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Baghdad Science Journal **Vol.14(2)2017**

DOI: http://dx.doi.org/10.21123/bsj.2017.14.2.0371

The Comparison Effect of Nickel (II) and Cadmium (II) Complexes with Aqueous Extract of *Tecrium polium.L* (Ja'adah) Plant on Hepatocellular Carcinoma Cell Line HeP2

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Received 8/11 /2016
Accepted 6/2 /2017

Abstract:

Cancer stay to be one of the leading causes of death throughout the world due to a limited success to use treatments. The new synthesized metal complexes with formula: [Ni L_2 $(H_2O)_2$]. 2.5 E t OH and [Cd L_2]. ½ H2O Where L = Bis [5 – (P – nitrophenyl) - 4 – phenyl 1 , 2 , 4 – trazol – 3 – dithiocarbamato hydrazide] and the aqueous extract of *Teucrium polium L.*(TP) plant (Ja'adahin Arabic) were examined against growth cells of hepatocellular Carcinoma ̀cell Line (HeP2). The cytotoxicity assay of cancer cell line was used for determination of inhibition rate with three concentrations; (62.5, 105 and 250 µg /200µl). The aqueous extract of TP plant induced death of cancer cells by significant elevation of the inhibition rate to 50.03% while the cytotoxic effect of Ni (II) complex reached 45.77% and Cadmium (II) was 35.73% at 125µg /200µl.The present study indicates that there are no significant differences between the two new complexes Nickel(II) and Cadmium(II) compared with anticancer drug Cis-pt for all doses .

Key words: Nickel(II) complexes, Cadmium (II) Complexes, Cancer cell line.

Introduction:

More than one thousand plants have been found to have significant anticancer properties; some of them are used as anticancer compounds to treat of cancer [1]. *Teucrium polium L.* (Lamiaceae) (TP) Plant comprises in excess 300 species commonly known as germanders and popular all over the continents [2]. This plant has been used for over two thousand years in traditional medicine, and its widely distributed in the greater part of the middle east as well as Mediterranean countries [3]. TP plant was known as Ja'adah and it's widely grown in Iraq - Alsodoor/Diyala. Several species of *Teucrium* are used for medical appearance with individual pathological purposes like anti- diabetic, anti-inflammatory, antiulcer, hypotensive, antispasmodic anorexic and antipyretic agents [4]. Medicinal application of metals can be investigated back to thousands years. The development of modern medicinal inorganic chemistry is excited by the discovery of Cis-platin

that has been accelerated by knowledge of the coordination and redox properties of metal ions [5]. The coordination chemistry has developed very rapidly mainly in the last years since many ligand of no or low biological activity become more active when transferred to their metal complexes [6]. The ahead of time reports on the therapeutic use of transition metal complexes in cancer and leukemia date from sixteenth century and in 1960. The anti-tumor activity of Cis-platin was discovered and it has improved in to one of the most frequently use [7]. Nickel and Cadmium compounds are famously carcinogens to humans and experimental animals, even though their DNA – damaging potentials are rather weak , they impede with the nucleotide and base excision repair at low, non-cytotoxic concentration [8]. The anticancer activity was studied for different metal complexes (Cu, Co, Ni and Y) and they were tested against HuH – 7 cell line[9]. Cadmium dichloride complex with semicarbazide were used in the cytostatic combination therapy together with cyclophosphamide drug and the survival was about 67% of mice with P-388 Leukemia [10] .

The goal of the current study is to assess the toxicity of aqueous extract of *Teucrium polium L*. and two new transition metals complexes with formula; [NiL2(H_2O)$_2$] . 2.5 ETOH and [Cd L2] . 1/2 H_2O where L= Bis [5 – (P – nitrphenyl) – 4 – phenyl – 1 , 2, 4, - traizol –3– dithiocarbamato hydrazide] on HeP 2 cell line with three doses of the treatment compared to the anti-cancer drug Cis – platin.

