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HYALURONIDASE (HAASE) INHIBITERS IN HUMAN SERUM

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ABSTRACT

The main target of this study was to investigate the natural compounds effecting on a Hyaluronidase enzyme (HAase) behaviour on human serum for creating new inhibitors to be used then as medications. In this work the activities of HAase enzyme were measured using Alfred Linker methods after mixed it separately with (i) 3,4-Diyhdroxy-3 chloro flavylium chloride, (ii) 3,4-diyhdroxy-3-acetoxy flavylium chloride) and (iii) heparin compounds at different concentrations: ($1x10^{-1}$,1x10-^{2},$1x10^{-3}$,$1x10^{-4}$) µM and incubation period times (0-25) minutes. The experimental works exhibited that, these compounds affecting on HAase enzyme activity in human serums from 60 to 90 % for 10 minutes' incubation period time and the competitive inhibition rates were (III>II>I)

KEYWORDS: Hyaluronidase, enzyme, and incubation.

1. INTRODUCTION

Hyaluronidase is one of the enzymes referring to a huge family of Hydrolases enzymes. this huge family named on1940[1] as HAases and currently as Hyases which discovered by Duran Reynals scantiest on 1928.[3] Numbers of papers and researches were published about these enzymes on time between 1936 to 1940 which explained there affects by several scantiest such as Duran Reynals.

Duran Reynals managed a robust studies and experimental works on Synovial fluids of Mammalia who then concluded that these enzymes have Spreading Factor which led to spreading the materials into the tissues. Number of published papers and articles emerged this enzyme usage in Anatomy for identifying the substrates materials that these enzymes can be worked inside tissue.

HAase family enzymes have some unknown specifications due to the difficulties in measuring it activities and also difficulties in purifying and separation as well. Because it presenting in low concentrations inside the tissues and it instability of it specific activity.[1-14]

Hyaluronidase enzymes were classified into three types according to their sources and functions: Testicular, Leech Hyaluronidase and Bacterial Hyaluronidase.

The main target of this work was to investigate the effect of natural materials on HAase enzyme behaviour to find new inhibitors compounds which can be used as a medication.

2. EXPERIMENTAL WORKS

2.1. *Materials and Equipments*

3`,4`-Diyhdroxy-3Chloro flavylium chloride, 3`,4`-diyhdroxy-3-acetoxy flavylium chloride), Heparin**,** sodium Bio-phosphate solution(PBS), Acetic acid solution and Sodium chloride were used in this study. All chemical compounds were supplied by Sigma company in the UK. Deionised water produced in the lab of chemistry department were also used in this study.

Sartorius three-digit balance supplied by Sartorius company was used. while, pH electrode instruments type Orion 720, Vortex-Genie, K550-GE and water bath equipments were used in this study. A Shimadzu, UV-210A, spectrophotometer equipment was also used and supplied by Japan company. Moreover, Janetzki, MSE, Minor-35 centrifuge and Virtis,12525 dryer equipments were used in this work.

2.2. Experimental work procedure

0.05 molar concentration solution at pH 4 was prepared by dissolving 3,4-Diyhdroxy-3choro chloride and 3,4-diyhdroxy-3-acetoxy flavylium chloride compounds in acetic buffer solution. A 0.1 molar concentration solution at pH 7 was also prepared by dissolving Heparin compound in sodium bio-phosphate buffer solution (PBS).

Afterward, the prepared solutions were added and mixed separately with human serum samples of three groups: breast endo carcinoma, uterine endo carcinoma and healthy people normal subjected) at different concentrations ($1x10^{-1}$,1x10-2^{3},$1x10^{-4}$) µMol and different incubation period times (0-25) min. Then HAase enzyme activity was determined in human serum samples by Alfred Linker method using spectrophotometer equipment at spectrum wavelength 560nm.[6] The optimal incubation time and concentration for added compounds

were determined in this work. The constant dissociation of enzyme complex with inhibitor (Ki) and superficial velocity (Vmax) were also determined using Lineweaver-BurK equation. all results are presented in tables.[1-5]

3. RESULTS AND DISCUSSION

From Tables 1-2 , it is clear for the breast and uterine carcinoma patients that the measured activity values of HAase was higher for Metastases patients compared with that in Non-Metastases patients. While from Table.3, The results of this experimental work exhibited that the compounds I,II and III have competitive inhibition behaviours to the HAase enzyme in human serum reach from 60% to 90% according to the following order (III > II> I). From Table 4, it was evident that, the 10 min is the optimum incubation time where the inhibition of compounds is happened. However, from Table 5, generally the Ki nearly doubled increased with compounds concentrations increases while the Vmax stay constant at value 25 for all compounds to give an indication that this parameter not effected at all. However,$1x10^{-3}$ was the optimum concentration.

