

Radiation Protection by Major Tea Polyphenol, Epicatechin

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ABSTRACT Whole body radiation exposure causes extensive physiological stress which may prove fatal if it is not appropriately managed. The current study is intended to evaluate the radioprotective effects of epicatechin (EC) in terms of amelioration of radiation induced hepatic and testicular oxidative stress. Swiss albino mice were administered with EC for three consecutive days before exposing them to a single dose of 5-Gy ^{60}Co gamma (γ) irradiation. Mice were necropsied and liver and testis were taken for biochemical tests for the detection of hepatic and testicular oxidative stress markers. To determine the oxidative stress developed after radiation SGOT, SGPT and ALP were measured to assess the alterations in liver function, reduced glutathione (GSH) content and lipid peroxidation (LPO) were also determined from liver homogenate. To evaluate oxidative stress of testis, LPO, Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP) were also evaluated. Whole body gamma radiation enhanced SGOT, SGPT and ALP level increased as also LPO and depleted GSH level in liver homogenate. Testicular damage was prominent since LPO and ACP level enhanced where as ALP level decreased. Epicatechin pretreatment ameliorated all these gamma radiation mediated alterations and improved the mice from the situation of oxidative stress. Thus, epicatechin pretreatment ameliorated radiation mediated systemic oxidative stress which also prevented liver and testis from further damage.

INTRODUCTION

Ionizing radiation can pass through living tissues and generate free radicals after radiolysis of water. The biomolecules those are hit by these free radicals can develop severe consequences and impair normal cellular functions. The cell pays most severe and permanent penalty if DNA structure is damaged and remains unrepaired by the radiation energy. The major free radicals resulting from aqueous radiolysis are hydroxyl radical ($\text{OH}^\bullet$), superoxide anion ($\text{O}_2^\bullet$), hydroperoxyl radical ($\text{HO}_2^\bullet$), etc. These free radicals react with cellular macromolecules and cause cellular dysfunction and mortality. Thus the search and development of radio protective agents has been the subject of intense research with respect to the side effects produced by ionizing radiation. This situation is particularly important in patients undergoing radiotherapy or people exposed to it at their work place. The radioprotectors have an important role for tolerance and/or increasing survival rate in such people. Antioxidants such as

vitamins (vitamin C, vitamin E), flavonoids, and phenolic acids play the main role in fighting against free radical species. In our recent study we have shown that quercetin prevents gamma radiation mediated RBC damage by scavenging reactive oxygen species (Das et al. 2013). In recent times, interest in exploring the radioprotective potentials of plants and phytochemicals has escalated owing to their natural origin, cost effectiveness and fewer side effects. The current study was intended to evaluate the radioprotective effects of epicatechin in terms of amelioration against radiation induced hepatic and testicular alterations. For this study the radioprotective property of such phytochemicals has been evaluated, Epicatechin (EC), belonging to the group of flavanol, one of the most potent antioxidants present in the human diet predominantly in grapes, tea, apples and cocoa (Natsume et al. 2003).

EC is also present in a common vegetable, *Moringa oleifera* leaf, which has been recently shown to protect the radiation hazards by affecting redox regulated transcription factor (Sinha et al. 2011-2012). EC is a major member of the flavanol family of polyphenolic antioxidant. EC is of particular interest because this compound and its metabolites have been identified as bioactive molecules *in vivo* and predominantly this monomeric flavanol (EC) is absorbed into circulation after ingestion of flavanol-containing foods (Schroeter et al.

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2006). Serum albumins functionally carry this substance through the circulatory system and eliminate reactive oxygen species (ROS) induced injury (Pal et al. 2012). EC possesses 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (the half maximal inhibitory concentration (IC_{50}) = 1.5 μ g), hydrogen peroxide (IC_{50} = 11.18 μ g) and superoxide anion scavenging (IC_{50} = 1.64 μ g) activities (Geetha et al. 2004). It can protect the DNA from gamma (γ)-radiation induced strand breaks (Nair et al. 2008).

