Radioprotective, antioxidant and antitumor efficacy of 
*Annona muricata* L. leaf extract

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Ionizing radiation is known to induce oxidative stress, which can damage critical cellular macromolecules and/or modulate gene expression pathways. This study investigated the effect of *Annona muricata* L. (AM) leaf extract against whole body gamma-irradiation induced oxidative damage in the lung and kidney tissues and determined the antitumor activity of AM using Ehrlich ascites carcinoma (EAC) bearing mice and different tumor cell line. Administration of AM (100 mg/kg, by tube) prior to irradiation (6 Gy) significantly attenuated serum lipid profiles, decreased malondialdehyde and total nitrate/nitrite levels, DNA fragmentation and significantly increased caspase-3 and superoxide dismutase activity, glutathione content and expression of glutathione peroxidase in the lung and kidney tissues compared with irradiated group. AM interestingly increased survival rate and decreased tumor volume of EAC-bearing mice more than the irradiated group. It was found to be selectively cytotoxic *in vitro* to tumor cell lines (PC3, MCF7, HELA, H1299 and A549). In conclusion, AM extract exhibits potential antitumor activity and attenuates radiation-induced toxicity by preventing oxidative stress and preserving the antioxidant activities.

**Keywords:** Antitumor activity ionizing radiation, Oxidative stress, Real-time polymerase chain reaction

Cancer has been considered as the major cause of mortality and morbidity globally as estimated by the World Health Organization. Radiotherapy is a cornerstone in cancer treatment\(^1\). However, ionizing radiation produces reactive oxygen species (ROS) which is known to damage cellular components in healthy cells, leading to damaged bases and DNA breaks, resulting in mutagenesis, carcinogenesis, and consequential cell death\(^3\). This resulting oxidative stress can damage critical cellular macromolecules and/or modulate gene expression pathways. ROS negatively impact the antioxidant defense mechanisms, leads to depletion of the endogenous antioxidants and finally to the development of systemic disease\(^3\).

*Annona muricata* L. (AM), (Soursop) a member of the Annonaceae family, is a fruit tree that is mostly distributed in tropical and subtropical regions of the world. It has been used to cure a wide range of human diseases, including inflammation, rheumatism, diabetes, hypertension, insomnia, and cancer\(^4\). AM leaves are known to be rich in flavonoids, tannins, lactone, coumarin, procyanidins, isoquinoline alkaloids and annonaceous acetogenins which have a therapeutic and chemopreventive effect\(^5\). Annonaceous acetogenins are derivatives of long chain fatty acids derived from the polyketide pathway that are selectively toxic to cancer cells, including multidrug resistant cancer cell lines\(^6\). They induce cytotoxicity by inhibiting the mitochondrial complex I, which is involved in ATP synthesis. As cancer cells have a higher request for ATP than the normal cells, mitochondrial complex I inhibitors have potential in cancer therapeutics\(^7\). Previous studies revealed that AM extract induced the necrosis of cancerous cells by inhibiting cellular metabolism\(^8,9\). The leaves of the plant are reported to possess enzymatic antioxidants, including catalase and superoxide dismutase, and non-enzymatic antioxidants, including vitamin C and E\(^10\).

Therefore, the current study was designed to evaluate the possible protective role of AM leaf extract as a natural product against gamma radiation-
induced oxidative stress and tissue injury in rats and to evaluate the antitumor activity and IC\textsubscript{50} of AM leaf extract using Ehrlich Ascites Carcinoma (EAC) bearing mice and different tumor cell lines.

**Material and Methods**

**Animals**

The present study was performed using adult male albino Wistar rats weighing (150–180 g). Rats were reared in the Organization for Biological Products and Vaccines (VACSERA, Egypt). Female Swiss albino mice weighing (18–20 g) were obtained from an animal facility, Pharmacology Unit, National Cancer Institute (NCI), Cairo University, Egypt. The animals were housed prior to and after irradiation at standard conditions; temperature (20–25°C), food and water were provided ad Libitum, and with 13/11 light/dark rhythm. The animals' treatment protocol has been approved by the animal care committee of the National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt, following the guidelines of NIH and the Institutional Animal Care and Use Committee Guidelines.

