RESEARCH ARTICLE

Anticancer and Radiosensitization Efficacy of Nanocomposite *Withania somnifera* Extract in Mice Bearing Tumor Cells

Nadia M Abdallah[1], Eman Noaman[2], Noaman A Eltahawy[2], Abdelfattah M Badawi[3], Eman Kandil[1]*, NA Mansour[3], Hebatallah E Mohamed[2]

Abstract

The objective of the present study was to evaluate the anticancer and radio-sensitizing efficacy of a *Withania somnifera* extract/Gadolinium III oxide nanocomposite (WSGNC) in mice. WSGNC was injected to solid Ehrlich carcinoma-bearing mice via i.p. (227 mg/kg body weight) 3 times/week during 3 weeks. Irradiation was performed by whole body fractionated exposure to 6Gy, applied in 3 doses of 2 Gy/week over 3 weeks. Biochemical analyses as well as DNA fragmentation were performed. Treatment of solid Ehrlich carcinoma bearing mice with WSGNC combined with γ-radiation led to a significant decrease in the tumor size and weight associated with a significant decrease in mitochondrial enzyme activities, GSH content and SOD activity as well as a significant increase in caspase-3 activity, MDA concentration and DNA fragmentation in cancer tissues. Combined treatment of WSGNC and low dose of γ-radiation showed great amelioration in lipid peroxidation and antioxidant status (GSH content and SOD activity) in liver tissues in animals bearing tumors. It is concluded that WSGNC can be considered as a radio-sensitizer and anticancer modulator, suggesting a possible role in reducing the radiation exposure dose during radiotherapy.

Keywords: *Withania somnifera* - gadolinium nanocomposite - tumor - mitochondria

Asian Pac J Cancer Prev, **17 (9)**, 4367-4375

Introduction

Although radiotherapy is one of the most effective strategies in the treatment of cancers, it is associated with short and long term side effects on normal tissues (Aghamohammadi et al., 2015). Enhancing of radioresponsiveness of tumors by using radiosensitizers is a promising approach to increase the efficacy of radiation therapy (Hematulin et al., 2014). Acquired radiation resistance is one of the major causes of radiotherapy failure and subsequent tumor relapse. Multiple approaches have been utilized to limit the radiation resistance while simultaneously enhancing the efficacy and safety of radiation therapy. The three major approaches for the improvement of radiation therapy involved (I) enhancing radio-sensitization of tumor tissue; (II) reversing the radiation resistance in tumor tissue; and (III) enhancing radio-resistance of the healthy tissue. Nanoparticles have played a key role in the enhancement of the radiation therapy by acting both as a therapeutic as well as a carrier for other therapeutics (Kwatra et al., 2013).

Radio-sensitizers are agents that sensitize the tumor cells to radiation. These compounds apparently promote fixation of the free radicals produced by radiation damage at the molecular level (Raviraj et al., 2014). The mechanism of action is similar to the oxygen effect, in which biochemical reactions in the damaged molecules prevent repair of the cellular radiation damage. Free radicals such as hydroxyl radicals are captured by the electron affinity of the radio-sensitizers, rendering the molecules incapable of repair (Raviraj et al., 2014).

Nanotechnology is a promising interdisciplinary field for developing improved methods of diagnosis and treatment of different diseases, including cancer (Roblero-Bartolon, and Ramon-Gallegos, 2015). Nanotechnology has been employed to enhance cancer therapy, via improving the bioavailability and therapeutic efficacy of anti-cancer agents (Basha et al., 2014). The use of nanotechnology in cancer treatment offers exciting opportunities, including the possibility of destroying tumors with minimal damage to healthy tissue by novel targeted drug delivery systems. The pH differences between healthy and tumor microenvironment provides pH responsive release of drugs at tumor site via smart nanoparticles (Unsoy et al., 2014). Metal-based compounds and nanoparticles (gadolinium in particular) if present in sufficiently high concentrations in the tumors, metals can act as a radiotherapy adjuvant: they possess an increased capability to absorb the X-ray (ionizing radiation) with respect to the water-based tissues. Low

*[1]Department of Biochemistry, Faculty of Science, Ain Shams University, [2]Department of Radiation biology, National Center for Radiation Research and Technology, (NCRRT), Egyptian Atomic Energy Authority (EAEA), [3]Petrochemical Department, Egyptian Petroleum Research Institute *For correspondence: kandil.eman@yahoo.com*

energy electrons will be then released close to the metal and, therefore, determine a local dose enhancement (Ceresa et al., 2014).

On the other side, natural compounds are emerging as effective agents for the treatment of malignant diseases (Lee et al., 2014). The plants used in Ayurvedic medicine, which has been practiced in India for thousands of years for the treatment of a variety of disorders, are rich in chemicals potentially useful for prevention and treatment of cancer. Withania somnifera (commonly known as Ashwagandha) is one of such medicinal plants with anti-carcinogenic properties (Senthil et al., 2015), radio-sensitization (Tiwari et al., 2014) and antioxidant activities (Ansari et al., 2013). Mitochondria are membrane-bound organelles found in most eukaryoted (Henze et al., 2003). Mitochondria have been shown to play an important role in regulating both programmed cell death and cell proliferation (Wallace, 2008). As the entry point for most electrons into the respiratory chain, NADH dehydrogenase or complex I has been suggested as the rate-limiting step in overall electron transfer. It plays a central role in oxidative phosphorylation (Sharma et al., 2009). Intracellular reactive oxygen species (ROS) may act as signaling molecules for carcinogenesis and cell death induction, a sudden intracellular raise in ROS accounting for a pro-death situation (Bras et al., 2005). They are produced by various enzymatic complexes including NADPH oxidase and mitochondrial electron transport chain, the latter being considered as the most important source of ROS (Fruehauf and Meyskens, 2007). In view of these considerations the aim of the current study was to evaluate the efficacy of WSGNC as an anticancer and radio-sensitizer agent.

