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ORIGINAL ARTICLE

# Mitigation of Radiation-Induced Oxidative Stress by Methanolic Extract of *Tragia involucrata* in Swiss Albino Mice

**Nivya Machettira Thimmaiah, Chandrashekhar Gajanan Joshi, Rajashekhar K. Patil[1], Ajay S. Khandagale[2], H. M. Somashekarappa[3], D. Ananda, H. M. Manjunath**

Department of Biochemistry, Mangalore University, Jnana Kavery, Chikka Aluvara, Kodagu, [1]Department of Applied Zoology, Mangalore University, [3]CARRT, Mangalore University, Mangalagangothri, [2]Department of Biochemistry, College of Fisheries, KVAFSU, Mangalore, Karnataka, India

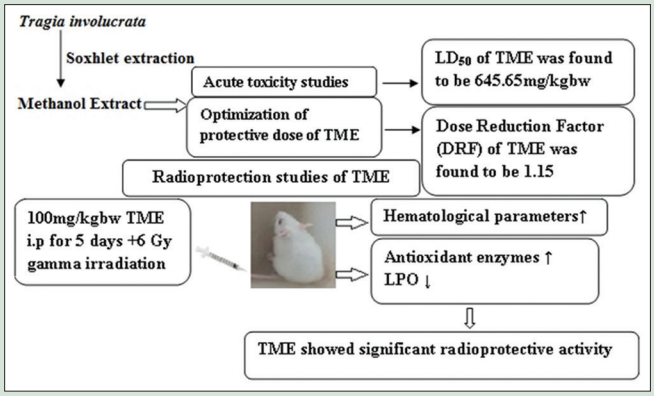
## ABSTRACT

**Background:** *Tragia involucrata* L. has been used in Indian traditional medicine since centuries to treat various ailments. The plant remains unexplored for its radioprotective properties and hence the present study. **Objective:** The main objective of the study is to ascertain the radioprotective effects of *T. involucrata* methanolic extract (TME) in Swiss albino mice against whole-body gamma radiation. **Materials and Methods:** The acute toxicity of TME was evaluated. The optimal protective dose of TME against radiation-induced mortality in mice was determined by survival analysis using the Kaplan–Meier curves. To understand the mechanism of radioprotection, TME was tested for hematological changes and antioxidant levels in mice by injecting100 mg/kg body weight intraperitoneally for 5 days and irradiated with a sublethal dose of 6 Gy gamma radiation. **Results:** The lethal dose 50%/of TME was about 645.65 mg/kgbw. The pretreatment of mice with 100 mg/kgbw of TME increased its survivability to 30% compared with the radiation control group and hence is considered as a radioprotective dose. The lethal dose 50%/30% of the irradiation alone group was found to be 9 Gy and 10.35 Gy for the TME + irradiation group. Hence, the dose reduction factor was about 1.15. The radiation decreased the hematological parameters in the blood, but on pretreatment with TME (100 mg/kgbw), increase in levels was noted. The antioxidant enzyme levels in the mice liver homogenate were found to be replenished in the test group compared with the radiation control. **Conclusion:** The present study indicates the protective role of TME against gamma radiation-induced mortality and oxidative stress. Hence, it is proposed as a candidate for radioprotection.

**Key words:** Antioxidant enzymes, ionizing radiation, methanolic extract, radioprotection, *Tragia involucrate*

## SUMMARY

- Radiotherapy, being one of the treatment modalities, is associated with side effects. It is evident that most research focuses on the use of natural products derived from plants as radioprotectors. Hence, the present study focused on to the use of *Tragia involucrata* against the damaging effects of radiation
- The administration of the methanolic extract of *T. involucrata* in mice offered protection against gamma radiation
- The decrease in the hematological parameters and antioxidant enzyme levels were found to be replenished by the TME.




**Abbreviations Used:** TME: *T. involucrate* methanolic extract; IAEC: Institutional Animal Ethical Committee; OECD: Organization for Economic Co-operation and Development; MDA: Malondialdehyde; GSH: Glutathione; SOD: Superoxide dismutase; GST: Glutathione S transferase; $LD_{50/30}$: Lethal dose 50%/30%.

**Correspondence:**
Dr. Chandrashekhar Gajanan Joshi,
Department of Biochemistry, Mangalore University, Jnana Kavery, Chikka Aluvara, Thorenur Post, Kushalnagar Hobli, Somwarpet, Kodagu - 571 232, Karnataka, India.
E-mail: josheejoshee@gmail.com

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## INTRODUCTION

Ionizing radiation finds applications in many fields of medical sciences and enhances the efficacy of diagnostic and therapeutic uses.[1] Radiotherapy has been used as a treatment modality for cancer patients during their course of illness.[2] Irradiation has associated side effects, as it induces damage to normal tissue resulting in acute and chronic toxicities or severe organ dysfunction.[3] Hence, the need arises to protect human beings from the effects of radiation even in a planned or unplanned accidental exposure.[4] The recent research focuses on the use of natural products derived from plants in offering protection against the harmful effects of radiation.[5,6]

