

A study of cytotoxic effect of Iraqi fenugreek seeds alkaloids extract on some cancer cell line.

Salim Dawood Salman
College of pharmacy, Al-Mustansyriah University

الخلاصة:

شملت هذه الدراسة تقييم التأثير السمي لمجموع القلويات المستخلصة من بذور عشب الحلبه العراقية على خطين من الخلايا السرطانية المختلفة ولفترات تعرض مختلفة. تم استخلاص المكونات الفعالة الرئيسية الموجودة في البذور باستخدام السوائل العضوية، وتم الحصول على مجموع القلويات التي هي أحد المكونات الرئيسية في البذور بعد تنقية المستخلص الخام وتم التعرف عليها باستخدام طرق الكشف المعتمدة عالميا. تمت دراسة التأثير السمي لمجموع القلويات المستخلصة على خطي الخلايا السرطانية (RD,REF) باستعمال تراكيز مختلفة من المستخلص المذكور (1000,500,250,125,62.0ug/ml) ولفترات تعرض مختلفة (24 hr , 48 hr, 72 hr). أظهرت النتائج ان هناك تأثير سمي تثبيطي معنوي لمستخلص القلويات على خط الخلايا السرطانية RD في التركيز العالي المستعمل في الدراسة (1000ug/hr) وفي فترة التعرض (24hr) بينما التأثير السمي غير معنوي في التراكيز الاخرى المستعملة وضمن نفس الفترة (24hrs). لم نتمكن من ملاحظة أي تأثير سمي معنوي للتراكيز المستعملة في فترة التعرض (48hr). اما في فترة التعرض (72hr) تم ملاحظة وجود تأثير سمي معنوي في التركيز (1000ug/hr) ولم نلاحظ تأثير سمي في التراكيز الاخرى ولنفس الفترة من التعرض. اما التأثير السمي على خط الخلايا السرطانية REF فقد اظهرت النتائج وجود تأثير سمي معنوي للمستخلص عند التركيز العالي (1000ug/hr) خلال فترة التعرض (48hr) بينما في فترات التعرض الاخرى (24hr,72hr) لم نلاحظ أي تأثير سمي للتراكيز المختلفة المستعملة في الدراسة. من ذلك تجد ان القلويات المستخلصة من البذور لها تأثير سمي على الخطوط السرطانية المختلفة التأثير يختلف باختلاف الخطوط المستعملة في الدراسة ولفترات التعرض ولنفس التراكيز.

Abstract:

The inhibitory activity of total alkaloids of Iraqi fenugreek extract on cancer cells was evaluated and its cytotoxicity on different cancer cell lines was measured.

Alkaloids were extracted from the seed, which contain the main active constituent, by organic solvent. The crude extract was purified and the total

alkaloids were obtained. Detection and identification of purified extract were carried out by using many assays.

Cytotoxic effects of different concentration (62.5, 125, 250, 500, 1000 µg/ml) of fenugreek alkaloids on two cell line (RD, REF) cancer line was studied after 24h, 48h and 72h of exposure to the alkaloids extract. It was found that there was a significant cytotoxic effect on this line at concentration (1000µg/ml) after 24hr of exposure while no significant effect seen in other concentration used in the study. Similarly it found after 72hr a significant effect could be detected at high concentration (1000µg/ml). No significant cytotoxicity after 48hr of exposure for all concentration used could be seen. On REF cancer cell line no significant cytotoxic effect could be seen after 24hr of exposure even that there is a slight none, significant effect in some concentration. After 48hr only at high concentration (1000µg/ml) a significant effect was seen. All concentration used have no cytotoxic effect after 72hr of exposure. So we can conclude that fenugreek alkaloids have a cytotoxic effect on different cancer cell line which differs in potency from cell line to other and different exposure times.

Introduction:

Cancer is one of the most dreaded diseases of the 20th century and increased in 21th century .globally more than 3000 people are expected to die of cancer each day, representing an estimate total mortality rate of about 650000^[1]. The rate of growth in cancer cases of overall population increase 1.7% per year^[2] .

