# Study of Cytotoxic Effect of Aqueous Extract Fenugreek(*Trigonella Foenum Graecum L.*S) Seeds and The New Complexes of Rh (III) and Pd (II) on Cancer Cell Lines

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## Abstract:

The effect of the aqueous extract of fenugreek seeds (*Trigonella Foenum Graecum L.),* Rhodium complex (III) with formula $[RhL_2CLH_2O]$.1 1/2 ETOH and palladium (II) $[pdl_2]$.2ETOH,where L=2-hydroxy phenyl piperonalidine was studied on two cancer cell lines. The first cell line was intestine cancer of female albino mice (L20B), the second one was Rhabdomysarcomas (RD)cell line in human. The activity of the new complexes and the aqueous extract was compared to the well-known anticancer drug (cis-platin) by utilizing the in vitro system. The cell lines were treated with four concentrations of cis-platin 31.25,62.5,125 and 250 μg/ml for 72 hour exposure time. The same concentrations were used with extract and the new complexes. This study showed that the aqueous extract, Rhodium (III) and palladium (II) complexes have a promising anticancer cell activity as noticed from their effect on inhibition of the cancer cells . Inhibition rate was increased with concentration for the three treatments and it was found to be significant differences ($p<0.05$) between them.The higher level of inhibition was 84.33% at the concentration 250 μg/ml of rhodium(III) complex. Comparing the cytotoxicity of the extract and the new complexes on cancer cell lines indicated that the L20B cell line was more effective than RD line and the cytotoxicity of aqueous extract fenugreek seeds and new complexes was similar to effect of the anticancer drug cis-pt.

**Key words: Fenugreek, rhodium (II) complex, palladium (II) complex.**

## Introduction:

Cis-platin or cisdiaminodichloro platinum (II) (CDDP) is one of the most effective antitumor agents for the treatment of variety of human malignancies including sarcomase, some carcinomase (e.g) small cell lung cancer and ovarian cancer[1].

The antitumor activity of the Cis-platin led to the development of other types of non-organic cytotoxic drugs, numerous platinum and non-platinum metal compounds were shown to be effective against animal model tumors as well as tumors in man [2]. The genotoxicity of Rh (II) complex in cultured human lymphocytes was studied using the chromosome aberrations assay (CA), which refered to the reduction in the frequencies of CA after 20 hour treatment[3]. Several rhodium and iridium complexes displayed different degrees of antitumor activity when they tested in mice carcinoma [4]. The progress in the field of anticancer chemistry of palladium-based transition metal complexes during the last ten years was well highlighted. The antitumor complex 1,2- Naphthoquinone-2-thiosemicarbazone-based palladium

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(Π) complex was investigated against MCF-7 human breast cancer cells [5]. Subhash padhye et al [6] have reported that the choloro,mono (phenanthrene quinine thiosemicarbozonato) palladium(Π) dimethyl formaide solvate compound exhibited remarkable activity against drug-sensitive and drug resistant breast cancer cell lines and was relatively nontoxic toward the normal mammry epithelial cells.The importance, necessity and potentiality of medicinal plants in practice of medicine today is well established and can not be over looked [7] .*Trigonella Foenum Graecum* L. commonly known as Fenugreek belongs to family fabaceae and flowering annual with autogamous white flowers occasionally visited by insects, *Trigonella Foenum Graecum* is extensively grown in the tropical and subtropical regions of India [8]. Fenugreek seeds contain active ingredients like vitamins, flavonoids (apigenin,luteolin, orientn, quercetin, vitexin and isovitexin), saponins, Glycoside like diosgenin and fixed, volatile oils and amino acids [9,10].Thirunavukkarasu et al [11] investigated the protective effect of fenugreek seeds from experimental ethanol toxicity.This study involved the evaluation of aqueous extract of Fenugreek (*Trigonella Foenum Graecum L.)* seeds and the new transition metals complexes Rhodium (Ш) and palladium (Π) with the ligand (L) which is (L=2-hydroxy phenyl piporonalidene) utitliing on in vitro system on two cancer cell lines like L20B and RD and compared their activity with anticancer drug Cis-platin.

