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Radiosensitizing effects of 2-deoxy-D-glucose and ferulic acid on mouse Ehrlich's Ascites Carcinoma in Swiss albino mice

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ABSTRACT:

The objective of the present study is to evaluate the radiosensitizing activity of combination of 2-deoxy-D-glucose (2DG) and ferulic acid (FA) against Ehrlich's Ascites Carcinoma (EAC) in Swiss albino mice. Extensive DNA damage has been observed in the cells collected from the peritoneal cavity of combined treatment group (2DG+FA+IR) than the other treatment modalities. Treatment of EAC tumor bearing mice with 2DG and FA before 8 Gy of hemi-body γ-radiation has resulted in the significant decrease of tumor volume and tumor weight compared with EAC control group. Further, combination of 2DG and FA along with radiation decreased the viability of tumor cells in the peritoneal fluid of EAC bearing mice. It has been also found that the percentage of EAC apoptotic cells in the peritoneal fluid has been significantly increased in the combined treatment group (2DG+FA+IR) compared with other treatment groups. Combined treatment of 2DG and FA activated caspase-3 and 9, when compared with the treatment given with radiation alone indicated radiosensitizing effect of this combination (2DG+FA). This has been further evidenced by the massive decrease of LDH activity in the 2DG+FA+IR group. On other hand, 2DG and FA combination protects the hematological changes occurred during radiation treatment. Histopathological examinations showed that the combination of 2DG and FA offers protection to normal tissues during radiation treatment. Taken together, the results of the study clearly suggested 2DG and FA combination sensitizes EAC bearing mice to radiation effects at the same time offers protection to normal tissues from radiation-induced damage.

Keywords:
Ehrlich ascites carcinoma; radiosensitization; apoptosis; 2-deoxy-D-glucose; ferulic acid.

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INTRODUCTION

Radiotherapy is one of the major treatment options in cancer management. About 52% of cancer patients receive radiotherapy at least once during their treatment period (Delaney *et al*., 2005).The goal of radiotherapy is to destroy or inactivate cancer cells while preserving the integrity of normal tissues within the treatment field. Many tumors develop resistance to radiotherapy when given alone for prolonged periods. In most cases radiotherapy is given along with surgery or chemotherapy. Newer approaches in 'combination therapy' are gaining momentum because of the beneficial effects offered. The most frequently used combination of chemotherapeutic agents includes cis-dichlorodiamine-platinum (II), paclitaxel, docetaxel, gemcitabine and topotecan (Dimitroulis *et al*., 2006). To reduce the toxic effects of combination regimens, new class of compounds are required which shall reduce the side effects and give optimum therapeutic benefits. Combination regimens using phytochemicals and metabolic analogs may be suited to alleviate the side effects of radiotherapy and chemotherapy and increase the effect of radiation on neoplastic cells, while protecting normal cells from radiation damage (Rao *et al*., 2008).

2-deoxy-D-glucose (2DG), an analog of glucose, is a glycolytic inhibitor. After the formation of glucose 6-phosphate (via hexokinase) the major pathways of glucose metabolism include glycolysis and the pentose phosphate cycle (Sharma *et al*., 2007). Glycolysis results in the formation of pyruvate and the pentose phosphate pathway results in the formation of NADPH (Sharma *et al*., 2007). Pyruvate, in addition to being a substrate for the formation of acetyl-CoA and energy metabolism via the tricarboxylic acid (TCA) cycle and mitochondrial oxidative phosphorylation, has been shown to scavenge hydrogen peroxide and other hydro peroxides (Ahmad *et al*., 2004). NADPH, by virtue of being the source of reducing equivalents for the glutathione/glutathione peroxidase/glutathione reductase system, has also been shown to participate in the metabolic decomposition of hydrogen peroxide and organic hydroperoxides (Ahmad *et al*., 2004). Therefore, in addition to its well known role in energy production, glucose metabolism appears to be integrally related to the metabolic detoxification of intracellular hydroperoxides formed as byproducts of oxidative metabolism. It was reported that glucose deprivation causes cytotoxicity in the MCF-7/ADR human multidrug-resistant breast carcinoma cell line (Lee *et al*., 1998). 2DG inhibits growth and induces apoptosis in a number of cancer cell lines (Lee *et al*., 1998). 2DG exhibits a cytotoxic effect in cancer cells, but the same dose spares normal cells (Reddy and Prasad, 2011). In addition, the cytotoxic effect of 2DG is greater in cancer cells that exhibit mitochondrial respiratory defects (Gogvadze *et al*., 2008). Many mechanisms are postulated to contribute to the antitumor effect of 2DG, including inhibition of glucose transport and hexokinase II activity, depletion of cellular ATP, blockage of cell cycle progression, induction of apoptosis, induction of endoplasmic reticulum stress, and/or induction of oxidative stress (Coleman *et al*., 2008). 2DG significantly sensitizes human osteosarcoma and non-small cell lung cancer to adriamycin and paclitaxel in mouse models (Maschek *et al*., 2004).

Plant phenolics could fit as promising adjuvants for radiotherapy by enhancing radiosensitivity of cancer cells and by decreasing radiation effects in normal cells (Kim *et al*., 2011). Radiosensitizing effects of many phytochemicals are thought to interact with several intracellular signaling molecules which then mediate signaling cascades including cell cycle arrest and cell death (Kim *et al*., 2011). Curcumin, a polyphenol, has been reported to confer radiosensitizing effect in relatively radio- resistant prostate cancer cell line PC-3 (Chendil *et al*., 2004). Ferulic acid (FA) (4-hydroxy-3-methoxycinnamic acid) ($C_{10}H_{10}O_4$) is a

Figure 1

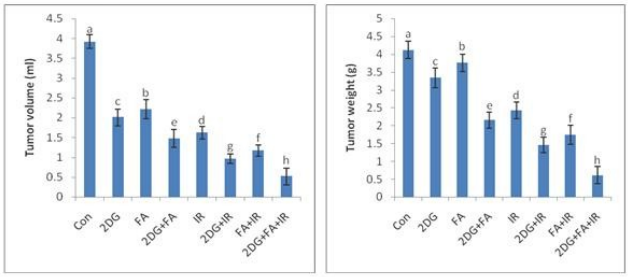


Figure 1 Effect of 2DG, FA and/or IR on (a) tumor volume and (b) tumor weight in EAC bearing mice. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a,b,c…etc) differ significantly at p<0.05 (DMRT).

