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

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ABSTRACT

Trigonella foenum-graceum extract either alone or combined with selenium nanoparticles exhibited antitumor effect. Ehrlich ascite carcinoma (EAC) cell line and four groups of female mice were used. Solid Ehrlich carcinoma (EC) was induced by inoculation of $2.5x10^6$ cells in left thighs of each animal. Mice were gavage orally by 2.5 µg/0.1 ml of *Trigonella foenum-graceum* extract either alone or combined with selenium nanoparticles daily during one month. Tumor size, serum tumor markers (TNF-α, IFN-γ, Granzyme-B and Caspase-3) were measured. Oxidative stress, antioxidant markers, Histopathological, apoptotic and necrotic examinations were determined in tumor tissues. Tumor size of experimental groups represents reduction. Caspase-3 as well as Granzyme-B activities were significantly elevated along with diminishing tumor size while, TNF-α and IFN-γ levels were decreased in serum. Meanwhile, oxidative stress marker (MDA) was significantly decreased in tumor tissue. The tumor GSH content and CAT activity were increased. Histopathological, apoptotic and necrotic examinations were context with previous conclusion. It could be concluded that *Trigonella foenum-graceum* extract either alone or combined with SeNPs exhibited antitumor effect which is reflected by a inhibition in tumor size, a decrease of serum TNF-α and IFN-γ, an increase in serum caspase-3 and Granzyme-B, reduction in tumor MDA and an increase in tumor GSH and CAT which cause regulate tumor regression.

INTRODUCTION

Fenugreek (*Trigonella foenum-graceum)* is a leguminous herb belonging to Fabaceae. It has pungent aromatic compounds in their seeds. It has therapeutic effects, including carminative, treat wounds, sore muscles, aphrodisiac as well as a lactation stimulant for women after childbirth **(Bano *et al.*, 2016)**. *Trigonella foenum-graceum* has antidiabetic, hypolipidemic, immunological, antibacterial, anthelmintic, anti-inflammatory, analgesic and antioxidant activity **(Yadav *et al.*, 2011)**. It also has gastro and hepatoprotective effects **(Pandian *et al.*, 2002)**.

Mature seeds of *Trigonella foenum-graceum* mainly contain amino acid, fatty acid, vitamins, saponins, flavonoids and a large quantity of folic acid (84mg/100g) **(Mohammed *et al.*, 2006)**. The chemical components of *Trigonella foenum-graceum* that has antitumor activity are flavonoids, catechins and saponins. Saponins reduce cell division in tumor cells and can activate apoptosis **(Shivangi *et al.*, 2016)**.

Selenium (Se) is an important element of health for humans and animals. Seleno-compounds act as chemopreventive and chemotherapeutic agents, which supported by epidemiological, preclinical as well as clinical studies **(El-Batal *et al.*, 2012a,b)**. Se has one of the narrowest ranges between dietary deficiency (<40mg/day) and toxic levels (>400mg/day) **(Saif-Elnasr *et al.*, 2018)**. Advantages of using nanoparticles are targets of drugs as well as enhanced safety profile **(Rasha *et al.*, 2016)**. Selenium nanoparticle (SeNPs) is a novel Se species with biological activities and low toxicity compared with other Se compounds like sodium selenite, selenomethinine, and methyl selenocysteine this makes SeNPs a good candidate to tumor treatment **(El-Batal *et al.*, 2012c)**.

AIM OF THE WORK

In the current study, There is sought to achieve the emerging nano-based approaches suitable to be used as imaging techniques for cancer treatment by *Trigonella foenum-graceum* extract either alone or combined with SeNPs.

MATERIALS AND METHODS

Animals

Outbred female Swiss albino mice (20-25g) taken from National Cancer Institute (NCI) that were utilized as experimental animals. Animals were housed in plastic cages and maintained under standard conditions of illumination, ventilation, temperature, humidity and a 12 light/dark cycle along the experimental period. They were provided with a pellet concentrated diet containing all the necessary nutritive elements throughout the experimental period. Mice were left to acclimatize for 1 week before starting the experimental period. The animals were maintained and used in accordance with the animal ethics and the guide for the care and use of laboratory animals (National Research Council, 1996).

Ehrlich Ascites Carcinoma Cell Line (EAC).

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

Preparation of *Trigonella foenum-graceum* extract

Trigonella foenum-graceum extract is produced by putting a flask contains 10 g of *Trigonella foenum-graceum* seeds with 20 ml D.W in autoclave at 120°C for 20 min. Then filtration to get its extract.

Preparation of Selenium nanoparticle:

Selenium dioxide 1mM solution was mixed with aqueous extract of *Trigonella foenum-graceum* powder 1:1 v/v. The previous mixture was stirred at room temperature then exposed to gamma ray at 40 kGy. This led to immediate formation of SeNPs visualized as a 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 was loaded with carbon-coated copper grids and solvent was left to evaporate by incubation at 37°C for 30min in an incubator. The size and morphology of SeNPs were estimated by TEM (JEOL electron microscope JEM-100 CX) operating at 80 KV accelerating voltages.

