Comparison of The Effect of Aqueous Extracts of two Plants, *Origanum Vulgare* L. and Fenugreek Seeds with Anticancer Drug Cis-Platin on the Growth of Cancer Cell Lines

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Abstract:
This study involved the effect of the aqueous extracts of two plants, *Origanum vulgare* L.(1), *Trigonella Foenum Graecum* L. (Fenugreek) seeds(2) on the growth of cancer cell lines. Rhabdomyosarcomas (RD) of human cell line and female intestine cells of Albino mice (L20B) in vitro System. These extracts were compared with the known anticancer drug Cis-platinum(Cis-Pt) as a positive control. The phytochemical tests were used for screening the active compounds in plants. The inhibition activity assay was used as a parameter of the cytotoxic effect of these extracts. Cancer 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 for the other extracts. This study found that the two aqueous extracts (1,2) have a cytotoxic effects on cancer cells as could be seen from their effects on inhibition percentage and the significant differences (p<0.05) which were observed for each extract (1,2) by the increased the inhibition percentage as the concentration was increased. The higher level of inhibition(51.63%) was obtained from 250 µg/ml of *Origanum vulgare* extract (1) on RD line and 51.41% on cell line L20B at the same concentration. The cytotoxic effects of extract 1 and 2 on cancer cell line L20B were similar to that on RD cell line. There are no significant differences between two cancer cell line in all used concentrations. The strong relationship which to be found between the concentrations and the two aqueous extracts (1,2) comparable with Cis-Pt drug.

Key words: Fenugreek, *Origanum vulgare*, Cis-platin, Cancer cell lines

Introduction:
According to the world health organization(WHO), cancer is a leading cause of death world wide. The most frequent types of cancer among women are breast, lung and stomach cancer [1]. Herbs were used as complementary medicine among women with cancer especially those with advanced cancer. The plant *origanum vulgare* L. belongs to the family Lamiacean that native to worm and Mediterranean region [2]. *Origanum vulgare* (Origano) contains naturally occurring substances such as phenols, carvacrol, terpines and flavonoids (quercetin, apigenin). Origano has demonstrated an activity against cancer cells through the inhibition of the development of induced colon cancer in rats [3]. The *origanum vulgare* extracts affect cancer proliferation and cell death on colon adenocarinoma( CaCO₂) cells, and leads to growth arrest and cell death in a dose and time dependent manner [4]. The fenugreek (*Trigonella Foenum Graecum* L.) belongs to the family Fabaceae and flowering annua (with autoganeous white

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flowers), grows native in Asia and southern Europe [5]. Fenugreek seeds contain active ingredients like vitamins, flavonoids (apigenin, luteolin, quercetin, vitexin) saponins, glycosides, volatile oils and amino acids [6,7]. Several compounds extracted from Fenugreek plant were reported to have antitumour activity and ability to induce cell death and morphological changes indicative of apoptosis in leukemic cell lines CCRF-HSB-2 and HL-60,10.[8]. Cis platin or Cis-diammino dichloro platinum (II) (CDDP) is a chemotherapy drug that is widely used to treat different types of cancer, including germ cell cancer, head and neck cancer and lung cancer [9]. At the centre of this drug is an atom of the metal platinum, that binds DNA throught cross linking and hence damaging the cancer cells [10]. The aim of this study is to compare between the cytotoxic effects of the aqueous extracts of two plants with that of the anticancer drug Cis-platin on growth cell lines (RD and L20B).