Liver is highly metabolically active tissue. Due to high dose of radiation exposure severe damage in hepatic tissue occurs. As regeneration of hepatocytes is very slow, radiation induced hepatic injury can be life threatening (Pyun et al. 2011; Reed et al. 1996; Ingold et al. 1965; Fajardo et al. 1978; Lewin et al. 1973). Irradiation also may have a profound effect on male reproductive function. Direct irradiation of the testis in lower doses, affect the germinal epithelium: doses of irradiation greater than 0.35 Gy cause aspermia, which may be reversible (Ogilvy-Stuart et al. 1993). Therefore, aim of this study is the exploration of an agent that can afford protection against radiation induced alterations of these important tissues in mechanistic viewpoint. Thus, the present study is the attempt to investigate the radio protective efficacy of EC against γ radiation induced inflammation and lipid per oxidation in the mouse liver and testis.

MATERIAL AND METHODS

Chemicals

Epicatechin, Bovine serum albumin (BSA), Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), 5, 5'-dithio-bis (2-nitro benzoic acid) (DTNB), 2, 4, 6-tripyridyl-s-triazine (TPTZ), were purchased from Sigma (St Louis, MO, USA). Ethylenediaminetetraacetic acid disodium salt (EDTA) was purchased from E Merck Co. (Darmstadt, Germany). Serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic- pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and Acid Phosphatase (ACP) kit were procured from Span Diagnostic Ltd, India. Other chemicals were purchased from either Sigma or E.Merck Co (Darmstadt, Germany).

Animals

Approximately 6–8 weeks old Swiss Albino inbred male mice (*Mus musculus*), weighing about 25 ± 1 g were used for the experiments. Animals maintained under controlled environment ($25 \pm 2^\circ\text{C}$ and photoperiod 12 h) were provided standard animal feed (Amrut Lab., Animal Feed, India) and water *ad libitum*. The rules and regulations of Institutional Animal Ethics Committee were strictly followed during experimentation.

Irradiation

Mice were irradiated with ^{60}Co (cobalt-60) source at Saha Institute of Nuclear Physics, Kolkata, India. Animals were restrained in well-ventilated perspex boxes and exposed whole body to gamma radiation (5 Gy), at a dose-rate of 1 Gy/min and a source-to-surface distance of 77.5 cm.

Experimental Design

Mice selected from the inbred colonies, were divided into four groups of 8 animals each. These groups were- 1) Control: The control mice received only distilled water. 2) IR: Mice were given distilled water for 3 days and then exposed to 5 Gy of gamma radiation. 3) EC: Mice were treated with 100 mg/kg body weight of EC through oral gavages for 3 consecutive days. 4) EC+IR: EC was given 100 mg/kg body weight orally for 3 days and 1 hr of the last dose, animals were exposed to gamma radiation (5 Gy). After 24 hrs of irradiation, blood was collected and animals were then sacrificed to collect liver, testis for different biochemical assays.

Determination of SGOT, SGPT, ALP and ACP

All these parameters were determined according to the protocols provided with the commercial kit.

Determination of Lipid Peroxidation (LPO)

Thiobarbituric acid reactive substance (TBARS) in the homogenate was estimated by using standard protocol (Beige et al. 1978). Briefly, the homogenate was incubated with

15% TCA, 0.38% TBA and 5 N hydrochloric acid (HCL) at 95°C for 15 min, the mixture was cooled, centrifuged and the absorbance of the supernatant measured at 535 nm against appropriate blank. The amount of lipid peroxidation was determined by using $\epsilon=1.56 \times 10^5$ M/cm and expressed as TBARS/g of tissue.

Determination of Reduced Glutathione (GSH)

Glutathione (reduced) was determined according to the method described by Moron et al (Moron et al. 1979). Liver homogenates were treated with 0.1 ml of 25% trichloroacetic acid (TCA) and the resulting precipitate was pelleted by centrifugation at 3900 g for 10 min. Free endogenous sulfhydryl was assayed in a total 3 ml volume by adding 2 ml of 0.5 mM 5, 5'-dithio-bis (2-nitro benzoic acid) (DTNB) prepared in 0.2 M phosphate buffer (pH 8) to 1 ml of the supernatant. The GSH reacts with DTNB and forms a yellow-colored complex with DTNB. The absorbance was read at 412 nm. The result was expressed as μ moles of GSH / mg protein. The protein concentration of the homogenate was determined according to Lowry et al (Lowry et al. 1951).

Statistical Analysis

The values are given as Mean $\pm$ standard error of the mean (SEM). One-way analysis of variance (ANOVA) with Tukey's post test was done for statistical evaluation of the data and for the determination of level of significance in various groups of animals. $P < 0.05$ being considered as level of significance.

