Modulation of Phosphatase Levels in Swiss Albino Mice by Genistein Treatment against Radiation-induced Effect

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Summary

Genistein, a soya isoflavone, is found naturally in legumes, such as soybeans and chickpeas. If taken an average of intervals of month periods (1st, 3rd, 7th, 15th and 30th days) into account, 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) subsides the radiation induced augmentation of the acid phosphatase (by 34.7 ± 12.39% and 28.38 ± 9.41% in pre, and post irradiated groups, respectively) and ameliorated the decline of the alkaline phosphatase (by 49.59 ± 13.82% and 45.44 ± 15.34% in pre and post irradiated groups, respectively) while comparing the experimental group with that control group (only irradiated) in Swiss albino mice. The results indicate that Genistein against radiation effect 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 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, acid and alkaline phosphatases.

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 in order 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. Radiation induced cellular degranulation of tissue damage was shown, as an increase in acid phosphatase activity was reported in animals[2-5]. An increased activity of acid phosphatase in mammals after sublethal doses of gamma radiation has been demonstrated[6]. The enhanced activity of acid phosphatase found in the liver could be due to either a direct effect of irradiation on the lysosomal membrane or some indirect effect such as liberation of thyroid hormone, it was proposed that radiation can cause the formation of lipid peroxides in lysosomes presumably due to direct oxidation of unsaturated fatty acids of the lysosomal membrane by free radical formed. This may be one of the reasons for the rupture of lysosomal membrane in the gamma irradiated mice liver. Acid phosphatase is localized in cellular lysosomes and its activity may be changed following to whole-body irradiation. An increased acid phosphatase level may also be attributed to an elevated Golgi activity and peroxidation of lysosomal membranes after irradiation causing lysis of membrane and oozing out of enzyme[7].

Scientists reported a dose dependent fall in alkaline phosphatase activity in rats exposed to lethal doses of radiations[8-10]. Similarly, a decline in alkaline phosphatase activity in male mice after irradiation with gamma rays has been reported[11-12].

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. Genistein can alter the expression of gangliosides and other carbohydrate antigens to facilitate their immune recognition[13-15].

In the present study liver is selected as a testing organ because some scientists reported it as highly radiosensitive organ[16] and in order to check ameliorating capacity of Genestein against radiation induced changes to phosphatase enzymes.

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 (1^{st}, 3^{rd}, 7^{th}, 15^{th} and 30^{th}). At least six observations were taken and spectrophotometer was used to measure the optical density. Acid phosphatase activity was estimated in serum by King's method[17], whereas alkaline phosphatase activity was estimated by Kind and King's method[18], using commercially accessible kits (Span diagnostics Ltd.). 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 acid and alkaline phosphatases.

Results

Acid Phosphatase

Genistein vs. Normal: Non significant lower activities of acid phosphatase were recorded in Genistein treated group as compared to those of normal (by 10.63%) .

Control vs. Normal: A sharp increase in acid phosphatase activity in control group was noticed till 7^{th} day (by 246.12%) followed by a decreasing trend upto 30^{th} day. A statistically highly significant increase ($p<0.001$) by 202.16%, 214.28%, 246.12%, 184.48% and 132.22% in acid phosphatase activities in control group has been noticed on 1^{st}, 3^{rd}, 7^{th}, 15^{th} and 30^{th} post-irradiation days, respectively, as compared to those of normal groups. An average increase in acid phosphatase activity of control group was approximately 195.85 ± 16.83% (±SD) (Table 1, Fig. 1).

Experimental-1 (G+IR) vs. Control: In Experimental-1 group, a sharp increase in acid phosphatase activity was recorded upto 7^{th} day and then a decline observed till 30^{th} day. As compared to those of control group, a statistically significant recovery ($p < 0.001$) by 24.01, 23.87%, 29.85%, 50.31% and 45.46% in acid phosphatase activity in Experimental-1 group was recorded on 1^{st}, 3^{rd}, 7^{th}, 15^{th} and 30^{th} post-irradiation days, respectively. An average recovery in acid phosphatase activity of Experimental-1 group was approximately 34.7 ± 12.39% (±SD). While comparing with those of normal, a highly significant increase ($p < 0.001$) in activity of acid phosphatase of Experimental-1 group was noticed on 1^{st} and 3^{rd} post-irradiation days which recovered and attained almost near normal value on 30^{th} day (Table 1, Fig. 1).

