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Research Article

Effects of radiation on Protein and DNA content in Genistein treated mice liver

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ABSTRACT
Genistein is a soya isoflavone, which is found naturally in legumes, such as soybeans and chickpeas. The intraperitoneal administration of optimum dose (200 mg/kg body weight) of Genistein before 24 hrs and 15 minutes of irradiation (8 Gy at a dose rate of 1.02 Gy/min) recovered the protein content (by 28.63 ± 1.4349%) and the DNA content (by 21.61 ± 8.1863%) in experimental group as compared to control group in liver of Swiss albino mice. Statistically analyzed survival data produced a dose reduction factor (DRF) = 1.24. The decrease of protein amount after irradiation may be due to its lysis by gamma-radiation or may be at the synthesis level or it may be due to the depression of enzymes involved in the activation of amino acids and transferring to tRNA or by the inhibition of release of synthesized polypeptides from polysomes. The decrease in DNA content after irradiation is due to an inhibition of replication of this compound in nucleus and accumulation of ribonucleotide in the cytoplasm, which is based on the inability, of irradiated cell to reduce ribonucleotide to DNA in the nucleus. The results indicate that Genistein against radiation effect may pave way to the formulation of medicine in radiotherapy for normal tissue and possible against radiomimetic drug induced toxicity.

Key Words: Genistein, Tyrosine kinase inhibitor, Radiation, Liver, Oxidative stress, protein, DNA.

INTRODUCTION

At present there is hardly any aspect of human welfare in which radiation does not play an important role. Radiations have cytotoxic and immunosuppressive effects. Hence, preventive methods to protect not only human but also animals and plants are necessary. Therefore, radioprotectors for use prior to exposure has been identified as one of the highest priority areas for research[1]. Recently Interest has been generated to develop potential drugs of plant origin which can quench the reactive energy of free radicals and eliminate oxygen and are capable of modifying radiation responses with minimum side effects especially during the radiotherapy where the necessity of protection of normal tissue occurs. Plants products appear to have an advantage over synthetic products in terms of low/no toxicity at effective dose.

Protein is essential for biosynthesis of glutathione, which provides the ultimate protection against the toxic effects of ROS, generated by radiation. Decrease in the protein content after exposure to irradiation might be due to either decline in the rate of protein synthesis or an increase in the consumption of protein. Radiation may also induce local defects in microstructure of protein molecules, which become center of thermal denaturation and cross linkage, thus causing tissue damage[2]. Increase in protein concentration in Experimental group is a beneficial effect. This process is important in the ribosomal activities, which enhance protein synthesis. This can be treated as an antiradiation effect. Reduction rate of the protein synthesis may be due to unfavourable condition like unavailability of one or more essential enzymes and /or reduction in the site of protein synthesis but if Genistein is available and accessible, many of these unpaired electrons get scavenged due to their excellent antioxidant property.

However, the protein synthesis is known to be unaffected by a low dose of radiation, but its rate of synthesis is reduced within a short period in tissue subjected to high doses[3-5]. The decrease of protein amount after irradiation may be due to its lysis by gamma- radiation or may be at the synthesis level or it may be due to the depression of enzymes involved in the activation of amino acids and transferring to tRNA or by the inhibition of release of synthesized polypeptides from polysomes. The decrease in protein is associated with high TBARS, could result in an increased production of reactive free radicals damaging cells and initiating lipid peroxidation.

Several mechanisms can be offered for the explanation of reduced content of DNA[6]. It has been shown that post-irradiation acute cell death could lead to loss of DNA in excess than is normally eliminated from the tissue. The prolonged interphase or delayed onset of DNA synthesis

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after irradiation also could lead to decreased content of DNA. One study has shown that ^{60}CO gamma radiations had more effect in DNA, RNA and protein function[7]. One Scientist also reported depressed rate of DNA synthesis in 1 or 10 days old rats after low dose of gamma radiation[8].

The drop in DNA content is due to an inhibition of replication of this compound in nucleus and accumulation of ribonucleotide in the cytoplasm, which is based on the inability, of irradiated cell to reduce ribonucleotide to DNA in the nucleus. There is also now general agreement that interference with DNA is one of the important biological effects of the irradiation. RNA, possibly synthesized in greater quantity in neurons is more radioresistant than DNA[9].

Genistein is a soya isoflavone, which is found naturally as the glycoside genistin and as the glycosides 6"-O-malonylgenistin and 6"-O-acetylgenistin. Genistein is the aglycone (aglucon) of genistin. Genistein and its glycosides are mainly found in legumes, such as soybeans and chickpeas. Some Scientists demonstrated that intraperitonial administration of Genistein increased the survival of mice against 8 Gy gamma irradiation[10,11].

Genistein a tyrosine kinase inhibitor, compete with the ATP binding site of the catalytic domain of several oncogenic tyrosine kinases. This is possible because of structural similarities between ATP and the TKIs. Kinases use ATP as a source of phosphate, but if a TKI binds to the enzyme instead of ATP, then the kinase can not phosphorylate proteins and signaling halts.

Liver is selected as a testing organ because some scientists reported it as highly radiosensitive organ[12]. The present study has been carried out in order to check ameliorating capacity of Genestein against radiation with respect to protein and DNA content of mice liver.

MATERIALS AND METHODS

Animals

Swiss albino mice (Mus musculus) obtained from All India Institute of Medical Sciences (AIIMS), New Delhi and kept at controlled condition of temperature (25 ± 2^o C) and light (light : dark, 12 : 12 hrs). They were provided standard mice feed (procured from Hindustan Uniliver ltd. Mumbai) and water ad libitium. For experimentation, healthy male mice of 6-8 weeks old with an average body weight of 22 ± 3 gm were selected from inbred colony.