Figure 2

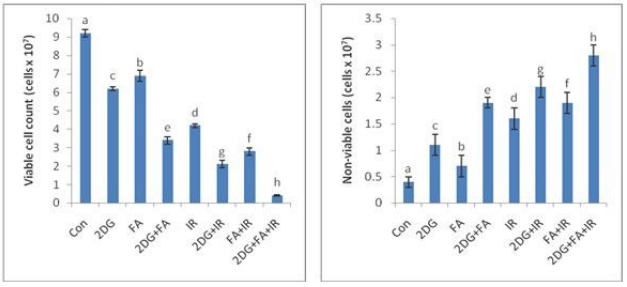


Figure 2 Effect of 2DG, FA and/or IR on (a) viable cell count and (b) nonviable cell count in EAC bearing mice. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a,b,c…etc) differ significantly at *p*<0.05 (DMRT).

ubiquitous phenolic compound in plant tissues; therefore, it constitutes a bioactive ingredient of many foods. Prooxidant activity of hydroxycinnamic acids in different experimental models has been previously reported (Zheng *et al*., 2008). It has been reported earlier from our laboratory that FA sensitizes human cervical carcinoma cells to radiation and this property is attributed to its pro-oxidant nature (Karthikeyan *et al*., 2011). Further, FA in combination with 2DG enhanced radiation effects in NCI-H460 cells *in vitro* (Reddy and Prasad, 2011). There were number of experimental models available to study *in vivo* anticancer effect of test compounds. EAC is one of the models still best suitable to study radiosensitizing mechanisms due to its rapid growth rate and undifferentiated nature (Ozaslan *et al*., 2011). Hence, in the present study, an attempt has been made to evaluate the radiosensitizing potential of 2DG and FA in EAC bearing experimental animals.

MATERIALS AND METHODS

Chemicals

2-deoxy-D-glucose, ferulic acid, ethidium bromide and acridine orange were purchased from Sigma Chemicals Co., St. Louis, USA. ApoAlert caspase 3 and 9 fluorescent assay kit was purchased from Clontech Laboratories, Inc., Canada. All other chemicals and solvents used in the study were of the highest purity and were purchased from Himedia and NICE chemicals, Mumbai, India.

Animal care and handling

Ten- to twelve-week-old female Swiss albino mice weighing 25 to 30 g were selected from an inbred colony maintained under the controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and light (14 and 10 h of light and dark, respectively). The animals had free access to sterile food and water. The animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. The study was approved by the Institutional Animal Ethical Committee of Annamalai University,

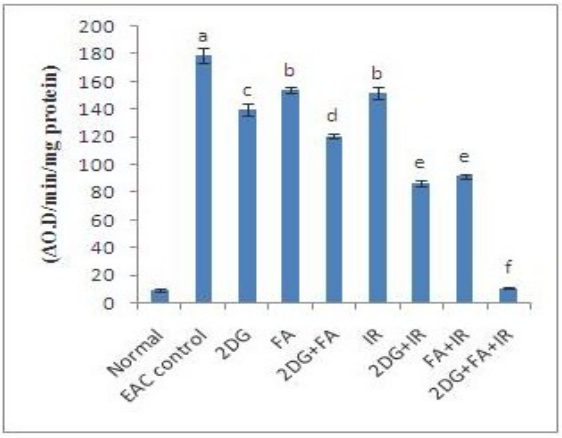


Figure 3 Effect of 2DG, FA and/or IR on serum LDH activity in EAC bearing mice. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a,b,c…etc) differ significantly at p<0.05 (DMRT).

