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Antitumor Activity of Resveratrol in Combination with Selenium in Ehrlich Ascites Carcinoma Bearing and/or Irradiated Mice

Mohamed R. Mohamed[1*], Soheir A. Osman[2], Neama M. El Fateh[2] and Mahmoud M. Refaat[2]

[1]*Department of Biochemistry, Faculty of Science, Ain Shams University, 11566 Abbassia, Cairo, Egypt,*
[2]*Radiation Biology Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt*

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ABSTRACT

Resveratrol (trans-3, 5, 4′-trihydroxystilbene) is a polyphenol non-flavonoid compound, particularly abundant in red grapes but it is also present in highly pigmented vegetables and fruits. It has been proven to be a potent antioxidant, anticancer, anti-inflammatory agent and recently it is proposed to have an antiangiogenic property. In this study, resveratrol 25 mg/kg body weight and sodium selenite 5µg/mice was investigated *in vitro* as well as *in vivo* via systemic intraperitoneal injection (i.p.) in female mice with or without γ-irradiation exposure targeting the improvement of cancer therapeutic protocols. Tumor necrosis factor-α (TNF-α) and matrix metalloproteinase 2 and 9 (MMP-2&-9) are the angiogenic regulators, lactate dehydrogenase (LDH), as well as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) are the antioxidant markers and lipid peroxide (LPx) is an oxidative stress marker were estimated to monitor efficacy of resveratrol and sodium selenite in cancer treatment strategy. All parameters were determined as a time course on days 16 and 22 after tumor volume reached 1 cm^3. The using of MTT assay on Ehrlich ascites carcinoma (EAC) cells *in vitro* showed that resveratrol and/or sodium selenite inhibit EAC cells proliferation. *In vivo*, administration of resveratrol and /or sodium selenite to mice bearing tumor and/or γ-irradiation reduced significantly the MMP-2 and 9 activities TNF-α level and LDH activity while, increase in the activities of liver antioxidant enzymes SOD, CAT and GSH concentration. It could be postulated that the combination of resveratrol and sodium selenite may used as modulators of cancer therapy via inhibit cancer growth through controlling the reactive oxygen species and angiogenic process.

Introduction

Cancer is a disease caused by a heterogeneous collection of dysregulated cellular signaling processes involved in cell proliferation and homeostasis and caused by a combination of genetic mutations and/or internal or external oncogenic stimuli [1]. Cancer etiology is a multistep process and cancer cells acquire the following characteristics: uncontrolled growth in the absence of growth signals, resistance to antiproliferative signals, evasion from apoptosis, limitless replication, development of new blood vessels [angiogenesis], and invasion to surrounding tissue and metastasis to distal organs. Metastatic spread of cancer cells depends on an adequate supply of oxygen and nutrients and removal of waste products. Angiogenesis is regulated by both activator and inhibitor molecules [2].

Angiogenesis is a fundamental process in reproduction and wound healing. Under these conditions, neovascularization is tightly regulated [3]; unregulated angiogenesis may result in different pathologies including cancer [4]. The construction of a vascular network requires different sequential steps including the release of proteases from "activated" endothelial cells with subsequent degradation of the basement membrane surrounding the existing vessel, migration of endothelial cells into the interstitial space, endothelial cell proliferation, and differentiation into mature blood vessels [5]. These processes are mediated by a wide range of angiogenic inducers, including growth factors, angiogenic enzymes, endothelial specific receptors, and adhesion molecules [5]. Many different proteins have been identified as angiogenic activators such as TNF-α,

* Corresponding author.
E-mail address: mohamed_elsotohi@sci.asu.edu.eg

MMP-2 and MMP-9. Levels of expression of angiogenic factors reflect the aggressiveness of tumor cells. The discovery of angiogenic inhibitors should help to reduce both morbidity and mortality from carcinomas [2]. Targeting inhibition of angiogenesis represents a potential approach in the treatment of solid tumors and such antiangiogenic strategies inhibiting the growth of endothelial cells may be more advantageous than targeting cancer cells [6].

Polyphenols are considered as major nutrients for improving general health and providing cure for certain specific pathological conditions [7]. They act via different mechanisms to inhibit the angiogenesis process by utilizing various components of tumor angiogenesis signaling pathway, which starts from the tumor cells secreting angiogenesis factors and ending in the formation of blood capillaries by endothelial cells [8].

Resveratrol [Res] is a polyphenol non-flavonoid compound, particularly abundant in red grapes [*Vitis vinifera*] and in highly pigmented vegetables and fruits [9]. It has been proven to be a potent antioxidant [10], anticancer [11]. Functionally, it belongs to phytoalexins, also called the plant antibiotics [12].

Selenium is an essential trace element existing in organic and inorganic chemical forms which have been shown to play an important role in maintenance of an optimal physiological state of mammalian cells. It has recognized a chemopreventive potential against various forms of environmental stress as well as against tumor development [13].

The present study evaluated the influence of the angiogenic regulators modification on the tumor growth targeting the improvement of cancer therapeutic protocols with or without γ-irradiation. Thus, the action of resveratrol and/or sodium selenite was examined *in vitro* on Ehrlich ascites carcinoma cells and *in vivo* in mice bearing Ehrlich cells a model of solid carcinoma tumor and/or γ-Irradiated.

Materials and Methods

Experimental animals

All animal procedures and experimental protocols were approved by the Research Ethics Committee and were carried out in accordance with the guide for the care and use of laboratory animals. Swiss albino mice weighting 20 - 25 g were obtained from the Egyptian Organization for Biological Products and Vaccines (Vacsera, Egypt) and housed under controlled conditioning 25±1°C constant temperature, 55% relative humidity and 12 hrs dark/light cycles. Food and water were allowed *ad libitum* during the study period.

Tumor cell line

Ehrlich ascites carcinoma (EAC) cell line was purchased from the Tumor Biology Department, National Cancer Institute, Cairo University. EAC is a murine spontaneous breast cancer that served as the original tumor from which an ascites variant was obtained. Intraperitoneal inoculation in female mice resulted in the production of ascites rich in tumor cells. The tumor cell line was maintained in our laboratory by serial i.p. passage in female Swiss albino mice at 7 or 10 days after passage. The EAC cells were prepared under aseptic conditions. EAC cells were tested for viability and contamination using Trypan blue dye exclusion technique [14]. EAC cells were suspended in normal saline so that each 0.2 ml contains 2.5×10^6 EAC cells. Cells were counted under the microscope using Neubauer hemocytometer.

