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# Modulation of radiation-induced biochemical alterations in mice by *Tylophora indica* leaf extract

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**Abstract**
Whole-body γ-irradiation (10 Gy) in mice induced marked oxidative stress in jejunal tissue, as evidenced by suppression of key antioxidants and enhanced lipid peroxidation. Superoxide dismutase (SOD) and catalase (CAT) activities were significantly reduced following radiation exposure, while malondialdehyde (MDA) levels, indicative of lipid peroxidation, showed a marked increase. Oral pre-treatment with *Tylophora indica* hydro-methanolic leaf extract (TE, 100 mg/kg b.wt.) for seven days prior to irradiation provided notable radioprotection. TE supplementation enhanced SOD activity by up to 23% on day 1 and improved CAT levels by ~67% at day 3 compared to irradiated controls. Similarly, reduced glutathione (GSH) content was preserved, showing a 39% higher level across days 1-15, while lipid peroxidation was reduced by ~58% over the same period. Furthermore, TE pre-treatment significantly prevented protein loss in intestinal tissues, with pronounced recovery observed on day 15 post-irradiation. Collectively, these findings demonstrate that *Tylophora indica* hydro-methanolic leaf extract mitigates γ-radiation-induced oxidative stress by reinforcing endogenous antioxidant defenses, reducing membrane lipid damage, and preserving protein integrity.

**Keywords:** Antioxidants, Gastrointestinal system, Ionizing radiation, Radioprotection, *Tylophora*

## Introduction
Ionizing radiation (IR) plays a pivotal role in modern medicine, particularly in diagnostic imaging and cancer therapy. Radiotherapy remains one of the primary modalities for treating malignant tumours, effectively controlling disease progression and improving patient survival [1, 2]. However, the therapeutic efficacy of radiation is often compromised by its detrimental effects on normal tissues surrounding the tumour. Such collateral damage may lead to acute side effects, including inflammation and oxidative stress, as well as chronic complications such as fibrosis, organ dysfunction, and secondary malignancies [3, 4]. These limitations highlight the urgent need to identify and develop effective radioprotective strategies that can minimize radiation-induced injury without compromising therapeutic benefits [5].
Radiation-induced cellular damage is largely mediated by the generation of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals, and hydrogen peroxide [6]. Although living organisms possess well-coordinated antioxidant defense systems comprising enzymes such as superoxide dismutase, catalase, and glutathione peroxidise [7]. Excessive ROS production following irradiation can overwhelm these protective mechanisms. This imbalance results in oxidative stress, which subsequently triggers lipid peroxidation, protein denaturation, DNA damage, and disruption of normal cellular metabolism [8]. Therefore, antioxidants and free-radical scavengers have gained considerable attention as potential radioprotective agents.
Medicinal plants have been traditionally employed in healthcare for centuries, offering therapeutic benefits with minimal side effects compared to synthetic drugs [9]. Recent research has emphasized the use of plant-derived bioactive compounds as natural radioprotectors owing to their antioxidant, anti-inflammatory and immunomodulatory properties [10]. Among these, *Tylophora indica* (Asclepiadaceae family), commonly known as Indian Ipecac, holds significant pharmacological potential [11, 12]. Traditionally used in Ayurveda and folk medicine, *T. indica* has been employed in the management of respiratory ailments (asthma, chronic cough, bronchitis), gastrointestinal disorders (diarrhoea, dysentery), arthritis, allergies, skin inflammations, and even cancer [13,14]. Phytochemical analysis reveals that *T. indica* contains diverse bioactive constituents, including alkaloids (tylophorine, tylophorinine, tylophorinidine, and isotylocrebrine), flavonoids, phenolics, terpenoids, saponins, and glycosides [15, 16]. Experimental studies have demonstrated its antioxidant, anti-inflammatory, antitumor,

immunomodulatory, hepatoprotective, antimicrobial, antidiabetic, and antidiarrheal activities underscoring its therapeutic versatility [17].

The intestine, being one of the most radiosensitive organs, plays a vital role in digestion, absorption, and barrier protection against toxins and pathogens [18]. Radiation-induced intestinal injury is primarily manifested through oxidative stress, mucosal damage, and biochemical alterations, ultimately impairing normal physiological functions [19]. Despite the well-documented pharmacological potential of *T. indica*, its role as a radioprotective agent in intestinal tissues remains largely unexplored.

Therefore, the present study was undertaken to investigate the modulation of radiation-induced biochemical alterations in the intestine of γ-irradiated mice by *Tylophora indica* hydro-methanolic leaf extract. This work aimed to provide novel insights into the potential application of *T. indica* as a natural radioprotective agent, thereby contributing to safer therapeutic strategies and preparedness against planned and unplanned radiation exposure.

## Materials and Methods

### Chemicals

All chemicals and reagents used in the present study were of analytical grade. These included Bovine Serum Albumin (BSA), Copper sulphate, 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB-Ellman's reagent), Folin-Ciocalteu reagent, Hydrogen peroxide ($H_2O_2$), Methanol, Pyrogallol, Reduced glutathione (GSH), Sodium carbonate, Sodium hydroxide, Sodium potassium tartrate, Thiobarbituric acid (TBA), Tris-cacodylic acid, and Trichloroacetic acid (TCA). All chemicals were procured from reputed suppliers in India and used without further purification.

