Original Research Article

Tumorcidal Effect of *Trigonella foenum-graceum* Extract and Selenium Nanoparticles on Ehrlich Carcinoma Bearing Mice

ABSTRACT

The present study was designed to examine the antitumor effect of *Trigonella foenum-graceum* either alone or combined with selenium nanoparticles. Ehrlich ascite carcinoma (EAC) cells and 4 groups of female mice were used. Solid Ehrlich carcinoma (EC) was induced by inoculation of $2.5x10^6$ cells in the left thighs of each animal. Mice were gavage orally by 2.5 µg/0.1 ml of fenugreek extract either alone or combined with selenium nanoparticles daily for one month. Tumor size, serum tumor markers (TNF-α, IFN-γ and Granzyme-B and Caspase-3) were evaluated. Oxidative stress and antioxidant markers, Histopathological, apoptotic and necrotic examination were determined in tumor tissues. Significant inhibition in tumor size. Caspase-3 and Granzyme-B activity were significantly elevated along with diminished tumor size while, TNF-α and IFN-γ levels were decreased in serum. Meanwhile, oxidative stress marker (MDA) was significantly decreased in tumor tissue. In addition, tumor GSH content and CAT activity were increased. Histopathological, apoptotic and necrotic examinations were context with the previous results. It could be concluded that Gg extract either alone or combined with SeNPs exhibited antitumor activity and this is reflected by reduction in tumor size, decrease of serum TNF-α and IFN-γ, increase in serum caspase-3 and Granzyme-B, reduction in tumor MDA and increase in tumor GSH and CAT which cause regulate tumor regression.

INTRODUCTION

Fenugreek (*Trigonella foenum-graceum)* is a leguminous herb belonging to fabaceae, cultivated throughout the world especially in the Asia and North African countries. It is best known for presence of pungent aromatic compounds in their seeds that gives color, flavor and aroma to food. It has been used as a medicinal plant since more than 4000 years in various parts of world. It has wide therapeutic applications including carminative, aphrodisiac and lactation stimulant in women after childbirth in traditional Chinese medicines. Its ability to treat wounds and sore muscles had made its use wide in science **(Bano *et al.*, 2016)**.

The main chemical components of *Trigonella foenum-graceum* are fibers, flavonoids, polysaccharides, saponins, fixed oils and some identified alkaloids. Mature seeds mainly contain amino acid, fatty acid, vitamins, saponins and a large quantity of folic acid (84mg/100g). It also contains disogenin, gitogenin, neogitogenin, homorientin saponaretin, neogigogenin, and trigogenin **(Mohammed *et al.,* 2006)**. The chemical constituents of fenugreek possessing anticancer activity are phytoestrogens and saponins. Saponins selectively inhibit cell division in tumor cells and can activate apoptotic programs that can lead to programmed cell death **(Shivangi *et al.*, 2016)**.

Apoptosis is a type of cell death. Flavonoids and catechins were first shown to be apoptotic in human carcinoma cells **(Ahmed *et al.,* 2000)**. Similar observation has since been extended to lung tumor cell lines, colon cancer cells, breast cancer cells, prostate cancer cells **(Hannan *et al.,* 2003)**, stomach cancer cells **(Zia *et al.,* 2001)**, brain tumor cells, head and neck squamous carcinoma **(Ramesh *et al.,* 2004)** and cervical cancer cells. They all induce apoptosis in tumor cells **(Thakran *et al.*, 2003)**. Fenugreek extract has also been shown to have stimulatory effects on macrophages.

The synthesis and application of selenium nanoparticles (SeNPs) attracted attention due to several advantages including chemical stability, biocompatibility and low toxicity **(Wang *et al.*,**

2007). With the growing interest in the issue of selenium intake in diet, nanoparticles are suggested as a novel nutritional supplement. A wide range of selenium compounds can be found in the environment and in living organisms ranging from simple inorganic forms (e.g. selenides, halides, oxyhalides, oxides, acids and salts of the oxyacids) up to the complex biogenic compounds such selenoenzymes and selenium nucleic acids **(Soda *et al.*, 2010)**. Huge family of selenium biogenic compounds consists of simple organic and methylated species, selenoamino acids, selenoproteins, selenoenzymes, selenoamino carboxylic acids, selenium peptides and also selenium derivate of pyrimidine, purine, cholines, sterioids, coenzyme A and many others. Most of these forms play a role in living organisms and have biological function by contributing to reduction of oxidative stress **(Kieliszek and Blazejak, 2013)**.

AIM OF THE WORK

In the current study, we sought to achieve the emerging nano-based approaches suitable to be used as imaging techniques for cancer treatment by fenugreek extract either alone or combined with SeNPs.

MATERIALS AND METHODS

Animals

Female outbreed Swiss albino mice originally obtained from National Cancer Institute (NCI) (20-25g) were used as experimental animals. All the studied animals were conducted in accordance with criteria of the investigation and ethics committee of the community laws governing the use of experimental animals.

Ehrlich Ascites Carcinoma Cell Line (EAC).

Ehrlich Ascites Carcinoma, were obtained from National Cancer Institute (NCI), Cairo university .The cells were propagated as ascites in female Swiss albino mice by weekly interperitoneal (ip) inoculation of 2.5 x 10^6 cells/ mouse **(Salem *et al.*, 2011)**.

Preparation of nanoparticle :

Selenium dioxide 1mM solution was mixed with aqueous extract of fenugreek powder 1:1 v/v . The mixture was stirred at room temperature and exposed to gamma ray at 40 kGy. This led to the immediate formation of SeNPs visualized as pink color solution. Then SeNPs were immediately characterized by Transmission electron microscopy **(TEM),** Dynamic light scattering measurement **(DLS)** and Fourier transform infrared spectroscopy **(FTIR)**.

Transmission electron microscopy (TEM)

SeNPs suspension were loaded on carbon-coated copper grids and solvent was allowed to evaporate by incubation at 37^{o}C for 30min in an incubator. The size and morphology of the SeNPs were estimated by TEM (JEOL electron microscope JEM-100 CX) operating at 80 kV accelerating voltage.

Dynamic light scattering measurement (DLS)

Average particle size and size distribution were determined by the dynamic light scattering (DLS).Technique (PSS-NICOMP 380-ZLS, USA); 250µl of suspension were transferred to a disposable low volume cuvette. After equilibration to a temperature of 25^{o}C for 2 min., five measurements were performed using 12runs of 10s each.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of the samples were recorded in KBr pellets using an FTIR spectrophotometer (JASCO FT-IR -3600) and spectrum was collected at a resolution of 4cm^{-1} in wave number region of 400 to 4000cm^{-1} to identify the possible molecules responsible for the reduction of selenium ions and to confirm FPP capped SeNPs.

- **In vitro study**

Chemosensitivity of nanoparticles (Cell viability):

Antitumor effect of fenugreek extract and/or SeNPs was assessed by observation of changes with respect to viable and nonviable tumor cell count. Cytotoxicity effects of the nanoparticles on tumor cells were determined according to the method of **El- Merzabani *et al.* (1979)**. In order to detect the cytotoxicity of nanoparticles, EACs were treated with nanoparticles at the concentrations of 1,2,3,4,5,6,7,8,9,10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml. The EACs were obtained by needle aspiration of ascites fluid from the preinoculated mice under aseptic condition using ultra violet laminar air flow system. The percentages of non-viable cells were determined by counting dead and viable EACs. To differentiate between dead and viable EAC cells, trypan blue stain was used. Then the percentages of non-viable cells (NVC) were calculated according to the following equations % NVC= C/T X 100, where (C) is number of non-viable cells and (T) is total number of viable cells.

Experimental Design

The animals were allowed 7 days for adaptation. 60 mice were then randomly distributed into 4 equal groups, 15 mice for each. The animal groups were recognized as follows:

G1: Normal control group. Normal mice neither injected nor treated.

G2: Ehrlich carcinoma (EC) bearing group. Mice were intramuscularly injected with 0.2ml of 2.5×10^{6} /ml/mouse viable Ehrlich ascite carcinoma cells in the left thigh.

G3: EC bearing fenugreek extract group. Mice were injected intramuscularly with 0.2ml of 2.5×10^{6} Ehrlich ascite carcinoma cells in the left thigh then after one day of tumor inoculation fenugreek extract gavage 2.5µg/0.1ml orally every day for one month.

G4: EC bearing fenugreek extract combined with SeNPs group. Mice were injected intramuscularly with 0.2ml of 2.5×10^{6} Ehrlich ascite carcinoma cells in the left thigh then after one day of tumor inoculation fenugreek extract combined with SeNPs gavage 2.5µg/0.1ml orally every day for one month.

Monitoring the tumor size

Tumor size was monitored twice or thrice weekly throughout the experiment. The tumor size being measured regularly using Vernier calipers and represented in terms of tumor size. The tumor size was estimated using the following formula: Tumor size (mm^3) = 4 (A/2) $(B/2)^2$=0.25 $A.B^2$, where A is the major axis and B is the minor axis **(Ghoneum *et al.*, 2008)**. The mean tumor size with the corresponding standard error was calculated in each experimental group. One month after treatments, experiment was terminated and all animals were sacrificed.

Sample preparation:

After one month of treatments, mice were anaesthetized using diethyl ether and sacrificed. Blood and tumor from animals of each group were collected and used for the proposed studies.

Preparation of serum:

Animals were sacrificed and the blood was collected from heart puncher using disposable plastic syringes, drained in tube, and left for coagulation. The blood was centrifuged and the upper layer (serum) was taken. TNF-α, IFN-γ, Granzyme-B and Caspase-3 were measured in serum of each group.

Tissue samples:

The EC tumor tissue of experimental animals were dissected out, washed and divided into two parts, one part was kept in 10% formalin for histopathological studies, apoptosis detection and the

other part was prepared in ice-cold saline (0.9%) using a potter-Elvehjem homogenizer to give a 10% homogenates which were used for determination of biochemical parameters.