## Materials and Methods:

1-Cis-platin (10 mg/ml) drug was provided by Ebew (Austria).

2-New complexes (Rhodium (Ш), palladium (Π))) were provided by chemistry department in College of Science for Women, then 10 mg of two complexes were dissoloved in 20 ml of normal saline (stock solution) and stored at (2-8) $C^o$ until used in tests.

3- Aqueous extract of Fenugreek seeds was prepared as following method [12]. 15 gm of plant was put in to the thimble of soxhlet apparatus which containing 100 ml of distilled water in a round bottom flask and boiled at 100 $C^o$ for 4 hours. The mixture was evaporated to give the product of Fenugreek powder, then 10mg the powder was dissolved in 20 ml of normal salin and stored at (2-8) $C^o$ until further using.

4-Phytochemical sereening of aqueous extract of Fenugreek plant were performed using standard procedure according to katsaros [13]. Tests of phenols, steroids, resins, alkaloids, terpenoids, tannis and saponins were according Ayoola et al method [14].

5- Study of cytotoxic effect on cancer cell lines. The method was used to investigate the cytotoxic effect aqueus extract solution Fenugreek seeds and the new complexes on Rhabdomyo Sarcomase (RD) in human cell line and females albino mice intestine cell line (L20B) was provided by center of biotechnology research of AL – Nahraine Uuniversity. All solutions have been prepared at the same center and the cultured tissues were studied in vitro under optimum conditions. The growth media used in tissue culture technique (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 cells washed with sterilized phosphate buffer solution (PBS) by autoclave at 121$C^o$ for 15 min. 2-3 ml of trypsin- versene solution was added for 3-5 min and moving, The culture flask kindness, trypsin- versene solution to discard and cells incubated at 37 $C^o$ until

separation from ground flask, added new growth medium and redistribution of cells at the microtiter plates and incubated at 37 $C^o$ [15].

**Cytotoxicity Assay:**

The cytotoxicity was tested for four solutions (aqueous extract, Rhodium complex, palladium complex and Cis-platin on growth cell lines (RD,L20B) by using four concentrations (31.25, 62.5, 125 and 250) µg/ml under sterilized conditions.

Trypsin-versen solution was added into the culture bottle and 20 ml of cultured medium which contain 10% of serum to provide the suspend cells, then mixed very well and added of 0.2 ml to each microtiter plates by the micropipette.The plates were incubated at 37 $C^o$ for 24 hour until to form monolayer, then the previous cultre medium which present in to the plates to discard. 0.2 ml of compounds under study was added and these three preparations repeated as negative control (cancer cell lines( RD, L20B) with buffer solution) and incubated at 37$C^o$ for 72 hour exposure time.The culture media to discard from microtiter plates. 0.2 ml of crystal violet stain solution was added to the plates and incubated for 20 min at 37$C^o$, then the plates were washed gently with distilled water and left to dry.In the end of assay the plates were examined by ELISA reader at 492 nm transmiting wave length. Only viable cells were able to take a stain while the dead cells were not.The inhibition rate was measured according to Gao et al [16] and as follows:

$$\text{Inhibition rate}\,\% = \frac{\text{Absorbance of Negative Control} - \text{Absorbance of test}}{\text{Absorbance of Negative Control}} \times 100$$

- Data were analyzed by analysis of variance ANOVA, investigation of variability between cis- platin and the relation with other groups by towards using the statistical program (SPSS) with significant level ($p < 0.05$) [17].

