Protection of Aspartate and Alanine Transaminase Levels in Swiss Albino Mice by Genistein Treatment against Radiation induced Augmentation

Ajay Gaur* and Arvind Lal Bhatia[1]

* L.B.S. College of Pharmacy, Jaipur-302004, Rajasthan, India.
[1] Radiation Biology Laboratory, Center for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur-302004, Rajasthan, India.

Summary

Genistein is a soya isoflavone, which is found naturally in legumes. 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) significantly ($p < 0.001$) inhibited the radiation induced augmented AST level (by 13.73 ± 3.28%) and ALT levels (by 33.52 ± 10.43%) 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 the Swiss albino mice. The results indicate that Genistein provide protection against radiation induced increase in enzyme activity and may pave way to the formulation of medicine in radiotherapy for normal tissue. Present study also establishes the fact that Genistein may be used as a radioprotector before and after radiation exposure. Hence, the possibility of its use as a radioprotectant and radiotherapeutic drug in accidental conditions or nuclear war conditions can not be ruled out.

Key Words: Genistein, Tyrosine kinase inhibitor, Radiation, Liver, Oxidative stress, AST, ALT.

Corresponding author E-mail: ajay_gaur11@yahoo.com
Mobile no.: 09414295110

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. 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[1]. Radiation brings about several biochemical alterations in the affected tissues by influencing the metabolic processes occurring in them. Aspartate Amino Transferase (AST or SGOT) is a mitochondrial enzyme that is also present in heart, muscle, kidney and brain etc. In many cases of liver inflammation, the ALT and AST activities are elevated roughly in a 1:1 ratio. The excessive production of free radicals and lipid peroxides due to irradiation might have caused the leakage of cytosolic enzymes such as aminotransferases (AST and ALT), lactate dehydrogenase (LDH), creatine kinase (CK) and phosphatase[2]. Radiation-induced increases in the levels of AST and ALT were significantly ameliorated by flaxseed oil pretreatment[3]. Alanine Amino Transferase (ALT or SGPT) is an enzyme produced in the liver cells (hepatocytes) attributed liver disease than some of the other enzymes. It is generally increased in situations where there is damage to the liver cell membranes. All types of liver inflammation can increased ALT. Liver inflammation can be caused by fatty infiltration due to some drugs/medications, alcohol, liver and bile duct disease. Scientist demonstrated that sunflower oil does not alter the activity of AST and ALT, whereas olive oil increased the level of these both enzymes[4]. Researchers observed the increasing amount of serum Alanine and Aspartate amino transferase (ALT and AST) after whole body irradiation in mice[5]. Isoflavones may prove multipurpose biochemicals that have several functions; the review reports some of the activities of Genistein. 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. 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[6-9]. Liver is selected as a testing organ because some scientists reported it as highly radiosensitive organ[10]. Our previous studies have reported that intrapertionally administration of Genistein 24 hrs before irradiation afforded better protection against irradiation[11-12] as well as the present study has been carried out in order to check ameliorating capacity of Genistein against radiation induced changes in AST and ALT activities.

Materials and Methods

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^{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 6-8 weeks old male mice with an average body weight of 22 ± 3 gm were selected from inbred colony.

Drug

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

Genistein solution: Genistein was dissolved in dimethyl sulfoxide and then different concentration solutions were prepared 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: Autopsies were performed by mean 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. Aspartate and alanine transaminase were estimated in serum by Colorimetric method[13]. The values are expressed as mean ± S.D. The difference between various groups was analyzed by Student's t-test.

Experimental Protocol

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 as worked out in our earlier experiments 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 were killed by cervical dislocation at various intervals ranging between 1-30 day and processed for biochemical estimation of AST and ALT.

Results

Aspartate Amino Transferase (AST or SGOT)

Genistein vs. Normal: AST levels of Genistein treated group did not show any appreciable difference (1.39%) as compared to those of normal group (Table 1, Fig. 1).