Biochemical analysis:

In serum, the levels of tumor necrosis factor-alpha, Interferon-gamma, Granzyme-B and Caspase-3 activities were assayed by the standard sandwich enzyme-linked immune-sorbent (ELISA) assay technique using ELISA kit (K0331186, KOMABIOTECH, Seoul, Korea) following the manufacturer's instructions, In Ehrlich carcinoma tumor tissues, lipid peroxidation, Reduced glutathione and Catalase were measured colorimetrically as described by **Yoshioka *et al.* (1979)**, **Beutler *et al.* (1963)** and **Sinha (1972)** respectively.

Statistical Analysis

The obtained data was expressed as mean±standard error (SE). All data were analyzed statistically using one-way analysis of variance (ANOVA) followed by Student's t-test. Statistical significance was considered at $P < 0.05$. Statistical Package for Social Sciences (SPSS) for Windows version 17.0 software was used for this analysis **(Harnett and Horrell, 1998)**.

Histopathological Examination:

Following mice sacrificing tumor tissues were rapidly dissected and excised, rinsed in saline solution and cut into suitable pieces, then fixed in neutral buffered formalin (10%) for 24 hours, following fixation, the specimens were dehydrated in ascending series of alcohol, then tissue specimens were cleared in xylene and embedded in paraffin at 60°C. Section of 5 microns thickness was cut by slidge microtome. The obtained tissue sections were collected on the glass slides and stained by haematoxylin and eosin stain for histopathological examination by the light microscope **(Banchroft *et al.*, 1996)**. Another tissue sections (2-4 µm thick) were cut from paraffin embedded blocks by microtome and mounted from warm water (40°C) onto charged adhesive slides. By using a mixture of 100 µg/ml acridine orange and 100 µg/ml ethidium bromide prepared in PBS, the apoptosis and necrosis staining were analyzed **(Ribble *et al.*, 2005)**. The tissue uptake of the stain was monitored under a fluorescence microscope.

RESULTS

Morphology of nanoparticles:

The distribution of the particles size, DLS was performed, and its outcomes were linked to the TEM results**.** The average particle size was defined by DLS technique and was determined as 117 nm in SeNPs as noted in Fig. 1.

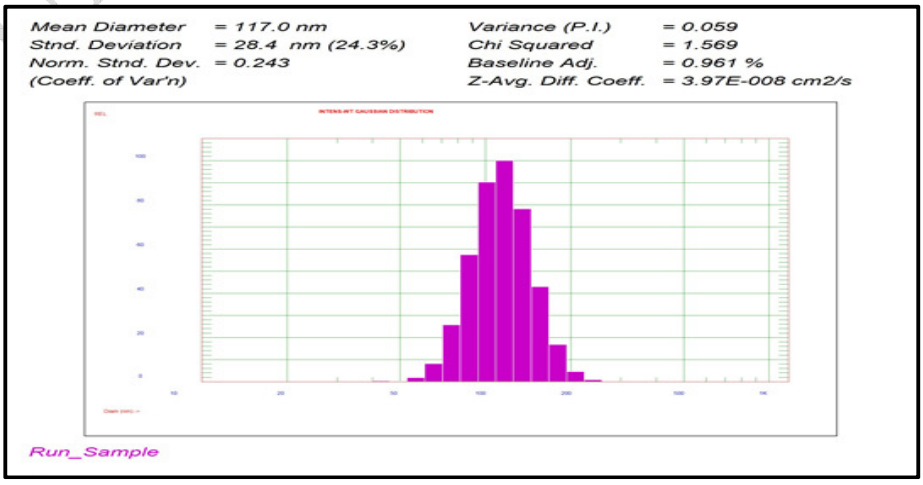

Fig. (1): Dynamic light scattering measurement (DLS)

Transmission Electron Microscope's result confirmed the spherical shapes of SeNPs within Nano range from 64.8 nm to 70.9 nm with the average mean diameter of 67.85 nm as explained in Fig. 2. The size of SeNPs received from DLS measures (117 nm) was greater than the TEM results (67.58 nm).

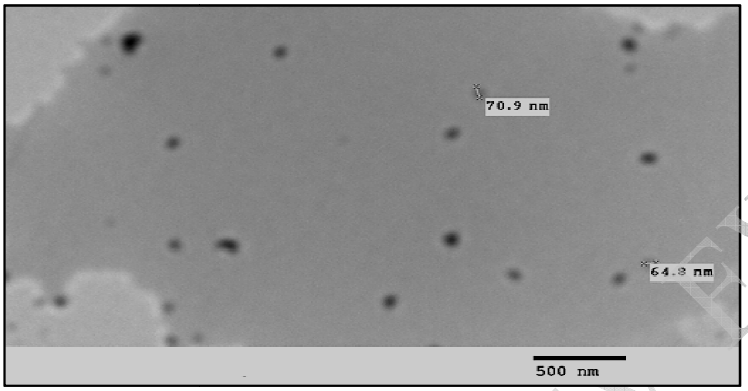

Fig. (2): Transmission Electron Microscopy (TEM)

The samples were recorded in KBr pellets using an FTIR spectrophotometer and spectrum was collected at a resolution of 4cm^{-1} in wave number region of 400 to 4000cm^{-1} to identify the possible molecules responsible for the reduction of selenium ions and to confirm FPP capped SeNPs as in Fig. 3.

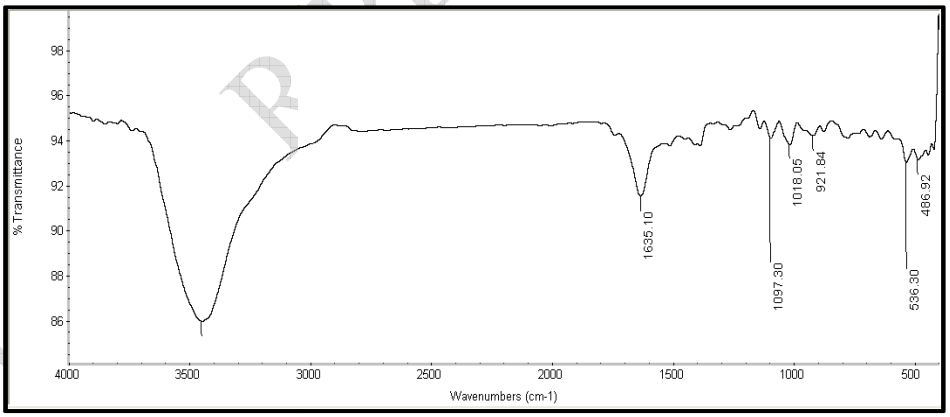

Fig. (3): Fourier transform infrared spectroscopy (FTIR)

In vitro studies:

Chemosensitivity of fenugreek extract either alone or combined with SeNPs on Ehrlich ascite carcinoma cells

The tumoricidal effect of different concentrations of fenugreek extract either alone or combined with SeNPs on Ehrlich cells viability is shown in Table (1). The low concentration (10 µg/ml) of fenugreek extract decreases the tumor cells viability by 15%.

The cytotoxicity of fenugreek extract led to the death of Ehrlich carcinoma cells. The median lethal concentration of fenugreek extract was 70 µg/ml for Ehrlich carcinoma cells. At a concentration of 20 µg/ml fenugreek extract led to the death of 20% of Ehrlich carcinoma cells and at a concentration of 90 µg/ml fenugreek extract led to the death of 65% of Ehrlich carcinoma cells.

The low concentration (10 µg/ml) of fenugreek extract combined with SeNPs decreases the tumor cells viability by 20%. The median lethal concentration of fenugreek extract was 60 µg/ml for Ehrlich carcinoma cells. For concentration of 20 µg/ml led to the death of 25% of Ehrlich carcinoma cells and at a concentration of 90 µg/ml fenugreek extract combined with SeNPs led to the death of 80% of Ehrlich carcinoma cells.

Table (1): The effect of fenugreek extract and fenugreek+SeNPs on the viability of Ehrlich ascites carcinoma cells.

Concentration (µg/ml)	Fenugreek extract		Fenugreek extract + SeNPs	
	% of viable cells	% of dead cells	% of viable cells	% of dead cells
0	99	1	99	1
9	90	10	90	10
10	85	15	80	20
20	80	20	75	25
30	75	25	70	30
40	70	30	65	35
50	65	35	60	40
60	60	40	50	50
70	50	50	40	60
80	40	60	30	70
90	35	65	20	80

In vivo studies

- **Ehrlich Carcinoma Tumor Size Monitoring:**

The size of solid Ehrlich carcinoma (EC) in the left thigh of mice was measured eight times along one month starting from EC tumor cells inoculation and beginning of tumor formation in control EC bearing mice. The delay of inhibition in tumor size in mice treated by fenugreek extract either alone or combined with SeNPs comparing with EC group is illustrated in Fig (4). It is clear that the mean size of the left thigh of healthy normal mice is 17.55 mm^3 and the inoculation of 2.5 million of EC cells in 0.2 ml physiological saline in the left thigh of healthy normal mice produced a solid tumor with a mean size of 95.67±3.83 mm^3 on the 7^{th} day after tumor inoculation after tumor inoculation. EC tumor size exceeds 400 mm^3 on the 10^{th} day after tumor inoculation. The increase of EC tumor size proceeds by days reaching 2583.33±35.7 mm^3 on the 30^{th} days after tumor inoculation.

The data obtained revealed lesser tumor size through the observation period in groups of experimental animals daily treated with fenugreek extract at the next day after tumor inoculation for

one month. At the 7th, 10th and 30th days after tumor inoculation tumor size were 60.5±4.42, 74±4.75 and 1682.5±48.36 mm^3 respectively. The tumor size of mice treated with fenugreek extract combined with selenium nanoparticles at the next day after tumor inoculation for one month every day showed (36.17±5.31, 136.33±5.07 and 1694.33±13.94 mm^3 on 7th, 10th and 30th days respectively).