**Drugs and Chemicals**

*Annona muricata* (leaf powder) extract capsules manufactured in GMP facility, USA. Each capsule contains 750 mg of AM leaf powder and was diluted to the required concentration before use. All other chemicals and solvents used were of the highest purity grade available.

**Human tumor cell lines**

Human tumor cell lines (prostatic small cell carcinoma; PC3, Breast carcinoma; MCF7, Lung carcinoma; H1299 & A549, Cervix carcinoma; HELA) were obtained frozen in liquid nitrogen (−180°C) from the American Type Culture Collection and grown as monolayer culture in RPMI-1640 medium supplemented with 10% FBS and 100 units/mL penicillin and 2 mg/mL streptomycin. The cell lines were incubated at 37°C in 5% CO\textsubscript{2}–95% air in a high humidity atmosphere in the water-jacketed incubator (Revco, GS laboratory equipment, RCO 3000 TVBB, USA). The tumor cell lines were maintained in the NCI, Cairo, Egypt, by serial sub-culturing.

Ehrlich Ascites Carcinoma Cell Line (EAC) was obtained from Cancer Biology Dept., NCI, Cairo University. EAC line was maintained by serial intraperitoneal injections (inoculation) in female Swiss albino mice at 7–10 day intervals. After the tumor inoculation, an ascites rich with tumor cells was produced. The inoculated mice lived for about 10–20 days.

**Irradiation**

Whole body gamma irradiation (IRR) was performed at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt, using an AECL (137 cesium) Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.012 Gy/s at the time of the experiment.

**Experimental design (in vivo)**

Effect of AM against Radiation-induced toxicity in rats

Forty male albino rats were divided randomly into four groups (n=10). Rats in group I served as a sham-treated group with 0.5 mL normal saline administered for 14 consecutive days. Rats in group II were administered AM extract (100 mg/kg, by tube) for 14 consecutive days\textsuperscript{11}. Rats in group III were administered vehicle by tube for 14 consecutive days, then exposed to single-dose γ-irradiation (IRR) (6 Gy). Rats in the group (IV) were received AM (100 mg /kg, by tube) for 14 consecutive days, one hour later after the last dose, rats were exposed to single-dose γ-irradiation (6 Gy).

Twenty-four hours after the last treatment, the animals were anesthetized through exposure to ether in desiccators kept in a well-functioning hood. Blood samples were harvested by heart puncture and serum samples were separated. Lungs and kidneys were quickly excised, washed with saline, blotted with a piece of filter paper and homogenized using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA). The homogenates were centrifuged at 800 × g for 5 min at 4°C to separate the nuclear debris. The obtained supernatant was centrifuged at 10500 × g for 20 min at 4°C to get the post-mitochondrial supernatant which was used for the determination of oxidative stress biomarkers.

The serum lipid profile was determined using a standard commercial kit (Spectrum diagnostics, Cairo, Egypt). Serum triglycerides (TG), total cholesterol (CH), high-density lipoprotein-cholesterol (HDL) were determined according to the methods described by Tietz et al.\textsuperscript{12}, Ellefson & Caraway\textsuperscript{13}, and Warnick & Wood\textsuperscript{14}, respectively. The low-density lipoprotein-cholesterol (LDL) was calculated according to Terpstra et al.\textsuperscript{15}.

Superoxide dismutase (SOD) activity, malondialdehyde (MDA) and reduced glutathione
(GSH) content in the lung and kidney homogenates were assessed according to the methods of Minami & Yoshikawa, Buege & Aust, and Ellman, respectively. The total nitrate/nitrite (NO$_3$) was measured according to Miranda et al.