TABLE 1: Variation in the acid phosphatase (KAU) level in liver of mice at various post irradiation days, with and without Genistein treatment

Normal = 2.15776 ± 0.3249 (100%)

Genistein = 1.9285 ± 0.2317 (89.37%) a^{NS}

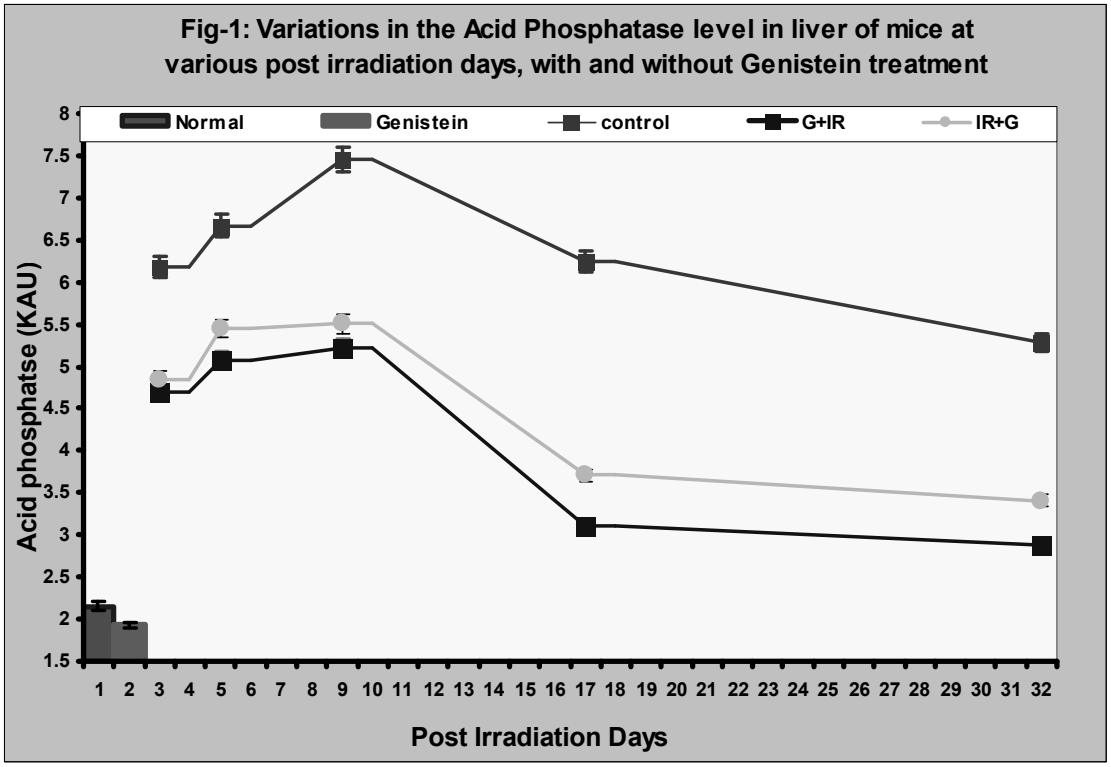
Group	Post Irradiation Days				
	1	**3**	**7**	**15**	**30**
Control (IR with 8 Gy only)	6.18 ± 0.3314 (302.16%) b***	6.666 ± 0.2160 (314.28%) b***	7.451 ± 0.4155 (346.12%) b***	6.25 ± 0.5290 (284.48%) b***	5.277 ± 0.2788 (232.22%) b***
Experimental-1 (Genistein+IR)	4.696 ± 0.2804 (229.62%) c***, d**	5.075 ± 0.4626 (239.28%) c***, d**	5.227 ± 0.6945 (242.81%) c**, d**	3.106 ±0.2476 (141.37%) c*, d***	2.878 ± 0.673 (126.66%) c^{NS}, d**
Experimental-2 (IR+Genistein)	4.848 ± 0.3847 (237.03%) e***, f**, g^{NS}	5.454 ± 0.4081 (257.14%) e**, f*, g^{NS}	5.503 ± 0.5961 (255.63%) e***, f**, g^{NS}	3.712 ± 0.4315 (168.96%) e**, f**, g^{NS}	3.409 ± 0.6426 (150%) e^{NS}, 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}