Materials and Methods:
1- Cis- platin (0.1mg / ml) drug was provide by Ebew (Austria)
2- Aqueous extraction of these two plants was prepared as following method. 15 gm of each plant (Origanum vulgare L. and Fenugreek seeds) were put into the thumble of soxhelt apparatus which contain 100 ml of distilled water in a round flask and boiled at 100 C° for 4 hours, then the mixture was evaporated by using the distillation apparatus to give weight of component for both origanum vulgare and fenugreek seeds) powder, then 10 mg of each powder extract was dissolved in 20 ml of normal salin as stock solution and stored at (2-8) C° until used [11].
3- Phytochemical screening for both aqueous extracts of (Origanum vulgare L. plant and fenugreek seeds) was preformed using standard procedure (qualitative measurement) according to Katsoros [12]. 1-Test of Tannins: 2ml of aqueous extract was used in two test tubes,1ml of ferric chloride(1%) was added to tube one and 1ml of lead acetate(1%) was added to tube two then a positive result was gelationous ppt.,green-blue solution respectively. 2- Glycosides:Benedict reagent was added to each extracts and put them in the boiling water bath for 5min ;then the red ppt.was a positive result. 3-Flavonoids: Ethanol,Potassium hydroxide solution( 10 %) were added in to extracts and mixing ,yellow solution was a positive test 4- Phenols: Ferric chloride (1%) was added,then the a greenish-blue ppt. was fomed .5- Resins: Ethanol 95% was added and put them in boiling water bath for 2 min then, added 1ml of 4% HCL, the turbid solution was formed. 6-Saponins: The two extracts were mixing very well ,a froth was a positive result 7-Terpenoids:Chloroform,anhydrous acetic acid and Sulfuric acide were added in to extracts , the brown solution was a positive test.8-Alkaloids:Mayers reagent was added to each extracts ,white ppt. was a positive result . 9-Stereoids: (The same of Trepneoids reagents after one day) the blueish solution was a negative result according to the method of Ayoola et al [13]method.
4- Study of inhibition percentage on growth cancer cell lines. The cytotoxic effects were tested for the three solutions (origanum vulgare aqueous extract, fenugreek seeds aqueous extract and Cis-platin) on growth cancer cell lines L20B (female intestine of albino mice) and RD (Rhabdomyo sarcomase in human cell line) which were provided by the center Biotechnology research center of Al-Nahraie University. All solutions were prepared at the same and cultured tissues were studied in vitro under optimum conditions.The growth media
used in tissue culture technique was MEM (Minimum Essential Media) which contains fetal calf serum (10%) to form a confluent monolayer, then subcultured to discard the previous growth medium and the cells washed with sterilized phosphate buffer solution (PBS) (autoclave at 121°C for 15 min) then 2-3 ml of trypsin versene solution was added for 3-5 min with stirring. The trypsin- versene solution was discarded and the cells were incubated at 37°C until the separation of the cells from the ground flask.

5- Cytotoxicity assay

In this assay, the cell lines L20B and RD were treated with two aqueous extract (Origanum vulgare, fenugreek) and cis-platin using four concentrations (31.25, 62.5, 125, 250)µg / ml. Immediately adding 25 ml of trypsin- versene solutions into culture bottle and 20 ml of culture medium which contains 10% of serum to provide the suspended cells, mixed very well and 0.2 ml was added to each microtiter. The plates were incubated at 37°C for 24 hour to form monolayer, then the previous culture medium which presents in to the plates was discarded. 0.2 ml of the solutions under study were added and these three preparations repeated as negative control (cancer cell line L20B, RD with buffer solutions) were and incubated at 37°C for 72 hour. The culture medium was discarded from microtiter plates, then 0.2 ml of crystal violet solution was added to wells and these three preparations repeated as negative control (cancer cell line L20B, RD with buffer solutions) were and incubated at 37°C for 72 hour. The plates were washed gently with distilled water and left to dry. At the end of assay the plates were examined by ELISA reader at 492nm (transmitting wave length). Only viable cells able to take the stain while the dead cells were not. The inhibition percentage was measured according to Gao et al [14] and as follows:

\[
\text{Inhibition percentage} \% = \frac{\text{Absorbance of negative control} - \text{Absorbance of test}}{\text{Absorbance of negative control}} \times 100
\]

- Statistical analysis

The data were analyzed using analysis of variance ANOVA. Investigation of differences and correlation factor(R) between Cis-platin and the two extracts were determined using the statistical program (SPSS) within significant level (P<0.05) [15].

Results and Discussion:

1-Screening of plant materials.

