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Original Article

Effects of Radiation on the Protein and DNA Content in Genistein Treated Swiss Albino Mice

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Abastract

Genistein is a soya isoflavone, which is found naturally in legumes, such as soybeans and chickpeas. The intraperitoneal administration of the optimum dose (200 mg/kg) of genistein 24 h and 15 minbefore irradiation (8 Gy at a dose rate of 1.02 Gy/min) recovered the deficit in protein content (28.63±1.44%) and the DNA content (21.61±8.19%) if an average of 5 intervals of autopsies 1st, 3rd, 7th, 15th and 30th days are taken into consideration as compared to those of control group in Swiss albino mice. 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.

Keywords: Genistein; Oxidative stress; Radiation; Tyrosine kinase inhibitor.
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1. 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 radiotherapy where the necessity of protection of normal tissue is needed. Plant products appear to have an advantage over synthetic products in terms of low/no toxicity at effective dose.

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Amino acids are essential for biosynthesis of proteins and 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]. Reduction rate of the protein synthesis may be due to unfavorable condition like unavailability of one or more essential enzymes and/or reduction in the site of protein synthesis, but if genistein is available, many of the unpaired electrons produced during oxidative stress is scavenged. 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.
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. One study has shown that 60CO 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. 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.

Our earlier studies showed that the intraperitoneal administration of genistein did not cause any toxic effect on mice and genistein treatment offers better survivability of mice. The maximum survival of mice (30%) has been recorded in the 200 mg/kg body weight dose of genistein and this was selected for further investigation against 8 Gy of gamma radiation. 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 [10, 11]. Liver is selected as a testing organ because some scientists reported it as highly radiosensitive organ [12] and the present study has been carried out in order to check ameliorating capacity of genistein against radiation with respect to protein and DNA content of mice liver.

2. Materials and methods

2.1. Animals

Swiss albino mice (*Mus musculus*) obtained from All India Institute of Medical Sciences (AIIMS), New Delhi were kept in controlled condition of temperature (25±2 °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 6-8 weeks old male mice with an average body weight of 22±3 g were selected from inbred colony.

2.2. Drug

Genistein was obtained as a gift sample from Palm Pharmaceuticals, Inc., USA, manufactured by L.C. Laboratories, 165 New Boston St. Woburn, MA01801 USA. Genistein was dissolved in dimethyl sulfoxide and then different concentration solutions were prepared so that the volume administered intraperitoneally was 0.5 ml.

Mice were administered intraperitoneally with an optimum dose of 200 mg/kg body weight of Genistein 24 h and 15 min before of irradition.

2.3. Biochemical Assays

Autopsies were performed by means of cervical dislocation of 6 mice from each group at each five post irradiation intervals (1st, 3rd, 7th, 15th and 30th). At least six observations were taken and 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.

2.4. Experimental protocol

Mice were divided into following five groups; Group-I normal: Mice of this group did not receive any treatment and kept under normal conditions; Group-II genistein treated: Mice of this group received an intraperitoneal 200 mg/kg body weight dose of genistein as worked out in our earlier experiments 24 h and 15 min. before study time; Group-III control: Mice of this group received intraperitoneal dimethyl sulfoxide as a vehicle 24 h and 15 min. before irradiation, equivalent to the optimum dose of genistein. Group-IV experiment-1 or G+IR: Mice of this group received an intraperitoneal 200 mg/kg body weight dose of genistein 24 h and 15 min. before irradiation. Group-V experiment-2 or IR+G: This group of mice was first exposed to gamma radiation and

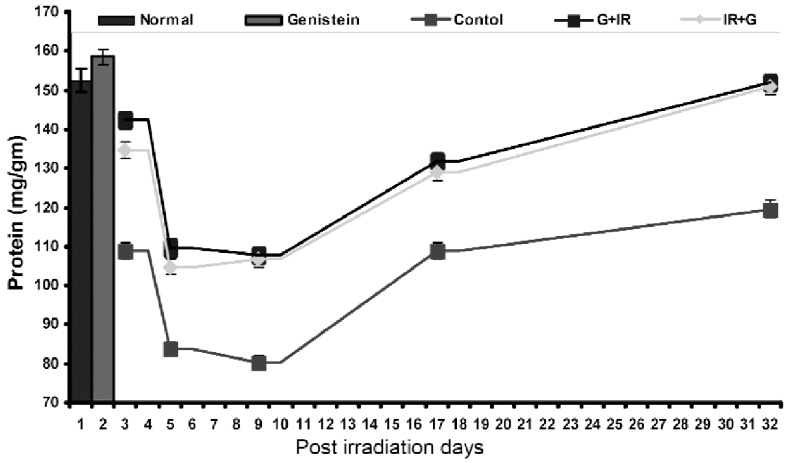

Figure 1. Variation in the protein content in liver of mice at various post irradiation days, with and without genistein treatment.

then received an intraperitoneal 200 mg/kg body weight dose of genistein 15 min. and 24 h after irradiation.

Mice of above treated groups 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.