Experimental-2 (IR+G) vs. Control: In Experimental-2 group, a sharp increase in acid phosphatase activity was recorded upto 7th day which is followed by a decline till 30th day. A statistical significant recovery ($p < 0.05$) by 21.56%, 18.19%, 26.15%, 40.61%, and 35.41% in acid phosphatase activities of Experimental-2 group were recorded on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively, as compared to those of control groups. From control, an average recovery in acid phosphatase activity of Experimental-2 group was approximately 28.38 ± 9.41% (±SD). While comparing with those of normal, though a highly significant level ($p < 0.001$) in activity of acid phosphatase of Experimental-2 group was noticed on 1st and 7th post-irradiation days, but this followed by a significant decline ($p < 0.05$) upto 15th day which attained almost near normal level on 30th day. In Experimental-2 group, an insignificant increased level of acid phosphatase has been noticed on all post-irradiation days in comparison to those of Experimental-1 group (Table 1, Fig. 1).

Alkaline Phosphatase

Genistein vs. Normal: Genistein administration of mice did not produce any appreciable difference in alkaline phosphatase (by 1.61%) (Table 2, Fig. 2).

Control vs. Normal: A sharp decline in control group was noticed upto 7th day followed by a slight recovery by 30th day. Statistically highly significant decreases ($p < 0.001$) by 49.13%, 59.62%, 61.19%, 47.22% and 31.3% in activities of alkaline phosphatase of control group were recorded on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively, as compared to those of normal groups. If mean of all the 5 intervals are taken into account, an average decrease by 49.69 ± 11.99% (±SD) in alkaline phosphatase activity of control group has been recorded (Table 2, Fig. 2).

Experimental-1 (G+IR) vs. Control: In Experimental-1 groups, a gradual decline in activities of alkaline phosphatase by 7th day followed by a recovery on 30th day have been recorded. Statistically highly significant recoveries by 43.61%, 69.31%, 58.27%, 40.94%, and 35.83% in activities of alkaline phosphatase in Experimental-1 group were recorded on 1st, 3rd, 7th, 15th and 30th post-irradiation days, respectively, as compared to those of control groups. An average recovery in alkaline phosphatase activity of Experimental-1 group was approximately 49.59 ± 13.82% (±SD). While comparing with those of normal, though a highly significant decreased level at $p < 0.001$ in activity of alkaline phosphatase of Experimental-1 group by 7th day was noticed, however, it was maintained upto 15th post-irradiation days which recovered on the later interval and attained almost near normal value by 30th day (Table 2, Fig. 2).

Experimental-2 (IR+G) vs. Control: A decrease in activity of alkaline phosphatase of Experimental-2 group was recorded by 7th day and which was followed by a slight recovery by 30th day. A statistical significant recovery of alkaline phosphatase of Experimental-2 group being maximum (by 67.19% on 3rd day) has been recorded which is followed by a lesser recovery by 55.92%, 36.13% and 31.83% on 7th, 15th and 30th post-irradiation days, respectively, as compared to those of control group. From control, an average recovery in alkaline phosphatase activity of Experimental-2 group was approximately 45.44 ± 15.3417% (±SD). While comparing with those of normal, a highly