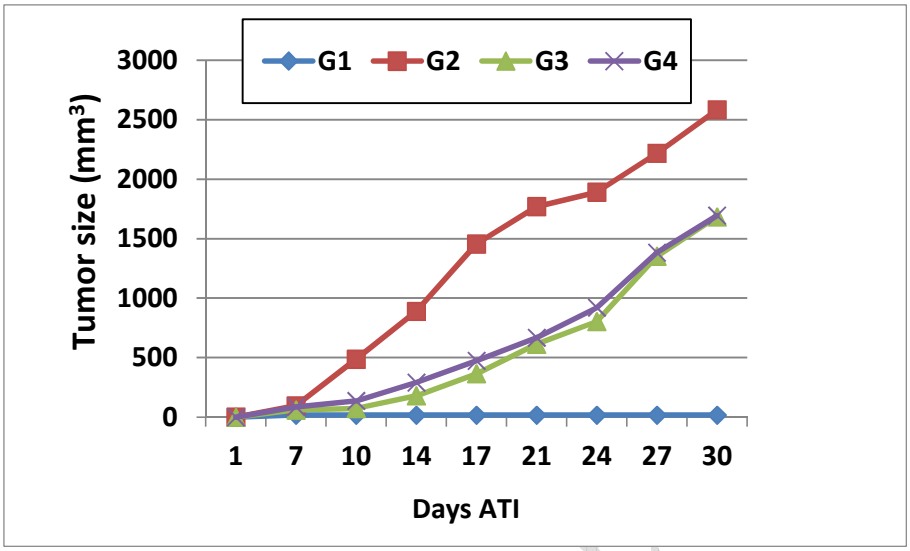

Fig. (4): Effect of fenugreek extract either alone or combined with SeNPs on EC tumor size.

➢ **Tumor markers responses**

Caspase-3, Granzyme-B, Serum tumor necrosis factor-alpha (TNF-α) and Serum Interferon gamma (IFN-γ) detection

Data revealed that female mice inoculated with EC and treated with fenugreek extract daily for one month recorded a significant increase in caspase-3 activity, a significant decrease in Granzyme-B activity, a significant decrease in TNF-α Level and a significant decrease in IFN-γ Level in compared to EC group. On the other hand, daily treatment of female mice inoculated with EC and treated with Licorice extract combined with SeNPs for one month predicts an increase in caspase-3 activity, an increase in Granzyme-B activity, an decrease in TNF-α level and an decrease in IFN-γ level compared to EC group.

Table (2): Effect of fenugreek extract either alone or combined with SeNPs on Caspase-3, Granzyme-B, TNF-α and IFN-γ levels of mice bearing EC.

Groups / Parameter	G1	G2	G3	G4
Caspase-3 (µmol pNA/min/ml)	2.2±0.03	2.83±0.07	3.1±0.23	7.69±0.06 ab
Granzyme-B (pg/ml)	78.63±2.16 b	14.1±0.62 a	13.63±1.01 a	46±1.89 ab
TNF-α (pg/ml)	30.89±0.88 b	113.47±4.02 a	39.5±1.85 ab	53.81±2.42 ab
IFN-γ (pg/ml)	17.47±0.48 b	85.96±2.35 a	18.59±0.67 b	35.4±0.95 ab

All data are the means of 10 records.

a: significant against N at $P \leq 0.05$ b: significant against EC at $P \leq 0.05$

➢ Oxidative stress and antioxidant markers in tumor tissues

Tumor tissue MDA, CAT and GSH activity are represented in Table (3) The data revealed that female mice bearing EC represents a highly significant increase in tumor MDA and a highly significant decrease in tumor GSH and CAT in compared to N group.

The oral gavages of female mice bearing EC by fenugreek extract daily for one month recorded decrease in tumor MDA and GSH activity and a significant increase in CAT in compared to EC bearing group. Treatment of female mice bearing EC with fenugreek extract combined with SeNPs daily for one month predicts decrease in tumor MDA, increase in tumor GSH and CAT in compared to EC group.

Table (3): Effect of fenugreek extract either alone or combined with SeNPs on MDA, CAT and GSH levels of mice bearing EC.

Parameter \ Groups	G1	G2	G3	G4
MDA (μM/gm tissue)	64.67±2.33 ^{b}	117.83±6.29 ^{a}	112.83±4.55 ^{a}	91.67±1.69 ^{ab}
Catalase (μM Catalase/gm tissue)	1.5±0.1 ^{b}	0.2±0.01 ^{a}	0.5±0.01 ^{ab}	1.5±0.5 ^{ab}
GSH (mg GSH/gm tissue)	2.33±0.09 ^{b}	1.6±0.14 ^{a}	1.36±0.11 ^{a}	1.75±0.09 ^{a}

All legends as in table (2)

-Histopathological examination of Ehrlich carcinoma (EC):

Histopathological examination possessed normal muscle histology (Fig. 5 A) of non-mice bearing Ehrlich carcinoma. Ehrlich carcinoma (EC) tissue section under light microscope showed compact and aggregation of the tumor tissue cells spread within the muscular tissues. EC showed groups of large, round and polygonal cells, with pleomorphic shapes, hyperchromatic nuclei and binucleation. Several degrees of cellular and nuclear pleomorphism were seen in (Fig. 5 B&C). EC of mice gavage orally by fenugreek extract daily for one month after 1 day of tumor inoculation represents extensive areas of necrotic EC cells and other areas contain of remnants, apoptotic and some pyknotic nuclei (Fig. 6 A, B&C). Photomicrographs in sections of Ehrlich carcinoma of mice gavage orally by fenugreek extract combined with SeNPs daily for one month represents extensive areas contain of remnants, apoptotic and some pyknotic nuclei after 1 day of tumor inoculation (Fig. 7 A&B).

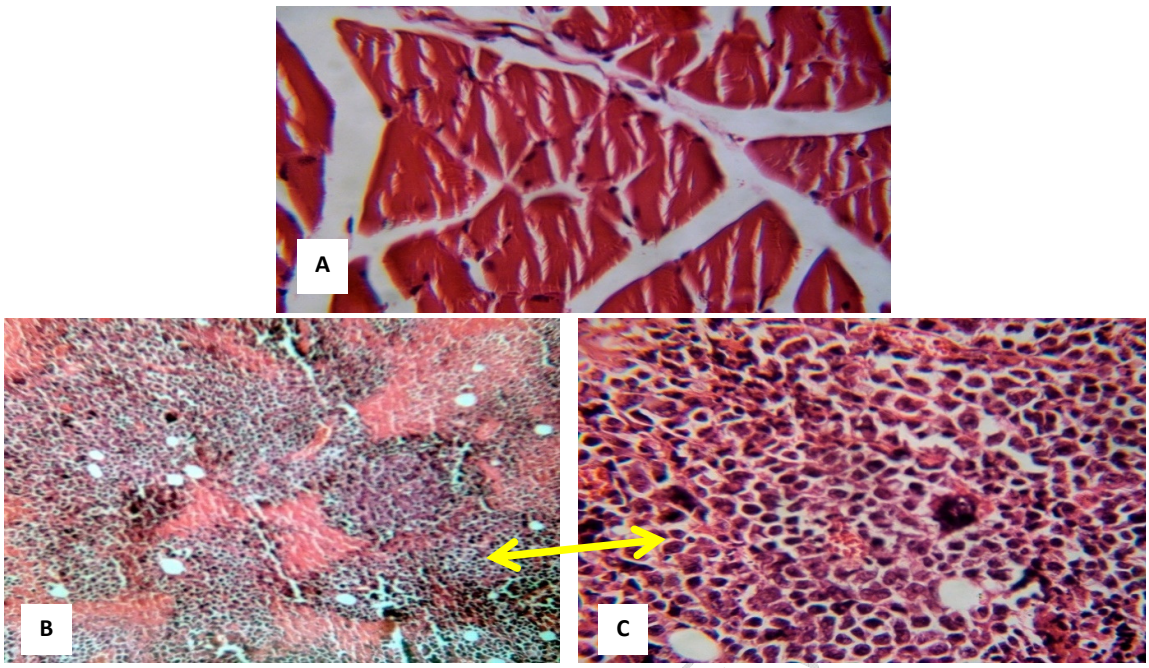

Fig. (5): Photomicrograph in sections of EC. **A:** Normal control muscle section in Albino mice represents normal muscular fiber. **B & C:** Control EC. Note: EC cells invaded muscular tissue; (↔) tumor cells encircled the muscles cells. (H and E stain, A&B X100- C X 400)

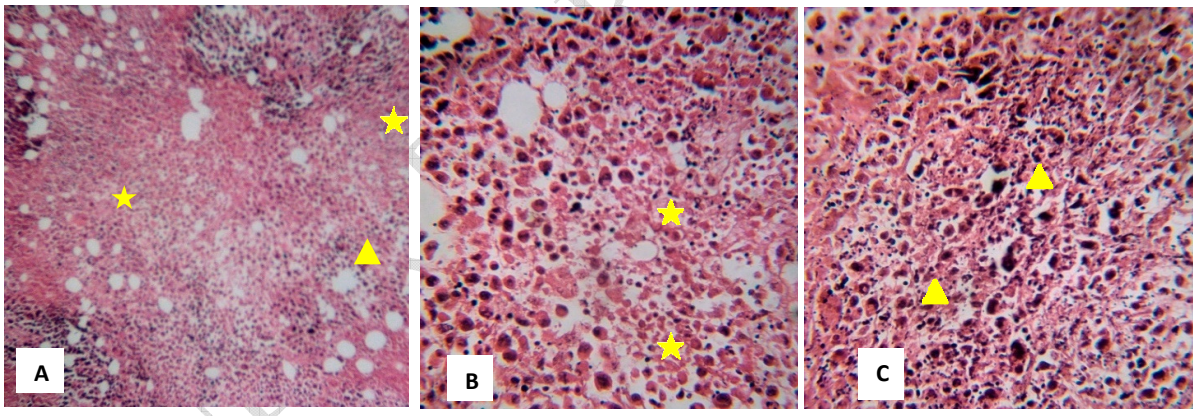

Fig. (6): Photomicrographs in sections of Ehrlich carcinoma of mice gavage orally by fenugreek extract daily for one month. **A, B& C:** gavage after 1 day of tumor inoculation represents extensive areas of necrotic EC cells (star) and other areas contain of remnants, apoptotic and some pyknotic nuclei (▲). (H and E stain, A X100- B& C X 400)

