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Biochemical and Spectral Analysis of Roridin A Toxin and Copper (I) Nicotinate Complex as Antidote on Male Rat Liver

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Abstract: This work has been designed to evaluate the curative effect of copper (I) –nicotinate complex on the hepatotoxicity of Roridin A in male rats as well as the study of the spectral analysis of this complex. Healthy young male albino rats (n=40) were exposed to a single dose of Roridin A (60 µg/kg body weight) and scarified and another group was treated with the copper (I) –nicotinate complex. Such intoxication resulted in some biochemical parameters such as ALP, GGT, TAS, Ferritin and AFP. Finally, Copper (I)-Nicotinate complex reduced many undesired changes of liver tissue and this improvement was predicted since this complex has been confirmed previously as a therapeutic agent against induced mycotoxins as Roridin A.

Keywords: Roridin A, Copper (I) - nicotinate complex, spectra, rat

1 Introduction

Roridin A mycotoxin is one of the important macrocyclic Trichothecenes, which is produced by growing fungi on foodstuffs such as corn, rice, wheat and other crops. All trichothecences have in common a 9,10 double bond and a 12,13 epoxide group, which is responsible for their toxicological actions (sudakin 2003) but the extensive variation exists relative to ring oxygenation patterns (Yang et al., 2000) Macrocyclic, e.g., Verrucarine A, B, J and Rordin A, D and E, etc. (Figure 1). Trichothecences mycotoxins prevent polypeptide chain initiation or elongation by interaction with eukaryotic co-s subunit (large nucleoprotein subunit of ribosome) and interact with the enzyme peptidyl transferase. Both human and animal suffer from several pathologien due to intoxication after consumption of foodstuffs contaminated with trichothecences and the conditions have been named differently according to the causative fungus and country where they occur. Toxicological evaluation of trichothecences in animal feed has been extensively reviewed (Eriksen and Petterssom 2004; van Egmond, 2004).

With regard to many of the biologically active copper chelating complexes (Sorenson et al., 1987) the copper (I) –nicotinate complex that exhibiting antioxidant activity as well as therapeutic pharmaceutical activity against Newcastle diseases (Mura et al, 1987), anti-inflammatory effect on gastric ulcer (EL-Saadani et al, 1993), reduction of adverse effects of 5-Fluorouracil in patients with hepatocelluler carcinoma (El-Saadani, 2004), curative effect against induction of fatty liver in experimental animals (Salama et al, 2007), curable clinical signs of rheumatoid arthritis in rats (Mandour et al, 2005), improved the skin burns in the experimental animals (Nassar et al., 2009), efficiently prevented induced nephrotoxicity by Aflatoxin B1 (AFB1) specifically by promoting phase II detoxificating glutathione S-transferase activity (Nassar et al., 2014), protected neurons and glial cells injured by 4-dimethylaminoazobenzene exposure (Youssef and Hassan 2014).

The objective of this study was to assess the effect of Roridin A on the levels of Alkaline phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), Total antioxidant status (TAS), Ferritin and Alpha-Fetoprotein (AFP) on hepatocytes cell line as a result of toxin administration on the liver of male rat in order to evaluate the possible role of copper (I)-nicotinate complex in treatment of Roridin A toxicity. To further understanding the structure-activity correlations, structural analysis is conducted here to elucidate the stoichiometric ratios of the prepared complexes.

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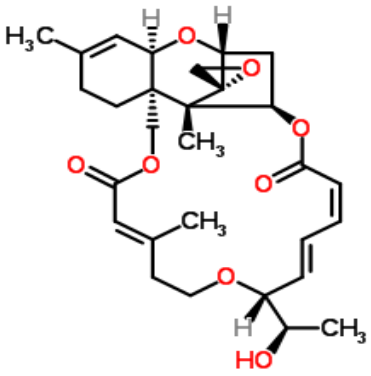

Figure 1. Roridin A

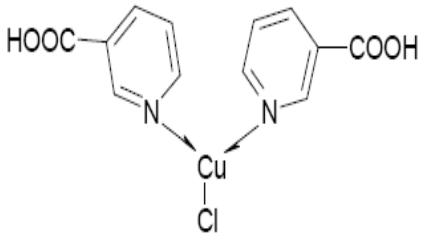

Figure 2. Copper (I)-Nicotinate complex

2 Materials and Methods

2.1 Chemicals

Roridin A (5 gm) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The Copper (I)- nicotinate complex was synthesized as described by **Gohar and Dratoviscky (1975)**.