Detection of glutathione peroxidase (GPx) gene expression by real-time-PCR in lung and kidney tissues

Total RNA was extracted from tissues using total RNA purification kit (Bioscience, Munich, Germany) according to the manufacturer's protocol. The quantity and integrity of total RNA were checked by a 260/280 optical density ratio. Total RNA (1 μg) was reverse-transcribed into complementary DNA (cDNA) and 1 μL of cDNA/sample was then amplified using qPCR Green Master from (Bioscience GmbH), using real-time cycler conditions of 95°C and 5 min (Initial denaturation), followed by 35 cycles of 95°C, 30 sec, 55°C, 1 min and 72°C, 30 sec for denaturation, annealing and extension steps, respectively. All primers used in this study were shown in Table 1. mRNA levels were calculated relative to β-actin as a housekeeping gene. Analysis of data was performed by using the 2$^{-\Delta\Delta Ct}$ method.

Determination of DNA fragmentation by agarose gel electrophoresis

The tissues were homogenized and lysed in a cold lysis buffer (10 mM Tris-HCl, 5 mM disodium EDTA, and 0.5% Triton X-100, pH 8.0) for 10 min at 4°C. Then DNA was sequentially extracted twice using half volumes of phenol/chloroform and incubated at 55°C for 10 min. After centrifugation at 800 × g for 20 min, the upper layer was incubated with proteinase K at 37°C for 60 min followed by incubation with ribonuclease at 37°C for 60 min. About 10 M ammonium acetate and 100% ethanol were added to precipitate the DNA, then maintained at −20°C overnight. The resulting DNA was electrophoresed through a 1.4% agarose gel containing ethidium bromide using TBE buffer (Tris-boric acid–EDTA buffer, pH 8.3) at 40 V for 5 h.

Equal quantities of DNA (based on optical density measurements at 260 nm) were loaded in each lane, and a molecular DNA marker was used as a molecular mass standard. DNA fragmentation was visualized and photographed under ultraviolet illumination.

Determination of apoptosis markers by western blotting

Lung and kidney were homogenized with phosphate buffer saline, then centrifuged at 105 000 × g at 4°C for 15 min. Protein content was determined in the supernatant using Bradford reagent (Thermo Scientific, USA). Equal amounts of proteins from each sample and SDS-reducing buffer were boiled for 5 min, electrophoresed on SDS-polyacrylamide gels then transferred to nitrocellulose membranes. The membrane was blocked with dilution buffer plus 1% Tween-20 (DBT) plus 1% bovine serum albumin (BSA) and the membrane blots were incubated with indicated primary antibodies (in DBT) at 4°C overnight, then washed three times with DBT buffer, then incubated with horseradish peroxidase-conjugated secondary antibody for 4 h at room temperature. After washing three times with DBT buffer, the protein bands were visualized by 3,3′-Diaminobenzidine chromogen which gives a brown precipitate at the reaction site. Specific protein bands on these transferred membranes were detected using the following antibodies: Anti-mouse caspase 3 monoclonal antibody and purified anti β-actin antibody were obtained from (Bioscience, USA). Relative expression of proteins was evaluated by normalizing the expression of proteins with quantitative housekeeping protein β-actin.

Histopathological study

Tissue specimens from kidney and lung were collected and fixed in 10% buffered formalin solution and fixed tissues were dehydrated, cleared and embedded in natural paraffin. Section of 5-micron thickness was prepared and stained routinely with hematoxylin and eosin and examined microscopically.

Antitumor activity

Percentage survival of animals

Forty female albino mice inoculated with EAC cells (2.5 × 10$^6$ cells/0.1 mL). Each mouse was injected i.p. with EAC cells. After 24 h of cell inoculation, 40 mice were classified into four groups. The group I; animals were injected with 0.2 mL of saline and served as control. Group II; animals were

| Table 1 — Primers of genes Gpx1, and β-Act used for real-time PCR |
|----------------|----------------|
| Primer         | Sequence      |
| Gpx (forward)  | 5’-AGTTCGGACATCAGGAGAATGGCA-3’ |
| Gpx (reverse)  | 5’-TCACCATTCACCTCGCACTTCTCA-3’ |
| β-Act forward  | 5’-TGTTTGAGACCTTCAACACC-3’   |
| β-Act (reverse)| 5’-TAGGAGCCAGGGCAGTAATC-3’   |
injected once with AM (100 mg/kg, i.p.). Group III; animals were exposed to a whole-body gamma radiation (6 Gy) Group IV; animals were injected once with AM (100 mg/kg, i.p.). After an hour, animals were exposed to a whole-body gamma radiation (6 Gy). The change in the percent survival of animals was recorded daily for a period of 45 days following treatment by using formula,

Percent survival = number of living animals/10 × 100.

