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Ameliorative Effect of Rutin against Oxidative Stress in Mice Induced By Gamma-Irradiation

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ABSTRACT

Rutin is a bioflavonoid, which is a potent antioxidant. In view of this the ameliorative effect of rutin administration prior to irradiation was analyzed in Swiss mice. Rutin (50 mg/kg body weight) were suspended in water using 0.5% w/v carboxy methyl cellulose and were given once daily (5 ml/kg body weight) for 5 days by oral route before γ-irradiation of female Swiss albino mice. γ Radiation was applied at levels 1, 2 Gy, 1 hour after the last dose of rutin administration. Animals were divided into control and 6 treated groups, and all groups were irradiated with γ-rays. Blood was collected 1 and 48 hours after irradiation and serum total protein, albumin, globulins, cholesterol, triglycerides, SGOT and SGPT were determined. Rutin was found to elevate the protein profile (total protein, albumin and globulin) and ameliorate the hyperlipidemic effects of γ- radiation. Rutin also improved the liver functions affected by γ-irradiation. The observations suggest that rutin may serve as a radioprotector.

Keywords: Rutin, Proteins, Cholesterol, triglycerides, Liver function, radioprotection.

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INTRODUCTION

It is now well established fact that there is significant need to develop a system that is capable of ensuring adequate protection of the human being against the harmful effects of ionizing radiation is currently debated [1-3].There is a need to reappraise the position taken by the International Commission on Radiological Protection (ICRP), which specifies that the regulations and standards it recommends for the radioprotection of humans implicitly ensures an adequate protection of other living organisms, and therefore of the environment [4,5].

The use of ionizing radiation in cancer therapy may lead to transient and/or permanent injury to normal tissues within the treatment field. The magnitude of damage depends both on the volume of tissue irradiated and the dose of radiation delivered. Radiotherapy plays a significant role in the management of head and neck cancer, either as the primary treatment modality or as a post-surgical adjuvant modality.

The development of radioprotective agents has been the subject of intense research in view of their potential for use within a radiation environment, such as space exploration, radiotherapy and even nuclear war. However, no ideal, safe synthetic radioprotectors are available to date, so the search for alternative sources, including natural dietary ingredients, synthetic vitamins, plants, has been ongoing for several decades. The traditional Indian food habits, we use many fruits and green-leafy vegetables in our main food course, which is main source for bioflavonoid, vitamins, essential minerals. Several dietary ingredients have been used to treat free radical-mediated ailments and, therefore, it is logical to expect that such dietary ingredients may also render some protection against radiation damage.

Rutin is one of a class of flavonoids that also includes hesperidin, quercetin, eriodictyl and citron and is essential for the absorption of vitamin C. Normally found in highly nutritious foods, such as citrus, red apples, teas, Broccoli and Onions, etc., it can be taken as a supplement form; ass the human body cannot produce bioflavonoid, they must be supplied through the diet. Rutin (RU), ellagic acid (EA) and quercetin (QU) are phenolic compounds. Structurally they have phenolic groups which serve as a source of readily available hydrogen atoms such that the subsequent radicals produced can be delocalized over the phenolic structure [6, 7]. Recent studies have shown that the bioflavonoid is powerful antioxidants that fight free radicals. Free radicals are said to be responsible for as much as 90 percent of all the human diseases including Cancer, arteriosclerosis, strokes, aging, etc [8-10].

Rutin, like all other bioflavonoid, is an antioxidant which can effectively scavenge free radicals that cause damage to tissues and cells [11, 12]. This activity may have a beneficial impact in building immunity against invasive infection, and current studies have asserted that ingesting supplemental flavonoids may be beneficial in combating harmful infectious attack. Current research claims that rutin supplements may promote healthy cholesterol levels in the blood, thereby promoting good circulation and possibly reducing hypertension [10-12]. In view

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of this, the present study was aimed at evaluating the ameliorative effect of rutin against radiation induced oxidative stress.

MATERIALS AND METHODS

Animals

Forty-two female Swiss albino mice weighting 40-50 g were used. Animals were kept under good ventilation and illumination conditions and allowed balanced diet and tap water was offered liberally. γ irradiation was performed by using ^{60}C source, installed in KMC Hospital, Mangalore. Animals were exposed to whole body γ-rays with dosage level of 1, 2 Gy as a single dose. The dose rate was 1.4 Gy/minute at the time of the experiment. Institutional animal ethics clearance was obtained and CPCSEA guidelines were followed throughout the study.