2.2 Animals and Diet

40 male albino rats (Wistar strain) weighing about 180 - 190 g were used for the experiments. They were maintained under standard experimental conditions, fed with standard pelleted diet, water and libitum. All animal experiments were carried out according to the guidelines of animal house ethics of Sohag University.

2.3 Experimental design

Animals were divided into 4 groups (10 rats each) and subjected to the following schedule of treatments.

Control group was gavage fed with 0.6 mg/kg B.W of DMSO saline solution and left for one week before dissection.

Treated (T_1) animals were gavage fed with a single dose of 0.6 mg/kg B.W of Roridin A (dissolved in 1% DMSO saline) to achieve 5% mortality (LD_{50}) (**Hughes et al., 1988**) and kept on normal feed for one week.

Treated (T_2) animals were gavage fed with a single dose of 0.6 mg/kg B.W of Roridin A but left for two weeks.

Treated (T_3) animals were gavage fed with Copper (I)-Nicotinate complex (0.4 mg/kg B.W) (**Shatat et al., 2013**) for two weeks after giving Roridin A for one week.

2.4 Sampling

After the end of each period, all the rats were anesthetized using ether. Blood samples from all groups were collected from the heart in plain tubes and centrifuged at 5000 rpm for 10 minutes to separate the serum.

2.5 Biochemical analysis

Serum samples were used for the measurement of ALP according to **Bowers and McComb, (1966)**, GGT according to **Szasz (1969)**, TAS was determined according to the method described by **(Koracevic, 2001)**. The determination of the antioxidative stress is performed by the reaction of antioxidants in the sample with defined amount of exogenously provide hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5-dichloro-2-hydroxybenzenesulphonate to a colored product, Ferritin according to **Young et al., (1995)**, and AFP was determined by ELISA kit which purchased from Elab Science.

2.6 Spectral analysis

2.6.1 Determination of the stoichiometry of the synthesized Cu-nicotinic acid complex

The stoichiometry of the Cu-nicotinic acid formed in solutions via the reaction of Cu ions with the nicotinic acid was determined by applying the spectrophotometric molar ratio and Job's method of continuous variation

2.6.2 Continuous variation method

Stock solutions of both the metal salt and ligand (1×10^{-2} mol dm^{-3}) were prepared by dissolving the calculated amount of each separately in the required volume of DMF. Then a set of solutions containing different independent mole fractions of the components; metal salt and the ligand, keeping the total concentration of the two constituents unvaried at 1×10^{-2} mol dm^{-3} were prepared. The absorbance value of each solution was measured at λ_{max} = 674 nm of the Cu-nicotinic acid. The Absorbance (Abs.) values for each compound were plotted against the mole fraction of the ligand ([L] / ([L] + [M])). **(Job, 1928; Issa et al, 2006)**

2.6.3 Molar Ratio method

In this method, stock solutions of both the metal salt and ligand (1×10^{-2} mol dm^{-3}) were prepared as described

previously in the continuous variation method section. Then a set of solutions containing a constant 2×10^{-3} mol dm^{-3} of the metal salt, where by varying concentrations of the ligand from 0.50×10^{-3} to $4.5x10^{-3}$ mol dm^{-3} were prepared. The absorbance values of the obtained solutions were measured at λ_{max} = 674 nm of the investigated complex. The absorbance (Abs) values of each complex were plotted against the ratio of the ligand to the metal in each solution of the previous prepared series ([L] / [M]). (**Yoe and Jones, 1944; El-Shiekh et al, 2011; Shah and Parmar, 2011**)

2.7 Statistical analysis

Data are expressed as the mean ±S.E. (standard error). Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey 's multiple comparison test using the Statistical Package for the Social Science (S.P.S.S. 11). The level of significance was set at $p<0.05$. (Values with $p<0.05$ were consider significant).

3 Results and Discussion

Table 1. Changes in Serum levels of ALP (U/ml), GGT (U/ml), TAS (mmol/l), Ferritin (µg/ml), and AFP (ng/ml) in control, untreated T_1, T_2 and treated T_3 with Copper (I)-Nicotinate complex.