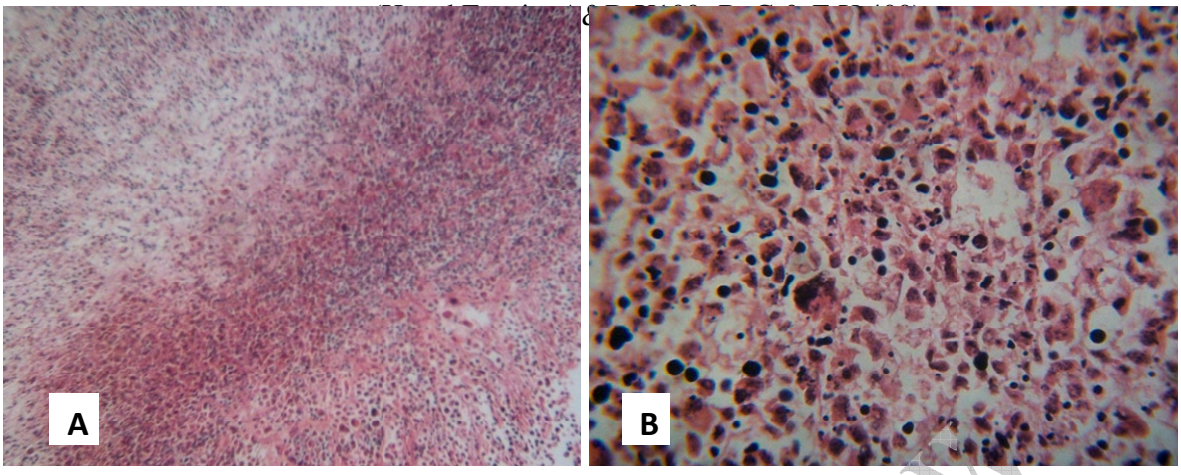

Fig. (7): Photomicrographs in sections of Ehrlich carcinoma of mice gavage orally by fenugreek extract combined with SeNPs daily for one month. **A& B:** gavage after 1 day of tumor inoculation represents extensive areas contain of remnants, apoptotic and some pyknotic nuclei (▲). (H and E stain, A X100- B X 400)

Apoptotic and necrotic examination of Ehrlich carcinoma (EC):

Apoptotic and necrotic stained by Acridine orange / propidium iodide stain and examined under a fluorescent microscope. Normal muscle tissue section represents vital tissue regions stained in green color (Fig. 8 A). Control section of EC represents vital tissue stained in green stain with no zones of necrosis (orange cells) or apoptosis (yellow cells) in addition to the presence of vital green regions and some vacuolated areas (Fig. 8 B&C).

Treatment of mice orally by fenugreek extract daily for one month represents extensive areas of necrotic EC cells and other areas contain of remnants of apoptotic nuclei and some vacuolated areas for gavage treatment after 1 day of tumor inoculation (Fig 9 A&B). Combined treatment of fenugreek extract with SeNPs daily for one month represents extensive areas of necrotic EC cells and other areas contain of remnants of apoptotic nuclei and some vacuolated areas for gavage after 1 day of tumor inoculation (Fig. 10 A&B).

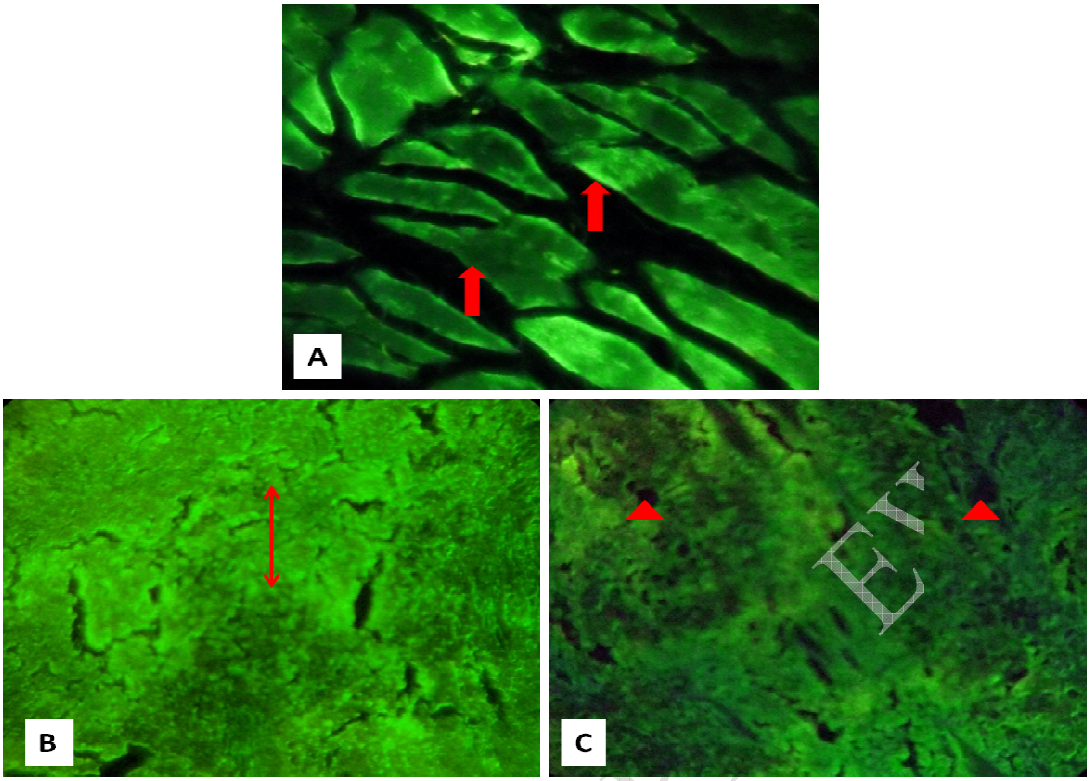

Fig. (8): Fluorescent imaging of sections in Ehrlich carcinoma stained by Acridine orange / propidium iodide stain. **A:** Normal muscle represents vital tissue regions stained in green (red blocked arrows). **B&C:** Control Ehrlich carcinoma represents vital green regions (↕) and some vacuolated areas (▲). (A&C X250, BX100)

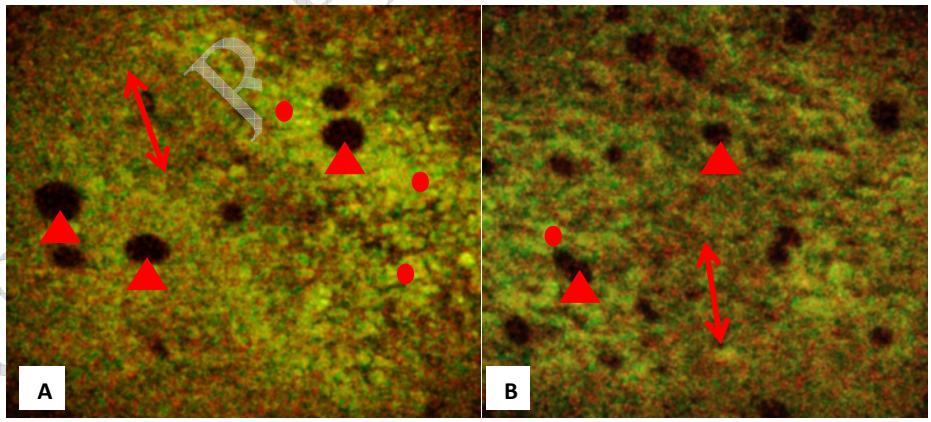

Fig. (9): Photomicrographs in sections of Ehrlich carcinoma Fluorescent imaging of sections in Ehrlich carcinoma stained by Acridine orange / propidium iodide stain of mice gavage orally by fenugreek extract daily for one month. **A& B:** gavage after 1 day of tumor inoculation represents extensive areas of necrotic EC cells (↕) and other areas contain of remnants of apoptotic nuclei (●) and some vacuolated areas (▲). (A& B x 250)

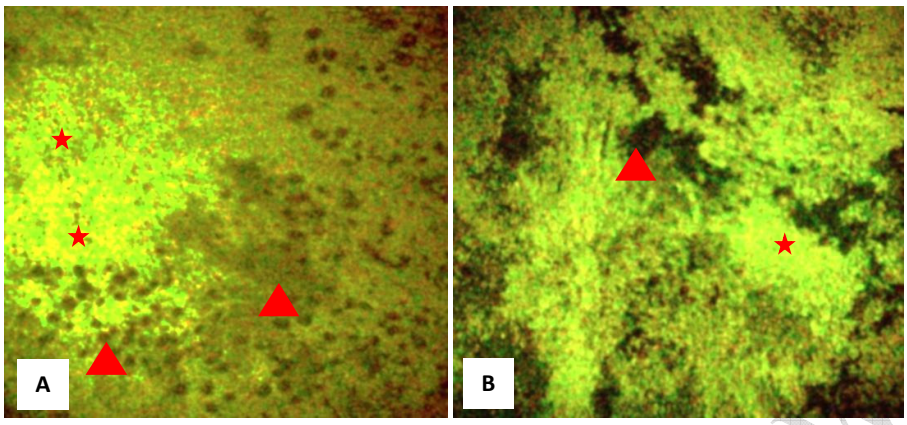

Fig. (10): Photomicrographs in sections of Ehrlich carcinoma Fluorescent imaging of sections in Ehrlich carcinoma stained by Acridine orange / propidium iodide stain of mice gavage orally by fenugreek extract combined with SeNPs daily for one month. **A& B:** gavage after 1 day of tumor inoculation represents extensive areas of necrotic EC cells (●) and other areas contain of remnants of apoptotic nuclei (star) and some vacuolated areas (▲). (A& B x 250)