RESULTS

Epicatechin Prevents Gamma Radiation Mediated Hepatic Toxicity

The normal functional status of liver was assessed by estimating the levels of liver enzymes SGPT, SGOT and ALP (Table 1). In the present study it was observed that gamma radiation induced a significant elevation in SGOT level. The serum ALP was much elevated by gamma ray irradiation (25.66 ± 1.19 IU/L) compared to normal values (19.38 ± 0.45 IU/L) and only EC treated group (17.84 ± 0.83 IU/L) sig-

nificantly ($p < 0.05$) after 24 hrs of irradiation. This elevation was reduced by treatment with epicatechin in EC+IR group (22.3 ± 1.63 IU/L). Serum GPT and GOT levels were elevated by gamma irradiated group (5.5 ± 0.56 IU/L and 39.5 ± 6.26 IU/L respectively) as compared with normal levels (5.0 ± 0.36 IU/L and 28.83 ± 2.14 IU/L correspondingly). EC treatment decreased elevated levels (5.36 ± 0.16 IU/L and 29.74 ± 0.83 IU/L) respectively.

Table 1: Liver toxicity study showing serum level of GOT, GPT and ALP

	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Control	5.0 ± 0.15	19.38 ± 0.45	28.78 ± 0.91
IR	5.58 ± 0.19	$25.66 \pm 1.19^{\#}$	$40.5 \pm 0.89^{\#}$
EC	4.94 ± 0.13	17.84 ± 0.82	24.38 ± 0.82
EC+IR	5.36 ± 0.15	$22.3 \pm 1.62^*$	$29.74 \pm 0.82^*$

Values are represented as Mean $\pm$ SEM (n= 8). Data were analyzed by One way ANOVA (analysis of variance). Statistical comparison: Significance level: $p < 0.05$. $^{\#}$ Control versus IR, * IR versus EC+IR.

Epicatechin Ameliorated Gamma Radiation Mediated Hepatic LPO

γ radiation (5 Gy) induced a significant ($p < 0.05$) increase in the level of TBARS (5.36 ± 0.48 nanomoles/g of tissue) compared to control (3.28 ± 0.19 nanomoles/g of tissue), EC treated (2.47 ± 0.20 nanomoles/g of tissue) (Fig. 1.A). Administration of EC for 3 consecutive days resulted in significant decrease (2.47 ± 0.20 nanomoles/g of tissue) in TBARS level compared to control group (3.28 ± 0.19 nanomoles/g of tissue). Whereas pre-treatment with EC ameliorated the effect of radiation exposure as TBARS level was significantly ($p < 0.05$) decreased (3.08 ± 0.25 nanomoles/g of tissue) compared to irradiated group.

EC Pretreatment Restored Hepatic GSH Level after Whole Body Gamma Radiation Exposure

Whole body γ -radiation exposure induced significant ($p < 0.05$) decrease (0.288 ± 0.013 μ moles/mg protein) in GSH level compared to control (0.435 ± 0.012 μ moles/mg protein), EC (0.472 ± 0.012 μ moles/mg protein) group (Fig. 1.B). Pre-administration of EC to radiation exposed mice showed significant ($p < 0.05$) increase (0.391 ± 0.013 μ moles / mg protein) in GSH content compared to irradiated group.