Cancer is a clonal disorder characterized by genetic instability and shift in the control mechanism that govern cell proliferation and differentiation^[3].

There are virtually dozen of different cancer type ,the most common in the USA is breast and lung cancers'. the main causes of cancer is the damage to DNA (DNA is found in every cell in body and regulate all of cell activities) .the body is usually able to repair DNA ,but unable to repair cancer cell. there are number of way to reduce the chance of getting cancer including eating diet rich in vegetables ,fruits and whole grains ,maintaining a healthy weight, avoiding over exposure to the sun, eating food low in fat etc.

Conventional cancer treatment can include surgery, radiation, biological therapy, hormones, stem cell transplantation and chemotherapy .the doctor may use one method or a combination of several depending on the type and location of the cancer, age and general health of patient. Unfortunately all these conventional treatment posses hazardous side effect making this conventional treatment not worthy. To overcome these side effect, the researchers searching for unconventional treatments to try to overcome these side effects^[4] .

A greater emphasis has been given toward the research on complementary and alternative medicine that deal with cancer management^[5]. So research is now goes toward plants natural product and found that these natural product play an important role in chemotherapy. Over 60 available chemotherapeutic

agent are plant derived^[6]. Several studies conduct on herb under a multitude of ethnobotanical ground. about 3000 plant, those of which posses anticancer properties, may used as potent anticancer drugs to prevent or suppressing various tumor^[6]. In USA, there are now four classes of plant derived anticancer agent on the market^[7]. many trials now conduct to discover additional novel natural product and their semi synthetic analog as potent anticancer drugs^[8].

Terrestrial plant, especially plants and herb, have long history of used in the treatment of different disease including anticancer agent, which play a critical role in cancer treatment and prevention due to that these agent yield chemicals useful in treatment of these diseases^[9,10]. These chemical agents also have a cytotoxic effect toward other cell as tumor cell^[11].

Note: Cytotoxic agent is toxic to tumor cell in vitro, and if its toxicity transfer through to tumor cell in vivo, the agent is said to have anticancer in clinical trials^[12].

In flora of Iraq, about 1500 medicinal plant species have been recorded, large number of these plants are used for medical purpose (97-medicinal plants belong to 43 families were identified as processing a medicinal properties)^[13].

There has been increasing realization in recent year that some plant natural products fight many disease with supreme especially result in cancer. plant derived from polyphenolic may possess anti microbial, antioxidant, anticancer, and apoptosis inducing properties^[14]. One of the useful herbs can used in this case is fenugreek.

Fenugreek: Is annual herb in the family of leguminosae or Fabaceae, a large and important family of flowering plant, which is commonly known as bean family, legume family or pulse family.

Fenugreek's binomial nomenclature is *Trigonella foenum-graecum* family leguminosae^[15] and is used as herb, leaves and seeds in cooking, medicine and as spice^[16]. It is cultivated worldwide as a semiarid crop, it frequently used in curry. It best known in the south Asian cuisines especially Indian cuisine. also it grows natively in the south Europe, north Africa and many region in the Asia as in Iraq, in Mousle, where it called hilba {the herb have different local names according to country where cultivated}^[17].

Fenugreek is used by ancient Egyptian to combat fever, the seed is yellow to amber colored used as spice, as tea in variety of sweet confection.

Fenugreek seeds are rich source of carbohydrate, polysaccharide galactomannan^[18] which is the main constituent of mucilage fiber in the seed, consist of galactose and mannose. seed also contain different form of glycoside, and low M.w carbohydrate as sucrose, glucose, fructose.

Fenugreek contain more than 25% of the seed as protein and amino acids^[19], 6-8% fat, fatty acid compose a fixed oil, different fatty acid as palmitic acid, stearic acid, arachidic acid and others. Fenugreek seed also contain 1.5% of steroidal saponin^[20] which upon hydrolysis yield saponin which the main is diosgenin, yamogenin, trigogenin, gitogenin, fenugreekin^[21].

Seed contain alkaloids especially trigonellin and choline, gentianine^[22], flavonoides, vitamines volatile compound and minerals also found in seed^[23].

