Effect of compounds 3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid ,2,3-di (acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid and 2,3,5,6-Tetra(acetyl Salicyloyl)-*L*-ascorbic acid on acid and Alkaline phosphatase activities in serum of different cancer patients.

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Abstract:

This research included a study the effect of some ascorbic acid derivatives on acid and alkaline phosphatase activitities . Blood samples have been taken from patients of different types of cancer after been diagnosis .The results revealed that the derivatives have an activation effect on the activity of acid and alkaline phosphatase in all concentrations and the activity percent increased as the concentrations of the derivatives were increased.

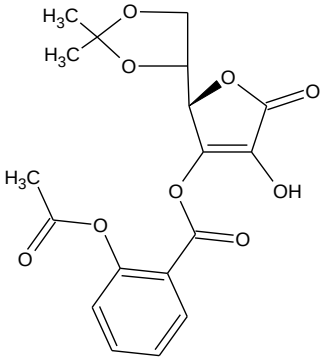
Introduction:

Alkaline phosphatase [EC 3.1.3.1];orthophosphoric monoester phosphohydrolase (alkaline optimum) ALP) catalyzes the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates.ALP is present in practically all tissues of the body,especially at or in the cell membranes, and it occurs at particularly high levels in intestinal epithelium, kidney tubules,bone,liver,and placenta.Several isoenzymes are knomn to exhibit optimal activity at a pH of about 10 in vitro, but the optimum pH and the activity observed vary with the nature and concentration of the substrate on which the action takes place ,the type of buffer or phosphate acceptor present ,and to some extent the nature of the isoenzymes.Although the exact metabolic function of the enzyme is not yet understood ,the enzyme appears to be associated with lipid transport in the intestine and the calcification process in bone.

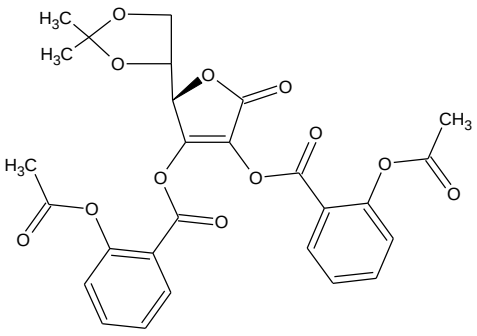
Under the name acid phosphatase (ACP) are included all phosphatases with optimal activity below a pH of 7.0 Thus the name refers to a group of simlar or related enzymes rather than to one particular enzyme species .However ,the ACP of greatest clinical importance[EC 3.1.3.2];orthophosphoric monoester phosphohydrolase[acid optimum] ;ACP) is the one derived from the prostate that has a pH optimum in the range of 5 to 6 .ACP is present in lysosomes,which are organelles in all cells, with the possible exception of erythrocytes. Extralysosomal ACPs allso are present in many cells.The greatest concentrations of ACP activity occur in the liver ,spleen,milk ,erythrocytes,platelets,bone marrow,and the prostate gland.The optimum pH for the indivdual ACPs varies ,depending on the tissues from which they are obtained .The observed pH optimum allso varies with the substrate on which the enzyme acts,the more acidic the substrate, the lower the pH at which maximum activity is obtained.In practice,differentiation specifically between increases in the concentration of the prostatic and nonprostatic forms is necessary . Certain inhibitors enhance the discriminaton between prostatic and non prostatic ACPs.For example ,the prostatic enzyme is inhibited strongly by dextrorotatory tartrate ions,wherase the erythrocyte isoenzym is not .Erythrocyte ACP is inhibited by formaldehyde and cupric ions , to which prostatic ACP is resistant .Thus these inhibitors, particularly tartrate , allow a deistinction to be made between prostatic and erythrocyte ACPs .Slight or moderate elevation in total ACP activity often occure in individuals with Paget's disease,in those with hyperparathyroidism with skeletal involvment,and in the presence of malignant invasion of the bones by cancers, such as breast cancer in women [1,2,3,4,5,6].