Radioprotectors are compounds intended to minimize damage in normal tissues caused by radiation. Many times, a compound with high antioxidant property can serve as radioprotector and must be present before or at the time of radiation.[7] As reported by Painuli and Kumar,[6] radioprotectors make sure the increase of non-protein sulfhydryl groups, reduce lipid peroxidation (LPO) and increase free-radical scavenging activity through transcription, and increase the regulation of antioxidant enzymes such as glutathione (GSH) transferase, catalase (CAT), superoxide dismutase (SOD), and GSH peroxidases. The damage induced by radiation can also be neutralized by escalation in DNA repair activity. Radioprotection is also offered by the inactivation



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of protein kinase (PK)-C, nitric oxide, and mitogen-activated PK, and downregulation of several other effectors responsible for molecular damage. The sulfhydryl group containing amino acid cysteine was used *in vivo* for exploring a potent radioprotector. Amifostine, synthesized by the Walter Reed Army Research Institute, approved by the Food and Drug Administration, is currently used as radioprotector in radiation therapy.[8] Due to associated side effects such as cephalalgia, nausea, sickness, vomiting, etc., and cost,[9] there is an urgent need for a radioprotector of plant origin.

Natural products isolated from plants have proven to be safe and cost-effective with least toxic effects. These plant products can be administered either before or after irradiation to offer protection to biological macromolecules and act as natural radioprotectors. Flavonoids are one such compound discovered in the early 1920s and are known to offer resistance to radiation, reduce inflammation, and delay the aging process.[10] *Tragia involucrata* L., an evergreen twiner of order malpighiales, belongs to the Euphorbiaceae family. It is the herb with scattered stinging hairs, can be found in the wastelands, and is distributed in dry places throughout India. The various parts of *T. involucrata* have been studied for their anti-inflammatory,[11,12] hepatoprotective, antioxidant,[13] antifertility,[14] cytotoxic,[15] antitumor,[16] antidiabetic,[17] antibacterial,[18,19] antimicrobial, and antiepileptic activities.[20] The plant contains flavonoids and many other bioactive molecules such as phenolics, and tannins.[21,22] Even though *T. involucrata* has been extensively studied with respect to its various medicinal properties, until date, there are no reports about the radioprotective property of this plant. Hence, the present study was carried out to evaluate the protective effects of *T. involucrata* methanolic extract (TME) against gamma-irradiated mice.

## MATERIALS AND METHODS

### Identification of plant material and preparation of *Tragia involucrata* methanolic extract

*T. involucrata* L., used for the study, was previously identified, and the sample specimen was deposited in the National Institute of Science Communication and Information Resource (NISCAIR) herbarium repository (NISCAIR/RHMD/Consult/2015/2927-120) dated 09/12/2015, Code Lab Reserve R07107, New Delhi. The TME was prepared using the Soxhlet extraction method, after sequential extraction with hexane, dichloromethane, and ethyl acetate. The extract thus obtained was concentrated using a rotary flash evaporator, evaporated to dryness, and stored at −4°C for further use. The TME was dissolved in 0.1% dimethyl sulfoxide (DMSO) and used for further studies.

### Ethical clearance

The Institutional Animal Ethical committee approved the study vide Cert. No. MU/AZ/504 (A)/IEAC/2015-16 dated 23/09/2015.

### Animals

In the present study, 6–8-week-old adult Swiss albino mice of either sex, weighing 25–28 g were used. The animals were housed in standard mice cages in a room with 12 h of light and dark cycle at 22°C ± 3°C with humidity of 50%–60%. The mice were given standard mouse chow and water *ad libitum*. The mice used for the experiment were allowed to adapt to the laboratory environment for a week before the start of the study. Animal care and handling was done according to the guidelines set by the Indian National Science Academy, New Delhi, and the World Health Organization.

### Gamma irradiation

The irradiation work was carried out at the Centre for Application of Radioisotopes and Radiation Technology (CARRT), Mangalore University, using the $^{60}$Co irradiator at dose rates of 9.709–9.772 Gy/min.

### Acute toxicity study

The acute toxicity of TME was determined as per the Organization for Economic Co-operation and Development 423 guidelines. The TME was administered intraperitoneally (i.p.) as described by Miller and Tainter.[23] The experimental setup comprised three female mice per step. The animals were randomly allocated into two groups: control group and treated group. The mice were weighed and the dose was calculated with reference to their respective body weight. A high dose of 2000 mg/kgbw of TME, followed by a series of doses (300, 500, and 1000 mg/kgbw) was administered IP. The dose volume was never >1 ml/100 gbw. The control groups received 0.1% DMSO. The animals were observed for signs of toxicity for the first 4 h, and daily for 14 days, up to 30 days. The survivors were weighed daily and monitored for mortality and radiation-induced sickness.