The phytochemical screening of the Origanum vulgare and Fenugreek was studied, and results the presented in table (1):
According to the results showed in table (1), the two aqueous extracts of *Origanum vulgare* plant and Fenugreek seeds contain phenols, flavonoids, terpenoids and tannins and others. The increased inhibition percentage when cancer cell lines (L20B and RD) were treated with extract (1) at different concentrations could be attributed to the phenolic compounds such as carvacrol and thymol commonly found in most plants which posses a wide spectrum of biological activities including effect on cell proliferation, differentions and apoptosis (a programmed cell death) [1]. It was reported that tannins compounds in aqueous extract led to apoptosis and stop one of the cell cycle phases (G, S, G2) on cancer cells [16]. *Origano* is widely used in natural medicine due to its many effective antioxidants such as Rosmarinic acid, caffeic acid and various flavonoids such as luteolin enderiodictyol [17]. The main chemical constituents of fenugreek were reported to be flavonoids, polysaccharidcs, tannins, phenols and saponins. The increased inhibition rates when cell lines (L20B and RD) were treated with extract (2) at different concentrations may be related to flavonoids including quercetin, apigenin, anthocanidine, flavonoids have antioxidant activity and thus protect against cancer [8].

**2- Study of cytotoxic effects on growth cell lines**

The cytotoxic effects of the aqueous extract of *origanum vulgare* (extract 1) and aqueous extract of fenugreek (extract 2) were studied on two cancer cell lines and compared with anticancer drug Cis-Pt.

**- Rhabdomyo sarcomas (RD) in human cell line**

The results showed in table (2) and (Fig 1) indicate the significant differences (P<0.05) as the concentrations increased when the cancer cells were treated with each extract (1,2) comparable with the positive control Cis-Pt after 72 exposure time. The higher inhibition percentage reached 51.63% at high concentration (250 µg/ml).
Figure (1): Inhibition percentage of human cancer cell line (RD) with different concentrations of two aqueous extracts (1,2) and Cis-Pt after 72 hour exposure time.

- Females intestine of albino mice (L20B) cell line.

The data of inhibition percentage of cancer cells treated with two extract (1,2) in L20B line are summarized in figure (2). The inhibition rates of the two aqueous extracts (1,2) have increased as the concentrations increased.

Figure (2): The inhibition percentage of females intestine of albino mice cell line (L20B) with different concentrations of two aqueous extracts (1,2) and Cis-Pt after 72 hour exposure time.
3- Comparison of the inhibition percentage between L20B and RD cell lines.

As shown in table (2), the inhibition percentage of both aqueous extracts (1) and (2) on growth cell line L20B was similar to that of RD cell line comparable with anticancer drug Cis-platin using different concentrations after 72 hour exposure time. The higher level of inhibition percentage was 72.13 % at 250 µg / ml.

Table (2): Comparison of the inhibition percentage between the two aqueous extracts (1,2) and Cis-Pt on L20B and RD cancer cell lines

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inhibition rates% (mean±standard deviation SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cis-platin</td>
</tr>
<tr>
<td></td>
<td>RD</td>
</tr>
<tr>
<td>31.25</td>
<td>a</td>
</tr>
<tr>
<td>62.5</td>
<td>a</td>
</tr>
<tr>
<td>125</td>
<td>a</td>
</tr>
<tr>
<td>250</td>
<td>a</td>
</tr>
</tbody>
</table>

* differences a,b are significant (P<0.05) to comparison row.

4- The correlation factor(R) of the extracts and the concentrations.

The results present in table (3) show the strong correlation range from medium to high between different concentrations and two aqueous extracts (Origanum vulgare 1 and Fenugreek 2). Comparable with anticancer drug Cis-Pt by using two cancer cell lines: RD and L20B, when cancer cells treated with Cis-Pt, extract 1 and 2 at different concentrations.