Materials and Methods

Chemicals

Gadolinium III oxide was purchased from sigma-Aldrich Company. The shade dried roots of Withania somnifera Dunal had been supplied from the Agricultural Seeds, Spices and Medical Plants Company, Egypt.

Experimental animals:

Sixty female Swiss albino mice, weighing 25g were used in the course of the present work. The animals were housed in especially designed plastic cages (15 mouse/ cage), under normal temperature, pressure, humidity, good ventilation and illumination conditions. Animals were fed on pellets diet supplied with excess of water. All animal procedures were performed in accordance with the Ethics Committee of the National Research Centre conformed to the "Guide for the care and use of Laboratory Animals" published by the National Institutes of Health (NIH publication No. 85-23, revised 1996).

Radiation processing

Whole body gamma irradiation of animals was carried out using an indoor shielded AECL/Cesium 137 Gamma cell-40 biological irradiator (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada), installed in the "National Centre for Radiation Research and Technology (NCRRT)", Egyptian Atomic Energy Authority (EAEA), Nasr City, Cairo, Egypt. The dose rate was 0.5 Gy/minute during the experimental period. The mice's whole body was exposed to gamma rays at a dose of 2 Gy/week up to a total dose of 6Gyduring 3 weeks.

Tumor transplantation

A line of Ehrlich ascites carcinoma cells (EAC) used in the present study had been kindly supplied from the National Cancer Institute (NCI), Cairo University, Egypt. It was maintained in female Swiss albino mice by weekly intraperitoneal injection of 2.5x106 tumor cells/ mouse (in the right thigh of the lower limb of each mouse). The solid form was obtained by the intramuscular inoculationof 0.2 ml of EAC, contained 2.5 x 106 viable EAC cells. Mice with a palpable solid tumor diameter ($10mm^3$) that developed within 10 days after inoculation were used in the study (Hamburger, 1981).

Determination of LD50 using experimental animals

In screening drugs, determination of LD50 (the dose which has proved to be lethal (causing death to 50% of the tested group of animals)is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations (provides information on health hazards likely to arise from short- term exposure of drugs) and is one of the initial screening experiments performed with all compounds (Akhila et al., 2007). The studied compound was administered in graduated doses and LD50 was determined (Narang and Desai, 2009). The optimum selected dose for evaluating the in vivo antitumor activity of the tested compound was calculated approximately as (1/5) of its LD50 value.

Treatment

Withania somnifera extract/gadolinium III oxide nanocomposite (WSGNC) was injected via intraperitoneal injection of animals at a dose of 227 mg/kg body weight for3times/week during 3 weeks.

Preparation of Withania somnifera extract/gadolinium III oxide nanocomposite (WSGNC)

The shade dried roots were powdered coarsely and the powder was extracted (soxhlet) with petroleum ether and 70% ethyl alcohol in 1:10 w/v ratio for 72 h according to (Wanger et al., 1984). 15 g of extracted Withania somnifera was placed in a beaker, 10 ml dimethyl sulphoxide (DMSO) were added (as solvent) and the reaction mixture put on the hot plate, after 10 minutes 5 g of Gadolinum III oxide nano powder were added. The proposed study was implemented both (in vitro and in vivo study):

Invitro study

MTT (3-[4,5-dimethylthiazole-2,5-diphenyltetrazolium bromide) Cell Viability Assay was carried out according to the method described by (Wilson et al., 1990). Invivostudy:

Experimental design

Female Swiss albino mice were divided into 5 groups

(n=15) as follows:

Group 1 (C):Normal healthy control animals

Group 2 (EC): Ehrlich Ascites Carcinoma inoculated mice.

Group 3 (EC+IR):EC bearing mice were exposed to fractionated whole body γ-irradiated with6Gy applied in 3 doses of 2Gy/week.

Group 4(EC+WSGNC): EC bearing mice were given 227 mg/kg body weight WSGNC via i.p. 3times/week during 3 weeks.

Group 5 (EC+WSGNC +IR): EC bearing mice were given 227 mg/kg body weight WSGNC via i.p.3times/week during 3 weeks and whole body γ-irradiated with 6Gy applied in 3 increments of 2Gy/week during 3 weeks.

Tumor volume and tumor weight monitoring

The volume of the formed solid tumor was measured using Vernir caliper after 10 days from inoculation of mice with EC twice a week. The tumor volume was calculated by the following equation. Tumor volume= ½(length × width2) × 1000 (Jensen et al., 2008). Where length is the greatest longitudinal diameter and the width is the greatest transverse diameter. Tumor weight was measured during samples collection after 3 weeks from starting treatment according to that suggested by (David et al., 1986).

Biochemical assays

DNA fragmentation was determined according to (Okamura et al., 2000). Caspase-3 activity was investigated in lysate cells of tumor tissue according to (Porter and Janicke, 1999). The mitochondria was isolated by the method of (Rickwood et al., 1978) and used for evaluation of enzyme assays. NADH-ubiquinone oxidoreductase (Complex I), (Complex II) and cytochrome c oxidoreductase (Complex III) were determined according to (Kwong and Sohal, 2000). Lipid peroxidation was estimated according to the method described by (Yoshioka et al., 1979). Reduced glutathione was determined in solid tumor according to (Beutler et al., 1963). Superoxide dismutase and catalase activities were determined according to (Sun et al., 1988) and (Johansson et al., 1988) respectively.

Statistical analysis

Data are reported as the mean ± SE. Data were analyzed using one-way analysis of variance (ANOVA) followed by LSD as a post-hoc test. The level of significance between mean values was set at $P \leq 0.05$. All statistical analyses were performed using SPSS software (Version 20.0).