	Control	**T_1**	**T_2**	**T_3**
ALP U/ml	3.699±0.002	4.9±0.003***	4.98±0.002***	3.7±0.002N.S
GGT U/ml	1.47±1.456	1.86±0.002***	1.56±0.003***	1.408±0.003 N.S
TAS mmol/l	±0.01059 0.229	±0.003*** 0.282	0.416±0.005***	0.329±0.003 N.S
Ferritin µg/ml	0.281±0.002	0.163±0.002***	±0.002*** 0.155	0.217±0.003 N.S
AFP ng/ml	133±0.3	138±0.3^{NS}	156.3±4.5***	98.6±0.2*

Data are expressed as mean ± SE. Number of samples in each group is 10.

^{NS} Non significant; *p<0.05; **p<0.01; ***p<0.001 represent significant differences as compared to control animals.

Trichothecene mycotoxins affected all parts of the world because they are detected as naturally occurring contaminants of agricultural product. The present study of Roridin A in male rats has been focused on the protective effect of the bioactive Copper (I)-nicotinate complex on liver function, lipid peroxidation and cancer markers. When liver tissue damage occurs, cellular enzymes may be released into the serum and elevation of certain enzymes such as ALP and GGT that often associated with hepatocellular diseases.

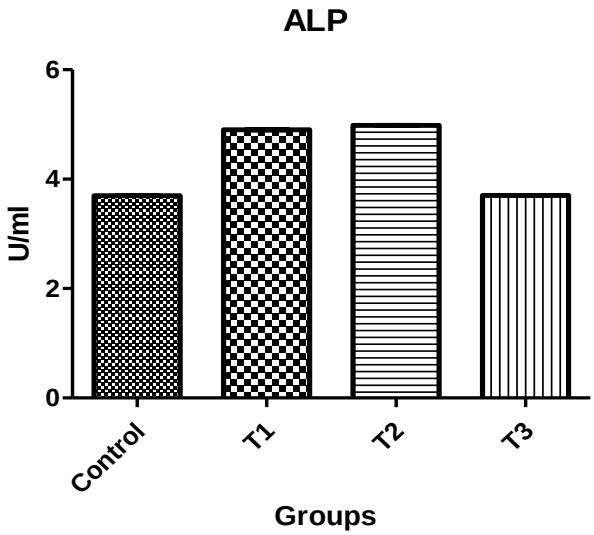

Figure 3. ALP levels in the sera of different groups

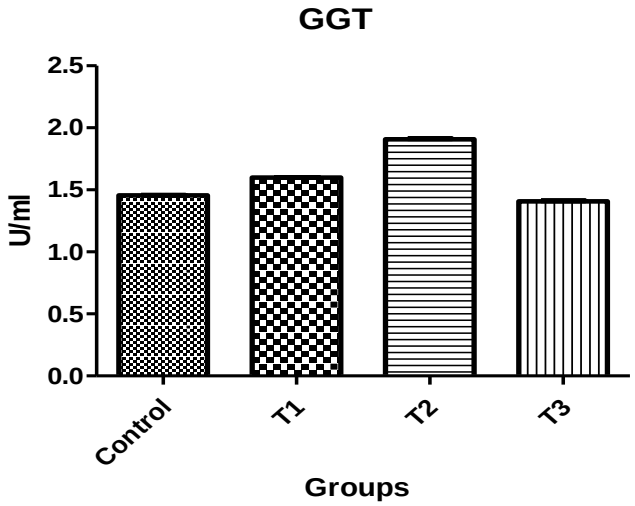

Figure 4. GGT levels in the sera of different groups

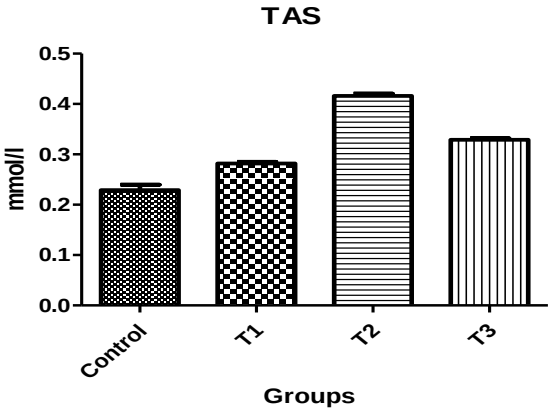

Figure 5. TAS levels in the sera of different groups.

