Possibility of Selenium Nanoparticles Manufactured by Glycyrrhiza glabra Extract and γ- irradiation to Suppress the Growth of Murine Tumor

Neamat Hanafi Ahmed[1], Ahmed I. El-Batal[2], Lamiaa Ali Barakat[3] and Salma M. Khirallah[3]

[1]Radiation biology Department, National Canter for Radiation, Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt.

[2]Drug Radiation Research Department, National Canter for Radiation, Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt.

[3]Biochemistry Department, Faculty of Science, Port Said University, Port Said, Egypt.

Corresponding author: neamathanafi@ymail.com +201001731786

Abstract

Glycyrrhiza glabra (Gg) is used in traditional medicine. The present study examine the possibility of Gg root extract and selenium nanoparticles (SeNPs) fabricate by Gg extract and γ- irradiation to suppress the growth of murine tumors. Ehrlich ascite carcinoma cells and 4 groups of Swiss albino mice were used. Solid Ehrlich carcinoma (EC) was induced by intramuscular injection of $2.5x10^6$ cells. Mice were gavages by Gg extract or SeNPs. Tumor size, serum TNF-α, IFN-γ, Granzyme-B and Caspase-3 were evaluated. Cat, GSH, MDA, histopathological, apoptotic and necrotic examinations were studied in tumor tissues. Either Gg extract or SeNPs significantly inhibit tumor size, TNF-α, IFN-γ and MDA. On the other hand, elevation in Caspase-3 activity and Granzyme-B were predicted. Meanwhile, the histopathological, apoptotic and necrotic examinations were context with the previous results.

From previous we conclude that Gg extract or SeNPs have the possibility to suppress the growth of Ehrlich carcinoma.

Key words: *Glycyrrhiza glabra*, selenium nanoparticles, Ehrlich carcinoma

Introduction

Glycyrrhiza glabra (Gg) the roots of *Glycyrrhiza glabra* (Leguminosae). Its slices are widely used in traditional Chinese medicine. Many modern studies have reported that Gg possesses various pharmacological activities, such as antitumor, antiviral, anti-inflammatory, and immunity-stimulating activities[1]. It is containing many natural active components, including more than 20 possess the antitumor activity[1] among ascites tumor liver cancer, gastric cancer, uterus tumor, melanoma, leukemia, bladder cancer, lung cancer, oral cancer, and a variety of solid tumors[1].

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[2, 3].

The synthesis and application of selenium nanoparticles (SeNPs) attracted attention due to several advantages including chemical stability, biocompatibility and low toxicity[2]. 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[3]. Huge family of selenium biogenic compounds consists of simple organic and methylated species, selenoamino acids, selenoproteins, selenoenzymes, selenoaminocarboxylic 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[4].

In the current study, we sought the possibility of Selenium nanoparticles (SeNPs) fabricate by *Glycyrrhiza glabra* extract and γ- irradiation to suppress the growth of murine tumor

Materials and methods

Animals

Female Swiss albino mice obtained from National Cancer Institute (NCI) (20-25g) were used as experimental animals. All the experimental procedures were carried out according to the principles and guidelines of the Ethics Committee of the National Research Centre conformed to "Guide for the care and use of Laboratory Animals" for the use and welfare of experimental animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

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 after intraperitoneal (i. p) inoculation.

Preparation of Selenium nanoparticle :

Selenium dioxide 1mM solution was mixed with aqueous extract of *Glycyrrhiza glabra* (Gg) 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 Dynamic light scattering measurement **(DLS),** Transmission electron microscopy **(TEM)** and Fourier transform infrared spectroscopy

(FTIR)

➢ **In vitro study**

according to the method of El- Merzabani et al.[5] the cytotoxic effect of Gg extract or SeNPs nanoparticles fabricate by Gg extract and γ- irradiation on tumor cells were determined. EACs were treated with Gg extract or SeNPs 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 tumor cells in the left thigh.

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

G4: EC bearing SeNPs group. Mice were injected intramuscularly with 0.2ml of 2.5×10^{6} Ehrlich carcinoma cells in the left thigh then after one day of tumor inoculation 2.5 µg/0.1ml Gg extract combined with SeNPs fabricate by Gg extract and γ- irradiation orally taken daily

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) = 0.52 (length × $width^2$) Where length is the greatest longitudinal diameter and width is the greatest transverse diameter[6].

