

Study Of The Cytotoxicity Effect Of New Cu(II) And Ni(II) Complexes Of Flavon On Cancer (Cell Line *L20b*) And Antimicrobial Activity

Omar H. Al-Obaidi

Chemistry Department , Education College for Women, Al-Anbar University, Ramadi , IRAQ

*Correspondence author email: dromaralobaidi@yahoo.com

ABSTRACT : A new series of Cu(II) and Ni(II) complexes with the Flavone ligand were studied on the growth of mutant mouse cells (Mice Transformed cell Line) (*L20B*) by using in vitro system and compared with anticancer drug cisplatin (cis-pt) as appositive control. The cancer cells were treated with different concentration and cis-pt after 72 hr. exposure time. The cytotoxic activity was tested by inhibition rate as parameter. The results showed significant differences ($p < 0.05$) for each three treatments when the inhibition rates were increased. The synthesized compounds were tested for antimicrobial activity by cup plate diffusion method. The results indicate the enhanced activity of metal complexes over the parent ligands.

Keywords: Flavone , complexes, antimicrobial activity, Cytotoxicity cisplatin.

INTRODUCTION

Flavonoids represent one of the largest groups of natural products. In addition to the various functions of flavonoids in plants, their widespread distribution in nature, their structural variability, their relatively low toxicity, and their antioxidant activities have increased the interesting flavonoids as beneficial for human health.

Several therapeutically interesting biological activities of certain flavonoids have been reported including antibacterial[1], antiviral, anti-inflammatory[2,3], antiallergic[4], antithrombotic[5], antimutagenic, antineoplastic[6], neuroprotective properties[7], and antioxidant properties[8,9], etc. The biological activity of flavones can be modified upon formation of metal complexes.

Flavonoid-metal complex compounds are the subject of our longtime research during which we investigated properties, composition, complex formation features, stability constants, as well as analytical appraisal of approximately 40 complexes of flavonoids from different flavonoid subclasses with a number of metal ions or metal groups [10,11]. The biological effects of flavonoid-metal complexes, confirmed by other authors in numerous studies, showed that complexes are more effective than free flavonoids. All these data are available in various previous publications [12,13].

The synthesized ligand and metal chelates have been screened for in vitro antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, and new prepared complexes were compared with anti cancer drug cis-platin.

Experimental

Instrumentation and Chemical

IR spectra were recorded Pye Unicam Sp. 3100 spectrophotometer, solid samples were measured as KBr disc. For UV measurement absolute methanol and ethanol were used as solvents. Rotary evaporator RE-120 Buchi. Gallenkamp (hot stage) determined M.P. BDH chemicals Ltd.-England, Fluka AG Buchs-Swaziland and Riedel Du Haen Germany supplied chemicals. (Cis-platin) (10mg/20ml) was provide by Ebew (Austria).

Preparation of Ligand and their Complexes

New complexes Ni-L and Cu-L were provide by our study [14]. 10 mg of each complexes dissolved in 20 ml of normal saline (stock solution) and were stored at (2-8) C° until processing.

General procedure for the preparation of Flavone – Mannose , 5-hydroxy-2-(4-(((2R,3S,4S,5S,6S)-3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methoxy)phenyl)-4H-chromen-4-one (L)

Take (1 mol) of Flavone 5-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one and dissolved in (200ml) of DMSO and placed inside round flask capacity (100ml) then add (1 mol) of dissolved Mannose in (20ml) of the same solvent and added drops of (5%) of HCl, the mixture heated for two hours in a water bath and then the solvent is evaporated and collects the output. spectral data of these compounds are given as follows: (yield

65%); FT-IR (KBr): 3401 (Ar-OH), due to presence of phenolic -OH group, 1600 (C=O pyrone ring) cm^{-1} ; UV-Vis shows max (EtOH) 250 nm, 280 nm and 350 nm. C. H. N. analysis cal.; C, 60.58; H, 4.84%. Found: C, 60.71; H, 4.33%.

General procedure for the preparation of the complexes

0.2 mmoles from L ($\text{C}_{21}\text{H}_{20}\text{O}_9$) was dissolved in MeOH (30 ml) then 0.1 mmole metal chlorides was added. The resulting mixture was refluxed for 30 min. and the volume of the final mixture was reduced under vacuum. The crude products were purified by recrystallization from MeOH to give a powder, yield 82%. Fig. 1.