### Preparation of Hydro-methanolic Leaf Extract

A specimen of *Tylophora indica* was collected during the winter season from the botanical garden of the Department of Botany, University of Rajasthan, Jaipur (26.8885° N; 75.8140° E), and authenticated at the Departmental herbarium (Accession No. RUBL211769). The hydro-methanolic leaf extract (TE) was prepared as described earlier and stored at 4°C until further use [20]. The prepared extract was employed for the evaluation of *in vivo* antioxidant activities and radioprotective potential in jejunum portion of small intestine.

### Experimental Animals

#### Animals used

Male Swiss albino mice (*Mus musculus*), aged 8-10 weeks and weighing 25 ± 5gm, were procured from the National Institute of Pharmaceutical Education and Research (NIPER), Mohali, India (Reg. No. 108/GO/ReRcBiBt/S/1999/CPCSEA). The animals were acclimatized and maintained in the animal facility of the Department of Zoology, University of Rajasthan, Jaipur (Reg. No. 1678/Go/Re/S/12/CCSEA), under standard laboratory conditions (temperature: 25 ± 2 °C; relative humidity: 50-60%; 12 h light/dark cycle). They were housed in groups of five in clean polypropylene cages with sterilized bedding and provided with standard pellet diet and water *ad libitum*. All experimental protocols were carried out in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA, formerly known as CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC), Department of Zoology, University of Rajasthan, Jaipur.

### Irradiation Procedure

Whole-body γ-irradiation was carried out using a Cobalt-60 teletherapy unit (Picker Advance Tele Cobalt (ATC) C/9, Advanced Medical Systems Inc., USA; Model No. 181361) installed at the Department of Radiological Physics, SMS Medical College and Hospital, Jaipur, Rajasthan, India. The source had an activity of 333 TBq (9000 Ci) and delivered radiation at a dose rate of 1.03 cGy/sec. Mice were placed in perforated polypropylene cages and irradiated in groups of five under ambient room temperature conditions.

### TE Treatment Regimen

The hydro-methanolic leaf extract of *T. indica* (TE) was freshly dissolved in sterile distilled water immediately prior to use. The required dosage was calculated according to body weight and expressed as mg/kg b.wt. Oral administration was performed using a gavage needle, with a maximum volume of 0.2 ml per mouse. Animals were randomly divided into the following four groups (n = 5 per group) for experimental evaluation (Figure 1):

a) **Control group:** Received saline orally without radiation exposure.
b) **Radiation only group:** Exposed to a single whole-body dose of 10 Gy γ-radiation.
c) **TE only group:** Administered TE orally at 100 mg/kg b.wt. for seven consecutive days without radiation.
d) **TE + Radiation group:** Administered TE orally at 100 mg/kg b.wt. for seven consecutive days, with the final dose given 2h before 10 Gy whole-body γ-radiation exposure.

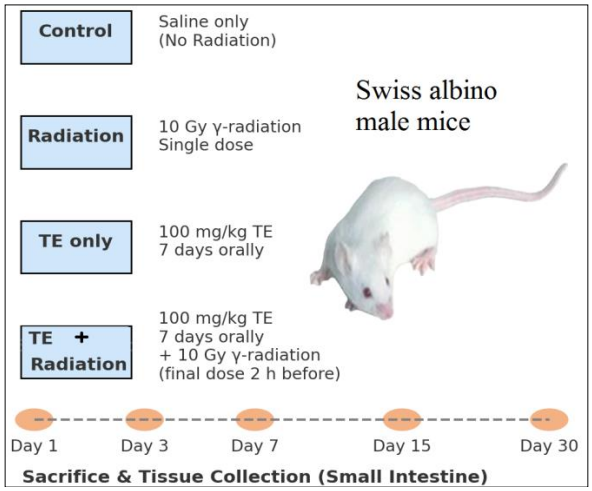


**Fig 1:** Development of an experimental plan to investigate biochemical parameters.

**Tissue Collection**
Animals were sacrificed at 1, 3, 7, 15, and 30 days post-irradiation. The jejunal portion of small intestine was perfused with buffered saline, carefully excised, weighed, and homogenized in phosphate-buffered saline for subsequent biochemical analysis.

**Biochemical Analysis**
Following the treatment schedule, animals from each experimental group were sacrificed by cervical dislocation at the designated time points. Intestinal tissues were excised, rinsed thoroughly with ice-cold phosphate-buffered saline (PBS, pH 7.4) to remove luminal contents and blood, and processed for biochemical investigations. A 10% (w/v) tissue homogenate was prepared in ice-cold PBS and centrifuged (Remi, India) to obtain the supernatant, which was subsequently used for the estimation of biochemical parameters, namely lipid peroxidation (LPO), catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD), and total protein content.