DISCUSSION

Classical very potent chemotherapeutic agents have been used against several tumor types for several decades. However, they have the disadvantage of affecting both tumor cells and normal cells, with the concomitant secondary effects including cardiotoxicity, cytotoxicity, neurotoxicity, nephrotoxicity, and ototoxicity **(Wang *et al.*, 2012)**. Some of these chemotherapeutic-associated problems have been solved by the use of nanoparticle formulations of these drugs. The most important advantage of these novel formulations is that they preferentially target tumor cells by the enhanced permeability and retention (EPR) phenomenon exhibited by solid tumors compared with normal tissues. In addition, nanoparticles as therapeutics carriers have other unique properties of higher therapeutic efficacy, lower toxicity and the ability to encapsulate and deliver poorly soluble drugs **(Wang *et al.*, 2012)**. The elaboration of nanoparticles of uniform shape, size and composition is a dynamic growing research field in cancer medicine. Novel improved biodegradable and biocompatible nanoparticle formulation with increasing bioavailability, in vivo stability, intestinal absorption, solubility, sustained and targeted delivery to site of action combined with therapeutic effectiveness, are being developed **(Ochekpe *et al.*, 2009)**. Since the size of the nanoparticles is significantly smaller than a cell, they can deliver a large payload of drugs, contrast agents or fluorescent probe onto the surface or interior of the cell, without disrupting its function **(Matteo *et al.*, 2006)**. These particles are able to deep penetrate tissues, going through the fenestration of the small blood-vessel epithelial tissue. They can enter the systemic blood circulation without forming blood platelet aggregates. Their reduced particle size entails high surface area and hence a strategy for faster drug release **(Matteo *et al.*, 2006)**.

Selenium (Se) is an essential trace element required by many organisms. It is a crucial cofactor of antioxidant enzymes such as glutathione peroxidases and thioredoxin reductases **(Srivastava and Mukhopadhyay, 2013)**. As the selenium nanoparticles (SeNPs) possess antimicrobial and anticancer properties, they can be used as nanomedicines **(Wadhwani *et al.*, 2016)**. Also, they exhibit less toxicity as compared to their inorganic and organic counterparts **(Shakibaie *et al.*, 2010)**.

On the other hand, many anticancer drugs exert adverse side effects, which can be severe or deadly. Thus, identification of novel anticancer compounds from natural products was proposed as a safer alternative and a promising strategy for cancer prevention or treatment. Many traditional herbal medicines and certain food constituents exhibit anti-inflammatory and antioxidant effects, suggesting their potential as chemopreventive or therapeutic agents **(Jasim, 2014)**.

In a number of studies, extract of fenugreek seeds and some of their constituents have shown anticarcinogenic potency. Consumption of fenugreek was accompanied with decreased polyamines (spermine, spermidine, putrescine) content in tumor tissue **(Jasim, 2014)**. The effect of biologically active constituent of fenugreek seeds on breast cancer cell lines caused G1 cell cycle arrest by down regulating cyclin D1, cdk-2 and cdk-4 expression in both estrogen receptor positive ER (+) and estrogen receptor negative ER (-) breast cancer cells resulting in the inhibition of cell proliferation and induction of apoptosis **(Jasim, 2014)**.

In the present study, the cytotoxicity of either fenugreek seeds extracts either alone or combined with selenium nanoparticles on Ehrlich carcinoma cell line was carried out.

The present study demonstrated that fenugreek extract could exert a high cytotoxicity against Ehrlich ascite carcinoma cell line. The median lethal concentration of fenugreek extract was 70 µg/ml and the median lethal concentration of fenugreek extract combined with SeNPs was 60 µg/ml.

Fenugreek extract were cytotoxic in vitro to a panel of cancers but not normal cells. Also, fenugreek extract have an inhibitory growth to breast, pancreatic and prostate cancer cell lines **(Shivangi *et al*., 2016)**.

Fenugreek extract selectively inhibit cell division in tumor cells and can activate apoptotic programs which can lead to programmed cell death **(Shivangi *et al*., 2016)**. Meanwile**,** the cytotoxicity effect of nanoparticles is due to their adherence to the cell membrane, particle internalization and degradation of products in the cell culture medium or inside the cells **(Abbasalipourkabir *et al*., 2011)**.

Our experimental data revealed that the positive control mice develop Ehrlich tumor bulb exceeded $1cm^3$ ($500\ mm^3$)14 days after tumor inoculation (ATI) of viable EAC cells. Also**,** microscopic investigations showed compact and aggregation of the tumor tissue cells spread within the muscular tissues with pleomorphic shapes, hyperchromatic nuclei and binucleation without necrosis or apoptosis. The effect of ROS production as a result of tumor growth, on other organs in the body can be explained as follows: ROS cause activation in nuclear factor κB (NF-κB) and phosphorylation of its inhibitor (IκB). Thus, they enable NF-κB to translocation in the cell nucleus and binds to DNA and regulates the transcription of various target genes (i.e inducible nitric oxide synthase, cyclooxygenase II, cytokines, etc.), which contribute to cell damage. Interestingly, in tumor cells cytokines activate NF-κB, which protects the tumor cells from TNF-α induced apoptotic cell death. NF-κB activation in cancer cells regulates transcription of genes involved in cell proliferation, antiapoptosis and invasion. Thus, activation of NF-κB induces tumor growth and metastasis and reduces cytokines-induced apoptosis **(Hanafi and Asmaa, 2015)**.

In the present study, regular and rapid increases in tumor volume were observed in EC tumor bearing mice, while in groups were taken the treatments, a decreased in tumor volume was observed supporting the beneficial anticarcinogenic effect of fenugreek. On the other hand EC of mice daily gavage orally by fenugreek extract either alone or combined with selenium nanoparticles represents histopathologically extensive areas contain of remnants, apoptotic and pyknotic EC cells.

Context with the findings of **Thippeswamy and Salimath (2006)** the tested extracts of fenugreek have potent proapoptotic effects on EC cells in vivo and the inhibitory effect of fenugreek on EC cell growth may be due to induction of apoptosis. The reduction in tumor volume was due to the treatment-induced inhibition in cell cycle progression **(Meikrantz and Schlegel, 1995)**.

In an in vivo study the effect of fenugreek seed powder along with its bioactive compound were able to inhibit the formation preneoplastic lesion **(Shivangi *et al*., 2016)**. Suppressed the expression of proapoptotic protein bcl-2 and there was an increase in the expression of caspase-3, an antiapoptotic protein **(Shivangi *et al*., 2016)**. Several studies on anticancer properties of chemical constituents of fenugreek have been done and have shown positive results. Some constituent of alkaloids, called "trigonelline," has revealed potential forusein cancer therapy **(Shivangi *et al*., 2016)**.

The chemopreventive activity of the methanolic extract of fenugreek seeds may be due to the rich chemical constituents (such as, saponins, flavonoids, alkaloids, galactomannans) that are present in the seed working synergistically at various stages of angiogenesis **(Shivangi *et al*., 2016)**.

Fenugreek was reported to have an ability to inhibit further growth of cancer cells without harming the healthy cells of the body.

The mechanism of selenium nanoparticles in reducing the tumor size may be through the long-circulating nanoparticulate carriers. They are able to efficiently deliver the chemotherapeutics to solid tumors by exploiting the enhanced permeability and retention effect and thus can significantly enhance the therapeutic index of the drug or improve reducing undesirable side effects. Studies recorded that ultra-low size particles can efficiently be targeted to the tumor tissue through the combined effects of extravagation and long circulation in blood **(Savita and Amarnath, 2009)**.

Our results demonstrated apoptosis suppression in solid EC tumors as evidenced by the significant reduction in the level of apoptotic molecules (caspase-3 and granzyme B), compared to non EC-bearing mice. Apoptosis is a programmed cell death that maintains the stability of the internal environment through removing genetic mutations and unstable cells. However, this process is inhibited in cancer, which leads to the accumulation of various genetically unstable cells. The disturbance in the apoptotic regulators leads to tumor proliferation and growth **(Medhat *et al*., 2017)**.

Caspases (**C**: cysteine protease mechanism, **aspase**: ability to cleave after aspartic acid) are aspartate-directed cysteine proteases that play a key role in the initiation and execution of apoptosis or PCD, necrosis and inflammation, failure of which may cause tumor development and several autoimmune diseases. Once activated, they cleave cellular substrates, leading to morphological hallmarks of apoptosis **(Hanafi and Asmaa, 2015)**.

In our study, treatment of experimental animals bearing EC with fenugreek extract either alone or combined with SeNPs represents significant increase in tumor caspase-3 levels when compared with their corresponding activity in EC bearing mice.

This increase in caspase-3 activities postulated the effect of apoptosis on MCF-7 cell line in Caspase 3, 8, 9, p53, Fas, FADD, Bax and Bak activation. There are various mechanisms through which apoptosis can be induced in cells such as the expression of pro and anti-apoptotic proteins. The mitochondrial apoptotic pathways and death receptor pathways are the two major pathways. The mitochondria have a central role in regulating the caspase cascade and apoptosis **(Shafi *et al.*, 2009)**. Caspases have a central role in the apoptotic process in that they trigger a cascade of apoptotic pathways **(Shah *et al.*, 2003)**.