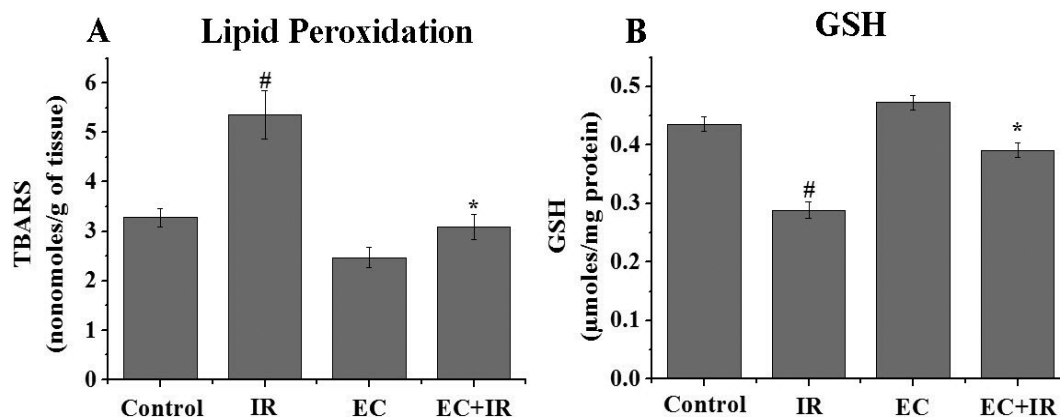


Fig. 1. A. The histograms show the hepatic LPO level of four different groups. B. It shows the hepatic GSH level of four different groups. Four different groups are Control, IR, EC, EC+IR. Values are represented as Mean $\pm$ SEM (n=8). Significance level: $p < 0.05$. [#]Control versus IR, ^{*}IR versus EC+IR.

Epicatechin Ameliorated Gamma Radiation Induced LPO of Testis

In irradiated group, LPO level significantly ($p < 0.05$) increased (4.88 ± 0.193 nanomoles TBARS/g of tissue) at 24 hrs of autopsy interval of observation in terms of thiobarbituric acid reactive substances (TBARS) when compared with control (1.62 ± 0.08 nanomoles TBARS/g of tissue), EC (1.5 ± 0.1 nanomoles TBARS/g of tissue) group (Fig. 2.A). On the other hand, in EC+IR (3.46 ± 0.074 nanomoles TBARS/g of tissue) group, a significant inhibition in LPO level was observed than IR group. However significant differences existed between control vs EC+IR.

Epicatechin Pretreatment Altered The Radiation Induced Testicular ACP and ALP Activity

In irradiated group, the acid phosphatase activity in testis showed significant ($p < 0.05$) increase (44.98 ± 0.74 IU/L) when compared with control (35.02 ± 1.17 IU/L), EC (29.98 ± 0.73 IU/L) (Fig. 2B). In EC (33.54 ± 1.70 IU/L) pretreated irradiated mice, a significant recovery in acid phosphatase activity was observed when compared to IR. Alkaline phosphatase activity in testis showed significant decline ($p < 0.05$) in irradiated group (33.18 ± 0.32 IU/L) after 24 hrs of observation than control (37.52 ± 0.95 IU/L), and EC (38.58 ± 0.89 IU/L) group (Fig. 2.C). In case of EC+IR (38.48 ± 0.84 IU/L) group, the

alkaline phosphatase activity was significantly increased compared to irradiated mice.

DISCUSSION

The chief rationale of finding a suitable radioprotector is that it should ameliorate oxidative and systemic stress after radiation exposure and itself should be friendly to the organism. Here we have chosen two organs to determine the amelioration of systemic stress by EC after radiation that is the radioprotective potential of EC. Liver is a major metabolic organ of the body; therefore, it sustains major challenge from the oxidative shock. On the other hand testis not only more sensitive to radiation, it also produces spermatozoa; thus it develops aspermia and can manifest infertility of the individual on repeated or prolonged exposure.

The chief intension of the present article was to seek whether EC can prevent oxidative stress after whole body radiation. It is important that these primary and secondary oxidative species after radiolysis are quenched before they trigger any reaction. These radicals and ions are responsible for peroxidation of the membrane. Peroxidation of this structure can lead to several anomalies of the cellular system. The antioxidants including many phytochemicals restore the endogenous antioxidant system, thus regulate the signalling cascade leading to liver injury. Our laboratory demonstrated this phytochemicals, EC prevents the cellular stress by eliminating ROS from the cellular system

