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Studies the Effected Microbiological, Biochemical Analysis and Histological Examination of Pendant Coumarin Thiocarbohydrazone and its Cobalt (II) Complex in Rats

[1]*Jehad. M. Yousef and* [2]*Amna. A. Saddiq*

[1]Department of Chemistry, King Abdul Aziz university, Faculty of Science,
P.O. Box 51459, Girl's Collage of Education, Jeddah- 21453, Saudi Arabia
[2]Department of Plant, Microbiology,, King Abdul Aziz university, Faculty of Science,
P. O. Box 51459, Girl's Collage of Education, Jeddah- 21453, Saudi Arabia

Abstract: The effects of Co(II) complex of the formula Co(L) Cl_2.S where L is 4-hydroxy coumarin-3-thiocarbohydrazon which have been prepared by the reaction of 4-hydroxy coumarine with dithiocarbohydrazide in molar ratio 1:1, S= H_2O or EtOH on some biochemical parameters and histological studies in serum and tissue liver and kidney in rats have been studied. The animals were scarified and blood was tested for some key enzymes and other non-enzymatic biochemical parameters at 3, 6 and 12 days of the experiment. At the end of the 12-day experiment, the rats were killed by cervical decapitation. Livers and kidneys from each group were removed, each organ was divided into 2 parts: parts of the liver and kidneys were used for the determination of some key enzymes and other non-enzymatic biochemical parameters. The other part of each organ was put in formalin solution (10 %) and stained by Hematoxyline and Eosine (H and E) to be used for histological examination. We see also the effect of *Aspergillus niger* and *Candida albicans* on the radial growth. The results of this study prove that the complex at low dose has a better effect than the complex at high dose and the ligand at both high and low doses has no effect on the biochemical analysis in serum, liver and kidney tissues and histological examination in rats. Also the free ligand showed positive results against *C. albicans* (100 μg/ml) although there was a sufficient increase in the fungi activity of free ligand compared to the complexes. The results also showed that *A.niger* was much more sensitive against all tested compounds compared with other filamentous fungi under identical experimental condition.

Key words: Coumarine Thiocarbohydrazone · Cobalt (II) · Liver · Kidney · Fungi

INTRODUCTION

Flavonoids and coumarins are naturally occurring compounds that are widely distributed in vegetables and have a broad pharmacological activity [1].

Coumarin used in the treatment of chronic venous diseases is mainly metabolized to non-toxic 7-hydroxy-coumarin [2]. Increasing evidence regarding free radical generating agent and inflammatory processes suggest that the accumulation of reactive oxygen species can cause hepatotoxicity and cell injury [3].

Ligands with potential sulfur and nitrogen donors, thiosemicarbazide and its derivatives, are interesting and have gained special attention due to their structural chemistry. Their importance in medical chemistry is increasing because these materials have been used as drugs and are reported to possess a wide variety of biological activities against bacteria, fungi and certain types of tumors [4].

Some thiosemicarbazone compounds have prophylactic activity against smallpox and therapeutic activity against vaccina virus (vv) infections [5]. Some derivatives of thiosemicarbazone have activities against protozoan parasite Trypanosoma cruzi [6]. Thiosemicarbazones and semicarbzones derivatives can enhance radiosensitivity of tumour cells in *vitro* and *vivo* which are the inhibition of DNA repair and antitumor activity towards kidney tumor cells, crystal structure and ligands [7,8].

Cobalt affects myocardial functions yet many clinical implications remain to be evaluated [9]. Cobalt has been

Corresponding Author: Jehad. M. Yousef, Department of Chemistry, King Abdul Aziz university, Faculty of Science, P.O. Box 51459, Girl's Collage of Education, Jeddah- 21453, Saudi Arabia.

shown to stimulate sodium transport across the distal nephron of the newt kidney [10]. The cobalt ion CO (II) complexes were found to be more active towards both gram positive and gram negative bacteria than the other metal complexes [4].

$CoCl_2$ affect the cytotoxic activity of various antioxidants and the cobalt ion stimulates the oxidation of antioxidants to their inactive products [11].

The metal inhibitions or enhancements of NO production may be pathogenic by suppression of defense mechanisms or induction of hypersensitivity, respectively [12].

Antimicrobial screening of the free ligand and its binary complex possesses antimicrobial activities towards four type of bacteria and five types of fungi and these results were compared with eleven types of known antibiotics [13].

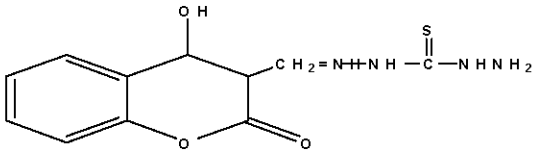
The tautomerism in this ligand and also the well known tendency of oxygen and sulfur donors to act as bridging sites allows various structural possibilities for the corresponding metal complexes [14].

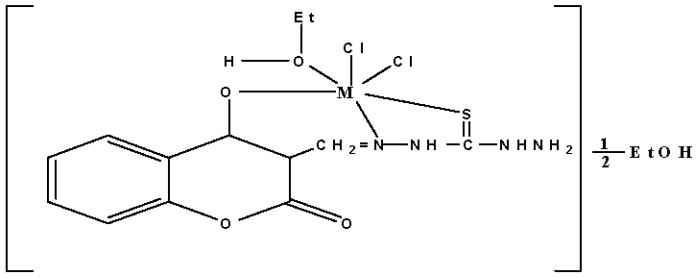
Fungi are considered very dangerous on cereals. The risk of contamination by mycotoxin is related to mycoflora associated with grains [15]. Wheat (*Triticum aestivum*) grains were infected with *Aspergillus niger* which caused black party of grains disease [16]. Genus *Aspergillus niger* was the most prevalent component of Wheat grains.

Experimental

Inorganic Preparations: 3-formyl-4-hydroxy coumarin [17] and thiocarbohydrazide[18] have been prepared by literature methods.