Results

1-Particle size determination

The new composite Withania somnifera extract/ Gadolinium III oxide nanocomposite was analyzed using transition electron microscope (TEM) and Fourier transform infrared spectroscopy (FTIR).The analysis was carried out by using transition electron microscope (TEM) for Gadolinium III oxide nanoparticles (Figure 1) and Withania somnifera extract/ Gadolinium III oxide nanocomposite (Figure 2) samples demonstrated that the particle size was about (25-35nm).

Fourier transform infrared spectroscopy (FTIR) spectra

FTIR is an important analysis technique that detects various characteristic functional groups present in Withania somnifera extract and Withania somnifera extract / Gadolinum III oxide nano composite sample. Upon interaction of infrared light with sample, chemical bonds can absorb infrared radiation in specific wavelength ranges regardless of the structure of the rest of the molecules. The different assignments of the FTIR spectra are summarized in Table (1).

FTIR spectra of the Withania somnifera extract/ Gadolinum III oxide nanocomposite

The structure of Withania somnifera extract/Gadolinum III oxide nanocomposite is characterized by absorption bands at 419.16 which correspond to the Gadolinum III oxide nanocomposite.

2-In vitro study

The results presented in Table (2) showed the treatment of Ehrlich ascites carcinoma (EAC) cells with WSGNC provoked a highly significantly decrease (44.3 and 39.7%) in the viability of cells at concentration 25 and 50μl, respectively, compared to untreated EAC cell line.

3-Invivo study

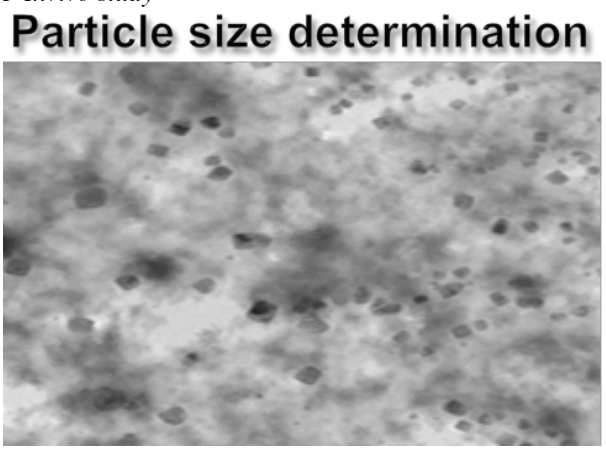

Figure 1. Gadolinium III Oxidenanoparticles

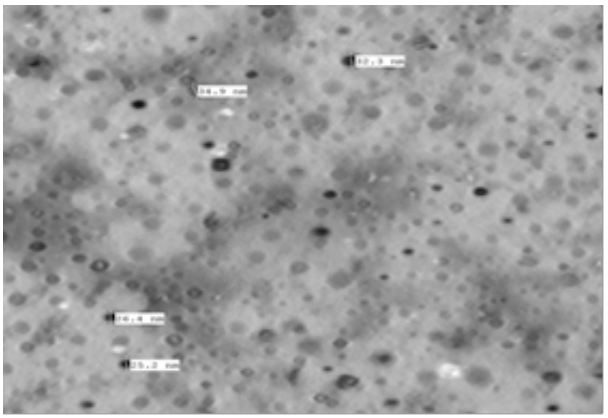

Figure 2. Particle Size and Particle Distribution of Gadolinium III Oxide in Withania Somnifera Extract Determined by Transmission Electron Microscope

Table 1. FTIR Assignment of *Withania somnifera* Extract

Assigned (cm-1)	Vibrational mode	Functional group
3393	OH stretching	Free OH
2939	C-H stretching	CH3-alkane
1627	C=O group	C=O Carbonyl α,β unsaturated
1385	C-H binding	CH3-alkane
1056	C-O stretching	C-O stretching of ether
923	C=C	Disubstitutedcis
777 &621	C-H	C-H stretching for different adjacent hydrogen atoms(5 adj.& 6 adj.)

Table 2. Percentage cell viability of Ehrlich Ascites Carcinoma (EAC) affected by Different Concentrations of *Withania somnifera*/Gadolinium IIi Oxide Nanocomposite (WSGNC) after 3 weeks of Treatment

Parameters	Conc. 10	Conc. 25	Conc. 50
Mean ±SE	1.5±0.01	1.06±0.05***	0.95±0.15***
% of cell viability	100	44.3	39.7
% of apoptosis	-	55.7	60.3

Each value represents the mean ± SE.*Significantly different compared to untreated EAC cell line. The % change calculated compared to

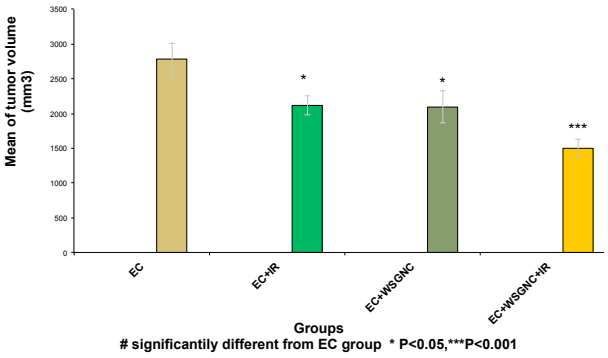

Figure 3. Effect of (WSGNC) on Tumor Volume (mm³) of Mice Bearing Tumor Cells after 3rd week of Treatments