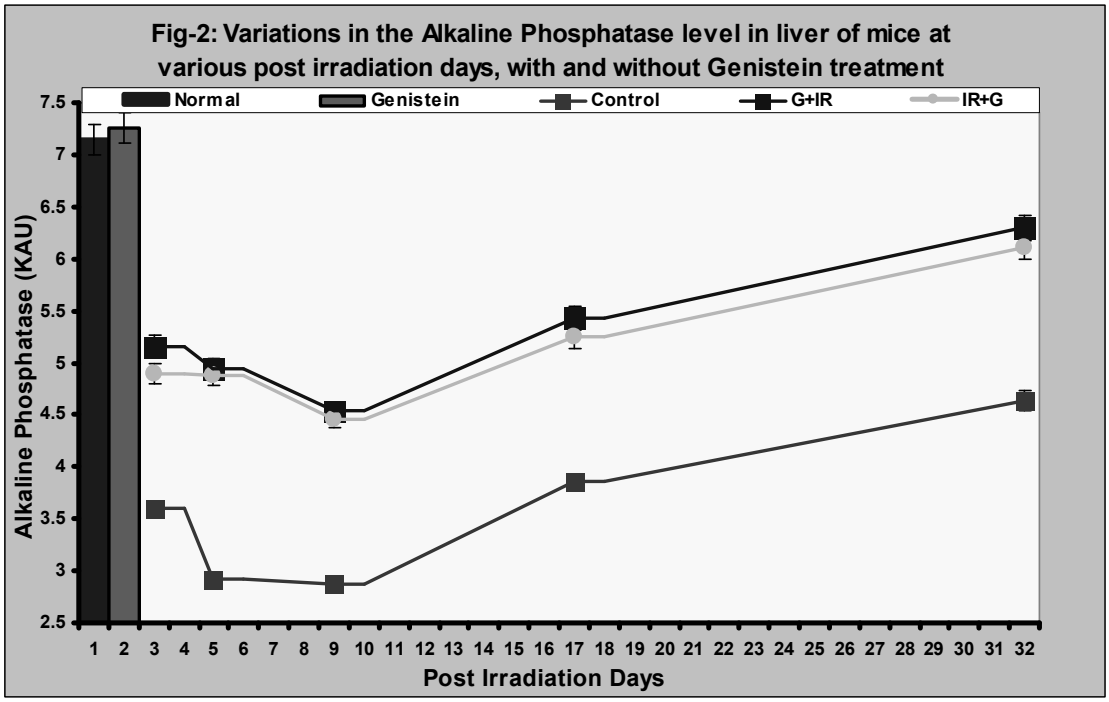
significant decrease ($p < 0.001$) in activity of alkaline phosphatase of Experimental-2 group was noticed on 7th day, which was maintained upto 15th post-irradiation day and later attained almost near normal value by 30th day. In Experimental-1 group, an insignificant increase in activities of alkaline phosphatase occurred on all post-irradiation days as compared to Experimental-2 group (Table 2, Fig. 2).

TABLE 2: Variation in the alkaline phosphatase (KAU) level in liver of mice at various post irradiation days, with and without Genistein treatment

Normal = 7.1428 ± 0.4591 (100%)
Genistein = 7.258 ± 0.3126 (101.61%) a^{NS}

Groups	Post Irradiation Days				
	1	**3**	**7**	**15**	**30**
Control (IR with 8 Gy only)	3.593 ± 0.1071 (50.87%) b***	2.916 ± 0.5036 (40.38%) b***	2.864 ± 0.1259 (38.81%) b***	3.854 ± 0.2645 (52.78%) b***	4.635 ± 0.2051 (68.71%) b***
Experimental-1 (Genistein+IR)	5.161 ± 0.3344 (73.06%) c**, d**	4.938 ± 0.3919 (68.37%) c**, d**	4.533 ± 0.3052 (61.41%) c***, d***	5.432 ± 0.4463 (74.34%) c**, d**	6.296 ± 0.1920 (93.33%) c^{NS}, d***
Experimental-2 (IR+Genistein)	4.892 ± 0.4833 (69.26%) e**, f**, g^{NS}	4.876 ± 0.5631 (67.52%) e**, f**, g^{NS}	4.466 ± 0.4568 (60.51%) e***, f**, g^{NS}	5.2469 ± 0.4645 (71.85%) e**, f**, g^{NS}	6.111± 0.3147 (90.58%) e^{NS}, 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

The acid phosphatase level was also found to be elevated after irradiation (in control group) by an average approximately 195.85 ± 16.83%. Genistein administration produced a significant recovery in acid phosphatase level in Experimental-1 group and in Experimental-2 group by approximately 34.7 ± 12.3949% and 28.38 ± 9.4116%, respectively from control.

An increasing value of acid phosphates enzyme was observed in present study on 7th day after irradiation which declined progressively, but it could not be restored to normal level even till the end of study. Pretreated irradiated animals exhibited a decline in acid phosphatase continuously (i.e. great recovery) from 7th day to last autopsy interval (30th day) just similar the trend that of control group. However, in case of Experimental-1 (pre-irradiation treated) group more protection was observed (an overall average recovery by 34.7 ± 12.39% in acid phosphatase activity, in comparison to that those of control group.