**Superoxide Dismutase (SOD) Activity**
SOD activity in intestinal tissue homogenates was assayed according to the method of Marklund and Marklund [21]. The reaction mixture contained 100 mM Tris HCL buffer (pH 8.2), 6 mM EDTA, and 0.6 mM pyrogallol in an air-equilibrated system. Appropriate controls were prepared, including a blank (without pyrogallol) and a control reaction (with pyrogallol but without tissue homogenate). The rate of pyrogallol auto-oxidation was monitored spectrophotometrically at 420 nm (Thermo Fisher, Finland), with absorbance recorded every minute for 4 min, beginning 30 s after initiation of the reaction. Enzyme activity was calculated from the kinetics of the reaction mixture and expressed as Units/mg tissue, where one Unit corresponds to 50% inhibition of pyrogallol auto-oxidation, using the formula:

**Percentage inhibition= $(A_1-A_0) / A_0$ X 100**

Where $A_0$ = OD/min of blank (Pyrogallol only); $A_1$ = O.D/min of treated samples

**Catalase (CAT) Activity**
Catalase activity in intestinal homogenate was determined following the procedure of Aebi [22]. Briefly, 0.1 ml of tissue homogenate was mixed with 1.9 ml of hydrogen peroxide solution (30 mM prepared in 50 mM phosphate buffer, pH 7.0), while distilled water served as blank. The rate of hydrogen peroxide decomposition was monitored spectrophotometrically at 240 nm (Thermo Fisher, Finland), for 1 min with 10-second interval readings. Catalase activity was calculated from the decline in absorbance, expressed as Units/mg tissue/min, where one Unit corresponds to the decomposition of one µmole of $H_2O_2$ per min. The following equation was used:

**CAT activity (U/mg of tissue/min)=$(A_1-A_0)/t$ x $V_t/\varepsilon$ x l x$V_s$**

Where $A_0$= Initial OD; $A_1$= Final OD; t = time (1 min); $V_t$ = Total reaction mixture (3ml); ε= Molar extinction coefficient of $H_2O_2$ (43.6 $M^{-1}cm^{-1}$); l=Path length of cuvette (1 cm); $V_s$= Volume of homogenate (0.1 ml).

**Reduced Glutathione (GSH) Content**
The level of reduced glutathione in intestinal tissue was estimated according to Moron *et al.* [23]. In brief, 0.5 ml of 10% homogenate (prepared in 1.15% KCl) was treated with 0.1 ml of 25% trichloroacetic acid (TCA) and centrifuged (Remi, India) at 2500 rpm for 5 min. The resulting supernatant (0.1 ml) was incubated with 2.0 ml of 0.6 mM DTNB and 0.9 ml of 0.2 M phosphate buffer (pH 8.0). Absorbance was measured at 412 nm against a TCA blank using a UV-Vis spectrophotometer (Thermo Fisher, Finland). GSH content was expressed as nmoles GSH/mg tissue using the following equation:

**GSH Activity = $A_{412}$ x $V_t/\varepsilon$ x l x $V_s$**

Where $A_{412}$= Absorbance at 412 nm; $V_t$ = Total reaction mixture (3ml); ε = Molar extinction coefficient of DNTB-GSH complex (13.6 x $10^3$ $M^{-1}cm^{-1}$); l= path length of cuvette (1 cm); $V_s$= Volume of tissue homogenate (0.1 ml)

**Lipid Peroxidation (LPO) Activity**
Lipid peroxidation was quantified by estimating malondialdehyde (MDA) levels following Ohkawa *et al.* [24]. A 0.1 ml aliquot of 10% homogenate was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.67% TBA reagent, followed by incubation in a boiling water bath (90°C) for 15 min. After cooling, the samples were centrifuged at 4000 rpm (Remi, India) for 10 min and diluted with 1.0 ml distilled water. The pink chromogen formed due to MDA-TBA adduct was measured spectrophotometrically (Thermo Fisher, Finland), at 532 nm against PBS blank. LPO levels were calculated as nmole MDA/mg tissue using the equation:

**LPO activity (nmole/mg of intestinal tissue) = $A_{532}$ x $V_t/\varepsilon$ x l x $V_s$**

Where $A_{532}$= Absorbance at 532 nm; $V_t$ = Total reaction mixture (3ml); ε = Molar extinction coefficient of MDA-TBA complex (1.56 x $10^5$ $M^{-1}cm^{-1}$); l= path length of cuvette (1 cm); $V_s$= Volume of tissue homogenate (0.1 ml)

**Total Protein Content**
Protein concentration was determined using the method of Lowry *et al.* [25]. Briefly, 10% homogenate was treated with reagent a (2% sodium carbonate in 0.1 N NaOH, 0.5% copper sulphate, and 1% sodium potassium tartrate) and incubated for 15 min. Thereafter, reagent B (freshly prepared Folin-Ciocalteu reagent diluted 1:1 with distilled water) was added, followed by 30 min incubation in the dark. The reaction, which involves the formation of a copper-protein complex and subsequent reduction of phosphomolybdate-phosphotungstate, produced a blue colour measured at 640 nm. Protein content was determined from a BSA standard curve (10 mg/ml stock).

**Statistical analysis**
All experimental values were expressed as mean ± standard deviation (SD) of three independent determinations. Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by post hoc testing to assess intergroup differences. Data were analyzed using IBM SPSS statistics for Window 10, SPSS Inc. and significance was set at $^{*}p< 0.001$, $^{\#}p< 0.01$, $^{\$}p< 0.05$ and $^{\#\#}p>0.05$ non-significant.

## Results

### Biochemical Analysis of Intestinal Tissue

To evaluate the radioprotective efficacy of *Tylophora indica* hydro-methanolic leaf extract (dose of TE; 100 mg/kg b.wt.), biochemical assays were performed on the jejunal portion of small intestine tissue of mice subjected to 10 Gy whole-body γ-irradiation. The parameters studied included SOD, CAT, GSH, LPO and total protein content.