Treatment:

Rutin was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Rutin (50mg/kg body wt.) were suspended in water using 0.5% w/v carboxy methyl cellulose and were given once daily (5 ml/kg body weight) for 5 days by oral route before γ-irradiation. Radiation processing was performed 1 hour after the last dose of rutin administration.

Experimental design:

The animals were divided into 5 groups,

- Group 1: Comprised 6 animals considered as control animals (not rutin administered or irradiated).
- Group 2: Comprised 6 animals were exposed to whole body γ irradiation at a dose of 1 Gy.
- Group 3: Comprised 6 animals administered orally rutin 5 days before whole body irradiation with 1 Gy.
- Group 4: Comprised 6 animals were exposed to whole body γ irradiation at a dose of 2 Gy.
- Group 5: Comprised 6 animals administered orally rutin 5 days before whole body irradiation with 2Gy.

Experimental Methods:

Blood samples were collected from orbital venous plexus after 1 and 48 hours after irradiation exposure. Serum samples were separated by centrifugation at 3000 rpm for 15 minutes and stored frozen until the biochemical analysis. Serum total proteins were determined by kinetic procedures using STANBIO® kits according to methods of Cannon DC [13].

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Albumin, [14]. Cholesterol, [15]. Triglycerides, [16]. Glutamic oxalacetic transaminase (GOT) [17]. Glutamic pyrovic transaminase (GPT) was determined by colorimetric method using Quimica Clinica Aplicada S.A. procedures [18]. Serum globulin was calculated.

Statistical analysis – Statistical significance were calculated. T-test was calculated for control group with all groups, and between irradiated groups to its identical (same dose of radiation and same time of blood collection, differ only in injection of rutin or not) rutin administered irradiated ones.

RESULTS

There was a significant decrease in serum total proteins in irradiated groups with 2 Gy and sacrificed after 1 and 48 hours in comparison with control group. There was also a significant increase in serum total proteins in groups administered with rutin pre-irradiation in comparison with its identical irradiated group.

There was a significant decrease in serum albumin of irradiated groups with 1 and 2 Gy compared to control group. There was also a significant increase in serum albumin in all groups that administered with rutin pre-irradiation in comparison with its identical irradiated group.

Fig. (1) Serum Total Protein, Albumin and Globulins with and without rutin administration after 1 hr of irradiation on (mean ± S.E. and T-test)