Also, the activity of caspase-3 is increased in tumor cells due to the inactivation of P53 (tumor suppressor protein), which is responsible for protecting cells from tumorigenic alterations **(Hanafi and Asmaa, 2015)**.

Caspase activation leads to apoptosis through two main pathways. One pathway involves a

tumor necrosis factor (TNF) receptor at the cell surface, which recruits caspase-8 through the adaptor protein FAS-associated death domain (FADD) leading to the activation of caspase-8. The intrinsic pathway involves the release of cytochrome c from mitochondria, a key intermediate step in the apoptotic process that leads to the activation of caspase 9 **(Hanafi and Asmaa, 2015)**. Cytosolic cytochrome c binds to Apoptotic protease-activating factor-1(Apaf-1) forming complex containing Apaf-1and cytochrome c **(Wang, 2001)**.

In the same direction, SeNPs inhibited cancer cell growth partially by caspase-mediated apoptosis, which was through the downregulation of androgen receptor (AR) phosphorylation expression at both transcriptional and translational levels. SeNPs treatment activated the Akt/Mdm2 pathway, and initiated AR phosphorylation, ubiquitination and degradation. The cancer suppression function of SeNPs consisted of at least two mechanisms, regulation of AR transcription and promotion of AR protein degradation **(Kong et al., 2011).**

Granzymes is a family of serine proteases is contained within the cytoplasmic granules of cytotoxic lymphocytes (CLs), and the pore-forming protein, perforin. According to the model of granule-mediated apoptosis, killing involves degranulation and subsequent transfer of these proteases into the cytoplasm of the target cell, where they rapidly induce apoptosis **(Birkedal and Taylor, 1982)**. This process is inhibited in cancer, which leads to the accumulation of various genetically unstable cells **(Medhat et al., 2017)**.

Our results demonstrated apoptosis suppression in solid EC tumors as evidenced by the significant reduction in the level of apoptotic molecules (caspase-3 and granzyme B), compared to non EC-bearing mice. Apoptosis is a programmed cell death that maintains the stability of the internal environment through removing genetic mutations and unstable cells.

Treatment of experimental animals bearing EC with fenugreek extract either alone or combined with SeNPs represents significant increase in granzyme B level when compared with their corresponding activity in EC bearing mice.

This increase in caspase-3 activities postulated that granzyme B has a similar preference as caspases for cleaving protein peptide bonds C-terminal to Aspartate residue. Granzyme B is capable of direct proteolytic processing and activation of the executioner procaspase-3 and -7. On the other hand, there are contradictory reports on the direct granzyme B-mediated procaspase-6 proteolytic activation **(Ilona and Evzen, 2010)**. Moreover, the apoptotic procaspases including procaspase-8, -10, -9, and -2 were reported to serve as substrates for the active granzyme B. However, it should be emphasized that granzyme B can proteolytically cleave these initiator procaspases but it cannot activate them. The initiator procaspases are activated exclusively by homodimerization in specific multiprotein activation platforms such as apoptosome, DISC and PIDDosome **(Ilona and Evzen, 2010)**.

There is mounting evidence that granzyme B can kill cells via a caspase-independent pathway **(Trapani and Sayers, 2003)**. The serine protease and the caspases appear to cleave some of the same cellular substrates, resulting in the demise of the cells **(Walker *et al.*, 1994)**. The granzyme B not only activates pro-death functions within a target, but also has a previously unidentified role in inactivating pro-growth signals to cause cell death **(Thomas *et al.*, 2000)**.

TNF-alpha is a cytokine produced by the innate immune cells and implicated in the promotion or inhibition of tumor development. It is produced by tumor cells or inflammatory cells in the tumor microenvironment. The role of TNF-alpha in chronic inflammatory diseases and tumor-promoting effects is well recognized as well as the role in promoting tumor cell survival through the induction of genes encoding NFκB- dependent antiapoptotic molecules. Other actions which may enhance tumor progression include; promotion of angiogenesis, metastasis and impairment of immune surveillance by suppressing T cell responses as well as cytotoxic activity of activated macrophages **(Hanafi and**

Asmaa, 2015).

Genetic poly morphisms which enhance TNF-alpha production are associated with increased risk of hepatocellular carcinoma (HCC) as well as other tumors such as multiple myeloma, bladder carcinoma, gastric carcinoma and breast carcinoma. Overall, TNF-alpha is an important factor involved in initiation, proliferation, angiogenesis, and metastasis of various types of cancers **(Hanafi and Asmaa, 2015)**.

The experimental data reveals that female mice bearing EC represents a significant increase in serum TNF-α level of tumor bearing mice in compared to normal control group.

The elevation in the TNF-α level in EC mice may be attributed to the increase in the production of ROS by macrophages which stimulate lipid peroxidation or initiating a potentially harmful immune response and stimulate neutrophil chemotaxis or activates transcriptional factor NF-κB which in turn increases the production of proInflammatory cytokines **(Hanafi and Asmaa, 2015)**.

Our data reveals that treatment of experimental animals bearing EC with fenugreek extract either alone or combined with SeNPs represents a significant decrease in serum TNF-α level in compared to EC group and a significant increase in compared to normal control level. Fenugreek extract inhibited TNF-induced invasion by inhibiting the proliferation of tumor cells and stopping the cells from progressing to G1 **(Liu *et al.*, 2005)**, downregulated the expression of antiapoptotic, proliferative, and angiogenic gene products **(Yin *et al.*, 2004)**. Also, Fenugreek extract suppressed TNF-induced invasion by tumor cells, and this inhibition correlated with the downregulation of MMP-9 and COX-2 **(Esteve *et al.*, 2002)**.

Mansour et al. (2010) postulated that use of selenium nanoparticle significantly decrease TNF-α concentration in the plasma of mice bearing EC.

Interferon gamma (IFN-γ) is a dimerized soluble cytokine that is the only member of the type II class of interferons **(Gray and Goeddel, 1982)**. IFN-γ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific immunity develops **(Schoenborn and Wilson, 2007)**. IFN-γ is also produced by non-cytotoxic innate lymphoid cells (ILC), a family of immune cells first discovered in the early 2010s **(Artis *et al.,* 2015)**.

EC bearing mice showed high increases in the activity of IFN-γ due to its role in systemic and local immunity and in almost all inflammatory responses **(Ikeda *et al.*, 2002)**.

Treatment of experimental animals bearing EC with fenugreek extract extract either alone or combined with SeNPs represents significant decrease in IFN-γ level when compared with their corresponding activity in EC bearing mice.

In the last years, many researches demonstrated the immunoregulatory activity of fenugreek extract. Among the compounds of them is believed to play an important role in stimulating the body's immune ability. It affects the body's nonspecific and specific immune functions and activates immune cells. In addition, it also showed immunoregulatory activity **(Fontes *et al.*, 2014)**.

Oxidative stress is occurred due to a disturbance in the balance between the production of ROS and the efficiency of the antioxidant defense. In other words, oxidative stress results if excessive production of ROS overwhelms the antioxidant defense system or when there is a significant decrease or lack of antioxidant defense **(Kang, 2002)**. Moreover, severe oxidative stress is not only known to cause DNA damage and mutations of tumor suppressor genes, which are initial events in carcinogenesis **(Kang, 2002)**, but can also play an important role in the promotion of multistep carcinogenesis **(Ahmed *et al.*, 1999)**.

The end product of lipid peroxidation, malondialdehyde, due to its high cytotoxicity and inhibitory action on protective enzymes, is suggested to act on tumor development **(Kang, 2002)**.

Lipid peroxidation plays an important role in the control of cell division **(Diplock *et al.*, 1994)** associated with pathological conditions of a cell. Moreover, it has been claimed that MDA acts as a tumor promoter and co-carcinogenic agent because of its high cytotoxicity and inhibitory action on protective enzymes. Also, the tumor development might be responsible for the antioxidant depletion and also the increased concentration of lipid peroxidation products **(Hanafi and Asmaa, 2015)**.

The increase in levels of lipid peroxidation in tumor tissue might be attributed to the deficiency of antioxidant defense mechanisms or probably due to the generation of reactive oxygen species (ROS) and altered redox statuses, which are common biochemical aspects in tumor cells. ROS can react with the polyunsaturated fatty acids of lipid membranes and induce lipid peroxidation. In addition, earlier studies observed increased lipid peroxidation and decreased antioxidant levels in the cancer patients **(Hanafi and Asmaa, 2015)**.

Our data revealed that experimental revealed that animals bearing EC represents a significant decrease in tumor GSH content in compared to EC group.

The depletion in GSH level in tumor tissue may be attributed to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by Ehrlich solid cells or to the diminished activity of glutathione reductase due to the deficiency or inactivation of glucose-6-phosphate dehydrogenase, the main supplier for NADPH which is necessary to change oxidized glutathione to its reduced form **(Hanafi and and Asmaa, 2015).**

Tirkey *et al.* (2005) indicated that oxidative stress causes depletion of intracellular GSH, a reducing agent with its sulhydryl group leading to serious consequences. The decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein caused by ROS generation which can in turn impair the antioxidant defense mechanism leading to increased lipid peroxidation **(Ohta *et al.*, 2004)**. Excessive lipid peroxidation can cause increased glutathione consumption **(Manda and Bhatia, 2003)**.

On the other hand, our data revealed that experimental animals bearing EC represents a significant decrease in tumor CAT content in compared to EC group.

When CAT activity is reduced, the level of hydrogen peroxide increased in cancer tissue. This may correspond with the report, which showed that some human cancer lines produced a large amount of hydrogen peroxide **(Szatrowski and Nathan, 1991)**. Indeed, the levels of glutathione, CAT and GSH-Px, have been shown to be significantly altered in malignant cells **(Oberley and Oberley, 1997)** and in primary cancer tissues homeostasis and stress adaptation in cancer cells or could also be due to exhaustion of the glutathione and antioxidant enzymes because of increased peroxidation **(Manimaran and Rajneesh, 2009)**. Alternatively, it is possible that the antioxidant system is impaired as a consequence of an abnormality in the anti-oxidative metabolism due to the cancer processes.

