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Study Of The Cytotoxicity Effect Of New Co(II), Mn(II), Ni(II), And Cu(II) Complexes Of Chalcone On Cancer (Cell Line L_{20}b) And Antimicrobial Activity

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ABSTRACT

A new series of Co(II) , Mn(II) ,Ni(II), and Cu(II) complexes with the chalcone ligand were studied on the growth of mutant mouse cells (Mice Transformed cell Line) ($L_{20}B$) 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: Chalcone, Complexes, antimicrobial activity, Cytotoxicity cisplatin.

INTRODUCTION

Chalcones are abundantly present in nature from ferns to higher plants [1]. They are aromatic compounds with an unsaturated side chain and often cytotoxic in vitro [2]. Chalcones have also been reported to be anti-inflammatory, analgesic and antipyretie [3]. Some chalcones possess bactericidal, antifungal and insecticidal activity and some of their derivatives are reported to be antimutagenic [4]. Chalcones are abundant in edible plants and are considered to be the precursors of flavonoids and isoflavonoids. 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.

Chalcone is a common natural pigment and one of the important intermediate in the biosynthesis of flavonoids [5]. Several therapeutically interesting biological activities of certain chalcones have been reported including antibacterial [3] anti-inflammatory activity [6], chemopreventive activity [7], cardiovascular disease [8], anticancer activity [9], cytotoxic activity [10], antiprolifirative activity [11], antimalarial activity [12], antiviral activity [13], anti-HIV activity [14], etc. The biological activity of flavones can be modified upon formation of metal complexes. 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.

MATERIALS AND METHODS

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 Co-L, Mn-L, 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^{o} until processing.

General procedure for the Preparation of chalcone, (E)-1-(2,6-dihydroxyphenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (L): To the mixture of 2,6-dihydroxy acetophenone (15.21 gm, 0.1 mol), alcohol (50 mL) and 4-(dimethylamino)benzaldehyde (14.91 gm, 0.12 mol), NaOH (40%,19mL)was slowly added with vigorous stirring (2-3 hrs), till orange solid mass was obtained and left it overnight at ambient temperature. Cold 5N, 42 mL HCl was poured on to it with constant stirring. The yellow solid was filtered, washed with water, dried and crystallized from alcohol. Yield 70%, brownish powder, M.P. >240 ºC, IR (KBr disc) shows absorption at 3400 cm^{-1} (OH), 1640 cm^{-1} (C=O). UV-Vis shows max (EtOH) 290 nm, 280 nm. C. H. N. analysis; C=72.00 (cal. 72.07), H=6.02 (cal. 6.05), N =4.89 (cal. 4.94).

General procedure for the preparation of the complexes: 0.2 mmoles from L ($C_{17}H_{17}NO_3$) 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 recrystallisation from MeOH to give a powder, yield 75%.

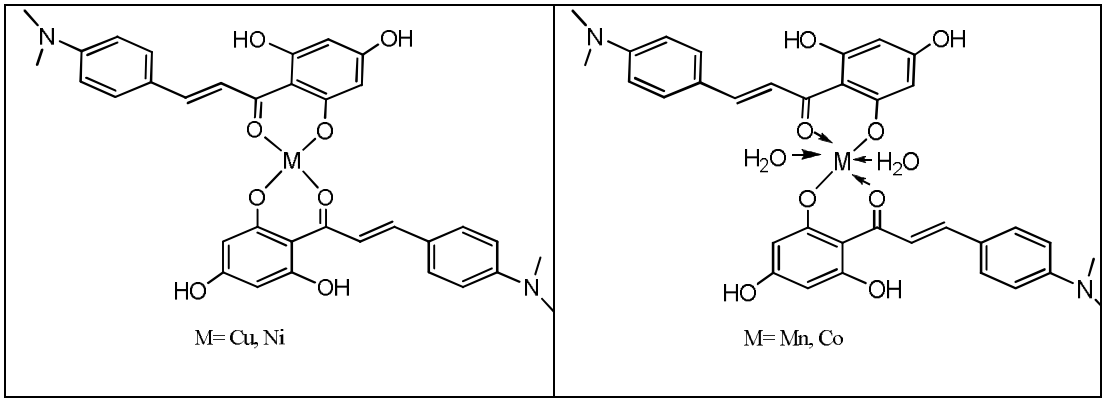


Fig 1. Suggested structure of the prepared complexes

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-tumor, 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° [16].

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) µg mL^{-1}, 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 [17] and as follows

Inhibition rate %= (Abs. of negative control –Abs. of Test / Abs. of negative control)x100

Antibacterial activity : Agar-well diffusion method followed as the using of Kirby Baauer method [18] 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 ager 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.