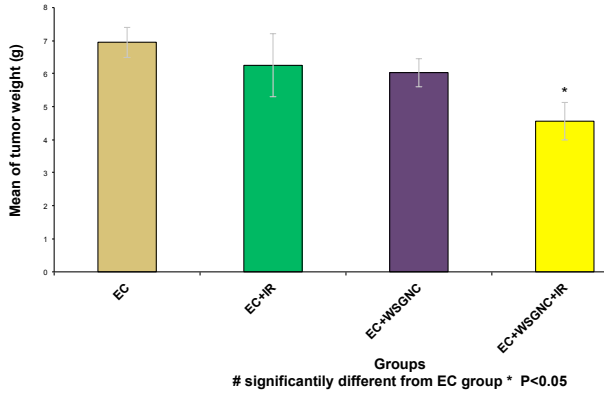

Figure 4. Effect of (WSGNC) on Tumor Weight (g) of Mice Bearing Tumor Cells after 3rd Week of Treatment

As shown in Figure 3 and Figure 4, the tumor volume in animals bearing tumor (EC group) was 2785.02 mm³. Animals bearing tumor treated with WSGNC recorded a significant decrease of -25%while animals bearing tumor treated with WSGNC and exposed to γ-radiation showed a highly significant decrease of -46% compared to EC

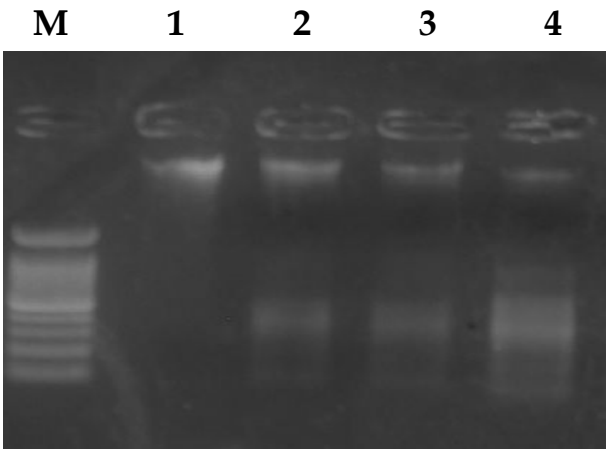

Figure 5. DNA Electrophoresis: EC(1),EC+WSGNC (2), EC+IR (3), EC+ WSGNC +IR (4)

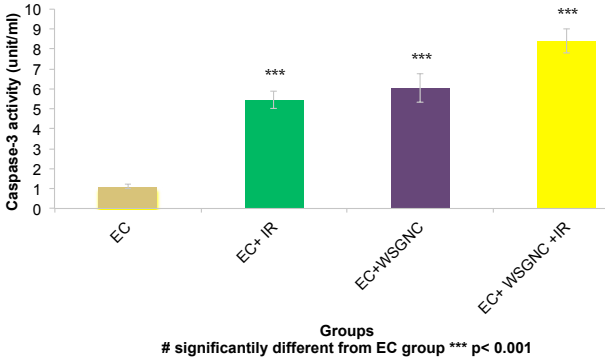

Figure 6. Effect of *Withania somnifera* Extract/ Gadolinium III Oxide Nanocomposite (WSGNC) on Caspase-3 activity (unit/ml) in Cancer Tissues of Mice Bearing Tumor Cells after 3 weeks of Treatment

group. The same group also recorded a significant decrease of -34% in tumor weight compared to EC group.

DNA fragmentation

The DNA fragmentation pattern was monitored for the different groups on agarose gel electrophoresis. Necrotic strand breaks/streaking DNA was observed in the treated groups EC+WSGNC (lane 3), EC+IR (lane 4) and EC+ WSGNC +IR (lane 5) but not in EC group (lane 2) (Figure 6).

DNA marker	EC	EC+WSGNC	EC+IR	EC+WSGNC+IR
1	2	3	4	5

Figure (3) showed the results of caspase-3 activity in cancer tissue. There was a highly significant increase

Table 3. Effect of *Withania somnifera* Extract/Gadolinium III Oxide Nanocomposite (WSGNC) on Mitochondrial Enzyme Activities in Cancer Tissue of Mice Bearing Tumor Cells after 3 Weeks of Treatment.

Parameters	EC	EC+ IR	EC+WSGNC	EC+WSGNC +IR
Complex I (U/μg mitochondrial protein)				
Mean±SE	113.8±13.10	41±4.30***	41±2.90***	38.4±3.80***
% change	---	-63	-63	-66
Complex II (U/μg mitochondrial protein)				
Mean±SE	26.70±3.66	13±1.90***	10.45±0.30***	9.60±1.36***
% change	---	-51	-60	-64
Complex III (U/μg mitochondrial protein)				
Mean±SE	7.32±0.30	1.31±0.15***	2.34±0.15***	3.14±0.52***
% change	---	-82	-68	-57

Each value represents the mean ± SE.*Significantly different from the EC group,***P<0.001.The % change calculated from EC group

Table 4. Effects of *Withania somnifera* Extract/Gadolinium III Oxide Nanocomposite (WSGNC) on MDA, GSH, SOD and CAT in Cancer Tissues of Mice after 3 Weeks of Treatment

Parameters	EC	EC+ IR	EC+WSGNC	EC+WSGNC+IR
MDA (μM/g tissue)	67.99±5.42	91.5±11.25	60.33±5.39	107±9.10b*
% change	---	-34.6	-11.3	57.4
GSH (mg/g tissue)	3.1±0.20	2.83±0.05	2.85±0.11	2.7±0.06b*
% change	---	-8.7	-8	-13
SOD (U/g tissue)	19.25±0.70	17.9 ±0.80	20.3±0.30	13±1.73b*
% change	---	-7	5.4	-32.5
CAT (μ M/g tissue)	13.78±1.10	13.74±0.50	12.94±0.80	12.92±0.80
% change	---	-0.3	-6	-6

Each value represents the mean ± SE. bSignificantlydifferentfromtheEC group. *p<0.05. The % change calculated from EC group

Table 5. Effect of *Withania somnifera* Extract/Gadolinium IIi Oxide Nanocomposite (WSGNC) on MDA, GSH, SOD and CAT in Liver Tissues of Mice Bearing Tumor Cells after 3 weeks of Treatment