Control vs. Normal: A sharp increase in AST level of control group from 1st day to 7th day (66.03%) and then slight decline occurred. Statistically a highly significant increase ($p < 0.001$) by 37.87%, 65.02%, 66.03%, 44.16% and 34.37% in AST level of control group was recorded on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively, as compared to those of normal groups. While taking the average of all the 5 intervals an increase in AST level of control group was approximately 49.49 ± 15.05% (±SD) (Table 1, Fig. 1).

Experimental-1 (G+IR) vs. Control: In Experimental-1 group, an increase in AST level was recorded upto 7th day (by 41.43%) and followed by a recovery nearby normal level till 30th day. As compared to those of control group, statistically a highly significant inhibition of the radiation induced increase ($p < 0.001$) by 8.85%, 17.6%, 14.82%, 12.44%, and 14.95% in AST level in Experimental-1 group was recorded on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively. The average recovery (taking the mean of all the 5 intervals) in AST level of Experimental-1 group was approximately 13.73 ± 3.2837% (±SD). While comparing with those of normal, a highly significant increase ($p < 0.001$) in AST level was noticed from day 1st to 15th post-irradiation days which attained almost near normal value on 30th day (Table 1, Fig. 1).

Experimental-2 (IR+G) vs. Control: A similar pattern of inhibition of radiation induced increase in AST level in Experimental-2 group as with that of Experimental-1 was recorded. As compared to those of control group, statistically a highly significant inhibition to the augmentation ($p < 0.001$) by 6.95%, 14.71%, 11.18%, 10.62%, and 23.67% in AST level of Experimental-2 group was noticed on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively. An average recovery in AST level of Experimental-2 group was around 13.43 ± 6.353% (±SD). If a mean of all the 5 intervals are taken while comparing with those of normal, a highly significant increase ($p < 0.001$) in the AST level was noticed from day 1st to 15th post-irradiation days, which declined and attained almost normal level by 30th day. In Experimental-1 group more recovery was noticed on 3rd and 7th post-irradiation days as compared to Experimental-2 group and then both Experimental groups attained almost normal level by 30th day (Table 1, Fig. 1).

TABLE – 1: Variation in the AST (U/L) level in mice at various post irradiation days, with and without Genistein treatment

Normal = 161.5335 ± 1.3134 (100%)
Genistein =159.3126 ± 1.7005 (98.61%) a^{NS}

Group	Post Irradiation Days				
	1	**3**	**7**	**15**	**30**
Control (IR with 8 Gy only)	221.777 ± 1.4008 (137.87%) b***	266.595 ± 1.4008 (165.02%) b***	269.396 ± 1.5345 (166.03%) b***	230.881 ± 1.6868 (144.16%) b***	218.97 ± 1.534 (134.37%) b***
Experimental-1 (Genistein+IR)	202.169 ± 1.5345 (125.68%) c***, d***	219.676 ± 1.2915 (135.97%) c***, d***	229.480 ± 1.7994 (141.43%) c***, d***	202.169 ± 1.5354 (126.23%) c***, d***	164.354 ± 1.5354 (100.85%) c^{NS}, d***
Experimental-2 (IR+Genistein)	206.371 ± 1.5345 (128.29%) e***, f***, g^{NS}	227..37 ± 1.5345 (140.74%) e***, f***, g**	239.284 ± 1.2915 (147.47%) e***, f***,g**	206.37 ± 1.5345 (128.85%) e***, f***, g*	167.155 ± 0.8859 (102.57%) e**, f***, g^{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}

