IN VITRO STUDY OF THE EFFECT OF SOME PREPARED OXADIAZOLE DERIVATIVES ON ALP, AST AND ALT ACTIVITIES IN SERA OF HEPATOCELLULAR CANCER PATIENTS AND HEALTHY CONTROL

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Abstract

The effect of some prepared oxadiazole compounds 5-[4-(p-methoxy phenyl) azo phenyl]-2-thiol-1, 3, 4-Oxadiazole [I], 5-[4-(methoxy phenyl) azo phenyl]-2-marcapto-alky-1, 3, 4-oxadiazole [II-V](where alkyl group is ethyl, propyl, butyl and pentyl respectively) were tested on the activity of ALP, AST, and ALT in sera of 30 patients with hepatocellular cancer and 30 healthy individuals as control group.

The results revealed significantly higher levels of ALP, AST, and ALT activity in patients group compared to control group.

Different concentrations (10^{-4}, 10^{-5}, and 10^{-6}M) of oxadiazole derivatives showed different percentage of inhibitions, but did not showed proportional with the concentration of derivatives.

The elevated values of ALP, AST, and ALT activity in sera of patients were returned to about normal values in most cases after addition of oxadiazole compounds to sera of patients *in vitro.*

Keyword: Oxadiazole compounds, Anticancer, Enzymatic Biomarker.

Introduction

Oxadiazoles are becoming of great interest, which stems mainly from their wide range of applications. It has been shown that the derivatives of 2-mercapto oxadiazole which, by virtue of incorporating the (-N=CS) group, are toxicities important in many drugs (1, 2), anticancer (3-5), fungicides (6,7) and insecticides (7). Diseases evidently alter the oxadiazole compounds in cells, tissues, and organelles then the diseases would be detected (8,9).

Hepatocellular carcinoma is one of the most common malignancies worldwide (1). Most cancer cases occur in individuals older than 40 years; with a shift toward a younger age group (10).

Human serum contains a number of enzymes; the variation in their activities was suggested to be of great value in the clinical diagnosis(11). Alkaline phosphatase (ALP) which is a general tumor marker that raised in nearly all cancer cases. Alanine transaminase (ALT) and aspartate transaminase (AST) of little importance were considered very sensitive indicator of liver damage (including cancer) (12-14).

Aim of the present study is to evaluate the effect of 5-[4-(p-methoxy phenyl) azo phenyl]-2-thiol-1,3, 4-Oxadiazole[I]and 5-[4-(methoxy phenyl) azo phenyl]-2-marcapto-alky-1,3, 4-oxadiazole [II-V] on the activity of some metabolic enzymes (i.e ALP, ALT and AST) in sera of hepatocancer patients and control.

Materials & Methods

The structures 0f oxadiazole and its derivatives which were prepared according to (15) are shown in Fig.(1). The R group, molecular formula and melting points of all compounds shown in Table (1).

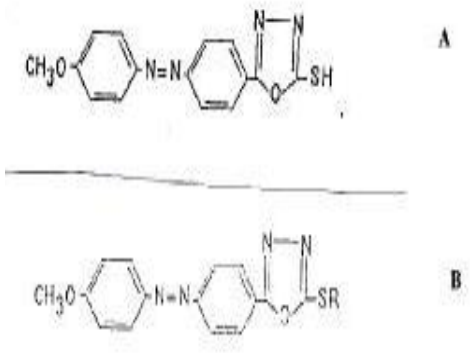

Fig. (1): A- Compound (I) Oxadiazole. B-Compounds (II–V): General formula of oxadiazole derivatives with different R groups.

Table (1)
Some oxadiazole derivatives and their physical properties.

No.of compound	R group	Molecular Formula	Melting Point(^{0}C)
I	H	$C_{15}H_{12}N_4O_2S$	229-231
II	C_2H_5	$C_{17}H_{16}N_4O_2S$	118-121
III	C_3H_7	$C_{18}H_{18}N_4O_2S$	108-110
IV	C_4H_9	$C_{19}H_{20}N_4O_2S$	94-97
V	C_5H_{11}	$C_{20}H_{22}N_4O_2S$	95-98

Selection of Subjects and Blood Sampling

Serum sample of two ml was obtained from 30 patients with hepatocellular cancer and 30 healthy individuals as a control group. The age of all subjects (patients & control) were ranged from (40-60) years.