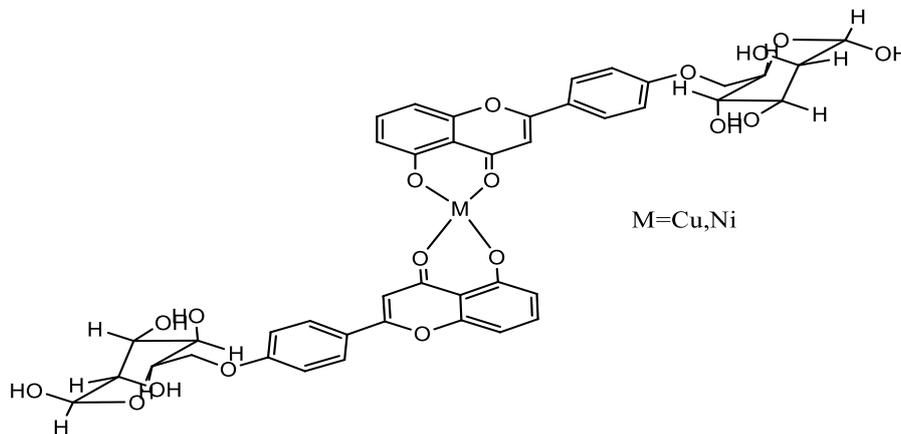


Figure 1. Suggested structure of the prepared Cu(II) and Ni(II) complexes .

Study of cytotoxic effect on cancer cell line

One type of cancer cell lines have been used to study the impact of the prepared compounds under study on the growth of cells in laboratory and thus know the specifications of extracts as an anti-tumors, this work done at Department of Cancer Research in Bio-technology Research Center, University of Nahrain.

All solutions are prepared at the same center and culturing tissues were studied in vitro under optimum conditions by the same center. The growth media used in tissue culture technique was MEM (Minimum Essential Media) was provided by Fetal Calf Serum (10%) to form a confluent monolayer, then Subculture to discard the previous growth medium and the cell washed with sterilized phosphate buffer solution (PBS) by autoclave at 121 C° for 15 min and addition 2-3 min and moving the culture flask kindness. The trypsin-versene solution to discard and cells incubated at 37 C° until the cell separation from ground flask, added new growth media and redistribution of cells at the microtiter and incubated at 37 C° [15].

Cytotoxic or cell growth inhibition Assay

In this assay, the cell line (L_{20}B) was treated with new complexes and cisplatin by using four concentrations (31.25, 62.5, 125, 250) $\mu\text{g}/\text{ml}$. immediately by adding of 25ml trypsin-versene solutions in to culture bottle and 20 ml of culture medium which contains 10% of serum to provide the suspend cells, mixed very well and addition of 0.2 ml to each microtiter. The plates were incubated at 37 C° for 24 hour until to form monolayer, then the previous culture medium which present in to the plates to discard 0.2 ml of compounds under study were added and these three preparation repeated as negative control (cancer cell line L_{20}B with buffer solutions) and incubated at 37 C° for 72 hour exposure time. The culture medium to discard from micro litre plates, about 0.2ml of crystal violet solution was added to wells and the plates were incubated for 20 min at 37 C° . the plates washed gently with distilled water and left to dry. In the end of assay the plates were examined by ELISA reader at 492 nm transmitting wave length. Only viable cells were able to take a stain while the dead cells were not. The inhibition rate was measured according to were Gao et al [16] and as follows: Inhibition rate % = $(\text{Abs. of negative control} - \text{Abs. of Test} / \text{Abs. of negative control}) \times 100$

Antibacterial activity

Agar-well diffusion method followed as the using of kirby Baauer method [17] in the measurement of the sensitivity of bacteria used in this research for various concentrations of compounds, Escherichia Coli and aurous Staphylococcus bacteria was obtained (isolated and diagnosed in culture laboratory in children's hospital in Ramadi). also we used Mueller Hinton agar to test the sensitivity of bacteria for compounds and it was prepared as company instruction process, then the dishes putted in incubator at 37 C° for (24) hours and inhibition diameter was then measure (Inhibition Zone) in each hole by ruler and record the results.