The administration of Roridin A for one and two weeks

produced a highly significant increase in the serum levels of ALP and GGT activities Table (1), Figure (3) and Figure (4) respectively, as compared to the control group ($P<0.001$). These results are in agreement with those obtained by **(Elsawi et al., 2010)** which is a common phenomenon in hepatocellular disease. On the other hand, there is no significant difference ($p>0.05$) after 2 weeks treated with Copper (I)-Nicotinate complex. So, this bioactive complex could be considered as a protective agent for hepatic cells against Roridin A toxicity.

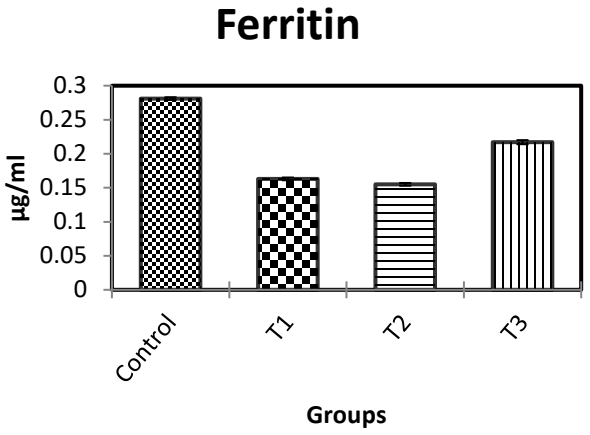

Figure 6. Ferritin levels in the sera of different groups

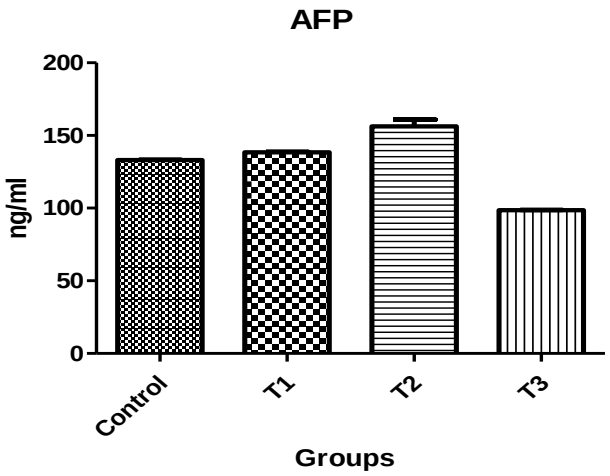

Figure 7. AFP levels in the sera of different groups

It was surprising to observe that total antioxidants status TAS activity table (1) and figure (5) were a highly significant increase in Roridin A treated groups as compared to the control ($P<0.001$). High level of TAS in Roridin A treated groups may reflect a body defense against the toxicity of Roridin A **(Elsawi et al., 2015)**. Normally, we can expect a decrease in the antioxidant levels after the administration of a drug that can be potentially hepatotoxic. Conversely, in the present study it was interesting to find a significant increase in TAS in Roridin A treated groups, it is suggested that an increase in antioxidant levels may be a defense mechanism in the liver to prevent Roridin A toxicity. In fact, **Abraham and Sugumar (2008)** support this view, who demonstrated a significant increase in glutathione and antioxidant enzymes after treated with cyclophosphamide. On the other hand, the group treated with the Copper (I)-Nicotinate complex showed also a highly significant increase in TAS activity compared to control ($P<0.001$) but it still less than those treated with Roridin A and this could be interpreted due to the anti-inflammatory and antioxidant effect of the Cu(I)-nicotinate complex on the cellular levels.

Iron stores in the body exist primarily in the form of ferritin. In the body, small amounts of ferritin are secreted into the plasma. The concentration of this plasma (or serum) ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation. A low serum ferritin value reflects depleted iron stores, but not necessarily the severity of the depletion as it progresses. Table (1) and Figure (6) show a highly significant decrease in serum ferritin in the group treated with Roridin A compared to the control group ($P<0.001$) and this is due to the toxicity of Riridin A which increases antioxidant production potential to mobilize ferritin iron and this suggestion is in agreement with **Reif (1992)**. The mean value of ferritin was significantly increased in copper (I)-nicotinate complex treated group as compared to the control group. This indicates that copper (I)-nicotinate complex reduces many undesirable changes in the liver tissue due to its ability for absorption or elimination of the mycotoxin or inhibiting its transformation, resulting in the increase of its toxicity.