Parameters	C	EC	EC+ IR	EC+WSGNC	EC+WSGNC+IR
MDA(μM/g tissue)	63.5±3.7	397.3±41.1a***	367.7±36.2	163±7.6b***	210.4±21b***
% change	---	290	-7.4	-59	-47
GSH(mg/g tissue)	4.5±0.16	2.9±0.17 a***	3.37±0.16	3.1±0.4	4.1±0.27b***
% change	---	-35	16	6.8	41
SOD(U/g tissue)	60.47±2.48	40.35±0.56a***	54.51 ±7.72b*	41.03±5.27	56.35±2.25b***
% change	---	-33	35	1.6	40
CAT(μ M/g tissue)	5.51±0.28	3.38±0.37a***	5.51±0.72b*	4.57±0.35b*	4.44±0.21
% change	---	-38.7	38	35	31

Each value represents the mean ± SE. aSignificantlydifferentfromthecontrol group.bSignificantlydifferentfromtheEC group. *p<0.05, ***p<0.001. The % change calculated from EC group

(P<0.001) of 396, 448 and 609% was recorded forcaspase-3 activity in cancer tissue of animals in the groups EC+ IR, EC+WSGNC and EC+WSGNC+IR, respectively compared to EC group.

As illustrated in Table 3 a highly significant decrease (P<0.001)was recorded in complex I, II & III activities in the mitochondria in cancer tissue of animals in the groups EC+ IR, EC+WSGNC and EC+WSGNC+IR, respectively compared to EC group.

As exhibited in Table 4 a significant increase of 57.4% in MDA level associated with a significant decrease of -13 % and -32.5 % in GSH content and SOD activity, respectively was occurred in cancer tissues of animals bearing tumor treated with WSGNC combined withγ-radiation compared to EC group.

As illustrated in Table 5 a highly significant increase of 290% in MDA level associated with a highly significant decrease of -35 %,-33 % and 38.7 % in GSH content, SOD and CAT activities, respectively in liver tissues of animals bearing tumor compared to control group. Also, a significant increase of 35% and 38% in SOD and CAT activities, respectively in animals bearing tumor combined with γ- radiation compared to EC group (see Figure 6).

Data obtained in the present study further revealed that a highly significant decrease of -59% in MDA level combined with a significant increase in SOD of 35% in animals bearing tumors treated with (WSGNC) compared to the EC group. In addition, the findings revealed that a highly significant decrease of -47% in MDA level was associated with a highly significant increase of 41% and 40 % in GSH content and SOD activities, respectively, in the animals bearing tumors treated with the WSGNC combined with γ- radiation as compared to in the EC group case.

Discussion

Numerous agents can interfere with redox cell signaling pathways have been identified, demonstrating, in preclinical models, selective toxicity towards the cancer cells, with increased endogenous ROS, raising oxidative stress over the threshold of toxicity. Association of chemical or radiation therapies with pharmacological agents that have pro-oxidant properties and/or are able to induce lipid peroxidation increases the effectiveness of the treatment. The increased intracellular antioxidant capacity is a common phenomenon in tumor cells resistant to many anticancer agents and radiation. The potential of using a redox-modulating strategy to eliminate malignant cells without killing normal cells represents a new therapeutic strategy even if a more detailed understanding of ROS-mediated signaling in tumor cells is necessary to develop the therapeutic intervention to selectively kill cancer cells and overcome drug and radiation resistance (Barrera,. 2012).

Withania somnifera ethanolic extract was reported to provoke significant arrest of cells at G2/M phase (G2/M arrest) and to increase sub-G0 phase indicating induction of apoptosis. Withania somnifera ethanolic extract act by cell cycle-specific mechanism inducing mitotic arrest and apoptosis in breast cancer cells (Maliyakkal et al., 2013).

The in vitro study showed that, treatment of EAC cells with Withania somnifera extract /gadolinium III oxide nanocomposite (WSGNC) induced a highly significant decrease in the viability of cells. The present results covalent with (Khazal et al., 2013) who reported that Withania somnifera inhibits viability of cultured breast cancer cells in association with reactive oxygen species (ROS)-dependent apoptosis induction. Because ROS production is implicated in induction of autophagy, which is an evolutionary conserved process for bulk degradation of cellular components including organelles (e.g., mitochondria) and considered a valid cancer chemotherapeutic target. The exposure of human lymphocytes to Gadolinum (Gd) resulted in a concentration- and time-dependent decrease in cell viability and an increase in micronuclei (MN) frequency, single strand DNA breakage, apoptotic cell death, and ROS production (Cho et al., 2014).

The root extract of the plant Withania somnifera has been reported to reduce tumor growth and tumor weight (Sinha and Ostrand-Rosenberg, 2013). A significant increase in the life span and a decrease in the cancer cell number and tumor weight were noticed in the tumor-induced mice after treatment with the ethanolic extract of the root of withania somnifera (Christina et al., 2004).In the current study, a pronounced delay in tumor volume associated with a significant decrease in tumor weight were recorded in the experimental animals treated with Withania somnifera extract/gadolinium III oxide nanocomposite (WSGNC) associated with irradiation which corroborates previous findings that the combination of Gadolinium-based nanoparticles (GBNs) with irradiation significantly delayed tumor growth with an increase in late apoptosis and a decrease in cell proliferation (Miladi et al., 2015).