Fenugreek seed has hypoglycemic and hypolipidemic effect due to high mucilage fiber content that inhibit glucose and fat absorption from G.I.T.^[24]. So it widely use as antidiabetic drugs, fenugreekin, steroidal saponin peptide ester isolated from seed showed diuretic effect^[25]. Seed have laxative properties^[26], also use in gastrointestinal ulcer^[27], use as appetite stimulation action^[28].

Seed also play role in increasing production of milk^[29] due to action of fenugreek oil principle^[30,31]. The seed also show a potential protective effect against breast cancer induced in experimental rat^[32] due to the activity of diosgenin component in the seed on mammary gland^[33]. Also ethanolic extract of fenugreek seed showed antineoplastic effect on the growth of MCF-7 cell by reducing cell viability inducing early apoptotic changes. but Fenugreek seed have received little attention as an agent in the treatment of cancer some. Studies have identified active agent amongst saponin and sapogenins that make up a considerable portion of the crude extract of Fenugreek. The exaggerated action of fenugreek in treating cancer has been demonstrated through various mechanism, the relevant clinical application of fenugreek is still in the queue of further study, so understanding of the mechanism is necessary.

The aim of our study is to study the cytotoxic effect of alkaloids, which is one of the fenugreek seed constituent, on different cancer cell line (we chose two, RD, REF) which there was very few studies of this chemical.

Keywords: Cancer. Cancer cell line (RD, REF).fenugreek seed, alkaloids, cytotoxicity, crystal violet staining (cvs) method.

Materials and methods:

Cell lines:

Cell lines were kindly provided by ICCMGR

1 - Rhabdomyosarcoma RD (NCI- DCTD,USA):

Human cell line was derived from biopsy specimen obtained from a pelvic Rhabdomyo sarcoma of 7-years old Caucasian girl (McAllister et al 1969)^[34]. Passage 245-247 RD cell line were used throughout this study and MEM medium (Minimal Essential Media) was used in propagation and maintenance.

2 - Primary rat embryo fibroblast r (REF):

(REF) cell line was established and kindly provided by Dr. Ahmed M. Al-Shamery^[35] from ICCMGR. cells of this normal murine cell line were a mixture of fibroblastic and epithelial cells with normal chromosomal picture. Tumorigenicity test of this cell line showed no tumor growth in injected rats during three months of monitoring. passage 15-17 of this cell line was used in the study and cells were maintained using RPMI-1640 medium.

Preparation of solution and mediums for in vitro cell culture:

- 1 - Phosphate buffer saline (PBS) Gibco. USA: this buffer was supplied as a sterile powder ready to dissolve in one liter of double distilled water (D.D.W), Sterilization was performed via autoclave for 20 minute and then cooled and immediately was use.
- 2 - Fetal calf serum (us biological, USA): the serum was already thermally inactivated, sterile and used directly for tissue culture media.
- 3 - Trypsin/Versin solution (US Biological, USA): it was prepared by dissolving 2g of Trypsin/Versin powder in the 100ml of distilled water and adjusted the pH:7 using 0.1g of sodium Bicarbonate , then sterilized by Millipore filter of 0.22 mm pore size and kept at 4⁰C till the time of use.
- 4 - Rosswel park Memorial Institute (RPMI-1640) (Gibco. USA): RPMI-1640 with HEPES buffer and L-glutamine, was prepared as follows:

| | |
|---|---------------------------|
| RPMI-1640 growth medium | 10.4g |
| sodium Bicarbonate 4.4% solution | 15ml (tomainle pH at 7.2) |
| Streptomycin (1g/5ml) | 0.5ml |
| Benzyl penicillin (G10 ⁶ I.V /5ml) | 0.5ml |
| Amphotericin B | 2.5ml |
| Fetal calf serum | 100ml |

The volume was made-up distilled to get (1 liter) the sterilized by Millipore sterile filter of 0.22mm pore size. The sterilization was done in a sterile environment, and then stored at 4⁰C for direct use .all antibiotics were freshly prepared.

