acid phosphatase activity (29). Enzymes acid phosphatase has several isoenzymes acting as common enzymes, breaking down hydrolytic of monophosphate esters. The purpose of this enzyme is hydrolysis a chemical bond.

Acid phosphatase is a protein polymer and its spatial structure after positioning the substrate in the active site- Paranitrophenol phosphate (PNPP) - will active the relevant reactions. Acid phosphatase can target bind phosphate groups and break the link between them ([11]11. Jencks, WP: Mechanism of enzyme action. During recent years several non-pharmaceutical treatments have been developed for different diseases (12-17). Herbal medicine is one of the main categories of such treatments. This study aims to comparatively investigate the inhibiting effect of the three extracts Aloe Vera, Anethum graveolens, and Kelussia Odoratissma on the activity of acid phosphatase.

Reversible Enzyme Inhibition

A reversible inhibitor dissociates very rapidly from its target enzyme because it becomes very loosely bound with the enzyme. Three general types of reversible inhibition are distinguished: competitive, non-competitive and UN competitive (18).

Phosphatase Enzyme

Studies on Phosphatase enzymes date from the early 1900's. They are one of the most ubiquitous enzymes and have been found in yeasts, molds, seeds, of higher plants, fruits and in the many animal tissues such as in prostate gland of man and monkeys , kidney, liver, spleen, erythrocytes, blood plasma, pig ailanthic fluid , etc (19).

Previously it was though that the difference between alkaline phosphatase and acid phosphatase rests only in the fact that they have completely different pH ranges.

However experiments using S- substituted monoesters of phosphorothioic acid and O-substituted monoester of orthophosphoric acid have shown that two hydroxyl groups are essential for activity of alkaline phosphatase whereas acid phosphatase an oxygen linkage and cannot be substituted by sulphur. Moreover acid phosphatase are not inhibited by metal complexing agents in contrast to alkaline phosphatase. However the structural and molecular properties of acid phosphatase are remarkably obscure compared with those of alkaline phosphatase. The acid and alkaline phosphatases proceed by way of phosphohistidine and phosphoserine E-P intermediates, respectively (20).

Acid phosphatase

Acid phosphatase or orthophosphoric monoester phosphohydrolases (Ec: 3.1.3.2) are a group of enzymes which catalyze hydrolysis of a variety of orthophosphate esters as well as transphosphorylation reactions in acid medium.

They are basic glycoproteins having broad and overlapping substrate specificities and so have been termed as acid phosphatases phosphoprotein phosphatases or ATPase.

Acid phosphatase catalyses an apparent transition state displacement and P-O cleavage.

The enzyme dephosphorylates phosphoproteins such as phosphocase in and auto phosphorylated protein kinas phosvitin, the egg yolk storage protein which contains phosphorylated serine. Potato acid phosphatase has a high activity and specificity for P-Tyr acid phosphatase are also found in poppy seed, wheat, maize seedling, etc. This suggests that the enzyme could function as a P-Tyr protein phosphatases in vivo (26).

Localization of Acid Phosphatases

According to de Duve (1969) acid and alkaline phosphatases are localized in distinct cell compartments. Using the Gomori technique for detecting phosphatases, cytochemists have studied mainly the cellular localizations of these hydrilases (27).

MATERIALS AND METHODS

One of the major problems in any assay for acid phosphatases is that that enzyme is subject to surface inactivation. Moreover there are discrepancies between the amount of inorganic phosphate produced and phenol liberated from phenolic phosphates if extensive phosphotransferase activity occurs (18).

The most common method employed by investigators to determine the activity of acid phosphatases makes use or p-nitrophenyl phosphate as the substrate. The rate of spectrophotometrically at 400 nm. Various references using different sources can be cited such as acid phosphatases from sweet potato (19), different plant and mammalian sources (20), red kidney bean (21), potato (22), uteroferrin (23), soybean (18), cultured tobacco cells (18) and beef spleen (19). The enzyme activity for a number of other phosphorylated compounds was determined by measuring the rate of liberation of orthophosphate by the method of Chen et al (24).Due to the non-specific nature of acid phosphatases, various substrates such as sodium-B-Glycerophosphate, inorganic pyrophosphate, ATP, metaphosphate, phenyl phosphate etc. A spectrofluorometric method using Alpha – naphthyl phosphate as substrate has been devised for estimation of acid phosphatase from prostate gland (19).