Drug

Genistein: Genistein was obtained as gift sample from Mr. M. Maniar (Palm Pharmaceuticals, Inc., USA). Genistein was manufactured by L.C. Laboratories, 165 New Boston St. Woburn, MA01801 USA.

Genistein solution: Genistein was dissolved in dimethyl sulfoxide and then prepared different concentration solutions so that the volume administered intraperitoneally was 0.5 ml.

Mode of administration: Mice were administered intraperitoneally optimum dose (200 mg/kg body weight) of Genistein before 24 hrs and 15 minutes of irradition.

Biochemical Assays: Five autopsies were performed by mean of cervical dislocation of 6 mice from each group at each post irradiation interval (1st, 3rd, 7th, 15th and 30th) were selected for the biochemical studies. Liver was removed at each autopsy interval from the sacrificed animal of each group and placed on a piece of filter paper to remove excess of moisture. At least six observations were taken. Spectrophotometer was used to measure the optical density. Protein was estimated by the Bradford's method[13] and DNA was estimated by the method of Ceriotti's method[14]. The values are expressed as mean ± S.D. The difference between various groups was analyzed by Student's t-test.

EXPERIMENTAL PROTOCOL

The experiment has been conducted in following 4 phases:

PHASE-I: Drug Tolerance Study

Mice were divided into six groups, each containing ten mice. First group of mice did not receive any treatment, second group were administered intraperitoneally dimethyl sulfoxide, as a vehicle before 24 hrs and 15 minutes of study time and other four groups of mice were administered intraperitoneally different doses 100, 200, 300 and 400 mg/kg body weight of Genistein before 24 hrs and 15 minutes of study time. All six groups were kept under normal conditions and then observed for 30 day for any sign of morbidity, mortality, body weight change and behavioral toxicity.

PHASE-II: Optimum Dose Selection

Mice were divided into five groups, each containing ten mice. First group of mice were administered intraperitoneally dimethyl sulfoxide, as a vehicle before 24 hrs and 15 minutes of irradiation and other four groups were administered intraperitoneally different doses 100, 200, 300 and 400 mg/kg body weight of Genistein before 24 hrs and 15 minutes of irradition. Finally, all the animals of five groups were exposed to 10 Gy of gamma radiation. Radiation sickness, mortality, behavioural toxicity and morbidity were observed for 30 days after irradiation. The dose of Genistein which show highest percentage of survival of mice against radiation has been selected as optimum dose for further experiment.

PHASE-III: $LD_{50/30}$ and Dose Reduction Factor

The protective action of any radio protective agent may be represented as a Dose Reduction Factor (DRF) and DRF can calculated as follows:

$$DRF = \frac{\text{LD50/30 of Experimental animals}}{\text{LD50/30 of Control animals}}$$

The DRF of Genistein was calculated by the aforementioned formula, by exposing a large number of Swiss albino mice to different doses of gamma rays in the presence or absence of Genistein. DRF of Genistein was calculated and for this mice were divided into two groups control and experimental, each containing 30 male Swiss albino mice.

Control Group: In this group, three subgroups (10 mice in each group) were made and then all mice were administered intraperitoneally dimethyl sulfoxide, as a vehicle before 24 hrs and 15 minutes of irradiation, equivalent to the optimum dose of Genistein. Now these three subgroups of mice were exposed to 6, 8, 10 Gy of gamma radiation and then observed for 30 days. Mortality and body weight were recorded every day.

Experimental Group: Mice of this group were administered intraperitoneally optimum dose (200 mg/kg body weight) of Genistein before 24 hrs and 15 minutes of irradition and then divided into 3 subgroups and then exposed to 6, 8, 10 Gy of gamma radiation.

PHASE-IV: Genistein against Radiation Damage

Mice were divided into following five groups:

Table1: Variations in terms of the maximum days of percentage survival of mice with and without Genistein after lethal gamma radiation

Groups	% Survival (Days)					
	50%	40%	30%	20%	10%	0%
Control (10 Gy)	4	5	6	7	10	11
Genistein+IR (100 mg/kg b.wt)	9	10	11	14	17	18
Genistein+IR (200 mg/kg b.wt)	21	26	30	Not found	Not found	Not found
Genistein+IR (300 mg/kg b.wt)	13	14	17	17.5	20	21
Genistein+IR (400 mg/kg b.wt)	10	12	15	16	17	18

Group-I Normal

Mice of this group were not received any treatment and kept under normal conditions.

Group-II Genistein Treated

Mice of this group were administered intraperitoneally optimum dose (200 mg/kg body weight) of Genistein before 24 hrs and 15 minutes of study time.

Group-III Control

Mice of this group were administered intraperitoneally dimethyl sulfoxide as a vehicle before 24 hrs and 15 minutes of irradiation, equivalent to the optimum dose of Genistein.

Group-IV Experiment-1 or or G+IR

Mice of this group were administered intraperitoneally optimum dose (200 mg/kg body weight) of Genistein before 24 hrs and 15 minutes of irradiation.

Group-V Experiment-2 or or IR+G

This group of mice was first exposed to gamma radiation and then intraperitoneally administered optimum dose (200 mg/kg body weight) of Genistein after 15 minutes and 24 hrs of irradiation.