- 5 - Minimal Essential Medium (MEM) (US biological, USA) .MEM with L-glutamine was prepared as follows:

| | |
|---|------------------------|
| MEM | 16.7g |
| HEPES buffer 1m | 10ml |
| Sod Bicarbonate 4.4%solution | 15ml (to maintaph 7.2) |
| Streptomycin (1g/5ml) | 0.5ml |
| Benzyl penicillin G (10 ⁶ 1.U/5ml) | 0.5ml |
| Amphotericin B | 2.5ml |
| Fetal calf serum | 100ml |

The volume was completed with distilled water to get (1 liter) then sterilized by Millipore sterile filter of 0.22mm pore size .the sterilization was done in a sterile environment, then stored at 20⁰for a long time or at 4⁰C for direct use. All antibiotic freshly prepared.

- 6 - Serum Free Media (SFM) (US Biological, USA):
The serum free media is either RPMI 1640 or MEM (as prepared previously) but without fetal calf serum (SFC) which mean media without serum.

In vitro cytotoxic assay:

Maintenance of cell line:

Following the protocol described by Yaseen (1990) ^[36] two lines used in this study were sub cultured when monolayer were confluent, the growth medium was removed and the cell sheet washed twice with phosphate buffer salin (PBS) (Gibco. USA).

Two to three ml of trypsin-versene solution added to the cell sheet and the flask rocked gently. After approximately 30 second, most of the trypsin poured off and the cell incubated at 30⁰C until they had detached from the flask, cell were further dispensed by pipetting in growth medium and then redistributed at the required concentration in to culture flask and re-incubated at 37⁰C in the presence of 5% co₂in air.

Cytotoxic assay of extract on cell lines:

This step was prepared under aseptic condition according to (Abdul-majeed, 2000)^[37], Freshney, 2001^[38]). The extract were prepared to micro titration assay by dissolving 0.01g extract in the 10ml of solvent (o.1ml DMSO+9.9ml DDW, the stock concentration is the 1000 mg/ml) and filtered by 0.22m Millipore filter, then the extract being ready to be used as stock s. from the stock five double fold concentration were made (62.5, 125, 250, 500, 1000mg/ml) when the cells are in the exponential phase exactly in population doubling time (PDT), which the cell in full of its activity (depending on the growth curve of each cell line).

The cell were collected after adding trypsin/versine (2-3ml)not more than 10min, and then concentrated into known volume with serum free media (SFM) US Biological, USA) (which is either PRMI-1640 or MEM but without fetal calf serum (FCS)which mean media without serum). Afterward, 0.2ml of prepared concentration were added to the 96-well micro titration lid and sealed with adhesive film, placed in co₂ incubator at 37⁰C for no more than 24hrs (for cells adherence). After cell attachment, the plate was checked-out for contamination and the media were removed. Serial concentration were added and the three replicates were used to each concentration and control (cell with SFM only), the exposure time was 72hrs. After the exposure time was finished, the extract and media were removed and a fresh SFM was added to all wells, and incubated for 24hrs at 37⁰C to give a chance if the affected cells and not damaged being repaire by self repairing system. The media wasremoved from the plate and washed with phosphate buffer salin (PBS) a known con. CV (cystalviolat).

In vitro, cytotoxic assays with cultured cell are common because they are rapid, inexpensive and have no ethical implication. Several method for determining cellular viability and/or growth following in vitro exposure to compounds have been reported. These methods used well known dye that develops a color , allowing colorimetric measurement of cell viability as crystal violet staining method (CVS) which measure the effect of compound on cell

growth through colorimetric evaluation of fixed cell stained with CVS. This method described by Saotome, *etal.* (1989) and modified by Itagaki, *etal.* (1991) briefly, in a 96-well tissue culture microplate (column 1 to 12) containing 0.1 mL of MEM in each well, 0.1 mL of the extract was inoculated in column 1. The content of column 1 was two-fold serial diluted until column 6. Subsequently, the wells from columns 1 to 8 were seeded with 0.1 mL of 10% MEM containing cell lines.

