STUDYING THE INHIBITORY EFFECTS OF ACETAMINOPHEN (PARACETAMOL), AND SALICYLATE DRUG (ASPEGIC) ON THE ACTIVITY OF CREATINE KINASE

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Abstract

We have studied for the first time in Iraq, the effect of aspegic and paracetamol on the activity of creatine kinase in the sera of 25 premenopausal health women our results revealed that in the presences of increasing concentrations of aspegic and paracetamol the activity of creatine kinase is inhibited and the inhibition is concentration dependant and independent respectively. The kinetic parameter of creatine kinase was measured (Vmax= 133.3.00 IU/L, Km=(2.13 ± 0.2 mM).Aspegic act as reversible competitive inhibitor for CK(150 ± 3.3 IU/L , Km= 2.4 ± 0.4mM) and paracetamol act as reversible uncompetitive inhibitor for creatine kinase (87.72 ± 2.1 IU/L, Km=2.02 ± 0.33 Mm).

Abbreviations

ATP: adinosine triphosphate; **ADP**: adinosine diphosphate **CK:** Creatine kinase; **PCr.** :Creatine phosphate;**G6PD**: Glucose 6-phoshate dehydrogenase **IU/L**; International units/Litter; **IV**; intravenous; **M** : molary; **mM** mille molar; **$NADP^+$**: nicotinamide adenine dinucleotide phosphate; **NADPH:** nicotinamide adenine dinucleotide phosphate, reduced.

Introduction

Aspecig is a salicylate drug, often used as and analgesic to relive minor aches and pain, as an antipyretic to reduce fever, and as an anti- inflammatory medication, it also has an anti- platelet effect [(1)], Fig.(1-a).

Paracetamol (acetaminophen) is a widely used analgesic and antipyretic. Unlike aspegic ,it is not a very effective anti-inflammatory agent, it also useful in the management of more severe pain[(2)] , Fig.(1-b).

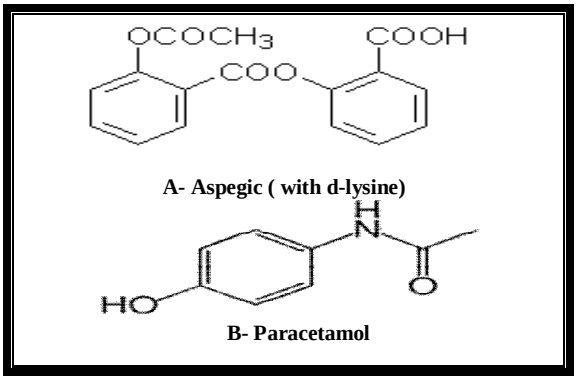


Fig. (1) : The Structures of: A- Aspegic [(25)] ; B- Paracetamol[(2)].

Antipyretic drugs shows to affect several enzymes like creatine kinase , and lactate dehydrogenase, acetyl choline esterase[(3,4,5)].

Serum creatine kinase (CK, ATP:creatine N-posphate transferase, EC 2.7.3.2)[(6)] . CK,a protein- product of chromosome 19, is an 86.000 molecular weight dimer molecule that produce adenosine triphosphate for use in muscle cells by catalyzing the transfer of a high energy phosphate bond from creatine phosphate, the major storage reservoir of energy during muscle at rest, to adenosine diphosphate[(7)]. Engelboroughs et al (1974)[(8)] have reported on initial product formation, and Gercken and Dőring (1974)[(9)] show that creatinine phosphate is not a substrate but is a competitive inhibitor of creatine kinase.Various divalent cations such as Mg^{+2}[(10)], Ca^{+2}[(11)], and Mn^{+2}[(12)] activate the enzyme. Various sulfhydryl reagents, chelating agents, some adenosine phosphate compounds, orthophosphate, pyro- and tripolyphosphate, adenosine,Cl^-, SO_4^{-2}, acetate (slighte), and other compounds such as dibenamine, phenothiazone, and 3,5-dinitro-o-

cresol. ADP strongly inhibite the forward reaction competitively with respect to creatine[8]. Creatine phosphate acts as competitive inhibitor with respect phosph-ocreatine[9].