The mononuclear complexes have the formula CoXS, X= Cl, NO_3, CH_3COO, where S= Ethanol, H_2O, have been previously prepared by dissolving 0.001 mol of metal chloride in 20 ml Ethanol then added gradually with stirring to 0.278 gm 0.001 mol of HL in 150-170 ml dioxane then reflux for 2h, the reaction tube closed with dry $CaCl_2$. The complexes have been formed, often after raising pH using 3 drops of ethanolic 1% KOH and investigated using elemental analysis magnetic moment, molar conductance, IR, vis- spectra and thermal analysis reference [19].





$$\left[CoLCl_2EtOH\right]\frac{1}{2}\ EtOH$$

4-Hydroxy Coumarin-3-Carboxaldhyde (1 m mol) + dithiocarbohydrzide (1 m mol) $\xrightarrow[Conc\ H_2SO_4]{-H_2O}$ 4-Hydroxy Coumarin-3-dithiocarbohydrzene)

HL

$$HL + CoCl_2.6H_2O \xrightarrow[EtOH\ reflex\ 2h]{dioxane} \left[CoLCl_2EtOH\right]\frac{1}{2}\ EtOH$$

Biochemical Analysis

Subject: In this study, eighty male Wister Albino Rats, weighing 100-150 gm were maintained in clean cages. The rats were fed with commercial pelleted diet obtained from King Fahad Medical Research Center in Jeddah. The duration of the experiment is 12 days. Rats were divided into seven groups:

Group (A) is a control group; they were given ethanol 25 mg/kg. Group (B) is a control group; they were given ethanol 5 mg/kg. Group (C) were given complex (COL $NO_3.2H_2O$) 25 mg/kg. Group (D) were given ligand (HL) 25 mg/kg. Group (E) were given complex (COL $NO_3.2H_2O$) 5 mg/kg. Group (F) were given ligand (HL) 5 mg/kg. All groups were injected by i.v from the first day to the end of the experiment [19].

MATERIALS AND METHODS

After 3, 6 and 12 days of the experiment, the rats were anesthetized with ether. Blood was collected to analyze some enzymatic and non-enzymatic biochemical parameters. Enzymatic biochemical parameters include some key enzymes such as aspartate aminotransferase (AST) [20], alanine aminotransferase (ALT) [21] and alkaline phosphatase (ALP) [22]. Non-enzymatic biochemical parameters include urea [23], uric acid [24] and creatinine [25] which were measured by Diminsion (DAD BEHRING Company, Germany). The rats were killed by cervical decapitation at the end of the experiment, livers and kidneys were removed from each group then divided into 2 parts. One part of the livers was used for the determination of Glutathione (GSH) [26], Glucose-6-Phosphate dehydrogenase (G-6-PDH) [27], Xanthine oxidase (XO) [28]. The other part was put in formalin solution (10 %) and stained by Hematoxyline and Eosine (H and E) to be used for the historical examination[29].

Statistical Analysis: Collected data were calculated by T-test and ANOVA using SPSS program version 15.

Microbiological Analysis: The HL (Ligand) and CoL $Cl2H_2O$ (Complex) was tested by studying the effect of *Aspergillus niger* and *Candida albicans* on the radial growth.

Step One: Collection of Sample: four samples of wheat seeds were brought from different farms at the south (w1), north (w2), east (w3) and west (w4) from Jeddah province. Wheat seeds were surface sterilized with 0.5% sodium hypochlorite solution for 5 minutes and rinsed several times in sterile distilled water.

Step Two: Isolation of Mycoflora: Wheat seeds were cultured on blotter test and malt extract agar plates [30, 31]. Isolated fungi were identified [32, 33] and *Aspergillus niger* was selected from the different isolated fungi. Afterwards, the effect of the HL and $CoLNO_3 2H_2O$ on the radial growth was studied (Table 5).

Step Three: Antifungal Screening: HL and $CoLCl 2H_2O$ were evaluated for their *vitro* antifungal activity by the agar-well diffusion method [34]. The *Candida albicans* were incubated in Malt Extract Broth (Difco) for 48 hours, in the agar-well diffusion fungi (10^3-10^4 per ml) for 24 hours. By using a sterilized cork borer (7 mm diameter), wells were dug in the culture plates. Compounds dissolved in DMF were left at 4°C for 2 hours then the plates were incubated at 25°C for 72 hours. At the end incubation of the period, inhibition zones formed on the medium were evaluated as millimeters (mm) in diameter. The control samples contained DMF only (Table 6).

Step Four: Effects of Hl and Colcl2h$_2$o on Radial Growth of *Aspergillus Niger*[34]: To test the effect of the two compounds on the growth of *Aspergillus niger*, two concentrations at 50.0 and 100.0 μm of the tested compounds were added to sabaroud dextrous agar. Afterwards, a disc of *A niger* cultured on a solid media was transferred to the middle of Petri dishes and incubated at 25°C for 6 days in the dark. The diameters of the fungal growth were measured after 3, 6 and 9 days (Table 6).

RESULTS AND DISCUSSION

1- Biochemical Parameters in Serum

A- Compared in One Group at Different Durations: Increases and decreases of some key enzymes and non-enzymatic biochemical parameters in one group at different duration were observed.

In group (A), the results showed an increase in the activity of AST, ALT but a decrease in the activity of ALP after 6 and 12 days. In addition, it showed a significant increase in the level of urea after 6 days and 12 days, but significant decrease in uric acid level after 6 and 12 days compared with 3 days.

In group (B), the results showed an increase in the activity of AST, ALT after 6 days but decrease in these activities after 12 days and decrease in the activity of ALP after 6 and 12 days. In addition, it showed a very significant decrease in the level of urea after 6 and 12 days. In addition, it showed a decrease in the level of uric acid after 12 days but a significant increase in the level of creatinine after 12 days compared with 3 days.

In group (C), the results showed a very significant decrease in the activity of AST after 6 and 12 days, a decrease in the activity of ALT after 6 and 12 days respectively. Also, it showed an increase in the activity of ALP after 6 days but a decrease after 12 days and a significant decrease in the level of urea after day 6 compared with 3 days.