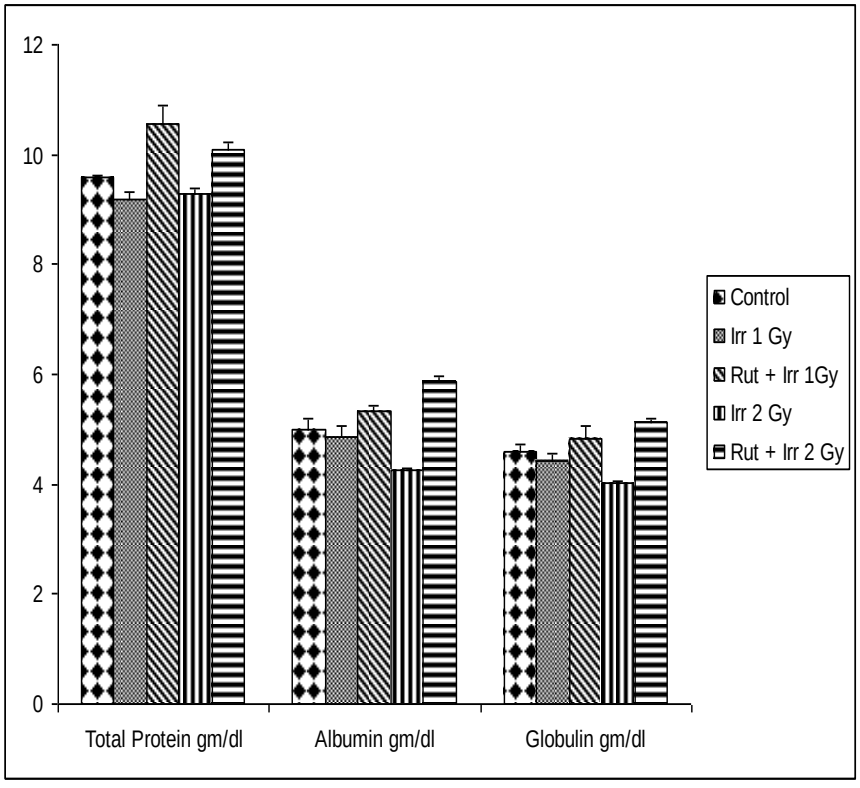

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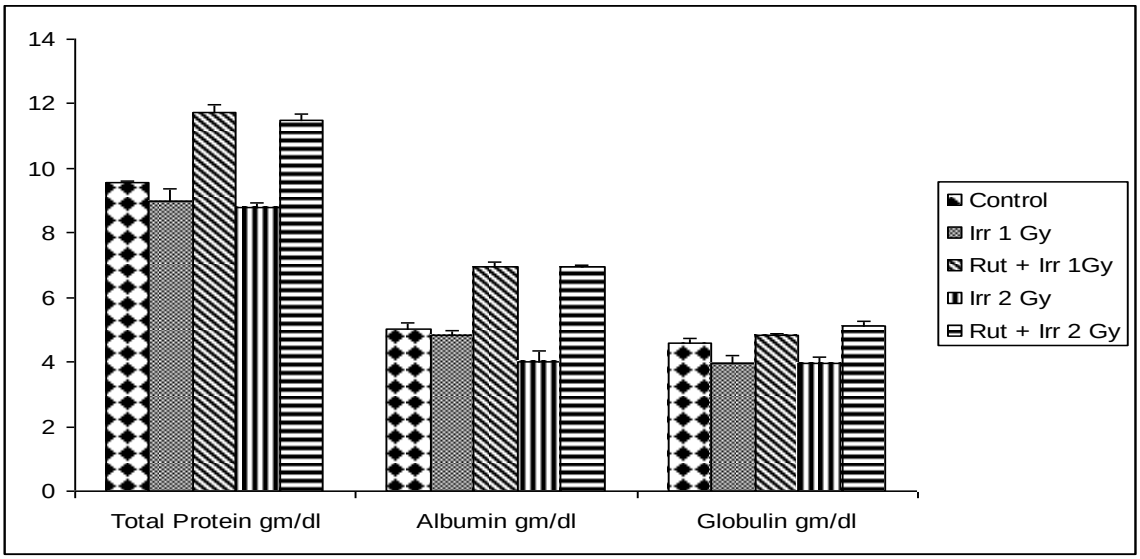
Fig. (2) Serum Total Protein, Albumin and Globulins with and without rutin administration after 48 hr of irradiation on (mean ± S.E. and T-test)



Data in table (1) showed the effect of rutin oral administration for 5 successive days pre-irradiation with doses 1, 2 Gy for each group on serum cholesterol and serum triglycerides. On comparison with control group cholesterol showed a significant increase in all irradiated groups, except that group irradiated with 1 Gy and sacrificed after 1 hour (non-significant increase). Also, there was a significant decrease in serum cholesterol in all rutin administered irradiated groups compared to those irradiated. Serum triglyceride showed a significant increase in all irradiated groups in comparison to control group except that group irradiated with 1 Gy and sacrificed after 1 hour (non-significant increase). Also, there was a significant decrease in serum triglycerides between all rutin administered irradiated groups in comparison with its identical irradiated group.

Table 1: Effect of irradiation on serum Cholesterol and Triglycerides with and without rutin administration (mean ± S.E. and T-test)

Group / Parameter	Control	Irr. 1 Gy		RUT + Irr. 1 Gy		Irr. 2 Gy		RUT + Irr. 2 Gy	
		1 hr	48 hr	1hr	48 hr	1hr	48 hr	1hr	48 hr
Cholesterol	188.0 ± 3.61 a	194.0 ± 4.39 b	221.8 ± 3.75 a, c	177.2 ± 1.14 a, b	168.2 ± 0.72 c	219.3 ± 4.20 a, f	228.3 ± 3.52 a, g	198.2 ± 2.83 a, f	207.7 ± 2.43 a, g
Triglycerides	121.7 ± 1.19 a	132.5 ± 1.59 b	129.2 ± 1.56 a, c	119.2 ± 1.06 a, b	127.8 ± 1.54 c	145.2 ± 0.65 a, f	155.1 ± 2.62 a, g	129.5 ± 1.75 a, f	134.7 ± 3.39 g

RUT. Irr. = Rutin administration irradiated Irr. = Irradiated only
Similar subscript litters mean significant change.

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Table (2) shows the effect of γ irradiation on SGOT/(AST) and SGPT/(ALT) in mice with and without oral rutin administration (50 mg/Kg) daily for 5 successive days then exposed to γ radiation with doses 1, 2 Gy for each group.