### Superoxide Dismutase (SOD) Activity

SOD serves as the first-line antioxidant defense by dismutating superoxide radicals generated during γ-radiolysis of water. A marked decline in SOD activity was observed in irradiated mice compared to controls. However, TE pre-treatment significantly restored enzyme activity at all post-irradiation intervals (1-15 days). The most pronounced enhancement (~23%) was recorded on day 1 in the TE pre-treated irradiated group compared to the radiation-only group, after which values gradually declined toward basal levels by day 30 (Figure2;Table 1 and 2). The partial restoration of SOD suggests that TE can enhance enzymatic dismutation of $O_2^{\bullet -}$, thereby reducing downstream ROS burden.

**Table 1:** Biochemical evaluation of parameters in the experimental group across different treatment durations

| | Days | SOD (Units/mg/min) | CAT (Units/mg/min) | GSH (nmol/mg) | LPO (nmol/mg) | Total Protein (mg/gm) |
|---|---|---|---|---|---|---|
| **Control** | | 0.374 ±0.004 | 0.334 ± 0.004 | 8.028 ±0.003 | 1.117 ± 0.004 | 2.623 ±0.105 |
| TE (100 mg/kg b. wt) | 1 D | 0.370 ±0.003 | 0.337 ± 0.002 | 8.031±0.006 | 1.119 ± 0.005 | 2.404 ±0.084 |
| | 3 D | 0.364 ± 0.005 | 0.329 ±0.005 | 8.018± 0.004 | 1.127 ± 0.005 | 2.605 ±0.024 |
| | 7 D | 0.378 ± 0.002 | 0.332 ± 0.005 | 8.024± 0.005 | 1.114 ± 0.003 | 2.638 ±0.030 |
| | 15 D | 0.362 ± 0.005 | 0.333 ± 0.004 | 8.016 ± 0.003 | 1.118 ± 0.005 | 2.504 ±0.203 |
| | 30 D | 0.366 ± 0.007 | 0.335 ±0.003 | 8.027 ± 0.004 | 1.112 ± 0.006 | 3.824 ±0.383 |
| Radiated (10 Gy) | 1 D | 0.246 ± 0.006 | 0.184 ±0.005 | 4.468 ± 0.005 | 6.242 ± 0.006 | 2.331 ±0.053 |
| | 3 D | 0.253 ± 0.004 | 0.154 ±0.005 | 4.452 ± 0.006 | 6.237 ± 0.006 | 2.583 ±0.112 |
| | 7 D | 0.262 ± 0.003 | 0.166 ±0.002 | 4.461 ± 0.004 | 6.224 ± 0.008 | 2.982 ±0.236 |
| | 15 D | 0.259 ± 0.008 | 0.175 ±0.003 | 4.457 ± 0.008 | 6.231 ± 0.007 | 1.853 ±0.143 |
| | 30 D | **Complete mortality in the radiation group of mice** | | | | |
| TE + (-2h) Radiation (10Gy) | 1 D | 0.304 ± 0.002 | 0.278 ±0.005 | 6.210 ± 0.003 | 3.672 ± 0.005 | 3.236 ±0.246 |
| | 3 D | 0.309 ± 0.005 | 0.257 ±0.004 | 6.206 ± 0.007 | 3.664 ± 0.008 | 2.703 ±0.113 |
| | 7 D | 0.314 ± 0.003 | 0.273 ±0.005 | 6.202 ± 0.006 | 3.647 ± 0.006 | 2.788 ±0.086 |
| | 15 D | 0.312 ± 0.003 | 0.266 ±0.003 | 6.214 ± 0.004 | 3.652 ± 0.004 | 2.427 ±0.107 |
| | 30 D | 0.316 ± 0.005 | 0.271 ±0.005 | 6.222 ± 0.005 | 3.638 ± 0.005 | 3.29 ±0.348 |

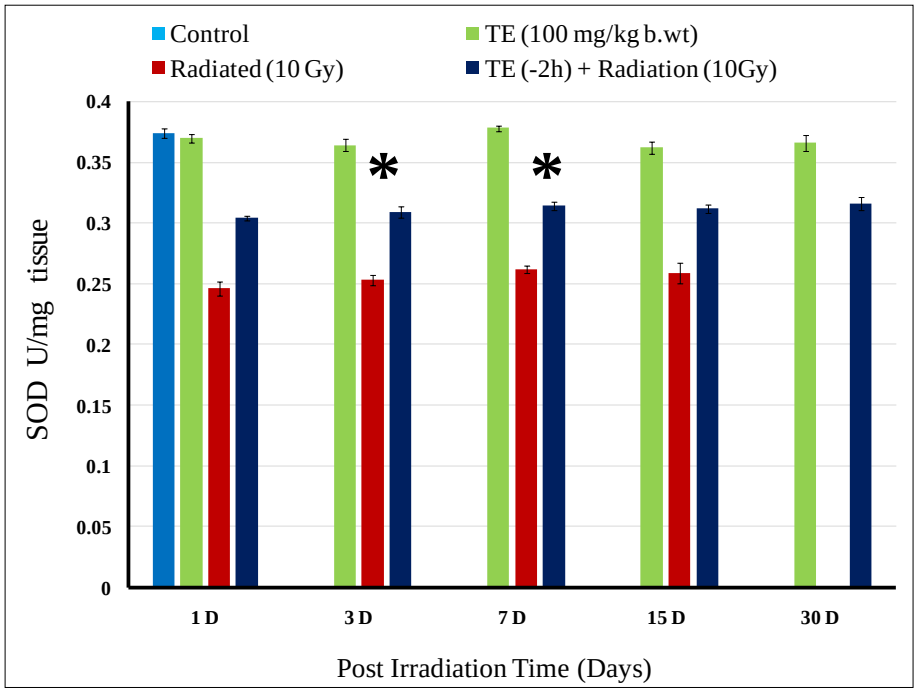


**Fig 2:** Effect of *Tylophora indica* leaf extract (TE) on superoxide dismutase (SOD) activity in jejunal portion of intestinal tissue of mice exposed to 10 Gy whole-body γ-radiations. Values are expressed as mean ± SD (n = 5).Significance: *$p$< 0.001 Radiation-only group *vs.* TE- Radiation (-2h) group.