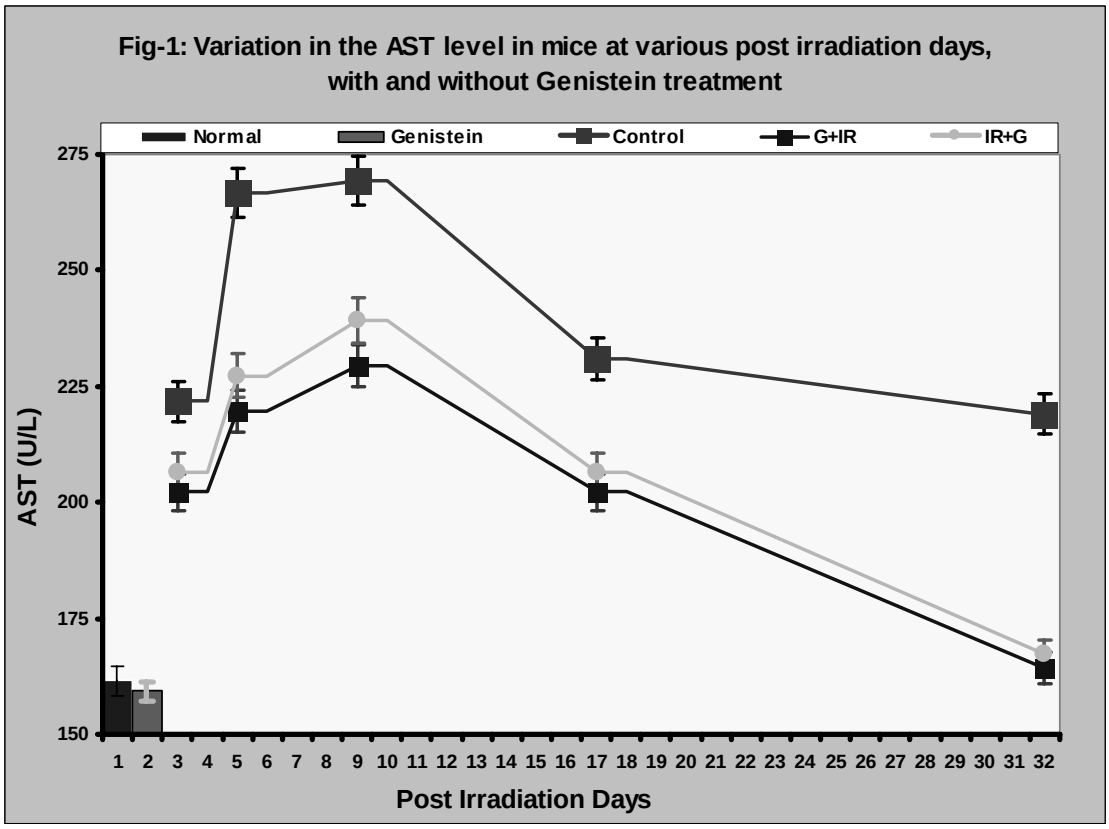

Fig-1: Variation in the AST level (Mean ± SEM) in mice at various post irradiation days, with and without Genistein treatment

Alanine Amino Transferase (ALT or SGPT)

Genistein vs. Normal: ALT level in Genistein treated mice did not show any difference as compared to those of normal group (by 2.34%) (Table 2, Fig. 2).

Control vs. Normal: Irradiation to whole body (control group) sharply enhanced the ALT level upto 7th day (152.68%). However, on later intervals it gradually declined upto 30th day. Statistically highly significant increases ($p < 0.001$) in ALT levels by 100.57%, 142.49%, 152.68%, 109.47% and 78.06% in control group on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively, were recorded as compared to those of normal groups. Taking into consideration the average of the values of all the foregoing 5 intervals, an increase in ALT level of control group was approximately 116.65 ± 30.6805% (±SD) (Table 2, Fig. 2).

