

# Ameliorating effects of genestein: Study on mice liver glutathione and lipid peroxidation after irradiation

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**Background:** Genistein is a soya isoflavone, which is found naturally in legumes, such as soybeans and chickpeas. Radiation-induced free radicals in turn impair the antioxidative defense mechanism, leading to an increased membrane lipid peroxidation that results in damage of the membrane bound enzyme and may lead to damage or death of cell. Hence, the lipid peroxidation is a good biomarker of damage occurs due to radiation and the inhibition of lipid peroxidation is suggestive of radioprotective action. Glutathione has been shown to protect cells against oxidative stress by reacting with peroxides and hydroperoxides and determines the inherent radiosensitivity of cells. **Materials and Methods:** For experimentation, healthy Swiss Albino male mice of 6-8 weeks old were selected from inbred colony. Genistein was dissolved in dimethyl sulfoxide and then prepared different concentration solutions so that the volume administered intraperitoneally was 0.5 ml. Lipid peroxidation was estimated by the method of Ohkawa and GSH was estimated by the method of Moron. **Results:** 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) reverted the increase in lipid peroxidation (by 18.01%  $\pm$  3.05) and decrease of Glutathione (by 62.05%  $\pm$  21.58) caused by irradiation in liver of Swiss albino mice. Statistically analyzed survival data produced a dose reduction factor (DRF) = 1.24. **Conclusion:** 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. **Iran. J. Radiat. Res., 2009; 7 (4): 187-199**

**Keywords:** Genistein, tyrosine kinase inhibitor, radiation, liver, oxidative stress

## 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<sup>(1)</sup>. 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.

Radiation-induced free radicals in turn impair the antioxidative defense mechanism, leading to an increased membrane lipid peroxidation (LPO) that results in damage of the membrane bound enzyme<sup>(2)</sup>. Radiation induced lipid peroxidation has been reported to be caused by superoxide radicals<sup>(3)</sup>. However, later studies indicated that the hydroxyl radical is the most active species involved in radiation induced lipid peroxidation<sup>(4-7)</sup>. Lipid peroxidation is a highly destructive process and cellular organelles and whole organism, lose biochemical function and/or structural architecture, which may lead to damage or death of cell. Hence, the lipid peroxidation is a good biomarker of damage occurs due to radiation and the inhibition of lipid peroxidation is suggestive of radioprotective action. LPO can be initiated by radiolytic products including hydroxyl and hydroperoxyl radicals<sup>(8)</sup>. Depletion of glutathione

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results in enhanced LPO<sup>(9)</sup>. It was observed that flaxseed oil pre-treatment significantly lower the radiation induced LPO in terms of malondialdehyde<sup>(10)</sup>. Inhibition of LPO in cell membrane can be caused by antioxidants<sup>(11)</sup>. Oxidative stress leads to lipid peroxidation, protein and carbohydrate oxidation and metabolic disorders<sup>(12-14)</sup>.

Therefore the measurement of LPO is a convenient method to monitor oxidative cell damage. The products of lipid peroxidation are toxic to cell. Lipid peroxidation within the membrane has a devastating effect on the functional state of the membrane because it alters membrane fluidity, typically decreasing it and thereby allowing ions such as Ca<sup>2+</sup> to leak into the cell. The peroxy radical formed from lipid peroxidation attacks membrane protein and enzymes and reinitiates lipid peroxidation. The preservation of cellular membrane integrity depends on protection on repair mechanism capable of neutralizing oxidative reactions. The best-characterized biological damage caused by °OH radical is its ability to stimulate the free radicals chain reaction, which results into the lipid peroxidation. This occurs when the °OH is generated close to membranes and attacks the fatty acid side chains of membrane phospholipids. In addition lipid hydroperoxide can decompose to yields a range of highly cytotoxic products such as malondialdehyde<sup>(15)</sup>. LPO plays a crucial role in inflammation, cancer and cardiac disease<sup>(16)</sup>. LPO has been suggested as one of the main causes of radiation induced membrane damage<sup>(17)</sup>. An extensive investigation has been presented the involvement of molecular damage to membrane as the loss of cellular functions after radiation exposure<sup>(18)</sup>. The gamma irradiation of liposomal membrane modal has shown significantly enhanced membrane rigidity possibly due to involvement of radicals of liquids<sup>(19)</sup>.