Irradiation

Irradiation was performed at the National Center for Radiation Research and Technology (**NCRRT**), Nasr city, Cairo, Egypt. The source of radiation was through Ceasium-137 (^{137}Cs) gamma cell-40 which ensured a homogenous dose distribution all over the irradiation tray. Mice were placed in a specially designed well-ventilated acrylic container and whole body irradiated at dose level of 6.5 Gy. A line of Ehrlich Ascites Carcinoma (EAC) cells was irradiated also at dose level of 6.5 Gy**.** The dose rate was 0.66 Gy/min single shot dose during the experimental periods.

In vitro study

The viability test 3-[4, 5-dimethylthiazol-2-y1]-2, 5-diphenyl tetrazolium bromide (MTT) Assay [15] is a sensitive, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate MTT into purple-blue formazan crystals determined spectro-photochemically and serves as estimation for the mitochondrial activity and hence the number of living cells in the sample [16].

In vivo study

Induction of solid tumors

Solid tumors were produced by intramuscular inoculation in the right thigh of the lower limb of each mouse with 0.2 ml of EAC cells, which contained 2.5 x 10^6 viable EAC cells. Mice with a palpable solid tumor mass 1 cm^3 that developed within 14 days after inoculation were used in the study. Tumor volume was measured at different time intervals during the experimental period days 6, 13, 16, and 22 using a vernier caliper and calculated [17].

Treatments

Resveratrol (Res) was dissolved in dimethyl sulphoxide (DMSO) and phosphate buffer saline (PBS) DMSO: PBS (1:20) and given to mice by daily i.p. injection dose of 25 mg/kg body weight [18] for 14 successive days. Sodium selenite (Sse) was dissolved in 0.9% saline and given to mice by i.p. injection of the maximal tolerated dose 5µg/mice [19], once every day for 14 successive days.

Work design

The animal groups were randomly categorized into 16 groups, 10 mice each, as follows:

I. control groups

Group (1) Control (C), mice received vehicle injection (DMSO in PBS).

Group (2) Resveratrol (Res), mice i.p. injected with Res for 14 consecutive days.
Group (3) Sodium selenite (Sse), mice i.p. injected with Sse for 14 consecutive days.
Group (4) Res+ Sse, mice i.p. injected with a mixture of Res and Sse for 14 consecutive days.

II. Irradiated groups

Group (5) Irradiated (Irr), mice were whole body *γ*–irradiated with 6.5 Gy, single dose.
Group (6) Res+Irr, mice i.p. injected with Res for 14 consecutive days and *γ*-irradiation 24 hrs. after the last Res injected dose**.**
Group (7) Sse+Irr, mice i.p. injected with Sse for 14 consecutive days and *γ*–irradiated with 6.5 Gy 24 hrs. after the last Sse injected dose.
Group (8) Res+Sse+Irr, mice (i.p.) injected with a mixture of Res and Sse for 14 consecutive days and γ-irradiated with 6.5 Gy 24 hrs. after the last injected dose.

III. Ehrlich groups

Group (9) Ehrlich (E), mice bearing solid Ehrlich tumor.
Group (10) E+Res, mice bearing were received 14 successive Res dose starting from the 15th day after EAC inoculation.
Group (11) E+Sse, mice bearing solid Ehrlich tumor were received 14 successive Sse dose starting from the 15th day after EAC inoculation.
Group (12) E+Res+Sse, mice bearing solid Ehrlich tumor were received 14 successive Res+Sse dose starting from the 15th day after EAC inoculation.

IV. Ehrlich- Irradiated groups

Group (13) E+Irr, mice bearing solid Ehrlich tumor and exposed to γ-irradiation 6.5 Gy on 29th day after EAC inoculation.
Group (14) E+Res+Irr, mice bearing solid Ehrlich tumor i.p. injected with Res for 14 consecutive days and exposed to γ-irradiation 6.5 Gy 24 hrs. after the last injected dose**.**
Group (15) E+Sse+Irr, mice bearing solid Ehrlich tumor i.p. injected with Sse for 14 consecutive days and exposed to γ-irradiation 6.5 Gy 24 hrs. after the last injected dose**.**
Group (16) E+Res+Sse+Irr, mice bearing i.p. injected with Res+Sse for 14 consecutive days and exposed to γ-irradiation 6.5 Gy 24 hrs. after the last injected dose**.**

Sample collections

Animals were fasted for 16 hrs before each sampling. Samples were collected on 16th and 22th days post the tumor volume reached 1 cm^3. Animals were sacrificed and the blood was collected from heart puncher and left for coagulation and was centrifuged for collecting serum. MMPs, TNF-α and LDH were measured in serum of each group. The tissues of liver and solid tumor of experimental animals were dissected out and divided into two parts: one part was dissected, weighed and homogenized in physiological saline for SOD, CAT, GSH and TBARS detection. Another portion of liver tissues was kept in 10% formalin for histopathological studies.

Total protein concentration was assayed in serum by means of Biuret reaction according to [20] and the presence and activity of specific MMP species MMP- 2 &- 9 were initially detected in the serum using substrate (gelatin) gel electrophoresis [21]. A buffer of 4% SDS, 0.15 mol/L Tris (pH 6.8), 20% glycerol and 0.5% (w/v) bromophenol blue was added to the serum sample. Volume serum samples mixed with buffer were directly added to 10% SDS–acrylamide gel containing 0.1% (w/v) gelatin (sigma) and separated by running on a mini gel apparatus at 15mA/gel, and then gels were gently rocked in a 2.5% Triton X-100 solution for 30 min at room temperature. Gels were then incubated overnight at 37 °C in substrate buffer containing 50 mmol/L Tris–HCl (pH 8), 5 m mol/L $CaCl_2$ and 0.02% NaN_3. Gel was subsequently stained for 30 min in 0.5% Coomassie Blue R-250 dissolved in a 1:3:6 solutions of acetic acid, isopropyl alcohol and water. The gel was scored for the presence/absence MMP activity by a blinded evaluator and photographed. MMP-2 and MMP-9 could be detected on the SDS gel as transparent bands.

Biochemical assays

Tumor necrosis factor-alpha (TNF- α) level

The levels of TNF- *α* in serum were assayed by standard sandwich enzyme-linked immune-sorbent assay technique (ELISA) using ELISA kit (K0331186, KOMABIOTECH, Seoul, Korea) following the manufacturer's instructions based on the principle of a solid phase ELISA [22].

Lactate dehydrogenase (LDH) activity

Serum LDH was detected according to the applied LDH kit test [23], (Puruvate. Kinetic UV. DGKC. Liquid), where one international unit (IU) is the amount of enzyme that transforms 1µmol of substrate per minute, in standard condition is expressed in units per litre of sample U/L.