In group (D), the results showed highly significant decrease in the activity of AST after 6 days and very highly significant decrease after 12 days, but increase in the activity of ALT after 6 and 12 days. Also, it showed a highly significant decrease in the activity of ALP after 6 and 12 days, a highly significant decrease in the level of urea on days 6 and 12. A very highly significant decrease in the level of uric acid after 6 days compared with 3 days.

In group (E), the results showed highly significant decrease in the activity of AST after 6 days and significant decrease after 12 days, but increase in the activity of ALT at 6 and 12 days. Decrease in the activity of ALP in 6 and 12 days. A highly significant decrease in the level of uric acid at 6 days and very highly significant decrease in 12 days compared with 3 days.

In group (F), the results showed an increase in the activity of AST at 6 days but significant decrease in 12 days. An increase in the activity of ALT in 6 days and highly significant increase in 12 days plus a significant decrease in the activity of ALP in 6, 12 days.

These results due to the presence of many components in the complex and its ligands caused the new interactions in different doses which affected the groups of this study. The compounds of coumarin may have played a chemopreventive role via reducing oxidative stress in living system, significantly decreased the leakage of AST and ALT. increasing evidence regarding free radical generating agents and inflammatory processes suggest that accumulation of reactive oxygen species can cause hepatotoxicity [3]. Also the coumarin compounds are reactive components of herbs used for the treatment of various diseases. The ability of coumarin compounds to lower plasma ALT was examined using mice induced with hepatitis [35]. For more than 40 years coumarin has been successfully used in the therapy of choronic venous insufficiency. The occurance of liver injuries is rather rare and happens predominantly when doses are administered significantly higher than necessary for therapeutical use and the release of the enzymes ALT [36]. Coumarin is a potent chemopreventive agent and suppresses ferric nitrilotriacetate induced nephrotoxicity and tumor promotions in Wister rats, lipid peroxidation, xanthine oxidase, blood urea nitrogen and serum creatinine [37]. This finding supports the hypothesis that cobalt affects myocardial function. Whether this finding has clinical implications, remains to be evaluated [9]. Cobalt has been shown to stimulate sodium transport across the distal nephron of the newt kidney. The mechanism of this action remains elusive [10] (Table 1).

B-Compared Between Groups at Different Duration: Tables 1 and 2 illustrate the results before we observed changes in the activities and the levels of enzymatic and nonenzymatic between groups at different durations. A very highly significant increase in the activity of AST at 3 days between A(C), B(C), C(D) and C(E), very highly significant increase in 12 days between A(D). A highly significant increase in 6, 12 days between E(F) and D(E) respectively. Significant increase in 12 days between A(C) and A(E). An increase in 3 days between A(D), A(E), B(D) and B(E). Also increase in 6 days between A(C) and B(C) and D(F) and increase in 12 days between B(E), C(E) and C(F). Very highly significant decrease in 3 days between C(F). Highly significant decrease in 3 days between D(E), highly significant decrease in 6 days between A(E), B(E) and C(E), highly significant decrease in 12 days between A(B), A(F) B(D), C(D) and E(F). Significant decrease in 3 days between D(F), Significant decrease in 6 days between C(D). A decrease in 3 days between A(F) and E(F). Also decrease in 6 days between A(B), A(D), A(F), B(D) and C(F) and decrease in 12 days between A(B), A(F), B(D), C(D), E(F), B(C) and D(F). Finally decrease in all duration between B(F). Highly significant increase in the activity of ALT at 6 days between A(C). Significant increase in 3 and 6 days between A(B). An increase in 3 days between A(C), A(D) and B(C), also increase in 6 days between A(D) and A(E), increase in 12 days between A(B), A(E), B(C), C(E), C(F), D(E) and D(F). An increase in all duration between C(D). Very highly significant decrease in 3, 6 days between B(F) C(F) respectively. Highly significant decrease in 6 days between B(F). Significant decrease in 3 days between B(E), C(F) and D(F), significant decrease in 6 days between C(E) and D(F). A decrease in 3 days between A(E), C(E) and D(F), decrease in 6 days between B(C), B(E), B(F) and D(E). Decrease in 12 days between A(C), A(D), B(E) and B(F). Additionally, decrease in all duration between A(F), B(D) and E(F). A significant increase in the activity of ALP at 3 days between C(D) and C(F). An increase in 3 days between A(B), A(D), A(F), B(D), B(F), C(F) and E(F), an increase in 6 days between B(C), D(E) and D(F). Significant decrease in 3 days between A(C), B(C), D(F),

Table 1: Effect of Transition Metal Complexes with of Pendant Coumarine Thiosemicarbazone on some key enzymes and non-enzymatic biochemical parameters, Aspartate Amino Transferase, Alanine Amino Transferase Alkaline Phosphatase(U/l), urea (mg/dl), Uric acid (mg/dl) and Creatinine (mg/dl), in Serum of different studied groups (A, B, C, D, E, F) at different duration

		Groups					
		A	B	C	D	E	F
Parameters	Days	X±S.D	X±S.D	X±S.D	X±S.D	X±S.D	X±S.D
AST(U/L)	3days	134±26.5	129±7.5	240.3±43.7	157.3±8.1	138.3±26.1	117.3±19.2
	6days	135.7±1.5	133.7±10.7	144.3±4.5***	105.7±15.5**	85.3±9.5**	130.7±
	12ays	137.3±41.9	99.3±14.2	99±15.5***	49.7±5***	100.3±16*	77±14.5*
ALT (U/L)	3days	54.7±3.8	75±6.6	61±13.5	63.7±6.5	53±10.1	44.3±3.5
	6days	60.3±10.1	77.3±5.7	84.3±7.5**	72.7±20.2	67.3±11.1	54±5
	12days	65±5.6	72.7±3.1	60±8.5	64.7±15.6	66.3±3.8	69.3±3.8**
ALP (U/l)	3days	334.7±68	335.7±27.6	242.7±27	352.7±35.2	296.3±112.5	337±61
	6days	301.7±95.3	270.3±11	287±9.6	224.3±38.6**	233.3±10.8	239.3±23.5*
	12days	262±56.3	267.7±40.6	233±43	223.3±54.6**	249.7±32.1	234.3±11.1*
Urea (mg/dl)	3days	12.3±3.1	30±1	28.3±1.2	24.7±3.1	23.3±5.9	15.7±2.1
	6days	20.7±3.2**	15±3.6***	20.3±1.2**	17.7±1.5**	19.7±1.5	15±2.6
	12ays	17±2*	17±1***	26.7±4.5	17.3±2.1**	16±2	16.3±2.1
Uric acid (mg/dl)	3days	3.1±0.5	3.2±0.5	1.8±0.8	1±0.1	3±0.3	3.1±0.3
	6days	1.7±0.2*	2.3±2.2	2.7±0.3	1±0.2	0.7±0.2***	1.3±0.8**
	12days	1.8±0.7*	1.2±0.3**	0.7±0.3	1.5±0.9	0.8±0.2***	0.6±0.3***
Creatinine (mg/dl)	3days	0.3±0.1	0.2±0.06	0.3±0.1	0.4±0.05	0.4±0.2	0.4±0.06
	6days	0.4±0.1	0.3±0.06	0.4±0.05	0.3±0.06	0.4±0.1	0.4±0.06
	12days	0.3±0.1	0.4±0.1*	0.4±0.05	0.4±0.2	0.3±0.06	0.4±0.06

