Effect of some ascorbic acid deriviteves on Acetyl Choline esterase (AChE) activities in different cancer patients.

Firas Taher Maher
College of Science , Tikrit University , Tikrit , Iraq
(Received 26 / 11 / 2007, Accepted 17 / 5 / 2009)

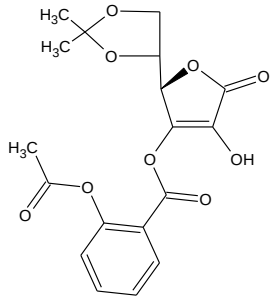
Abstract:
This research included a study the effect of some ascorbic acid derivatives on Acetyl Choline esterase (AChE) activities in different cancer patients activitities . Blood samples have been taken from patients of different types of cancer (Breast cancer, Lung cancer, Leukemia, Prostate cancer).The results revealed that the derivatives have an inhibition effect on the activity of Acetyl choline esterase activity .

Introduction:
Cholinesterase (choline esterase) EC 3.1.1.7), which is also referred to as plasma or serum cholinesterase, pseudocholinesterase, and butyrylcholinesterase, is synthesized by the liver and is present in plasma. refs Its true physiological function is unknown. Although it can hydrolyze acetylcholine, it is less specific for this substrate than is the red blood cell enzyme acetylcholinesterase its function may hydrolyze other choline esters. The enzyme activity is usually measured for one of two reasons. The most common reason is as an indicator of exposure to organophosphorus compounds, including many pesticides or reason is to identify the presence of inherited abnormal variants of cholinesterase[1,2,3,4]. Variants are identified by assaying both total activity and the extent of inhibition by either Dibucaine or fluoride; some of these variants lead to prolonged apnea in patients receiving the anesthetic succinylcholine drug. Measurement of acetylcholinesterase activity can be performed during amniotic fluid analysis for neural tube defects to confirm an elevated amniotic fluid alpha-fetoprotein (AFP) level[5].

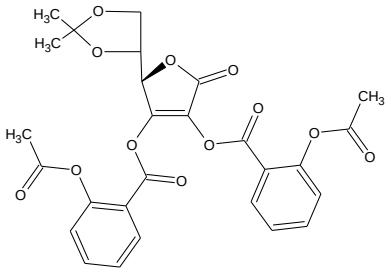
Since pesticide poisoning can present as an acute case, an assay for cholinesterase must be simple enough to be performed "stat" but accurate enough for determination of cholinesterase phenotyping. Most current assays are spectrophotometric and use propionylthiocholine or acetylthiocholine as substrate 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) is added to react with the released thiocholine to form the yellow compound 5-thio-2-nitrobenzoic acid (absorption maximum, 412 nm). The reactions may be followed as a rate or end-point procedure[6,7].

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 identified according to the literature[8,9,10].

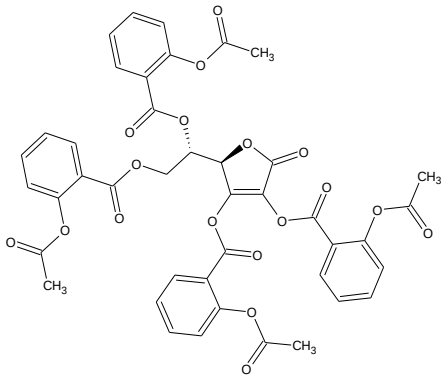
Sample
Samples from 20 patients (Blood serum) : four of them of Breast cancer; five lung cancer ;six of Leukemia; and the last five patients of Prostate cancer .The different cancer been taken from the medical center of cancer disease in Baghdad.



(1)



(2)



(3)

Determination of Choline Esterase (ChE) activity :

materials:

1- Buffer solution: The buffer solution (Phosphate buffer), (pH= 7.2, 0.2 M) was prepared by dissolving (2.89 gm) from (Na_2HPO_4) in (100 ml) distilled water (some drops of H_3PO_4 was added to fixe the pH) .

2- Indicator DTNB (Ellman's reagent) : The indicator DTNB (5,5-Dithio-2-nitrobenzoic acid , 0.001 M) was prepared by dissolving (0.01gm) DTNB (MWt = 396.36gm/mol) in 25 ml distill water , stirring , heating and a small amount of ($NaHCO_3$) was added to complete the dissolve . The solution was stored in amber flask to protect from light.

3- Substrate solution (S-Acetyl thiocholine Iodide) : (0.01735 gm) of (S-Acetyl thiocholine Iodide) was dissolved in (1 ml) distill water .