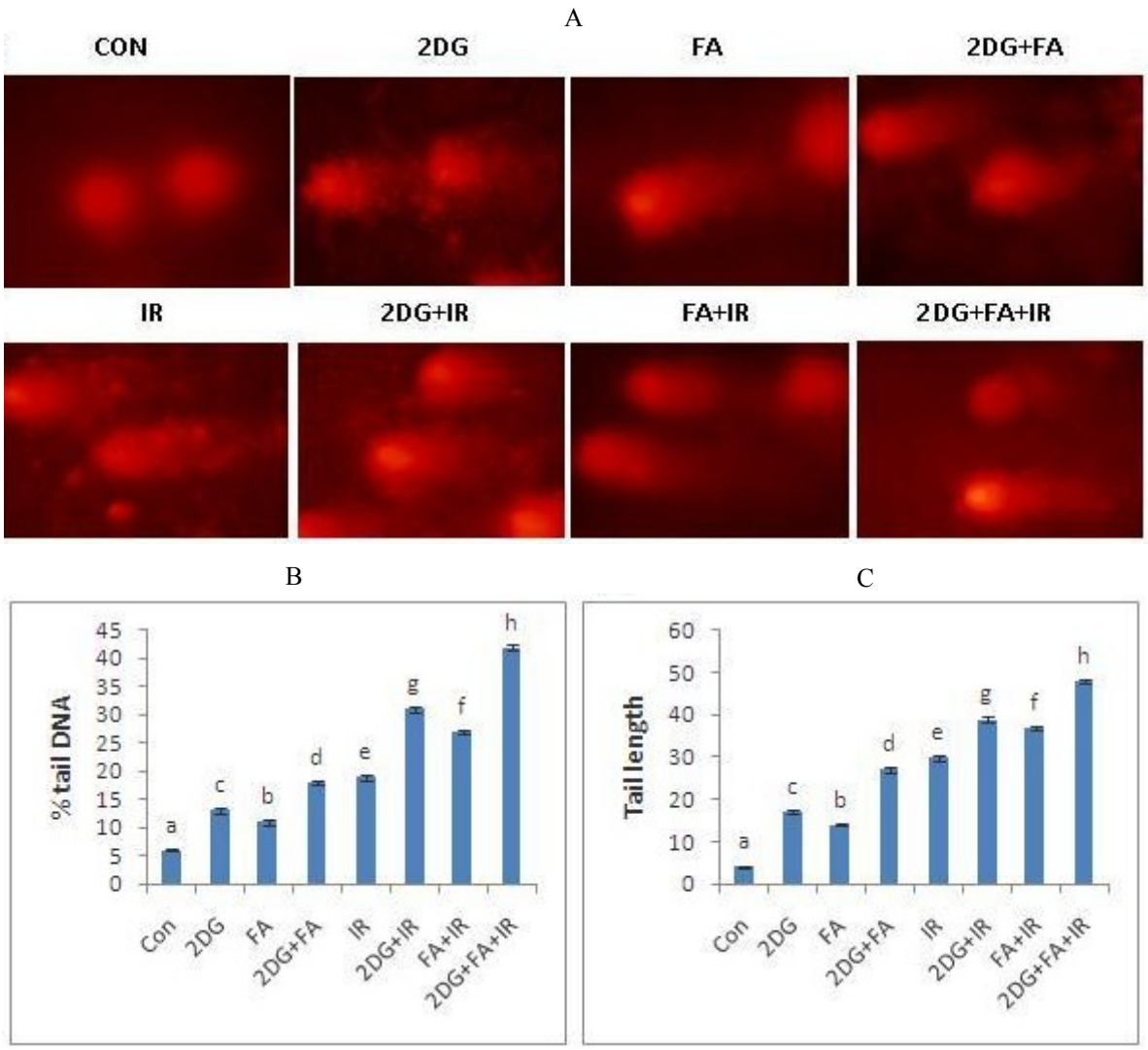


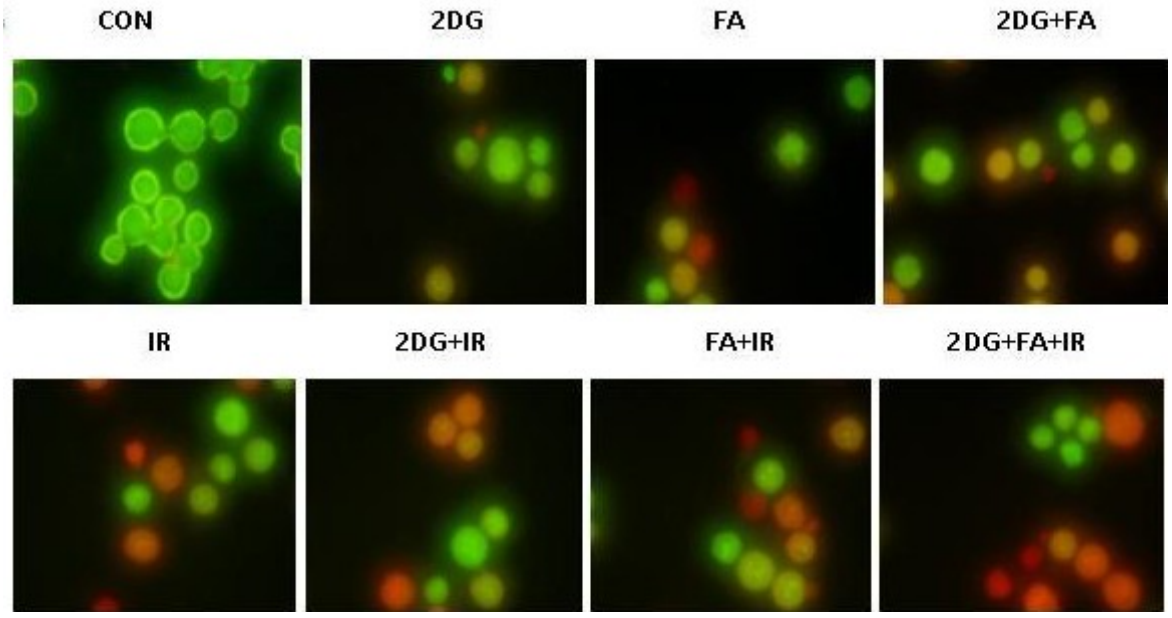
Figure 4 Effect of 2DG, FA and/or IR on (a) DNA damage (b) Percentage tail DNA (c) tail length in EAC bearing mice. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a,b,c…etc) differ significantly at $p<0.05$ (DMRT).

Chidambaram, India.

Transplantation of tumor and treatment schedule

EAC cells were obtained from Amala Cancer Research Institute, Thrissur, Kerala, India. The EAC cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation of 2 x 10^6 cells per mouse every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7-8 of tumor bearing) of the tumor cells. Each animal received 0.1 ml of tumor cell suspension containing 2 x 10^6 tumor cells intraperitoneally. On the 7th day after EAC tumor inoculation, when the tumors were in the exponential growth phase, the animals were randomly divided into nine groups (each group=6 animals) as follows: 54 Swiss albino mice were divided into nine groups (n=6) and given food and water *ad libitum*. All the animals in each groups except Group I received EAC cells (2 x 10^6 cells/mouse i.p.). Group III and GroupVII received 0.5 g/kg body weight of 2DG through i.p. injection on every alternate day for 14 days. Group IV and Group VIII received 50 mg/kg body weight of FA through i.p. injection on every alternate day for 14 days. Group V and Group IX received 0.5 g/kg body weight of 2DG and 50 mg/kg body weight of FA through i.p.

A



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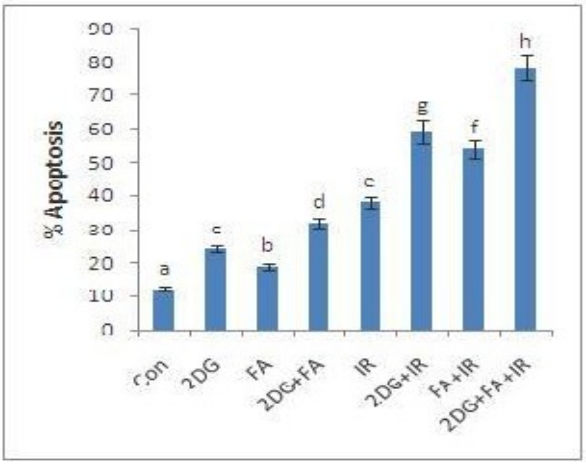

Figure 5 Effect of 2DG, FA and/or IR on (a) apoptotic morphology (b) percentage of apoptosis in EAC bearing mice. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a,b,c…etc) differ significantly at $p<0.05$ (DMRT).

injection on every alternate day for 14 days. On the 15th day, animals from Group VI to IX received 8 Gy hemi-body γ-radiation (2 Gy/min). 24 hours after the exposure of animals to γ-radiation and 18 h of fasting, the animals were anesthetized and sacrificed by cervical dislocation. Peritoneal fluid containing EAC cells was collected and used to estimate DNA damage and apoptotic morphological changes and tumor cell count. Blood was collected to analyze the hematological changes. Serum was collected to analyse the LDH activity. Tissues (Liver and spleen) were dissected to observe the histopathological changes.

Tumor volume

The ascitic fluid was collected from the peritoneal cavity of the mouse. The volume was measured by taking it in a graduated centrifuge tube (Bhattacharya *et al*., 2011).

Tumor weight

The tumor weight was measured by taking the weight of the animal before and after the collection of

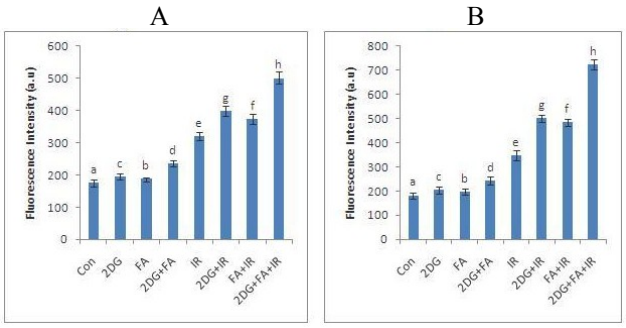

Figure 6 Effect of 2DG, FA and/or IR on (a) caspase 3 and (b) caspase 9 expressions in EAC bearing mice. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a,b,c…etc) differ significantly at p<0.05 (DMRT).

the ascetic fluid from peritoneal cavity (Bhattacharya *et al*., 2011).