Materials and Methods:

1-Extract preparation of plant

Aqueous extract of *TeucriumPolium L*. plant was prepared according to the method of Salmman [11]. This plant was purchased from a local market in Baghdad City and weighted 15gm of it, then added 100 ml of boiling distilled water (100 ^{0}C) on a plant and the mixture was left for about 45min at room temperature (drenching method). The mixture was filtrated by filter paper (whatman (No.1) and the extract was dried at least at 37 ^{0}C to obtain of powder. Stock solution (10mg/20ml) of this plant extract was prepared in cell culture medium and applied to the culture.

2-Phytochemical Assay

The aqueous extract of *TeucriumPolium L*. plant was examined by using a standard procedure according to Ayoola *et al*., [12] .Several tests have been done for the detection of some compounds like Phenols, Resins, Steroids, Alkaloids, Terpenoids, Sapnins and Tannis.

3-Anti-cancer drug

One vial of Cis-diamminedichloroplatine (CDDP) drug which contains10mg/20ml was provided by Ebew company .

4-New transition Complexes:

Nickel (II) and cadmium (II) Complexes were prepared by Hashim [13]. 10mg of each complex was dissolved in 20ml of normal saline (0.9 % NaCl) to make stock solution and it was stored at 2-8 ^{0}C until used for tests.

5-Tissue Culture technique

The cancer cell line (Hepatocellular Carcinoma) (Hep 2) was obtained from Biotechnology Center in Al-Naharin University. It was plated in 96-well. All solutions were prepared with culturing tissues (*in vitro*) under optimum conditions by the same center. The growth media used in tissue culture technique was Minimum Essential media (MEM) supplied with fetal calf serum (10%) to form a running together

monolayer, then sub cultured to discard the prior growth medium and the cells were washed with decontaminated phosphate buffer solution (PBS) by autoclave at 121^0Cfor 15min. 2-3 ml of trypsin versene solution was added to culture flask gently and it is shaking gently for 3-5min. The trypsin versene solution was discarded and the cells were inputted at 37 ^{0}C until the cells were separated from ground flask. Then adding new growth media and redevising of cells at the microliter and they were incubated at 37^0C [14] .

6-Cytotoxicity Assay

The Cancer cell line (Hep2) was treated by aqueous extract of *TeucruimPolium L*. plant and new complexes (Nickel (II) and Cadmium (II)) and Cis-platin by using three doses (62.5 , 125 and 250) µg/200µl for each complex under sterilized conditions . Trypsin - versen solution was added in the culture bottle , then adding 20 ml of cultured medium that contains 10% of serum to culture medium to obtain the suspension cells. After that mixing the mixture very well and adding of 0.2ml to each micro titer plates by the micropipette. The plates were inputted at 37 ^{0}C for 24 hours until form monolayer, then the prior culture medium was presented in to the plates to be discarded. 0.2 ml of complexes under study were added and these three preparations restated are used as negative control (Cancer cell line with buffer solution) and were incubated at 37^0C for 48 hour exposure time .

The culture medium was discarded from micro titer plates and 0.2ml of crystal violet stain solution was added to the plates and were inputted for 20min at 37^0C , then the plates were washed gently with distilled water and they left to dry . At the end of this assay the plates were examined by ELISA reader at 492 nm transmitting wave length. Only viable cells able to take a stain while the dead cells are not. The inhibition rate was measured according to the following equation [15].

$$\text{Inhibition rate} = \frac{\text{Absorbance of negative control} - \text{Absorbance of Test}}{\text{Absorbance of negative control}} \times 100$$

Statistical analysis

In this study , statistical analysis system SAS (2012) program was used to estimate the different factors and findings . Least significant difference (LSD) test was used to compare between means with probability ($P \leq 0.05$) [16] .

Results and Discussion:

The qualitative analysis of the aqueous extract of *Teucrium polium L*. (TP) plant was examined to reflect the presence of various classes of compounds in this plant by Phytochemical screening. The results indicated that the aqueous extract contains; Flavonoids, Phenols , Trepenoids, Saponins, Tannins and Resins as shown in Table (1) .