Determination of Alkaline Phosphatase Activity(16)

Colorimetric determination of ALP activity was performed using a kit from Biomerieux, France according to the following reaction :

$$\text{Phenylphosphate} \xrightarrow{pH\ 10} \text{Phenol+ Phosphate}$$

The phenol liberated was measured in the presence of amino-4-antipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stop the enzymatic reaction. The absorbance of the sample was measured at 510 nm against reagent blank.

Determination of AST and ALT Activity(12)

Colorimetric determination of AST and ALT activates were measured using a kit from Biomegreb according to the following reactions:

$$\text{Aspartate+}\alpha\text{–ketoglutarate} \xleftrightarrow{AST} \text{oxaloacetate + glutamate}$$

$$\text{Alanine+}\alpha\text{–ketoglutarate} \xleftrightarrow{ALT} \text{Pyruvate + glutamate}$$

The pyruvate or oxaloacetate formed were measured in its derivative form, 2,4–dinitrophenylhydrozone.The absorbance of samples were recorded against the reagent blank at 546 nm.

Determination of Biological Activity of Oxadiazole Compounds as Anticancer:

The oxadiazole compound and their derivatives listed in Table (1) were tested for their effect on the activity of ALP, AST and ALT.

A liquate (0.1ml) of each concentrations (10^{-4}, 10^{-5}, 10^{-6} M) of oxadiazole compounds which were dissolved in ethanol, added to serum of patients with hepatocellular cancer. Then the activity of enzymes were determined in the presence of the above compounds using the methods mentioned in references (12&16). The effect of ethanol which used as solvent was found to be negligible.

Results and Discussion

Tables (2,3,4) summarizes ALP, AST and ALT activities, respectively ,which expressed as (mean ± SD) before and after addition of different concentrations (10^{-4}, 10^{-5}, 10^{-6} M) of oxadiazole compounds to sera of patients with hepatocellular cancer and healthy individual as control group, *in vitro* study.

An elevated values of all enzymes in patients with liver cancer compared to control group was found. Markers of liver damage (transaminases and alkaline phosphatase) showed predominate rise in patients with different hematological disorders cancer (17-19).

After addition of different concentrations of oxadiazole compounds to serum of liver cancer patients, the levels of ALP, AST and ALT activities were decreased, the results agreed with the results of other studies (20).

All the oxadiazole compounds showed inhibitory effects on enzymes under consideration. This inhibitory effects did not showed a concentration gradient. The effect of ethanol was ignored. Recently demonstrated that oxadiazole compounds prevent, treat and management of disease that is associated with a gene exhibiting premature translation termination and /or nonsense mediated mRNA decay. In one embodiment, cancer is due lack of expression of the gene resulting from a premature step colon (21-23).

A research indicate that oxadiazole derivatives reduce pathological symptoms of infection in the liver (24).

Other studies reported inhibition effect of the oxadiazole compounds on the activity

enzymes could be due to the facts that molecular interactions between the atoms N, O and S of the oxadiazole moieties with R group of the amino group of active site of enzymes (25).

The original activity of ALP, AST and ALT were (61.6, 31.2, 30.55) for control respectively and (159.2, 150.37, 235.25) for patients respectively which considered to be 100%. The inhibition percentage of oxadiazole and its derivatives on ALP, AST and ALT.

A conclusion from the present study could be made that oxadiazole derivatives showed an inhibitory effect on ALP, AST and ALT. This inhibitory effect did not showed a concentration gradient dependent.

Table (2)
Levels of ALP activity before (B) and after (A) addition of oxadiazole compounds in sera of patients with liver cancer and normal control.

Groups	***ALP activity(B) U / L***	***Oxadiazole Compounds***	***ALP activity (A) U / L***		
			10^{-4} M	***10^{-5} M***	***10^{-6} M***
Control	61.1±16.44	Compound I	72.3±16.05	77.6±4.28	59.7±18.3
Patients	159.2±36.06	Compound II	86.6±27.5	87.3±21.6	86.8±28.1
		Compound III	99.6±20.02	111.8±27.5	97.8±31.5
		Compound IV	93.8±20.3	124±28.8	134.3±32.11
		Compound V	128.5±45.5	139±39.9	146.8±31.9

Table (3)
Levels of AST activity before (B) and after (A) addition of oxadiazole compounds in sera of patients with liver cancer and normal control.