Withaferin A, a steroidal lactone purified from Withania somnifera, has been shown to inhibit the proliferation, metastasis, invasion and angiogenesis of cancer cells. Withaferin Amarkedly increased the sub-G1 cell population and the cleavage of poly ADP-Ribose polymerase (PARP), which are markers of apoptosis (Park et al., 2015).Treatment with withaferin A provoked a marked inhibition of the tumor growth factor (TGF-β)-induced increase in expression and activity of matrix metalloproteinase(MMP)-9. In addition, treatment with withaferin A induced inhibition of TGF-β induced phosphorylation of a serine/threonine-specific protein kinase (Akt), involved in the downregulation of expression of MMP-9 at the protein level demonstrating that withaferin A may be an effective strategy for control of metastasis and invasiveness of tumors (Lee et al., 2013). Combined treatment with radiation has improved the outcome in various cancers and many radiosensitizers are used to enhance the therapeutic efficiency of radiotherapy. Withaferin A-enhanced ionizing radiation induced apoptosis is associated with the poly ADP ribose polymeraseenzyme (PARP) cleavage, caspase-3 activation, as well as specifically down-regulation of anti-apoptotic protein Bcl-2, suggesting that Withaferin A may be a potential radiosensitizer. Generation of reactive oxygen species (ROS), Bcl-2 down-regulation and activation of Mitogen-activated protein kinases (MAPKs) pathway were critically involved in the apoptosis induced by Withaferin A and radiation (Yang et al., 2011).

In the course of the present study, treatment of EC bearing mice with Withania somnifera extract/gadolinium III oxide nanocomposite (WSGNC) and irradiation led to a highly significant decrease in mitochondrial enzymes activities, with activation of caspase-3 and significant increase in DNA Fragmentation. These results correlates with (Kroemer et al., 2009) who reported that the mitochondria pathway of apoptosis is characterized by mitochondrial membrane permeabilization and release of pro-apoptotic proteins (cytochrome c) from the intermembrane space to the cytosol. These events launch activation of the initiator caspase 9, which in turn triggers the caspase cascade leading to DNA condensation/ fragmentation and cell death.

A variety of oxidative stress causes the mitochondrial dysfunction, which induces the loss of mitochondrial membrane potential. The Bcl2 family of proteins is involved in pro or anti apoptotic processes by interacting with mitochondria (Ekert et al., 1999). Bcl-2 proteins levels were markedly decreased by the combined treatment. These results suggest that the mitochondria apoptotic pathway may be involved in the enhancement of ionizing radiation induced apoptosis.

Compounds interfering with the respiratory chain may promote leakage of electrons and increase intracellular ROS production. Thus, it is presumed that inhibition of the respiratory chain complex I may provoke a radiosensitizing effect by elevating ROS generation and eliciting mitochondrial oxidative stress. Mitochondrial respiratory chain complex I may be a potential target to improve X-ray radiation therapy for enhanced cancerous cell killing, but the underlying mechanism remains to be illustrated (Zhang et al., 2012). Withaferin A may thus

stimulate O2.- production by activating NADPH oxidase of the plasma membrane or by inhibiting complex I or complex III of the electron transport chain (Bras et al., 2005).

Withaferin A -induced apoptosis is mediated by reactive oxygen species (ROS) production due to inhibition of mitochondrial respiration. Withaferin A -mediated ROS production as well as apoptotic histone-associated DNA fragment release into the cytosol was significantly attenuated by ectopic expression of Cu, Zn-superoxide dismutase in both MDA-MB-231 and MCF-7 cells. ROS production resulting from Withaferin A exposure was accompanied by inhibition of oxidative phosphorylation and inhibition of complex III activity (Hahm et al., 2011). Enhanced ROS generation induces dissociation of the I-III supercomplex with the consequent lack of efficient electron channeling from complex I to complex III; moreover, the concomitant disruption of complex I assembly induced by supercomplex dissociation might also account for the decrease of NAD-linked respiration and ATP synthesis (Aurelio et al., 2006).

In all cell lines, the apoptotic process triggered by withaferin A involves the mitochondrial pathway and was associated with Bcl-2 down regulation, Bax mitochondrial translocation, cytochrome c release into the cytosol, transmembrane potential dissipation, caspase 9 and caspase 3 activation and DNA fragmentation. Withaferin A cytotoxicity requires early reactive oxygen species (ROS) production and glutathione depletion (Mayola et al., 2011).

In the current study, treatment of EC bearing mice with Withania somnifera extract/gadolinium III oxide nanocomposite (WSGNC) combined with irradiation prompted to a significant increase in lipid peroxide concentration concomitant with a significant decrease in GSH content and SOD activity in tumor tissue. These results correlates with (Arora, 2008) who reported that a number of mechanisms are involved in radiosenstization, some of the commonly reported mechanisms include: enhanced generation of ROS/RNS, selective depletion of tumor cell antioxidants and antioxidant enzymes, increased of lipid peroxidation, depletion of GSH, elevated levels of lipid peroxidation and DNA damage of tumor cells, formation of DNA adducts, inhibition of DNA repair, inhibition of DNA synthesis, induction of cell cycle arrest, induction of apoptosis and depletion of protein kinase c.

MnSOD is a key antioxidant enzyme that regulates cell transformation, tumor growth, and cell response to stress-inducing therapeutic regimens (Wang et al., 2002). Previous studies have shown that radiation induces cellular reactive oxygen species levels and MnSOD expression. Thus the inhibition of MnSOD expression can enhance the radiosensitivity of tumor cells (Josson et al., 2005).

Mitochondrial transmembrane potentials were dramatically decreased by the combined treatment, with increases in pro-apoptotic Bcl-2-family proteins Bid and Noxa, and downregulation of antiapoptotic Bcl-2 and Mcl-1. Withaferin A enhances hyperthermia-induced apoptosis via a mitochondria-caspase-dependent pathway; its underlying mechanism involves elevated intracellular oxidative stress, mitochondria dysfunction, and JNK activation (Cui et al., 2014).