### Catalase (CAT) Activity

As a critical enzyme for detoxifying hydrogen peroxide, CAT activity was substantially suppressed in irradiated mice. In contrast, TE pre-treatment significantly preserved CAT activity throughout the study period. The maximum recovery (~67%) was observed on day 3 post-irradiation in the TE + radiation group relative to radiation-only mice. By day 30, activity values approached stable. TE with and without irradiated at all time points except 30$^{th}$ day as no survival was seen after 14$^{th}$ day post irradiation (Figure3; Table 1 and 2). Restoration of CAT implies that TE supplementation supports the enzymatic removal of peroxides, thereby minimizing hydroxyl radical (•OH) formation via Fenton reactions.

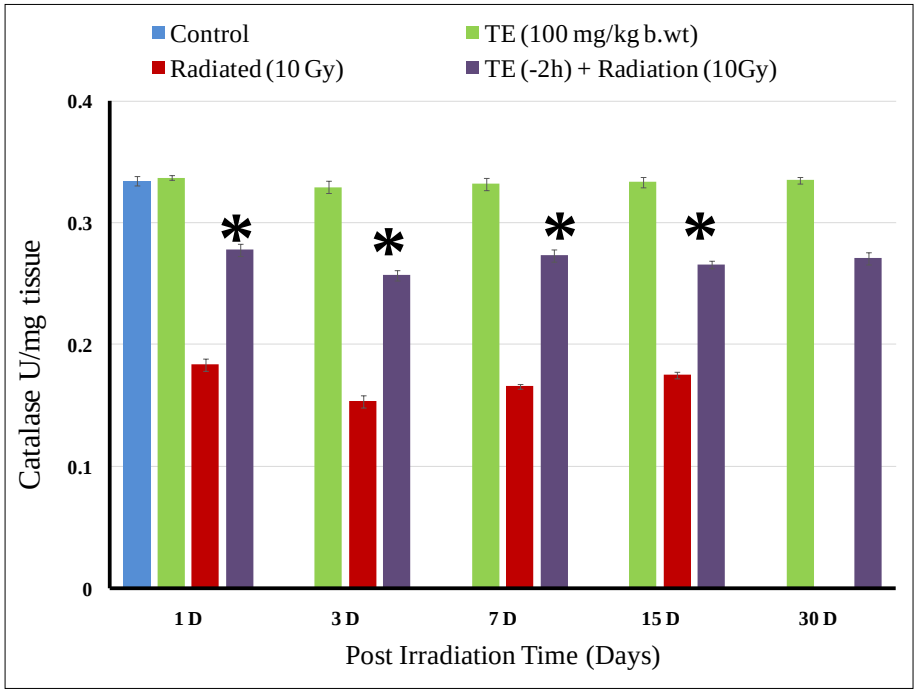


**Fig 3:** Effect of TE on catalase (CAT) activity in jejunal portion of intestinal tissue of irradiated mice. TE pre-treatment significantly preserved CAT activity compared to the radiation-only group. Values are expressed as mean ± SD (n = 5).Significance: *$p$< 0.001 radiation-only group *vs.* TE- radiation (-2h) group.

**Reduced Glutathione (GSH) Level**

Radiation exposure drastically depleted GSH pools, consistent with its rapid utilization for ROS detoxification. TE pre-treatment significantly elevated GSH levels (~39% increase relative to the radiation-only group), particularly between days 1-15. This effect suggests that TE either promotes de novo synthesis of GSH or prevents its depletion by reducing oxidative load. Maintenance of thiol balance underpins TE's ability to stabilize intracellular redox homeostasis (Figure 4; Table 1 and 2).