Table (1): The Phytochemical Screening of Aqueous Extract of *Teucrium Polium L.*

Active compound	Reagent	Indicators	Result
Flavonoids	Ethanol potassium Hydroxide	Yellow solution	+
Phenols	Ferric chloride	Greenish – blue ppt.	+
Trepenoids	Chloroform , glacial acetic acid and sulfuric acid	Brown solution	+
Saponins	Convulse solution	Forth	+
Tannins	Lead acetate and ferric chloride	Gelatinous ppt. with green blue solution	+
Resins	Ethanol → 95% Boiling → 40% HCL	Turbid Solution	+
Alkaloids	Mayer' s reagent	White ppt.	-
Steroids	The same of Trepenoids reagent after one day	Bluish solution	-

According to the results as shown in Table (1), the presence of these classes of constituents in this plant may play a role in the observed cytotoxic effects. The current study examined the effect of the aqueous extract of TP on cell proliferation on cancer cell line (Hep2). The treatment with TP plant extract inhibited the cell proliferation in comparison with untreated (control) cells .This may attribute to the phenolic compounds that are most responsible for anti –oxidative specific acts modulate carcinogenesis through two main mechanisms :Adaptation redox status and interference of basic cellular functions (cell cycle, apoptosis invasion, inflammation, angiogenesis and metastasis) [17]. On the other hand, flavonoid compounds in *TP* plant extract is thought to be true to be apoptosis – inducers through p53 and other regulators of apoptosis [18]. Al Bahtiti [19] illustrated the ability of flavonoids that are important bioactive compounds of *TP* plant extract to the inhibition of human prostate cancer cells (DU145 and P (3) Proliferation, decrement of the cancer cells invasion and metastasis, induction of differentiation to an epithelial phenotype. In addition, Flavonoids influence a variety of cell functions by modulating cell signaling and inhibiting cancer cell proliferation and migration [20]. Tannins compounds in aqueous extract are effected by the apoptosis and they stopped one of cell cycle phases (G1, S1, G2) on cancer cells [21]. The effect of Trepenoids in the TP plant was agreement with the effect of it in *Dophnemucronata* plant on human myelogenous leukemia cell line K562 and it has been reported to stop action of G1-Phase in the cell cycle [22].

In vitro cytotoxicity screening

The new Nickel (II) complex was tested on cancer cell line Hep2 with various concentrations as compared with chemotherapy drug Cis-platin as a positive control after 48 hour exposure time. The highest inhibition rates reached 50.03% and to 45.77% when the cancer cells were treated with aqueous extract of TP and Nickel (II) complex respectively while the inhibition rate of anti-cancer drug was 42.23% at the 125 µg / 200 µl. The results showed that there were no significant differences between aqueous extract and new Nickel (II) complex in each concentration as shown in Table (2)

Table (2) : The Inhibition Rates Of The Aqueous Extract of *Teucriumpolium L.* plant, Nickel (II) Complex And Anti-cancer Drug After 48 Hour Exposure Time.

Concentration (µg / 200 µl)	Inhibition rate (%) : Mean ISD			LSD Value
	Ni(II) complex	TP extract	Cis-plant	
62.5	24.96 ± 6.91 C a	19.46 ± 3.70 C a	22.07 ± 3.12 B a	5.982 NS
125	45.77 ± 4.24 A ab	50.03 ± 3.07 A a	42.23 ± 4.69 A b	6.731*
250	33.33± 2.25 B a	33.81± 7.23 B a	29.54 ± 3.05 B a	6.446 NS
LSD value	10.389*	10.121*	8.983*	-------

* ($P \leq 0.05$)
NS: Non Significant
- Different capital letters (A, B, C) are significant ($P \leq 0.05$) to compare between columns
- Different small letters (a , b, c) are significant ($P \leq 0.05$) to compare between rows.