Antioxidant and oxidative stress markers

Liver and tumor superoxide dismutase (SOD) activitie was estimated by detection of superoxide anions using nitroblue tetrazoluim formazan color development according to [24], where One unit (50% inhibitory level of the enzyme) corresponds to 7.47 µg/ml of reaction mixture of SOD**.** Liver and tumor catalase (CAT) activity was assayed according to [25]. Liver and tumor reduced glutathione (GSH) concentration was assayed according to [26]. Liver and tumor lipid peroxide (LPx) content was determined by quantifying the thiobarbituric acid reactive substances (TBARs) according to [27].

Statistical Analysis

Statistical analysis of results including the mean and standard error (SE) values were performed using Statistical Package for Social Science (SPSS) for windows, version 15. Chicago, SPSS Inc. Released **2006.** All data are given as means ± SE. Data were analyzed statistically using one-way analysis of variance (ANOVA) followed by Post Hoc LSD test. Differences were considered significant at $p < 0.05$.

Results

In Vitro study

The MTT test shows significant alteration in the EAC cells viability when incubated with different concentrations of resveratrol and/or sodium selenite (**Table 1**). Res and Sse inhibit cells growth in a dose dependent manner. The maximal inhibitory concentration of Res and Sse were aproximately 100 µM and 5 µg/ml, respectively, after 24 hrs of incubation. When cells were incubated with 80 µM Res and 5 µg Sse, synergistic anti-proliferative effect was observed. The Sse and Res combination induced 91%. **Figure (1)** showed inhibition of cell growth**.** The radiation exposure of EAC cells treated with 80 µM Res and 5µg Sse showed dramatic cell growth inhibition 96%.

Table 1: Effect of different concentrations of resveratrol and resveratrol accompanied with 5 µg /ml sodium selenite on the viability of EAC or irradiated EAC cells, after 24 hrs incubation, using the MTT assay.

EAC cells		***Irradiated EAC cells***	
Concentrations	**% of cell inhibition**	**Concentrations**	**% of cell inhibition**
Resveratrol		**Resveratrol**	
0	0%	0	0%
30 µM	12%	30 µM	23%
50 µM	25%	50 µM	35%
60 µM	34%	60 µM	48%
80 µM	45%	80 µM	54%
100 µM	51%	100 µM	69%
Resveratrol with 5 µg /ml sodium selenite		**Resveratrol with 5 µg /ml sodium selenite**	
0	0%	0	0%
30 µM	70%	30 µM	82%
50 µM	79%	50 µM	85%
60 µM	83%	60 µM	92%
80 µM	91%	80 µM	96%
100 µM	94%	100 µM	98%

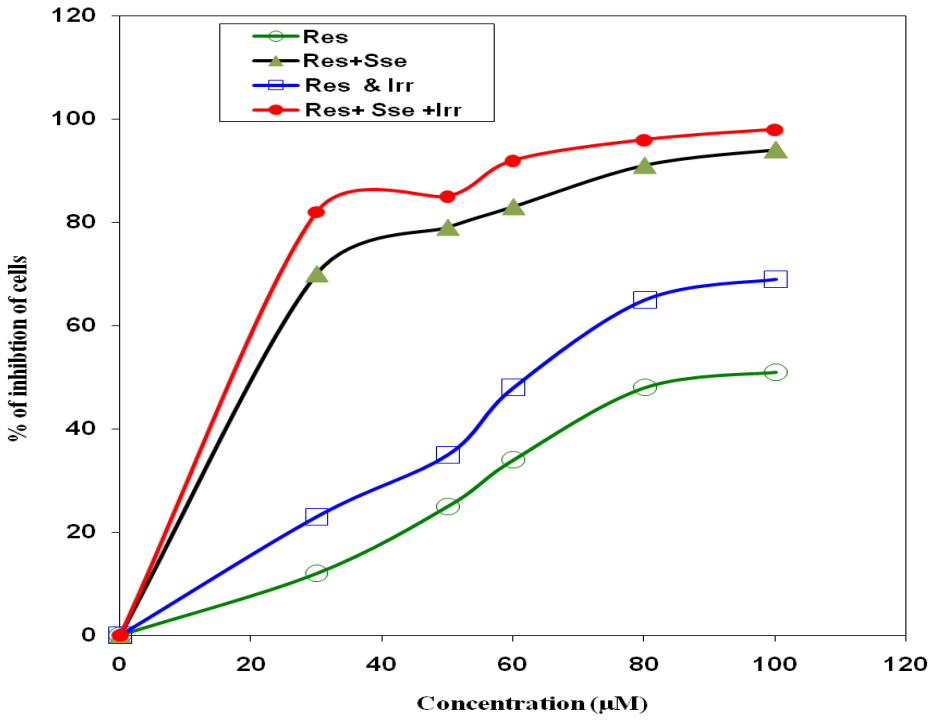

Fig. 1: Cell viability at different resveratrol doses (µM) and resveratrol accompanied with 5 µg/ml sodium selenite.

***In Vivo* study**

Tumor volume (mm^3)

Data obtained in **Table (2)** revealed a consecutive treatment of the animals with Res, Sse or their combination for 14 days starting after the tumor volume reached 1 cm^3 at 14 days after tumor inoculation, caused a marked suppression of tumor growth at days 6, 13, 16 and 22 from the day of tumor volume reaching 1 cm^3. Whole body γ-irradiation of mice bearing a tumor and treated with Res and/or Sse for 14 days showed a marked reduction in tumor volume at 16 days, and a further significant suppression at 22 days, compared to that in the non treated mice bearing Ehrlich. The E +Res + Sse +Irr mice reveal the most reduced tumor volume as compared with other groups.

Serum MMP-2 and MMP9 activities

Table (3) and **Fig 2** (A&B) demonstrate that the activity of serum MMP-2 and MMP-9 in Irr, E or E+Irr mice group was significantly increased, compared to control mice group, also the activity of serum MMP-2& -9 in E+Irr mice group was significantly increased, compared to Ehrlich mice group on 1st day and 1st week post irradiation. In contrast, a significant reduction in the serum activity of MMP-2 and MMP-9 in EAC bearing mice treated with Res, Sse or Res + Sse before exposure to γ-irradiation, compared to Ehrlich mice group. The results revealed that Res + Sse combination showed more pronounced decrease in serum MMP-2&MMP-9 activities than Res or Sse alone.

Serum TNF-α level

Table (4) revealed that serum TNF-α of Irr, E or E+Irr mice group showed significant increase, compared to control group on the 1st day and 1st week post irradiation, also E+Irr showed significant increase in serum TNF-α, compared to Ehrlich group. In contrast γ-irradiated mice group (Irr) showed significant decrease in serum TNF-α, compared to Ehrlich group (E).

Treatment of mice bearing tumor (E) mice group with Res, Sse or Res+ Sse had a significant decrease in TNF-α concentration, compared to Ehrlich group on the 1st day and 1st week post irradiation. Furthermore, E+Irr mice group treated with Res, Sse or Res+Sse showed significant decrease in TNF-α concentration, compared to Ehrlich group on the 1st day and 1st week post irradiation. The result revealed that Res + Sse induced more pronounced decrease in TNF-α concentration than Res or Sse alone.