Tumor cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer's counting chamber and the numbers of cells in the 64 small squares were counted (Bhattacharya *et al*., 2011).

Viable/nonviable tumor cell count

The viability and nonviability of the cells were checked by trypan blue assay. The cells were stained with trypan blue (0.4 % in normal saline) dye. The cells that did not take up the dye were viable and those that

$$\text{Cell count} = \frac{\text{Number of cells X Dilution factor}}{\text{Area X Thickness of liquid film}}$$

took the dye were nonviable. These viable and nonviable cells were counted (Reddy and Prasad, 2011).

Lactate dehydrogenase (LDH) assay

The serum sample was added to a freshly prepared NADH solution (2.5 mg/ml in 0.1 M phosphate buffer; pH 7.4), and a sodium pyruvate solution (2.5 mg/ml in 0.1 M phosphate buffer, pH 7.4) was added to it after 20 min. The absorbance of the mixture was noted at 340 nm for 5 min at regular intervals (Wroblewski and LaDue, 1955). The unit activity of LDH was expressed as the change in optical density/min/mg of protein in serum sample. Total protein content was determined at 650 nm using freshly prepared solutions of 2% alkaline sodium carbonate, 0.5% copper sulphate in 1% sodium potassium tartrate and Folin–Ciocalteu's phenol reagent (Lowry *et al*., 1951).

Measurement of DNA damage

DNA damage was estimated by alkaline single cell gel electrophoresis (comet assay) according to the method of Singh *et al*., 1988 (Singh *et al*., 1988) with slight modification (Reddy and Prasad, 2011). The extent of DNA damage was estimated by fluorescence microscopy using a digital camera and analyzed by image analysis software, CASP. For each sample, 100 cells were analyzed and classified visually into one of five classes according to the intensity of fluorescence (DNA) in the comet tail. DNA damage was quantified as % of tail DNA and tail length.

Apoptotic morphology

EAC cells were collected from the peritoneal cavity and washed with PBS and fixed on microscopic slides with 3:1 ratio of methanol:acetic acid. Later, these slides were stained with 1:1 ratio of ethidium bromide and acridine orange as described elsewhere (Reddy and Prasad, 2011). Stained cells were washed with PBS and viewed under a fluorescence microscope. The number of cells showing features of apoptosis was counted as a function of total number of cells present in the field. During apoptosis DNA becomes condensed and fragmented. This could be observed when several small spots of DNA are visible in the nucleus stained by acridine orange. Later, the cell membrane becomes permeable to dyes like ethidium bromide. The DNA spots turn orange red after intercalation of ethidium bromide with DNA.

Caspase 3 and 9 activity assays

ApoAlert caspase-3 and 9 assays were performed according to the manufacturer's instructions using DEVD-AFC and LEHD-AMC as substrates,

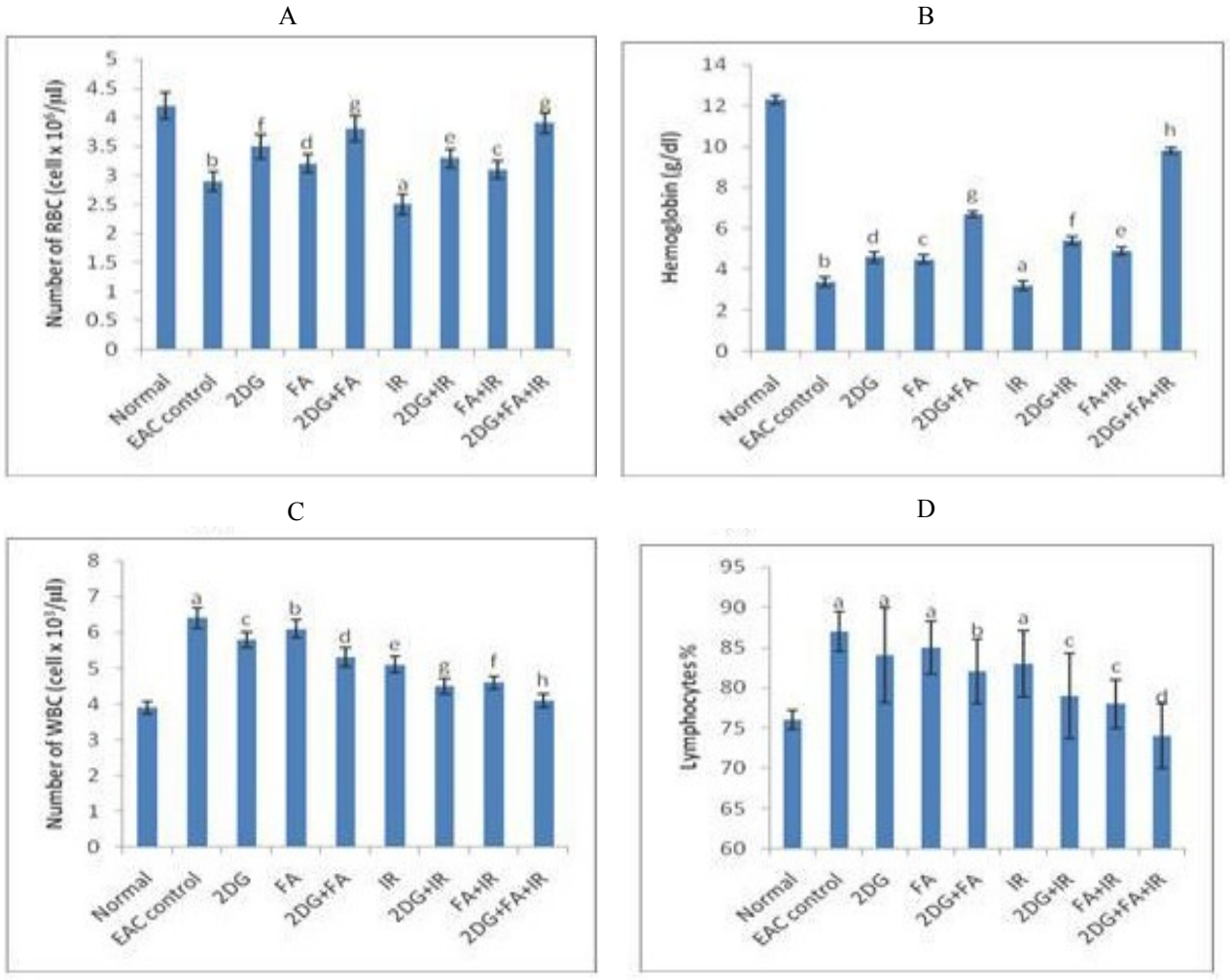

Figure 7 Effect of 2DG, FA and/or IR on (a) RBC count (b) hemoglobin content (c) WBC count (d) Percentage of lymphocytes in EAC bearing mice. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a,b,c…etc) differ significantly at p<0.05 (DMRT).

respectively. Fluorometric detection for caspase 3 is performed using a 400 nm excitation filter and 505 nm emission filter. Fluorometric detection for caspase 9 is performed using 380 nm excitation filter and 460 nm emission filter. Emissions of samples were compared with uninduced control cells to determine the increase in caspase activities.