RESULT AND DISCUSSION

The flavone have been synthesized through on cyclization in alkaline H_2O_2 yielded 5-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one, which react with mannose to produce, 5-hydroxy-2-(4-(((2R,3S,4S,5S,6S)-3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methoxy)phenyl)-4H-chromen-4-one(L). Complexes formation between Cu(II) or Ni(II) chlorides and 5-hydroxy-2-(4-(((2R,3S,4S,5S,6S)-3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methoxy)phenyl)-4H-chromen-4-one(L) performed in ethanolic dimethylformamide show the formation of mononuclear complexes with 1:2 metal to ligand stoichiometry (Fig 1). Prepared ligand have been characterized by spectroscopic methods (UV-Vis, IR) and C. H. N. analysis.

FT-IR spectra

The characteristic infrared spectral assignment of ligand (L) and their complexes are reported in experimental section. In the FT-IR spectra of ligand (L), the presence of phenolic OH and carbonyl group are confirmed by peaks at 3401 and 1600 cm^{-1} respectively [17]. However, in the spectra of the complexes there is complete disappearance of peak at 3401 cm^{-1} suggesting absence of phenolic OH group indicates its coordination. The band assigned to the carbonyl group is shifted to a lower wave number comparing with that of the free ligand, proving its coordination.

UV-Vis Spectra and Conductivity Measurements

The UV-Vis spectra of the complexes expected differences in the position of the absorption bands between the ligand and the related complexes, which are due to the coordination between the ligand and the transition metals. Appearance of new absorption maxima is considered as a hint for the formation of complexes. The bathochromic shift in band I upon coordination is due to the electronic transition ($n \rightarrow \pi^*$) of the lone pair of electrons of the hydroxyl group in the complex.

Band II, which caused by the transition ($\pi \rightarrow \pi^*$) of the aromatic ring, exhibit absorption maxima at 280 nm. This measured wavelength reflect the effect of substitution by auxochromes (hydroxyl group) caused a bathochromic shift in bands I and II, of the complexes[18].

The measurements of the molar electrical conductivity of the complexes in DMSO are indicated that the results clearly show values for the molar conductivity of the complexes of bivalent metals are non-electrolyte. From the above spectroscopic results (IR, UV-VIS) and conductivity measurement the following general structure can be proposed for the metal-Flavone complexes Fig.(1).

Antimicrobial Tests

The activity have been studying for the complexes, in different concentrations and using two types of pathogenic bacteria Escherichia Coli and aureus Staphylococcus. Inhibition zone larger than 6mm indicated antimicrobial activity. The complexes showed better efficiency Fig. (2) for Staphylococcus aureus and Escherichia Col.

A comparison of the value of flavone Ligand with these of the complexes indicates that the metal complexes exhibited higher antibacterial activity than ligand. Such increased activity of the complexes can be explained based on Overtones concept[19] and the tweedy chelation theory[20]. Moreover, the copper complexes were more active than the other complexes against the tested microorganisms as shown in scheme (2).

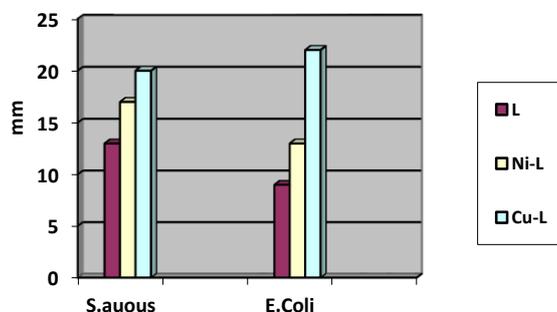


Figure 2. Effect of ligand and their complexes on growth of pathogenesis bacterial

Study of Cytotoxic Effect

Cancer cell lines have been used to study the effect of ligand and their complexes on the growth of cells in laboratory to know the specifications of extracts as anti-tumors. cancer cell line type mice transformed cell

line (L₂₀B) used with different concentrations comparable with anticancer drug cisplatin as a positive control after 72 hr. exposure time.