## Results and Discussion:

- Test of screening plant materials

The phytochemical screening of the *Trigoneella foenum graecum L.* seeds was studied. The results are presented in Table (1)

**Table (1) : Tests of screening aqueous extract materials**

| Active Compound | Reagent | Indicators | RESULT |
|---|---|---|---|
| Tannins | Lead Acetate | Gelationus PPt | + |
| | Ferric Chloride | Green-Blue Solution | + |
| Glycosides | Bendicat | Red PPT | + |
| Flavonoids | Ethanol, Potassium, Hydroxide | Yellow Solution | + |
| Phenols | Ferric Chloride | Greenish-Blue PPt | + |
| Resins | Ethanol 95% Boiling 4% HCL | Turbid Solution | + |
| Saponins | Convulse Solution | Forth | + |
| Alkaloids | Mayer's Reagent | White PPt | + |
| Terpenoids | Chloroform, Anhydrous AceticAcid and Sulfuric Acid | Brown Solution | + |
| Steriods | The Same of Terpenoids Reagent After One Day | Blueish Solution | _ |

(+) Indicate the positive test
(-)Indicate the negative test

The result listed in table (1) indicated that aqueous extract of Trigonella Foenum Graecum L. Seeds plant contion flavonoids, phenols, trepenoids and tannins, etc. The increased of inhibition values when cancer cells treated (L20 B, RD) with extract plant different concentrations could be attributed to the flavonoids such as quercetin and taxifolin have antiporliferative on growth cancer cell

lines (squamous cell carcinoma and leukemia HL-60 [18].

The diosgenin (glycoside yielding steroidal sapogenins on hydrolysis) which have inhibitor role on colon cancer cell line HT-29 IN human and caused the apoptosis [19]. Trepenoide and tannins which found in extract seeds have acytotoxic effects on cancer cells (inti-oxidant) by the free radical scavenging [20].

## Study of the inhibition rates:

- Cell line L20B

The inhibition rates of cell line treated with the new complexes and aqueous extract of seeds fenugreek comared with cis-platin listed in table (2). The results gave an evidence that the elevated of inhibition rates when cancer cells treated with the new complexes and aqueous extract at different concentrations after 72 hour exposure time.

There were no significant differences between three treatments and cis-platin at all concentrations. The higher level of inhibition was reached to 84.33% at high concentration (250 µg/ ml) when the cell line L20B was treated with rhodium (III) complex.

The results which showed the effect of Rhudium complex was similar to the effect of palladium (Π) complex at three concentrations (31.25, 62.5, 125)µg/ml.

**Table (2): the inhibition rates on growth cancer cell line L20B with different concentrations of aqueous extract and new complexes comparable with cis-platin after 72 hour exposure**

| Treatment / Conc. µg/ml | Inhibition Rates% (Mean ±Standard Deviation SD) | | | |
|---|---|---|---|---|
| | cis-pt | Ru- Complex | Pd-Complex | Aqueous extract |
| 31.25 | C,a 26.33±6,659 | C,a 25.55±4.497 | C,a 18.60±3.841 | B,a 15.81±4.433 |
| 62.5 | C,a 29.26±5.565 | B.C,a 34.72±13.993 | B.C,a 27.56±7.426 | A,a 35.767±6.505 |
| 125 | B,a 44.56±7.999 | B,a 48.18±10.165 | AB,a 40.54±4.64 | A,a 37.88±6.370 |
| 250 | A,a 72.13±7.965 | A,a 84.33±6.948 | A,ab 52.327±11.97 | Ab 42.97±7.421 |

-Different letters A,B,C significant differences ($p<0.05$) as comparasion between column and a,b,c between row.

- Cell line RD

The data of inhibition rates on cancer cell line RD when the cancer cells treated with the new complexes and aqueous extract compared with cis-platin were showed in table (3). The results gave an evidence that was significant ($p<0.05$) enhanced after 72 hour exposure time between each new complexes, aqueous extract comparable with cis-platin. The inhibition values were reached to 45.45% for Rhodium complex and 38.54% for palladium complex, while the inhibition rate of aqueous extract was reached to 43.12% at 250 µg/ ml. The results also present the inhibition values of cancer cell line when their treat with new complexes and aqueous extract there were no significant differences between them at different concentrations.