Mice of above treated group were observed from the day of treatment till their autopsy time with respect to body weight changes, sickness, general activity, mobility and other visible abnormalities. Mice were killed by cervical dislocation at various intervals ranging between 1-30 day and whole liver was removed and processed for biochemical estimation of protein and DNA content.

RESULTS

The intraperitoneal administration of Genistein did not caused any toxic effect on mice and Genistein treatment

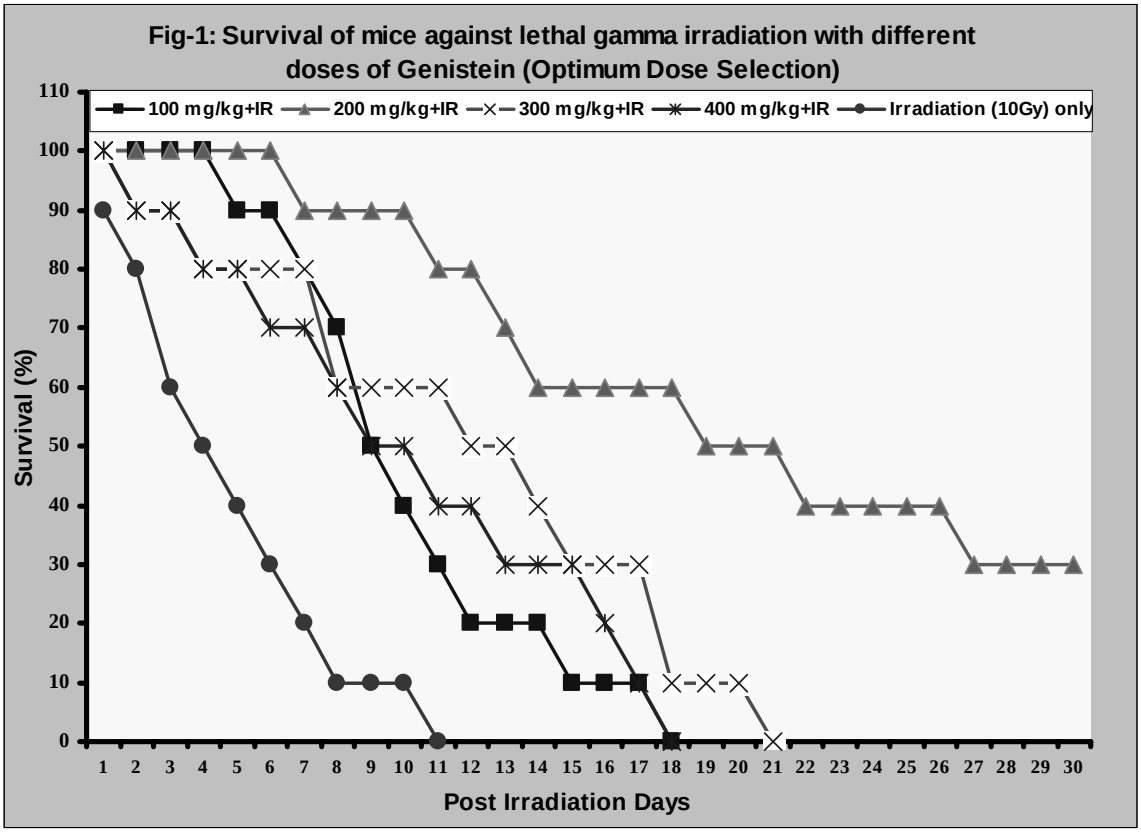

Fig-1: Survival of mice against lethal gamma irradiation with different doses of Genistein (Optimum Dose Selection)

offers better survivability of mice. All irradiated mice without Genistein treatment have shown 100% mortality within 11 days. However, maximum survival of mice (30%, even beyond 30 days) has been recorded in the 200 mg/kg body weight dose of Genistein. On the basis of this survivability experiment, 200 mg/kg body weight dose of Genistein was found as the optimum dose and this was selected for further investigation against 8 Gy of gamma radiation (Table 1, Fig. 1).

Table 2: Regression analysis of percentage survival of mice ($LD_{50/30}$ estimation)

Groups	Intercept (b)	Slope (m)	Y = mx + b	$LD_{50/30}$	DRF
Control (IR 6, 8, 10 Gy)	196.67	-20	50 = (-20x) + 196.67	7.25	1.24
Experimental (Genistein+IR)	206.67	-17.5	50 = (-17.5x) + 206.67	9	