Glutathione (GSH), a tripeptide composed of glutamate, cysteine and glycine, is present in most plants and animal tissues and is the most important

and ubiquitous low molecular weight thiol compound. Working intra and extra-cellularly in its reduced form, L-glutathione, abbreviated as "GSH", is the body's key antioxidant and protectant. GSH has multiple functions in disease prevention and in detoxification of chemicals and drugs while its depletion is associated with increased risks of toxicity and disease. GSH works synergistically with the other cellular antioxidants to neutralize and scavenge oxygen and other free radical species and thereby prevent or diminish "oxidative stress".

Glutathione may have clinical importance since enzyme deficiencies of the glutathione metabolism may cause hemolytic anemia and neurologic symptoms in children<sup>(20)</sup> and decreased glutathione levels have been reported in several disease including acquired immune deficiency syndrome<sup>(21, 22)</sup>, diabetes<sup>(23)</sup>, adult respiratory distress syndrome<sup>(24)</sup> and Parkinson disease<sup>(25)</sup>. Glutathione with its sulfhydryl group functioned in the maintenance of sulfhydryl groups of other molecules (especially proteins), as a catalyst for disulfide exchange reactions, and in the detoxifications of foreign compounds, hydrogen peroxide, and free radicals<sup>(26, 27)</sup>.

Thiols such as GSH and dihydrolipoate support vitamin C and E recycling<sup>(28, 29)</sup>. GSH-transferase and reductase, involved in the termination of lipid peroxidation<sup>(30, 31)</sup>. GSH has been shown to protect cells against oxidative stress by reacting with peroxides and hydroperoxides<sup>(32)</sup> and determines the inherent radiosensitivity of cells<sup>(33)</sup>. Changes in glutathione level after first fraction of conventional radiotherapy may help in protecting the tumor response to radiotherapy and also in identifying radio-resistant tumors<sup>(34)</sup>.

GSH status affects the synthesis of two major cellular polymers, i.e. proteins and DNA. Oxidation or depletion of GSH may decrease protein biosynthesis. Numerous enzymes are GSH dependent, e.g. glutathione synthetases, glutathione peroxi-

dase, glutathione transferases, glutaredoxin and glyoxylase. The activity of further enzymes may be regulated by thiodisulfide exchange, and thus depend on the GSH status. Therefore, conclusion can be drawn that cells not only become more susceptible to any further challenge, but their basic functions are also perturbed by the extensive GSH depletion<sup>(35)</sup>.

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 has been found to increase the activities of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase<sup>(36-38)</sup>. Genistein up-regulates expression of antioxidant genes: involvement of estrogen receptors, ERK1/2, and NFB<sup>(39)</sup>. Some Scientists demonstrated that intraperitoneal administration of Genistein increased the survival of mice against 8 Gy gamma irradiation<sup>(40, 41)</sup>.

Liver is selected as a testing organ because some scientists reported it as highly radiosensitive organ<sup>(42)</sup>. The liver is the primary organ responsible for drug metabolism and mainly detoxifies damaging electrophiles generated during oxidative stress. Above evidences demonstrated that Genistein has a wide range of activities like antiestrogenic, anticancer and potent protein tyrosine kinase inhibiting activities. The present study has been carried out in order to check ameliorating capacity of Genestein against radiation with respect to glutathione and LPO 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 ( $25 \pm 2$

°C) and light (light:dark, 12:12 hrs). They were provided standard mice feed (procured from Hindustan Uniliver ltd. Mumbai) and water ad libitum. For experimentation, healthy male mice of 6-8 weeks old with an average body weight of  $22 \pm 3$  gr 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 irradiation.