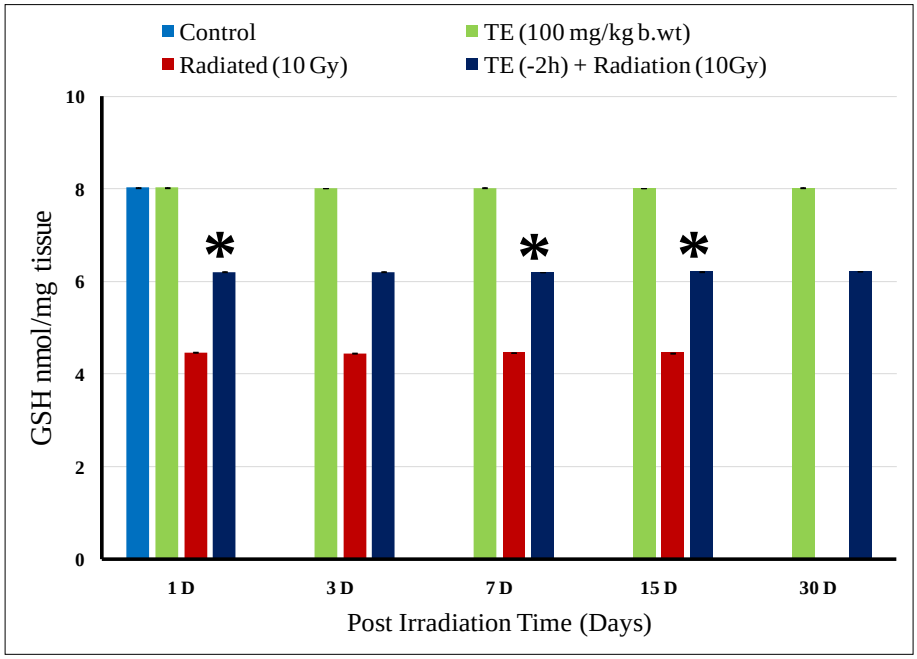


**Fig 4:** Effect of TE on reduced glutathione (GSH) levels in jejunal portion of intestinal tissue following radiation exposure. TE supplementation enhanced GSH recovery compared to irradiated controls. Values are expressed as mean ± SD (n = 5).Significance: *$p$< 0.001 radiation-only group *vs.* TE- radiation (-2h) group.

**Lipid Peroxidation (LPO) Activity**

A pronounced rise in MDA levels was observed post-irradiation, reflecting lipid membrane damage. TE pre-treatment significantly suppressed LPO (~58% lower than radiation-only group), particularly during the acute phase (1-15 days). This attenuation of lipid damage indicates that TE-derived antioxidants effectively neutralize free radicals before they propagate chain reactions in membrane lipids (Figure. 5; Table 1 and 2).

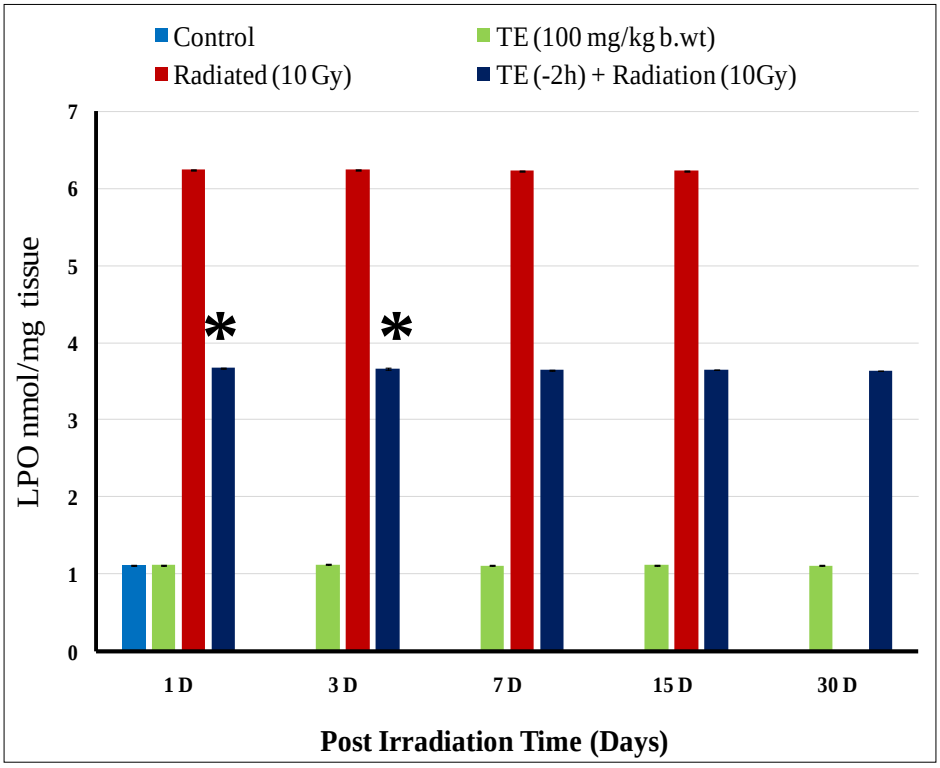


**Fig 5:** Effect of TE on lipid peroxidation (LPO; measured as MDA equivalents) in jejunal portion of intestinal tissue of mice. Radiation markedly increased MDA levels, while TE pre-treatment significantly suppressed lipid peroxidation. Values are expressed as mean ± SD (n = 5).Significance: *$p$< 0.001 radiation-only *vs.* TE- radiation (-2h) group.

**Total Protein Content**

Radiation caused a substantial decline in intestinal protein content, attributable to oxidative modification of amino acid residues and impaired protein synthesis. TE-treated irradiated mice exhibited marked protection, with protein levels significantly higher than irradiated-only animals across all intervals. By day 30, protein content in TE-treated mice nearly approached baseline values, highlighting its capacity to preserve structural and functional proteins under radiation stress (Figure. 6; Table 1and 2). Significant reduction (30%; p<0.001) in total protein content was observed in the jejunal portion of tissue of only irradiated mice compare to irradiated mice pre-treated by TE (-2h) at 15$^{th}$ day.