X±S.D = Mean ± Standard deviation

* significant P < 0.05

highly significant P < 0.01 **

*** very highly significant P < 0.001

Table 2: Effect of Transition Metal Complexes with of Pendant Coumarine Thiosemicarbazone on some key enzymes, Aspartate Amino Trasferase, Alanine Amino Trasferase and Alkaline Phosphatase(U/l) in Serum between different studied groups (A, B, C, D, E, F) at different durations

	AST(u/l)						ALT(u/l)						ALP (mg/dl)					
	3day		6 day		12 day		3 day		6 day		12 day		3 day		6 day		12 day	
Duration																		
Groups	P	Sig.	P	Sig	P	Sig	P	Sig	P	Sig	P	Sig	P	Sig	P	Sig	P	Sig
A (B)	0.789	N. S	0.902	N. S	0.024	*	0.01	*	0.03	*	0.315	N. S	0.981	N. S	0.453	N. S	0.892	N. S
A (C)	0.000	***	0.593	N. S	0.022	*	0.405	N. S	0.003	**	0.510	N. S	0.032	*	0.724	N. S	0.487	N. S
A (D)	0.155	N. S	0.070	N. S	0.000	***	0.239	N. S	0.11	N. S	0.965	N. S	0.665	N. S	0.069	N. S	0.355	N. S
A (E)	0.789	N. S	0.003	**	0.027	*	0.826	N. S	0.36	N. S	0.86	N. S	0.359	N. S	0.107	N. S	0.767	N. S
A (F)	0.307	N. S	0.758	N. S	0.001	**	0.178	N. S	0.405	N. S	0.568	N. S	0.955	N. S	0.140	N. S	0.507	N. S
B (C)	0.000	***	0.511	N. S	0.984	N. S	0.071	N. S	0.358	N. S	0.10	N. S	0.030	*	0.689	N. S	0.407	N. S
B (D)	0.094	N. S	0.09	N. S	0.004	**	0.14	N. S	0.539	N. S	0.29	N. S	0.683	N. S	0.273	N. S	0.290	N. S
B (E)	0,593	N. S	0.005	**	0.951	N. S	0.006	*	0.192	N. S	0.41	N. S	0.347	N. S	0.376	N. S	0.665	N. S
B (F)	0.448	N. S	0.853	N. S	0.173	N. S	0.000	***	0.004	**	0.7	N. S	0.974	N. S	0.458	N. S	0.425	N. S
C (D)	0.000	***	0.021	*	0.004	**	0.725	N. S	0.129	N. S	0.539	N. S	0.011	*	0.138	N. S	0.816	N. S
C (E)	0.000	***	0.001	**	0.934	N. S	0.294	N. S	0.030	*	0.405	N. S	0.232	N. S	0.202	N. S	0.689	N. S
C (F)	0.000	***	0.401	N. S	0.180	N. S	0.033	*	0.000	***	0.222	N. S	0.028	*	0.256	N. S	0.974	N. S
D (E)	0.003	**	0.214	N. S	0.003	**	0.165	N. S	0.483	N. S	0.826	N. S	0.181	N. S	0.829	N. S	0.528	N. S
D (F)	0.018	*	0.129	N. S	0.098	N. S	0.014	*	0.018	*	0.539	N. S	0.707	N. S	0.719	N. S	0.791	N. S
E (F)	0.2	N. S	0.008	**	0.155	N. S	0.257	N. S	0.085	N. S	0.692	N. S	0.331	N. S	0.885	N. S	0.713	N. S

*** The mean difference is very highly significant at p < 0.001

** The mean difference is highly significant at p< 0.01

*The mean difference is significant at p< 0.05, N.S Not significant

Table 3: Effect of Transition Metal Complexes with of Pendant Coumarine Thiosemicarbazone on some non-enzymatic biochemical parameters, urea (mg/dl), Uric acid (mg/dl) and Creatinine (mg/dl) in Serum between different studied groups (A, B, C, D, E, F) at different durations.