Also the results indicated that there was no significant differences between two treatments: Cadmium (II) complex and anti-cancer drug at 62.5 and 250µg / 200µl as shown in Figure (1). The highest inhibition rates was 35.73% when the cancer cells were treated with cadmium (II) complex at 125 mg / 200 ml as compared with aqueous extract of TP and Cis-platin. The significant difference was clearly between two treatments of Cadmium (II) complex and Cis-platin at 62.5and 125 µg /200µl .

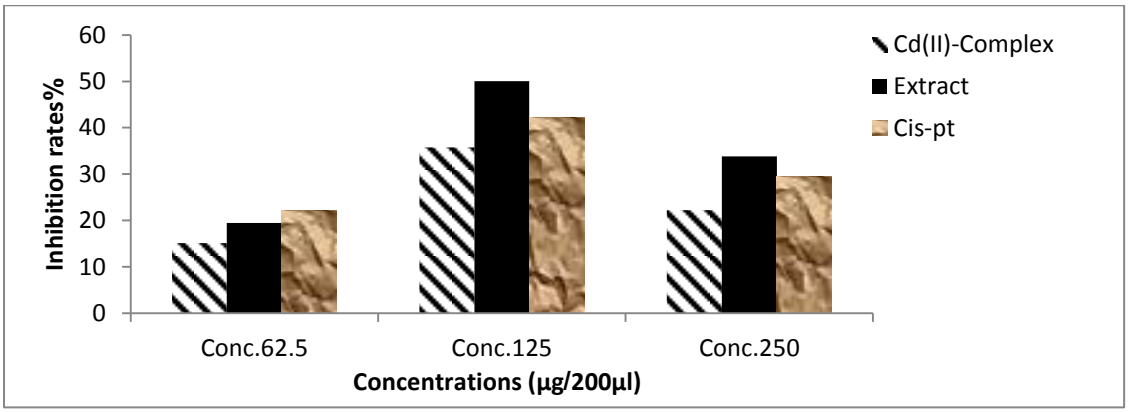

Fig. (1) : The Inhibition Effect Of Aqueous Extract TP Plant And Cadmium (II) Complex on Growth Cell Line (Hep 2) Comparison With Anti-cancer Drug Cis-platin After 48 Hour of Exposure Time.

Figure (2) illustrates the higher ability of Nickel (II) complex to inhibit on growth cancer cell line Hep2 compared with cadmium(II) complex. Also it shows the significant difference between the two new complexes at each concentration.

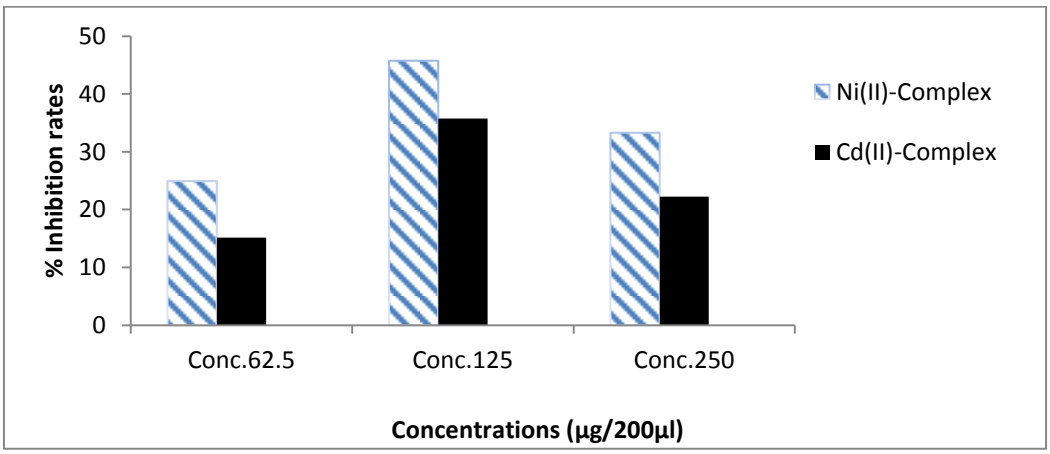

Fig. (2) :Comparison Between Two New Complexes :Nickel (II) and Cadmium (II) on Growth Cell Line (Hep 2) With Different Concentrations After 48 hour Exposure Time.