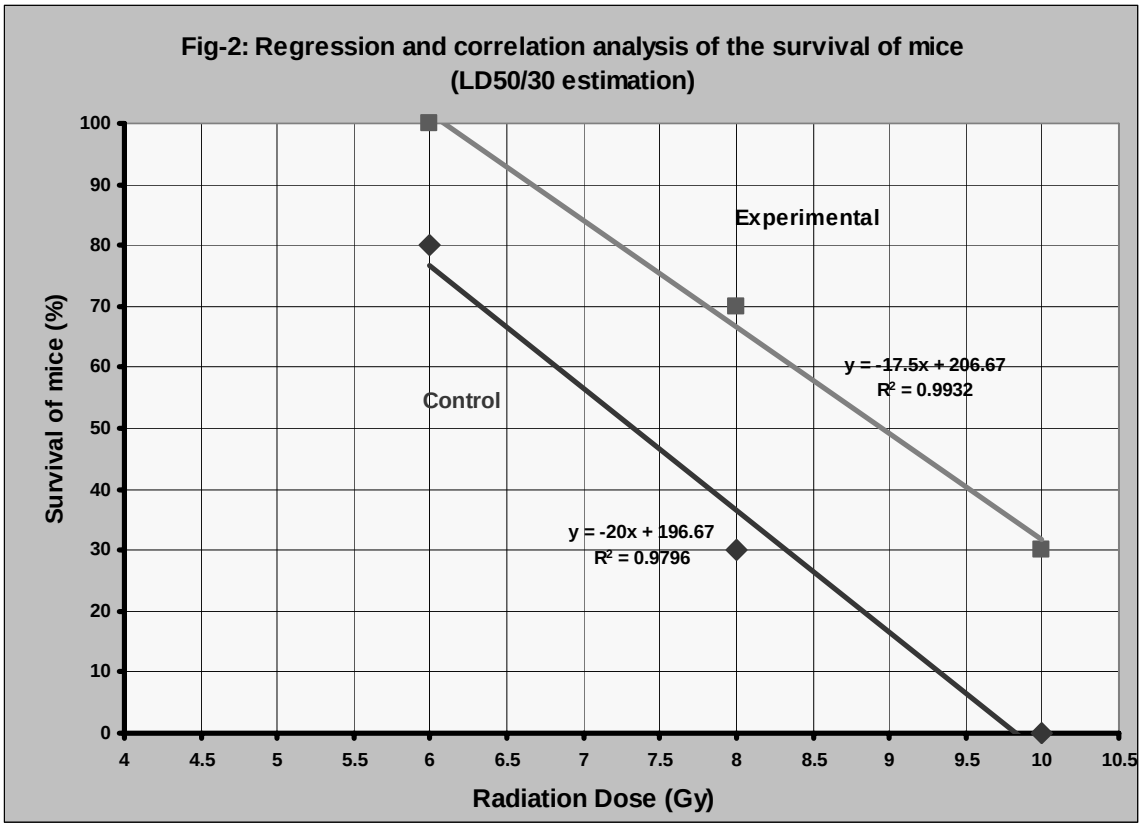

Fig-2: Regression and correlation analysis of the survival of mice ($LD_{50/30}$ estimation)

The $LD_{50/30}$ values for control group and for pre-irradiation administration of Genistein (G+IR) group were computed as 7.25 Gy and 9 Gy, respectively. The dose reduction factor has been 1.24 (Table 2, and Fig. 2).

Protein

Genistein vs. Normal: A significant increase (by 4.1%) in the protein content has been noticed in Genistein treated group as compared to those of normal groups (Table-3, Fig.- 3).

Control vs. Normal: Protein content declined upto 7th day (by 47.62%) of post-irradiation which tended to recover on later days. However, it did not reach the normal level by 30th day. Statistically a highly significant decrease ($p < 0.001$) by 25.7%, 44.89%, 47.62%, 29.52%, and 23.15% in protein content in control group was noticed on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively, as compared to those of normal group. The average decrease in protein content of control group was approximately 34.18 ± 11.2985% (±SD) (Table-3, Fig.- 3).

Experimental-1 (G+IR) vs. Control: The protein content tended to decline upto 7th day in Experimental-1 group, which is followed by an increase till 30th day. In Experimental-1 group, the average recovery in protein content was approximately 28.63± 1.4349% (±SD) as compared to that of irradiated (control) group. Statistically a highly significant recovery ($p < 0.001$) by 30.64%, 30.6%, 34.01%, 20.93%, and 26.98% in Experimental-1 group has been recorded on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively, as compared to those of control groups. While comparing with normal, still a highly significant decrease ($p < 0.001$) in protein content in the animals in Experimental-1 group has been noticed on 3rd, 7th and 15th post-irradiation days; however, it became less significant by 30th day interval (Table-3, Fig.- 3).

Experimental-2 (IR+G) vs. Control: In Experimental-2 group, also a decrease in protein content recorded upto 7th day and then a recovery occurred till 30th day. From those of control, statistically a highly significant recovery ($p < 0.001$) in protein content by 23.58%, 24.87%, 32.82%, 18.28%, and 26.17% in Experimental-2 group was noticed on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively. Maximum recovery being on 7th day, the average recovery in protein content of Experimental-2 group was approximately 25.14 ±

TABLE 3 Variation in the protein content (mg/gm) in liver of mice at various post irradiation days, with and Without Genistein treatment

Groups	Post Irradiation Days				
	1	3	7	15	30
Control (IR with 8 Gy only)	108.92 ± 1.7857 (74.3%) B***	83.92 ± 1.785 (55.11%) b***	80.35 ± 1.7857 (52.38%) b***	108.92 ± 1.785 (70.48%) B***	119.64 ± 1.7857 (76.85%) b***
Experimental-1 (Genistein+IR)	142.30 ± 1.5701 (97.07%) c*, d***	109.61 ± 1.110 (71.98%) c***, d***	107.69 ± 1.570 (70.19%) c***, d***	131.73 ± 1.841 (85.23%) C***, d***	151.92 ± 1.1102 (97.58%) c*, d***
Experimental-2 (IR+Genistein)	134.61 ± 1.570 (91.83%) e***, f***, g**	104.8 ± 0.9615 (68.82%) e***, f***, g**	106.8 ± 1.8412 (69.57%) e***, f***, g^{NS}	128.84 ± 1.110 (83.37%) E***, f***, g^{NS}	150.96 ± 0.9615 (96.96%) e**, f***, g^{NS}

Normal = 152.50 ± 1.3153 (100%) Genistein = 158.75 ± 1.2417 (104.1%) a^{**}

Each value represents Mean ± SEM.