One month after tumor inoculation, experiment was terminated and all animals were sacrificed. After one month from EC cells challenge, mice were anaesthetized using diethyl ether and sacrificed. Blood and tumor tissue from animals of each group were collected and used for the proposed studies.

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 alpha, IFN-γ, Granzyme-B and Caspase-3 were measured in serum of each group by the standard sandwich enzyme-linked immune-sorbent (ELISA) assay technique using ELISA kit (K0331186, KOMABIOTECH, Seoul, Korea) following the manufacturer's instructions

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.

In Ehrlich carcinoma tumor tissue lipid peroxidation[7], Reduced glutathione[8] and Catalase[9] were measured calorimetrically. EC tissue sections were collected on the glass slides and stained by haematoxylin and eosin stain for histopathological examination by the light microscope[10]. Another tissue sections (2-4 µm thick) were stained by using a mixture of 100 µg/ml acridine orange and 100 µg/ml ethidium bromide prepared in PBS, for apoptosis and

necrosis staining[11] and monitored under a fluorescence microscope.

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 12.0 software was used for this analysis[12].

Results

Characterization of nanoparticles:

The distribution of the SeNPs and its size was defined by DLS technique and was determined as 117 nm as noted in **fig. 1**

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).

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 Se NPs as in **fig. 3.**

Cytotoxicity of *Gg* extract and SeNPs fabricate by Gg extract and γ- irradiation on Ehrlich ascite carcinoma cells

In vitro study the cytotoxic effect of different concentrations of Gg extract or SeNPs on Ehrlich cells viability is shown in Table [1]. The low concentration (10 µg/ml) of Gg extract decreases the tumor cells viability by 20%. The median lethal concentration of Gg extract was 40 µg/ml

for Ehrlich carcinoma cells. At a concentration of 20 µg/ml Gg extract led to the death of 30% and at a concentration of 90 µg/ml led to the death of 100% of Ehrlich carcinoma cells.

10 µg/ml of SeNPs fabricate by Gg extract and γ- irradiation decreases the tumor cells viability by 20%. The median lethal concentration of SeNPs fabricate by Gg extract and γ- irradiation was 40 µg/ml and 90 µg / ml SeNPs fabricate by Gg extract and γ- irradiation led to the death of 100% of Ehrlich carcinoma cells.

Monitoring of Ehrlich carcinoma tumor size

The tumor size of mice treated by Gg extract or SeNPs fabricate by Gg extract and γ- irradiation comparing with EC group is illustrated in Fig. (4). It is clear that 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. 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. In groups of experimental animals daily treated with Gg extract at the next day after tumor inoculation for one month. The tumor sizes were 34.17±2.36, 63.17±2.81 and 1448±22.63 mm^3 at the 7^{th}, 10^{th} and 30^{th} days after tumor inoculation respectively. The tumor size of mice daily treated with SeNPs fabricate by Gg extract and γ- irradiation at the next day after tumor inoculation for one month showed 85.33±1.84, 166.67±8.9 and 964±14.5 mm^3 on 7^{th}, 10^{th} and 30^{th} days respectively.

Cytokines and apoptotic responses

In table (2) data revealed that female mice inoculated with EC and treated with either Gg extract or SeNPs daily for one month recorded a significant increase in activities of Caspase - 3 and a significant decrease in Granzyme-B activity and in levels of TNF-α and IFN-γ in

compared to EC group levels. Meanwhile, treatment with SeNPs recorded less effect.

Oxidative stress and antioxidant markers in tumor tissues

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

The oral gavages of female mice bearing EC by Gg extract daily for one month recorded decrease in tumor MDA, GSH levels and CAT activity in compared to EC bearing group.

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 (Fig. 5 B&C). Ehrlich carcinoma of mice gavage orally by Gg extract daily for one month represents extensive areas of necrotic EC cells and other areas contain of remnants, apoptotic and some pyknotic nuclei when mice bearing EC treated with Gg extract after 24hrs of tumor inoculation for one month (Fig. 6 A,B& C). In the same direction sections of Ehrlich carcinoma of mice gavage orally with SeNPs daily for one month represents extensive areas contain of remnants, apoptotic and some pyknotic nuclei and large arias contain dead tumor cells when experimental animals begin to gavage the previous treatment after 1 day of tumor inoculation (Fig.6 D, E&F).

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. 7 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. 7 B&C).

Regarding the effect of orally gavage Gg extract on mice bearing EC daily for one month after 1 day of tumor inoculation represents extensive areas of necrotic EC cells and some vacuolated areas (Fig. 8 A&B).