Table 2: Tumor volume measurements (mm^3) in the different animal groups.

Experimental Days of Tumor Measurement (mm^3)	Ehrlich animal groups				Ehrlich-irradiated animal groups			
	E	E + Res	E + Sse	E+ Res + Sse	E+Irr	E+ Res + Irr	E + Sse+ Irr	E + Res+ Sse+ Irr
	G9	G10	G11	G12	G13	G14	G15	G16
6 day	916±53	490^{b}±25	365^{b}±25	278^{b}±26				
13 day	1592±45	874^{b}±59	657^{b}±43	558^{b}±39				
16 day	1900^{c}±46	1545^{bc}±47	1285^{bc}±10	1136^{bc}±54	901^{b}±19	823^{bc}±33	737^{bc}±25	674^{bc}±17
22 day	2066^{c}±54	1869^{bc}±55	1723^{bc}±24	1512^{bc}±43	1397^{b}±52	1219^{bc}±39	1112^{bc}±51	939^{bc}±46

Irr: Irradiation; E: Ehrlich; Res: resveratrol; Sse: sodium selenite. Each value represents Mean± SE of 6 observations, Values with dissimilar super script letters are considered significantly different at $p<0.05$. b: significant against Ehrlich group (G9). c: significant against E+Irr.

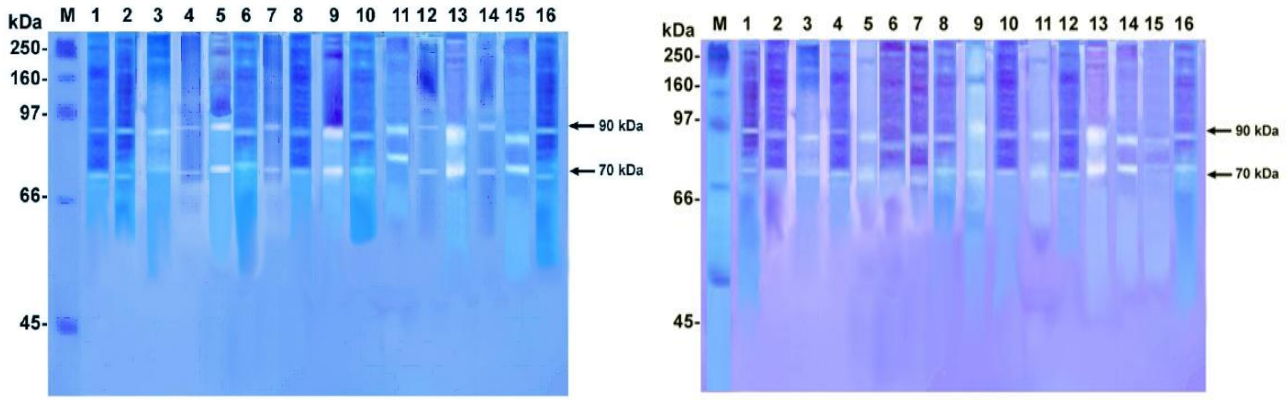

Fig. 2 (A&B): Gelatin zymography of serum MMP-(2 and 9) activities (70 & 90 kDa) in all studied groups on 1st day and 1st week post irradiation, respectively. Lane (M): Protein marker; Lanes (1-16) are groups from control to E, Res, Sse, Irr as ordered in **Table (4)**.

Table 3: Serum MMP-2&-9 activities in the different animal groups on 1st day and 1st week post irradiation.

parameter	Control groups				Irradiated groups				Ehrlich groups				Ehrlich- Irradiated groups			
	C	Res	Sse	Res+ Sse	Irr	Res+ Irr	Sse+ Irr	Res+ Sse+ Irr	E	E+ Res	E+ Sse	E+ Res+ Sse	E+ Irr	E+ Res+ Irr	E+ Sse+ Irr	E+ Res+ Sse+ Irr
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
MMP-2	(U/mg)															
1st day post irradiation	79.9^{b} ± 5.2	83^{b} ± 6.2	81^{b} ± 2.2	81.8^{b} ± 7.5	231^{ab} ± 15.4	165^{ab} ± 4.8	199^{ab} ± 5.5	149^{ab} ± 2.3	179^{a} ± 6.8	123^{ab} ± 3	148^{ab} ± 2.2	110^{ab} ± 3.8	284^{ab} ± 14.9	241^{ab} ± 4.9	255^{ab} ± 2.5	190^{ab} ± 3.2
% Change	0	4	1	2	189	106	149	87	124	53	86	37	255	195	219	138
1st week post irradiation	81.7^{b} ± 1.7	78.5^{b} ± 4.4	76.8^{b} ± 3.5	84.9^{b} ± 3.6	359^{ab} ± 7.9	243^{ab} ± 7.14	274^{ab} ± 4.9	231^{ab} ± 4.6	285^{ab} ± 5	176^{a} ± 4.3	195^{ab} ± 6.1	154^{ab} ± 2.5	337^{ab} ± 9.8	282^{ab} ± 3.8	288^{ab} ± 5.2	234^{ab} ± 9.3
% Change	0	-4	-6	4	340	198	235	182	248	116	138	39	312	244	252	188
LSD	29.9															
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
MMP-9	(U/mg)															
1st day post irradiation	176^{b} ± 2.1	177^{b} ± 1.7	174^{b} ± 2.3	182^{b} ± 4.1	495^{ab} ± 2.2	302^{a} ± 1.8	325^{ab} ± 2.8	244^{ab} ± 7.5	312^{a} ± 4.9	227^{ab} ± 9.1	266^{ab} ± 6.9	206^{ab} ± 2.6	369^{ab} ± 4.5	248^{ab} ± 4.8	306^{a} ± 3	240^{ab} ± 5.2
% Change	0	1	-1	3	181	72	85	38	78	29	51	17	110	41	74	36
1st week post irradiation	177^{b} ± 1.5	175^{b} ± 2.6	182^{b} ± 2.7	189^{ab} ± 2	602^{ab} ± 5.9	348^{ab} ± 5.3	396^{ab} ± 7.1	303^{a} ± 7.1	323^{a} ± 3.7	273^{ab} ± 2	288^{ab} ± 1.7	242^{ab} ± 1.9	537^{ab} ± 17.3	316^{a} ± 11.9	345^{ab} ± 5.6	286^{ab} ± 3.0
% Change	0	-1	3	7	241	97	125	72	83	55	63	38	204	79	96	62
LSD	12.6															

C: Control; Irr: Irradiation; E: Ehrlich; Res: Resveratrol; Sse: Sodium Selenite; LSD: Least significant difference. Each value represents Mean± SE of 6 observations, %: Percent change from the values of control mice. Values with dissimilar super script letters are considered significantly different at $p<0.05$. a: significant against normal Control group (G1). b: significant against Ehrlich group (G9).