In comparison to control group, all irradiated groups showed a significant increase in SGOT (Serum Glutamate Oxaloacetate Transaminases) and SGPT (Serum Glutamate Pyruvate Transaminase), with 2 Gy γ-irradiation dose levels while the change was insignificant with the use of 1 Gy. Comparison between irradiated groups at the same dose, sacrificed in the same day showed significant increase in SGOT with the rutin administered irradiated groups.

Table 2: Effect of irradiation on SGOT and SGPT with and without rutin administration (mean ± S.E. and T-test)

Group / Parameter	Control	Irr.1 Gy		RUT + Irr. 1 Gy		Irr. 2 Gy		RUT + Irr. 1 Gy	
		1 hr	48 hr	1 hr	48 hr	1 hr	48 hr	1 hr	48 hr
SGOT (AST)	177.0 ± 1.75 a	183.0 ± 2.05 b	198.3 ± 4.91 c	173.7 ± 3.60 a, b	176.7 ± 2.88 a, c	200.7 ± 1.35 a, f	210.3 ± 5.47 a, g	186.7 ± 4.89 f	197.8 ± 2.13 g
SGPT (ALT)	8.98 ± 0.35 a	9.17 ± 0.20 b	9.63 ± 0.14 c	8.77 ± 0.13 a, b	8.90 ± 0.09 a, c	10.02 ± 0.20 a, f	10.13 ± 0.05 a, g	9.02 ± 0.34 f	9.25 ± 0.14 g

RUT. Irr. = Rutin administration irradiated Irr. = Irradiated only
Similar subscript litters mean significant change.

DISCUSSION

Bioflavonoids may be found in herbal plants, fruits and fruit rinds (especially citrus fruits: orange, grapefruit, lemon, lime and contained mainly in the edible pulp of the fruits, rather than in the strained juices), vegetables and nuts, etc., and because they cannot be manufactured by the body, they must be supplied through the diet [19-23].

Total proteins, albumin and globulins decrease with irradiation of mice. Administration of rutin possibly tolerated this hypoproteinemic effect of γ radiation as shown in table 1. De Whalley et al. [24, 25] suggested that the decrease in total protein of irradiated mice might be referred to either damage of vital biological processes or to changes in permeability of liver, kidney and other tissue cells leading to leakage of proteins via the kidney. The present study revealed a significant decrease in serum total protein and albumin accompanied by marked alterations in serum protein fractions following exposure of mice to 2 Gy whole-body gamma irradiation. Keren [26] stated that the decrease in concentration of serum albumin might result from excessive loss through injury to the kidneys or gastrointestinal tract or from thermal injury to the skin. It also occurs in hypercatabolic states [27]. In addition, this decrease could be related in part to hepatic dysfunction and decreased protein synthesis [28]. These phenomena might be, at least partially responsible for protein loss after irradiation.

The present study reveals that all groups irradiated with γ radiation showed an increase in cholesterol and triglycerides significantly in relation to control group, which justify the hyperlipidemic effect of γ radiation. This hyperlipidemic effect of γ radiation was ameliorated with the treatment of mice with rutin, which reflected on the significant decrease in the level of cholesterol and triglycerides in all groups.

As we know that both SGOT (Serum Glutamate Oxaloacetate Transaminases) and SGPT (Serum Glutamate Pyruvate Transaminase) are enzymes produced by liver cells of which SGPT is very much liver specific. When the liver cells get damaged these enzymes get released and their levels in the blood go high. The groups of rats that irradiated at all doses of γ radiation induced an increase in the level of SGOT and SGPT but the groups that rutin administered showed decrease in those levels. This indicates that rutin ameliorated the oxidative hazardous effect of γ radiation. The data revealed a significant increase in transaminases activity (AST & ALT) in irradiated animals at 1 and 48 hours post irradiation periods. These increased levels could be referred to the destruction of the radiosensitive tissues of the hepatocytes. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed in serum and anti-oxidant parameters were assessed in liver tissues. Rutin is believed to harbor strong antioxidant properties.

CONCLUSION

Rutin elevates the protein profile (total protein, albumin and globulins) of the body that caused by whole body γ-irradiation of mice. Rutin ameliorates the hyperlipidemic effects of γ radiation effects. Rutin improves the liver functions that had been affected by γ irradiation of animals. Rutin can be used as a radioprotector in professional radiation workers who may be expose to low level of ionizing radiation.

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