Meanwhile mice gavage orally by Gg extract combined with SeNPs daily for one month recorded extensive areas of necrotic EC cells and other areas contain remnants of apoptotic nuclei (Fig. 8 C).

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[13]. Some of these chemotherapeutic-associated problems have been solved by the use of nanoparticle formulations of these drugs. Nanoparticles as therapeutics carriers have other unique properties of higher therapeutic efficacy, lower toxicity and the ability to encapsulate and deliver poorly soluble drugs[13].

Selenium (Se) is an essential trace element required by many organisms. As the selenium nanoparticles (SeNPs) possess antimicrobial and anticancer properties, they can be used as nanomedicines[14]. On the other hand, many anticancer drugs exert adverse side effects. Thus, identification of novel anticancer compounds from natural products was proposed. Also, Glycyrrhiza glabra (Gg) was reported to induce apoptosis of various cancer cell types.

In the present study, the cytotoxicity of Gg and SeNPs on Ehrlich carcinoma cell line. The cytotoxicity effect of Gg extract is act by inducing apoptosis by the expression of factor associated suicide ligand (FasL) and activated caspases 8 and 3 and PARP[15]. Meanwile, the

cytotoxicity effect of nano particles is due to their adherence to the cell membrane, particle internalization and degradation of products in the cell culture medium or inside the cells[16].

The experimental data revealed that the positive control mice develop Ehrlich tumor bulb exceeded 1cm^3 (500 mm^3)14 days ATI. ROS production cause activation in nuclear factor κB 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[17].

The obtained data revealed that there is significant decrease in mean of tumor size of the two groups (Gg extract group and SeNPs group) with the presence of areas contain of remnants, apoptotic and some pyknotic nuclei were also predicted in tumor tissue sections.

Gg extract inhibit cancer cell growth through the induction of apoptosis, cell cycle arrest and autophagy[18].

The mechanism of SeNPs 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[19].

The present 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.

Caspases play a key role in the initiation and execution of apoptosis, necrosis and inflammation once activated, they cleave cellular substrates, leading to morphological hallmarks of apoptosis[17].

treatment with Gg extract or SeNPs predicts a significant increase in caspase-3 levels

indicating increase in apoptosis and represents great areas contain remnants, apoptotic and some pyknotic nuclei. Activity of caspase-3 was significantly higher in carcinomas tissue due to the inactivation of P53[17]. Caspase activation leads to apoptosis through a tumor necrosis factor (TNF) receptor at the cell surface[17].

Treatment of female mice bearing EC with Ga glabra extract or SeNPs predicts a significant increase in caspase-3 levels compared to EC group through the blockade of NF-κB activation[15]. In the same direction, nano-Se inhibited cancer cell growth partially by caspase-mediated apoptosis[20].

Granzymes is a family of serine proteases is contained within the cytoplasmic granules of cytotoxic lymphocytes (CLs), and the pore-forming protein. 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[21]. This process is inhibited in cancer, which leads to the accumulation of various genetically unstable cells[22].

Article results demonstrated apoptosis suppression in the level of apoptotic molecules (caspase-3 and granzyme B) in solid EC tumors. Apoptosis is a programmed cell death that maintains the stability of the internal environment through removing genetic mutations and unstable cells.

Treatment with selenium nanoparticles represents significant increase in granzyme B level and caspase-3 activities has a similar preference. Granzyme B can kill cells via a caspase-independent pathway[23]. 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[24].

TNF-alpha is a cytokine produced by the innate immune cells and implicated in the promotion or inhibition of tumor development by suppressing T cell responses as well as cytotoxic

activity of activated macrophages[17].

In the present study a significant increase in serum TNF-α level of tumor bearing 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[17].

Gg extract either alone or combined with SeNPs predicts a significant decrease in serum TNF-α level context with the findings of Mansour et al.[25]. On the other hand, Gg exerts its antitumor activity by attenuating the level of TNF-α, and inducing cancer cell apoptosis through the caspase- and mitochondria-dependent pathways[26].

Interferon gamma (IFN-γ) is a dimerized soluble cytokine[27] 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[28].

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[29].

Treatment with Gg extract or SeNPs represents significant decrease in IFN-γ level Through its affects the body's nonspecific and specific immune functions and activates immune cells[30] and immunoregulatory activity[31].

Oxidative stress is occurred due to excessive production of ROS overwhelms the antioxidant defense system or when there is a significant decrease or lack of antioxidant defense[32].