Statistical comparison: Normal vs. Genistein = a, Normal vs. Control = b, Normal vs. Exp.-1 = c, Control vs. Exp.-1 = d, Normal vs. Exp.-2 = e, Control vs. Exp.-2 = f, Exp.-1 vs. Exp.-2 = g.

Significance levels: p < 0.1 = *, p < 0.05 = **, p < 0.001 = ***, Not significant = ^{NS}

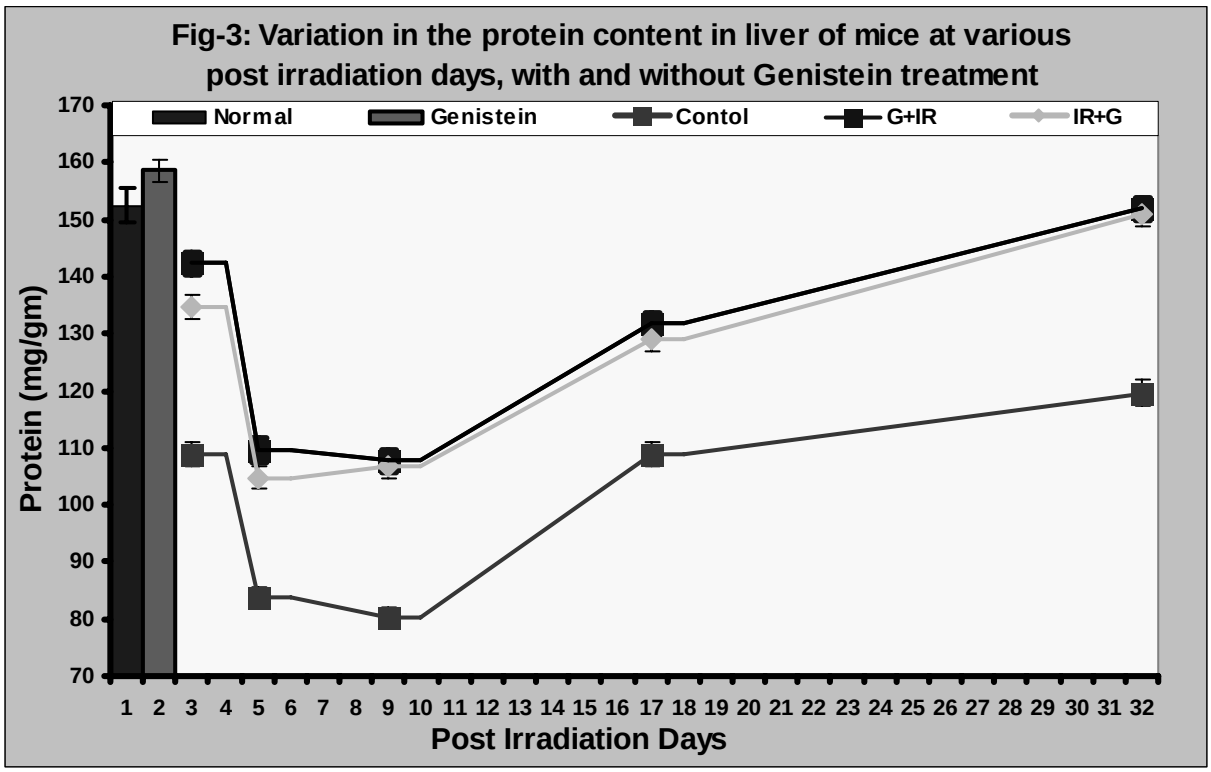

Fig-3: Variation in the Protein content in liver of mice at various post irradiation days, with and without Genistein treatment

5.2347% (±SD). While comparing with those of normal, though a highly significant decrease (p < 0.001) in the protein content was noticed from day 1st to 15th post-irradiation days, it became less significant by the 30th day, showing its tendency to attain as normal. The protein content in Experimental-1 group on later intervals, 7th, 15th and 30th days showed almost no difference to those of Experimental-2 groups (Table-3, Fig.- 3).

Deoxyribose Nucleic Acid (DNA)

Genistein vs. Normal: The DNA content of Genistein treated group was insignificantly higher than those of normal groups (by 0. 78%) (Table-4, Fig.-4).

Control vs. Normal: A sharp decline by 7th day (by 42.57% of the normal) has been observed in control group with a sign of recovery in the following intervals upto 30th day. A Statistically significant decrease (p < 0.05) by 30.75%, 38.63%, 42.57%, and 30.75% in DNA content in control group was noticed on 1st, 3rd, 7thand 15th post-irradiation days, respectively, as compared to those of normal group, which became insignificant on 30thday. The average deficit in DNA content of control group was approximately 30.86 ± 11.9204% (±SD) (Table-4, Fig.-4).

Experimental-1 (G+IR) vs. Control: In Experimental-1 group, a decrease in DNA content was noticed upto 7th day (by 31.87% of the normal) and then a recovery occurred till 30th day. From control group, statistically significant recovery (p < 0.05) in DNA content in Experimental-1 group by 17.88%, 28.44%, 29.4% and 22.76% on 1st, 3rd, 7th

Table 4: Variation in the DNA content (mg/gm) in liver of mice at various post irradiation days, with and without Genistein treatment