Tumor volume
Forty female mice were used and the solid tumor was transplanted subcutaneously in the right thigh of the lower limb of each mouse. Mice with a palpable tumor mass (100 mm³) that developed within 7 days after implantation were divided into 4 groups: Group I; 10 mice were injected with 0.2 mL of saline and served as controls. Group II; ten mice were injected once with AM (100 mg/kg, i.p.). Group III; ten mice were exposed to a whole-body gamma radiation (6 Gy). Group IV; ten mice were injected once with AM (100 mg/kg, i.p.), then after an hour, animals were exposed to a whole-body gamma radiation (6 Gy). The change in tumor volume was measured every other day using a vernier caliper and calculated by following formula according to Osman et al. 25.

Tumor Volume (mm³) = 4 π(A/2)²X (B/2)/3, where, A and B indicated the minor and major tumor axis, respectively.

Cytotoxicity Assay: (In vitro study)
Cytotoxicity was measured by the Sulphorhodamine-B (SRB) method according to Skehan et al. 26 and expressed as the survival fraction compared with untreated control cells. PC3, HELA, MCF7, A549, and H1299 cell lines were seeded in 96-well microtiter plates at a concentration of 5 × 10³ cell/well in a fresh medium and left to attach to the plates for 24 h. After 24 h, cells were either treated with AM at various concentrations ranging from 50–500 μg and completed to 200 μL volume/well using fresh medium and incubated for 48 h. Control cells were treated with vehicle alone. For each drug concentration, 3 wells were used. The cells were fixed with 10% trichloroacetic acid for 1 h at 4°C. Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The wells were then washed 4 times with 1% acetic acid. The plates were air-dried and the dye was solubilized with 10 mm Tris base (pH 10.5) for 5 min on a shaker (Orbital shaker OS 20, Boeco, Germany) at 1600 rpm. The optical density was measured spectrophotometrically at 564 nm with an ELISA microplate reader (Meter tech. Σ 960, U.S.A.). The percentage of cell survival was calculated as follows:

Survival fraction = O.D. (Treated cells)/O.D. (Control cells)

The IC₅₀ values (the concentrations required to produce 50% inhibition of cell growth) were calculated from the equation of the dose-response curve. The experiment was repeated 3 times for each treatment.

Statistical analysis
Differences between the obtained values (mean ± SEM, N = 6) were carried out by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison tests. AP value of 0.05 or less was taken as a criterion for a statistically significant difference.

Results

Effect on serum lipid profile
Exposure to γ-irradiation (6 Gy) induced a significant increase in the serum levels of TG, CH, and LDL and the significant decrease in the level of HDL compared to the control group (Table 2). On the other hand, the administration of AM extract for 14 consecutive days had no significant effect on serum lipid profiles compared to the control group. The administration of AM extract prior to irradiation resulted in a significant recovery in TG, CH, and LDL levels, and increased HDL levels compared to the irradiated group.

Oxidative stress markers
Figure 1 shows the effects of AM, IRR and their combination of levels of GSH, MDA, and NO(x) and the activity of SOD in lung and kidney tissues.

Table 2 — Effect of gamma-irradiation (IRR), Annona Muricata(AM) and their combination on the level of triglycerides (TG), cholesterol (CH), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in the serum of male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TG (mg/dL)</th>
<th>CH (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.16±5.02</td>
<td>97.39±7.47</td>
<td>48.49±3.35</td>
<td>45.60±2.16</td>
</tr>
<tr>
<td>IRR</td>
<td>105.22±6.41</td>
<td>123.03±11.41</td>
<td>31.48±2.63</td>
<td>81.55±8.08</td>
</tr>
<tr>
<td>AM</td>
<td>65.17±5.69</td>
<td>96.88±7.48</td>
<td>50.93±4.55</td>
<td>48.95±4.51</td>
</tr>
<tr>
<td>AM+IRR</td>
<td>75.41±3.79</td>
<td>97.79±6.63</td>
<td>43.79±4.81</td>
<td>60.99±3.66</td>
</tr>
</tbody>
</table>