Table (3): The correlation factor (R) between the concentrations and each groups (Origanum vulgare extract 1, Fenugreek extract 2) and Cis-Pt and between the same groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Conc. µg /ml</th>
<th>R (RD)</th>
<th>Cis- Pt Extract 1</th>
<th>R(L20B)</th>
<th>Cis-Pt Extract 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis-platin</td>
<td>31.25,62.5,125,250</td>
<td>0.920</td>
<td>0.781</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract 1</td>
<td>31.25,62.5,125,250</td>
<td>0.982</td>
<td>0.974</td>
<td>0.928</td>
<td>0.954</td>
</tr>
<tr>
<td>Extract 2</td>
<td>31.25,62.5,125,250</td>
<td>0.971</td>
<td>0.984</td>
<td>0.992</td>
<td>0.999</td>
</tr>
</tbody>
</table>

R= correlation factor
The results in table 2,3 and figure 1,2 show an evidence that the two extracts (1,2) have a cytotoxic effects on cancer cell lines (L20B and RD) as shown by the elevated inhibition percentage with the increased concentrations (31.25, 62.5, 125, 250) µg/ml compared with that of anticancer drug cis-platine. In this study, we suggest that Origanum extract have cytotoxic effect, this effect was similar to Srihari et al [3] that show the Origanum extract have antimutagenic, antigenotoxic, and antiproliferation properties. Origanum extract protect cells from oxidative stress, mitogen, and radition induced DNA damage. Carvacrol and thymol have been reported to protect DNA from variety damaging agents and suppress proliferation of cancer cells [18]. Tatjana [1] show the relationship activity between extract and two human breast cancer cell lines (MDA-MB-361) and (MDR-MB-453), which exhibited significant antiproliferation after treatment with the extract. The elevated inhibition percentage with increased concentration increased when cell lines (L20B and RD) were treated with fenugreek extract. This effect attributable to the fenugreek seeds components such as phenols, saponins and alkaloids, flavonoids, trepenoids, these extracts active ingredients contribute to its prevention effects on cancer [19].

Alarcon et al [20] studied the effect of fenugreek extract on human neoplastic cells, their results showed the cytotoxic effect of the extract against this cell line which resulted in growth inhibition, cell death and apoptosis. Flavonoids such as quercetin and taxifolin have antiproliferative effects on growth cancer cell lines (squamous cell carcinoma and leukemia HL-60) [21]. Thomson et al[10] have studied the effect of the cisplatin binding and cross linking of DNA which ultimately triggers apoptosis.

**Conclusion:** The study showed that the two aqueous extract (Origanum vulgare and Fenugreek) have a cytotoxic effects on that two cancer cell line (L20B and RD) at different concentrations after 72 hour exposure time, these effects were similar to the effect of anti-cancer drug Cis-platin.

**References:**


مقارنة تأثير المستخلصات المائية لنباتي المردقوش

وردوز الحلبة مع العقار المضاد للسرطان السز بلاتين

على نمو الخطوط الخلوية السرطانية

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الخلاصة:

في هذه الدراسة تم مقارنة تأثير المستخلص المائي لنباتي المردقوش (1) ونبات الحلبة (2) على نمو الخط الخلوي السرطاني لكل نوع من الخلايا السرطانية في الأنسان (RD) والخط الخلوي لامعاء الفئران L20B في نظام خارجي حي في vitro وقرون تأثير المستخلصين مع العقار المعروف المضاد للسرطان السز بلاتين كمثيله. هذه الدراسة أظهرت أن العصارين المائيين لنباتي المردقوش والفول السوداني قد أظهرت فعالية في مكافحة الخلايا السرطانية. تم تعاين العصارين المائيين لكتبتين رابطين، وتم استعمال نفس التراكيز من العصارين المائيين. أظهرت هذه الدراسة أن المستخلص المائي لنبات المردقوش دفع 51.63% و51.41% على RD و L20B، على الترتيب، حيث لم تظهر فروق معنوية بين الخطيتيين لعدم التراكيز المختلفة، وهذا تأكيده جيد لتأثير المستخلص المائي لنبات المردقوش.

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Origanum vulgare L. و F. trilobata L. مع العقار المعروف المضاد للسرطان السز بلاتين

المستخلص المائي لنبات المردقوش ونبات الحلبة في مكافحة الخلايا السرطانية على نمو الخطات الخلوية السرطانية

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