The end product of lipid peroxidation act on tumor development[32] might be responsible for the antioxidant depletion and also the increased concentration of lipid peroxidation products[17].

The significant decrease in tumor GSH content in our data may be attributed to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by Ehrlich solid cells[17]. Also, a significant decrease in tumor CAT content in compared to EC group may be that some cancer lines produced a large amount of hydrogen peroxide[33]. Indeed,

the levels of glutathione, CAT and GSH-Px, have been shown to be significantly altered in malignant cells[34].

Treatment of experimental animals bearing EC with Gg extract or SeNPs represents a decrease in levels of lipid peroxidation and un-significant change in catalase and reduced glutathione in tumor tissue 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[18].

Catalase is mainly catalyzing 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[35].

When CAT activity is reduced, the level of hydrogen peroxide increased in cancer tissue[33]. Indeed, the level of glutathione and CAT have been shown to be significantly altered in malignant cells[34] and in primary cancer tissues[35]. 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.

Conclusion:

From the previous discussed results, we postulated the antitumor action of Gg extract or SeNPs. Either of them led to the death of 100% of Ehrlich carcinoma cells at consentration 90 µg / ml. Significantly inhibit nearly to half the tumor size after 30 days of tumor inoculation. Also, significantly inhibit TNF-α, IFN-γ and MDA. On the other hand, elevation in Caspase-3 activity and Granzyme-B were predicted. Meanwhile, the histopathological, apoptotic and necrotic examinations were context with the previous results.

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Table (1): The effect of Gg extract or SeNPs on the viability of Ehrlich ascites carcinoma cells.

Concentration (µg/ml)	Gg 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	80	20	80	20
20	70	30	65	35
30	60	40	55	45
40	50	50	50	50
50	40	60	40	60
60	30	70	25	75
70	20	80	20	80
80	10	90	10	90
90	0	100	0	100

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

Groups / Parameters	G1	G2	G3	G4
TNF-α (pg/ml)	30.89±0.88 b	113.47±4.02 a	50.71±0.39 ab	74.84±1.19 ab
IFN-γ (pg/ml)	17.47±0.48 b	85.96±2.35 a	56.79±2.11 ab	48.17±1.86 ab
Caspase-3 (µmol pNA/min/ml)	2.2±0.03	2.83±0.07	12.54±0.63 ab	8.37±0.04 ab
Granzyme-B (pg/ml)	14.1±0.62 b	78.63±2.16 a	35.08±0.9 ab	28.54±2.01 ab

All data are the means of 10 records ±SE. a: significant against N b: significant against EC $P \leq 0.05$ G1: Normal control group. G2: Ehrlich carcinoma (EC) bearing group. G3: EC bearing Gg extract group. G4: EC bearing SeNPs group.

Table (3): Effect of Gg extract or SeNPs on MDA, CAT and GSH levels of mice bearing EC.

Groups / Parameters	G1	G2	G3	G4
MDA (μM/gm tissue)	64.67±2.33 b	117.83±6.29 a	103.67±2.96 ab	98.33±2.06 ab
Catalase (μM Catalase/gm tissue)	0.2±0.01 b	1.5±0.1 a	1.2±0.1 a	1±0.06 ab
GSH (mg GSH/1 gm tissue)	2.33±0.09 b	1.6±0.14 a	0.93±0.06 ab	1.5±0.14 a

All legends as in table (2)