Serum LDH activity

Results presented in **Table (4)** revealed significant increase in LDH activity in serum of Irr, E or E+ Irr mice group, compared to control mice group, also serum LDH activity in E+Irr mice group was significantly increased, compared to Ehrlich mice group on 1st day and 1st week post irradiation. On the other hand, treatment of mice bearing tumor with Res or Res + Sse before exposure to γ-irradiation had a significant decrease in serum LDH activity, compared to Ehrlich bearing mice on 1st day and 1st week post irradiation. Furthermore, combined treatment with Res & Sse to E+ Irr mice group showed significant reduction in serum LDH activity, compared to Ehrlich bearing mice on 1st day and 1st week post irradiation.The results revealed that Res + Sse combination showed more reduction in serum LDH activity than resveratrol or sodium selenite alone.

Antioxidant and oxidative stress markers

Liver tissue antioxidants

Table (5) revealed significant decrease in the liver of Irr, E or E + Irr mice groups for antioxidant enzymes (SOD and CAT) activities and GSH concentration, respectively, on 1st day post and 1st week post irradiation, compared to control mice group, also the liver of E+Irr mice group revealed significant decrease in CAT activity and GSH concentration, compared to Ehrlich group on 1st day and 1st week post irradiation. On the other hand, treatment of mice bearing tumor with Res, Sse or Res + Sse before and after exposure to γ-irradiation revealed significant increase in liver SOD activity, compared to Ehrlich bearing mice on 1st day post and 1st week post irradiation, while treatment of mice bearing tumor with Res, Sse or Res + Sse revealed significant increase in liver CAT activity, compared to Ehrlich bearing mice on 1st day post irradiation and only Res + Sse on 1st week post irradiation. Furthermore, liver CAT activity showed significant increase in E+ Irr treated groups with Res + Sse, compared to Ehrlich bearing mice on 1st day post irradiation.

Treatment of mice bearing tumor with Res, Sse or Res + Sse revealed significant increase in liver GSH concentration, compared to Ehrlich bearing mice on 1st day and 1st week post irradiation, while treatment of E+ Irr treated groups with Res or Res + Sse revealed significant increase in liver GSH content, compared to

Ehrlich bearing mice. The results revealed that Res + Sse combination showed higher increase in liver antioxidant markers than Res or Sse alone.

Tumor tissue antioxidant enzyme

Data represented in **Table (6)** revealed that the activities of tumor antioxidant enzymes (SOD and CAT) and tumor GSH concentration were significantly decreased in E +Irr mice group, compared to Ehrlich bearing mice on 1st day and 1st week post irradiation in tumor SOD and CAT activities and on 1st week post irradiation in tumor GSH content. Whole body γ- irradiation to mice bearing tumor treated with Res, Sse or Res + Sse had a significant decrease in tumor antioxidant markers on 1st day and 1st week post irradiation, compared to Ehrlich group, this decline was more evident 1st week post irradiation.

Administration of Res, Sse or Res + Sse to EAC bearing mice before γ- irradiation exposure (E) mice groups had a significant decrease in tumor CAT activity and GSH concentration on 1st day and 1st week post irradiation, compared to Ehrlich group, while Treatment of E mice groups with a mixture of Res and Sse had a significant decrease in tumor SOD activity on 1st day and 1st week post irradiation, compared to Ehrlich group. The results revealed that Res + Sse showed a significant decrease in tumor antioxidant markers than Res or Sse alone.

Liver TBARS level

Results presented at **Table (5)** revealed significant increase of TBARS concentration in liver of Irr, E or E+Irr mice group, compared to control mice group, also liver TBARS concentration of E+Irr mice group was significantly increased, compared to Ehrlich mice group on 1st day and 1st week post irradiation. In contrast, treatment of EAC bearing mice with Res, Sse or Res + Sse before exposure to γ-irradiation had a significant decrease in liver TBARS concentration, compared to Ehrlich bearing mice group on 1st day and 1st week post irradiation. Furthermore, liver TBARS concentration was significantly decreased in E+Irr mice group treated with Res on the 1st week post irradiation, as well as Res or Res + Sse on the 1st day post irradiation, compared to Ehrlich mice group. The results revealed that Res+ Sse exert more obvious decrease in liver TBARS concentration than Res or Sse alone.

Table 4: Serum TNF-α level and LDH activity in the different animal groups on 1st day and 1st week post irradiation.

parameter	Control groups				Irradiated groups				Ehrlich groups				Ehrlich- Irradiated groups			
	C	Res	Sse	Res+ Sse	Irr	Res+ Irr	Sse+ Irr	Res+ Sse+ Irr	E	E+ Res	E+ Sse	E+ Res+ Sse	E+ Irr	E+ Res+ Irr	E+ Sse+ Irr	E+ Res+ Sse+ Irr
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
TNF-α	(pg/ml)															
1st day post irradiation	88.3[b] ± 5.9	93[b] ± 3.39	97[b] ± 3.1	92[b] ± 1.4	217[ab] ± 9.9	175[ab] ± 3.7	185[ab] ± 8.8	137[ab] ± 7.5	288[a] ± 8.8	190[ab] ± 5.3	197[ab] ± 9.3	144[ab] ± 2	334[ab] ± 16.3	209[ab] ± 5.3	214[ab] ± 7.5	187[ab] ± 5.4
% Change	0	6	10	4	146	99	110	55	227	116	123	63	280	137	143	112
1st week post irradiation	87[b] ± 2.4	96[ab] ± 1.8	98[ab] ± 3.1	94[ab] ± 3	230[ab] ± 4.7	191[ab] ± 3.2	197[ab] ± 2.7	149[ab] ± 8.4	319[ab] ± 13	211[ab] ± 3.1	217[ab] ± 9.8	180[ab] ± 5.4	365[ab] ± 13.3	247[ab] ± 6.1	259[ab] ± 12.8	231[ab] ± 6.1
% Change	0	-2	13	8	164	118	126	71	265	142	149	106	319	183	197	165
LSD	48															
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
LDH	(µmol/L/min)															
1st day post irradiation	37.4[b] ± 0.90	34.3[b] ± 0.20	33.7[b] ± 1.65	38.3[b] ± 0.23	103[a] ± 7.1	58.6[b] ± 5.3	62.5[b] ± 3.18	40.1[b] ± 0.35	140[a] ± 6.3	77.7[b] ± 3.29	83.2 ± 2.6	56.8[b] ± 1.37	216[ab] ± 7.5	113[a] ± 5.1	120[a] ± 3.12	62.8[b] ± 4.47
% Change	0	-8	-10	2	176	57	67	7	274	108	122	52	477	202	220	68
1st week post irradiation	38.1[b] ± 0.41	40.0[b] ± 2.52	44.6[b] ± 2.54	37.5[b] ± 0..85	118[ab] ± 6.3	77.9[b] ± 1.52	77.8[b] ± 3.9	61.9[b] ± 0.72	144[a] ± 12.5	82.1[b] ± 1.73	87.6 ± 3.47	74.3[b] ± 4.8	239[ab] ± 4.8	154[a] ± 7.6	162[a] ± 3	76.3[b] ± 3.5
% Change	0	5	17	-2	207	104	107	62	277	115	130	95	529	305	324	100
LSD	65.7															