Hematological parameters

At the end of the experimental period, the next day after an overnight fasting blood was collected from freely flowing tail vein and used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) count, white blood cell (WBC) count and lymphocyte count by standard procedures using cell dilution fluids and haemocytometer (Mukherjee, 1988).

Histopathological studies

Liver and spleen of sacrificed mice were fixed in formaldehyde-saline and processed to prepare slides of the tissues stained with hematoxylin-eosin for microscopic observation of their histopathological status.

Statistical analysis

Statistical analysis to evaluate the significance of any differences between the data of two chosen groups was done by using one-way ANOVA followed by DMRT (Duncan Multiple Range Test) taking $p<0.05$ to test the significant difference between groups. Values are given as means ± S.D. of experiments in each group.

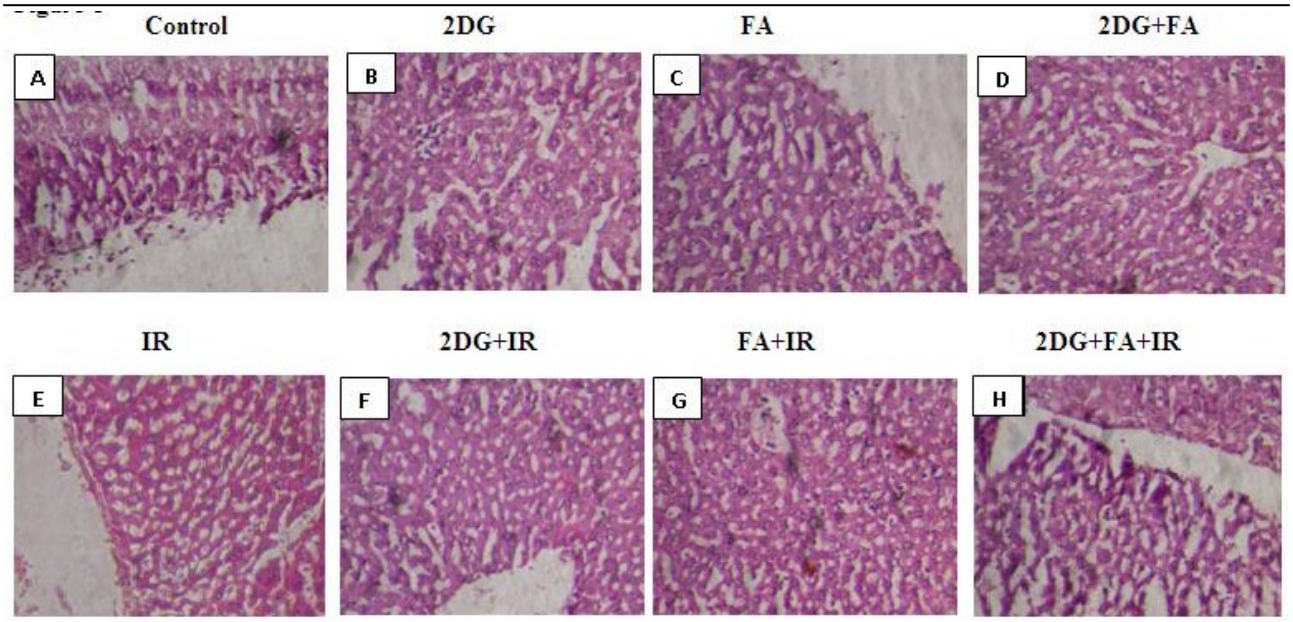

Figure 8 Effect of 2DG, FA and/or IR on histopathological changes in the liver of EAC bearing mice. (A) migration of tumor cells close to the capsule (B) appearance of tumor cells near capsules minimized, nuclear disintegration observed (C) presence of tumor cells near the lining of the capsule minimized (D) tumor cells showed necrosis, nuclear disintegration observed (E) tumor cells appeared adherent to the lining of the capsule (F) necrosis of the tumor cells and blast formation of the liver cells observed (G) tumor cell neurosis and blast formation of the liver cells observed (H) Viable tumor cells not seen on the lining of the capsule.

Values not sharing a common marking (a,b,c.. etc.) differ significantly at $p<0.05$.

RESULTS

Tumor volume and tumor weight

Figure 1 shows the changes in tumor volume and tumor weight in untreated and treated EAC bearing mice. EAC control animals showed significant tumor volume and tumor weight (3.9 ml and 4.1 g, respectively). Tumor volume and tumor weight has been reduced significantly upon the treatment with 2DG (2 ml and 3.4 g, respectively), FA (2.1 ml and 3.6 g. respectively) and IR (1.5 ml and 2.4 g, respectively) alone when compared with EAC control mice. Combination of 2DG and FA (2DG+FA) (1.4 ml and 2.1 g, respectively) significantly decreased the tumor volume and tumor weight compared with 2DG or FA alone treated groups. Combined treatment group (2DG+FA+IR) (0.5 ml and 0.5 g, respectively) has showed a further decrease in tumor volume and tumor weight when compared with all other treatment modalities.

Tumor cell viability

Figure 2 shows the effect of 2DG and FA pretreatment on tumor cell viability in γ-radiation treated EAC bearing mice. Compared with control group, the viable cell count has been decreased in 2DG, FA and IR treatment groups. Combined treatment of 2DG and FA has resulted in the significant decrease in viable cell count compared with 2DG or FA alone treated groups. Combined treatment group (2DG+FA+IR) has showed a further decrease in viable cell count compared with 2DG and FA treatment group.

Lactate dehydrogenase activity

Figure 3 shows the effect of 2DG and FA pretreatment on LDH activity in γ-radiation treated EAC bearing mice. Compared with normal group, EAC control mice have shown high LDH activity. LDH activity has decreased significantly in 2DG, FA and 2DG+FA treated groups compared to EAC control. 2DG+FA+IR treatment group has showed decreased activity of LDH compared to IR alone treated group. The LDH activity is near to normal in 2DG+FA+IR treatment group.