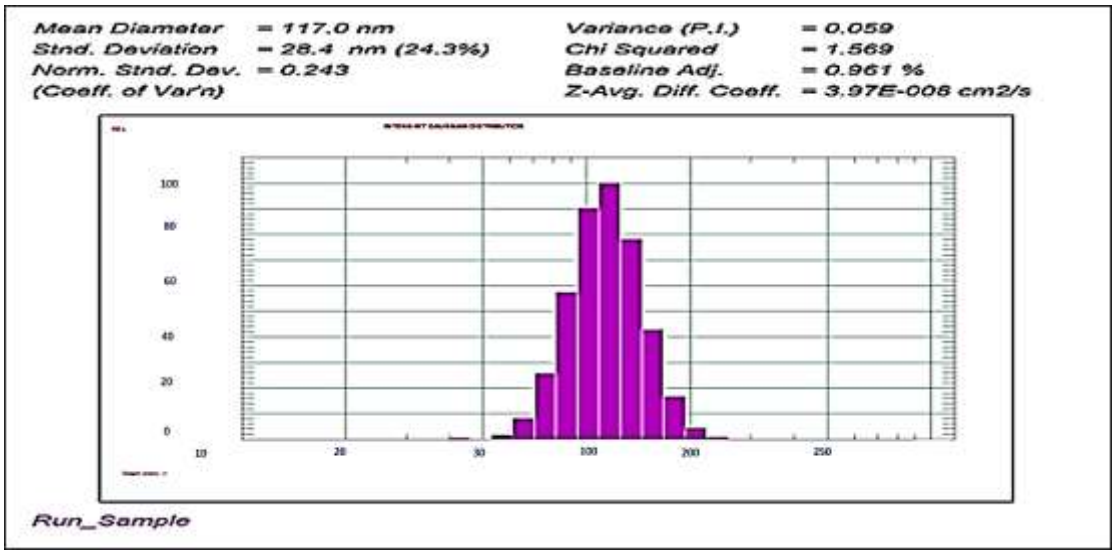

Fig. (1): Dynamic light scattering measurement (DLS)

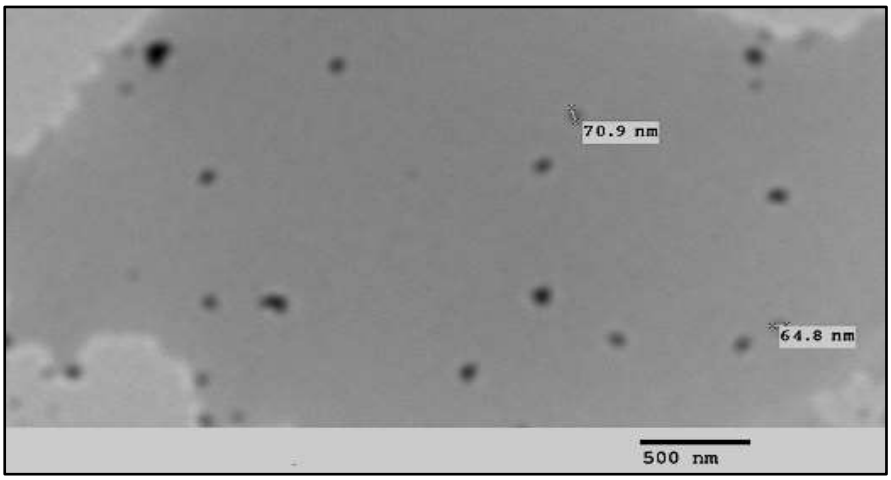

Fig. (2): Transmission Electron Microscopy (TEM)

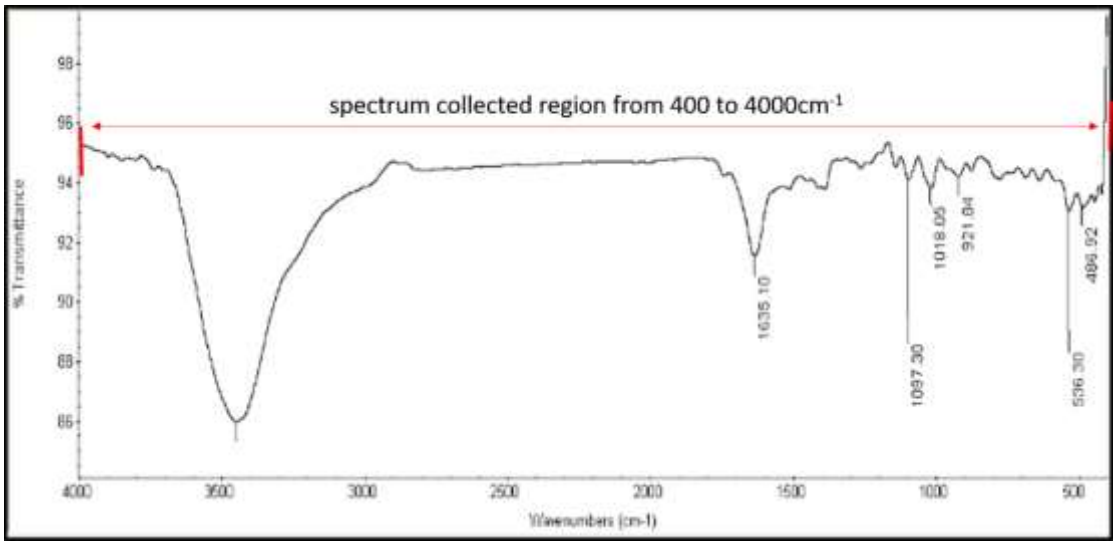

Fig. (3): Fourier transform infrared spectroscopy (FTIR)