In the present study, treatment of EC bearing mice with Withania somnifera extract/gadolinium III oxide nanocomposite (WSGNC) combined with irradiation prompted to a highly significant decrease in lipid peroxide concentration a companied with a highly significant increase in GSH content and SOD activityin liver tissue compared to EC group. This result correlates with (Mansour andHafez, 2012) who reported that Withania somnifera pretreatment showed significant decrease in serum hepatic enzymes, malondialdehyde (MDA) levels and DNA damage, and significant increase in antioxidant status. These observations suggest that Withania somnifera could be developed as a potential preventive drug for ionizing irradiation induced hepatotoxicity disorders via enhancing the antioxidant activity. The methanolic extracts of Withania somnifera showed comparable antioxidant activity with standard reference. Withania somnifera ameliorate oxidative stress induced mitochondrial dysfunction in an animal model (Bhaskar and Chintamaneni, 2014).

From the aforementioned results, it is possible to conclude that, Withania somnifera extract gadolinium III oxide nanocomposite has anticancer and radiosensitization effect which could lead to reducing the treatment irradiation dose during radiotherapy.

References

Aghamohammadi A, Hosseinimehr SJ, Ghasemi A, et al (2015). Radiosensitization effects of a *Zataria multiflora* extract on human glioblastoma cells. *Asian Pac J Cancer Prev*, **16**, 7285-90.

Akhila JS, Deepa S, Alwar MC (2007). Acute toxicity studies and determination of median lethal dose. *J Curr Sci*, **93**, 917-20.

Ansari AQ, Ahmed SA, Waheed MA, et al (2013). Extraction and determination of antioxidant activity of *Withania somnifera* Dunal. *Eur J Experimental Biol*, **3**, 502-7.

Arora MS, (2008). Herbal Radiomodulators, Applications in Medicine, Home and Defence & Space. Edited By Rajesh Arora, Cabi International, Biddlesltd, Kings Lynn.

Aurelio M, Gajewski CD, Lenaz G, et al (2006). Respiratory chain supercomplexes set the threshold for respiratory defects in human mtDNA mutant cybrids. *J Hum Mol Gene*t, **15**, 2157-69.

Basha R, Sabnis N, Heym K, et al (2014). Targeted nanoparticles for pediatric leukemia therapy. *J Front Oncol*, **13**, 101.

Bhaskar M, Chintamaneni M, (2014).Withania somnifera and eclipta alba ameliorate oxidative stress induced mitochondrial dysfunction in an animal model of alzheimer's disease. *Am J Phytomedicine Clinical Therapeutics*, **2**, 140-52.

Bras LM, Clement MV, Pervaiz S, et al (2005). Reactive oxygen species and the mitochondrial signaling pathway of cell death. *J Histol Histopathol*, **20**, 205-19.

Barrera G, (2012). Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy. *J ISRN Oncol*, **137289**, 21.

Beutler E, Duron O, and Kelly MB, (1963). Improved method for the determination of blood glutathione. *J Lab Clin Med*, **61**, 882-88.

Ceresa C, Bravin A, Cavaletti, G, et al (2014). The combined therapeutical effect of metal-based drugs and radiation therapy: the present status of research. *J Curr Med Chem*,