In this method, we calculate the proportion of cells number within the optimal conditions for growth without the addition of compounds so the output is the control group (control). Then compounds are added for the purpose of knowing their effects on cell growth in elected lines.

The result statistically analyzed by one way ANOVA. the following results as Fig.(3) which demonstrates the impact of compounds on cells number ratio when using cell line (L₂₀B), it is clear that hot alcoholic extract have the greatest influence on the proportion of growth cell number and the effect was significantly ($P < 0.05$). This result is identical to those published in literature [21].

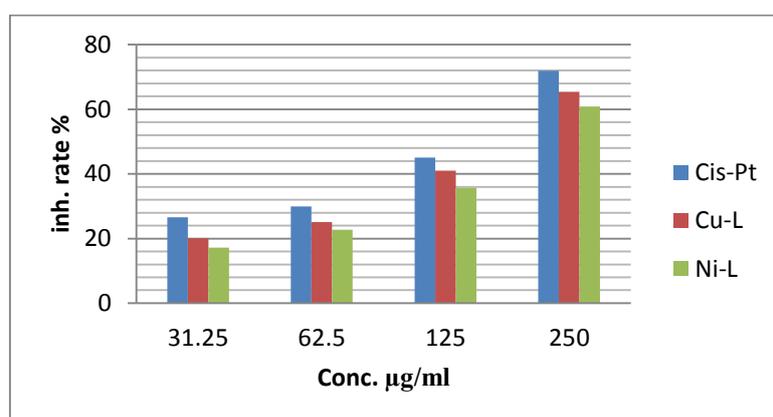


Figure 3. the comparison of inhibition rates between three treatments with cis-pt drug in cell line (L₂₀B)

The results in Fig. (3) shows an evidence that new complexes have cytotoxic effect on cancer cell line by elevated of inhibition rates with concentration increased, this effect was similar to effect of anti-cancer cisplatin. In this study, we suggest the new complexes of chalcone ligand have inhibition effect, this effect was similar to Tsuda et al [22] studied on colon and liver cancer in mice and result in DNA damage after shortly administration of relatively high dose, while carcinogenicity was detected after prolong treatment with low doses. Kenyon et al [23] showed the type of organic ligand (bis-8-hydroxyquinolin) coupled to tumor cellular copper forming potent proteasome inhibitors and apoptosis inducers at copper concentration found in tumor tissues. Saadiyh et al [24] showed the new copper complexes and aqueous origanum vulgare extract have a cytotoxic effect on RD cell line in four concentrations. This study similar to our results which rise the inhibition rates with elevation of concentration. The new complexes were similar effect to cis-pt that could be attributed to the cis-pt binding to and cross linking of DNA which ultimately triggers opoptosis (programmed cell death)[25].