Dynamic light scattering measurement (DLS)

Average particle size as well as size distribution was 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°C for 2 min., five measurements were performed using 12runs of 10s each.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of 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 molecules responsible for reduction of selenium ions and to confirm FPP capped SeNPs.

- **In vitro study**

- Chemosensitivity of *Trigonella foenum-graceum* extract either alone or combined with SeNPs on Ehrlich ascite carcinoma cells (Cell viability):

The antitumor effect of *Trigonella foenum-graceum* extract and/or SeNPs was assessed by observation of changes with respect to viable as well as nonviable tumor cell count. Cytotoxicity effects of the nanoparticles on tumor cells were determined according to the method of **El- Merzabani *et al.* (1979)**. To detect the cytotoxicity of SeNPs, EACs were treated with SeNPs 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 airflow system. The percentages of non-viable cells were measured by counting viable as well as dead EACs. To differentiate between viable and dead EAC cells, trypan blue stain was used. Then the percentages of non-viable cells (NVC) were measured according to the following equation % NVC= C/T X 100, where (C) is the number of non-viable cells and (T) is the total number of viable cells.

Experimental Design

Mice were allowed 7 days for adaptation. 60 mice were then randomly distributed into 4 equal groups, 15 mice for each group. 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 left thigh.

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

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

Monitoring the tumor size

Tumor size was measured twice or thrice every week during experiment. Tumor size measured 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 corresponding standard error was measured in each group. One month after treatments, the experiment was terminated and all mice were sacrificed.

Sample preparation:

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

Preparation of serum:

Mice were sacrificed and blood was collected from heart puncher using disposable plastic syringes, drained in tube, and left in order to coagulation. The blood was centrifuged and 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 mice were dissected out, washed and divided into two parts, one part was kept in 10% formalin for histopathological examinations, apoptosis detection then the other part was prepared in ice-cold saline (0.9%) using a potters-Elvehjem Homogenizer to give a 10% homogenates which were used for determination of biochemical parameters.

Biochemical analysis:

The levels of tumor necrosis factor-alpha, Interferon-gamma, Granzyme-B and Caspase-3 were assayed in serum 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 and Kawada (1979)**, **Beutler and Duron (1963)** and **Sinha**

(1972) respectively.

Statistical Analysis

The obtained data were 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, 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 an 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 glass slides and stained by haematoxylin and eosin stain for histopathological examination by the light microscope **(Banchroft *et al.*, 1996)**. Another tissue section (2-4 µm thick) was 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 / propidium iodide 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 Selenium nanoparticles:

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

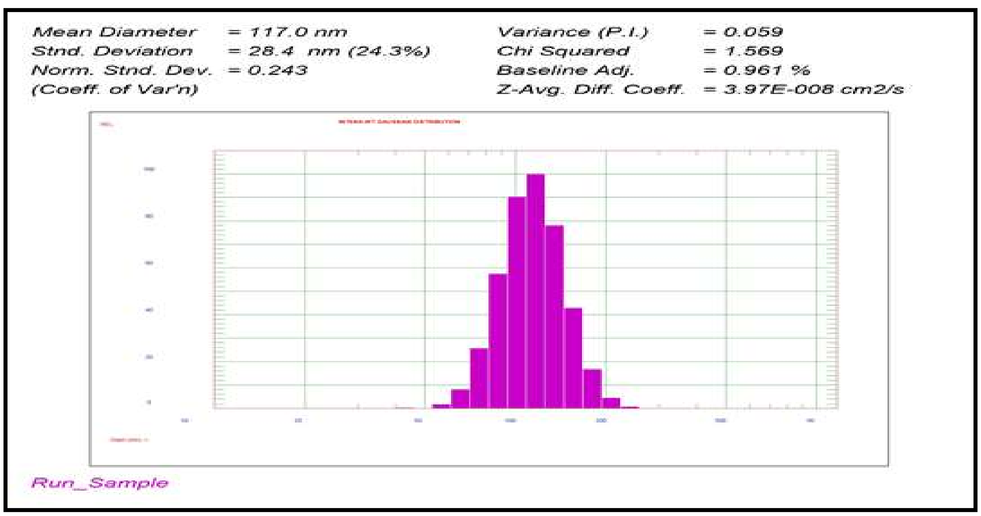

Fig. (1): Dynamic light scattering measurement (DLS)

Transmission Electron Microscope's result confirmed 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).

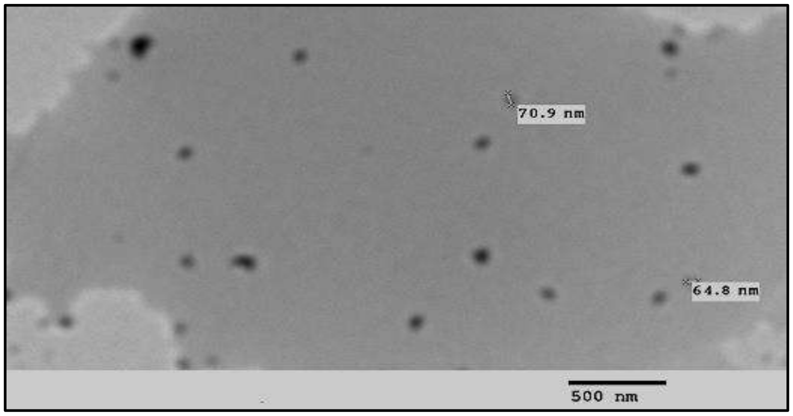

Fig. (2): Transmission Electron Microscopy (TEM)

SeNPs was 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.

