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 activities. 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 known 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 similar 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. Extramyosomal ACPs also 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 individual ACPs varies depending on the tissues from which they are obtained. The observed pH optimum also 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 discrimination between prostatic and nonprostatic ACPs. For example, the prostatic enzyme is inhibited strongly by dextrorotatory tartrate ions, whereas the erythrocyte isoenzyme is not. Erythrocyte ACP is inhibited by formaldehyde and cupric ions, to which prostatic ACP is resistant. Thus these inhibitors, particularly tartrate, allow a distinction to be made between prostatic and erythrocyte ACPs. Slight or moderate elevation in total ACP activity often occurs in individuals with Paget’s disease, in those with hyperparathyroidism with skeletal involvement, 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).
Determination of Alkaline phosphatase (ALP) activity:
Colorimetric determination of alkaline phosphatase activity was carried out according to the following reaction:

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

The phenol liberated is measured in the presence of amino 4-antipyrene 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 Belfield. 

Procedure:
1: Reagent:
- Reagent No. 1: Substrate Buffer
- Disodium phenyl phosphate
- Carbonate-bicarbonate buffer pH 10
- 5 mmol/L
- 50 mmol/L
- Reagent No. 2: Standard
- Phenol
- Equal to 20% of Kind and King U
- Reagent No. 1: Inhibitor
- Amino-4-antipyrene
- Sodium arsenate
- 60 mmol/L
- 75 g/L
- Reagent No. 1: Color reagent
- Potassium ferricyanide
- 150 mmol/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°C

Mix well, incubate for exactly 15 min at 37°C

| R3 | 0.5ml | 0.5ml | 0.5ml | 0.5ml |

Mix, let for 10 min in the dark.
Measurement at 510 nm against reagent blank, the color intensity is stable for 45 min.

Calculation:

\[
A_{\text{serum sample}} - A_{\text{serum blank}} \times 20
\]

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: \( \rho \) - nitrophenyl phosphate (5.5 mmol/L)
3- Sodium tartrate (200 mmol/L)
4- NaOH (200 mmol/L)
5- The contents of bottle 2 (substrate) were reconstituted with 10 ml buffer (1). They were stable for (5) days at +2 to +8°C
6- Sodium 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 | 1.0 ml | 1.0 ml |
| Tartrate (3) | - | - | 0.1 ml |

Incubate for 5 min at 37°C
Incubate exactly for 30 min at 37°C
Dilute NaOH
20 ml
20 ml
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 phosphatases (ALP) activity in patient's serum:
The effect of the new compounds were calculated at fixed concentrations (5.2x10^{-3} M) for (1), (3.7x10^{-4} M) for (2) and (2.42x10^{-3} 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), (3) on the Acid phosphates (ACP) activity in patient's serum:
The effect of the new compounds were calculated at fixed concentrations (5.2x10^{-3} M) for (1), (3.7x10^{-4} M) for (2) and (2.42x10^{-3} 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 compounds 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), 2,3,5,6-(acetyl Salicyloyl)-5,6 –O-isopropylidene-L-ascorbic acid (3) on Alkaline phosphatase activity. in patients

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

**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), 2,3,5,6-(acetyl Salicyloyl)-5,6 –O-isopropylidene-L-ascorbic acid (3) on Acid phosphatase activity. in patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total acid pho. Without any compounds</th>
<th>Total acid pho. Compound (1)</th>
<th>Total acid pho. Compound (2)</th>
<th>Total acid pho. Compound (3)</th>
</tr>
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<tbody>
<tr>
<td>Breast cancer</td>
<td>49.21±2.5</td>
<td>68.82±3.2</td>
<td>78.56±3.5</td>
<td>83.24±4</td>
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<td>Lung cancer</td>
<td>22.42±1.5</td>
<td>29.51±1.16</td>
<td>34.33±1.5</td>
<td>44.60±2.2</td>
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<tr>
<td>Leukemia</td>
<td>27.11±1.5</td>
<td>32.3±1.85</td>
<td>39.11±1.5</td>
<td>48.47±2.5</td>
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<tr>
<td>Prostate cancer</td>
<td>44.30±2.5</td>
<td>61.85±3</td>
<td>82.64±3.8</td>
<td>89.55±4.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Prostatic acid pho. Without any compounds</th>
<th>Prostatic acid pho. Compound (1)</th>
<th>Prostatic acid pho. Compound (2)</th>
<th>Prostatic acid pho. Compound (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>15.6±1.5</td>
<td>22.82±2</td>
<td>31.56±2.5</td>
<td>42.24±2.5</td>
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<tr>
<td>Lung cancer</td>
<td>7.42±0.5</td>
<td>11.5±1</td>
<td>19.33±1.5</td>
<td>28.60±2.2</td>
</tr>
<tr>
<td>Leukemia</td>
<td>9.11±1</td>
<td>16±1.85</td>
<td>21.11±1.5</td>
<td>33.47±2.5</td>
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<tr>
<td>Prostate cancer</td>
<td>13.30±1.5</td>
<td>18.85±3</td>
<td>28.64±3.8</td>
<td>39.55±2.5</td>
</tr>
</tbody>
</table>
Reference:
(2) M.Elaine., Biochem.J. 244(1987)725
(9)M.Vandekar,WHO/VBS,78,692(1978)

تأثير مركبات 3- (إستياتل ساليسنويل)- 6- ايزوبروبيلدين- اسكوريك أسد 0- 3- دي- (إستيتيل ساليسنويل)- 5- اوزوبروبيلدين- اسكوريك أسد و 5-3،5،3،6،5-رابعي- (إستيتيل ساليسنويل)- 5-0
ايوزوبروبيلدين- اسكوريك على فعالية إنزيمي الفوسفيتز القاعدي والحامضي في مصل المرضى المصابين
بأنواع مختلفة من السرطان

فراس طاهر ماهر و سوزان جميل علي
كلية العلوم، جامعة تكريت، تكريت، جمهورية العراق
كلية التربية، جامعة تكريت، تكريت، جمهورية العراق

الملخص:
تم في هذه الدراسة دراسة تأثير بعض من مضادات حمض الأسكوريك على فعالية إنزيمي الفوسفيتز القاعدي والحامضي. تم اخذ عينات مصل الدم من
أشخاص مصابين بأمراض مختلفة من مرض السرطان بعد اخذ المعلومات الكاملة عن حالاتهم الصحية. أظهرت الدراسة أن المركبات المختبرة تاثيراً مشطعاً
على فعالية إنزيمي الفوسفيتز القاعدي والحامضي. وجميع الترتيبات المختبرة حيث كانت النسبة الملونة للتنشيط تتناسب طريقاً مع تركيز المادة المختبرة.