Groups	Post Irradiation Days				
	1	**3**	**7**	**15**	**30**
Control (IR with 8 Gy only)	1.23 ± 0.0123 (69.25%) b**	1.09 ± 0.0765 (61.37%) b**	1.02 ± 0.0897 (57.43%) b**	1.23 ± 0.0654 (69.25%) b**	1.57 ± 0.0567 (88.4%) b^{NS}
Experimental-1 (Genistein+IR)	1.45 ± 0.0765 (81.64%) c**, d**	1.4 ± 0.0999 (78.82%) c**, d**	1.32 ± 0.078 (74.32%) c**, d**	1.51 ± 0.0768 (85.02%) c*, d**	1.72 ± 0.0786 (96.84%) c^{NS}, d^{NS}
Experimental-2 (IR+Genistein)	1.42 ± 0.0765 (79.95%) e**, f**, g^{NS}	1.35 ± 0.0689 (76.01%) e**, f**, g^{NS}	1.25 ± 0.0122 (70.38%) e**, f**, g^{NS}	1.42 ± 0.0334 (79.95%) e**, f**, g^{NS}	1.66 ± 0.0897 (93.46%) e^{NS}, f^{NS}, g^{NS}

Normal = 1.776 ± 0.0986 (100%) Genistein = 1.79 ± 0.0241 (100.78%) a^{NS}

Each value represents Mean ± SEM.

Statistical comparison: Normal vs. Genistein = a, Normal vs. Control = b, Normal vs. Exp.-1 = c, Control vs. Exp.-1 = d, Normal vs. Exp.-2 = e, Control vs. Exp.-2 = f, Exp.-1 vs. Exp.-2 = g.

Significance levels: p < 0.1 = *, p < 0.05 = **, p < 0.001 = ***, Not significant = ^{NS}

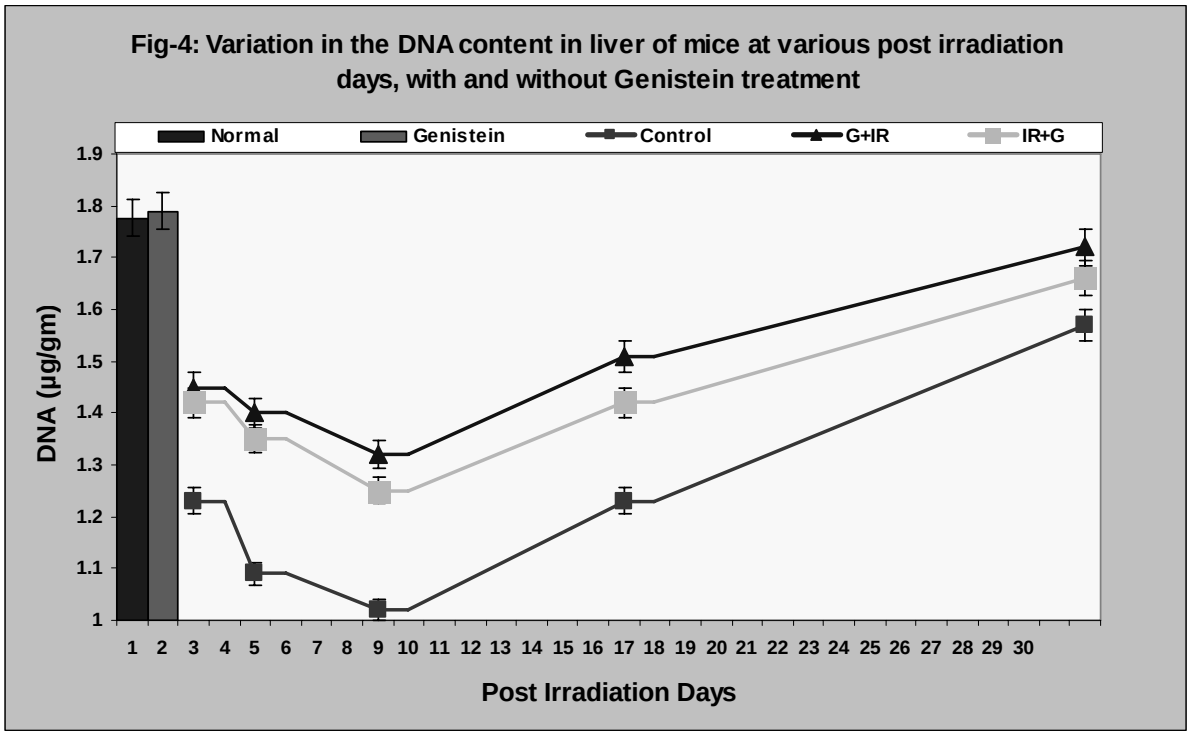

Fig-4: Variation in the DNA content in liver of mice at various post irradiation days, with and without Genistein treatment

and 15th post-irradiation days, respectively, were noticed which became insignificant by 30th day. Nevertheless the average recovery in DNA content of Experimental-1 group was approximately 21.61 ± 8.1863% (±SD) of control. As compared to those of normal group, a significant decrease ($p < 0.05$) in DNA content in Experimental-1 groups was noticed upto 7th post-irradiation day and then became insignificant on 30th day (Table-4, Fig.-4).

Experimental-2 (IR+G) vs. Control: In Experimental-2 group a similar pattern to that of Experimental-1 group in DNA content occurred. Statistically a significant recovery ($p < 0.05$) in DNA content in Experimental-2 group by 15.44%, 23.85%, 22.56% and 15.4%, respectively, on 1st, 3rd, 7th and 15th post-irradiation days was noticed which became insignificant on 30th day, as compared to those of control. Nevertheless the average recovery in DNA content of Experimental-2 group has been approximately 16.61 ± 7.2242% (±SD). As compared to those of normal, a significant decrease ($p < 0.05$) in DNA content in Experimental-2 groups was noticed upto 15th post-irradiation day and then became insignificant on 30th day. In both Experimental groups no significant variations in DNA content were noticed on all post-irradiation days (Table-4, Fig.-4).