Treatment of experimental animals bearing EC with fenugreek extract either alone or combined with SeNPs represents a decrease in levels of lipid peroxidation, an increase in catalase activity and un significant change in reduced glutathione in tumor tissue in compared to EC group.

The decrease in MDA level when compared with their corresponding level in EC bearing mice explain the more pronounced delay in tumor size and the protective activity of fenugreek aqueous extract against tumor progression and the return of muscular tissue to its normalization . The fenugreek extract inhibited the promoter of LPO by blocking the production of thiobarbituric acid reactive substances (TBARS) **(Umesh and Najma, 2014)**.

According to the results obtained, it could be postulated that lipids, especially polyunsaturated fatty acids (PUFA) in the membranes, are very susceptible to free radical attack which can initiate lipid

peroxidation **(Halliwell and Gutteridge, 1999)**. Lipid peroxidation plays an important role in the control of cell division **(Diplock *et al*., 1994)**.

Glutathione (GSH), the most abundant non-enzymatic antioxidant present in the cell, plays an important role in the defense against oxidative stress-induced cell injury. In the cells, glutathione is present mainly in its reduced form. Reduced GSH can be converted to oxidized glutathione (GSSG) which is revertible to the reduced form with the glutathione reductase (GR). Cells are also equipped with the enzymatic antioxidant mechanisms that play an important role in the elimination of free radicals **(Hanafi and Asmaa, 2015)**.

Indeed, the level of glutathione have been shown to be significantly altered in malignant cells and in primary cancer tissues**,** suggesting aberrant regulation of redox homeostasis and stress adaptation in cancer cells or could also be due to exhaustion of the glutathione and antioxidant enzymes because of increased peroxidation**.** Alternatively, it is possible that the antioxidant system is impaired as a consequence of an abnormality in the anti-oxidative metabolism due to the cancer processes **(Hanafi and Asmaa, 2015)**.

Our data revealed that treatment of experimental animals bearing EC with fenugreek extract either alone or combined with SeNPs represents un-significant change in tumor GSH content in compared to EC group.

The depletion in glutathione level has been reported to enhance the cell death and apoptosis of the tumor cells along with the loss of essential sulfhydryl groups that result in an alteration of the calcium homeostasis and eventually loss of cell viability **(Hanafi and Asmaa, 2015)**.

GSH is essential for cell survival and its depletion increases the cellular susceptibility to apoptosis **(Morales *et al.*, 1998)**. High intracellular GSH levels have been related to apoptosis resistance **(Cazanave *et al.*, 2007)**, and GSH depletion has been shown either to induce or potentiate apoptosis **(Tormos *et al.*, 2004)**.

Moreover, the inhibition of antioxidant enzymes activities and a reduction in glutathione level as a result of tumor growth were also reported **(Gupta *et al.*, 2004)**. This phenomenon could be attributed to the exhaustion of these antioxidants especially glutathione and glutathione-containing enzymes in the detoxification of free radicals and peroxides generated due to tumor inoculation. These free radicals conjugate with GSH and ultimately protect the cells and organs from oxidative stress.

Catalase is mainly catalyzes the dismutation of hydrogen peroxide (H_2O_2) into water and molecular oxygen and used by cells to defend against the toxic effects of hydrogen peroxide, which is generated by various reactions and/or environmental agents or by the action of superoxide dismutase while detoxifying superoxide anion **(Michiels *et al.*, 1994)**.

When CAT activity is reduced, the level of hydrogen peroxide increased in cancer tissue. This may correspond with the report, which showed that some human cancer lines produced a large amount of hydrogen peroxide **(Szatrowski and Nathan, 1991)**. Indeed, the level of glutathione and CAT have been shown to be significantly altered in malignant cells **(Oberley and Oberley, 1997)** and in primary cancer tissues **(Murawaki *et al.*, 2008)**, suggesting aberrant regulation of redox homeostasis and stress adaptation in cancer cells or could also be due to exhaustion of the glutathione and antioxidant enzymes because of increased peroxidation **(Manimaran and Rajneesh, 2009)**. Alternatively, it is possible that the antioxidant system is impaired as a consequence of an abnormality in the anti-oxidative metabolism due to the cancer processes.

From the previous discussed results, we postulated the antitumor action of fenugreek extract either alone or combined with selenium nanoparticles.

683

REFERENCES

Abasalipourkabir, R., Salehzadeh, A. and Abdullah, R. (2011): Cytotoxicity effect of solid lipid nanoparticles on human breast cancer cell lines. *Biotechnology*, 10: 528–533.

Ahmed, M. I., Fayed, S. T., Hossein, H. and Tash, F. M. (1999): Lipid peroxidation and antioxidant status in human cervical carcinoma. *Disease Markers*, 15: 283-291.

Ahmed, N., Gupta, S. and Mukhtar, H. (2000): "Green tea polyphenol epigallocatechin – 3 - gallate differentially modulates nuclear factor kappa B in cancer cells versus normal cells". Arch Biochem Biophys., 376: 338-346.

Artis, David, Spits and Hergen (2015): The biology of innate lymphoid cells. *Nature*, 517: 293–301.

Banchroft, J. D., Stevens, A. and Turner, D. R. (1996): Theory and practice of histological techniques, Fourth Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.

Bano, D., Tabassum, H., Ahmad, A., Mabood, A. and Zareen, I. (2016): The medicinal significance of the bioactive compounds of *trigonella foenum-graecum*: a review. *International Journal of Research in Ayurveda and Pharmacy*, 7: 84–96.

Beutler, E. and Duron, O. (1963): Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine*, 61: 882–888.

Birkedal-Hansen, H. and Taylor, R. E. (1982): Detergent activation latent collagenase and resolution of its molecules. *Biochemical and Biophysical Research Communications*, 107: 1173–1178.

Cazanave, S., Berson, A., Haouzi, D., Vadrot, N., Fau, D., Grodet, A., Letteron, P., Feldmann, G., El-Benna, J., Fromenty, B., Robin, M. A. and Pessayre, D. (2007): High hepatic glutathione stores alleviate fas-induced apoptosis in mice. *Journal of Hepatology*, 46: 858–868.

Diplock, A. T., Rice-Evans, A. C. and Burton, R. H. (1994): Is there a significant role for lipid peroxidation in the causation of malignancy and for antioxidants in cancer prevention? *Cancer Research*, 54: 19525–19526.

El-Merzabani, M. M., El-Aaser, A. A., Atia, M. A., El-Duweini, A. K. and. Ghazal, A. M. (1979): Screening system for Egyptian plants with potential anti-tumour activity. *Journal of plant Medica*, 36: 150.

Esteve, P. O., Chicoine, E., Robledo, O., Aoudjit, F., Descoteaux, A. and Potworowski, E. F. (2002): *Journal of Biological Chemistry*, 277: 35150– 35155.

Fontes, L. B., Dos Santos, D. D., de Carvalho, L. S., Mesquita, H. L., da Silva, R. L., Dias, A. T., Da Silva, F. A. and do Amaral, C. J. (2014): Immunomodulatory effects of licochalcone A on experimental autoimmune encephalomyelitis. *Journal of Pharmacy Pharmacology*, 66: 886–894.

Ghoneum, M., Badr El-Din, N. K., Noaman, E. and Tolentino, L. (2008): Saccharomyces cerevisiae, the Baker's Yeast, suppresses the growth of Ehrlich carcinoma-bearing mice. *Cancer Immunology Immunotherapy*, 57: 581–592.

Gray, P. W. and Goeddel, D. V. (1982)*: Structure of the human immune interferon gene. Nature, 298: 859–63.*

Gupta, M., Mazumder, U. K., Kumar, R. S. and Kumar, T. S. (2004): Antitumor activity and antioxidant role of Bauhinia racemosa against Ehrlich ascites carcinoma in Swiss albino mice. *Acta Pharmacologica Sinica*, 25: 1070–1076.

Halliwell, B. and Gutteridge, J. M. C. (1999): Free radicals in biology and medicine, 3rd ed. Oxford: Oxford University Press, New York. 617–783.

Hanafi, N. and Asmaa, A. (2015): Role of irradiated tumor cell lysate vaccine and low doses of gamma irradiation in tumor regression. *International Scientific Research Journal*, 1–7.

Hannan, J. M., Rokeya, B., Faruque, O., Nahar, N., Mosihuzzaman, M., Khan, A. A. and Ali, L. (2003): Effect of soluble dietary fibre fraction of *Trigonella foenum graecum* on glycemic, insulinemic, lipidemic and platelet aggregation status of Type 2 diabetic model rats. *Journal of Ethnopharmacol*, 88: 73–77.

Harnett, D. L. and Horrell, J. F. (1998): Data, Statistics and Decision Models with Excel. John Wiley & Sons, INC. Chapter 10 (Analysis of Variance). 450–455.

Ilma, R. and Evzen, K. (2010): Granzyme B-induced apoptosis in cancer cells and its regulation (Review). *International Journal of Oncology*, 37: 1361–1378.

Ikeda, H., Old, L. J. and Schreiber, R. D. (2002): The roles of IFN gamma in protection against tumor development and cancer immunoediting. *Cytokine Growth Factor Reviews*, 13: 95–109.

Jasim, N. A. (2014): Therapeutic uses of fenugreek (Trigonella foenum-graecum L.). *American Journal of Social Issues and Humanities*, 2276–6928.

Kang, D. H. (2002): Oxidative stress, DNA damage breast cancer. *American Association of Critical-Care Nurses clinical Issues*, 13: 540–549.