RESULTS AND DISCUSSION

Claisen Schmidt condensation was chosen for the synthesis of chalcone (L). The reaction partners are 2-hydroxy acetophenone and benzaldehyde, which condense in the presence of base in aqueous alcoholic solution, (E)-1-(2,6-dihydroxyphenyl)-3-(4-(dimethylamino) phenyl) prop-2-en-1-one (L) (Scheme 1). Complexes formation between Co(II), Mn(II), Ni(III) and Cu(II) chlorides and (E)-1-(2,6-dihydroxyphenyl)-3-(4-(dimethyl amino) phenyl) prop-2-en-1-one (L) performed in ethanolic dimethylformamide show the formation of mononuclear complexes with 1:2 metal to ligand stoichiometry (fig 1). The complexes are stable at room temperature, non-hygroscopic, insoluble in water and soluble in EtOH, DMF and DMSO. Prepared ligand has 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 3400 and 1640 cm^{-1} respectively [15]. However, in the spectra of the complexes there is complete disappearance of peak at 3400 cm^{-1}as 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 ligands and the related complexes, which are due to the coordination between the ligands and the transition metals. Appearance of new absorption

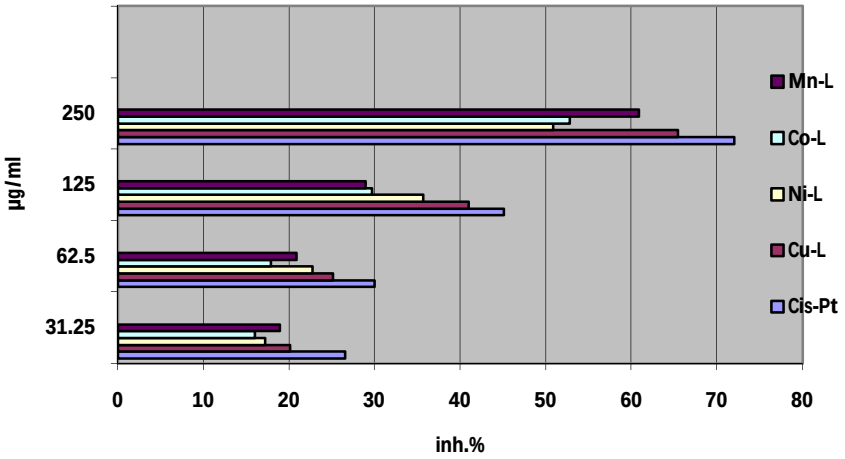
maxima is considered as a hint for the formation of complexes. The bathochromic shift in band I upon coordination is due to the electronic transition (n→π*) of the lone pair of electrons of the hydroxyl group in the complex.

Band II, which caused by the transition (π→π*) of the aromatic ring, exhibit absorption maxima at 290 nm. This measured wavelength reflect the effect of substitution by auxochromes (hydroxyl group) caused a bathochromic shift in bands 1 and 2 of the complexes[16].

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–Chalcone complexes fig.1.

APPLICATIONS

Study of Cytotoxic Effect: Cancer cell lines have been used to study the effect of complexes on the growth of cells in laboratory to know the specifications of extracts as anti-tumors. Cancer cell line type mutant mouse cells (Mice Transformed cell Line) ($L_{20}B$) 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 scheme (1) which demonstrates the impact of compounds on cells number ratio when using cell line ($L_{20}B$), 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 [19].

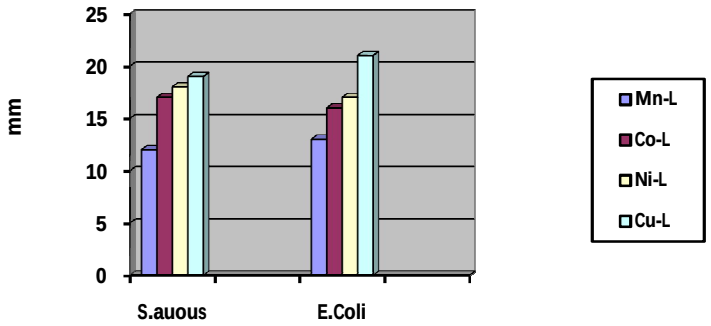


Scheme1. The comparison of inhibition rates between three treatments with cis-pt drug in cell line ($L_{20}B$)

The results in scheme 1 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 cis-platin. In this study , we suggest the new complexes of chalcone ligand have inhibition effect, this effect was similar to Tsuda et al [20] 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 [21] showed the type of organic ligand (bis-8-hydroxyquinolin) coupled to tumor cellular copper forming potent protoeasome inhibitors and apoptosis inducers at copper concentration found in tumor tissues. Saadiyh et al [22] 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)[23].

Antimicrobial Tests: The activity have been studying for the complexes, in different concentrations and using two types of pathogenic bacteria Escherichia Coli and aurous Staphylococcus. Inhibition zone larger than 6mm indicated antimicrobial activity. The complexes showed better efficiency scheme (2) for Staphylococcus aurous and Escherichia Col.
A comparison of the value of chalcone Ligand with these of the complexes indicates that the metal complexes exhibited higher antibacterial activity then ligand. Such increased activity of the complexes can be explained based on Overtones concept [24] and the tweedy chelation theory[25]. Moreover, the copper complexes were more active than the other complexes against the tested microorganisms as shown in scheme 2.



Scheme 2. The effect of complexes on the growth of bacteria.

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