**Biochemical Assays:** Five autopsies were performed by mean of cervical dislocation of 6 mice from each group at each post irradiation interval (1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup>) 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. Lipid peroxidation was estimated by the method of Ohkawa<sup>43</sup> and GSH was estimated by the method of Moron<sup>44</sup>. The values are expressed as mean  $\pm$  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 irradiation. Finally, all the animals of five groups were exposed to 10 Gy of gamma radiation. Radiation sickness, mortality, behavioral 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<sub>50/30</sub> and dose reduction factor**

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

$$DRF = \frac{LD_{50/30} \text{ of Experimental animals}}{LD_{50/30} \text{ of Control animals}}$$

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 irradiation 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:

**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 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 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 LPO and Glutathione.

**RESULTS**

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

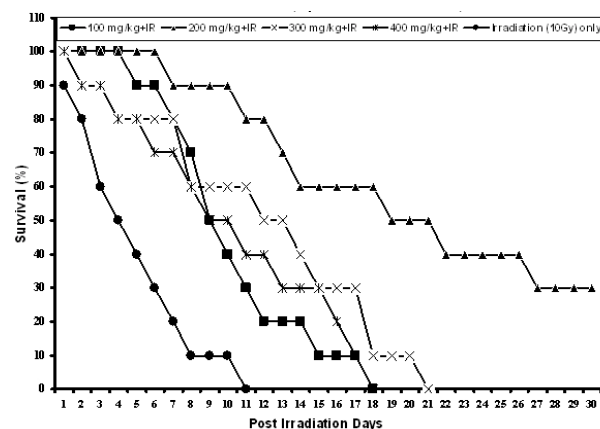


Figure 1. Survival of mice against lethal gamma irradiation with different doses of Genistein (Optimum Dose Selection).

investigation against 8 Gy of gamma radiation (table 1, figure 1).

The LD<sub>50/30</sub> 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, figure 2).

Table 1. 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) | 9                 | 10   | 11   | 14        | 17        | 18        |
| Genistein +IR (200 mg/kg) | 21                | 26   | 30   | Not found | Not found | Not found |
| Genistein +IR (300 mg/kg) | 13                | 14   | 17   | 17.5      | 20        | 21        |
| Genistein +IR (400 mg/kg) | 10                | 12   | 15   | 16        | 17        | 18        |

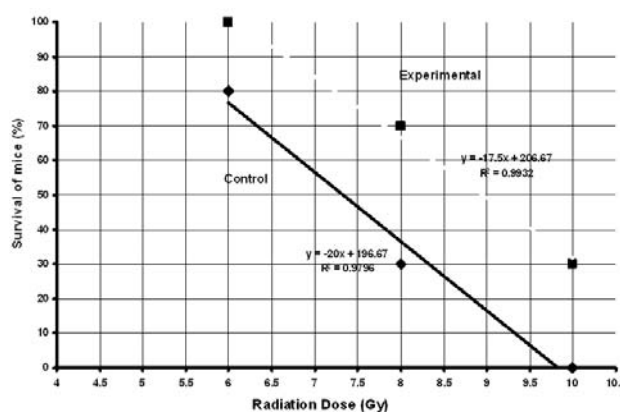


Figure 2. Regression and correlation analysis of the survival of mice (LD<sub>50/30</sub> estimation).

Table 2. Regression analysis of percentage survival of mice (LD<sub>50/30</sub> estimation).

| Groups                      | Intercept (b) | Slope (m) | Y = mx + b             | LD <sub>50/30</sub> | 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                   |      |

**Lipid Peroxidation (LPO)**

**Genistein vs. Normal:** In Genistein administered group the MDA content (TBARS levels) did not show any appreciable loss than those of normal groups (by 0.64%), (table 3, figure 3).

**Control vs. Normal:** The values of lipid peroxidation continuously increased after irradiation upto 7<sup>th</sup> day (by 57.2%) and then MDA content start to decline, which continued till 30<sup>th</sup> day. Statistical high significant increases ( $p < 0.001$ ) by 28.52%, 46.51%, 57.2%, 37.6%, and 26.16% in MDA content in control groups were noticed on 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> post-irradiation days, respectively, as compared to those of normal groups. The average increase in MDA content of control group was approximately  $39.2 \pm 12.8850\%$  ( $\pm$ SD) (table 3, figure 3).