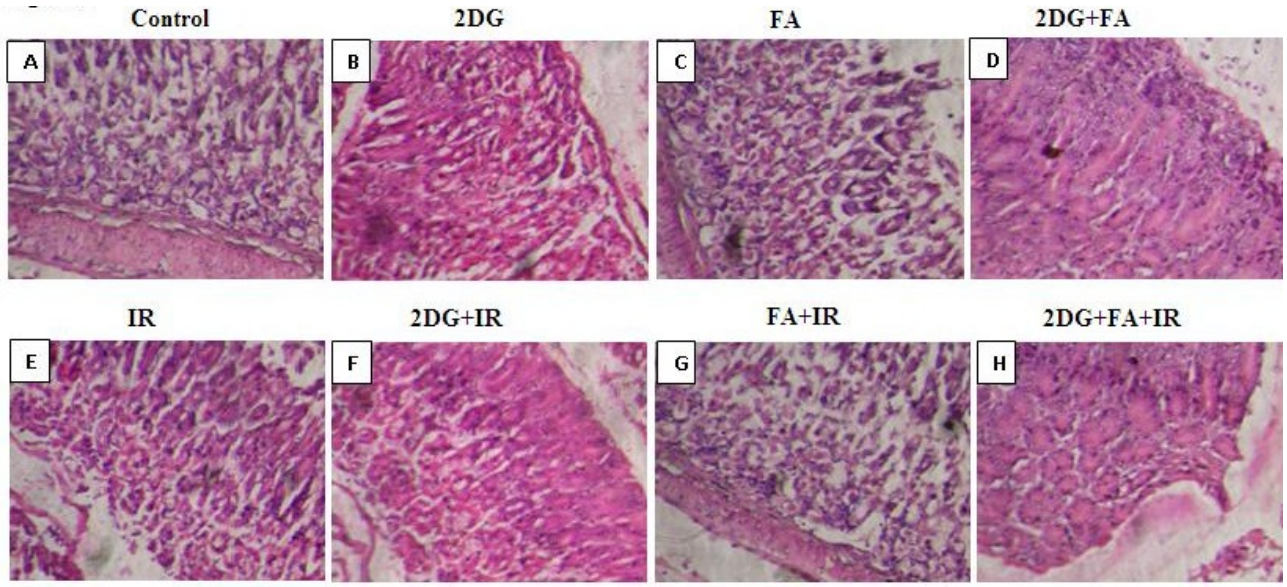

Figure 9 Effect of 2DG, FA and/or IR on histopathological changes in the spleen of EAC bearing mice. (A) tumor cells seen peripheral to the spleenic capsule (B) spleen shows necrosis and fibrosis around the capsules (C) viable tumor cells were seen around the lining of the spleenic capsule (D) tumor cells seen decreased around the capsule and fibrosis observed (E) tumor cells present around the spleenic capsule shows necrosis (F) less number of tumor cells around the capsule observed (G) tumor cells shows necrosis and fibrinolytic changes observed (H) viable tumor cells not seen on the lining of the spleenic capsule.

DNA damage

Figure 4a shows photomicrographs of DNA damage (comet assay) in 2DG, FA and IR treatment groups. The control cell showed largely non-fragmented DNA. We observed fragmented DNA in 2DG, FA and radiation treated mice which appear as a comet during single cell gel electrophoresis. Figure 4b & 4c shows the changes in the levels of % DNA in the tail and tail length of different treatment groups in EAC bearing mice. There were no changes in levels of DNA damage in the untreated control cells. Treatment with 2DG, FA and IR alone increased DNA damage compared to untreated control cells. 2DG+FA+IR treatment group shows increased % of DNA in the tail and tail length compared to any other treatment group.

Apoptotic morphology

Cells with condensed or fragmented chromatin, indicative of apoptosis, were observed in different treatment groups as compared to control cells which showed evenly distributed green fluorescent chromatin (Figure 5a). Figure 5b shows the quantitative result of apoptosis in the different treatment groups. Percentage of apoptotic cells has increased in the combined treatment of 2DG and FA compared with 2DG and FA alone treatment groups. Combined treatment group (2DG+FA+IR) has showed 79% of apoptotic cells.

Caspase-3 and 9 expression

Figure 6a and b shows the spectrofluorometric readings for the analysis of caspase 3 and 9 in 2DG, FA and IR treatment groups. The activity of apoptotic enzymes caspase-3 and 9 were significantly increased in 2DG, FA and 2DG+FA treatment groups. The group with the treatment of radiation alone has also showed a significant increase in the activity of caspase-3 and 9 compared with EAC control group. Treatment with 2DG and FA before irradiation has resulted in the increased expression of caspase 3 and 9, compared with the group treated with radiation alone.

Hematological changes

Figure 7 shows the effect of 2DG, FA and IR on hematological parameters in EAC bearing mice. RBC count and hemoglobin content have been increased in the

2DG and FA treatment group compared with 2DG and FA alone treated groups. WBC count and % of lymphocytes have been decreased in the 2DG and FA treatment group. In the combined treatment group (2DG+FA+IR), RBC count and hemoglobin content had significantly increased and WBC count had decreased compared with IR treatment group alone.

Histopathological changes

Figure 8 shows the histopathological results of the liver of treated and untreated EAC bearing mice. In EAC control mice, clumps of EAC cells were observed close to the capsule of liver. In radiation alone treated mice, few cells were seen adherent to the capsule and appeared viable. But there were no tumor cells outside the capsule and the rest of the hepatocytes exhibited nuclear disintegration. In 2DG and FA treated group, the tumor cells were not seen both inside the capsule and outside the capsule and the nuclear disintegration of the hepatocytes was also observed.

The histopathological results of the spleen of treated and untreated EAC bearing mice is shown in the figure 9. The tumor cells were seen peripheral to spleenic capsule and the anaphylatic cells were observed deeper to the capsule, but in the radiation group, the tumor cells were seen peripheral to the capsule with necrosis. Animals received 2DG and FA together showed that, 2DG and FA increased the radiation effects on tumor cells by increasing the tumor cell necrosis.