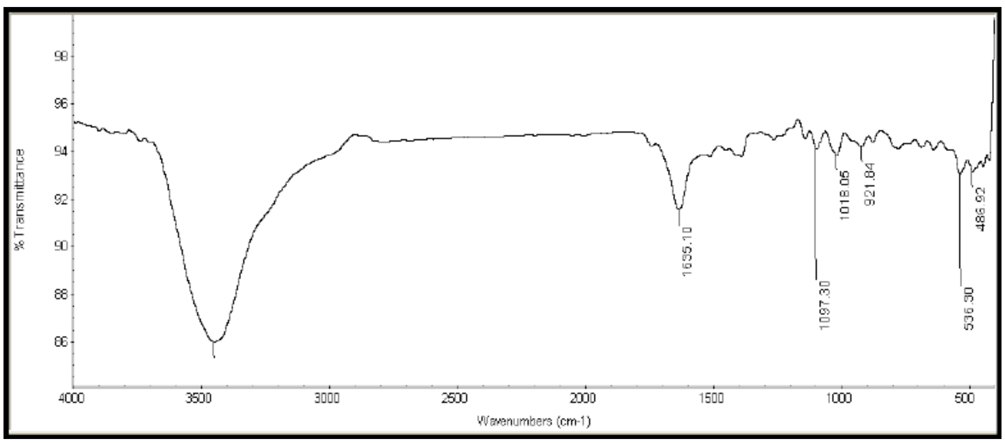

Fig. (3): Fourier transform infrared spectroscopy (FTIR)

- ***In vitro studies:***

- Chemosensitivity of *Trigonella foenum-graceum* extract either alone or combined with SeNPs on Ehrlich ascite carcinoma cells

The tumoricidal effect of varying concentrations of *Trigonella foenum-graceum* extract either alone or combined with SeNPs on Ehrlich cell viability is shown in Table (1). The low concentration (10 µg/ml) of *Trigonella foenum-graceum* extract reduces the tumor cell viability by 15%.

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

The low concentration (10 µg/ml) of *Trigonella foenum-graceum* extract combined with SeNPs reduces the tumor cell viability by 20%. The median lethal concentration of *Trigonella foenum-graceum* 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 *Trigonella foenum-graceum* extract combined with SeNPs led to the death of 80% of Ehrlich carcinoma cells.

Table (1): The effect of *Trigonella foenum-graceum* extract and *Trigonella foenum-graceum* extract combined with SeNPs on the viability of Ehrlich ascites carcinoma cells.

Concentration (µg/ml)	*Trigonella foenum-graceum* extract		*Trigonella foenum-graceum* extract combined with 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 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 with *Trigonella foenum-graceum* extract either alone or combined with SeNPs compared with EC group is illustrated in Fig (4). The mean size of 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 7th day after tumor inoculation after tumor inoculation. EC tumor size exceeds 400 mm^3 on the 10th day after tumor inoculation. The increase of EC tumor size proceeds by days reaching 2583.33±35.7 mm^3 on the 30th days after tumor inoculation.

The data obtained revealed the lesser tumor size through the observation period in groups of experimental animals daily treated with *Trigonella foenum-graceum* extract on the next day after tumor inoculation for one month. On the 7th, 10th and 30^{the} 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 *Trigonella foenum-graceum* extract combined with selenium nanoparticles on the next day after tumor inoculation for one month every day showed (86.17±5.31, 136.33±5.07 and 1694.33±13.94 mm^3 on 7th, 10th and 30th days respectively).