Isolation and purification

Acid phosphatase has been purified to homogeneity from several microbial, plant and animal sources (Initial steps of purification involved the use of conventional techniques of protein purification such as acetone / ethanol extraction, ammonium sulphate precipitation low salt dialysis, gel permeation.

Dialysis

Dialysis was performed in the dialysis bags, made out of cellophane casting, placed in distilled water at the appropriate temperature. Prior to use, the bags were soaked in sodium bicarbonate (1.0 % W/W) and EDTA (0.15%, W/W) at 70-80 co. for thirty minutes and then thoroughly washed with water. Rubber tags, treated in the same manner, were used to tie end of the dialysis bags.

Protein estimation

The protein was determinate by Bradford's method (REf). Using Bovine serum albumin as standard. The results of a typical experiment are shown in figure 1.

Chromatography

A glass column, (2.5 x L 45.0 cm) with a capacity of 210.0 ml, was packed with Sephadex G_{50} and equilibrated by pumping degasses normal saline (at 12ml/h) at 4 oc for ten hours. The void volume (VO) was determination by blue dextran 2000. The absorbance of the blue dextran 2000 was determinated at625nm. The total volume (VT) was determinate by bromophenol blue at 623nm. 3 ml of precipitate sulphate ammonium (after dialysis approximately 5 mg/ml) was loaded elute 3ml fraction. Acid phosphatase activity was checked for each collected fraction and the protein content monitored (spectrophotometrically) are 280 nm. The active fraction was pooled and freeze at 4 p C (Fig. 2).

Extraction Mountain celery, dill and Aloe Vera

Mountain celery (*Kelussia Odoratissma*), dill (*Anethum graveolens*) and Aloe Vera (*Aloe Vera*) were collected and identified in the Department of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences. Each of these plants are separately dry in shade and become powder. Forty-three g of each plant become wet in flask that containing solvent hydroalchohol (70% ethanol) like immersed powder.(Extraction by maceration or soaking), then all of them held for 72 hours in vitro and in that time, to avoid sinking, 4 to 5 times a day are well mixed. Then filtered extract by filter paper. The resulting material located in oven for 72 h at 37 ° C, then extracts obtained until the test in temperature of - 20 ° C at the freezer.

Enzyme detection study

Assay acid phosphatase activity

Precipitate sulphate ammonium (after dialysis) and fraction's chromatography are tested for presence of acid phosphatase activity. Assay acid phosphatase activity, 200 µl substrate PNPP (0.1M pH 6.8), phosphate buffer was incubated with 100 µl precipitate sulphate ammonium (after dialysis) or fraction's chromatography for 1hr. 3ml of NaOH was added to arrest the reaction.

Effect of concentrations of ethanol extract of Aloe Vera, dill and Celery Mountain on acid phosphatase activity:

One hundred µl of ethanol extract of Aloe Vera, dill and Celery Mountain incubated in room temperature with 100 µl fraction's chromatography for 16hr. then 100 µl substrate PNPP (0.1M pH 6.8), was incubated with above solution for 1hr. 3ml of NaOH was added to arrest the reaction .

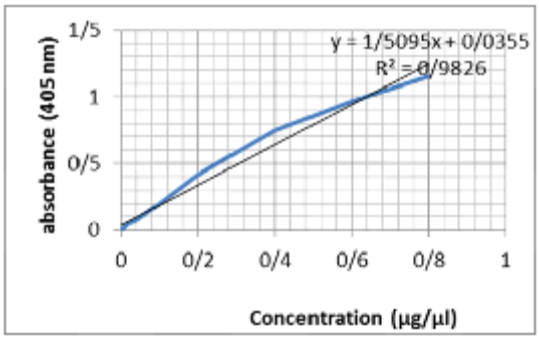

Fig. 1: Calibration Curve for Bovine serum Albumin at 280 (Bradford's method)

RESULT

Isolation of acid phosphatase

Isolation of acid phosphatase activity from Rat's Liver was blended in normal saline. N- Butanol treatment was then to the slurry and acetone precipitate. This procedure removes lipids and coloring matter which are likely to interference during extraction and ammonium sulphate fractionation.