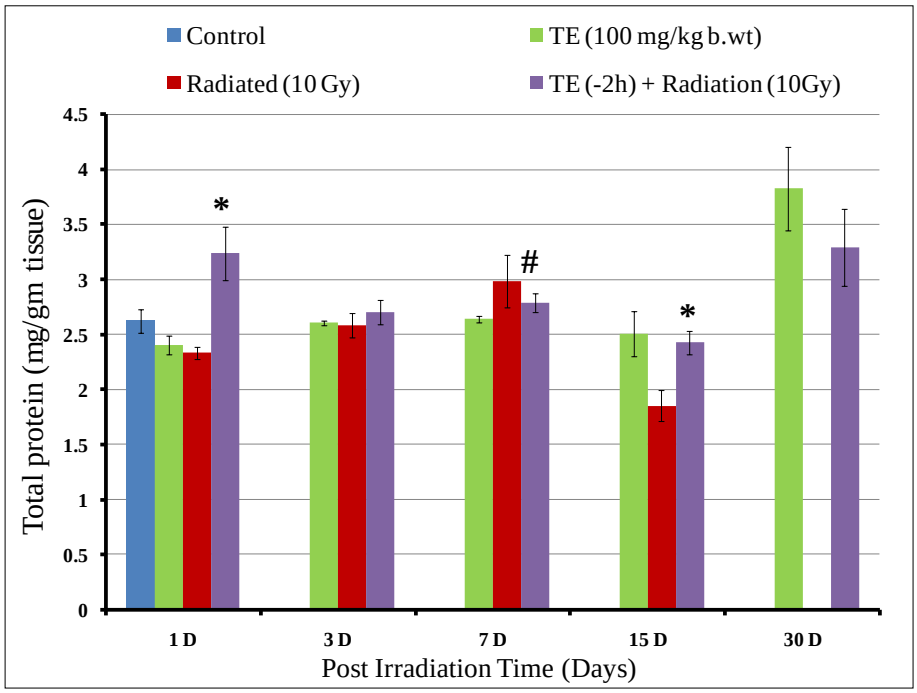


**Fig 6:** Effect of TE on total protein content in jejunal portion of intestinal tissue of γ-irradiated mice. Radiation significantly reduced protein levels, whereas TE pre-treatment restored protein content toward near-normal values. Values are expressed as mean ± SD (n = 5).Significance: $^{*}p$< 0.001 and $^{\#}p$< 0.01 Radiation-only group *vs.* TE- Radiation (-2h) group.

**Table 2:** Relative percentage of enzyme activity (-2h) (%) in group the TE pre-treated (-2h) + radiation group compared to the only radiation group.

| Days | Percentage increase (%) SOD | Percentage increase (%) CAT | Percentage increase (%) GSH | Percentage decrease (%) LPO | Percentage increase (%) Total Protein |
|---|---|---|---|---|---|
| 1 D | 123.577 | 151.283 | 138.989 | 58.827 | 138.817 |
| 3 D | 122.134 | 167.178 | 139.398 | 58.746 | 104.638 |
| 7 D | 119.847 | 164.937 | 139.027 | 58.596 | 93.488 |
| 15 D | 120.463 | 151.899 | 139.421 | 58.610 | 130.994 |
| 30 D | Complete mortality in the Radiation group of mice | | | | |

The overall biochemical profile indicates that γ-radiation induces severe oxidative stress, as evidenced by suppression of enzymatic (SOD, CAT) and non-enzymatic (GSH) antioxidants, coupled with elevated LPO and protein degradation. Pre-treatment with TE markedly attenuated these changes, restoring SOD, CAT, and GSH activities, reducing LPO, and preserving protein content, suggesting that its phytochemical constituents act both as direct free radical scavengers and as modulators of endogenous antioxidant defense systems. Administration of TE alone did not induce any significant alterations in either antioxidant status or protein content, which clearly indicates its non-toxic and biologically safe nature under the experimental conditions**.**

The early recovery of SOD and CAT suggests that TE provides immediate enzymatic stabilization against ROS surge, while the sustained preservation of GSH and proteins indicates long-term protection against cumulative oxidative damage. The strong reduction in LPO emphasizes TE's role in safeguarding membrane integrity of gastrointestine (GI). Collectively, these data provide strong evidence that TE confers time-dependent radioprotection by mitigating oxidative injury and preserving biochemical homeostasis in intestinal tissue. The protective effect was most prominent during early post-irradiation intervals (1-15 days)**,** suggesting TE supports acute-phase antioxidant defense and cellular recovery.

## Discussion

Exposure to IR is well known to trigger excessive production of ROS, causing oxidative stress and cellular injury in radiosensitive organs- small intestine [26]. In this study, a single 10 Gy dose of whole-body γ-irradiation led to pronounced oxidative damage, as evidenced by elevated lipid peroxidation (MDA levels) and marked depletion of endogenous antioxidants, including SOD, CAT, and GSH [22-24]. These findings are consistent with earlier reports that radiation disrupts the balance between ROS generation and antioxidant defense, ultimately resulting in membrane damage, protein oxidation, and altered cellular metabolism [27, 28].

Pre-treatment with *Tylophora indica* hydro-methanolic leaf extract (TE) provided significant radioprotection. Animals receiving TE (-2h) prior to irradiation showed reduced lipid peroxidation and restoration of SOD, CAT, and GSH activity toward near-normal levels when compared with irradiated-only controls (Table 3). This protective effect can be attributed to bioactive phytoconstituents of TE—such as alkaloids, flavonoids, and phenolics—known for their free radical scavenging and antioxidant-enhancing properties [20]. Similar protective effects of plant-based extracts rich in phytochemicals have been documented, reinforcing the role of natural antioxidants in mitigating radiation-induced oxidative injury [29].