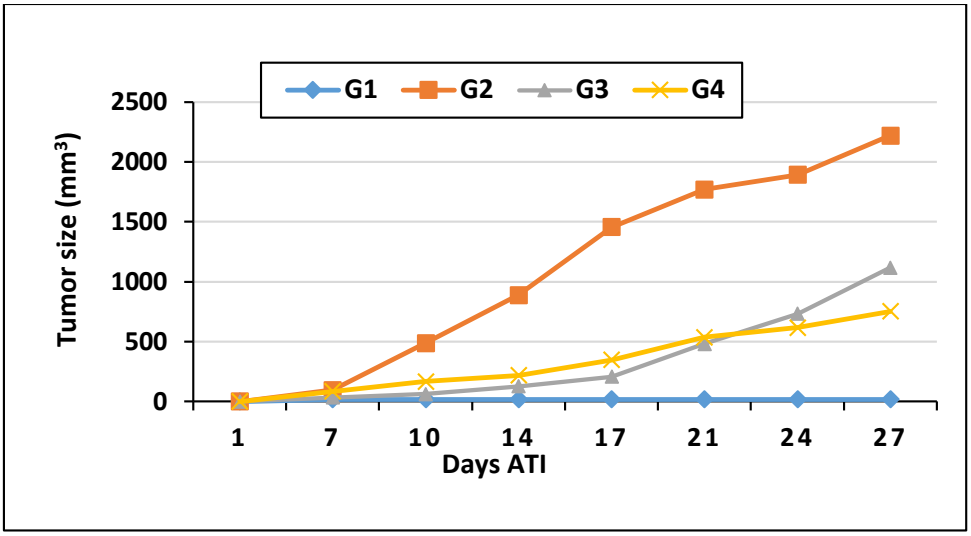

Fig. (4): Effect of Gg extract or SeNPs on EC tumor size.

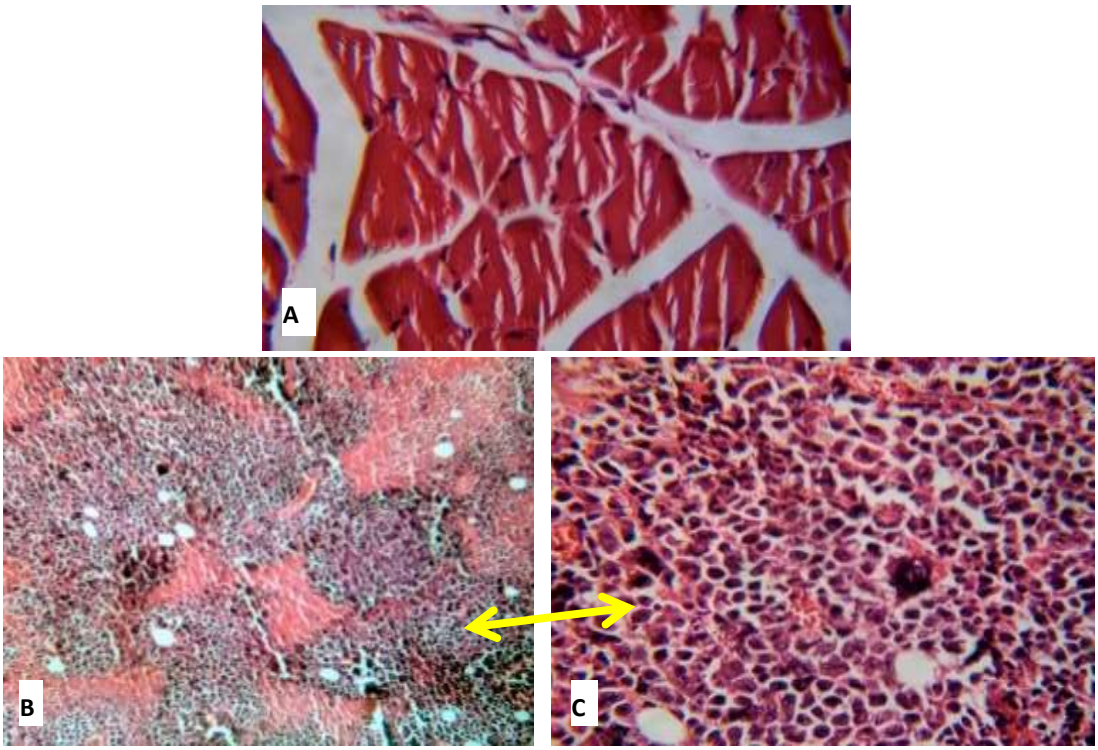

Fig. (5): Photomicrographs in sections of EC. **A:** Normal control muscle section in Albino mice represents normal muscular fiber. **B & C:** Control EC. Note: EC cells invaded muscular tissue; (↔) tumor cells encircled the muscles cells. (H and E stain, A&B X100- C X 400)