3. Results

3.1. Protein

Genistein *vs*. Normal: A significant increase (4.1%) in the protein content was noticed in genistein treated group as compared to those of normal groups (Table 1 and Figure 1).

Genistein *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, compared to those of normal group. The average decrease in protein content of control group was approximately 34.18±11.30% (Table 1 and Figure 1).

Experimental-1 (G+IR) *vs*. Control: The protein content tended to decline upto 7th day in experimental-1 group, which was followed by an increase till 30th day. In experimental-1 group, the average recovery in protein content was approximately 28.63±1.44% compared to that of irradiated (control) group. Statistically a highly significant recovery (p<0.001) by 30.64%, 30.60%, 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, 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 1 and Figure 1).

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

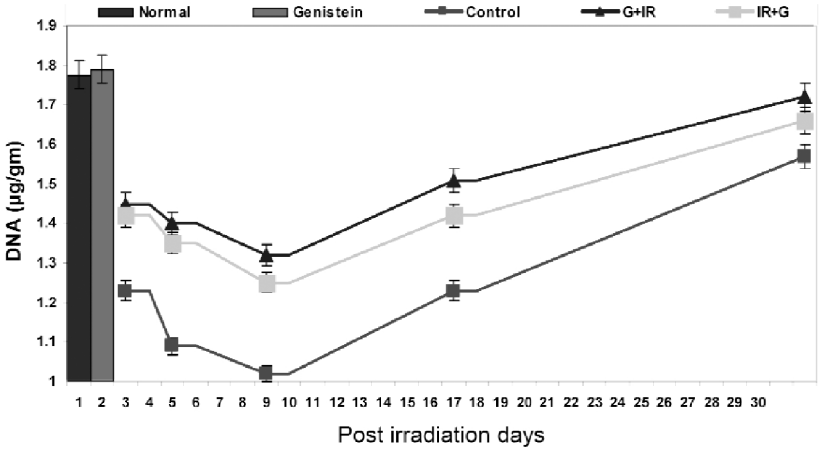

Figure 2. Variation in the DNA content in liver of mice at various post irradiation days, with and without genistein treatment.

Table 1. Variation in the protein content (mg/g) 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±1.79 (74.3%) ^{b***}	83.92±1.79 (55.11%) ^{b***}	80.35±1.79 (52.38%) ^{b***}	108.92±1.79 (70.48%) ^{b***}	119.64±1.79 (76.85%) ^{b***}
Experimental-1 (Genistein+IR)	142.30±1.57 (97.07%) $^{c*, d***}$	109.61±1.11 (71.98%) $^{c***, d***}$	107.69±1.57 (70.19%) $^{c***, d***}$	131.73±1.84 (85.23%) $^{c***, d***}$	151.92±1.11 (97.58%) $^{c*, d***}$
Experimental-2 (IR+Genistein)	134.61±1.57 (91.83%) $^{e***, f***, g**}$	104.8±0.96 (68.82%) $^{e***, f***, g**}$	106.8±1.84 (69.57%) $^{e***, f***, gNS}$	128.84±1.11 (83.37%) $^{e***, f***, gNS}$	150.96±0.96 (96.96%) $^{e**, f***, gNS}$

Normal = 152.50±1.32 (100%); ^{a**}Genistein = 158.75±1.24 (104.1%); Each value represents Mean±SEM. Statistical comparison: ^{a}Normal *vs*. Genistein; ^{b}Normal *vs*. Control; ^{c}Normal *vs*. Exp.-1; ^{d}Control *vs*. Exp.-1; ^{e}Normal *vs*. Exp.-2; ^{f}Control *vs*. Exp.-2; ^{g}Exp.-1 *vs*. Exp.-2; Significance levels: **p*<0.1; ***p*<0.05; ****p*<0.001; ^{NS}Not significant.

recovery in protein content of experimental-2 group was approximately 25.14 ± 5.24%. 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 1 andFigure 1).

3.2. Deoxyribose nucleic acid (DNA)

Genistein *vs.* Normal: The DNA content of genistein treated group was not significantly higher than those of normal groups (0.78%) (Table 2 and Figure 2).

Control *vs.* Normal: A sharp decline by 7th day (by 42.57% of the normal) was 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, 7th and 15th post-irradiation days, respectively, compared to those of normal group, which was not significant on 30th day. The average deficit in DNA content of control group was approximately 30.86±11.92% (Table 2 and Figure 2).

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 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±8.19% 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 2 and Figure 2).