DISCUSSION

In the present study, we observed the radiosensitizing potential of 2DG and FA in EAC bearing Swiss albino mice. Tumor volume and tumor weight has been reduced significantly upon the treatment with 2DG (2 ml and 3.4 g, respectively), FA (2.1 ml and 3.6 g. respectively) and IR (1.5 ml and 2.4 g, respectively) when compared with EAC control mice (3.9 ml and 4.1 g, respectively). In combined treatment group (2DG+FA+IR), administration of 2DG and FA before 8 Gy hemi-body γ-radiation caused retardation in the tumor volume (0.5 ml) and tumor weight (0.5 g). The decreased tumor volume might be because of the inhibition of EAC cell proliferation by the combination of 2DG and FA. Reduction in viable cell count in the peritoneal fluid of tumor host suggests the radiosensitizing effect of combination of 2DG and FA in EAC bearing mice. It has been reported earlier that the combination of 2DG and FA decreases the cell proliferation of NCI-H460 cells *in vitro* (Reddy and Prasad, 2011).

The induction of apoptotic pathway is crucial for the anti-proliferative potential of treatment compounds administered during cancer therapy (Essack *et al*., 2011). In this study, we observed an increase in the percentage of EAC apoptotic cells in the radiation treated EAC mice. Administration of 2DG+FA before irradiation has resulted in the maximal increase of apoptotic cells in the peritoneal fluid. Recently, specific intracellular proteases belonging to the protease family have emerged as crucial effectors of apoptosis (Eriksson *et al*., 2009). It is well established that activated caspase 9 triggers a cascade that culminates in the activation of caspase 3 resulting in chromosomal DNA fragmentation and cellular morphologic changes characteristic of apoptosis (Thornberry and Lazebnik, 1998). The present study results demonstrated that initiator caspase 9 from intrinsic pathway and effector caspase 3 participated in the apoptotic process following radiation; these results are well correlated with the earlier findings (Eriksson *et al*., 2009). It was demonstrated that glucose deprivation promoted the activation of mitochondria-dependent pathways leading to apoptosis (Munoz-Pinedo *et al*., 2003). It has been reported earlier that phytochemicals induce reactive oxygen species, which mediate apoptosis in human cervical cancer cells (Javvadi *et al*., 2008). The induction of DNA strand breaks is often used to predict the radiosensitivity of tumor cells. Increased DNA damage observed during

radiation treatment in the cells isolated from the peritoneal cavity of EAC mice. In the combined treatment group (2DG+FA+IR), the extent of DNA damage was more, as is evident by the increased tail length and % of DNA in tail. 2DG inhibits DNA repair pathways in cancer cells after exposure to ionizing radiation (Sinthupibulyakit *et al*., 2009). Damage to DNA by ROS and RNS is reported to initiate signaling cascades and results in activation of transcription of specific groups of genes which may lead to apoptosis (Valko *et al*., 2007). Phenolic acids are reported to cause DNA damage by increasing the ROS production in cancer cells (Kim *et al*., 2011; Chendil *et al*., 2004).

The inclusion of phytochemicals in radiotherapy regimens will improve the therapeutic index by killing neoplastic cells and reducing radiation toxicity to normal tissues. The major problem that arises with radiation treatment is anemia due to reduction in RBC. In this study, elevated WBC count, reduced hemoglobin and RBC count were observed in EAC bearing mice. It was observed there was a decrease in the RBC count, hemoglobin content and WBC count in the radiation alone treated EAC mice. Myelosuppression and anemia (reduced hemoglobin) have been frequently observed in ascites carcinoma (Price *et al*., 1950). Anemia encountered in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number (Fenninger *et al*., 1954). Treatment with 2DG and FA before irradiation restored the RBC count and hemoglobin content to near normal levels, which indicates the protective action of 2DG and FA against the radiation induced damage. It has been reported earlier that ferulic acid offers protection against γ-radiation induced damage in Swiss albino mice (Maurya and Nair, 2006). Intraperitoneal administration of 2DG and FA before irradiation restored the WBC count to near normal levels. The observed change in hematological parameters supports the hematopoietic protecting activity of 2DG and FA. It has been also observed that the combination of 2DG and FA also offers protection against myelotoxicity, the most common side effect of cancer radiotherapy. It was reported earlier that 2DG offers protection against perchloroethylene induced alterations in hematological parameters (Ebrahim *et al*., 2001). Earlier reports also highlighted the protective effect of FA on hematopoietic cell recovery in γ-irradiated mice (Ma *et al*., 2011).

The multitude of pathological changes caused by the progression of tumor as well as its inhibition through treatment compounds are expected to be reflected in the biochemical parameters of the host system, particularly pertaining to the liver. In the present study, increased LDH activity in EAC bearing mice might be because of two reasons; 1) progression of tumor 2) high rate of glycolysis observed in malignant conditions (Hazra *et al*., 2005). Administration of 2DG+FA before irradiation helps in restoring the activity of LDH to near normal. Taken together with the decreased viable cell count in the combined treatment (2DG+FA+IR), the reason for restoration of LDH activity could partly be explained. In the present study, experimental animals exposed to γ-radiation alone have showed comparatively elevated levels of LDH in serum. In alignment with earlier reports, it is assumed that the radiosensitive organs thymus and spleen might be the possible sources of increased amounts of serum LDH after γ-radiation exposure (Hori *et al*., 1970). It was reported earlier that the level of LDH release was reduced significantly by a treatment with 2DG in neural progenitor cells (Park *et al*., 2009). Microscopic examination of tissues from EAC control mice showed marked alterations in the structure of liver and spleen. Presence of tumor cells along the lining of the capsules of liver and spleen are seen in EAC bearing mice. Treatment with 2DG and FA decreased the viable tumor cells along the lining of the capsules and showed near to normal pathological status despite being challenged by the tumor cells.

CONCLUSION

In conclusion, the present study findings indicated the radiosensitizing potential of combination of 2DG and FA, by decreasing the tumor weight, tumor volume, tumor cell count and increasing the expression of caspase family proteases. It has also been understood that the combination of 2DG and FA restored the hematological and histopathological changes to near normalcy. Further studies highlighting the molecular mechanisms involved in the radiosensitizing activity of 2DG and FA are warranted.

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REFERENCES

Ahmad IM, Aykin-Burns N, Sim JE, Walsh SA, Higashikubo R, Buettner GR. 2004. Mitochondrial O2*- and H2O2 mediate glucose deprivation-induced stress in human cancer cells. *J Biol Chem.,* 280:4254-4263.

Bhattacharya S, Prasanna A, Majumdar P, Kumar RB, Haldar PK. 2011. Antitumor efficacy and amelioration of oxidative stress by Trichosanthes dioica root against Ehrlich ascites carcinoma in mice. *Pharm Biol.,* 49:927-935.

Chendil D, Ranga RS, Meigooni D, Sathishkumar S, Ahmed MM. 2004. Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. *Oncogene* 23:1599-1607.