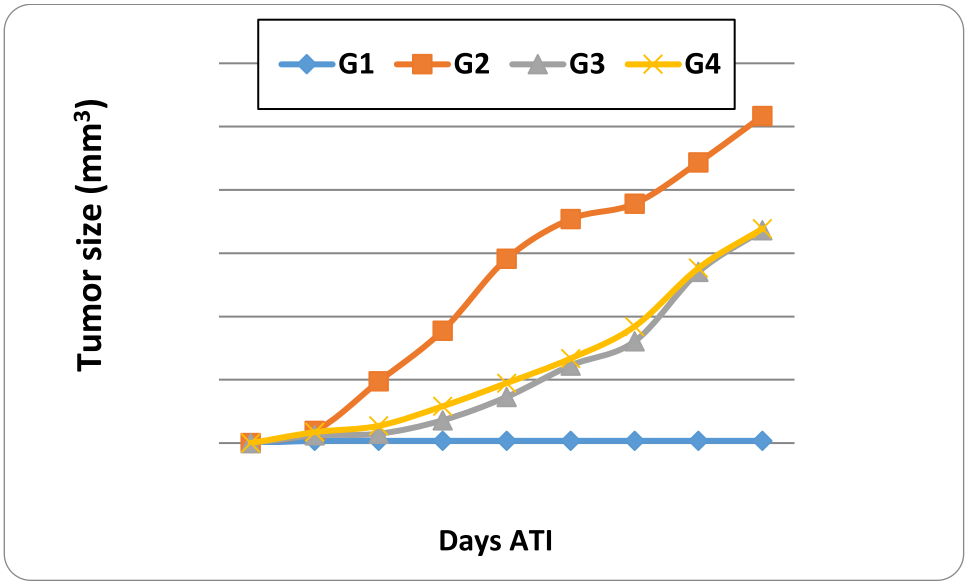

Fig. (4): Effect of *Trigonella foenum-graceum* extract either alone or combined with SeNPs on EC tumor size.

- **Tumor markers responses**

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

The data revealed that female mice inoculated with EC and treated with *Trigonella foenum-graceum* extract daily for one month recorded an increase in caspase-3 activity, a decrease in Granzyme-B activity, a decrease in TNF-α Level and a decrease in IFN-γ Level in compared to EC group. While, daily treatment of female mice inoculated with EC and treated with *Trigonella foenum-graceum* extract combined with SeNPs for one month predicts an increase in caspase-3 activity, an increase in Granzyme-B activity, a decrease in TNF-α level and a decrease in IFN-γ level compared to EC group.

Table (2): Effect of *Trigonella foenum-graceum* 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 represent an increase in tumor MDA and a decrease in tumor GSH and CAT in compared to N group.

The oral gavages of female mice bearing EC by *Trigonella foenum-graceum* extract daily for one month recorded decrease in tumor MDA and GSH activity and an increase in CAT in comparison to the EC bearing group. Treatment of female mice bearing EC with *Trigonella foenum-graceum* extract combined with SeNPs daily for one month predicts decrease in tumor MDA, an increase in tumor GSH and CAT in comparison to EC group.

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

Groups / Parameter	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 pleomorphic were seen in (Fig. 5 B&C). EC of mice gavage orally by *Trigonella foenum-graceum* 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 *Trigonella foenum-graceum* 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).

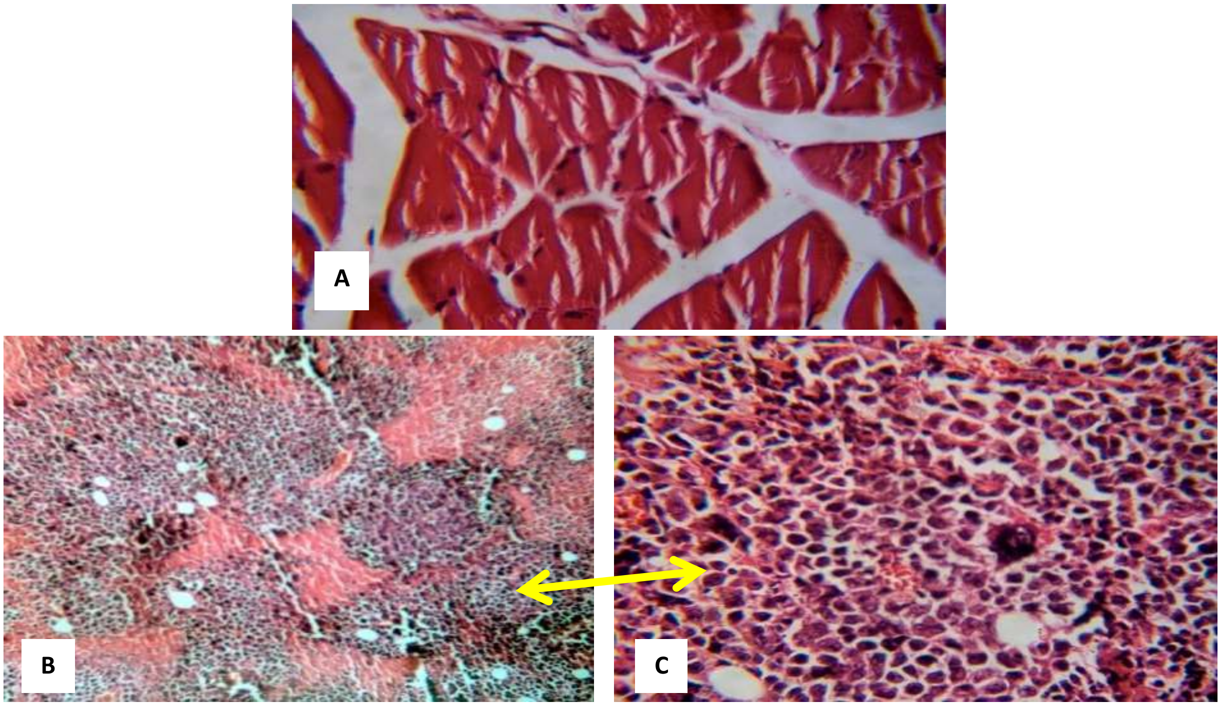

Fig. (5): Photomicrograph in sections of EC. **A:** Normal control muscle section in Albino mice represents normal muscular fiber. **B & C:** Control EC. Note: EC cells invaded muscular tissue; (↔) tumor cells encircled the muscles cells. (H and E stain, A&B X100- C X 400)