Experimental-2 (IR+G) *vs.* Control: In experimental-2 group a similar pattern to that of experimental-1 group in DNA content occurred. A statistically 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±7.22%. As compared to those of normal, a significant decrease (p<0.05) in DNA content in

Table 2. Variation in the DNA content (mg/g) 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±0.01 (69.25%) b**	1.09±0.08 (61.37%) b**	1.02±0.09 (57.43%) b**	1.23±0.07 (69.25%) b**	1.57±0.06 (88.4%) bNS
Experimental-1	1.45±0.08	1.4±0.10	1.32±0.08	1.51±0.08	1.72±0.08
(Genistein+IR)	(81.64%) c**, d**	(78.82%) c**, d**	(74.32%) c**, d**	(85.02%) c*, d**	(96.84%) cNS, dNS
Experimental-2	1.42±0.08	1.35±0.07	1.25±0.01	1.42±0.03	1.66±0.09
(IR+Genistein)	(79.95%) e**, f**, gNS	(76.01%) e**, f**, gNS	(70.38%) e**, f**, gNS	(79.95%) e**, f**, gNS	(93.46%) eNS, fNS, gNS

Normal = 152.50±1.32 (100%); [a]**Genistein = 158.75±1.24 (104.1%); Each value represents Mean±SEM. Statistical comparison: [a]Normal *vs*. Genistein; [b]Normal *vs*. Control; [c]Normal *vs*. Exp.-1; [d]Control *vs*. Exp.-1; [e]Normal *vs*. Exp.-2; [f]Control *vs*. Exp.-2; [g]Exp.-1 *vs*. Exp.-2; Significance levels: *p<0.1; **p<0.05; ***p<0.001; [NS]Not significant.

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 2 and Figure 2).

4. Discussion

Amino acids are essential for biosynthesis of proteins and glutathione, which is an important agent for 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 synthesis and concentration by external compounds is a beneficial 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 or by effects of free radicals in oxidative stress, and antioxidants such as genistein can prevent these effects by scavenging free radicals.

However, the protein synthesis is known to be unaffected by low doses 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 7th day then it gradually increases by 30th day in both the experimental groups and control group, but experimental groups achieved normal level by 30th 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±1.44% and 25.14±5.25% occurred in experimental-1 group and experimental-2 group, respectively, from that of control group. In experimental groups, a highly significant recovery in protein content was 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 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 may has 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].

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 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 protection against radiation in experimental groups and achieved normal level on 30^{th} 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 protein and 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.

5. 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.

6. Significance of finding

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.

References

[1] Pellmar TC, Rockwell S. Meeting report: Priority list of research areas for radiological nuclear threat countermeasures. *Radiat Res* 2005; 163: 115-23.

[2] Old MG, Stocken LA. The biochemical sessions *in vivo in vitro.* In: Erreca M, Forsberg A, (editors). *Mechanisms in radiobiology*. New York: Academic Press, 1961; p. 259.

[3] Ermolazeva NV. Enzymic degradation of deoxyribonuleoprotein (DRNP) isolated from tissue of normal and after X-irradiation. *Biokhimua* 1967; 32: 639.

[4] Ivanov II, Radakov VV. Effects of whole body gamma-rays irradiation on tissue protein synthesis. *Bull Expt Biol Med* 1968; 66: 1340.

[5] Kim ST, Kim JD, Ahn SH, Ahn GS, Lee YI, Jeong YS. Hepatoprotective and antioxidant effects of *Alnus japonica* extracts on acetaminophen-induced hepatotoxicity in rats. *Phytother Res* 2004; 18: 971-5.

[6] Sit KH, Wong KP, Bay BH. Effects of genistein on ATP induced DNA synthesis and intracellular alkalinization in chang liver cells. *Japan J Pharmacol* 1991; 57: 109-11.

[7] Egana E, Liona I, Ramimirej MT. Effect of whole body gamma radiation in DNA, RNA and protein function in developing CNS. *Proc VII Int Cong Radiat Res* 1983; 1-7.

[8] Cerda H. Effects of low doses of gamma radiation on DNA synthesis in the developing rat brain. *Acta Radiol Oncol* 1983; 3: 233-9.

[9] Egana E. Some effects of ionizing radiations on the metabolism of the neuron system. *Ind J Neurol* 1962; 3: 631-47.

[10] Bhatia AL, Gaur A, Sharma A. Radiation protection by an isoflavone genistein: A study on the survivability of mice. *Nucl Technol Radiat Protection* 2007; 22: 34-9.

[11] Gaur A, Sharma A, Bhatia AL. Genistein against radiation damage: A study on Swiss albino mice. *Asian J Exp Sci* 2006; 20: 269-73.

[12] Bhatia AL, Gupta ML, Singh RP. Response of mice liver to continuous irradiation from tritiated water. *J Radiat Res* 1978; 19: 197-204.

[13] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye binding. *Anal Biochem* 1976; 72: 248-54.

[14] Ceriotti G. Determination of nucleic acid in animal tissue. *J Biol Chem* 1955; 214: 59-70.

[15] Donato NJ, Talpaz M. Clinacal use of tyrosine kinase inhibitors: Therapy for chronic myelogenous leukemia and other cancers. *Clin Cancer Res* 2000; 6: 2965-6.

[16] Matsuoka S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* 1998; 282: 1893-7.

[17] Ruiqiong Y, Bodero A, Zhou BB, Khanna KK, Lavin MF, Miller SPL. The plant isoflavenoid genistein activates p53 and Chk2 in an ATM-dependent manner. *J Biol Chem* 2001; 276: 4828-33.

[18] Simon MA. Receceptor tyrosine kinases: Specific outcomes from general signals. *Cell* 2000; 103: 13-5.