Experimental-1 (G+IR) vs. Control: In Experimental-1 group, an increase in ALT level was observed upto 7th day (by 95.6%) followed by a recovery nearby normal level is attained till 30th day. Statistically a highly significant recovery ($p < 0.001$) in ALT level

by 41.68%, 21.61%, 22.59%, 40.83% and 40.87% in Experimental-1 group was noticed on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively, as compared to those of control groups. The average recovery in ALT level of Experimental-1 group was approximately 33.52 ± 10.4326% (±SD). While comparing with those of normal, a highly significant increase ($p < 0.001$) in the ALT level was noticed from day 1st to 15th post-irradiation days which attained almost normal value on 30th day (Table 2, Fig. 2).

Experimental-2 (IR+G) vs. Control: In Experimental-2 group similar pattern of recovery of ALT level was noticed as in Experimental-1 group. As compared to those of control group, statistically a highly significant recovery ($p < 0.001$) in ALT level by 39.98%, 19.3%, 20.05%, 37.24% and 39.02% in Experimental-2 group were recorded on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively. The average recovery in ALT level of Experimental-2 group was approximately 31.12 ± 10.4954% (±SD). While comparing with those of normal, a highly significant increase ($p < 0.001$) in the ALT level was noticed from day 1st to 15th post-irradiation days, which is followed by a slight decrease on 30th day. In Experimental-1 group, more recovery obtained on 7th and 15th days as compared to those of Experimental-2 groups and then both Experimental groups attained almost normal level by 30th day (Table 2, Fig. 2).

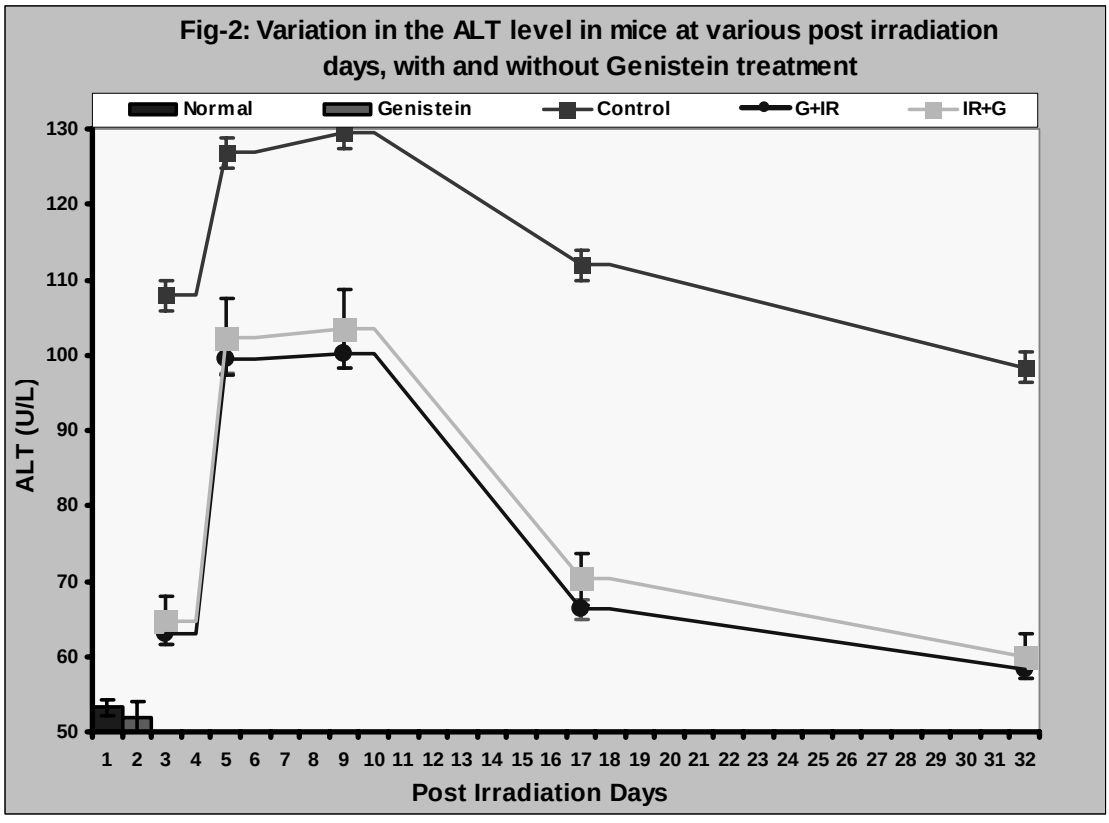

Fig-2: Variation in the ALT level (Mean ± SEM) in mice at various post irradiation days, with and without Genistein treatment