In Experimental-2 group (post irradiation treated), the activity of enzyme was similar to Experimental-1 group (an average recovery by 28.38 ± 9.41% from the control). This indicates that Genistein administration is also protective against radiation damage even when given after radiation and prior to radiation too.

Several researchers reported a radiation induced cellular degradation of tissue damage. Acid phosphatase is localized in cellular lysosomes and its activity may be changed following to whole-body irradiation. The enhanced activity of acid phosphatase found in the liver could be ascribed either to a direct effect of irradiation on the lysosomal membrane or some indirect effect such as liberation of thyroid hormone. It was proposed that radiation can cause the formation of lipid peroxides in lysosomes presumably due to direct oxidation of unsaturated fatty acids of the lysosomal membrane by free radical formed. This may be one of the reasons for the rupture of lysosomal membrane in the gamma irradiated mice liver. An increased acid phosphatase level may also be attributed to an elevated Golgi activity in addition to peroxidation of lysosomal membranes after irradiation causing lysis of membrane and oozing out of enzyme[19].

It is well known that radiation increases the permeability of membranes of several cellular organelles and hence an increase in serum acid phosphatase activity was seen after irradiation. A rise in acid phosphatase activity can be attributed to gastrointestinal syndrome however; further rise can be assigned to other factors like hematopoitic injury.

Whole-body gamma-irradiation of 8 Gy dose revealed a decrease in the serum alkaline phosphatase activity by an average approximately 49.69 ± 11.99% (in control group). Pre-irradiation administration and post-irradiation administration of Genistein recovered the level of alkaline phosphatase significantly by an average approximately 49.59 ± 13.82% and 45.44 ± 15.34%, respectively, as compared to those of control group, though the level of alkaline phosphatase could not reach the normal value at last autopsy interval. This result corroborates the therapeutic action of Genistein against radiation damage. Concomitantly, it also proves to be prophylactic as well as preventive against radiation action.

Scientists suggested that lesions are produced in the membrane lipids, possibly by peroxides, due to irradiation leading to the activation of latent acid hydrolase, which could result in the digestion of the membrane itself with the consequent activation and release of the other lysosomal enzymes. Although, the activation of lysosomal enzymes in tissues with interphase death is well documented, information on lysosomal enzymes in liver, kidneys and brain are meagre and often contradictory. It is known that lysosome from different cell types or even from the same tissue varies greatly in their susceptibility to damage by irradiation. An increase in lysosomal enzymes has also been reported at sublethal doses of radiation. The release of enzymes from lysosome may be due to activation of pre-existing latent enzymes or due to synthesis of new lysosome as a consequence of radiation[20-21].

Researchers observed that alkaline phosphatase activity decreased after whole body irradiation to the mice, whereas acid phosphatase activity increased. Alkaline phosphatase plays an important role in maintenance of cellular permeability and acts as monophosphoesters. Damage to cell membrane caused by radiation may be the reason for declined activity of alkaline phosphatase. Post irradiated damage to liver can be another reason attributing to increased level of acid and alkaline phosphatase. Radiation induced stress also accounts for an increased activity of these enzymes. Alkaline phosphatase, a brush border enzyme splits various phosphate esters in an alkaline medium and mediate membrane transport. Thus acid phosphatase helps in early recovery from radiation damage by removing debris and alkaline phosphatase helps in stabilizing the membrane. One of the causes of radiation damage is due to lipid peroxidation. This alters the lysosomal membrane permeability resulting into release of hydrolytic enzymes. So, an increase in acid phosphatase was noticed after radiation treatment. The alkaline phosphatase activity is associated with membrane permeability and on account of membrane damage and depletion of hepatocytes after irradiation the enzyme activity was decreased[22-23].

In our earlier studies the intraperitoneal administration of Genistein did not cause any toxic effect on mice and Genistein treatment offered 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[24-25]. The current findings along with those of previous ones prove that Genistein provide protection against radiation damage.

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 and there is likelihood that Genistein may exert an antiradiation influence in the body. It would further pave way to the formulation of medicine against radiation induced toxicity 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 prove worth-heeding for formulation of medicine in radiotherapy for normal tissue against possible 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. Hence the possibility of using Genistein as a radioprotectant and radiotherapeutic drug in accidental conditions or nuclear war conditions can not be ruled out.

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