Procedure :

1- (2.25 ml) from the buffer solution was poured in the test tube, added (50 µL) of DNTB and (10µL) from the serum were added and mixed.

2- (2 ml) from solution (1), poured in the test tube, then (34µL) from substrate solution (3) was added, the difference in the wave length (412 nm) absorbance after and before adding was recorded in each (3 min.). The enzyme activity was calculated using (µmol/ml/min) unit.[11]

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

The effect of the new compounds were calculated at fixed concentrations (0.005gm/25ml)for (1) , (2) and (3) . The concentration of the compounds were prepared by serial dilution in DMSO from the stock solution (0.5gm/25ml) .

The measurement of enzyme activity was carried out using the method described in section (A procedure), by adding (1 ml) from the compound to the substrate buffer .The substrate concentration was (0.1 M)

The inhibition percentage was calculated by comparing the activity with and without using the test compound under the same conditions .

$$\% \text{ Inhibition} = \left[\frac{}{\text{The activity without inhibitor}} \right] \text{X100}$$

Result and Discussion :

effect of new compounds (1),(2),(3) on the (AChE) activity in serum :

The activity of (AChE) without copounds 1,2 and 3 in patients were (3.24 ,2.85,2.91and 3.12) µmol/ml/min for Breast cancer, Lung cancer, Leukemia and Prostate cancer respectively comparison with the normal value . The value of (AChE) in normal blood serum was between (4.41±0.61–8±1.14 µmol/min/ml) this value is in the normal value of AChE activity in Iraqi population (men and women) .[12],[13]

The evaluated compounds (1),(2),(3) were found to inhibit of (AChE) in patients .:

1- Compound 3-(acetyl Salicyloyl)-5,6 –O-isopropylidene -*L*-ascorbic acid (1) was found to have inhibition effect on activity of (AChE) in patients. The activity of (AChE) and The inhibition percent was (2.1 µmol/ml/min, 35 %), (1.81 µmol/ml/min ,37 %) , (1.78 µmol/ml/min ,39 %) and (2.21 µmol/ml/min ,29%) for Breast cancer, Lung cancer, Leukemia and Prostate cancer respectively .The compound 1 in the same concentration (0.005 g/25ml) was assay in the (AChE) activity in normal blood serum and was found to be inhibitor of (AChE) activity (2.38 ±0.125µmol/ml/min), inhibitory percentage ٥٢,١٦% and % Recovery was ٤٧,٨ %[14].

The inhibitory percentage of the compound (1) in patients are summarized in table (1)

2- Compound 2,3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic (2) acid was found to have inhibition effect on activity of (AChE) in patients. The activity of (AChE) and inhibition percent was (1.45 µmol/ml/min,55.25 %), (1.26 µmol/ml/min ,55.78 %) , (1.24 µmol/ml/min ,57.36 %) and (1.291 µmol/ml/min ,58.62%) for Breast cancer, Lung cancer, Leukemia and Prostate cancer respectively. The compound 2 in the same concentration (0.005 g/25ml) was assay in the (AChE) activity in normal blood serum and was found to be inhibitor of (AChE) activity (1.48±0.125µmol/ml / min), inhibitory percentage 62.34% and % Recovery was 36.66 %[14].

The inhibitory percentage of the compound (2) in patients are summarized in table (2).

3- Compound 2,3,5,6-Tetra(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic (3) acid was found to have inhibition effect on activity of (AChE) in patients. The activity of (AChE) and inhibition percent was (1.15 µmol/ml/min ,64.33 %), (0.92 µmol/ml/min ,67.49 %) , (0.901 µmol/ml/min ,69.2 %) and (0.951 µmol/ml/min ,69.49%) for Breast cancer, Lung cancer, Leukemia and Prostate cancer respectively .The compound 3 in the same concentration (0.005 g/25ml) was assay in the (AChE) activity in normal blood serum and was found to be inhibitor of (AChE) activity (1.38±0.125µmol /ml / min), inhibitory percentage 72.27% and % Recovery was 27.73 %[14].

The inhibitory percentage of the compound (3) in patients are summarized in table (3).