In the present work for the first time in Iraq we would try to study the effect of aspegic and paracetamol on the activity of the enzyme and on the kinetic parameter of it.

Material and Methods

Materials:

1- **Creatine kinase kit** were provided dy Spinreact (Spain).
2- **Drugs:** wrere provided from different sources
   - Apegic (inectable 0.5IM.IV/5 ml water) from Laboratories Synthelabol/Synth labo Group (France).
   - Hayamol (paracetamol)(injection 375 mg/ 5ml water) from IBn Hayan Pharmaa-ceuticals.R.Faysal & Co. (Syria).
3- **Subjects:** twenty five healthy pre-meopausal female subjects with age ranging (20-40year) were included in this study. There was not complaining from any illness or using any medication.
4- **Blood sampling:** Blood was sampled by venipuncture, allowed to colt, and centrifuged (1500g, 5min, room temperature). the supernatant serum was collected for analysis , and stored at -20C0 until the assay day, although all the samples used in the study were almost collected freshly.(Note: the serum is stable for 7 days at 2-8C^0 protected from light. The creatine kinase activity 10% after 1 day at 2-5C^0 or after 1 hour at 15-25 C^0 [13].

Methods:

1. Creatine Kinase(CK) Measurement:

The activity of sera creatine kinase was measured by using coupled reaction system. The reaction of creatine kinase is coupled to those catalysed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD). The rate of NAPDH formation, measured photometically is propotional to the catalytic concentration of CK present in the sample[14].

The experiment was performed at a fixed concentration of creatine phosphate 30Mm ; ADP 2mmol /L; HK 2500U/L ; G6PD 1500 U/L. the final volume of the reaction was 1040 µl at 30 C^0. The initial absorbance (A) of the was read, and then after 1 min intevral therafter for 3min.s.

Calculations:

The difference between the absorbances were calculated, and the average absorbance difference per min ($\Delta A/min$), at 37 C^0

$\Delta A/min \times 8095 = U/L$ CK

2. Effect of Aspegic and Paracetamol:

The effect of both aspegic and paracetamol on the activity of CK were examined in the presence of increasing concentration of of each drug:

- Aspegic (4.5-22.5x10 $^{-4}$ M)
- Paracetamol (0.5 -2.5 $X10^{-3}$ M)

The experiment was performed as in 1.

Calculations:

The differences between the absorbance were calculated as in 1.

3. Kinetic parameters:

- **In the absence of drugs**

The Km and Vmax of CK for in the sera of premenopausal healthy females were determined by using increasing concentrations of creatine kinase (2.0-2.25mmol) (2.0mM was the optimum concentration of substrate in the assay to determination activity), at pH 7.0.the initial rate of the reaction was measured by the following the increasing in A_{340} nm associated with reduction of $NADP^+$.

- **In the presence of Aspegic and Paracetamol**

The Km and Vmax of CK was determined as shown previously but in the presence of fixed concentrations of aspegic and paracetamol ($2.5x10^{-3}$Mm) and ($1x10^{-3}$mM) respectivily

Note: Those fixed concentrations gave the highest activities of CK.

Results and Discussion

1- CK level

The level of the sera CK activity of 25 healthy premenopausal women were determined by using coupled reaction and the mean concentration of the CK was (70.83 ± 40 IU/L). This result is in disagreement with Athayde et al[15] who reported a CK level of 15 normal subject with (mean age, 29.6±10.3 years;), and Szasz who reported a CK Activity in human serum(28 ±1 IU/L)[16]. and agreed

with Carl *et al* who reported that ck values rang (26-140 IU/L) for female at $37C^{0}$ [13].The most definitive reference inteverals are those established for our patient population.