**Table (3): The cytotoxic effects of the new complexes and aqueous extract on cancer cell line RD after 72 hour exposure time**

| Treatment / Conc.µg/ml | Inhibition Rates% (Mean ±Standard Deviation SD) | | | |
|---|---|---|---|---|
| | Cis-PT | Ru (III) | Pd (III) | Aqueous extract |
| 31.25 | C,a 10.50±2.36 | C,a 13.86±4.09 | C,a 11.37±2.31 | C,a 11.29±3.55 |
| 62.5 | B,a 23.72±3.10 | BC,a 20.45±7073 | B.a 24.00±4.26 | B,a 26.49±5.14 |
| 125 | B,a 29.99±4.81 | B,a 31.87±3.54 | AB,a 28.77±3.14 | AB,a 33.11±1.74 |
| 250 | A,a 46.64±6.97 | A,a 45.45±5.40 | A,a 38.54±6.46 | A,a 43.12±6.06 |

-Different letters (A,B,C) significant differences $p<0.05$ as comparable between column and (a,b,c) between row.

-The correlation between concentrations and inhibition rates on cancer cell lines (L20B,RD).Shows in table (4), The correlation between concentrations and inhibition rates on cancer cell lines (L20B, RD) for each new complexes (Rhodium (Ш), palladium (П)) and aqueous extract comparison with anti cancer drug Cis-pt.The results were showed strong relation between new complxes and cis- platin for both cell lines (L20B, RD), while there was moderate relation between aqueous extract and new complexes.

**Table (4): The correlation P<0.05 between all concentrations and each groups**

| GROPS | L20 B | RD |
|---|---|---|
| Cis- pt | + 0.999 | + 0.920 |
| Ru (Ш) | + 0.998 | + 0.932 |
| Pd (П) | + 0.97 | +0. 988 |
| Aqueous extract | + 0.777 | + 0.971 |

*Direct relation( +)
* Moderaterelation (0.5 – 0.7)
* Strong relation (0.8- 0.9)

According to our results which gave the higher inhibition rates when cancer cell lines (L20B and RD) were treated with Rhodium (Ш), palladium (П) complexes at higher concentration compared to Cis-platin, could be attributed to the palladium (П) complex has capacity to induce apoptosis in tumor cells, the apoptotic process was triggered due to the interaction of this complex with secondary structure of DNA in treated cells [21].This study showed that palladium (П) complex has biologic activity, attributable to the methodologies for application of bulky aromatic or aliphatic nitrogen, ligands,chiral organic moieties, chelates contaning other donar atoms that nitrogen and multinuclear palladium complex [5].The cytotoxic effect of Rhodum complex on cancer cell lines (L20B, RD) was similar to the effect of anti-cancer drug Cis-platin which was the most effective antitumar agents for the treatment of avariety human malignancies [22].The elevated of inhibition vates with concentrations increased that could be attributed to antitumar compounds bind to DNA and inhibit DNA replication and protein synthesis in amanner akin to cis-platin [23]. Sadiq et al [3] showed the cytotoxicity effect of Rhodium complexes in cultured human lymphocytes by using chromosomal aberration (CA) assay, the cytotoxicity of Rhodium complex can be explained by the effects they exert on the cellular control mechanisms that temporarily arrest the cell cycle following damage to the DNA.Conclusion: Aqueous extract of fenugreek seeds and the new complexes, Rhodium (Ш) and palladium (П) showed to have a cytotoxic effects by increased of the inhibition rates of growth cell lines (L20B and RD) at different concentrations, these effects were similar to the effect of anti- cancer drug Cis-platin at the same concentrations. Rhodium complex was more effective on cancer cell lines than palladium complex and aqueous extract.

## References:

1. Pruefer, F.G.; Lizarraga F.; Maldonada, v. and Meledez_-zajgla, j. 2008. Participation of Omi Htra2 serine- protease activity in the apoptosis induced by cisplatin on SW 480 colon cancer cells, j.chemother. 20 (3): 348- 54.
2. Zofia, G.; Catherine, N.and Steven, H.2006. Impact of platinum group metals on the environment atoxicological, gentotoxic and analytical chemistry study, J.