Table (1) effect of 3-(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (1) on Acetyl Choline Esterase activity in patients

Subject	No.	AChE activity µmol/ml/min Without inhibitor	AChE activity µmol/ml/min With inhibitor	Inhibition %	Recovery%
Breast cancer	4	3.24	2.1	35%	65%
Lung cancer	٥	2.85	1.81	37%	63%
Leukemia	٦	2.91	1.78	39%	61%
Prostate cancer	٥	3.12	2.21	29.2%	70.8%

Table (2) effect of 2,3-di(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (2) on Acetyl Choline Esterase activity in patients

Subject	No.	AChE activity µmol/ml/min Without inhibitor	AChE activity µmol/ml/min With inhibitor	Inhibition %	Recovery%
Breast cancer	4	3.24	1.45	55.25%	44.75%
Lung cancer	٥	2.85	1.26	55.78%	44.22%
Leukemia	٦	2.91	1.24	57.36%	42.64%
Prostate cancer	٥	3.12	1.291	58.62%	41.38%

Table (3) effect of 2,3,5,6-Tetra(acetyl Salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (3) on Acetyl Choline Esterase activity. in patients

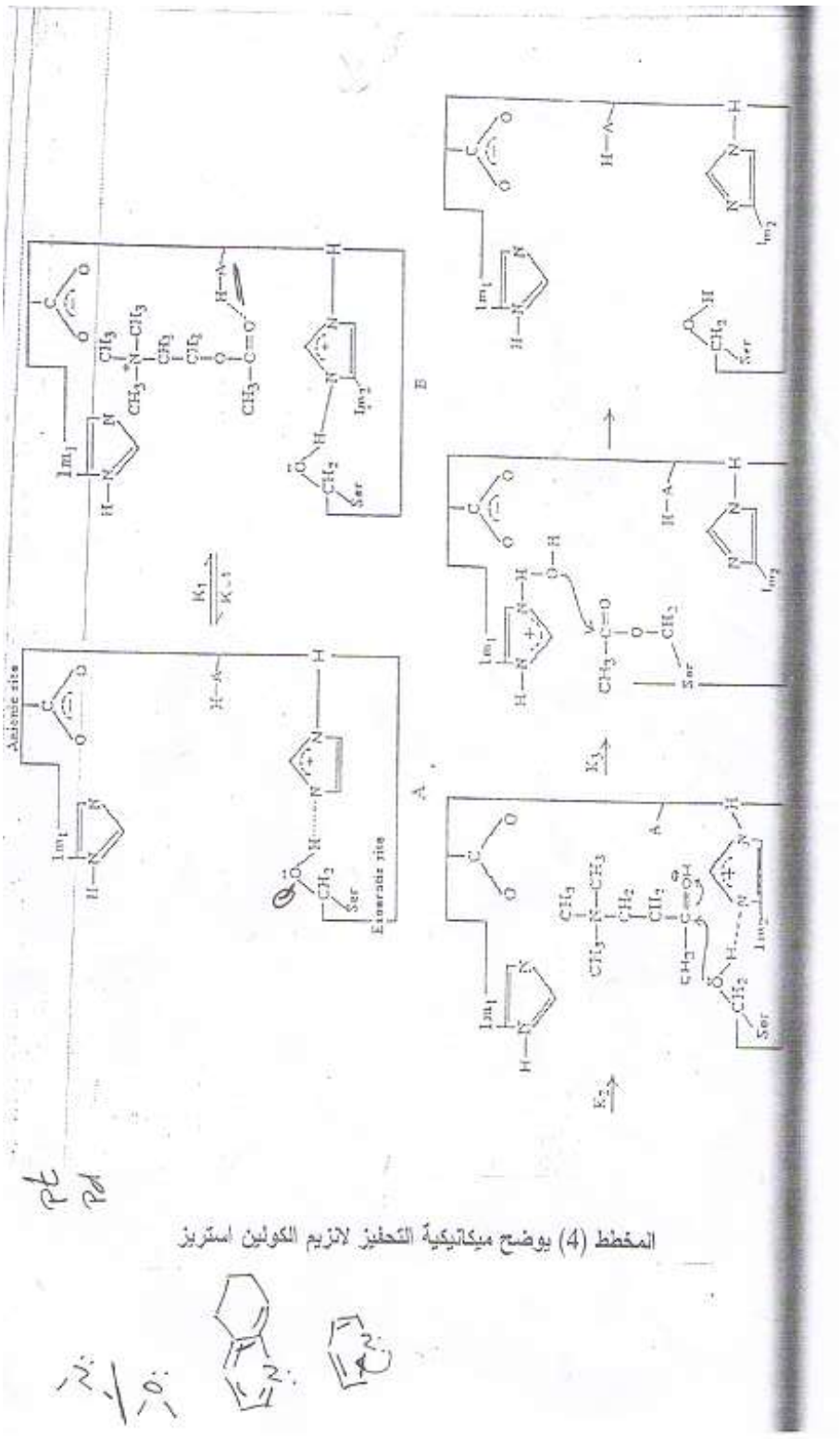
Subject	No.	AChE ctivity µmol/ml/min Without inhibitor	AChE activity µmol/ml/min With inhibitor	Inhibition %	Recovery %
Breast cancer	4	3.24	1.155	64.33%	35.67%
Lung cancer	٥	2.85	0.92	67.49%	32.51%
Leukemia	٦	2.91	0.901	69.2%	30.98%
Prostate cancer	٥	3.12	0.951	69.49%	30.51%