Experimental:

compounds 3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (1) ,2,3-di(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (2) and 2,3,5,6-Tetra(acetyl Salicyloyl)-*L*-ascorbic acid (3) were synthesized and identification according to the literature[6,7,8]



(1)



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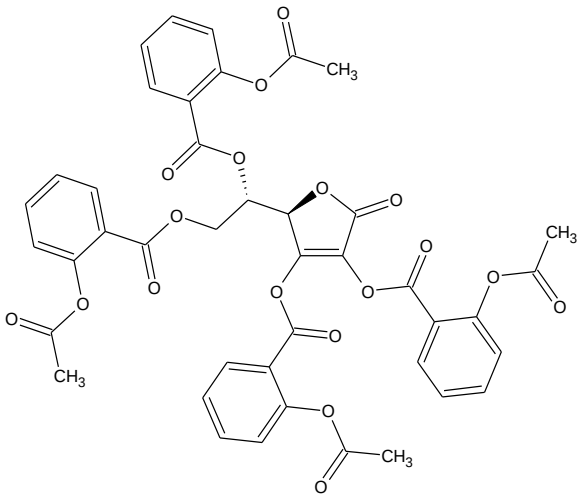

(3)

Determination of Alkaline phosphatase (ALP) activity:

Colorimetric determination of alkaline phosphatase activity was carried out according to the following reaction:

$$\text{Phenyl phosphate} \xrightarrow[\text{pH 10}]{\text{Alkaline phosphatase}} \text{phenol} + \text{phosphate}$$

The phenol liberated is measured in the presence of amino 4-antipyrine and potassium ferricyanide.The presence of sodium arsenate in the reagent stops the enzymatic reaction

The ALP activity was measured in serum according to the method of Kind and Belfleld .[8,9]

Procedure :

1 : Reagent :

Reagent No. 1 Substrate Buffer	Disodium phenyl phosphate Carbonate-bicarbonate buffer pH 10	5 mmol/L 50mmol/L
Reagent No. 2 standard	Phenol	Equal 20 kind and king U
Reagent No. 1 Inhibitor	Amino-4-antipyrine Sodium arsenate	60 mmol/L 75 g/L
Reagent No. 1Color reagent	Potassium ferricynide	150mmol/L

1-Assay :

Set up the following tubes

Table (1): measurement of total ALP activity in serum

	Serum Sample	Serum blank	Standard	Reagent blank
R1	2ml	2ml	2ml	2ml

Incubate for 5 min at 37^{0}C

Serum	50µl	-	-	-
R2	-	-	50µl	-

Mix , incubate for exactly 15 min at 37^{0}C

R3	0.5ml	0.5ml	0.5ml	0.5ml

Mix well

R4	0.5ml	0.5ml	0.5ml	0.5ml
Serum	-	50µl	-	-
Distilled water	-	-	-	50µl

Mix, let for 10min in the dark .

Measurement at 510 nm against reagent blank ,the color intensity is stable for 45 min.

$$\textbf{Calculation:} \quad \frac{\textbf{A}\text{ serum sample} - \textbf{A}\text{serum blank}}{\textbf{A}\text{Std}} \times 20$$

Normal Range : Children : 10-20 KAU / dl

Adults : 3-13 KAU /dl

2 : Determination of Acid phosphatase (ACP) activity

Solutions :

1- Buffer solution: Citrate buffer(5.5 mmol /L) , pH = 4.8

2- Substrate : ρ – nitro phenyl phosphate (5.5 mmol/L)

3- Sodium tartrate (200 mmol/L)

4- NaOH (200mmol/L)

5- The contents of bottle 2 (substrate) were reconstitute with 10 ml buffer (1).They were stable for (5)days at +2 to +8 ^{0}C

6-Sodum hydroxide was diluted (10 ml NaOH + 90 ml distilled water)

1- procedure of assay :

The following tubes were set up as follow :

Table (2): measurement of total ACP activity in serum

	Reagent blank	Sample 1	Sample 2
Substrate (2)	1.0 ml	1.0ml	1.0ml
Tartrate (3)	-	-	0.1 ml

Incubate for 5 min at 37^{0}C

Serum	-	0.2 ml	0.2 ml

Incubate exactly for 30 min. at 37^{0}C

Dilute NaOH	10 ml	10 ml	10 ml
Serum	20 ml	-	-

Mix , read the absorbance of the sample against the reagent blank at 405 nm .

Calculation :

Total acid phosphate : 101X A Sample 1

Prostatic acid phosphates: 101X (A Sample 1– A Sample 2)

Normal value :

Total acid phosphate : up to 11 u/L

Prostatic acid phosphates: up to 4 u/L

Effect of the new compounds (1), (2), (3) on the Alkaline phosphates(ALP) activity in patient's serum:

The effect of the new compounds were calculated at fixed concentrations (5.2×10^{-4} M)for (1) , (3.7×10^{-4} M) for (2) and (2.42×10^{-4} M) for (3) . The different concentration of the compounds were prepared by serial dilution in DMSO from the stock solution (0.5gm/25ml) . The measurement of enzyme activity was determined by the method described, (1 ml) from each compound was added to the substrate buffer .