**Table 3:** Summary of biochemical alterations in intestinal tissue following γ-radiation exposure and TE administration

| Parameter | Control | TE (100 mg/kg b. wt) | Radiation (10 Gy) | TE (-2h) + Radiation (10 Gy) |
|---|---|---|---|---|
| SOD | Normal | No Changes | Marked decline 1-15 D | Significant recovery; max at 1 D |
| CAT | Normal | No Changes | Sharp decline at 1-15 D | Partial restoration; peak at 3 D |
| GSH | Normal | No Changes | Sustained depletion 1-15 D | Improved levels; ~39% recovery vs. Irradiated only |
| LPO | Baseline | No Changes | 2-3 fold rise; Persistent | Suppression by ~58% at 1-15 D |
| Total Protein | Normal | No Changes | progressive loss; minimum at 15 D; no survival by 30 D | Restored toward normal; survival observed up to 30 D |

Importantly, TE administration in non-irradiated animals did not significantly alter biochemical parameters, suggesting its non-toxic and biocompatible nature at the tested dose. The recovery pattern in TE-pre-treated groups, particularly evident at later intervals (15-30 days), indicates that TE not only provides acute antioxidant support but also contributes to long-term tissue repair. Such dual action is relevant in managing radiation-induced gastrointestinal syndrome, where both immediate oxidative damage and delayed tissue dysfunction contribute to morbidity and mortality.

The GI tract is inherently vulnerable to oxidative insults, as it is continuously exposed to endogenous and exogenous oxidants. Under normal physiological conditions, ROS are neutralized by intrinsic defense mechanisms. However, radiation and other stressors (e.g., metal toxicity, pollutants, etc.) overwhelm these systems, leading to enhanced ROS activity, lipid peroxidation, and cellular injury [30]. Among ROS, hydroxyl radicals, hydrogen radicals, and hydrogen peroxide are considered the primary mediators of radiation-induced cytotoxicity. Thus, the scavenging of these reactive intermediates forms the cornerstone of radioprotective strategies employed by synthetic, microbial such as SQGD, CBLB-502 and herbal agents [31, 32]. Among herbal radioprotectors, extracts of *Podophyllum hexandrum*, *Ocimum sanctum* (Tulsi), *Panax ginseng*, and *Withania somnifera* (Ashwagandha) etc. have demonstrated notable radioprotective potential primarily mediated through mechanisms such as ROS scavenging, DNA stabilization, and immunomodulation supports TE mediated radioprotective potential [33].

To counteract ROS, cells rely on an integrated antioxidant defense system comprising enzymes such as SOD, CAT, and GSH. In line with previous findings, our data demonstrate that TE pre-treatment significantly enhanced the activity of these

enzymes in irradiated mice, thereby reducing oxidative burden [34]. SOD catalyzes the dismutation of superoxide anions into hydrogen peroxide, which, if not cleared, can accumulate and propagate oxidative stress. Catalase then detoxifies hydrogen peroxide into water and oxygen, preventing its harmful accumulation [35]. Restoration of CAT, along with elevated GSH and SOD activity in TE-pre-treated animals, suggests that the extract effectively reinforced this defense cascade and minimized oxidative injury.

Taken together, the results indicate that hydro-methanolic leaf extract of *Tylophora indica* offers substantial protection against γ-radiation-induced oxidative damage in intestinal tissue. Its ability to attenuate lipid peroxidation, restore antioxidant enzyme activity, and support long-term recovery underscores its promise as a safe and effective natural radioprotector. Further investigations focusing on phytochemical characterization, molecular pathways, and pharmacokinetics are warranted to establish its translational potential and to compare its efficacy directly with currently available standard radioprotectors.

## Conclusion

The hydro-methanolic leaf extract of *Tylophora indica* (TE) showed significant radioprotective effects against γ-radiation-induced intestinal oxidative stress in mice. Whole-body exposure to 10 Gy elevated lipid peroxidation and depleted SOD, CAT, GSH, and total protein, whereas TE pre-treatment restored these parameters and preserved tissue integrity. TE was safe at the tested dose, with no adverse effects in un-irradiated animals. Its phytoconstituents, rich in alkaloids, flavonoids, and phenolics, likely mediate free radical scavenging and antioxidant enhancement. Thus, TE represents a promising natural radioprotector, warranting further mechanistic and comparative studies for translational application.

## Author Contribution Statement

Mr. Sunil Kumar Meena conducted the experiments, analyzed the results, and contributed to manuscript preparation. Mr. Ronit Parashar assisted in performing the experiments and contributed to manuscript preparation. Dr. Priyadarshi Meena and Dr. Dev Dutt Patel conceptualized and designed the experimental plan reviewed the results and critically revised the manuscript.

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## Ethical Declaration

This study was ethically approved from Institutional Animal Ethical Committee (IAEC), Department of Zoology, University of Rajasthan, Jaipur (Protocol approval no. UDZ/IAEC/IV/05 dated 18 December 2021) and management were carried out in compliance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA now known as CCSEA), Ministry of Environment, Government of India, India.

## Conflicts of Interest

The authors state no conflicts of interest.

## Approval

This study is part of the PhD thesis and approved by the Departmental Research Committee (DRC), Department of Zoology, University of Rajasthan, Jaipur.

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