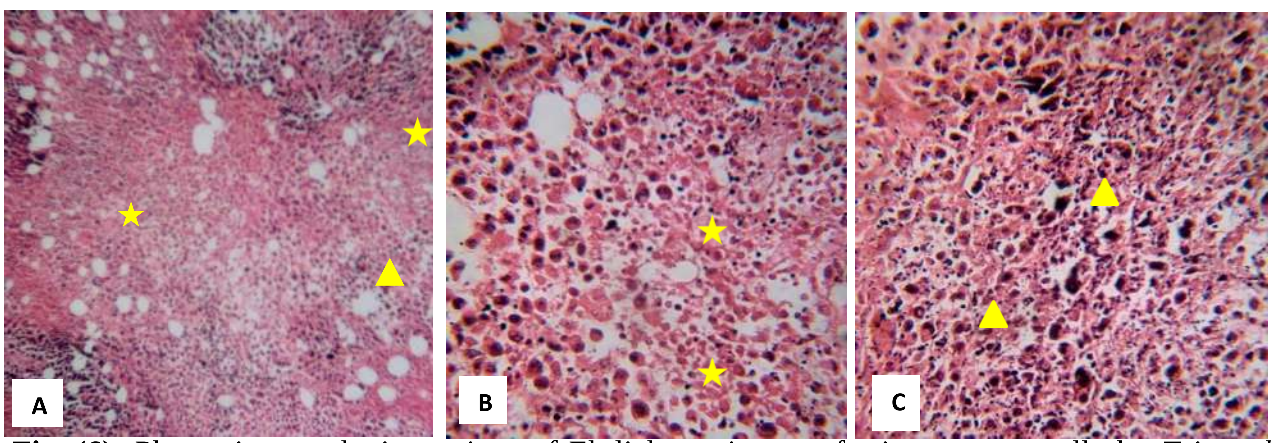

Fig. (6): Photomicrographs in sections of Ehrlich carcinoma of mice gavage orally by *Trigonella foenum-graceum* extract daily for one month. **A, B& C:** gavage after 1 day of tumor inoculation represents extensive areas of necrotic EC cells (star) and other areas contain of remnants, apoptotic and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

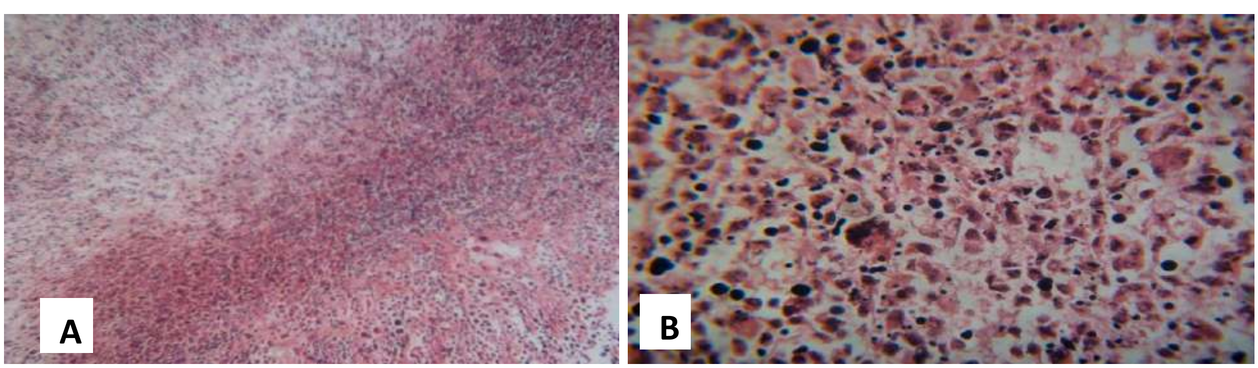

Fig. (7): Photomicrographs in sections of Ehrlich carcinoma of mice gavage orally by *Trigonella foenum-graceum* extract combined with SeNPs daily for one month. **A& B:** gavage after 1 day of tumor inoculation represents extensive areas contain of remnants, apoptotic and some pyknotic nuclei (▲). (H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