**Experimental-1 (G+IR) vs. Control:** In Experimental-1 group, an increase in MDA

content was noticed upto 7<sup>th</sup> day (by 24.82%). This is followed by a recovery by 30<sup>th</sup> day. As compared to those of control group, statistically a highly significant recovery ( $p < 0.001$ ) by 19.17%, 18.49%, 20.6%, 12.73% and 19.06% in MDA content in Experimental-1 group was noticed on 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> post-irradiation days, respectively. The average recovery in MDA content from that of control group was approximately  $18.01 \pm 3.0522\%$  ( $\pm$ SD). While comparing with those of normal, a highly significant increase ( $p < 0.001$ ) in the MDA contents were noticed from day 1<sup>st</sup> to 15<sup>th</sup> post-irradiation days, which attained normal level by 30<sup>th</sup> day (table 3, figure 3).

**Experimental-2 (IR+G) vs. Control:** A similar pattern of recovery of MDA content in Experimental-2 group was noticed to that of Experimental-1 group. Statistically a highly significant recovery in MDA content by 17.03%, 17.58%, 19.79%, 12.31% and 18.97% in Experimental-2 group has been

**Table 3.** Variation in the TBARS (n mol/gm) level in liver of mice at various post irradiation days, with and without Genistein treatment.

| Groups                               | Post Irradiation Days                                |  |   |   |   |
|--------------------------------------|--|--|---|---|---|
|                                      | 1  | 3  | 7   | 15  | 30  |
| <b>Control (IR with 8 Gy only)</b>   | 332.34 $\pm$ 1.11<br>(128.52%)<br>b***               | 392.62 $\pm$ 0.7790<br>(146.51%)<br>b***                       | 404.17 $\pm$ 0.2835<br>(157.20%)<br>b***            | 355.00 $\pm$ 1.5176<br>(137.60%)<br>b***                        | 325.68 $\pm$ 0.740<br>(126.16%)<br>b***                       |
| <b>Experimental-1 (Genistein+IR)</b> | 268.66 $\pm$ 0.783<br>(103.89%)<br>c***, d***        | 320.05 $\pm$ 1.8478<br>(124.4%)<br>c***, d***                  | 320.94 $\pm$ 1.3329<br>(124.82%)<br>c***, d***      | 309.83 $\pm$ 0.6398<br>(120.09%)<br>c***, d***                  | 263.62 $\pm$ 1.710<br>(102.12%)<br>c*, d***                   |
| <b>Experimental-2 (IR+Genistein)</b> | 275.77 $\pm$ 1.25<br>(106.64%)<br>e***, f***,<br>g** | 323.6 $\pm$ 0.5058<br>(125.79%)<br>e***, f***, g <sup>NS</sup> | 324.200 $\pm$ 0.6568<br>(126.09%)<br>e***, f***, g* | 311.31 $\pm$ 0.9209<br>(120.66%)<br>e***, f***, g <sup>NS</sup> | 263.92 $\pm$ 1.245<br>(102.23%)<br>e**, f***, g <sup>NS</sup> |

Each value represents Mean  $\pm$  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 = <sup>NS</sup>

noticed on 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> post-irradiation days, respectively, as compared to those of control groups. The overall average recovery in MDA content of Experimental-2 group was around  $17.14 \pm 2.9114\%$  ( $\pm$ SD). While comparing with those of normal, a highly significant increase ( $p < 0.001$ ) in the MDA contents were noticed from day 1<sup>st</sup> to 15<sup>th</sup> post-irradiation days, which diminished on 30<sup>th</sup> day. As compared to those of Experimental-2 group, a significant variation (decreased) in MDA content was noticed on 1<sup>st</sup> day in Experimental-1 group which became insignificant by 30<sup>th</sup> post-irradiation day (table 3, figure 3).