	Urea (mg/dl)						Uric acid (mg/dl)						Creatinine (mg/dl)					
	3day		6 day		12 day		3 day		6 day		12 day		3 day		6 day		12 day	
Duration																		
Groups	P	Sig.	P	Sig	P	Sig	P	Sig	P	Sig	P	Sig	Sig	P	Sig	Sig	P	Sig
A (B)	0.000	***	0.05	*	1	N. S	0.810	N. S	0.234	N. S	0.223	N. S	0.377	N. S	0.657	N. S	0.188	N. S
A (C)	0.000	***	0.882	N. S	0.000	***	0.024	*	0.078	N. S	0.054	N. S	1	N. S	0.377	N. S	0.377	N. S
A (D)	0.000	***	0.187	N. S	0.882	N. S	0.001	**	0.234	N. S	0.510	N. S	0.377	N. S	1	N. S	0.657	N. S
A (E)	0.000	***	0.656	N. S	0.656	N. S	0.875	N. S	0.088	N. S	0.061	N. S	0.058	N. S	0.377	N. S	0.188	N. S
A (F)	0.143	N. S	0.015	*	0.766	N. S	1	N. S	0.549	N. S	0.036	*	0.657	N. S	1	N. S	0.082	N. S
B (C)	0.459	N. S	0.022	*	0.000	***	0.013	*	0.549	N. S	0.455	N. S	0.377	N. S	0.657	N. S	0.657	N. S
B (D)	0.022	*	0.239	N. S	0.882	N. S	0.000	***	0.021	*	0.569	N. S	0.082	N. S	0.657	N. S	0.377	N. S
B (E)	0.005	**	0.043	*	0.656	N. S	0.675	N. S	0.05	**	0.491	N. S	0.011	*	0.188	N. S	1	N. S
B (F)	0.000	***	1	N. S	0.766	N. S	0.810	N. S	0.078	N. S	0.355	N. S	0.188	N. S	0.657	N. S	0.657	N. S
C (D)	0.108	N. S	0.239	N. S	0.000	***	0.173	N. S	0.005	**	0.192	N. S	0.377	N. S	0.377	N. S	0.657	N. S
C (E)	0.031	*	0.766	N. S	0.000	***	0.036	*	0.001	**	0.952	N. S	0.082	N. S	0.082	N. S	0.657	N. S
C (F)	0.000	***	0.022	*	0.000	***	0.024	*	0.021	*	0.857	N. S	0.657	N. S	0.377	N. S	0.377	N. S
D (E)	0.553	N. S	0.375	N. S	0.553	N. S	0.001	**	0.590	N. S	0.212	N. S	0.377	N. S	0.377	N. S	0.377	N. S
D (F)	0.000	***	0.239	N. S	0.656	N. S	0.001	**	0.549	N. S	0.139	N. S	0.657	N. S	1	N. S	0.188	N. S
E (F)	0.001	**	0.043	*	0.882	N. S	0.857	N. S	0.258	N. S	0.810	N. S	0.188	N. S	0.377	N. S	0.657	N. S

*** The mean difference is very highly significant at $p < 0.001$

** The mean difference is highly significant at $p < 0.01$

*The mean difference is significant at $p < 0.05$, N.S Not significant

Table 4: Effect of Transition Metal Complexes with of Pendant Coumarine Thiosemicarbazone on Glutathion (GSH) (µ mol/gm tissue), Glucos-6-phosphatase (G-6-PDH) (µ mol/gm tissue), Glucos-6-phosphatase (G-6-PDH) and Xanthine oxidase (XO) (n mol/min/mg) in Liver, Malondialdehyde (MDA) (n mol/gm tissue), Nitric oxide (NO) (µ mol/gm tissue) and Xanthine oxidase (XO) (n mol/min/mg), in kidney in different studied groups (A, B, C, D, E, F) at different duration

Groups	A	B	C	D	E	F
Parameters	X±S.D	X±S.D	X±S.D	X±S.D	X±S.D	X±S.D
GSH (µ mol/gm tissue)	3±o.3	4±0.5***	2.9±0.2	3.9±0.3	3.7±0.3**	3.7±0.2
G-6-PDH (µ mol/min/mg protein)	0.05±0.00	0.08±0.01***	0.05±0.01	0.08±0.01	0.08±0.01***	0.08±0.06
XO (n mol/min/mg)	2.7±0.2	2.3±0.2	2.8±0.2	2±0.04	2.3±0.2*	2.3±0.2
MDA (n mol/gm tissue)	12.9±1.2	10.4±1*	15.5±0.9*	10.6±0.9	18.1±1.4***	11.6±1.6
NO (µ mol/gm tissue)	20.6±2.3	11.2±1.2***	19.1±1.6	11.6±0.7	17±1.4**	11.6±0.9
XO (n mol/min/mg)	5.2±1.1	3.4±0.4**	5.9±0.7	3.2±0.4	7.1±0.5**	3.6±0.5

X±S.D = Mean ± Standard deviation

* significant $P < 0.05$

highly significant $P < 0.01$ **

*** very highly significant $P < 0.001$

C(E) and D(E). A decrease in 3 days between D(E) and D(F), decrease in 6 days between A(B), A(C), A(D), A(F), B(D), B(F), C(D), C(E), C(F) and E(F). Also, decrease in 12 days between A(B), A(C), A(D), A(F), B(C), B(D), B(F), C(D), C(F) and E(F). Significant increase in 6 days between A(B), B(D) and C(D). Significant increase in 3 days between C(D) and E(F). An increase in 3 days between C(E) and D(F), increase in 6 days between A(C) and A(F), also increase in 12 days between C(E), C(F) and E(F). Slightly increase in 6 days between C(F). Very highly significant decrease in 3 days between B(C), B(D) and B(F), very highly significant decrease in 6 days between D(E) and D(F). Additionally, very highly significant decrease in 12 days between A(E), A(F), B(C), B(D) and B(F). A very highly significant decrease in all duration between B(E). Highly significant decrease in 3 days between A(C). Significant decrease in 3 days between D(E). Significant decrease in 6 days between B(C), B(F) and C(E). Significant decrease in 12 days between D(E) and D(F). Decrease in 3 days between A(D) and A(E), Also decrease in 6 days between A(E), but similar in 3 days between A(F).