Coleman MC, Asbury CR, Daniels D, Du J, Aykin-Burns N, Smith BJ. 2008. 2-deoxy-D-glucose causes cytotoxicity, oxidative stress, and radiosensitization in pancreatic cancer. *Free Radic Biol Med.,* 44:322-331.

Delaney G, Jacob S, Featherstone C, Barton M. 2005. The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. *Cancer* 104:1129-1137.

Dimitroulis J, Toubis M, Antoniou D, Marosis C, Armenaki U, Stathopoulos GP. 2006. Alternate paclitaxel-gemcitabine and paclitaxel-vinorelbine biweekly administration in non-small cell lung cancer patients: a phase II study. *Anticancer Res.,* 26:1397-1402.

Ebrahim AS, Babu E, Thirunavukkarasu C, Sakthisekaran D. 2001. Protective role of vitamin E, 2-deoxy-D-glucose, and taurine on perchloroethylene induced alterations in ATPases. *Drug Chem Toxicol.* 24:429-437.

Eriksson D, Löfroth PO, Johansson L, Riklund K, Stigbrand T. 2009. Apoptotic signalling in HeLa Hep2 cells following 5 Gy of cobalt-60 gamma radiation. *Anticancer Res.* 29:4361-4366.

Essack M, Bajic VB, Archer JA. 2011. Recently confirmed apoptosis-inducing lead compounds isolated from marine sponge of potential relevance in cancer treatment. *Mar Drugs* 9:1580-1606.

Fenninger LD, Mider GB. 1954. In: Greenstein JP, Haddow A (ed) Advances in cancer research. Academic Press, New York. 244.

Gogvadze V, Orrenius S, Zhivotovsky B. 2008. Mitochondria in cancer cells: what is so special about them? *Trends Cell Biol.,* 18:165-173.

Hazra B, Kumar B, Biswas S, Pandey BN, Mishra KP. 2005. Enhancement of the tumour inhibitory

activity, in vivo, of diospyrin, a plant-derived quinonoid, through liposomal encapsulation. *Toxicol Lett.,* 157:109-117.

Hori Y, Takamori Y, Nishio K. 1970. The Effects of X-Irradiation on Lactate Dehydrogenase Isozymes in Plasma and in Various Organs of Mice. *Radiat Res.* 43:143-151.

Javvadi P, Segan AT, Tuttle SW, Koumenis C. 2008. The chemopreventive agent curcumin is a potent radiosensitizer of human cervical tumor cells via increased reactive oxygen species production and overactivation of the mitogen-activated protein kinase pathway. *Mol Pharmacol.* 73:1491-1501.

Karthikeyan S, Kanimozhi G, Prasad NR, Mahalakshmi R. 2011. Radiosensitizing effect of ferulic acid on human cervical carcinoma cells in vitro. *Toxicol In Vitro.,* 25:1366-1375.

Kim W, Seong KM, BuHyun Youn. 2011. Phenylpropanoids in radioregulation: double edged sword. *Exp Mol Med.* 43:323-333.

Lee YJ, Galoforo SS, Berns CM, Chen JC, Davis BH, Sim JE. 1998. Glucose deprivation-induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. *J Biol Chem.,* 273:5294-5299.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 193:265-275.

Ma ZC, Hong Q, Wang YG, Tan HL, Xiao CR, Liang QD. 2011. Effects of ferulic acid on hematopoietic cell recovery in whole-body gamma irradiated mice. *Int J Radiat Biol.* 87:499-505.

Maschek G, Savaraj N, Priebe W, Braunschweiger P, Hamilton K, Tidmarsh GF. 2004. 2-deoxy-D-glucose increases the efficacy of adriamycin and paclitaxel in human osteosarcoma and non-small cell lung cancers in vivo. *Cancer Res.,* 64:31-34.

Maurya DK, Nair CKK. 2006. Preferential radioprotection to DNA of normal tissues by ferulic acid under ex vivo and in vivo conditions in tumor bearing mice. *Mol Cell Biochem.* 285:181-190.

Mukherjee KL. 1988. Medical laboratory technology. a procedure manual for routine diagnostic tests. Tata McGraw Hill, New Delhi.

Munoz-Pinedo C, Ruiz-Ruiz C, Ruiz DA, Palacios C, Lopez-Rivas A. 2003. Inhibition of glucose metabolism sensitizes tumor cells to death receptor-triggered apoptosis through enhancement of death-inducing signaling complex formation and apical procaspase-8 processing. *J Biol Chem.,* 278:12759-12768.

Ozaslan M, Karagoz ID, Kilic IH, Guldur ME. 2011. Ehrlich ascites carcinoma. *Afr J Biotechnol.,* 10:2375-2378.

Park M, Song KS, Kim HK, Park YJ, Kim HS, Bae MI, Lee J. 2009. 2-Deoxy-d-glucose protects neural progenitor cells against oxidative stress through the activation of AMP-activated protein kinase. *Neurosci Lett.,* 449:201-206.

Price VE, Greenfield RE. Anemia in cancer. In: Greenstein JP, Haddow A (ed) 1950. Advances in cancer research. Academic Press, New York. 199-200.

Rao SK, Rao PS, Rao BN. 2008. Preliminary investigation of the radiosensitizing activity of guduchi (Tinospora cordifolia) in tumor-bearing mice. *Phytother Res.,* 22:1482-1489.

Reddy BV, Prasad NR. 2011. 2-deoxy-D-glucose combined with ferulic acid enhances radiation response in non-small cell lung carcinoma cells. *Cent Eur J Biol.,* 6:743-755.

Sharma S, Guthrie PH, Chan SS, Haq S, Taegtmeyer H. 2007. Glucose phosphorylation is required for insulin-dependent mTOR signalling in the heart. *Cardiovasc Res.,* 76:71-80.

Singh NP, McCoy MT, Tice RR, Schneider EL. 1988. A simple technique for quantification of low levels of DNA damage in individual cells. *Exp Cell Res.,* 175:184-191.

Sinthupibulyakit C, Grimes KR, Domann FE, Xu Y, Fang F, Ittarat W. 2009. p53 is an important factor for the radiosensitization effect of 2-deoxy-D-glucose. *Int J Oncol.* 35:609-615.

Thornberry NA, Lazebnik Y. 1998. Caspases: Enemies within. *Science* 281:1312-1316.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.,* 39:44-84.

Wroblewski F, LaDue JS. 1955. Lactic dehydrogenase activity in blood. *Proc Soc Exp Biol Med.,* 90:210-213.

Zheng LF, Dai F, Zhou B, Yang L, Liu ZL. 2008. Prooxidant activity of hydroxycinnamic acids on DNA damage in the presence of Cu (II) ions: mechanism and structure-activity relationship. *Food Chem Toxicol.,* 46:149-156.