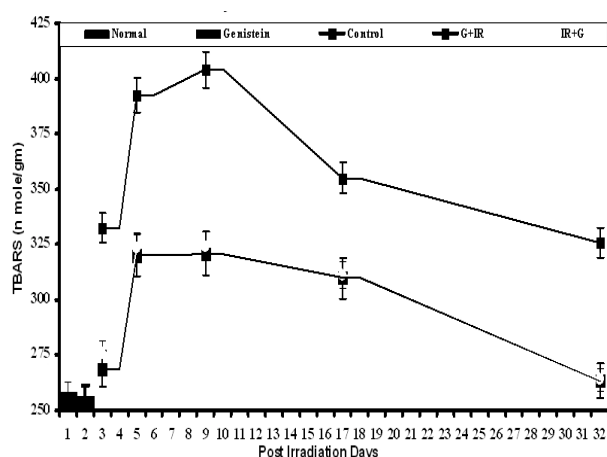


Figure 3. Variation in the TBARS (MDA) content in liver of mice at various post irradiation days, with and without Genistein treatment.

### Glutathione (GSH)

**Genistein vs. Normal:** The GSH content of mice liver was higher (statistically insignificant) in Genistein treated group than those of normal group (by 3.33%) (table 4, figure 4).

**Control vs. Normal:** The GSH decreased after irradiation upto 7<sup>th</sup> day (59.76%) and then increases upto 30<sup>th</sup> day. Statistical high significant decreases ( $p < 0.001$ ) in GSH level by 32.19%, 57.95%, 59.76%, 41.79%, and 37.85% in control groups were observed on 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> days, post-irradiation, respectively, as compared to those of normal groups. The trend shows

a dip on day 7<sup>th</sup> with a rise by 30<sup>th</sup> day which never attained normally. The average decrease in GSH level of control group was  $45.71 \pm 12.1357\%$  ( $\pm$ SD) of the normal (table 4, figure 4).

**Experimental-1 (G+IR) vs. Control:** In Experimental-1 group, GSH level decreased upto 7<sup>th</sup> day and then gained to normally upto 30<sup>th</sup> day. As compared to those of control group, statistically a highly significant recovery ( $p < 0.001$ ) in GSH level by 37.29%, 76.24%, 91.21%, 49.18% and 56.34% in Experimental-1 groups was noticed on 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> post-irradiation days, respectively. The average recovery in GSH level of Experimental-1 group was around  $62.05 \pm 21.5810\%$  ( $\pm$ SD). While comparing with those of normal, though a highly significant decrease ( $p < 0.001$ ) in GSH level was noticed on 3<sup>rd</sup> and 15<sup>th</sup> days, which became insignificant and attained almost near normal value on 30<sup>th</sup> day (table 4, figure 4).

**Experimental-2 (IR+G) vs. Control:** In Experimental-2 group, a similar pattern of recovery of GSH level to that of Experimental-1 group has been noticed. Statistically a highly significant recovery by 31.68%, 73.49%, 84.94%, 47.52% and 54.17% in GSH level in Experimental-2 group was noticed on 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> post-irradiation days, respectively, as compared to those of control group. The average recovery in GSH level of Experimental-2 group has been approximately  $58.36 \pm 21.1086\%$  ( $\pm$ SD). While comparing with those of normal, though a highly significant decrease ( $p < 0.001$ ) in GSH level was noticed from day 1<sup>st</sup> to 15<sup>th</sup> post-irradiation days, it tended to attain normal and became less significant on 30<sup>th</sup> day. Both the Experimental groups did not differ in their results and an insignificant increase in GSH level in Experimental-1 group occurred on all post-irradiation days, as compared to those of Experimental-2 groups (table 4, figure 4).

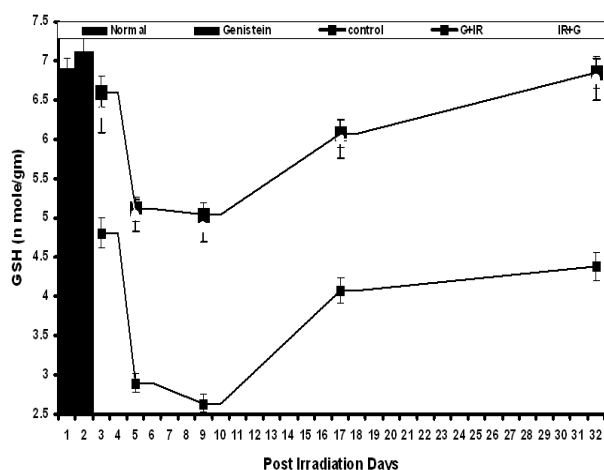
**Table 4.** Variation in the glutathione (n mol/gm) level in liver of mice at various post irradiation days, with and without Genistein treatment.