Gel – filtration of Precipitate sulphate ammonium (after dialysis) of Rat's Liver on Sephadex G_{50}.

Attempts were made to resolve acid phosphatase activity. Precipitate sulphate ammonium (after dialysis) on Sephadex G_{50}.

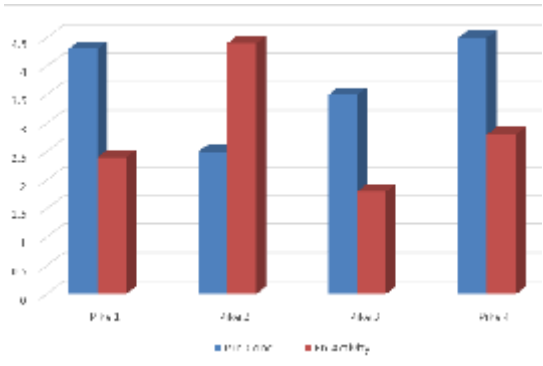

Fig. 2: filtration profile of acid phosphatase activity on Sephadex G_{50}

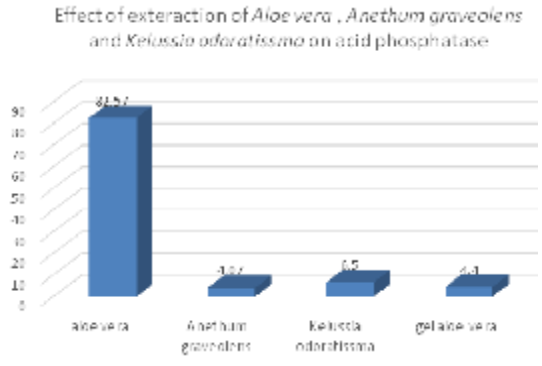

Fig. 3: Effect of *Aloe Vera*, *Anethum graveolens* and *Kelussia Odoratissma* on acid phosphatase

Analysis of the fractions for enzymes activity revealed the presence of only acid phosphatase. Acid phosphatase, has been partial purified by successive chromatography separations on sephadex G50. This enzyme was purified and a final specific activity of 479.35 U/mg.

Effect of Mountain *celery*, *dill,* and *Aloe Vera* on acid phosphatase activity

In this experiment, activity of acid phosphatase on the absorption changes was studied in different concentrations of hydro-alcoholic extract of aloe Vera, dill and celery radish (as inhibitor). The results of this study showed that with increasing concentration of *Aloe Vera* from 1 to 10 mg per hundred milliliters reduce 82.57% activity of acid phosphatase. With increasing concentration of *Dill* from 1 to 10 mg per hundred milliliters, reduce 4.07%activity and increase concentration of Celery Mountain reduce 6.5% of enzyme activity was. In the event that with *aloe Vera gel* with a concentration of 5 mg, 4.4% of enzyme activity was reduced (Figure 3).

DISCUSSION AND CONCLUSION

As respect that *Aloe Vera* extract reduce 82.57%, *Dill* extract 4.07%, *Celery mountain* extract 6.5% and *Aloe Vera gel* 4.4%, the amount of acid phosphatase activity; The plant that reduce more activity of acid phosphatase could recommend for prevent degradation phosphatic compounds by acid phosphatase in those diseases that which increased amount of acid phosphatase. Disease that increase amount of acid phosphatase are include: Bone Diseases, Prostate Infarctions, Prostatitis and Benign Prostatic Hyperplasia (BPH), Myelocytic leukemia, Gaucher disease and Niemen-pick disease, Prostate carcinoma, Multiple Myeloma, Paget's Disease, Sickle Cell Crisis, Recent Manipulation of the Prostate, Prostatitis, Breast and Bone cancer, Cirrhosis, Parathyroid Abnormalities, Thrombocytosis and Cancer Metastasis to Bone. The diseases listed as acid phosphatase makes removing phosphate from the phosphate compounds, creates many problems, including loss of phosphate from bone which increases bone disease and also by dephosphorylation of some enzymes, acid phosphatase causes more active and dephosphorylation make them disabled that creates many problems in vivo. On the other side excessive acid phosphatase increased too much phosphate in the blood and cells and the buffer set disrupted. The study showed that using *Aloe Vera* extract reduce 82.57% acid phosphatase activity and can control activity of acid phosphatase in the above diseases.

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