Effect of the new compounds (1) , (2) , (21) on the Acid phosphates (ACP) activity in patient's serum :

The effect of the new compounds were calculated at fixed concentrations (5.2×10^{-4} M)for (1) , (3.7×10^{-4} M) for (2) and (2.42×10^{-4} M) for (3) . The different concentration of the compounds were prepared by serial dilution in DMSO from the stock solution (0.5gm/25ml) . The measurement of enzyme activity was determined by the method described, (1 ml) from each compound was added to the substrate buffer .

Result and discussion :

Alkaline phosphatase and acid phosphatase:

Compounds 3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (1) , compound 2,3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (2) , 2,3,5,6 -

(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (3) was found to have activation effect on activity of (ALP) ,(ACP) .

These counpuonds were not useful to treatment cancer patients because they increase the activity of ALK and ACP (the activity of ALK and ACP in this case were higher and these compounds were increase the activity)

The activation effect of the 3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (1), 2,3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (2) ,2,3,5,6-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (3) on (ALP) and (ACP) is shown in table (3 and 4):

Table (3): Effect of 3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (1) , 2,3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (2) , 2,35,6-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (3) on Alkaline phosphatase activity. in patients

Subject	**Enzyme Activity** KAU/dl Without any compounds	**Enzyme Activity** KAU/dl Compound (1)	**Enzyme Activity** KAU/dl Compound (2)	**Enzyme Activity** KAU/dl Compound (3)
Breast cancer	71.23±3.66	152.33±6.8	211±7.5	271.24±8
Lung cancer	33.25±2.5	71.25±4.16	102.2±5	145.60±6.2
Leukemia	37.81±3	77.3±4.85	115.42±5.5	162.47±6.5
Prostate cancer	77.43±4	164.2±7	228.2±8	281.55±8.5

Table (4): Effect of 3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (1) , 2,3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (2) , 2,35,6-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (3) on Acid phosphatase activity. in patients

Subject	**Total acid pho.** Without any compounds	**Total acid pho.** Compound (1)	**Total acid pho.** Compound (2)	**Total acid pho.** Compound (3)
Breast cancer	49.21±2.5	68.82±3.2	78.56±3.5	83.24±4
Lung cancer	22.42±1.5	29.51±1.16	34.33±1.5	44.60±2.2
Leukemia	27.11±1.5	32.3±1.85	39.11±1.5	48.47±2.5
Prostate cancer	44.30±2.5	61.85±3	82.64±3.8	89.55±4.5
Subject	**Prostatic acid pho.** Without any compounds	**Prostatic acid pho.** Compound (1)	**Prostatic acid pho.** Compound (2)	**Prostatic acid pho.** Compound (3)
Breast cancer	15.6±1.5	22.82±2	31.56±2.5	42.24±2.5
Lung cancer	7.42±0.5	11.51±1	19.33±1.5	28.60±2.2
Leukemia	9.11±1	16±1.85	21.11±1.5	33.47±2.5
Prostate cancer	13.30±1.5	18.85±3	28.64±3.8	39.55±2.5

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تاثير مركبات ٣-(اسيتايل ساليسلويل)-٥،٦- ايزوبروبايلدين- اسكوربك اسد ،٢،٣- داي-(اسيتايل ساليسلويل)-٥،٦- ايزوبروبايلدين- اسكوربك اسد و ٢،٣،٥،٦-رباعي -(اسيتايل ساليسلويل)-٥،٦- ايزوبروبايلدين- اسكوربك على فعالية انزيمي الفوسفتيز القاعدي والحامضي في مصل المرضى المصابين بانواع مختلفة من السرطان

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الملخص:

تم في هذه الدراسة دراسة تأثير بعض من مشتقات حامض الاسكوربيك على فعالية انزيم الفوسفيتيز القاعدي و الحامضي. تم اخذ عينات مصل الدم من اشخاص مصابين بانواع مختلفة من مرض السرطان بعد اخذ المعلومات الكاملة عن حالتهم الصحية. اظهرت الدراسة ان للمركبات المختبرة تاثيرا منشطا على فعالية انزيمي الفوسفتيز القاعدي والحامضي وبجميع التراكيز المختبرة حيث كانت النسبة المئوية للتنشيط تتناسب طرديا مع تركيز المادة المختبرة .