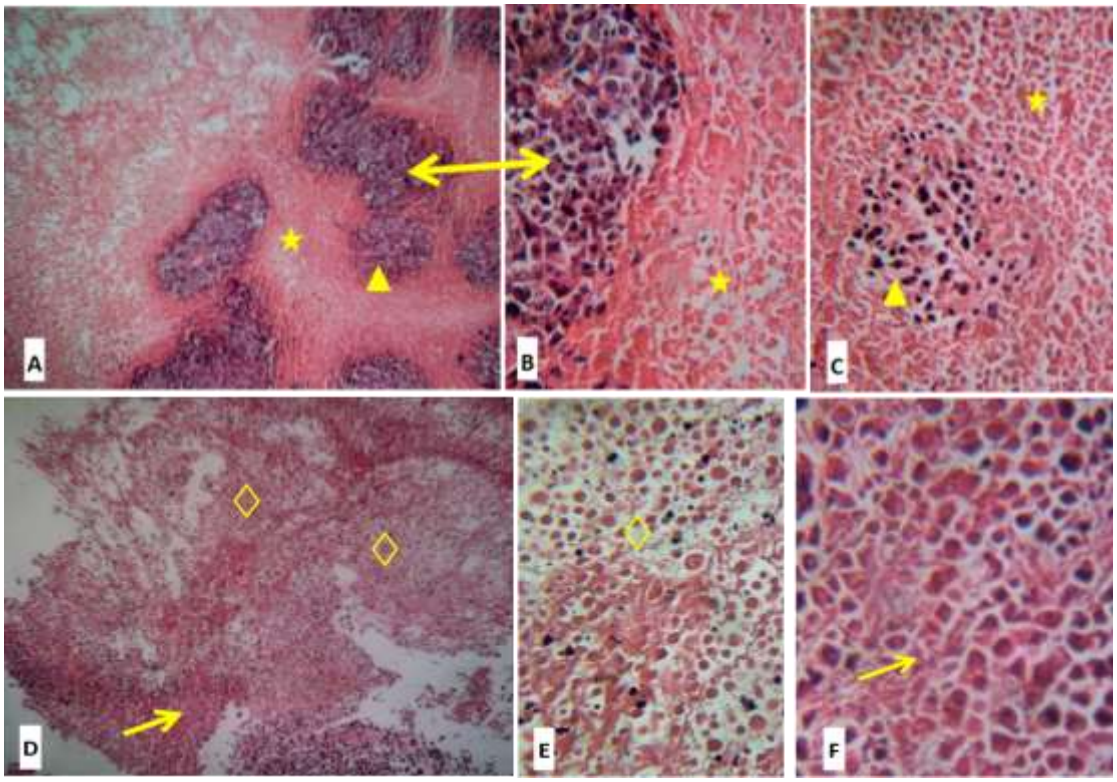

Fig. (6): Photomicrographs in sections of Ehrlich carcinoma in mice. **A, B& C:** gavage orally by Gg extract represents extensive areas of necrotic EC cells (star) and other areas contain of remnants, apoptotic and some pyknotic nuclei (▲). **D, E&F:** gavage orally by SeNPs represents extensive areas contain of remnants, apoptotic and some pyknotic nuclei (→) and large arias contain dead tumor cells. (H and E stain, A &D X100 – B, C, D &F X 400)