The values are expressed as mean±S.E.M of 10 rats/ group. P value is significant ≤ 0.05 using ANOVA followed by Tukey–Kramer as a post ANOVA test. a, b Significantly different from the control group and IRR treated group, respectively.
Gamma-irradiation exposure resulted in a significant decrease by (48.7% and 49.8%) in SOD activity and significant decrease by (26.1% and 21.2%) in GSH content and a significant increase by (56% and 94.7%) in MDA and significant increase by (154.4% and 237.8%) in NO(x), in lung and kidney tissues, respectively, as compared to the corresponding control group. Treatment with AM for 14 consecutive days prior to irradiation resulted in a significant increase by (81.7% and 67.6%) in the activity of SOD and by (19.4% and 23%) in GSH content in lung and kidney tissues, respectively, as compared to the corresponding control group. Treatment with AM for 14 consecutive days prior to irradiation resulted in a significant increase by (81.7% and 67.6%) in the activity of SOD and by (19.4% and 23%) in GSH content in lung and kidney tissues, respectively, as compared to the corresponding control group.

DNA fragmentation

Figure 3A & B shows that γ-irradiation induced significant DNA fragmentation in lung and kidney tissues (lane 3). Control sample and AM sample (lane 1 and lane 2) did not show any band indicating no DNA fragmentation. Pretreatment with AM extract prior to irradiation showed a small release of DNA (lane 4) compared to lane 3 in kidney tissue while there is still an observed DNA fragmentation in lung tissue.

Effect on the level of caspase 3 protein

Exposure of rats to 6 Gy γ-rays showed an increase in the caspase-3 activation in lung and kidney tissues that executes the apoptosis while pretreatment with AM partly reversed the activation of caspase-3 indicating inhibition of apoptosis (Fig. 3C & D).

Histopathological findings

Histological examinations of sections of the kidney of the control animals have revealed the normal structure of nephrotic tissues. Numerous tubules (proximal & distal) lie adjacent to renal corpuscles; contain a single layer of cuboidal epithelium (Fig. 4A). Rats in irradiated group, renal cortex showed atrophied glomeruli, the rest of glomerular tuft is shrinking due to slight fibrosis. Widened Bowman’s space and thickened basement membrane were seen. The convoluted tubules showed distinctive patterns of ischemic renal injury represented by losing its details with partial or complete obstruction of its lumens, the cuboidal epithelium cells of proximal and distal renal tubules showed nuclear changes mainly pyknosis and karyolysis with hyalinization of some tubules (Fig. 4B & C). The kidney in treated group...
was used as control. In the treated irradiated group, mostly, the histological structure of kidney showed relatively well-preserved architecture without degenerative changes but in few cases showed slightly congested blood vessels (Fig. 4D).

Histopathological examination of the lung in the irradiated group showed normal histological structure as a control, while in a few cases slightly histological changes in form of a collapse of some alveoli with mild proliferative pneumocytes type II, dilated alveolar capillary with a thickness of inter-alveolar septa (Fig. 4F & G). Lung of the AM-treated group showing normal histological structure as a control. Lung of the treated irradiated group showing normal histological structure (Fig. 4H).

**Percent survival of animals**

Figure 5A shows the percent survival of EAC-bearing mice treated with AM (100 mg/kg single i.p.) and/or irradiation (6 Gy). None of the control untreated tumor-bearing mice survived on day 22, while on day 38 of the experiment, none of the animals survived in the AM treated group. On the other hand, on day 26, none of the irradiated mice survived, whereas none of the mice treated with AM and irradiated survived at day 30.