C: Control; Irr: Irradiation; E: Ehrlich; Res: Resveratrol; Sse: Sodium Selenite; LSD: Least significant difference. Each value represents Mean± SE of 6 observations, %: Percent change from the values of control mice. Values with dissimilar super script letters are considered significantly different at $p<0.05$. a: significant against normal Control group (G1). b: significant against Ehrlich group (G9).

Table 5: Liver SOD & CAT activities and liver GSH & TBARS concentrations in the different animal groups on 1st day and 1st week post irradiation.

parameter	Control groups				Irradiated groups				Ehrlich groups				Ehrlich- Irradiated groups			
	C	**Res**	**Sse**	**Res+ Sse**	**Irr**	**Res+ Irr**	**Sse+ Irr**	**Res+ Sse+ Irr**	**E**	**E+ Res**	**E+ Sse**	**E+ Res+ Sse**	**E+ Irr**	**E+ Res+ Irr**	**E+ Sse+ Irr**	**E+ Res+ Sse+ Irr**
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**
SOD	**(U/min/mg)**															
1st day post irradiation	9.2^{b} ± 0.5	9.1^{b} ± 0.5	9.13^{b} ± 0.82	8.3^{b} ± 0.43	4.2^{a} ± 0.5	7.7^{ab} ± 0.4	6.8^{ab} ± 0.3	6.9^{ab} ± 0.2	5.4^{a} ± 0.42	7.3^{ab} ± 0.35	7.5^{ab} ± 0.07	8.1^{b} ± 0.29	4.5^{a} ± 0.29	7.5^{ab} ± 0.4	7.3^{ab} ± 0.2	8.3^{b} ± 0.55
% Change	0	-2	-1	-10	-55	-17	-27	-25	-41	-21	-18	-12	-51	-19	-21	-10
1st week post irradiation	9.1^{b} ± 0.3	9.6^{b} ± 0.1	8.6^{b} ± 0.09	8.2^{b} ± 0.18	3.7^{ab} ± 0.1	4.3^{a} ± 0.18	4.1^{ab} ± 0.22	5.5^{a} ± 0.2	5.1^{a} ± 0.58	6.8^{ab} ± 0.29	6.5^{a} ± 0.81	6.4^{a} ± 0.74	5.2^{a} ± 0.32	7.6^{ab} ± 0.8	7.5^{ab} ± 0.4	7.7^{ab} ± 0.5
% Change	0	5	-5	-10	-59	-53	-55	-40	-44	-25	-28	-30	-43	-17	-18	-15
LSD	**1.5**															
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**
CAT	**(µmoles consumed H_2O_2 /min/mg)**															
1st day post irradiation	245^{b} ± 2.8	251^{b} ± 1.36	243^{b} ± 2.19	249^{b} ± 0.99	180^{ab} ± 5.66	202^{a} ± 1.27	202^{a} ± 1.07	212^{a} ± 4.66	207^{a} ± 2.38	231^{ab} ± 1.8	229^{ab} ± 2.4	237^{b} ± 0.71	139^{ab} ± 0.95	203^{a} ± 1.04	198^{ab} ± 1.26	215^{a} ± 3.03
% Change	0	2	-1	1	-27	-18	-18	-14	-15	-6	-7	-3	-43	-17	-19	-13
1st week post irradiation	240^{b} ± 2.64	246^{b} ± 3.69	239^{b} ± 3.47	245^{b} ± 2.23	146^{ab} ± 1.71	181^{ab} ± 1.92	178^{ab} ± 5.45	196^{ab} ± 6.09	170^{ab} ± 2.78	192^{ab} ± 5.6	188^{ab} ± 3.3	200^{a} ± 3.49	118^{ab} ± 1.36	161^{ab} ± 2.4	133^{ab} ± 1.32	170^{ab} ± 3.4
% Change	0	3	-0.4	2	-39	-25	-26	-18	-29	-20	-22	-17	-51	-33	-45	-30
LSD	**14.4**															
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**
GSH	**(mg GSH/ gm)**															
1st day post irradiation	36.5 ± 1.6	34.7 ± 0.4	33.7 ± 1.7	37.1^{b} ± 0.8	29.2^{ab} ± 2.1	33.7 ± 0.9	32.1^{a} ± 0.9	35.9 ± 2.0	33.7 ± 0.9	35.7 ± 0.5	34.7 ± 0.5	37.3^{b} ± 1.3	24.9^{ab} ± 2.1	31.2^{a} ± 0.9	30.4^{a} ± 1.5	34.2^{b} ± 0.6
% Change	0	-5	-8	2	-20	-8	-12	-2	-8	-2	-5	2	-32	-15	-17	-6
1st week post irradiation	36.8 ± 1.4	35.1 ± 0.2	34.4 ± 0.5	37.7^{b} ± 0.8	24.8^{ab} ± 0.5	30.5^{a} ± 0.4	30^{ab} ± 0.7	32.2^{a} ± 2.1	33.9 ± 1	35.2 ± 0.3	34.7 ± 0.2	36.5 ± 0.9	20.9^{ab} ± 0.9	30.2^{a} ± 1.3	29.4^{a} ± 2.0	33.3^{b} ± 0.6
% Change	0	-5	-7	3	-33	-17	-18	-13	-8	-4	-6	-1	-43	-18	-20	-10
LSD	**4.4**															
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**
TBARS	**(nmol/g)**															
1st day post irradiation	118^{b} ± 2.89	106^{ab} ± 1.89	112^{b} ± 1.87	117^{b} ± 3.2	235^{ab} ± 6	176^{ab} ± 3.9	202^{ab} ± 2.14	212^{ab} ± 12.5	272^{a} ± 13.1	210^{ab} ± 3.4	217^{ab} ± 1.6	244^{ab} ± 2.89	334^{ab} ± 9.8	243^{ab} ± 5.24	285^{ab} ± 5.4	255^{ab} ± 8.4
% Change	0	-10	-5	-1	99	49	72	80	131	78	85	107	184	106	142	117
1st week post irradiation	119^{b} ± 3.61	117^{b} ± 2.48	119^{b} ± 2.45	122^{b} ± 5	283^{ab} ± 4.9	212^{ab} ± 0.33	223^{ab} ± 5.1	254^{ab} ± 6.6	304^{ab} ± 9.5	241^{ab} ± 6.7	264^{ab} ± 2.58	309^{ab} ± 8.4	357^{ab} ± 5.4	236^{ab} ± 6.9	289^{ab} ± 18.6	281^{ab} ± 8.16
% Change	0	-2	-0.3	2	137	77	87	113	154	102	121	159	199	98	142	136
LSD	**11.7**															