Two-fold serial cell dilutions were made from columns 8 to 11. The microplate was incubated for 72 h at 37°C in 5% CO₂ atmosphere. After this period, the medium was removed and the cells were stained with 0.4% crystal violet solution in methanol for 30 min and carefully washed with distilled water. The quantitative analysis (colorimetric evaluation of fixed cells) was performed by absorbance measurements by Elisa readers at 492 nm. The effect on cell growth was calculated as the difference in absorbance percentage in presence and absence of the plant material.

At the end of the last incubation period the dye was removed from the plate and the wells washed with worm PBS twice, then 0.2ml of DMSO was added to each well dissolved the CV crystal during that we added 25ml of glyceine buffer492nm .

The percentage of inhbiition calculate according to the following equation:

Percentage inhibition: % I = (mean of control–mean of test) x*100/mean of test

Taxonomy and preparation:

Plant material: dried seeds of Iraqi fenugreek was obtain from the markets in Mousel, Iraq. Taxonomic identification of the genus and species of the seed was done by department of pharmaceutical chemistry and pharmacognosy in college of pharmacy University of Al-Mustansyria as *Trigonella foenum-graecum*, F. legumenosea. The seeds were cleaned from dust and dried at room temperature.

Extraction :

Preparation of ethanolic extract:

Ethanolic extract Of fenugreek seed was prepared in the department Of pharmaceutical chemistry and pharmacognosy; college of pharmacy; Al-Mustansiriya University according to Simandi et al (2001e)^[39], procedure by the soxhlet apparatus which yield the crude alkaloidi present in the extract:

- 1 - Take 1kg of trignella foenum seed, after cleaning it and dried at room temperature, crushed and milled by mortar.
- 2 - Defatted by sokhelt apparatus with 2 liters of n-hexane until disappearance of color (yellowish) which spend about 6hrs or more.
- 3 - The remaining residue which is oil free was left at room temperature for 24hrs.

- 4 - The defatted residue of seed was extracted by alcohol through reflex method with 2 liters of 80% ethanol for 6hrs at 40⁰C, then left the mixture to cool and filtered by filter paper.
 - 5 - The resultant filtrate was evaporate on the steam bath to syrup liquid containing the total constituent of active ingredient in FGS.
 - 6 - added to concentrated filtrate 2%HCL, remove all the remaining alcohol by evaporating in large evaporating dish on the steam bath.
 - 7 - Acidic extract is then cooled ,filtered and placed in separatory funnel where it is made alkaline by adding ammonium hydroxide solution (using litmus paper).
 - 8 - The alkaline solution is then extracted three time with 100ml of chloroform.
 - 9 - The chloroform solution is the concentrated to give crude alkaloid.
- All chemical used is manufacture by BDH (England)

Identification of the alkaloids in the extract using a:

- 1 - Modified dragendroff test: drop of test was mixed with 5ml from extract, positive result is orange test residue ^[40].
- 2 - Myer's test: Alkaloid identify in extract by taking a drop from the test and mixed with 5ml from extract, the positive result is white residue ^[41].

All instrument s and chemical agents provide by ICCMGR

| Instrument | Manufacturer |
|---------------------------------|--|
| Autoclave | Hospital management supply (Germany) |
| Co ₂ incubator | Gallenkamp U.K |
| Hot plate with magnetic stirrer | Gallenkamp U.K |
| Water bath | Précis term (Germany) |
| Vortex | Buchi (switzer land) |
| Ph meter | Orient research U.S.A |
| Laminar air flow cabinet class2 | Gelaire class100 Gelman instrument U.K |
| Elisa | Asays (Belgium) |
| Soxhelt | |

Table-1: Instrument used in the study with its manufacturer.

| Dimethyl sulfoxide(DMSO) | BDH (UK) |
|---|-------------------|
| Rosswell park memorial institute medium PRMI-1940 | Gibco USA |
| Phosphate buffer salin (PBS) | US biological USA |
| Fetal calf serum | Flow lab(U.K) |
| Trypsin /versine solution | Gibo USA |
| Sodium bicarbonate Benzyl | BDH (England) |