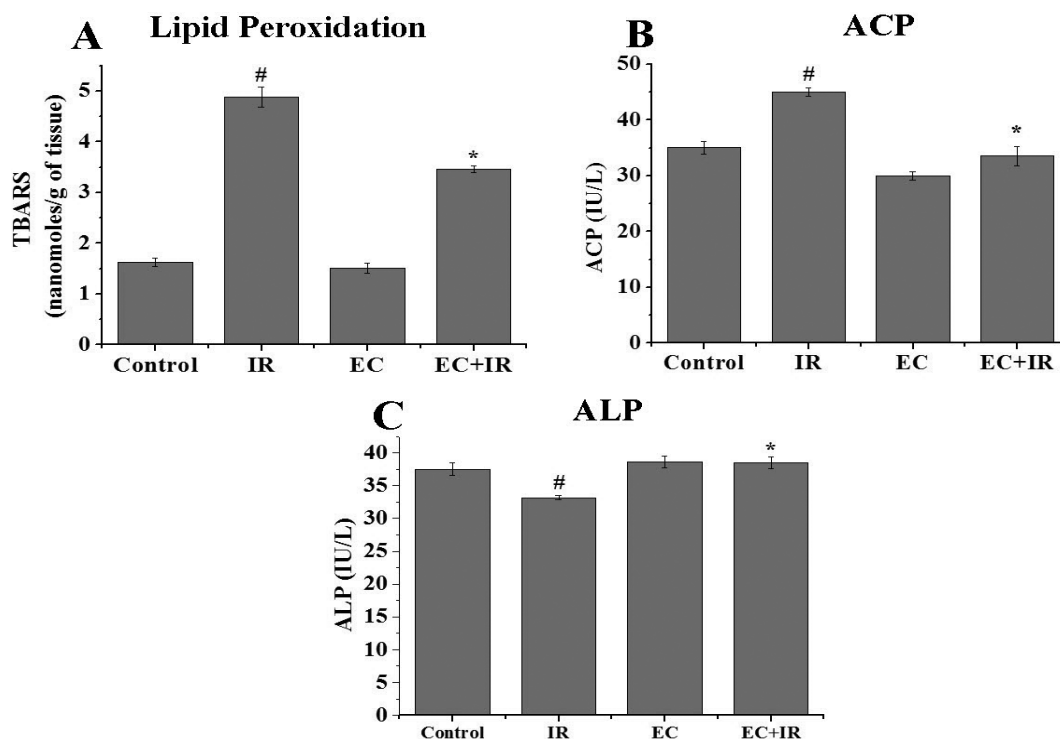


Fig. 2. A. The histograms show the testicular LPO level of four different groups. B. This panel shows testicular ACP level of different groups. C. It shows ALP level of testis of different groups. Values are represented as Mean $\pm$ SEM (n = 8). Level of significance is $p < 0.05$. [#]Control versus IR, ^{*}IR versus EC+IR.

and thus prevents nuclear translocation of NF- κ B (p^{65}) (Sinha et al. 2012). The later is a crucial redox sensitive element for cellular inflammation and other anomalies after gamma irradiation. In the current study it was also observed that 5 Gy gamma radiation caused increase in SGPT, SGOT, serum ALP levels. The radiation also increased testis ACP level but reduced ALP levels. Lipid peroxidation was also increased in testes due to irradiation. The radiation has thus aggravated reactive oxygen species including the lipid peroxidation and thus deranged the physiological function as manifested by various defence mechanisms. The lipid peroxidation has been reported to be directly proportional to oxidative stress, where the efficacy becomes weaker (Jagetia et al. 2005). It has been observed that lipid peroxidation causes degradation of fine structures, alteration of integrity, fluidity, permeability and functional loss of biomembranes, modifies low density lipoprotein (LDL) to proatherogenic and proinflammatory forms and generates potential toxic products (Greenberg

et al. 2008). ROS also negatively impacts the antioxidant defence mechanism, reduces the intracellular concentration of GSH. This could be due to an enhanced utilization of the antioxidant system during detoxification of the free radicals generated by irradiation.

In our study EC treatment prior to radiation was found to protect the liver and testis from radiation induced oxidative shock. EC prevented systemic and cellular oxidative stress. The prevention of the systemic shock reflects the prevention of development of inflammation of organs. Previously, it was also reported that bioactive components from the tea polyphenols influence on endogenous antioxidant defence system after total-body irradiation in mice (West et al. 2006).

CONCLUSION

Therefore, our present and previous reports strengthen the evidences in favour of the usefulness of epicatechin rich diet to prevent higher

levels of radiation induced oxidative shock, inflammation and lipid peroxidation. This is particularly important for workers in nuclear plant, defence personals or space travellers who succumb to the possibility of radiation exposure. However, EC treatment prior to radiotherapy warrants further clinical trials regarding the selection of dose and other issues for individual cases. However, with the present set of experiments and data we establish that the epicatechin qualifies as an effective radio protective agent in low dose of ionizing radiation exposure which prevents systemic organ stress and infertility development.

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