Alpha-fetoprotein (AFP) is used as a tumor marker to help detect and diagnose cancers of the liver, testicles, and ovaries. Increased AFP levels may indicate the presence of cancer, mostly in the liver **(Furukawa et al., 1984)**. In Table (1) and Figure (7), the mean value of AFP concentration was increased by 1st and 2nd weeks compared to the control group and this is due to the toxic effect of Roridin A on the liver of the animals. On the other hand, AFP in the group treated with Copper (I)-Nicotinate complex was less than the value of control and treated groups with roridin A indicating that Copper (I)-Nicotinate complex reduced many undesired changes of liver tissue and this improvement was predicted since this complex has been confirmed previously as a therapeutic agent against induced mycotoxin as Roridin A (ref).

3.1 Electronic Spectra of the copper-nicotinic acid complex

Electronic spectra is a valuable tool for coordination chemists to draw important information about the structural aspects of the complexes. The ligands, which are mainly organic compounds, have absorption bands in the ultraviolet region of the electromagnetic spectrum and in

some cases these bands extend over to the higher wavelength region due to conjugation. But, upon complexation with metal ions, changes will take place in the electronic properties of the system. New features or bands in the visible region due d-d absorption and charge transfer spectra from metal to ligand (M→L) or ligand to metal (L→M) can be observed.

These data can be processed to obtain information regarding the structural and geometry of the complexes. The absorption region, the molar extinction coefficient and band assignment of the different bands in the recorded spectra of the copper-nicotinic acid complex in the range 200 – 900 nm and at 298 K, c.f. Fig. (8) and Table (2). As shown in Table (2), the designed copper-nicotinic acid complex display a characteristic band centered at ≈ 674 nm and ≈ 815 nm, these bands could be mainly attributed to d-d transition.

Table (2). λ_{max} (nm) of the copper-nicotinic acid complex at and 298 K.

Compound	**λ_{max}, nm**	**Assignment**
copper-nicotinic acid complex	815 674	d-d band d-d band

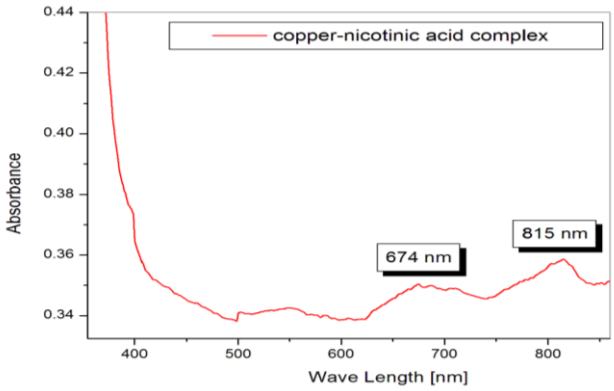

Fig. (8). UV.Vis. Spectrum of the copper-nicotinic acid complex

3.2 Determination the stoichiometry of the copper-nicotinic acid complex

The stoichiometry of the copper-nicotinic acid complex formed in solutions via the reaction of Cu(I) with nicotinic acid was determined by applying the spectrophotometric molar ratio and continuous variation methods. The results from both molar ratio method and contentious variation method supports each other and confirm the formation of the complex in 1:2 molar ratio (M:L).

3.3 Continuous variation method

The data of continuous variation of the copper-nicotinic acid complex, *c.f.* table (3), are plotted as; the Absorbance (Abs.) for copper-nicotinic acid complex vs. the mole fraction of the ligand ([L] / ([L]+[M])), *c.f. fig. (9)*.

The curve of continuous variation method displayed maximum absorbance at mole fraction **X ≈ 0.6-0.7**, *c.f. fig. (9)*, which indicates that, the formation of the complex with the metal ion to ligand in 1:2 molar ratio.