Table-2: Chemical used in the study with its manufacture.

| | |
|--------------------|---------|
| Soxhelt | |
| Reflex | Germany |
| Rotary evaporator | GERMANY |
| Water bath | Germany |
| PH-meter | USA -S- |
| Filter paper | |
| ETHANOL | BDH |
| 1 Hexane | BDH |
| Chloroform | BDH |
| HCL | BDH |
| Ammonium Hydroxide | BDH |

Table-3: Chemical and instrument use in extraction

Statistical analysis:

Analysis of variance (Anova) and descriptive was done by SAS system and followed by least significant difference LSD statistical significance was defined as $P < 0.05$

Result and Discussion:

1 - Fenugreek extraction:

Eathonolic extract of fenugreek seed was prepared. Seeds are extract due to the fact that the major chemical constituents of the herb were present in the seed, very small amount present in leaves^[42]. Ethanol 80% were used in extraction as done with many bioactive constituent from plant material. The use of aqueous alcohol allow the extraction of both polar and non-polar compound^[43], as in extraction of flavonoids, carotenoid^[44], saponin alkaloids (trigonelline and other), amino acid^[45] and other components.

In our study we extracted the seed to get the total crude alkaloid and this was done according to procedure mentioned previously (in the methodology) which used many steps and different chemicals to get the total alkaloid needed. The extract was found to have a positive result of modified Dragendroff test and Mayer's test indicating that the extract contained alkaloid^[46], and this is compatible with positive result of phytochemical analysis^[47], then the extract evaluated to show whether it efficient in their inhibitory effect in cancer cells in vitro, using two cell lines (RD, REF)^[48].

2 - Cytotoxic effect of fenugreek seed alkaloids on some cells line:

In vitro, cytotoxicity assays with cultured cells, several method were used for determining cellular viability and/or inhibition.

These methods used well known dyes that develop color allowing colorimetric measurement of cell viability.

In our study, to investigate the relationship between the concentration of the extract used and their cytotoxic effect on the cancer cell lines (RD, REF) we used crystal violet staining method (CVS) which is very effective and reproducible.

A set of two folds in five concentration (62.5, 125, 250, 500, 1000 $\mu\text{g/ml}$) was made for the extract. The exposure times of the assay were 24hrs, 48hrs, 72hrs and two types of cancer cell lines were tested (RD, REF) in our study as follows:

1 - Effect of fenugreek alkaloid extract on RD cell line:

The cytotoxic activity of fenugreek seed extract was evaluated against RD cell line as in fig-1. Statistically the cytotoxic effect of the extract was detected on this cell line in a dose dependent manner after 24hrs, 48hrs and 72hrs. After 24hrs exposure, there were a reduction in cell viability in almost all tested concentration of fenugreek alkaloid used. A significant cytotoxic effect was detected at higher concentration (1000 $\mu\text{g/ml}$, $p > 0.05$) in comparison with control where the % of inhibition was 38.35 after 24hrs while at other concentration used we detected 23.24% at 500 $\mu\text{g/ml}$, 15.41% at 250 $\mu\text{g/ml}$, 9.36% at 125 $\mu\text{g/ml}$, 8.63% at 62.5 $\mu\text{g/ml}$. No significant cytotoxic effect of extract was detected at all concentration that used in our study at the end of the 48hrs exposure. Similary a significant reduction in cell viability detected only at higher concentration (1000 $\mu\text{g/ml}$, $p < 0.05$) with 32.56% inhibition but at 500 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$, 125 $\mu\text{g/ml}$, 62.5 $\mu\text{g/ml}$. no significant reduction detected were the% of inhibition are (19.24%, 12.73%, 3.93%, 1%) respectively after 72 hrs of exposure.