2- The Effect of Aspegic and Pracetamol

The effect of two types of anti-pyretic drugs on the activity of CK were examined by using increasing concentrations of aspegic (4.5-22.5x10^{-4} M) and paracetamol (0.5 -2.5 x10^{-3} M) as shown in Fig.(2, 3).

As shown in Fig.(2) , aspegic (4.5x 10^{-4}M) decreases the activity of CK for 4-folds (36.42 IU/L), and for approximant 1-fold (145.71 IU/L) at (22.5X10^{-4} M) as compared to control (149.74 IU/L), this result revealed that the effect of spegic on the activity of CK is concentration dependant. Our result disagreed with van Werkum[17] who reported an activation in the activity of CK reaches (6743/4230IU/L) after given a patient aspegic 900 mg, and agreed with Sanae[18] who reported an inactivation of CK by salicylic acid that is easily produced from aspirin and aspegic.

In the presence of paracetamol as shown in Fig.(3) the activity of the sera CK shows maximum decrement (64.76 m) for approximately 2.3 fold at (0.5x10^{-3}M), and for 1.1 fold (141.66 IU/L)at a concentration 1x10 $^{-3}$M. as shown in the fig the effect of the drug on enzyme activity is not concentration dependant. This result is disagreed with Yanpallewar [19] who reported that there were an increase in serum marker enzymes of hepatic damage after paracetamol administration, and agreed with Demitri[20] who reported an inhibition in CK activity after administration paracetamol but the decrement was not significant.

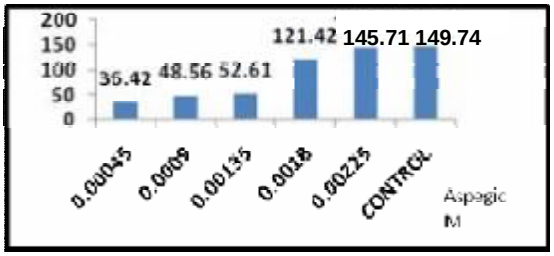


Fig.(2) : The Effect of Aspegic on the Activity of CK.

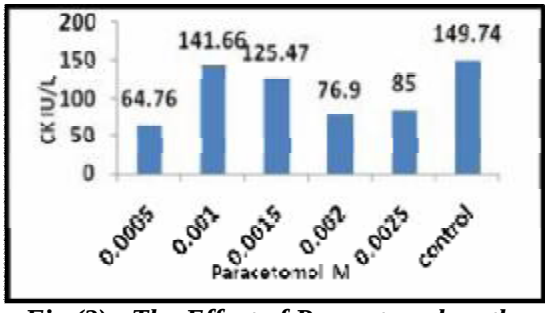


Fig.(3) : The Effect of Paracetamol on the Activity of CK.

3- Kinetic parameter:

- **Km and Vmax for CK in the absences and presence of aspegic and paracetamol**

The kinetic parameter of creatine kinase for the conversion of Pcr→Cr, were determined in the absence of any effectors .Initially a plot of a reaction velocity (v) as a function of substrate concentration (creatine phosphate) [S], Fig.(4-a).

The Vmax, and km values were determined by using Lineweaver –Burk plot Fig. (4-b) Table (1).

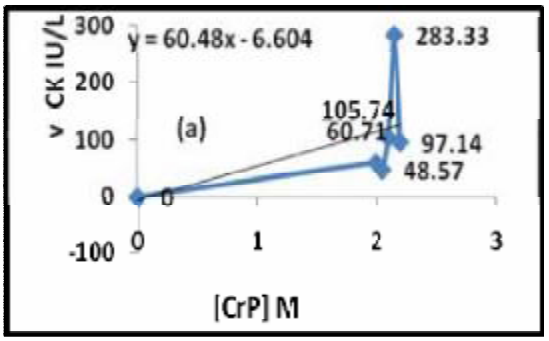
The Vmax and Km values were determined by using Michaels-Menten equation (1)[21].