C: Control; Irr: Irradiation; E: Ehrlich; Res: Resveratrol; Sse: Sodium Selenite; LSD: Least significant difference. Each value represents Mean± SE of 6 observations, %: Percent change from the values of control mice. Values with dissimilar super script letters are considered significantly different at $p<0.05$. a: significant against normal Control group (G1). b: significant against Ehrlich group (G9).

Tumor TBARS level

Data represented in **Table (6)** demonstrate the concentration of TBARS was increased on 1st week post irradiation when compared with its corresponding level on 1st day post irradiation. The concentration of TBARS in E+Irr mice group was significantly increased as compared to Ehrlich mice group. Treatment of E and E+Irr mice groups with Res, Sse or Res+Sse induced significant increase in tumor TBARS concentration on 1st day and 1st week post irradiation, compared to Ehrlich group. The increase was more significant on 1st week post irradiation. The results revealed that Res + Sse mice group have the most tumor TBARS concentration increment compared to Res or Sse mice group alone.

Table 6: Tumor SOD & CAT activities and tumor GSH & TBARS concentrations in the different animal groups on 1st day and 1st week post irradiation.

parameter	Ehrlich animal groups				Ehrlich-Irradiated animal groups			
	E	E+ Res	E+ Sse	E+ Res+ Sse	E+ Irr	E+ Res+ Irr	E+ Sse+ Irr	E+ Res+ Sse+ Irr
	G9	G10	G11	G12	G13	G14	G15	G16
SOD	**(U/min/mg)**							
1st day post irradiation	5.8 ± 0.26	5.5 ± 0.22	5.7 ± 0.15	4.1[b] ± 0.03	4.9[b] ± 0.025	3.9[b] ± 0.1	4.2[b] ± 0.04	3.3[b] ± 0.04
% Change	0	-6	-2	-29	-16	-33	-28	-43
1st week post irradiation	6.1 ± 0.04	6 ± 0.15	6.4[b] ± 0.06	3.9[b] ± 0.03	3.7[b] ± 1.03	3.2[b] ± 0.06	3.4[b] ± 0.09	1.9[b] ± 0.03
% Change	0	-1	6	-36	-39	-47	-43	-68
LSD	**0.6**							
CAT	**(µmoles consumed H_2O_2 /min/mg)**							
1st day post irradiation	126.30 ± 0.51	92.17[b] ± 4.71	110.6[b] ± 1.91	87.73[b] ± 2.88	62.7[b] ± 2.09	56.1[b] ± 3.56	48.63[b] ± 4.05	41.43[b] ± 2.14
% Change		-27	-12	-31	-50	-56	-61	-67
1st week post irradiation	134.5 ± 2.37	114[b] ± 2.7	123.53 ± 0.55	111.3[b] ± 1.45	39.5[b] ± 2.91	42.37[b] ± 2.25	46.37[b] ± 1.76	31.1[b] ± 0.98
% Change		-16	-8	-17	-71	-69	-66	-77
LSD	**8.2**							
GSH	**(mg GSH/ gm)**							
1st day post irradiation	30.7 ± 1.4	21.6[b] ± 1.5	22.8[b] ± 1.46	18.9[b] ± 0.86	29.6 ± 0.97	19.63[b] ± 0.87	20.87[b] ± 1.21	16.20[b] ± 0.61
% Change	0	-30	-26	-39	-4	-36	-32	-47
1st week post irradiation	32.80 ± 1.01	25.1 ± 1.1	29.2 ± 1.34	22.73[b] ± 1.62	21.1[b] ± 1.04	16.97[b] ± 0.99	19.4[b] ± 0.81	14.9[b] ± 0.74
% Change	0	-23	-12	-31	-36	-48	-41	-55
LSD	**7.97**							
TBARS	**(nmol/g)**							
1st day post irradiation	71.7 ± 4.2	130.6[b] ± 2.16	100.6[b] ± 3.35	112.2[b] ± 3.1	134.4[b] ± 4.42	178.9[b] ± 4	155.3[b] ± 4.6	265.6[b] ± 7.7
% Change	0	82	40	56	87	149	117	270
1st week post irradiation	82.5 ± 4.2	158.2[b] ± 2.7	130.9[b] ± 5.4	162.7[b] ± 5.4	173.8[b] ± 4.2	264.9[b] ± 5.14	225.4[b] ± 3.5	287.1[b] ± 2.9
% Change	0	92	59	97	110	222	173	249
LSD	**10.7**							

Irr: Irradiation; E: Ehrlich; Res: Resveratrol; Sse: Sodium Selenite; LSD: Least significant difference. Each value represents Mean± SE of 6 observations. Values with dissimilar super script letters are considered significantly different at $p<0.05$. a: significant against normal Control group (G1). b: significant against Ehrlich group (G9). c: significant against E+Irr.

Discussion

Metastatic spread of tumor cells cause cancer deaths. Moreover, clinical findings providing a pathway for tumor cell dissemination, so tumor associated neovascularization [angiogenesis] is a key component of metastatic spread [28]. Angiogenesis, the growth of new blood vessels from an existing vasculature, is a critical process in the formation of solid tumor growth beyond 1-2 mm in diameter. The Angiogenic process is a balance between stimulatory and inhibitory switch allowing tumor to induce microvessels formation from the surrounding host vasculature [2].

Data represented in this study revealed a significant increases [p<0.05] in TNF-α level and MMP-2, MMP-9 and LDH activities. The increase in TNF-α expression could be concerned in its role in neovascularization process. TNF-α is a major inflammatory mediator that induces multiple changes in Endothelial cell [EC] including induction of adhesion molecules, integrins, and matrix metalloproteinases [MMPs] [29].

Altered levels of pro-inflammatory and pro-angiogenic factors are observed in various forms of cancer, TNF-α expression was related to differentiation, invasiveness, and angiogenesis of various tumor [30].

Matrix metalloproteinases [MMPs] are a family of enzymes involved in many physiological processes involving matrix remodeling, and appear to be essential in angiogenesis, tumor cell invasion and metastasis [31]. These are zinc-dependent responsible for extracellular matrix [ECM] degradation and secreted in inactive pro-enzymatic forms. MMP-2 & 9 [gelatinases A & B] were found to be over-expressed in many invasive tumor cells. Several experiments have confirmed the key role of these enzymes in angiogenesis [32].