| Groups                        | Post Irradiation Days                                    |  |  |  |  |
|-------------------------------|--|--|--|--|--|
|                               | 1  | 3  | 7  | 15   | 30   |
| Control (IR 8 Gy only)        | 4.810 ± 0.028<br>(67.91%)<br>b***                        | 2.900 ± 0.191<br>(42.93%)<br>b***                        | 2.639 ± 0.176<br>(40.24%)<br>b***                        | 4.07 ± 0.246<br>(58.21%)<br>b***                         | 4.387 ± 0.225<br>(62.15%)<br>b***                      |
| Experimental-1 (Genistein+IR) | 6.605 ± 0.130<br>(93.24%)<br>c**, d***                   | 5.111 ± 0.156<br>(75.66%)<br>c***, d***                  | 5.047 ± 0.1816<br>(76.94%)<br>c**, d***                  | 6.074 ± 0.058<br>(86.84%)<br>c***, d***                  | 6.859 ± 0.057<br>(97.17%)<br>c <sup>NS</sup> , d***    |
| Experimental-2 (IR+Genistein) | 6.335 ± 0.079<br>(89.43%)<br>e***, f***, g <sup>NS</sup> | 5.031 ± 0.174<br>(74.48%)<br>e***, f***, g <sup>NS</sup> | 4.88 ± 0.0289<br>(74.42%)<br>e***, f***, g <sup>NS</sup> | 6.007 ± 0.054<br>(85.88%)<br>e***, f***, g <sup>NS</sup> | 6.764 ± 0.050<br>(95.82%)<br>e*, f***, g <sup>NS</sup> |

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



**Figure 4.** Variation in the GSH content in liver of mice at various post irradiation days, with and without Genistein treatment.

## DISCUSSION

### Lipid Peroxidation (LPO)

The radiation caused an immediate increase in lipid peroxidation. In contrast to reactive oxygen species (ROS) generation and glutathione depletion, the lipid peroxidation rather persisted by 7<sup>th</sup> day after irradiation. ROS themselves are relatively short-lived molecules however,

the ROS can attack polyunsaturated fatty acid in the vicinity that are present in many membranes and initiate lipid peroxidation within the cells. Once initiated the lipid peroxidation is self-propagated due to generation of peroxy radicals even after the ROS dissipate. The lipid peroxidation results in the formation of aldehyde by products such as malondialdehyde. These molecules have longer half-lives than ROS and have the potential to diffuse from their site of origin to reach distant intracellular and extra cellular targets, thereby amplifying the effects of oxidative stress. The formation of MDA occurs only through the peroxidation of PUFAs, which is preferentially oxidized, owing to decreased carbon-hydrogen bond strength in methylene groups between unsaturated carbon pairs<sup>(45, 46)</sup>.

Results show that radiation induced the lipid peroxidation as reflected by the TBARS equivalents content, increased on 7<sup>th</sup> post irradiation day which is followed by a gradual decrease by 30<sup>th</sup> day, both in irradiated and Genistein treated groups, though only Genistein treated groups

achieved almost normal MDA content on 30<sup>th</sup> day. The Genistein treatment significantly prevented the radiation-induced lipid peroxidation in liver, as statistically there is a highly significant difference ( $p < 0.001$ ) between control and Experimental groups. In control group the MDA content increased by an average approximately  $39.20 \pm 12.89\%$ , on account of the radiation damage; from this an average approximately  $18.01 \pm 3.05\%$  recovered in Experimental-1 group and  $17.14 \pm 2.91\%$  in Experimental-2 group from that of control. Therefore, pre-irradiation administration of Genistein as well as post irradiation administration significantly lower the radiation induced LPO as compared to those of control group. It indicates that Genistein not only prevents the oxidative stress prior to radiation exposure but is also effective after irradiation. This is a great unravel that there is a drug that can be used after sudden radiation exposure. Present study shows that Genistein can be used as a radioprotector even after radiation exposure.