$$v = \frac{Vmax+[S]}{Km+[S]} \quad \text{.....(1)}$$

Table (1)

	***V max* IU/L**	**Km mM**
Control (no drug)	133.3 ± 4.0	2.13 ± 0.2
Aspegic (22.5 x 10^{-4}M)	150 ± 3.3	2.4 ± 0.4
Paracetamol (1 x 10^{-3} M)	87.72 ± 2.1	2.02 ± 0.33

as shown in (Table (1)) the mean value of Km for CK activity was (2.13 ± 0.2 mM) and *Vmax* 133.3.00 ± 4.0 IU/L this result in disagreement with Stepanov [22] who reported a km value (2.4±0.1 mM) of CK activity for the conversion of PCr→Cr, and with Michael[23] who reported a Km value (0.9±0.12mM) for the conversion of PCr→Cr



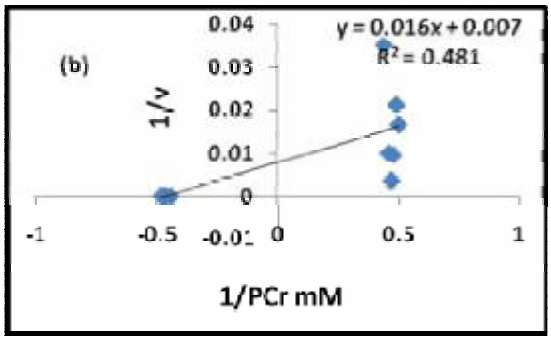
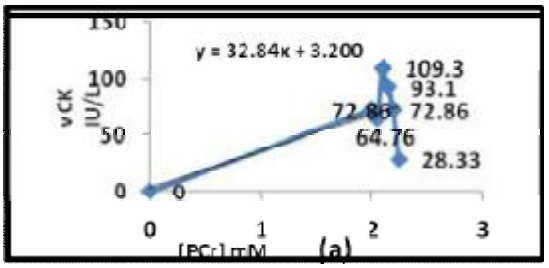


Fig.(4-a) :The Michaels-Menten plot of CK Fig.(4-b) :The Leinwever-Burk plot of ck in the absence of drug

The Km and Vmax values for CK in the presences of aspegic,and paracetamol were determined as shown in Fig.(5-a,b) and Fig.(6-a,b) respectively, and (Table(1)).



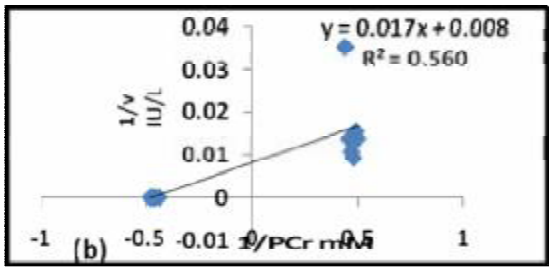
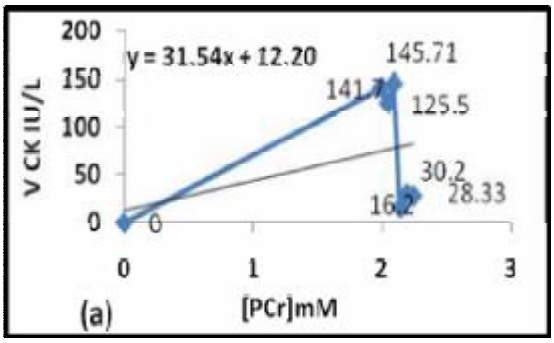


Fig. (5-a) :The Michaels-Menten plot for CK in the presence of Fig.(5-b) :The Leinwever-Burk plot of CK in the presence Apegic 22.5 10^{-4} M of Aspegic 22.5 x 10.