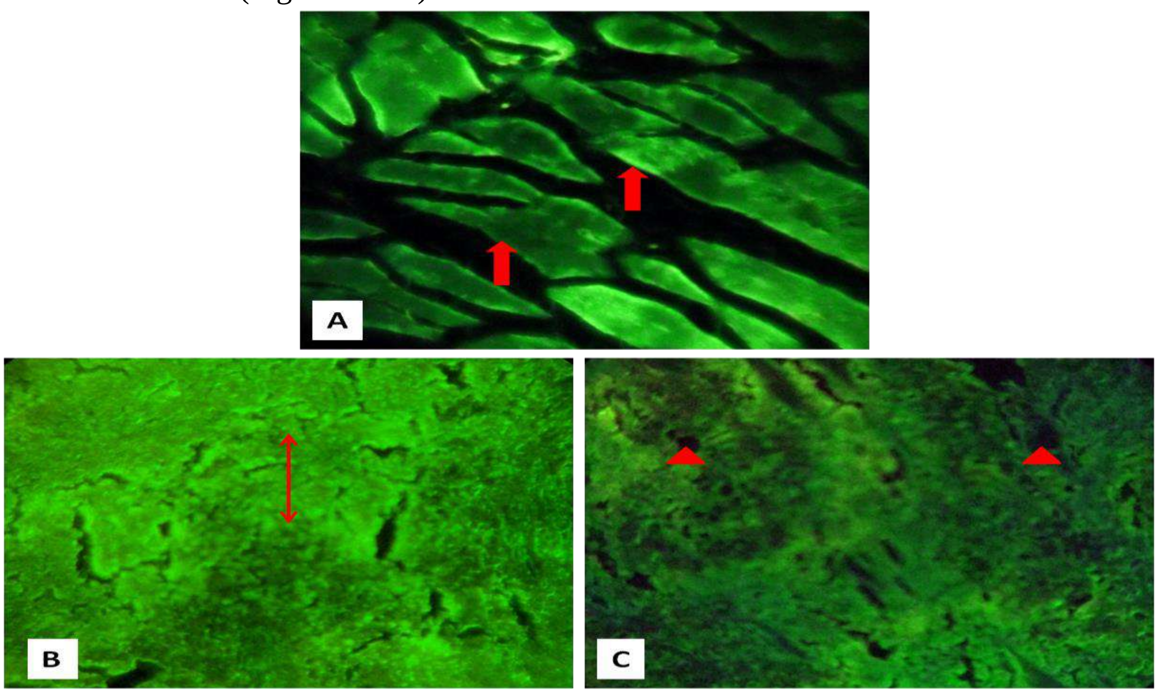

Fig. (8): Fluorescent imaging of sections in Ehrlich carcinoma stained by Acridine orange / propidium iodide stain. **A:** Normal muscle represents vital tissue regions stained in green (red blocked arrows). **B&C:** Control Ehrlich carcinoma represents vital green regions (↕) and some vacuolated areas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

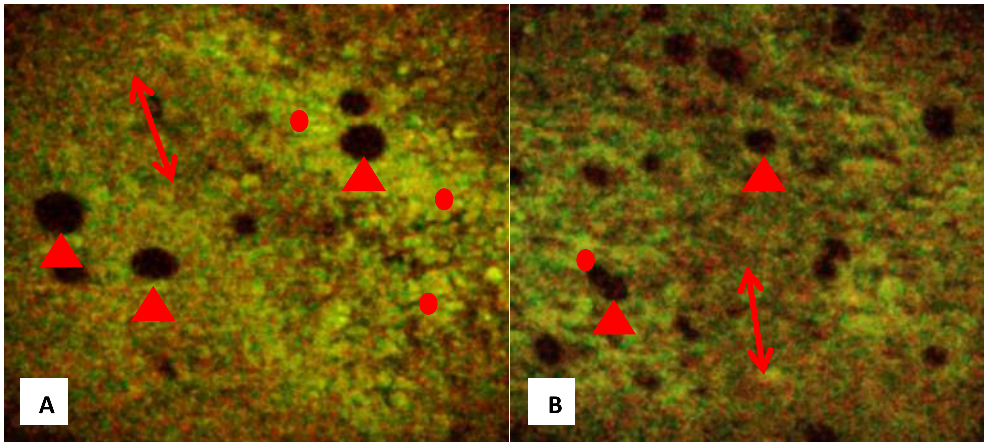

Fig. (9): Photomicrographs in sections of Ehrlich carcinoma Fluorescent imaging of sections in Ehrlich carcinoma stained by Acridine orange / propidium iodide stain of mice gavage orally by *Trigonella foenum-graceum* extract daily for one month. **A& B:** gavage after 1 day of tumor inoculation represents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