Plant flavonoids, which show antioxidant activity *in vitro* also, function as antioxidants *in vivo*, and their radioprotective effect may be attributed to their radical scavenging activity<sup>(47)</sup>. Due to antioxidant property of Genistein it is suggested that hepatic cells can be protected from radiation induced free radical damage. In present study the reduction in MDA level in the Genistein treated animals suggests that Genistein has the potential to scavenge the free radicals formed during oxidative stress.

In lipid peroxidation, a hydrogen atom is liberated from the fatty acid, a reactive free radical and there is formation of lipid radical<sup>(48)</sup>, which on attack by molecular oxygen produces a lipid peroxy radical, which can either form a lipid hydroperoxide or endoperoxide. The formation of lipid endoperoxide in unsaturated fatty acids leads to formation of MDA as breakdown product. This MDA interacts with DNA and other cell materials leading to chronic occurrence of mutagenesis and carcinogenesis<sup>(49)</sup>.

Radiation damage to biological membranes has been extensively studied *in vitro* systems<sup>(50, 51)</sup>. Lipid peroxidation of both model and intact membranes has been studied by various authors from different points of view, with special emphasis on the radiation chemistry of lipids<sup>(52)</sup>. In Experimental lipid peroxidation, polyunsaturated fatty acid residues forming the membrane phospholipids components are peroxidized by radiation-induced free radicals. Latest studies indicated that the hydroxyl radical is the most active species involved in radiation induced lipid peroxidation<sup>53</sup>.

The preservation of cellular membrane integrity depends on protection on repair mechanism capable of neutralizing oxidative reactions. It was reported that LPO could be inhibited by flavonoids possibly through their activity as strong superoxide scavengers<sup>(54)</sup> and singlet oxygen quenchers<sup>55</sup>. Recently, the ability of 8 structurally related naturally occurring flavonoides to inhibit LPO and mitochondrial membrane permeability transition was described<sup>(56)</sup>.

The measurement of TBARS gives an index of free radical activity. Radical scavenging by protectors results in inhibition of TBARS. The basic effect of radiation on cellular membrane is believed to be the peroxidation of membrane lipids, LPO can be initiated by hydrogen abstraction from lipid molecules by lipid radiolytic products, including hydroxyl and hydroperoxyl radicals. The ROS induce cell degeneration by increasing LPO of cell membrane lipids. The toxic end products of peroxidation induce damage of the structural and functional integrity of cell membranes, break DNA strands and denature cellular protein.

In a non-specific biological activity, partitioning and distribution of the drug in a certain membrane compartment is responsible for the activity<sup>(57)</sup>. Lipophilicity and ionization parameters are the most important regarding transport, distribution and binding drugs in biological systems and may be equally responsible for changes in

lipid contents and lipid peroxidation phenomenon. Depletion of glutathione also results in enhanced LPO<sup>(58)</sup>.

### **Glutathione (GSH)**

The present study demonstrates a significant reduction in GSH level after irradiation i.e. in control group (by an overall average of  $45.71 \pm 12.14\%$  from normal,); this could be attributed to enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. Genistein treatment significantly recovered GSH from the damage by radiation in Experimental-1 group and Experimental-2 group by an average  $62.05 \pm 21.5810\%$  and  $58.36 \pm 21.1086\%$ , respectively, as compared to control group.

The temporal change of glutathione depletion was immediately following the ROS generations suggests that the cellular glutathione content is a sensitive and early marker of the oxidative stress as results of its reaction either enzymatic or nonenzymatic. Glutathione reacts with ROS and then gets oxidized to disulphide form (GSSG). The GSSG is then reduced by glutathione reductase at the expense of NADPH<sub>2</sub> or exported from cells<sup>(59-61)</sup>. Hence, the decrease in cellular glutathione content is a sensitive indicator of the oxidative stress. Reduction in the cellular glutathione contents is expected to significantly compromise the cellular antioxidant capacity.

In Experimental-1 group the higher value of GSH as compared to those of Experimental-2 suggests that pre-irradiation administration of Genistein is more effective to cope up the free radicals generated by irradiation.