Table 5: The percentage of fungi isolated from wheat seeds cultured on malt extract agar (1gm / 1000distal water)

Sample	Isolation name	%of growth on blotter test	%of growth on malt extract agar	Total
w1	*Aspergillus niger*	11.7	3.32	331
	Ulocladium alternariae	3.32	0.60	
	Aspergillus fumigates	5.74	1.21	
	Fusarium oxysporum	1.81	0	
w 2	*Aspergillus niger*	28.88	3.70	135
	Penicillium sp	8.88	1.48	
	Fusarium moniliforme	14.07	2.96	
	Alternaria alternata	4.44	1.48	
w 3	*Aspergillus niger*	12.50	3.12	128
	Alternaria	7.03	2.34	
	Penicillium sp	8.59	0	
	Mucar sp	3.12	1.56	
w 4	*Aspergillus niger*	24.17	9.89	91
	Ulocladium alternariae	7.69	2.19	
	Penicillium sp	3.29	0	
	Aspergillus flavus	5.49	1.09	

Tables 1 and 3 illustrate A very highly significant increase in the level of urea at 3 days between A(B), A(C), A(D) and A(E). Very highly significant increase in 12 days between A(C) and B(C). Highly significant increase in 3 days between B(F). Significant increase in 3 days between C(E) and significant increase in 6 days between B(C) and C(E). Very highly significant decrease in 3 days between B(F), C(F) and D(F), very highly significant decrease in 12 days between D(E) and D(F), also very highly significant decrease in 12 days between C(D), C(E) and C(F). Highly significant decrease in 3 days between E(F). Significant decrease in 3 days between B(D). Significant decrease in 6 days between A(B), A(F), E(F), C(F) and B(E). Highly significant increase in the level of uric acid at 3 days between D(E) and D(F). Significant increase in 3 days between C(E). Very highly significant decrease in 3 days between B(D). A highly significant decrease in 3 days between A(D), highly significant decrease in 6 days between B(E), C(D) and C(E). Significant decrease in 3 days between A(C), B(C) and C(F). Significant decrease in 6 days between B(D) and C(F). Also, significant decrease in 12 days between A(F). A significant increase in the level of creatinine 3 days between B(E).

Biochemical Parameters in Tissues: Table 5 shows some key enzymes and non-enzymatic biochemical parameters. Very highly significant increase in the level of GSH between C(D) and A(B), highly significant increase between A(D), A(E), A(F), C(E) and C(F). Very highly significant decrease between B(C). A decrease between A(C), B(C), B(E), B(F), D(E) and D(F)), but similar between E(F). Also, very highly significant increase in the activity of G-6-PDH between A(B), A(D), A(E), A(F), C(D), C(E) and C(F). A very highly significant decrease between B(C), but similar between A(D), B(D), B(E), B(F), D(F), E(F) and D(E). A very highly significant increase in the activity of XO between A(D) and C(D). Highly significant increase between B(C). Significant increase between D(F). An increase between A(C) and D(F). Highly significant decrease between C(E) and C(F). Significant decrease between A(B), A(E) and A(F). A decrease between B(D). Similar between B(E), B(F) and E(F). A very highly significant increase in the level of MDA between A(E), B(C), B(E) and D(E). Significant increase between A(C) and C(E). An increase between B(F) and D(F). Slightly increase between B(D). Very highly significant decrease between C(D), C(F) and E(F). Significant decrease between A(B) and A(D). A decrease between A(F). Very highly significant increase in the level of NO between B(C), B(E) and D(E). Slightly increase between B(E) and B(F). Very highly significant decrease between A(B), A(D), A(E), A(F), C(D), C(F) and E(F). A decrease between A (C) and C(E). Similar between D(F). Additionally, very highly significant increase in the activity of XO between B(C) and B(E). Highly significant increase between A(C) and A(F). Significant increase between C(E). Slightly increase between B(F) and D(F). Very highly significant decrease between A(B), A(D) and A(F). A decrease between B(D).

Derivatives of coumarin cause inhibition of xanthine oxidase (XO) activity and the structure activity relationship of these derivatives against XO activity and suppression of reactive oxygen speicies [38].

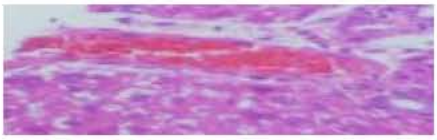

1-a

Fig (2-a). A part of liver from control group (A) showing laminal of hepatic cells and blood sinusoid. Hematoxyline & Eosine (H&E) (X 400)


1-b

Fig (2-b). A part of liver from control group (B) showing increase in the number and size of nucleus also found binucleated hepatic cells and necrotic of the nuclei (arrow). Hematoxyline & Eosine (H&E) (X 400)


1-c

Fig (2-c). A part of liver from group (C) showing congestion in the centralvein and necrosis in some liver cells. Hematoxyline & Eosine (H&E) (X 400)


1-d

Fig (2-d). A part of liver from group (D) showing increase in the number of kupffer cells and necrotic of the nucleus. Hematoxyline & Eosine (H&E) (X 400)


1-e

Fig (2-e). A part of liver from group (E) showing dilatation in portal area, congestion and fibrous cells. Hematoxyline & Eosine (H&E) (X 400)

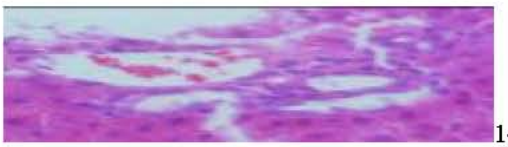

1-f

Fig (2-f). A part of liver from group (F) showing dilation and degenerative of blood vessels (arrow), more hemorrhage and necrotic of nucleus. Hematoxyline & Eosine (H&E) (X 400)

Fig. 1:

Fig (1-a). A part of liver from control group (A) showing kuppfer cells around the portal area, nucleus, blood sinusoid but found hemorrhage in the portal area. Hematoxyline & Eosine (H&E) (X 400)

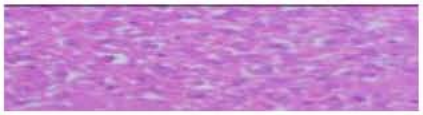

2-a

Fig (1-b). A part of liver from control group (B) showing degenerative of blood vessels. Hematoxyline & Eosine (H&E) (X 400)

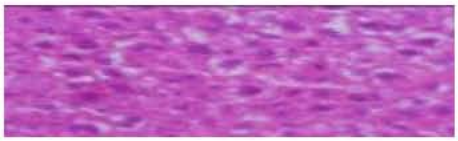

2-b

Fig (1-c). A part of liver from group (C) showing congestion in the portal area. Hematoxyline & Eosine (H&E) (X 400)