Table 1: Illustrates haase activity for breast carcinoma patients.

Metastasis	***No.of sample***	***HAase activity lNAG/min/l***	***±S-D***
	11	70.81	±17.54
Non- Metastasis	16	38.12	±5.67

Table 2*:* Illustrates HAASE activity for uterine carcinoma patients.

Metastasis	***No. of samples***	***HAase activity µM /NAG/min/l***	***±S-D***
	6	62.03	±15.47
Non- Metastasis	7	41.97	±5.51

Table 3: Effect of compounds (I,II,III) on HAase activity in serum of normal and patients groups.

Case type	**Inhibition compounds**	**I µM**	**Mean Activity of Inhibited (HAase) values µmo lNAG/min/l**	**% Inhibition**
Normal	I	$1x10^{-3}$	5.40 ± 3.6	76.2
	II	$1x10^{-3}$	5.40 ± 2.01	76.2
	III	$1x10^{-3}$	3.6 ± 1.23	84.1
Breast carcinoma	I	$1x10^{-3}$	8.10 ± *2.01*	64.2
	II	$1x10^{-3}$	*5.85 ± 1.23*	74.2
	III	$1x10^{--3}$	*3.15 ± 1.23*	86.1

Uterine carcinoma	I	$1x10^{-3}$	8.10 ± *1.23*	64.2
	II	$1x10^{-3}$	5.85 ± *1.23*	74.2
	III	$1x10^{--3}$	3.60 ± *1.23*	84.1

Table 4: Effect of compounds (I,II,III)on HAase activity in serum of normal and patients groups.

Incubation time min	Enzyme Activity µmolNAG/min/l	Activity of Inhibited (HAase) values µmolNAG/min/l	% Inhibition	% activity Recovery
Compound I				
0	0	0	100	0
5	11.3	11.3	50	50
10	22.6	9.0	60.2	39.8
15	11.3	11.2	50.5	49.5
20	11.3	11.2	50.5	49.5
25	11.2	11.2	50.5	49.5
Compound II				
0	0	0	100	0
5	11.3	9.0	62.2	39.8
10	22.6	6.75	70.2	29.8
15	11.3	6.75	70.2	29.8
20	11.3	11.2	50.5	49.5
25	11.2	11.2	50.5	49.5
Compound III				
0	0	0	100	0
5	11.3	4.5	80.1	19.9
10	22.6	2.25	90.05	9.95
15	11.3	9.0	60.2	39.8
20	11.3	9.0	60.2	39.8
25	11.2	11.2	50.5	49.5

Table 5: Kinetic properties of inhibited (HAase) enzyme by compounds (I,II,III)using Lineweaver-Burk plot.

Compounds type	[I]. (mM)	Ki (mM)	Vmax µmol.NAG/min/l
I	$4X10^{-5}$	1.68	25.0
	$8X10^{-5}$	2.85	25.0
II	$4X10^{-5}$	2.63	25.0
	$8X10^{-5}$	4.34	25.0
III	$4X10^{-5}$	5.00	25.0
	$8X10^{-5}$	9.10	25.0

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3. CONCLUSIONS AND RECOMMENDATIONS

The findings of this study show, that these three compounds have inhibition behaviour for enzyme in percentage 60-90% and take the following order according to their inhibition affect III>II>I and these added compounds behave as completive inhibitor to HAase enzyme which increased rapidly when cancer patients reach to metastatic stage. According to that, these compounds can be used as medications to stop or prolonged of the cancer patients to reach metastases stage. For future work it is recommended to identify the more reliable inhibitors compounds via using new natural compounds such as some types of flyfunedate or any natural materials have the same essential structures with HAase.

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