TABLE – 2: Variation in the ALT (U/L) level in mice at various post irradiation days, with and without Genistein treatment

Normal = 53.2127 ± 0.8525 (100%)
Genistein =51.9697 ± 0.63192 (97.66%) a^{NS}

Group	Post Irradiation Days				
	1	**3**	**7**	**15**	**30**
Control (IR with 8 Gy only)	107.90 ± 1.9139 (200.57%) b***	126.91 ± 1.6756 (242.49%) b***	129.47 ± 1.4626 (252.68%) b***	111.924 ± 1.850 (209.47%) b***	98.398 ± 0.9393 (178.06%) b***
Experimental-1 (Genistein + IR)	62.93 ± 1.6676 (116.98%) c***, d***	99.49 ± 1.2666 (190.11%) c***, d***	100.226 ± 0.9250 (195.6%) c***, d***	66.227 ± 1.3183 (123.94%) c***, d***	58.18 ± 1.4349 (105.29%) c^{NS}, d***
Experimental-2 (IR + Genistein)	64..76 ± 1.4349 (120.38%) e***, f***, g^{NS}	102.419 ± 1.3484 (195.69%) e***, f***, g^{NS}	103.516 ± 1.3183 (202.02%) e***, f***, g*	70.248 ± 1.6676 (131.47%) e***, f***, g*	60.012 ± 1.7913 (108.6%) e**, f***, g^{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}

Discussion

AST is a mitochondrial enzyme that is also present in heart, muscle, kidney and brain etc. In many cases of liver inflammation, the ALT and AST activities are elevated roughly in a 1:1 ratio. The excessive production of free radicals and lipid peroxides due to irradiation might have caused the leakage of cytosolic enzymes such as aminotransferases (AST and ALT), lactate dehydrogenase (LDH), creatine kinase (CK) and phosphatase. In our study irradiation produced a sharp increase in AST level by an average approximately 49.49 ± 15.06% in control group.

From this, Genistein treatment significantly ($p < 0.001$) recovered AST level in Experimental-1 group and Experimental-2 group by an average approximately 13.73 ± 3.28% and 13.43 ± 6.4%, respectively, from the damage in control group.

The ALT is an enzyme produced in the liver cells (hepatocytes) attributed liver disease than some of the other enzymes. It is generally increased in situations where there is damage to the liver cell membranes. All types of liver inflammation can increased ALT. Liver inflammation can be caused by fatty infiltration due to some drugs/medications, alcohol, liver and bile duct disease. In Genistein treated group ALT level was significantly ($p < 0.001$) recovered than those of control group. Scientist observed the increasing amount of serum Alanine and Aspartate amino transferase (ALT and AST) after whole body irradiation in mice. In our study an overall average increase in ALT level by 116.65 ± 30.68% approximately occurred in control group. Genistein treatment before irradiation (Experimental-1 group) and after irradiation (Experimental-2 group) significantly recovered ALT levels by approximately 33.52 ± 10.43% and 31.12 ± 10.49%, respectively, from the damage impinged upon by radiation (control).

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. All irradiated mice without Genistein treatment have shown 100% mortality within 11 days. However, maximum survival of mice (30%) 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. 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. So Genistein checks the depletion of the radiation induced augmentation of AST, ALT activities.

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 maintained the normal levels of acid phosphatase, alkaline phosphotase and 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

Present study established the fact that Genistein may be used as a radioprotector before and after radiation exposure.

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