2-c

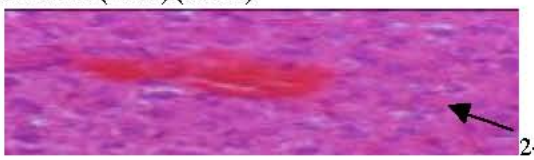
Fig (1-d). A part of liver from group (D) showing degenerative and necrotic of hepatic cells (arrow). Hematoxyline & Eosine (H&E) (X 400)


2-d

Fig (1-e). A part of liver from group (E) showing many necrosis in some liver cells and hemorrhage (arrow). Hematoxyline & Eosine (H&E) (X 400)


2-e

Fig (1-f). A part of liver from group (F) showing many hepaocytes were binucleated accompanied by granular degeneration and fatty changes activation of kupffer cells (arrow) and necrosis in some liver cells. Hematoxyline & Eosine (H&E) (X 400)


2-f

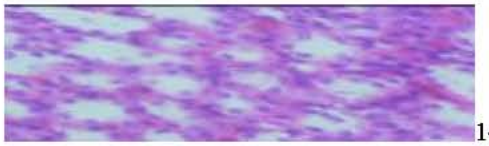

1-a

Fig (2-a). A part of kidney from control group (A) showing degenerative; necrotic changes in kidney tubules and glomerulus. Nuclear pleomorphism in kidney tubules. Hematoxyline & Eosine (H&E) (X 400)

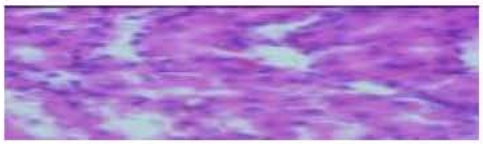

1-b

Fig (2-b). A part of kidney from control group (B) showing nearly normal distal convoluted tubule with diluted luman and low epithelial height and nuclei in some proximal epithelial cells. Hematoxyline & Eosine (H&E) (X 400)


1-c

Fig (2-c). A part of kidney from group (C) showing widening of tubular lumen, extruded nuclei, shortening, damage of brush border in proximal tubules, hyalinization and hypertrophy of glomerulus. Hematoxyline & Eosine (H&E) (X 400)

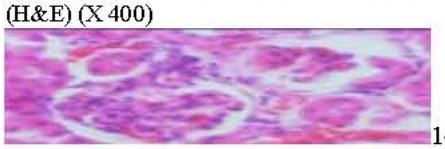

1-d

Fig (2-d). A part of kidney from group (D) showing hypertrophy of glomerular cells, tubularlysis, tubular necrosis, pyknotic nuclei and highly eosinophlic cytoplasm in necrotic tubules. Hematoxyline & Eosine (H&E) (X 400)

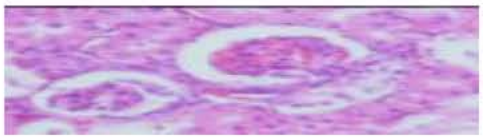

1-e

Fig (2-e). A part of kidney from group (E) showing nearly normal distal convoluted tubule with diluted luman and low epithelial height and nuclei in some proximal epithelial cells. Hematoxyline & Eosine (H&E) (X 400)


1-f

Fig (2-f). A part of kidney from group (F) showing atrophoid tubules with separated from basement membrane, deformed and stained intensely. Hematoxyline & Eosine (H&E) (X 400)

Fig. 2:

Fig (1-a). A part of kidney from control group (A) showing nearly normal distal convoluted tubule with diluted luman and low epithelial height; fragmented glomerular tuft; extruded nuclei in some proximal epithelial cells and lymphocytic infiltration. Hematoxyline & Eosine (H&E) (X 400)

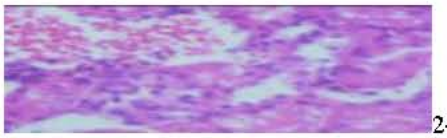

2-a

Fig (1-b). A part of kidney from control group (B) showing windening of tubular lumen, extruded nuclei, shortening, damage of brush border in proximal tubules. Hematoxyline & Eosine (H&E) (X 400)


2-b

Fig (1-c). A part of kidney from group (C) showing hypertrophy, hypercellulatory of glomerulus cells, cellular infiltration and hypertriophy of tubular nuclei. Hematoxyline & Eosine (H&E) (X 400)

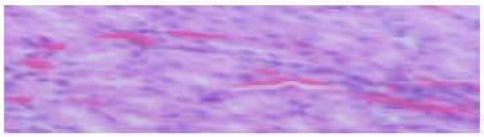

2-c

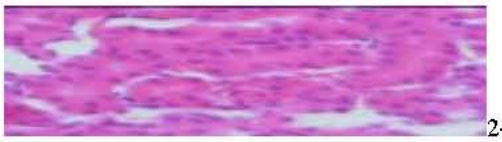
Fig (1-d). A part of kidney from group (D) showing obliteration of urinary space, reflex of proximal tubule into urinary space. Hematoxyline & Eosine (H&E) (X 400)


2-d

Fig (1-e). A part of kidney from group (E) showing hypertrophoid, fragmented, congested glomeruli slightly swollen proximal tubules with disturbed with nuclei and cellular infiltration. Hematoxyline & Eosine (H&E) (X 400)


2-e

Fig (1-f). A part of kidney from group (F) showing necrotic tubule separated from basement membrane and fragmented glomeruli with congested capillaries. Hematoxyline & Eosine (H&E) (X 400)


2-f

Derivative of coumarin treated diabetic rats by decreasing the level of glucose-6-phosphate dehydrogenase activity and shows promising potential for restoration of normal blood glucose levels erythrocyte lipid peroxidation antioxidants and lipid profile [39].