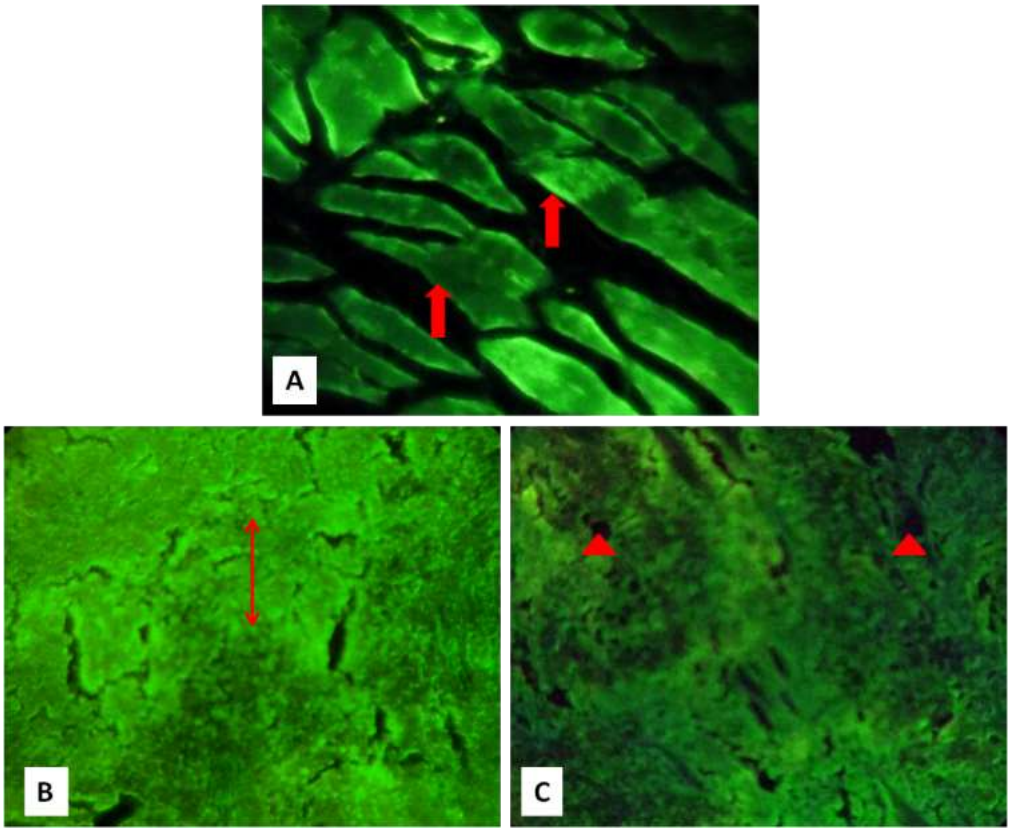

Fig. (7): Fluorescent imaging of sections in Ehrlich carcinoma stained by Acridine orange / propidium iodide stain. **A:** Normal muscle represents vital tissue regions stained in green (red blocked arrows). **B&C:** Control Ehrlich carcinoma represents vital green regions (↕) and some vacuolated areas (▲). (A&C X250, BX100)

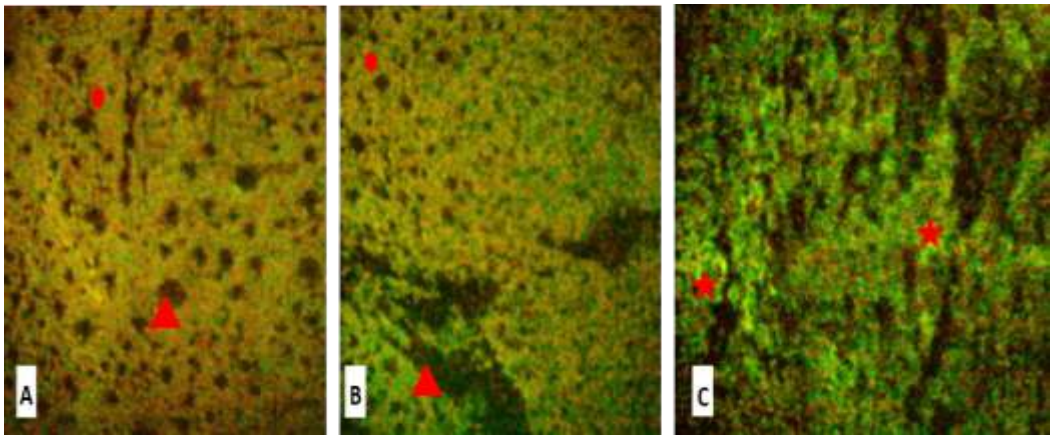

Fig. (8): Photomicrographs in sections of Ehrlich carcinoma Fluorescent imaging of sections in Ehrlich carcinoma stained by Acridine orange / propidium iodide stain. **A&B:** mice gavage orally by Gg extract represents extensive areas of necrotic EC cells (●) and some vacuolated areas (▲). **C:** mice gavage orally with SeNPs represents necrotic EC cells and remnants of apoptotic nuclei. (A, B &C x 250)