DISCUSSION

Protein

Protein is essential for biosynthesis of glutathione, which provides the ultimate protection against the toxic effects of ROS. Decrease in the protein content after exposure to irradiation might be due to either decline in the rate of protein synthesis or an increase in the consumption of protein. Radiation may also induce local defects in microstructure of protein molecules, which become center

of thermal denaturation and cross linkage, thus causing tissue damage[2]. Increase in protein concentration in Experimental group is a beneficial effect. This process is important in the ribosomal activities, which enhance protein synthesis. This can be treated as an antiradiation effect. Reduction rate of the protein synthesis may be due to unfavourable condition like unavailability of one or more essential enzymes and /or reduction in the site of protein synthesis but if Genistein is available and accessible, many of these unpaired electrons get scavenged due to their excellent antioxidant property.

However, the protein synthesis is known to be unaffected by a low dose of radiation, but its rate of synthesis is reduced within a short period in tissue subjected to high doses. The decrease of protein amount after irradiation may be due to its lysis by gamma- radiation or may be at the synthesis level or it may be due to the depression of enzymes involved in the activation of amino acids and transferring to tRNA or by the inhibition of release of synthesized polypeptides from polysomes. The decrease in protein is associated with high TBARS, could result in an increased production of reactive free radicals damaging cells and initiating lipid peroxidation[3-5].

In irradiated mice, the amount of protein was found to be significantly lower than their corresponding Genistein pre-treated and post-treated group of mice at all the irradiation intervals. There was a sharp decline in protein content in liver after irradiation on 7^{th} day then it gradually increases by 30^{th} day in both the Experimental groups and control group, but Experimental groups achieved normal level by 30^{th} day. In control group an average depletion in protein content was approximately 34.18 ± 11.30%, in which a recovery by an average 28.63 ± 1.4349% and 25.14 ± 5.2347% occurred in Experimental-1 group and Experimental-2 group, respectively, from that of control group. In Experimental groups, statistically a highly significant recovery ($p < 0.001$) in protein content has been noticed on all post-irradiation days as compared to those of control group.

Up to 30% of all proteins may be modified by kinase activity, and kinases are known to regulate the majority of cellular pathways, especially those involved in signal transduction, the transmission of signals within the cell. The chemical activity of a kinase involves removing a phosphate group from ATP and covalently attaching it to one of three amino acids that have a free hydroxyl group. Most kinases act on both serine and threonine, others act on tyrosine, and a number (dual specificity kinases) act on all three. An important feature of kinases is that a single molecule is able to activate many substrate molecules, thus allowing for amplification of the initial signal. So the kinases are of interest to researchers involved in drug discovery, because of their broad relevance to health and diseases.

A primary benefit of using TKIs in cancer treatment is that they can be directed to kill tumor cells without harming healthy cells. This is due to the dependence of cancerous cells on uninterrupted signaling from tyrosine kinases, whereas the non-cancerous cells only use these signals occasionally[15]. Hence due to their site of action dependent or tissue dependent effect of TKIs, it was hypothesized that it might prove an extraordinary radioprotector, which may may provide shield to normal tissue but may synergistically to cancerous tissue.

The higher value of protein content in Experimental-1 and Experimental-2 groups as compared to control group also suggested that Genistein worked as antioxidant that quenched the free radicals, so that DNA and mRNA can be protected from free radicals and protein synthesis occurred.

Tyrosine kinases use ATP as a source of phosphate, but if Genistein a tyrosine kinase inhibitor binds to the enzyme instead of ATP, then the kinase can not phosphorylate proteins and signaling halts and ultimately prevent transmition of the extracellular signal to the nucleus and prevent changes in gene expression[6]. Genistein inhibits protein tyrosine kinase, which is involved in phosphorylation of tyrosyl residues of membrane-bound receptors leading to signal transduction, and it inhibits topoisomerase II, which participates in DNA replication, transcription and repair. By blocking the activities of PTK, topoisomerase II and matrix metalloprotein and by down-regulating the expression of about 11 genes, including that of vascular endothelial growth factor, Genistein can arrest cell growth and proliferation, cell cycle at G2/M, invasion and angiogenesis.

It has been reported that the exposure of cells to ionizing radiation results in activation of Ataxia Telangiectasia Mutated Protein (ATM) dependent signaling pathways that leads to activation of p53 and the protein kinase Chk2[16]. Treatment of ATM-positive cells with Genistein was found to dramatically increase the ability of p53 to bind to its specific DNA sequence. Binding of p53 to DNA was significantly reduced in A-T cells compared with normal cells, again indicating that the effect of Genistein on p53 is very similar to that of ionizing radiation. Activation of the sequence-specific DNA binding properties of p53 suggests that Genistein might activate the transcriptional activation activity of p53 and result in induction of downstream genes such as p21[17].

Deoxyribose Nucleic Acid (DNA)

It was observed from the study, that the DNA content showed a continuous decline after radiation exposure till 7^{th} day followed by a slight recovery observed on 30^{th} day. In control group an average decrease in DNA content was approximately 30.86 ± 11.92%. From this overall an average recovery by 21.61 ± 8.19% and 16.61 ± 7.22% in Experimental-1 group and Experimental-2 group, respectively, occurred from that of control group. Therefore, Genistein treatment, both prior and after radiation exposure provided significant ($p < 0.05$) protection at all the autopsy intervals as indicated by increased DNA content as compared to those of control group.