Increasing evidence regarding free radical generating agent and inflammatory processes suggests that accumulation of reactive oxygen species can cause hepatotoxicity. Compounds of coumarin may play a chemopreventive role via reducing oxidative stress in living system, significantly decrease the leakage of LDH and decrease the formation of MDA and reduce oxidative stress in the liver [35]. Coumarin has been successfully used in the therapy of choronic venous insufficiency where LDH increases and there is a measurable reduction of perfusion flow, oxygen consumption and rate of bile secretion. Additionally, the concentrations of hepatic ATP and oxidized and total glutathione (GSSG/ GSH) decrease. In the livers of fasting animals, coumarin doubles the concentration of hepatic MDA. The cofactor in coumarin (Troxerutin) preparations is used for the therapy of chronic venous insufficiency [1]. Maybe, thiosmecarbazone derivatives and its metal complex act as antioxidants and pro-oxidants which affect the level of MDA [40].

Histological Examination in Tissues: Figures 1-a, 2-a, 1-b, 2-a, 1-c, 2-c, 1-d, 2-d, 1-e, 2-e, 1-f and 2-f show the liver tissues. In group (A), many kupffer cells around the portal area, nuclei, but found hemorrhage in the portal area. In group (B), degeneration of blood vessels was found and binucleated hepatic cells and necrotic of the nucleus. In group (C), the figures show congestion in the portal area, congestion and necrosis. In group (D), degenerative, necrotic of hepatic cells and nucleus, increase in the number of kupffer cells. In group (E), necrosis, hemorrhage, congestion and fibrous cells. In group (F), many hepatocytes were binucleated fatty changes activation of kupffer cells, necrosis and dilation and degeneration of blood vessels.

Figures 1-a, 2-a, 1-b, 2-b, 1-c, 2-c, 1-d, 2-d, 1-e, 2-e and 1-f, 2-f show the kidney tissues. In group (A), it shows almost normal distal convoluted tubule with diluted luman and low epithelial height, extruded nuclei in some proximal epithelial cells lymphocytic infiltration and necrotic changes in kidney tubules. In group (B), extruded nuclei and damage of brush border in proximal tubules, also nearly normal distal convoluted tubule with diluted luman. In group (C), hypertrophy, hypercellulatory of glomerulus cells, cellular infiltration, hypertrophy of tubular nuclei, extruded nuclei, shortening and damage of brush border in proximal tubules. In group (D), obliteration of urinary space, reflex of proximal tubule into urinary space, hypertrophy of glomerular cells, tubularlysis, tubular necrosis and pyknotic nuclei. In group (E), hypertrophoid, fragmented, congested glomeruli slightly swollen proximal tubules with disturbed with nuclei, cellular infiltration and nearly normal distal convoluted tubule with diluted luman. In group (F), necrotic tubule separated from basement membrane and fragmented glomeruli with congested capillaries. Also the morphological changes in the liver and kidney caused by thiosmecarbazone derivative and it's metal complex. Hestopathological evaluation of rat liver lesions induced by t-BHP [40], including hepatocyte swelling leukocyte infiltration and necrosis [3]. From all the results, we show that group (E) gave a better effect in all parameters and histological examination than group (C), this group better than Group (D) but this group gave close results to Group (F). Also Group (E) was better than Group (F).

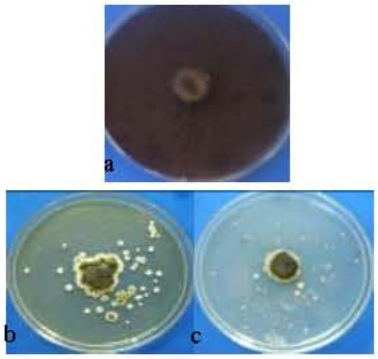


Fig. 3: Effect of the HL (b) and COL $NO_3.2H_2O$ (c) on at 100.0 μm on the Radial growth of Aspergillus niger grown on the solid media (a)control

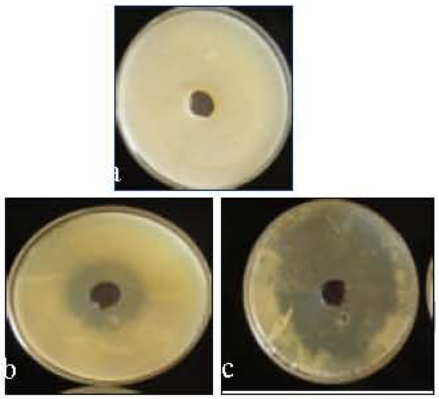


Fig. 4: Antifungal activities of the Hl and COL $NO_3.2H_2O$: (a) control (b) C. albicans grown on the solid media (c).

Table 6: Effect of Different Concentrations of Hl and COL $NO_3 2H_2O$ on the Radial Growth and Inhibition of *Aspergillus niger* Grown on Solid Media (mm/ disc ; Mean of Replicates±SE)

		Incubation (days)					
		3		6		9	
Treatment	Concentration % (p.p.m)	Radial growth	Inhibition %	Radial growth	Inhibition %	Radial growth	Inhibition %
Control	0.0	3.83±0.28	0.00	8.63 ±0.15* *	0.00	9.00±00**	0.00
HL	50.0	4.43±3.09	15.67	5.66±1.87**	34.41	7.50±0.86**	16.67
	100.0	2.96±0.65	22.72	3.43±0.40	60.25	8.83±0.35	1.89
COL NO3.2H2O	50. 0	3.73±0.40	2.60	5. 50±2.17	36.26	5.83±0.76	50.00
	100.0	3.83±3.61	0.00	2.83±0.76	67.20	7.83±1.15**	46.34

* significance at 5% ** significance at 1%

The results of this study demonstrated that the complex at a low dose has a better effect than at a high dose and ligand at high and low doses on the biochemical analysis in serum and liver, kidney tissues and histological examinations.

Microbiological Activity: In This study, the experiment was carried out on four samples of wheat seeds brought from different parts of Jeddah area. Results of parasitic fungi isolates were got from blotter test: 10 species belong to 5 genera and 11 species belong to 3 genera were isolated from samples cultivated on malt extract agar medium. Genus *A. niger* was the most prevalent component of samples.

The free ligand showed positive results against *C. albicans* (100 μg/ml) although there was a sufficient increase in the fungi activity of free ligand compared to the complexes. The results also showed that *A.niger* was much more sensitive against all tested compounds compared with other filamentous fungi under identical experimental condition (Table 6), (Figure 3,4)

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