21, 2237-65.
Cho S, Lee Y, Lee S, et al (2014). Enhanced cytotoxic and genotoxic effects of gadolinium following ELF-EMF irradiation in human lymphocytes. *J Drug Chem Toxicol*, **37**, 440-7.
Christina AJM, Joseph DG, Packialakshmi M, et al (2004). Anticarcinogenic activity of withania somnifera dunal against daltons ascitic lymploma. *J Ethnopharmacol*, **93**, 359-61.
Cui ZG, Piao JL, Rehman MU, et al (2014). Molecular mechanisms of hyperthermia-induced apoptosis enhanced by withaferin A. *Eur J Pharmacol*, **15**, 99-107.
David M. Euhus MD, Charles Hudd MB, et al (1986).Tumor measurement in the nude mouse. *J Surgical Oncol*, **31**, 229-34.
Ekert PG, Silke J, Vaux DL, (1999). Caspase inhibitor. J Cell Death Differ, 6, 1081-86.
Fruehauf JP, Meyskens FL, (2007). Reactive oxygen species: a breath of life or death? *J Cin Cancer Res*, **13**, 789-94.
Hahm ER, Moura MB, Kelley EE, et al (2011). Withaferin A-induced apoptosis in human breast cancer cells is mediated by reactive oxygen species. *J PLoS One*, **6**, 23354.
Hamburger AW, (1981). Use of in-vitro testes in predictive cancer chemotherapy. *J NCI*, **66**, 981.
Hematulin A, Ingkaninan K, Limpeanchob N, et al (2014). Ethanolic extract from Derris scandens Benth mediates radiosensitzation via two distinct modes of cell death in human colon cancer HT-29 cells. *Asian Pac J Cancer Prev*, **15**, 1871-77.
Henze K, Martin W, Martin W, (2003). "Evolutionary biology: essence of mitochondria". *J Nature*, **426**, 127-8.
Jensen MM, Jorgensen J, Binderu PT, et al (2008). Tumour volume in subcutaneous mouse xenografts measured by micro CT is more is more accurate and reproducible than determined by F-FDG-micro PET or external caliper. *J BMC Medical Imaging*, **8**, 16
Johansson LH, Borg, LA, (1988): A spectrophotometric method for determination of catalase activity in small tissue samples. *J Anal Biochem*, **174**, 331-36.
Josson S, Xu Y, Fang F, et al (2005). RelB regulates manganese superoxide dismutase gene and resistance to ionizing radiation of prostate cancer cells. *J Oncogene*, **25**, 1554-59.
Khazal KF, Simon L, Odemuyiwa S, et al (2013).Withania somnifera extract inhibits invasion and metastasis of MDA-MB-231 cells by inhibiting CCL2 and CD44 high/CD24low stem cells. *J Res*, **73**.
Kroemer G, Galluzzi L, Vandenabeele P, et al (2009). Classification of cell death: recommendations of the nomenclature committee on cell death. *J Cell Death Differ*, **16**, 3-11.
Kwatra D, Venugopa A, Anant S, (2013). Nanoparticles in radiation therapy: a summary of various approaches to enhance radiosensitization in cancer. *J Transl Cancer Res*, **2**, 330-42.
Kwong k, Sohal RS, (2000). Age-related changes in activities of mitochondrial electron transport complexes in various tissues of the mouse. *J Arch Biochem Biophs*, **307**, 16-22.
Lee DH, Lim, IH, Sung EG, et al (2013). Withaferin A inhibits matrix metalloproteinase-9 activity by suppressing the Akt signaling pathway. *J Oncol Rep*, **30**, 933-38.
Lee WH, Loo CY, Young PM, et al (2014).Recent advances in curcumin nanoformulation for cancer therapy. *J Expert Opin Drug Deliv*, **24**, 1-19.
Maliyakkal N, Udupa N, Pai KSR, et al (2013). Cytotoxic and apoptotic activities of extracts of Withania somnifera and Tinosporacordifolia in human breast cancer cells. *Int J Applied Research in Natural Products*, **6**, 1-10.
Mansour HH, Hafez HF, (2012). Protective effect of Withania somnifera against radiation-induced hepatotoxicity in rats. *J Ecotoxicology Environmental Safety*, **80**, 14-19.
Mayola E, Gallerne C, Espoti DD, et al (2011). Withaferin A induces apoptosis in human melanoma cells through generation of reactive oxygen species. *J Apoptosis*, **16**, 1014-27.
Miladi I, Aloy MT, Armandy E, et al (2015). Combining ultrasmall gadolinium-based nanoparticles with photon irradiation overcomes radioresistance of head and neck squamous cell carcinoma. *J Nanomedicine*, **11**, 247-57.
Narang AS, Desai DS, (2009). Anticancer drug development unique aspects of pharmaceutical development. Y. LU, R. I. Mahato (eds), pharmaceutical perspectives of cancer therapeutics, 49-92.
Okamura T, Miura T, Takemura G, et al (2000). Effect of caspase inhibitors on myocardial infarct size and myocyte DNA fragmentation in the ischemia-reperfused rat heart. *J Cardiovascular Res*, **45**, 642-50.
Park JW, Min KT J, Kim DE, et al (2015). Withaferin A induces apoptosis through the generation of thiol oxidation in human head and neck cancer cells. *Int J Mol Med*, **35**, 247-52.
Porter AG, Janicke RU, (1999). Emerging role of caspase-3 in apoptosis (review). *J Cell Death Differ*, **6**, 99-104.
Raviraj J, Bokkasam VK, Kumar et al (2014). Radiosensitizers, radioprotectors, and radiation mitigators. *Ind J Dent Res*, **25**, 83-90.
Rickwood D, Wilson, MT, Darley-Usmar V M, (1978). Isolation and characteristic of intact mitochondria In: V. M. Darley-Usmar, D. Rickwood, M. T. Wilson, (eds). Mitochondria: A practical approach. I. R. L. press, Washington, D. C. pp 4-5.
Roblero-Bartolon GV, Ramon-Gallegos E, (2015). Use of nanoparticles (NP) in photodynamic therapy (PDT) against cancer. *J Gac Med Mex*, **151**, 85-98.
Senthil K, Jayakodi M, Thirugnanasambantham P, et al (2015). Transcriptome analysis reveals *in vitro* cultured Withania somnifera leaf and root tissues as a promising source for targeted withanolide biosynthesis. *J BMC Genomics*, **22**, 14.
Sharma LK, Lu J, Bai Y, (2009). Mitochondrial respiratory complex I: structure, function and implication in human diseases. *J Curr Med Chem*, **16**, 1266-77.
Sinha P, Ostrand-Rosenberg S, (2013). Myeloid-derived suppressor cell function is reduced by Withaferin A, a potent and abundant component of Withaniasomnifera root extract. *J Cancer Immunol Immunother*, **27**, 1663-73.
Sun Y, Oberley LW, Li Y, (1988). A simple method for clinical assay of superoxide dismutase. *J Clin Chem*, **34**, 497-500.
Tiwari R, Chakraborty S, Saminathan M, et al (2014). Ashwagandha (Withania somnifera): Role in safeguarding health, immunomodulatory effects, combating infections and therapeutic applications: A review. *J Biological Sciences*, **14**, 77-94.
Unsoy G, Yalcin S, Khodadust R, et al (2014). Chitosan magnetic nanoparticles for pH responsive Bortezomibrelease in cancer therapy. *J Biomed Pharmacother*, **68**, 641-8.
Wallace DC, (2008). Mitochondria as chi. *J Genetics*, **179**, 727-35.
Wang T, Zhang X, Li JJ, (2002). The role of NF-κB in the regulation of cell stress responses. *Int J Immunopharmacol*, **2**, 1509-20.
Wanger H, Bladt S, Zagaiwski EM, (Eds.) (1984). Plant drug analysis. Springer-verlag, Berlin/New YORK, PP. 126-169
Wilson JK, Sargent JM, Elgie AW, et al (1990). A feasibility for chemosensitivity testing in ovarian malignancy. *Br J Cancer*, **2**, 189-194.
Yang ES, Choi MJ, Kim JH, et al (2011). Withaferin A enhances radiation- induced apoptosis in Caki cells through induction

of reactive oxygen species, Bcl-2 downregulation and Akt inhibition. *Chem-Biol Interact*, **190**, 9-15.

Yoshioka T, Kawada K, Shimada T, et al (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated oxygen toxicity in the blood. *Am J Obstet Gynec*, **135**, 372-76.

Zhang X, Zhou X, Chen, R, et al (2012). Radiosensitization by inhibiting complex I activity in human hepatoma HepG2 cells to X-ray radiation. *J Radiat Res*, **53**, 257-63.