REFERENCES

- Birt, F.D, Hendrich, S, Wang, W., " Dietary Agents in Cancer Prevention Flavonoids and Isoflavonoids Pharmacology and Therapeutics", USA, pp 157-177, 2001.
- Cazarolli, L.H.; Zanatta, L.; Jorge, A.P.; de Sousa, E.; Horst, H.; Woehl, V.M.; Pizzolatti, M.G.; Szpoganicz, B.; Silva, F.R.M.B. Follow-up studies on glycosylated flavonoids and their complexes with vanadium: Their anti-hyperglycemic potential role in diabetes. *Chemico-Biol. Interact.* 2006, 163, 177–191.
- Cushnie, T.; Lamb, A. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 2005, 26,343-356.
- Ferguson, P.J.; Kurowska, E.M.; Freeman, D.J. In vivo inhibition of growth of human tumor lines by flavonoid fractions from cranberry extract. *Nutr. Cancer* 2006, 56, 86-94.
- Freshney, R.I., " Cultured of Animal Cells: A manual for Basic Techniques ", Inc. Publication, New York, pp64-69, 2000.
- Grazul, M.; Budzisz, E. Biological activity of metal ions complexes of chromones, coumarins and flavones. *Coord. Chem. Rev.* 2009, 253, 2588–2598.
- Hertog, M.G.L.; Feskens, E.J.; Hollman, P.C.; Katan, M.B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *Lancet* 1993, 342,1007-1011.
- Jovanovic, S.V.; Steenken, S.; Simic, M.G.; Hara, Y.; Antioxidant properties of flavonoids: Reduction potentials and electron transfer reactions of flavonoid radicals. In *Flavonoids in Health and Disease*; Rice-Evans, C.A., Packer, L., Eds.; Marcel Dekker, Inc.: New York, NY, 1998; pp. 137-161.
- Jun, T.; Bochu, W.; Liancai, Z. Hydrolytic cleavage of DNA by quercetin zinc(II) complex. *Bioorg. Med. Chem. Lett.* 2007, 17, 1197-1199.
- K.Nakmato, *Infrared and Raman spectra of Inorganic and coordination compounds*, 4th ed., wiley, New York(1986).
- Kenyon, G., Puja, G., Harbach, R., Wayne, G., Ping Dou, Q., "Organic Copper Complexes as new Class of Proteasome Inhibitors and Apoptosis Inducers in Human Cancer Cells," *Bioc. Pharm.* 67, pp1139-1151, 2004.
- Knekt, P.; Kumpulainen, J.; Jarvinen, R.; Rissanen, H.; Heliovaara, M.; Reunanen, A.; Hakulinen, T.; Aromaa, A. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.* 2002, 76,560-568.
- L. Mishra, V. H. Singh, *Indian J. Chem.* 32A, 1993, pp. 446 .
- Lever, A.B.P. *Inorganic Electronic Spectroscopy*; Elsevier: Amsterdam, The Netherlands, 1986; pp. 385–392.

- Omar H. Al-Obaidi, Synthesis , Spectral, Characterization, Theoretical Evaluation Of New Cu(II) and Ni(II) Complexes Of Flavone , (under published).
- Pereira, R.M.S.; Andrades, N.E.D.; Paulino,N.; Sawaya, A.C.H.F.; Eberlin, M.N.; Marcucci M.C.; Favero, G.M.; Novak, E.M.; Bydlowski, S.P. Synthesis and Characterization of a Metal Complex Containing Naringin and Cu, and its Antioxidant, Antimicrobial, Antiinflammatory and Tumor Cell Cytotoxicity, *Molecules* 2007, 12, 1352-1366.
- Pietta, P.G. Flavonoids as Antioxidants. *J. Nat. Prod.* 2000, 63, 1035-1042.
- Saadiyah, A.D., Jinan H., and Iman H., *J. of Al-Nahrain Univ.* ,15(2), pp23-28, 2012.
- Stordal B., Davey, M., " Vander Standing cisplatin Resistance Using Cellular Models", *IUBMB Life*, 59, pp696-9, 2007.
- Teixeira S.; Siquet, C.; Aloes, C.; Boal, I.; Marques, M.P.; Borges, F.; Lima, J.L.F.C.; Reis, S. Structure-property studies on the antioxidant activity of flavonoids present in diet. *Free Radic. Biol. Med.* 2005, 39, 1099-1108.
- Tsuda, S., Matsusaka, N., Madaram H., Ueno, S., Susa, N., Ishida, K., Kawamura, N., Sekihashi, K., Sasak, Y., " the Comet Assay in Eight Mouse Organs Results with 2 Azo Compounds", *Mutat. Res.*, 465, pp1139-1151, 2000.
- van Acker, S.A.B.; van Balen, G.P.; van den Berg, D.J.; Bast, A.; van der Vijgh, W.F.F. Influence of iron chelation on the antioxidant activity of flavonoids. *Biochem. Pharmacol.* 1998, 56,935-943.
- Van Acker, S.A.B.E.; van Den Berg, D.-J.; Tromp, M.N.J.L.; Griffioen, D.H.; van Bennekom, W.P.; van der Vijgh, W.J.F.; Bast, A. Structural aspects of antioxidant activity of flavonoids. *Free Radic. Biol. Med.* 1996, 20, 331-342.
- Vandpitte J. , et al.,(1991), "Basic laboratory procedures in clinical bacteriology "WHO., Genevap.pp78-110).
- Y. Anjaneyalu, R. P. Rao, *Synth. React. Inorg.-Org. chem.*. 16, 1986, pp. 257.