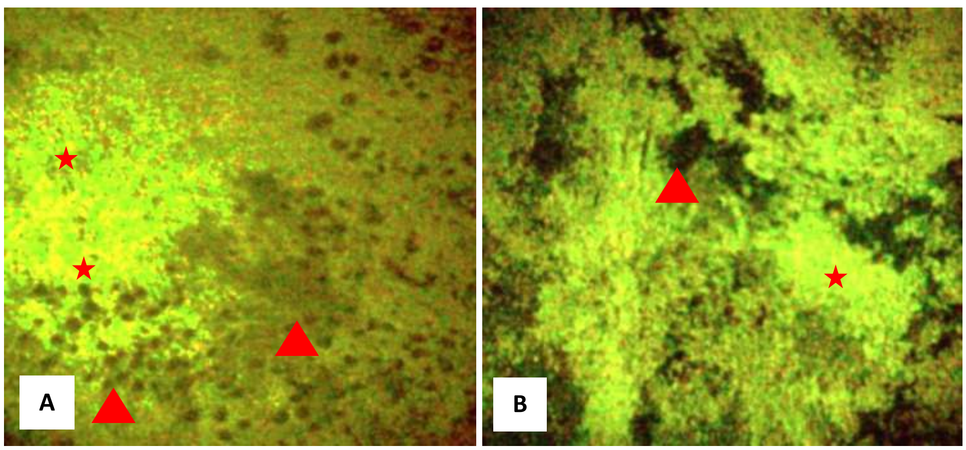

Fig. (10): Photomicrographs in sections of Ehrlich carcinoma Fluorescent imaging of sections in Ehrlich carcinoma stained by Acridine orange / propidium iodide stain of mice gavage orally by *Trigonella foenum-graceum* extract combined with SeNPs daily for one month. **A& B:** gavage after 1 day of tumor 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

Nanotechnology holds a promise for medication because materials at the nanometer dimension exhibit properties that differ from those of bulk material **(El-Ghazaly *et al.*, 2017)**. Chemotherapy-associated problems have been solved by using nanoparticles of drugs. Most important advantage of novel formulations is that they preferentially target tumor cells by the enhanced permeability and retention (EPR) phenomenon exhibited by solid tumors. In addition, nanoparticles as therapeutics carriers have other unique properties of the higher therapeutic effect, lower toxicity, ability to encapsulate and deliver poorly soluble drugs **(Wang *et al.*, 2012)**. Their reduced particle size entails high surface area and hence a strategy for faster drug release **(Matteo *et al.*, 2006)**.

Selenium is a trace element found in materials of the earth's crust **(El-Batal *et al.*, 2014)**. Selenium is an essential trace element for the animal, plant, and human tendency. Selenium level in men is about 82.0 mg and the dietary needed is about 56.0 mg by day **(Mosallam *et al.*, 2018)**. 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)**.

Many traditional herbal medicines and certain food constituents exhibit anti-inflammatory and antioxidant effects, suggesting potential as chemopreventive agents **(Jasim, 2014)**. An extract of *Trigonella foenum-graceum* seeds and some of their constituents have shown anticarcinogenic potency. Consumption of *Trigonella foenum-graceum* was accompanied by decreased polyamines (spermine, spermidine, and putrescine) content in tumor tissue **(Jasim, 2014)**. The effect of biologically active constituent of *Trigonella foenum-graceum* 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)**.

The cytotoxicity of either *Trigonella foenum-graceum* seeds extracts either alone or combined with selenium nanoparticles on Ehrlich carcinoma cell line was carried out. The present study demonstrated that *Trigonella foenum-graceum* extract could exert a high cytotoxicity against Ehrlich ascite carcinoma cell line. The median lethal concentration of *Trigonella foenum-graceum* extract was 70 μg/ml and the median lethal concentration of *Trigonella foenum-graceum* extract combined with SeNPs was 60 μg/ml.

Trigonella foenum-graceum extract has an inhibitory growth to breast, pancreatic and prostate cancer cell lines. *Trigonella foenum-graceum* extract selectively inhibits cell division in tumor cells and activate programmed cell death **(Shivangi *et al*., 2016)**. Meanwhile**,** the cytotoxicity effect of nanoparticles is due to their adherence to the cell membrane, particle internalization and degradation of products in cell culture medium or inside cells **(Abbasalipourkabir *et al.*, 2011)**.

The experimental data revealed that the positive control mice develop an 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 tumor tissue cells spread within muscular tissues with pleomorphic shapes, hyperchromatic nuclei and binucleation without necrosis or apoptosis. ROS production as a result of tumor growth, on other organs in the body can be explained as follows: ROS activate nuclear factor κB (NF-κB) as well as phosphorylation of its inhibitor (IκB). Thus, they enable NF-κB to translocation in the cell nucleus and binds to DNA and regulates transcription of various target genes (i.e. Inducible nitric oxide synthase, cyclooxygenase II, cytokines, etc.), which cause cell damage. Interestingly, cytokines activate NF-κB in tumor cells that protects tumor cells from TNF-α induced apoptosis. NF-κB regulates transcription of genes involved in cell proliferation, antiapoptosis and invasion. Thus, activation of NF-κB induces tumor growth, metastasis as well as reducing cytokines induced programed cell death **(Hanafi and Asmaa, 2015)**.

Regular as well as rapid increases in tumor volume were represented in EC tumor bearing mice, while groups were taken the treatments, a decreased in tumor volume was represented supporting beneficial anticarcinogenic effect of *Trigonella foenum-graceum*. On the other hand EC of mice daily gavage orally by *Trigonella foenum-graceum* 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 *Trigonella foenum-graceum* have potent proapoptotic effects on EC cells in vivo as well as inhibitory effect of *Trigonella foenum-graceum* on EC cell growth may be due to induction of programed cell death. The inhibation in tumor volume was due to treatment-induced reduction in cell cycle progression **(Meikrantz and Schlegel, 1995)**.