GSH acts as a reducing agent; so it is oxidized and forms a disulfide link with other molecules of GSH, oxidizes glutathione (GSSG), in turn it can be reduced to GSH by the action of GSSG reductase enzyme, in a reaction using NADPH. NADPH is recycled by glucose 6-phosphate dehydrogenase via the pentose phosphate pathway<sup>(62)</sup>. Radiation exposure

as well as a number of natural or synthetic radioprotectors can alter the balance of endogenous protective systems, such as glutathione and antioxidant enzyme systems. This could be due to the enhanced utilization of the antioxidant system in an attempt to detoxify the free radicals generated by radiation. GSH offers protection against oxygen-derived free radicals and cellular lethality following exposure to ionizing radiation<sup>(63)</sup>.

The GSH/GST detoxification system is an important part of cellular defense against a large array of injurious agents. Under normal conditions the inherent defense system including glutathione and antioxidant enzymes protects against the oxidative damage. Glutathione with its sulphhydryl group functions in the maintenance of sulphhydryl groups of other molecules (especially proteins) and in the detoxification of foreign compounds, hydrogen peroxide and free radicals. The depletion of GSH promotes generation of reactive oxygen species and oxidative stress with a cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes<sup>(64)</sup>. The increased GSH level suggests that protection by the Genistein may be mediated through the modulation of cellular antioxidant levels as revealed in glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase activities.

Decrease in GSH content has been observed following gamma radiation. Scientist reported that the radioprotective action of aminothiols is mediated through their capacity to release glutathione<sup>(65)</sup>. It is well known that, in addition to its free form, glutathione also occurs in the cells in relatively large amounts bound to protein in the form of mixed disulphide<sup>(66, 67)</sup>. The oxidative stress due to the radiation induced free radicals can cause a dramatic fall in the hepatic GSH and enzymes, which overwhelms the cellular defense and lead to membrane lipid peroxidation and loss of protective thiols<sup>(68-70)</sup>.

Glutathione is found at high concentrations in various cells, and has multiple biological roles. The reduced form (GSH) has direct or indirect roles in many biological processes, including protein and DNA synthesis, amino acid transport, activation of enzyme activities, activation of metabolism, and protection of cells from damage caused by ROS<sup>(71)</sup>. Most of the cell damage caused by ionizing radiation is mediated by ROS generated from the interaction between radiation and water molecules in cells. Intracellular GSH scavenges these ROS and protects the cells from radiation toxicity. Several authors have suggested that GSH is involved in the resistance of many cells to radiation; the higher level of cellular GSH, the greater the resistance to radiation. Cells with low contents of GSH are generally sensitive to radiation. High doses of radiation generate large amounts of ROS, resulting in a decrease of cellular GSH and a corresponding increase of oxidized glutathione (GSSG).

Thus, it has been suggested that redox ratio (GSH/GSSG) in tissues, e.g., blood, can be used as an index of radiation-induced oxidative stress. Depletion of intracellular GSH has been implicated as one of the causes of radiation-induced damage, while increased levels of intracellular GSH are responsible for the radioprotective action. The present study demonstrates that intraperitoneally administration of Genistein to Swiss albino mice did not influence significantly the endogenous GSH level in liver, but its mere presence during the radiation exposure protects the endogenous GSH depletion due to irradiation. The lower depletion of liver GSH in the Genistein pretreated irradiated animals could be due to the higher availability of GSH, which increases the ability of the cell to cope up with the free radicals produced by radiation.

GSH is a versatile protector and executes its radioprotective function through free radical scavenging, restoration of damage molecule by hydrogen donation,

reduction of peroxides and maintenance of protein thiols in the reduced state. Exposure to cells to radioprotective drug increases the intracellular concentration of NPSH group. It is also known that cells rich in NPSH are radioresistant. It is therefore suggested that the release of NPSH group after drug might play a role in the radioprotection of the cells<sup>(72)</sup>.

It is evident from the figure 1 on surviving curves that Genistein treated group had a pronounced protective effect against the radiation mortality. The protection offered by the Genistein can in part be attributed to quenching of singlet oxygen and variety of free radical species. In present study the reduction in MDA level and increase in GSH, in the Genistein treated animals suggests that Genistein has the potential to scavenge the free radical formed during oxidative stress and to maintain the antioxidant defense system.

In conclusion 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.

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