Kieliszek, M. and Blazejak, S. (2013): Selenium: Significance, and outlook for supplementation. *Nutrition*, 29: 713-718.

Kong, L., Yuan, Q., Zhu, H., Li, Y., Guo, Q., Wang, Q., Bi, X. and Gao, X. (2011): The suppression of prostate LNCaP cancer cells growth by selenium nanoparticles through Akt/MDM2/AR controlled apoptosis. *Biomaterials*, 32: 6515–6522.

Liu, M. J., Wang, Z., Ju, Y., Wong, R. N. and Wu, Q. Y. (2005): Diosgenin induces cell cycle arrest and appoptosis in human leukemia K562 cells with the disruption of Ca^{2+} homeostasis. *Cancer Chemotherapy and Pharmacology*, 55: 79–90.

Lord, S. J., Rajotte, R. V., Korbutt, G. S. and Bleackley, R. C. (2003): Granzyme B: a natural born killer. *Immunological Reviews*, 193: 31–38.

Manda, K. and Bhatia, A. L. (2003): Pre-administration of beta-carotene protects tissue glutathione and lipid peroxidation status following exposure to gamma radiation. *Radiation Environmental Biology*, 24: 369–372.

Manimaran, A. and Rajneesh, C. P. (2009): Activities of antioxidant enzyme and lipid peroxidation in ovarian cancer patients. *Academic Journal of Cancer Research*, 2: 68–72.

Mansour, S. Z. Anis, L. M. and El- Batal, A. I. (2010): Antitumor Effect of Selenium and Modified Pectin Nano Particles and Gamma Radiation on Ehrilch Solid Tumor in Female Mice. Journal of Radiation Research and Applied Sciences. *Journal of Radiation Research Applied Sciences*, 3: 655–676.

Matteo, C., Valeria, T., Cesare, B., Gianni, P., Lorenzo, P. S. and Ugo, D. G. (2006): Anticancer Drug Delivery with Nanoparticles. *IN VIVO*, 20: 697–702.

Medhat, M. A., Khaled, S. A., Mahmoud, M. S., Neama, M. E. and Nermeen, M. E. (2017): Antitumor and radiosensitizing synergistic effects of apigenin and cryptotanshinone against solid Ehrlich carcinoma in female mice. *Tumor Biology*, 1–13.

Melkrantz, W. and Schlegel, R. (1995): Apoptosis and the cell cycle. *Journal of Cellular Biochemistry*, 58: 160–174.

Michiels, C., Raes, M., Toussaint, O. and Remacle, J. (1994): Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. *Free Radical Biology and Medicine*, 17: 235–248.

Mohamed, Z. (2006): Biochemistralstudy of anti diabetic action of the egyption plants Fenugreek and alanites. *Molecular and cellular biochemistry*, 173–183.

Morales, A., Miranda, M., Sanchez-Reyes, A., Biete, A. and Fernandez-Checa, J. C. (1998): Oxidative damage of mitochondrial and nuclear DNA induced by ionizing radiation in human hepatoblastoma cells. *International Journal of Radiation Oncology Biology Physics*, 42: 191-203.

Murawaki, Y., Tsuchiya, H., Kanbe, T., Harada, K., Yashima, K. and Nozaka, K. (2008): Aberrant expression of telomeroproteins in the progression of colorectal cancer. *Cancer letters*, 259: 218–230.

Oberley, T. D. and Oberley, L. W. (1997): Antioxidant enzyme levels in cancer. *Histology and Histopathology*, 12: 525–535.

Ochekpe, N. A., Olorunfemi, P. O. and Ngwuluka, N. (2009): Nanotechnology and Drug Delivery Part 1: Background and Applications. *Tropical Journal of Pharmaceutical Research*, 8: 265–274.

Ohta, Y., Kongo, N., Nishimra, M., Matsura, T., Yamada, K., Kitagawa, A. and Kishikaw, T. (2004): Melatonin prevents disruption of hepatic reactive oxygen species metabolism in rats treated with carbon tetrachloride. *Journal of Pineal Research,* 36: 10–17.

Ramesh, H. P. and Srinivasan, K. (2004): "Isolation of galactomannan-rich endosperm of Fenugreek and study of its anti-diabetic influence in streptozotocin-induced diabetic rats." Trends in *Carbohydrates Chemistry*, 9: 99–103.

Riddle, D., Goldstein, N. B., Norris, D. A. and Shellman, Y. G. (2005): A simple technique for quantifying apoptosis in 96-well plates. *BMC Biotechnology*, 10: 5–12.

Salem, F. S., Badr, M. O. and Neamat-Allah, A. N. (2011) : Biochemical and pathological studies on the effects of levamisole and chlorambucilon Ehrlich ascites carcinoma bearing mice. *Veterinaria Italiana*, 47: 89–95.

Savita, B. and Amarnath, M. (2009): Dextran–doxorubicin/chitosan nanoparticles for solid tumor therapy.Wiley Interdisciplinary Reviews: *Nanomedicine and Nanobiotechnology*, 1: 415–425.

**Schoenborn, J. R. and Wilson, *C. B. (2007): Regulation of interferon-gamma during innate and adaptive immune responses. Advances of Immunology, 96: 41–101.*

Shafi, G., Munshi, A. and Hasan, T. N. (2009): Induction of apoptosis in HeLa cells by chloroform fraction of seed extracts of Nigella sativa. *Cancer Cell International*, 27: 9–29.

Shah, S., Gapor, A. and Sylvester, P. W. (2003): Role of caspase-8 activation in mediating vitamin E-induced apoptosis in murine mammary cancer cells. *Nutrition and Cancer*, 45: 236–46.

Shakibaie, M., Khorramizadeh, M., Faramarzi, M., Sabzevari, O. and Shahverdi, A. (2010): Biosynthesis and recovery of selenium nanoparticles and the effects on matrix metalloproteinase-2 expression. *Biotechnology and Applied Biochemistry*, 56: 7–15.

Shivangi, G., Nidhi, G. and Sreemoyee, C. (2016): Investigating therapeutic potential of trigonella foenum-graecum l. as cell defense mechanism against several human diseases. *Journal of Toxicology*, 3: 1–10.

Sinha A. K. (1972): Colorimetric assay of catalase. *Journal of Analytical andBiochemistry*, 47: 389–94.

Soda, K., Tanaka, H. and Esaki, N. (2010): Biochemistry of physiologically active selenium compounds. In: *Organic Selenium and Tellurium Compounds (1987).* John Wiley & Sons, Inc. 349-365.

Srivastava, N. and Mukhopadhyay, M. (2013): Biosynthesis and structural characterization of selenium nanoparticles mediated by Zooglea ramigera. *Powder Technology*, 244: 26–29.

Szatrowski, T. P. and Nathan, C. F. (1991): Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Research*, 51: 794–798.

Thakran, S., Salimuddin and Baquer, N. Z. (2003): Oral administration of orthovanadate and *Trigonella foenum-graecum* seed power restore the activities of mitochondrial enzymes in tissues of alloxan- induced diabetic rats. *Molecular and Cellular Biochemistry*, 247: 45–53.

Thippeswamy, G. and Salimath, B. P. (2006): Curcuma aromatica extract induces apoptosis and inhibits angiogenesis in Ehrlich Ascites Tumor cells in vivo. *my SCIENCE*, 1: 79–92.

Thomas, D. A., Du, C., Xu, M., Wang, X. and Ley, T. J. (2000): DFF45/ICAD can be directly processed by Granzyme B during the induction of apoptosis. *Immunity*, 12: 621–632.

Turkey, N., Pilkhwl, S., Khad, A. and Chopra, K. (2005): Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharmacology*, 5: 2.

Tsomos, C., Javier C. F., Garcia, M. J., Garrido, F., Jover, R., O'Connor, J. E., Iradi, A., Oltra, A., Oliva, M. R. and Saez, G. T. (2004): Role of glutathione in the induction of apoptosis and c-fos and c-jun mrnas by oxidative stress in tumor cells. *Cancer Letters*, 208: 103-113.

Umesh, C. S. Y. and Najma, Z. B. (2014): Pharmacological effects of Trigonella foenum-graecum L. in health and disease. *Pharmaceutical Biology*, 52: 243–254.

Wadhwani, S. A., Shedbalkar, U. U., Singh, R. and Chopade, B. A. (2016): Biogenic selenium nanoparticles: current status and future prospects. *Applied Microbiology and Biotechnology*, 100: 2555–2566.

Walker, N. P., Talanian, R. V. and Brady, K. D. (1994): Crystal structure of the cysteine protease interleukin-1 beta-converting enzyme: a (p20/p10)2 homodimer. *Cell*, 78: 343–352.

Wang, A. Z., Langer, R. and Farokhzad, O. C. (2012): Nanoparticle delivery of cancer drugs. *Annual Review of*

Medicine, 63: 185–198.

Wang, H., Zhang, J. and Yu, H. (2007): Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: Comparison with selenomethionine in mice. *Journal of Free Radical Biology & Medicine*, 42: 1524–1533.

Wang, X. (2001): The expanding role of mitochondria in apoptosis. *Genes and Development*, 15: 2922–2933.

Yan, J., Tezuka, Y., Kouda, K., Le Tran, Q., Miyahara, T. and Chen, Y. (2004): New diarylheptanoids from the rhizomes of Dioscorea spongiosa and their antiosteoporotic activity. *Planta Medica*, 70: 220–226.

Yoshioka, T. and Kawada, K. (1979): lipid peroxidation inmaternal, cord blood, and protective mechanism against activated oxygen toxicity in the blood. *American Journal of Obstetrics and Gynecology,* 135: 372–376.

Zafar, T., Hasnain, S. N. and Hasan, S. K. (2001): Evaluation of the oral hypoglycemic effect of *T. foenum- graecum* L. (Methi) in normal mice. Journal of Ethnopharmacology, 75: 191–195.