An in vivo study reflects effect of *Trigonella foenum-graceum* seed powder along with its proactive compound was able to inhibit formation preneoplastic lesion. Suppressed expression of proapoptotic protein bcl-2 and there was an increase in expression of caspase-3, an antiapoptosis protein. The chemopreventive effect of extract of *Trigonella foenum-graceum* seeds may be due to the rich chemical components (like, saponins, flavonoids, alkaloids, galactomannans) that are present in the seed working synergistically at various stages of angiogenesis **(Shivangi *et al*., 2016)**. *Trigonella foenum-graceum* inhibits 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 long-circulating nanoparticulate carriers. They can deliver chemotherapeutic to solid tumors by the enhanced permeability and retention effect and thus can enhance the therapeutic index of the drug or improve reducing undesirable side effects. Studies recorded that ultra-low size particles can be targeted to tumor tissue through combined effects of extravagation and long circulation in blood **(Savita and Amarnath, 2009)**.

Caspases are aspartate-directed cysteine proteases that play role in 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)**.

Treatment of experimental animals bearing EC with *Trigonella foenum-graceum* extract either alone or combined with SeNPs represents a significant increase in tumor caspase-3 levels when compared with their corresponding activity in EC bearing mice.

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 activation of caspase-8. The intrinsic pathway involves 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).**

Granzyme 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 genetic unstable cells **(Medhat et al., 2017)**.

The 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.

Treatment of experimental animals bearing EC with *Trigonella foenum-graceum* extract either alone or combined with SeNPs represents an increase in the granzyme B level when compared with their corresponding activity in EC bearing mice.

There is mounting evidence that Granzyme B can kill cells via a caspase-independent pathway **(Lord *et al.*, 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. Tumor cells or inflammatory cells in the tumor microenvironment produce it. The role of TNF-alpha in chronic inflammatory diseases and tumor-promoting effects are 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 the cytotoxic activity of activated macrophages **(Hanafi and Asmaa, 2015)**.

The experimental data reveal that female mice bearing EC represent a significant increase in serum TNF-α level of tumor bearing mice in comparison to a 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)**.

The data reveals that treatment of experimental animals bearing EC with *Trigonella foenum-graceum* extract either alone or combined with SeNPs represents a decrease in serum TNF-α level in compared to EC group and an increase in compared to normal control level. *Trigonella foenum-graceum* 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, *Trigonella foenum-graceum* 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)**.

EC bearing mice showed 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 *Trigonella foenum-graceum* extract either alone or combined with SeNPs represents a 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 *Trigonella foenum-graceum* 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)**.

The end product of lipid peroxidation, malondialdehyde, due to its high cytotoxicity and inhibitory action of protective enzymes, are suggested to act on tumor development **(Kang, 2002)**. Lipid peroxidation plays an important role in the control of cell division **(Diplock *et al*., 1994)**.

Treatment of experimental animals bearing EC with *Trigonella foenum-graceum* extract either alone or combined with SeNPs represents a decrease in the levels of lipid peroxidation, an increase in catalase activity and a significant change in reduced glutathione in tumor tissue in comparison 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 *Trigonella foenum-graceum* aqueous extract against tumor progression and the return of muscle tissue to its normalization. The *Trigonella foenum-graceum* extract inhibited the promoter of LPO by blocking the production of thiobarbituric acid reactive substances (TBARS) **(Umesh and Najma, 2014)**.

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

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)**.

The data revealed that experiments revealed that animals bearing EC represents a decrease in tumor GSH content in comparison 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 Asmaa, 2015)**. **Tirkey *et al*. (2005)** indicated that oxidative stress causes depletion of intracellular GSH, a reducing agent with its sulfhydryl group leading to serious consequences. The decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein caused by ROS generation that 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)**.

The data revealed that treatment of experimental animals bearing EC with *Trigonella foenum-graceum* extract either alone or combined with SeNPs represents un-significant change in tumor GSH content in comparison to EC group. 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 catalyzed dismutation of hydrogen peroxide (H_2O_2) into water and molecular oxygen and used by cells to defend against toxic effects of hydrogen peroxide, which is generated by various reactions and/or environmental agents or by action of superoxide dismutase while detoxifying superoxide anion **(Michiels *et al.*, 1994)**.

The data revealed that experimental animals bearing EC represent a decrease in tumor CAT content in comparison 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)**.

CONCLUSION

It is clear that *Trigonella foenum-graceum* extract either alone or combined with selenium nanoparticles exhibited antitumor activity reflected by reduction in tumor size, Inhibition of tumor cytokine profile (TNF-α, INF-γ), increased tumor apoptotic profile (caspase-3, Granzyme-B), decreased lipid peroxidation and increased antioxidant markers (GSH, CAT) in tumor tissue, Histopathological, apoptotic and necrotic examinations of tumor tissue.

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