Environ sci. Health A. 41 (3): 397-414

3. Sadiq, M.F; Zaghal,M. H. and EL-shanti, H.E. 2000. Induction of chromosomal aberrations by the rhodium (III) complex cis- [RH-$(biq)_2$ $Cl_2$] Cl in cultured human lymphocytes, Mutagenesis. 15(5): 375- 378.
4. Giraldi, T.; Sava, G.; Mestroni, G.and Stolfa, D. 2002 Antitamor action of rhodium (1) and iridium (1) complexes, Chemico Biological intraction. 22:231- 238.
5. Abu- Surrah, A.S.; AL- sadoni, H.H.; Abdalla, M.Y. 2008. Palladium-based chemotherapeutic agents: Routes toward complexes with good antitumor activity, Cancer therapy. 6:1-10.
6. Padhye, S.; Afrasiabi, Z.;sinn, E.;fok,J.; Mehta, k. and Rath, N. 2005. Antitumor Metallothiosemicarbuzonates: Structure and antitumor activity of palladium complex of phenanthrene quinone thiosemicarbazone, Inorg. chem. 44 (5): 1154- 1156.
7. Mukhtar, M.H.; Ansari, S.H.;Ali,Mand Wani, F.A. 2002. Antimicrobial activity of Betula Pendula, Hamdard Medicn, 45:41-43.
8. Dangi, R.S.; Lagu, M.D.; Choudhary,L.B.; Ranjekar, P.K. and Gupta, V.S. 2004. Assessment of genetic diversity in Trigonella Foenum – graecum and Trigonella Caerulea using ISSR and RAPD marker, BMC Plant biol. 4:13-16.
9. Blumenthal, M.; Goldberg, A. and Brinckmann, J. 2000. Herbal Medicine: Expanded commission E Monographs, American botanical council. pubI. by integrative medicine communications, USA, pp 130- 133.
10. Shang, M; cai, S; Han,J.Li; Zhao, Y.; Zheng, J.; Namba, T.;Kadota,S.; Tezuka, Y. and fan, w. 1998. Studies on flavonoids from fenugreek (Trigonella Foenum-Graecum L.), Zhongguo zhong yao za zhio. 23 (10): 614- 6, 639.
11. Thirunavuk karasu, v.; Anuradha, C.V. and Viswanathan, p. 2010. Protective effect of fenugreek (Trigonella Foenum Graecum) seeds in experimental ethanol toxicity, Phytotherapy Research. 17 (7): 737- 743.
12. Salmman, I. 2008. Effect of crude silybum marianum on cancer and normal cell lines,Thesis, Education college for women, Iraq, pp 2, 39, 59.
13. Katsaros, N. 2009. Rhodium and its compounds as potential agents in cancer treatment, On Cology Hematology. 42 (3): 297.
14. Ayoola, G.A; Coker. H.A.B.; Adesegun,S. A. Adepoju-Belllo, A.A; Obaweya, k.; Ezennia, E.C. and Atangbayila. B. 2008. Phytochemical screening and antioxidant activities of some select medicinal plants used for malaria therapy in south western Nigeria, topical journal of pharmace utical research. 7 (3): 1019- 1024.
15. Freshney,R.I. 2000. Cultured of animal cells: Amanual for basic techniques Publication, New York, pp 64- 69.
16. Gao, X.; X, Y. Janakiraman, N. and Gautan, s.c. 2002. Disparate in vitro and in vivo antileukemic effect of resveratrol a natural polyphenolic compound found in grapes, Journal of Nutr. 132: 2076- 2081.
17. AL-Mohammed, N.T; AL-Rawi, K.M.; Younis, M.A. and AL-Morani, w.k. 1986. Principle of statistics, Journal of AL- Mousl university.
18. piantelli,M.; Rinelli, A.; Macri, E.; Maggiano, N.; Larocca, L.M.; Scerrati.M., Roselli, R.; laconngeli, M.; Seambi, G.; Capelli, A. and Ranelletti, F.O. 1993. Type II

estrogen biniding sites and antiproliferative activity of Quercetin in human meningiomas, J. of Cancer. 71: 193- 198.