Groups	***AST activity(B) U/L***	***Oxadiazole Compounds***	***AST activity(A) U/L***		
			10^{-4} M	***10^{-5} M***	***10^{-6} M***
Control	31.2±0.76	Compound I	42.6±3.30	47.01±31.0	48±8.1
Patients	150.37±2.61	Compound II	64.5±6.5	61.5±1.45	54.6±2.41
		Compound III	74.3±4.3	76.8±3.2	82.01±4.0
		Compound IV	93.7±1.75	83.42±4.5	93.4±2.41
		Compound V	111.1± 18.16	124.6± 28.3	118.2± 23.05

Table (4)
Levels of ALT activity before (B) and after (A) addition of oxadiazole compounds in sera of patients with liver cancer and normal control.

Groups	*ALT activity(B) U/L*	*Oxadiazole Compounds*	*ALT activity (A) U/L*		
			10^{-4} M	*10^{-5} M*	*10^{-6} M*
Control	30.55±17.39	Compound I	48.9±2.07	54.6±2.4	51.3±2.75
Patients	235.25±4.11	Compound II	76.3±0.79	58.9±1.54	64.5±6.5
		Compound III	77.6±1.67	70.0±1.69	77.4±9.2
		Compound IV	87.0±1.63	85.0±1.39	80.8±2.02
		Compound V	93.9±2.70	91.0±2.3	92.7±1.19

Table (5)
Inhibition percentage of oxadiazole and its derivatives on ALP activity in sera of patients with liver cancer.

Compound Con.(M)	*Inhibition Percentage %*				
	I	*II*	*III*	*IV*	*V*
10^{-6}	62.5	45.4	38.5	15.6	7.78
10^{-5}	51.2	44.8	29.7	22.1	12.6
10^{-4}	45.6	54.4	37.4	41.08	19.2

Table (6)
Inhibition percentage of oxadiazole and its derivatives on AST activity in sera of patients with liver cancer.

Compound Con.(M)	*Inhibition Percentage %*				
	I	*II*	*III*	*IV*	*V*
10^{-6}	38.14	63.6	45.4	37.7	27.2
10^{-5}	68.6	59.05	54.7	44.3	17.09
10^{-4}	71.8	57.05	50.5	37.6	26.06

Table (7)
Inhibition percentage of oxadiazole and its derivatives on ALT activity in sera of patients with liver cancer.

Compound Con.(M)	*Inhibition Percentage %*				
	I	*II*	*III*	*IV*	*V*
10^{-6}	78.1	72.5	67.07	65.6	60.5
10^{-5}	76.7	74.9	70.2	63.8	61.2
10^{-4}	79.1	67.5	66.9	62.9	60.06

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الخلاصة

تم دراسة تاثير بعض مركبات الاوكساديازول المحضرة 5-(4-(بارا – ميثوكسي فنيل) ازو فنيل -2- ثايول 4,3,1 –اوكساديازول (I)، و 5-(4- (بارا–ميثوكسي فنيل) -2- مركبتو–الكيل 4,3,1 اوكساديازول (II-V) على فعالية كل من الانزيمات ALP, AST, ALT في مصل ثلاثون شخصا مصابا بسرطان الكبد و ثلاثون شخصا من الاصحاء كمجموعة سيطرة.

اظهرت النتائج قيم معنوية عالية لفعالية كل من في مجموعة المرضى مقارنة مع مجموعة السيطرة.وقد تبين ان جميع مركبات الاوكساديازول لها فعل تثبيطي مختلف في مدى التراكيز(M 10^{-6},10^{-5},10^{-4}) ولنفس المركب على الانزيمات اعلاه.

ان فعالية الانزيمات المدكورة قد انخفضت وتراجعت الى مستوياتها الطبيعية تقريبا في معظم الحالات بعد اضافة مركبات الاوكساديازول في امصال المرضى خارج المضيف.