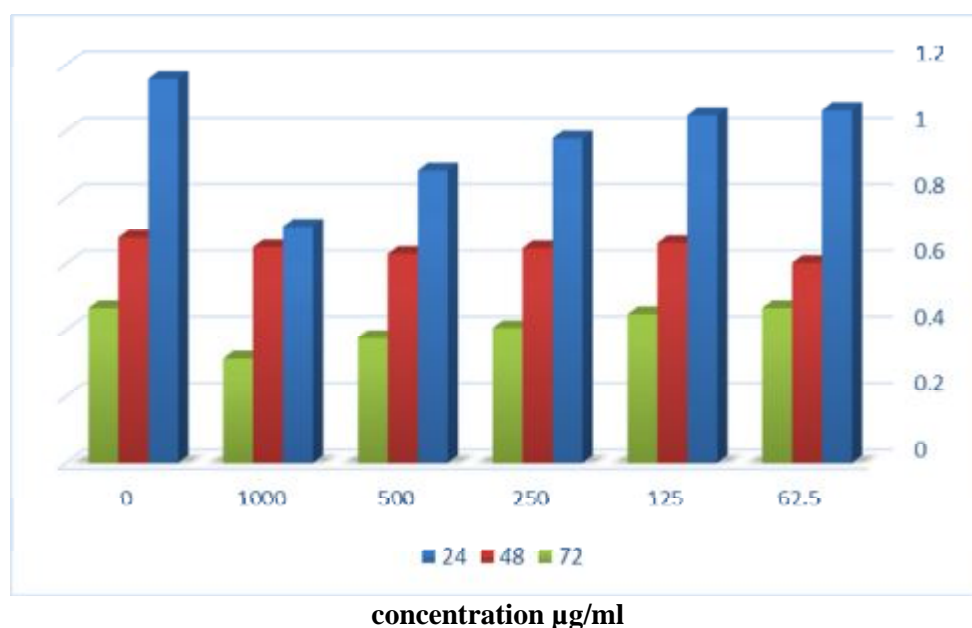


Fig-1: The cytotoxic effect of different concentration of fenugreek alkaloid on RD cell line.

2 - Effect of fenugreek alkaloid extract on REF cell line:

The normal fibroblastic primary cells (REF) were cultured and used for evaluation the cytotoxicity of fenugreek alkaloid extract after 24hr, 48hr and 72hr, as shown in fig-2.

There is no significant cytotoxic effect after 24hrs exposure for all concentration of extract that used in our study while after 48hrs of the exposure there was a slight inhibition start at concentration 500 μ g/ml with percentage of inhibition 23.2% and last to concentration 1000 μ g/ml where the percentage is 59.3%. After 72hrs of exposure, no inhibition can be detected for all concentration used in this study.

From the result, we can conclude that there is a very slight non-significant cytotoxic effect of extract on that particular primary cell line.

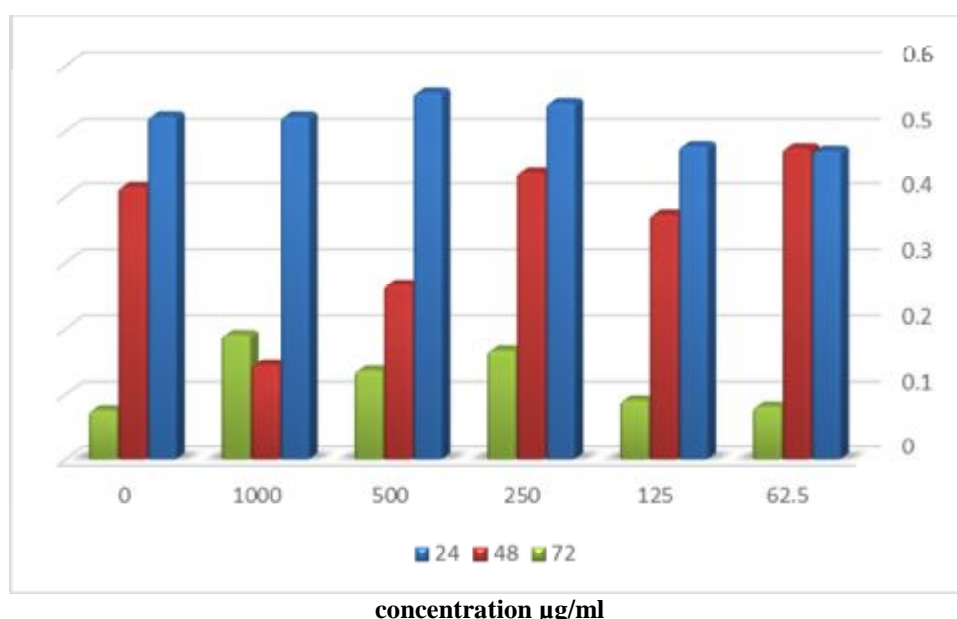


Fig-2: The cytotoxic effect of different fenugreek alkaloid on REF cell line.