19- Raju, J.;Patlolla, J.M.; Swamy, M.V. and Rao, C.V. 2004. Disogenin, a steroid saponin of Trigonella Foenum Graecum (fenugreek); Inhibits. Azoxymethane -induced aberrant crypt foci formation in 344 rats and induces apoptosis in HT-29 human colon cells, cancer epidemiology, Biomarkers and prevention.18:17-34

20- Abdel-Massih, R.M.; Fares, R.; Bazz, S., EL-Chami, N. and Baydoun, E. 2009. The apoptotic and anti- proliferative activity of origanum majorana extracts on human leukemic cell line, Leuk. Res. 22: 41-48.

21- Natalia, M.; Eva, F.F; Peter, L.; Maria, p.s.; piroska, V.Corina, T.; Valentin, I.C.; Evamarie, H.H. and luminita, S.D. 2009. Antiproliferative effect and genotoxicity of novel synthesized palladium complexes with organoarsenic ligands, Journal of Inorganic biochemistry. 103 (12): 1739- 1747.

22- Shuichi, K., Kenji, M., Yoko, T., Hiromi, A.; Aya, O;S shoji, F.; and Yoshikazu, T.2000. Cytotoxicity of Cis-[((IR,2R)-1,2-cyclohexanediamine - N, Ń) bis (myristato)]- platinum (II) suspended in lipiodol in anewly established cis platin – resistant rath Hepatoma cell line, JPN. J. Cancer Res. 91: 1326- 1332.

23- Helene, T. and kim, R. 2005. Intraction of metal- metal- bonded antitumor active complexe with DNA Fragment and DNA, ACC.Chem. Res. 38: 146- 156.

# دراسة التأثير السمي الخلوي للمستخلص المائي لبذور الحلبة Fenugreek (*Trigonella Foenum Graecum L.*) ومعقدات الروديوم والبلاديوم الجديدة على الخطوط الخلوية السرطانية

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**الخلاصة:**

تم دراسة تأثير المستخلص المائي لبذور الحلبة Fenugreek (*Trigonella Foenum Graecum L.*) مع معقد الروديوم (III)بصيغته $(RhL_2ClH_2O)$ 1 1/2ETOH ومعقد البلاديوم (II) $(PdL_2).2ETOH$ حيث2-hydroxy Phenyl piperonalidin على خطين خلويين سرطانيين الاول. الخط الخلوي لسرطان امعاء اناث الفئران (L20B) والثاني الخط الخلوي لسرطان العضلة البشرية (RD ). تم مقارنة فعالية المعقدات الجديدة والمستخلص المائي مع العقار المعروف (السزبلاتين) المضاد للسرطان وذلك بتوظيف نظام خارج جسم الكائن الحي in vitro . حيث تم معاملة الخطوط الخلوية بأربعة تراكيز للسزبلاتين هي , 62.5 , 125 , 250 31.25 مايكروغرام/مل لمدة تعريض 72 ساعة ، وقد استخدمت التراكيز نفسها للمستخلص المائي والمعقدات الجديدة . بينت الدراسة امتلاك المستخلص المائي والمعقدات الجديدة فعالية مضادة للسرطان من خلال تأثيرها التثبيطي على الخلايا السرطانية، ان معدل التثبيط تزايد مع التركيز للمعاملات الثلاث حيث ظهرت بينهم فروق معنوية ($p<0.05$). وقد بلغ اعلى مستوى للتثبيط 84.33% عند التركيز 250 مايكروغرام \ مل لمعقد الروديوم (III). ان مقارنة السمية الخلوية للمستخلص والمعقدات الجديدة على الخطوط السرطانية بينت ان التثبيط عند الخط الخلوي L20B كان اكثرها فاعلية من الخط الخلوي RD وان السمية الخلوية للمستخلص المائي لبذور الحلبة والمعقدات الجديدة كانت مشابهة لتأثير عقارCis-pt المضاد للسرطان.