Table (3). Continuous variation data of copper-nicotinic acid complex

Continuous variation method	
[L] / ([M]+[L])	Abs. of copper-nicotinic acid complex
0.9	0.46
0.8	0.486
0.7	0.514
0.6	0.506
0.5	0.476
0.4	0.446
0.3	0.416
0.2	0.39
0.1	0.375

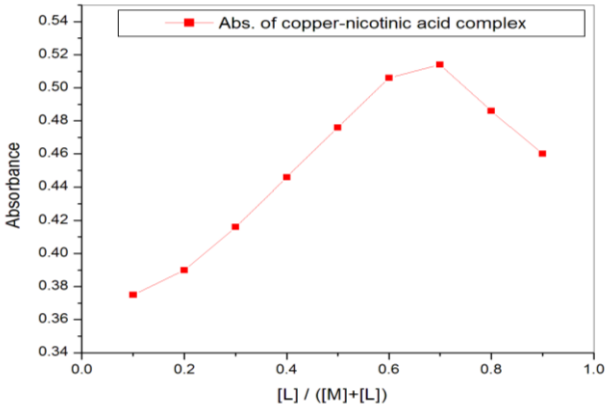

Fig. (9). Continuous variation plot for copper-nicotinic acid complex

3.4 Molar Ratio method

Table (4). Molar Ratio method of copper-nicotinic acid complex

Molar Ratio method	
[L]/[M]	Abs. of copper-nicotinic acid complex
0.25	0.22
0.5	0.286
0.75	0.396
1	0.506
1.25	0.616
1.5	0.726
1.75	0.836
2	0.876
2.25	0.912

The data of molar ratio plot of the copper-nicotinic acid complex, *c.f. table (4)*, are plotted as; the absorbance (Abs.) of copper-nicotinic acid complex vs. the ratio of the ligand to the metal in each solution of the previous series ([L] / [M]), *c.f. fig. (10).*

The graph of the molar ratio plot of the copper-nicotinic acid complex consists of two linear portions intersecting at a value of ([L] / [M]) ≈ 2, *c.f. fig. (10),* which indicate the formation of the complex between copper (I) and nicotinic acid in the ratio 1:2.

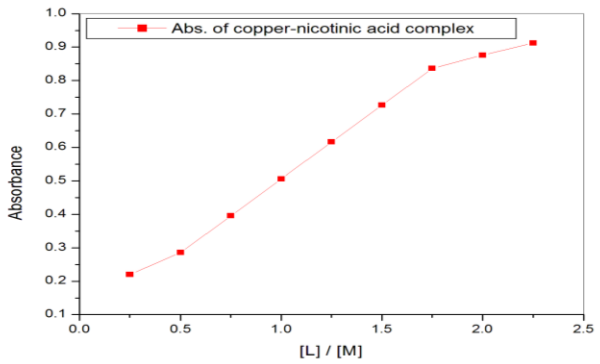

Fig. 10. Molar ratio plot for copper-nicotinic acid complex

3.5 Evaluation of the apparent formation constants of the copper-nicotinic acid complex.

The formation constants (K_f) of the copper-nicotinic acid complex formed in solution were obtained from the spectrophotometric measurements by applying the continuous variation method according to the following relations,

$$K_f = \frac{\left(\frac{A}{A_m}\right)}{\left(1-\left(\frac{A}{A_m}\right)\right)^2 C} \quad \text{..... In the case of 1:1 complex}$$

And

$$K_f = \frac{\left(\frac{A}{A_m}\right)}{4C^2\left(1-\left(\frac{A}{A_m}\right)\right)^3} \quad \text{..... In the case of 1:2 complex}$$

Where A_m is the absorbance at the maximum formation of the complex, A is the arbitrary chosen absorbance values on either sides of the absorbance pass and C is the initial concentration of the metal.

As mentioned in *table (5),* the obtained K_f values indicate the high stability of the prepared complexes. The negative values of Gibbs free energy mean that the spontaneous and favorable.

In conclusion, the majority of the present study indicates that the biochemical effect of rordin A was observed In liver and hepatocytes tools by different degrees to the time of exposure .Basically, these obtained results .By using copper (1) nicotinate complex as a therapeutic agent against Rordin A and also ameliorates the biochemical functions of liver.

Table (5). The formation constant (K_f), stability constant ($\log K_f$) and Gibbs free energy ($\Delta G^{\neq}$, KJ/mol) values of the copper-nicotinic acid complex at 298 K.

Complex	Type of the complex	Formation constant (K_f)	Stability constant ($\log K_f$)	$\Delta G^{\neq}$ (kJ/mol)
copper-nicotinic acid complex	1 : 2	8.484 x 10^7	7.92	-45.18

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