**Tumor volume**

Figure 5B shows the effect of AM (100 mg/kg), gamma-irradiation (6 Gy) and their combination on the growth of solid Ehrlich carcinoma. The tumor volume of the control group showed a progressive increase, whereas, treatment of mice with AM resulted in a significant decrease in tumor volume as compared to control group. Irradiation alone induced a significant decrease in tumor volume compared to control group. Pre-treatment with AM in the
combined group resulted in a significant decrease in tumor volume compared to control group.

**Cytotoxicity**

In this study AM induced 50% survival in PC3 cell line at (IC$_{50}$ =80 ug), in MCF7 cell line at (IC$_{50}$ = 220 ug). However, only 50% survival was achieved in HELA cell line at (IC$_{50}$ =100 ug), in H1299 cell line at (IC$_{50}$ = 146 ug) and in A549 cell line at (IC$_{50}$ = 194 ug) (Fig. 6). In the present study, AM administration prior irradiation ameliorated the lipid profile level compared to control and irradiated rat groups. These alterations could be valuable in preventing irradiation complications as well as in improving lipid metabolism. This effect could be due to its ROS-scavenging activity.$^{30}$

In this study, a significant increase in the levels of MDA and NO(x) and a significant decrease in the activity of SOD and GSH content and GiPx gene expression were observed in the kidney and lung tissues of γ-irradiated rats, indicating the presence of radiation-induced oxidative damage. These results comply with Liu et al.$^{31}$ study, who have revealed that the ionizing radiation affects negatively on the antioxidant enzymes and then induces the ROS. Consequently, increases the lipid peroxidation, which leads to increase in the MDA level, which may contribute to impaired cellular function and necrosis.

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**Discussion**

It is well known that ionizing radiation induces oxidative stress through the generation of ROS resulting in an imbalance in pro-oxidant/antioxidant status in the cells.$^1$ In the present study, whole body γ-irradiation of rats induced the significant increase in the levels of serum TC, TG and, LDL associated with a decline in the HDL level. This might be due to the attack of free radicals on the fatty acid components of membrane lipids.$^{27}$ The whole body γ-irradiation of rats produced high levels of serum cholesterol fractions through its release from tissues, destruction of cell membranes and increases the rate of cholesterol biosynthesis in the liver and other tissues, as an early reaction necessary for the restoration of biomembranes.$^{28}$ In addition, radiation could modify LDL and HDL metabolism indirectly through the action of various inflammatory products and might decrease the lipoprotein lipase activity in adipose tissues, leading to a reduction in the uptake of lipids.$^{29}$

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The decreased level of GSH in γ-irradiated rats may be due to their utilization by the enhanced production of ROS$^3$. Glutathione peroxidase plays an important role in the defense mechanisms of mammals against damage by catalyzing the reduction of H$_2$O$_2$ and hydroperoxides to water and alcohols respectively, consuming GSH as the hydrogen donor and its depletion leads to GiPx inactivation. The significant decrease in the expression of GiPx of irradiated rats could be attributed to its inactivation by lipid peroxidation byproducts.$^{32}$ The enhancement of NO production following exposure to a high dose (6 Gy) of γ-irradiation might be due to direct DNA damage where DNA damage-activated poly (ADP-ribose) polymerase (PARP), which induced nuclear factor kappa B (NF-kB) activation, finally resulting in increased inducible nitric oxide synthase expression and NO production$^3$.

In this study, administration of AM induced a significant increase in the SOD activity, GSH content and GiPx expression and significantly decreased the level of MDA and NO(x) in the lung and kidney tissues compared to the irradiated group. Based on these results, the AM prevent the oxidative injury which has agreed with previous studies revealed that AM has antioxidant effects and ROS-scavenging activity.$^{4,30,33}$ The effect could be due to the antioxidant properties of its acetogenins.

Many studies demonstrated that the exposure to ionizing radiation induces the cellular DNA lesions including bases and sugars damage, single and double
strand breaks and DNA-DNA or DNA-protein cross-links\(^\text{27,34}\). The present study showed an increase in the cellular DNA damage in the lung and kidney tissues of \(\gamma\)-irradiated rats detected by the elevation the percentage of DNA fragmentation and the visualization of DNA ladders upon gel electrophoresis which is an indicator of radiation-induced DNA damage. Furthermore, our results showed that the treatment of rats with the AM prior to \(\gamma\)-irradiation prevented the DNA damage in the kidney induced by irradiation compared to the control group; while there is still an observed DNA fragmentation in lung tissue.