Statistically, it can be documented that the ethanolic fenugreek alkaloids extract possess a cytotoxic effect toward different cancer cell lines, but the severity of this cytotoxicity was varied from one cell line to another.

The relationship between the concentration of fenugreek alkaloids extract and their cytotoxic effects on the two cell lines (RD, REF) were investigated by CVS method. The five concentrations were used (62.5, 125, 250, 500, 1000 μ g/ml) are the best range to give a least significant inhibitory effect of the fenugreek alkaloids extract in vitro. The final outcomes from CVS assay were variable cytotoxic effects with significant differences on these two cell lines (RD, REF) in a dose and time-dependent manner. This different cytotoxic effect on cancer cell lines may be attributed to the presence of some specific component in the extract used which was found in other herbs that have the same mechanism as in fenugreek and the significance was varied from one cell type to another [49, 50]. Several reports have shown that alkaloids (one of the fenugreek active groups) extracted from other plants or herbs possess cytotoxic, antitumor or anticancer

effects ^[51,52,53]. Alkaloids extracted from lobelia (*Lobelia inflata*), L-lobeline, Lobelanine, Norlobelan, capable to reverse P-g P (P-glyco-protein) dependent multidrug resistance property of two types of cancer cell lines caco-2 (human colon carcinoma) and CEM ADR 5000 (leukemia cell line). The alkaloids fraction affect the P-g p expression by enhancing rhodamine 123 retention in both caco-2 cells and CEM ADR 5000 because the rhodmine 123 is a substrate of P-g p, alkaloid thus inhibit P-g p function probably by substrate competition^[54]. From study made on alkaloids from Harmaline, Harmine, Telepathine, Banisterine, was found that alkaloids possess an inhibitory effect on cell proliferation in cervical carcinoma (HeLa) cell line and colon carcinoma (SW460) cell line, in a dose dependent manner^[55].

The observed inhibition could related to the DNA binding properties of this chemical and to its ability to interact with DNA topoisomerases, which inhibit the DNA relaxation activities of topoisomerase I which is the molecular target for many antitumor drug. Many reports state that the primary cytotoxic effect of Vinca alkaloids is their ability to stop cellular division; also, this is same done by alkaloid of other plant as in colchicin, taxol. Vinca alkaloid stops cellular division by preventing microtubule function which is vital to cell division. Vinca alkaloid bind to specific site on tubulin, inhibiting the assembly of tubulin in to microtubules (Mphase of the cell cycle). Several in vitro studies have demonstrated the selective tumoricidal action of natural products without harming the normal cells ^[56,57,58,59]. The selectivity properties may comes from the specification of one or more of the chemical constituent of fenugreek alkaloids which also see in the different effect of other natural plant product on different cancer cell lines.

Flavoereirine (PB-100), a plant derived alkaloids, selectively destroy sixteen cancer cell lines even when these cell line are multidrug resistance rather than normal cell. This may due to that Malignant DNA is relaxed destabilized molecules because many of its H-bond have been broken, these abnormally and permanently separated strand areas .Many other endogenous or exogenous molecules can easily gain access to open DNA chain and cause disruption.

Furthermore, permeability of cancer cell is different from normal cell so the efflux pumping system may be a suitable target to overcome the drug resistance. Fenugreek seeds contain alkaloids that are closely structurally related to alkaloids found in many plants and herbs, thus it may cause acytotoxic effect by similar mechanism on the cell line as that caused by these (Foster et al 2005).

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