Data represented in this work demonstrate similar pattern in MMP-2 and MMP-9 changes. The significant increase in MMP-2 and MMP-9 activities in EAC bearing mice could be attributed to TNF- α level. TNF-α stimulated MMP-2 and MMP-9 activities in human corneal epithelial cells via the activation of focal adhesion kinase [FAK]/ extracellular regulated protein kinase [ERK] signaling [33]. In addition, the experimental data revealed a significant increase [p<0.05] in LDH concentration of EAC bearing mice group during 1st day and 1st week post irradiation. As a diagnostic and prognostic marker, serum LDH has previously been reported mainly as a marker of ominous outcome in cancer patients, including a variety of solid tumors [34].

The presented data shows that the increases in TNF-α, MMP-2, MMP-9 and LDH are accompanied with increases in tumor volume. The neovasculaturization enhances the ability of the tumor to grow as well as increases its invasiveness and metastatic ability [35]. Moreover, the increase in tumor volume in mice bearing EAC is associated with significant depletion in antioxidant parameters SOD, CAT and GSH. Tumor GSH depletion may have increased the sensitivity of tumor to radiation so, the initial complete cell killing might have occurred at the early stages of tumor development [36]. Depletion of GSH can lead to increase lipid peroxidation and cell damage while an increase in GSH level enhances antioxidant protection and cell function [37].

The present data reveal marked depletion in GSH content accompanied by significant inhibition of GSH dependent enzymes of tumor-bearing mice group, either exposed or not to irradiation. The depletion in GSH content has been previously reported in tissues of animals exposed to irradiation [38] and in irradiation-treated mice bearing tumor [39]. In addition, there is a close correlation between depletion of GSH and antioxidant enzymes and the increase in lipid peroxidation [38].

The loss of mitochondria in tumor host could be responsible for the decrease in total SOD activity in liver tissues of the tumor host [40]. When the oxidative damage is extreme as a result of tumor growth ROS scavenging system such as SOD, GSH and CAT are degraded which in turn lead to increase in free radicals which cause oxidative stress. Free radicals and oxidative stress in turn increase the expression of TNF-α which responsible for the successive steps in the angiogenesis process leading to continuous tumor growth.

The present study interests in control of angiogenic process as a promising approach in overwhelming cancer, the antiangiogenic capacity of Resveratrol in combination with Sodium selenite was examined, and administration of Resveratrol and /or Sodium selenite to mice bearing tumor and/or γ-irradiated induced improvement in the level of angiogenic activators TNF-α, MMP2, MMP9 and LDH when compared with their correspondence values in EAC mice.

The present results are in harmony with those of **Liu *et al.*,** who reported that resveratrol inhibits the invasiveness of diverse cancer cells by reducing the expression and activity of matrix metalloproteinase [MMP-2& MMP-9], involved in ECM degradation [41], and with those of **Garvin *et al.*,** who affirmed that intratumoral, peritumoral or intraperitoneal administration of resveratrol significantly arrested tumor growth *in vivo* [42].

Resveratrol is a fat-soluble compound exists in *cis*-and *trans*-stereoisomeric forms *trans*–resveratrol is more active and can undergo isomerization to the *cis*-form when heated or exposed to ultraviolet irradiation [43].

The present data are in accordance with those of **Luo *et al.*,** who examined the pre-treatment with resveratrol prior to ionizing radiation exposure of resveratrol radiosensitives human cervical tumor cell lines and non-small cell lung cancer [NSCLC] cells enhances tumor cell killing by ionizing radiation in a dose dependent manner [44].

Resveratrol possesses three phenolic groups and acts as a free radical scavenger by transferring the proton from its phenolic group to the free radicals [45]. It has documented antitumor function but also acts synergistically with other agents from the same class [45], and contributed to a putative anticancer action, such as antioxidant activity [46], proapoptotic capacity [47], antiproliferative [48] and

antiangiogenic activities [49].

Zhang *et al.* reported that resveratrol inhibited TNF-α-induced apoptosis and rescued the inhibition of TNF-α in osteogenic differentiation of BM-MSCs at an early stage, when the TNF-α-activated NF-κB signaling was suppressed by resveratrol, and it is a good candidate for further research as an anti-inflammatory or anti TNF-α agent in bone repair under *in vivo* inflammatory micro-environment [50].

Selenium could inhibit the angiogenesis of hepatocarcinoma in rats, by down regulating the expression of TNF-α and VEGF [41], and it also leads to inhibition of TNF-α in human umbilical vein endothelial cells [HUVECs] and in turn lead to suppression of MMP-2 &MMP-9 activities [51]. Sodium selenite and other different selenium forms could be able to inhibit cancer metastasis and primary tumor growth in multiple types of cancer in animals [52].

Many investigators have reported that the inhibition of antioxidant systems in blood and tissues of mice and rats accompanied by an increase in lipid peroxide products after irradiation exposure [53] and in irradiated mice bearing EAC [39]. On the other hand, a number of studies have indicated that tumor growth can cause antioxidant disturbances in certain tissues of the tumor host [39].

In fact, tumor development might be responsible for the liver antioxidant depletion and also the increased concentration of lipid peroxidation products [54]. Furthermore, the generation of lipid peroxide in mice liver after exposure to γ-irradiation could result from inactivation of antioxidant activities by irradiation-induced production of ROS. Elapsed Lipid peroxidation apparently can be initiated by hydrogen abstraction from lipid molecules by lipid radiolysis products [55]; this leads to permeability changes due to alterations of membrane proteins and polysaccharides. It was also reported that the level of increase in [LPx] after irradiation is in proportion to radiation dose and elapsed time [56]. When the oxidative damage is extreme as a result of tumor growth and/or γ-irradiation, ROS scavenging enzymes such as (SOD& CAT) and GSH are degraded [57]. Free radical and oxidative stress in turn increase the expression of TNF-α which is responsible for the successive steps in the angiogenesis process leading to continuous tumor growth.

Gamma-radiation (3 Gy) exposure increased the levels of [ROS], percent apoptotic cells and decreased the mitochondrial membrane potential in human peripheral blood lymphocytes [58].

The present data also, showed significant amelioration of the antioxidant parameters CAT, SOD and GSH in EAC mice treated with resveratrol and/or sodium selenite γ-irradiated or not when compared with their equivalents values in EAC mice group. It could be postulated that, resveratrol and/or sodium selenite could inhibit cancer growth through controlling the angiogenic process. The increase in the activities and concentration of these antioxidants after the administration of resveratrol may be due to the direct effect of resveratrol.

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