The results of the present study demonstrate that the new complexes: Nickel (II) and Cadmium (II) have a cytotoxic activity on cancer cell line Hep2 . It's important to note that heavy metals able to inhibit that specific act of membrane bound enzymes and thereby affect the cell function, metabolism and signal transduction. The interaction of metal

ions with the lipids of biological membranes might have significant consequences for the structural and functional properties of cells [23]. The inhibition rate of Nickel (II) complex in this study is higher than cadmium (II) complex on cancer cell which may be attributed to the ability of Nickel to cause a significant depression in protein levels, also Nickel reduces the DNA and RNA polymerase activity and decreases DNA replication fidelity which in turn can reduce the protein [24]. Cadmium toxicity occurs through the interaction with proteins that subsequently may cause the dysfunction of protein complex and organelles [25]. Also Cadmium has the ability to produce reactive radical, bring about DAN destroy, lipid peroxidation, reduction of protein, sulfhydryl's and other effects and chelates of amino acids , peptides and protein completed with toxic metal [26]. The cytotoxic effect of Nickel (II) complex in this study agrees with the same effect that it was previously studied by Ascar [27]. On liver enzymes activity (GOT, GPT) and creatinine level in female mice, the complex has been able to inhibit these parameters compared with cyclophosphamide. Also, the same effect of Cadmium (II) complex was studied on GPT and AIP activity [28].

Conclusion:

The new synthesized complexes of Nickel (II), Cadmium (II) and aqueous extract of *Teucrium Polium L.* plant were evaluated against the cancer cell line (Hep2). These treatments have the cytotoxicity effects on cancer cells for three concentrations after 48 hour exposure time by the highest inhibition rate at 125 µg/200 µl as constricted with chemotherapy drug Cis- platin.

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مقارنة تأثير معقدات النيكل (II) و الكادميوم(II) مع المستخلص المائي لنبات الجُعدة على الخط الخلوي لسرطان الخلايا الكبدية (Hep2)

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الخلاصة:

يبقى السرطان سببا لموت البشرية نتيجة لوجود علاجات محدودة النجاح تم فحص المعقدات الفلزية المصنعة حديثا ذات الصيغة$NiL_2(H_2O)_2$] .2.5 ETOH و [$CdL2].1/2H_2O$وان-p) -5] Bis =L: nitrophenyl– 4 – phenyl - 1,2,4 - traizol – 3 –dithiocarbamotohydrazide)] والمستخلص المائي لنبات الجُعدة على نمو الخلايا الكبدية السرطانية للخط الخلوي .(Hep2)تم استخدام اختيار السمية الخلوية لتقدير نسبة التنشيط في ثلاثة تراكيز هي (250,125,62.5) مايكروغرام / 200 مايكروليتر أحدث المستخلص المائي لنبات الجعُدة تأثيرا معنويا مميتا للخلايا السرطانية وذلك من خلال زيادة نسبة التنشيط الى 50.03% بينما وصل التأثير السمي الخلوي لمعقد لنيكل (II) الى 45.7% ومعقد الكادميوم(II) الى 35.73% عند التركيز 125 مايكروغرام / 200 مايكروليتر. بينت الدراسة الحالية عدم وجود فروق معنوية بين المعقدين الجديدين : النيكل (II) و الكادميوم(II) مقارنة مع العقار المضاد للسرطان السزبلاتين لكل الجرع .

الكلمات المفتاحية : معقدات النيكل (II)، معقدات الكادميوم (II)، الخط الخلوي السرطاني.