In agreement with our results, George et al.\(^\text{30}\) reported that aqueous leaf extract of AM revealed marked antioxidative activity accompanied by DNA protective effects against gamma-irradiation-induced toxicity. The ability of AM to prevent DNA fragmentation may be due to their radical scavenging potentials of its acetogenins.

Apoptosis (a programmed cell death) is a common mode of action of chemotherapeutic agents, including the natural product-derived drugs\(^\text{35}\). Apoptosis includes specific biochemical and morphological changes such as chromatin condensation, membrane blebbing, cell shrinkage and DNA fragmentation. Loss of mitochondrial membrane potential (MMP) is a distinctive evidence for apoptosis occurs during the early stage of apoptosis before the cell morphology changes\(^\text{35}\). The sharp decrease in the MMP indicates the irreversible incidence of early apoptosis due to an increase in the permeability of the mitochondrial membrane followed by the release of apoptotic factors, including cytochrome c\(^\text{36}\).

Radiotherapy, induces cell death in different forms, including mitotic catastrophe, apoptosis, autophagy, and senescence\(^\text{34}\). Generation of ROS within the mitochondria after irradiation has been shown to release cytochrome c, activate caspase and mediate apoptosis. Excessive ROS caused oxidative damage and loss of MMP, impaired the membrane integrity, and activate mitogen-activated protein kinases (MAPK) pathways\(^\text{37}\).

Treatment of rats with AM before irradiation partly inhibited \(\gamma\)-irradiation-induced apoptosis. Phytochemical investigation of the leaf of AM showed the presence of alkaloids, essential oils and acetogenins. These acetogenins demonstrated to be selectively toxic against various types of the cancerous cells without harming healthy cells\(^\text{38}\). Previous studies revealed that acetogenins act as a DNA topoisomerase I poison, arrested cancer cells at the G1 phase and induced apoptotic cell death in a Bax and caspase-3-related pathways, and inhibited NADH-ubiquinone oxidoreductase (complex I) in mitochondria. It can be suggested that the synergistic effects of phytochemicals present in this plant extracts including acetogenins might be responsible for their antiproliferation activity through the significant decrease in the MMP induction of apoptosis\(^\text{39}\). Acetogenins are inhibitors of enzyme processes that are only found in the membranes of cancerous tumor cells\(^\text{1,39,40}\). This is why they showed cytotoxicity towards cancer cells, but have no toxicity to healthy cells.

Radiotherapy used in cancer treatment destroys the tumor through the induction of the DNA damage in the tumor cells. The damage to the malignant cells leads to induction of the cell death and subsequent reduction in the tumor volume\(^\text{41}\). The current study revealed that the whole-body irradiation suppresses the EAC growth and as a result, the tumor volume is decreased and induced a significant difference in the survival rate compared to the control group.

Our study demonstrated that the AM decreased tumor volume and increased survival rate compared with the untreated control. These results agree with the previous studies which demonstrated that AM has selective toxicity to tumor cells at very low dosages\(^\text{5,40}\). The combined effect of the AM and IRR on the percent survival and tumor volume in our study was less than the individual drug. Interestingly, AM increased survival rate and decreased tumor volume of EAC-bearing mice more than the irradiated group. Also, this effect might be due to the antioxidant activity of AM. Therefore, AM could be a good candidate with irradiation to enhance tumor cell killing without harming the healthy cells.

**Conclusion**

The present study demonstrates that the AM induces certain cytotoxicity on different tumor cell lines, increase the percent survival, decrease tumor volume of EAC-tumor bearing mice, and ameliorated IRR-induced lung and kidney toxicities. All these parameters suggest that AM exhibits potential antitumor and antioxidant activities and has a radioprotective effect against gamma-irradiation by diminishing oxidative stress, scavenging free radicals and enhancing antioxidant enzymes.
References