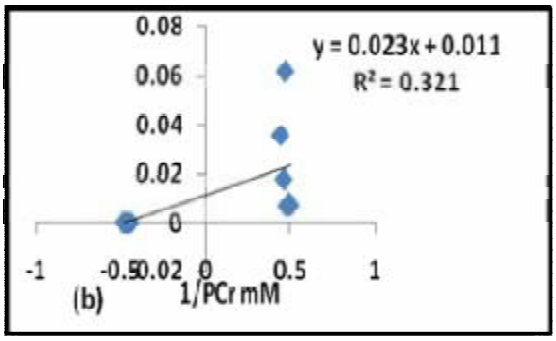


Fig. (6-a) : The Michaels-Menten plot for CK in the presence Fig.(6-b) : The Leinwever-Burk plot of CK in the presence of Paracetamol ($1x10^{-3}$M) of Paracetamol ($1 x 10^{-3}$ M).

As shown in Table (1) aspegic lowers the km (2.4 ±0.4 mm) while the *Vmax* remain high. These data revealed that the aspegic act as a reversible competitive inhibitor for the CK. Because the inhibitor binds to the enzyme, the competition can be biased to favor the substrate simply adding more substrate. When [S] far exceeds [I], the probability that a molecule will bind to the enzyme is minimized, and the reaction exhibits a normal V max. However the [S] at which v_0 =1/2 *V max*, the apparent Km, will increase in the presences of inhibitor by factor α (equation -2,3)

$$V_0 = \frac{Vmax[s]}{\alpha Km + [s]} \quad \text{.....(2);}$$

$$\alpha = 1 + \frac{[I]}{K_i} \quad \text{.....(3)}$$

On the other hand,as shown in Fig (6-a,b) and Table (1) Paracetamol decreases both the V max and Km values ,this results revealed that paracetaoml act as a reversible uncompetitive inhibitor for CK. This type of inhibitors observed only with enzyme having two or more substrates[24] like CK which has

two substrates (PCr. and ADP) .An uncompetitive inhibitor bind at a site distintinct from the substrate active site and, unlike a competitive inhibitor, bind only to the ES complex [24]. In the presence of uncompetitive inhibitor, the Michaelis – Menten equation is altered to (eq(4)):

$$V0 = \frac{Vmax\,[S]}{Km+\alpha`[S]} \quad \text{.....(4)}$$

$$\alpha` = 1 + \frac{[I]}{K`i} \quad \text{.....(5)}$$

As describe by equation (4) , at high concentration of substrate ,v_0 approaches *Vmax*/α`. Thus an uncompetitive inhibitor lowers the measured *Vmax*. The apparent Km also decreases, because [S] required to reach one-half *Vmax* decreases by factor $\alpha`$ [24]. The authors didn't find an in vitro researches that could confirmed their results.

Conclusions

1. The activity of creatine kinase is decreased by using increasing concentration of aspegic, and effect is concentration dependant.
2. The activity of creatine kinase is decreased by using increasing concentration of paracetamol, and effect is not concentration dependant.
3. Aspegic act as a reversible competitive inhibitor for creatine kinase.
4. Pracetamol act as a reversible uncompetitive inhibitor for creatine kinase.

Acknowledgments

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الخلاصة

تم في هذه البحث دراسة تاثير كل من الاسبيجك والباراسيتامول على فعالية الكرياتين كاينيز قي امصال 25 امراة من النساء الاصحاء واظهرت النتائج ان التراكيز المتزايدة من كل من الاسبيجك والباراسيتامول تثبط فعالية الانزيم وبصورة معتمدة على التركيز وغير معتمدة على التوالي. كما تم قياس الدالات الحركية للكرياتين كاينيز (Vmax= 133.3±4.0 Km=2.13±0.2 mM IU/L,). لوحظ من الدراسات الحركية ان الاسبيجك يعمل كمثبط تنافسي للكرياتين فوسفيت (Vmax=150±3.3 IU/L , Km= 2.4±0.4mM)، اما الباراسيتامول فيعمل كمثبط غير تنافسي للمادة الاساس (Vmax=87.72±2.1 IU/L , Km=2.02±0.33mM).