The mechanism of activation : the active site of AChE includes anionic and estratic site .The anionic site includes(C=O) group of γ-carcarboxlate group (COO^-) of glutamic acid .the active site of AChE has A-H of tyrosine in addition to two imidazole groups (Im_1& Im_2) and hydroxyl group of serine.

The OH group of serine will bind with imidazole by H-bond which increases the nucleophilic of serine OH while ,the H^+ of A-H group will bind the oxegen atom of (C=O)of choline ester which increases the electophilic effect of choline ester .

The OH group of serine will attach the (C=O) group of choline ester ,then the enzyme will be turned over sterometrically to repeat the same mechanism with Im_1.The hydrolysis will produce acetic acid and active enzyme .The delay in the mechanism of hydrolysis would lead to inhibit the enzyme action .

The experimental compounds which have been used in this research are those which contain ester group .The mechanism of activation and the hydrolysis of acetyl choline include the binding of A-H group of tyrosine with OH group .The used compounds contain the same carbonyl group which is combined with A-H group will decrease the activity of choline esterase and The inhibitory effect was found to increase as the acetyl Salicyloyl group was increase in the structure of compounds.



Mechanism of Acetyl choline estar ease

المخطط (4) يوضح ميكانيكية التحفيز لانزيم الكولين استريز

Reference:

(1) Golz, H.H. and C.B. Shaffer. *Toxicological information on cyanamid Insecticides. American Cyanamid Co., Princeton,* NJ 1960..

(2) Paul, Jane. *Commercial pesticide applicators may get*

(1) M.Roth; (1974)Clin.Biochem.J.; (21)164

(3) A. Kaplan., Clinicalchemistry, (1989),"*theory analysis and correlation* "2nd ed .The C.V Mosby Co., 387.

(4) M.Elaine.,*Biochem.J*;244(1987)725

(5) D.W. Moss, R.H. Eaton, J.K. Smith and L.G. Whity., (1967) *J. Biochm*; (102) 53 .

(6) H.Fleish , R.G.Russell and F.Strauman., (1966), "*Effect of pyrophosphate on hydroxyl a patite and its implications in calcium hemostasis" Nature*; (212) 901

(7) W.H. Fisman , S. Green and N.I. Inglis ., (1962). Decliue in rat serum ALP following bile duct ligalion , *Biochem . Biophys. Acta*; (62) 429 .

(8) Jusko. W.J.and Lewis. G.P., *J.Pharm.Sci.*, 64(1975) 181.

(9) A.IVogel, "*A TEXTBOOK OF PARTICAL ORGANIC CHEMISTIRY* " . Long man group limited , London , 3rd .ed., (1965) .

(10Sinkula.A.A., Merozowich. W. and Rowe.E.L., *J. Pharm.,* Sci.,62(1973)1106.

(11)Vandekar.M , *WHO/VBS/*78,692(1978)

(12)Al-Alazzawi. M.J , S.M.Al-Shafi and M.A.Ali ; *J.Saddam* University ; 1,185 (1997).

(13) AlRawi. I.A ; R.M. Hebeeb and H.R. Rabbfat ; *J.Bulletin of Health research* ; 28, 73(1987) .

(14) Maher. F. T.(2005), Biocamical study of some derevedives of L-ascorbic acid ,PhD, College of Science, University of Al-Nahrain ,P 99-105.

الملخص

تم في هذه البحث دراسة تأثير بعض من مشتقات حامض الاسكوربيك على فعالية انزيم الاسيتايل كولين استريز في عينات مصل الدم لاشخاص مصابين بانواع مختلفة من مرض السرطان(سرطان الثدي ،سرطان الرئة ،سرطان البروستات ،سرطان الدم). اظهرت الدراسة ان المركبات المختبرة تظهر تاثيرا مثبطا على فعالية انزيم الكولين استريز .