Several mechanisms can be offered for the explanation of reduced content of DNA[6]. It has been shown that post-irradiation acute cell death could lead to loss of DNA in excess than is normally eliminated from the tissue. The prolonged interphase or delayed onset of DNA synthesis after irradiation also could lead to decreased content of DNA. The drop in DNA content is due to an inhibition of replication of this compound in nucleus and accumulation of ribonucleotide in the cytoplasm, which is based on the inability, of irradiated cell to reduce ribonucleotide to DNA in the nucleus. There is also now general agreement that interference with DNA is one of the important biological effects of the irradiation. Our findings indicate that Genistein a tyrosine kinase inhibitor provide significant ($p <$

0.05) protection against radiation in Experimental groups and achieved normal level on 30th day.

Tyrosine Kinases are an important target as they play an important role in the modulation of growth factor signaling. By blocking the tyrosine kinases receptor, the goal is to prevent the cascade of reactions and inhibit proliferation, survival, invasion, and angiogenesis. Due to their involvement in various diseases like cancers, TKs have become prominent targets for therapeutic intervention. The adapter Grb2 contains one phosphotyrosine binding src homology 2 domain (SH2) and two proline-rich binding src homology 3 domains (SH3). Autophosphorylation of a receptor (e.g., Epidermal Growth Factor Receptor) or phosphorylation of a receptor associated adapter such as Shc allows Grb2 to bind to these proteins via its SH2 domain. The SH3 domain of Grb2 then binds to the proline-rich C-terminal tail of Sos and recruits Sos to the membrane-bound complex. Sos, a GTP/GDP exchange factor, activates Ras by exchanging GTP for GDP on the Ras molecule. The GTP-bound form of Ras then binds to Raf protein kinase (a MAPK kinase kinase) isoforms, including C-Raf-1, B-Raf and A-Raf. This interaction results in targeting of Raf to the membrane where its protein kinase activity is increased by phosphorylation, thereby allowing it to activate other signaling molecules[18].

Genistein a tyrosine kinase inhibitor, compete with the ATP binding site of the catalytic domain of several oncogenic tyrosine kinases. This is possible because of structural similarities between ATP and the TKIs. Kinases use ATP as a source of phosphate, but if a TKI binds to the enzyme instead of ATP, then the kinase can not phosphorylate proteins and signaling halts. Tyrosine kinases use ATP as a source of phosphate, but if Genistein binds to the enzyme instead of ATP, then the kinase can not phosphorylate proteins and signaling halts and ultimately prevent transmition of the extracellular signal to the nucleus and prevent changes in gene expression which disturb homeostasis conditions of cells[6].

The treatment of irradiated cells or ATM-positive cells with Genistein was found to dramatically increase the ability of p53 to bind to its specific DNA sequence. Binding of p53 to DNA was significantly reduced in A-T cells compared with normal cells, again indicating that the effect of Genistein on p53 is very similar to that of ionizing radiation. Activation of the sequence-specific DNA binding properties of p53 suggests that Genistein might activate the transcriptional activation activity of p53 and result in induction of downstream genes such as p21[17].

Our results show that Genistein treatment increased the DNA content in normal and Experimental group as compared to those of control group. So Genistein provide protection against radiation induced oxidative stress on mice which mediate through genes and can prove as follows.

(1) Genistein treatment increases protein, DNA in Experimental and normal group as compared to those of control group. As Genistein treatment do not affect normal cell whereas it increased the readiosensitivity of tumor cells. Therefore, Genistein a tyrosine kinase inhibitor may afford protection not only before the radiation exposure but also after the radiation exposure.

(2) The treatment of irradiated cells with Genistein was found to dramatically increase the ability of p53 to bind to its specific DNA sequence. Binding of p53 to DNA was significantly reduced in A-T cells compared with normal cells, again indicating that the effect of Genistein on p53 is very similar to that of ionizing radiation. Activation of the sequence-specific DNA binding properties of p53 suggests that Genistein might activate the transcriptional activation activity of p53 and results in induction of downstream genes such as p21.

CONCLUSION

Man is exposed to a number of toxic substances in the environment including radiation as well as to toxic metabolites and ROS generated within the body. From the present study it is obvious that Genistein prevent the toxic effects of ROS, there is likelihood that Genistein may exert an antiradiation influence in the body. So, it would further pave way to the formulation of medicine against radiation and toxicity induced during radiotherapy. Owing to this property, the Genistein known for its functional properties can be further extended to exploit its possible application for various health benefits as nutraceuticals and food ingredient in radiotherapy to protect the normal tissue.

Genistein, a potent protein tyrosine kinase inhibitor increased protein, DNA content and maintained the normal levels of other biochemical parameters against the oxidative stress produced by radiation in normal tissue of mice. The results indicate that Genistein against radiation effect may pave way to the formulation of medicine in radiotherapy for normal tissue and possible against radiomimetic drug induced toxicity.

SIGNIFICANCE OF FINDINGS

At present there has not been a single effective radioprotectant drug which could show its efficacy with respect to a longer retention in the body in relation to time of exposure either as radioprotective or radiosensitive agent. Till now the body-load of the drug has been first created either before irradiation through chronic administration or the drug has been administered just shortly before or after administration. The findings of proposed work will likely to cover up such shortcomings which exist in drug testing studies for and against radiation. Present study established the fact that Genistein may be used as a radioprotector before and after radiation exposure. Hence the possibility of using Genistein as a radioprotectant and radiotherapeutic drug in accidental conditions or nuclear war conditions can not be ruled out.

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