### Optimization of *Tragia involucrata* methanolic extract

Female mice were used for the optimization of TME. The mice were segregated into three major groups, including a test group divided into four subgroups consisting of 10 animals each, in order to determine the lethal dose 50% ($LD_{50}$) of radiation as well as TME. The animals were pretreated with different concentration (10, 50, 100, and150 mg/kgbw) of TME (i.p.) for 5 days, and on the 5$^{th}$ day, 1 h after the administration of the last dose, they were exposed to a high dose (10 Gy) of gamma radiation. The mice were monitored for 30 days for the development of radiation sickness or mortality, if any. A survivability curve was then constructed using the Kaplan–Meier method. The experimental groups ($n$ = 10) were as follows:

- Group I: Control – without any treatment
- Group II: Radiation control – animals were treated with 0.1%DMSO i.p. for 5 days + 10 Gy gamma radiation and
- Group III: Test Group – animals received 10, 50, 100, and 150 mg/kgbw TME i.p. for 5 days + 10 Gy gamma radiation.

A vehicle control (DMSO, 0.1%) along with the experimental groups was maintained. The animals were regularly monitored for 30 days for the development of radiation sickness such as epilation, irritability, weight loss, reduction in food and water intake, weight loss, lethargy, behavioral toxicity, and mortality. The results obtained after a 30-day survival study determines the optimum concentration of the TME to be used for further investigations.

### Determination of dose reduction factor

To ascertain the protective role of TME against a lethal dose of gamma radiation, the dose reduction factor (DRF) was calculated. The experiment was designed by dividing the animals into the following three major groups of six animals each.

- Group I: Control – without any treatment
- Group II: Radiation control – animals were treated with 0.1% DMSO i.p. for 5 days, and on the 5$^{th}$ day exposed to 7, 9, and 11 Gy gamma radiation and
- Group III: Test group – animals were pretreated with 100 mg/kgbw TME i.p. for 5 days, and on the 5$^{th}$ day, were exposed to 7, 9, and 11 Gy gamma radiation.

The animals were observed daily for up to 30 days for any sickness and mortality due to radiation. The DRF was calculated as per the method of Miller and Tainter:[23]

$$DRF = \frac{\text{Lethal dose } 50\%/30\%\left(LD_{50/30}\right) \text{ of Drug}\left(TME\right) + \text{Irradiation group}}{LD_{50/30}\text{of Control} + \text{Irradiation group}}$$

## Radioprotection studies

To gain insight into the mechanism of radioprotection, the male mice ($n$ = 6) were treated with optimum protective dose of TME (100 mg/kgbw) and exposed to a sub-lethal dose of radiation (6 Gy). The experiment was conducted with the following 5 groups of 6 animals each:

- Group I – Normal control-untreated
- Group II – Radiation control-animals received 0.1% DMSO i.p. for 5 days, and on the 5$^{th}$ day, irradiated with 6 Gy gamma radiation
- Group III – TME control-animals received 100 mg/kgbw TME i.p. for 5 days
- Group IV – Test Group-animals received 100 mg/kgbw TME i.p. for 5 days and on the 5$^{th}$ day, irradiated with 6 Gy gamma radiation.

## Animal dissection

At the end of the experimental study, on day 6, all animals were sacrificed by cervical dislocation (within 24 h of irradiation). The hematological estimations were performed with whole blood obtained by cardiac puncture. The liver was excised and homogenized and used for antioxidant studies.

## Blood hematology

The whole blood was collected in tubes containing 2% ethylenediaminetetraacetic acid. The hematological studies of the whole blood were then done using the Erma Veterinary blood cell counter (PCE-210VET).

## Liver homogenization

The liver was washed and perfused with ice-cold phosphate-buffered saline (PBS). About 10% homogenate was prepared in ice-cold PBS (pH 7.4) using a homogenizer (Remi, RQT-127A). The homogenized samples were then centrifuged for 20 min at 10,000 rpm and 4°C in cooling centrifuge (Remi, C-24BL), and the supernatants were used for biochemical estimations. The total protein in the liver homogenate was determined by Lowry's method.[24]

## Antioxidant studies

### *Estimation of catalase*

The CAT activity in the liver homogenate was estimated as per the protocol of Aebi.[25] The reaction mixture consisted of 10 µl liver homogenate and 3 ml of 60 mM hydrogen peroxide. About 3 ml of distilled water served as blank and 3 ml of 60 mM hydrogen peroxide as the reagent blank. A sample blank was maintained with 3 ml distilled water and 10 µl of the sample. The kinetic measurement was taken at 240 nm with an interval of 15 s for 2 min. A decrease in absorbance was noted. The CAT activity was expressed in U/mg protein.

### *Estimation of lipid peroxidation*

The formation of malondialdehyde (MDA) was estimated as devised by Ohkawa *et al.*[26] In brief, the liver homogenate was mixed with trichloroacetic acid (TCA)-thiobarbituric acid reagent. The reaction mixture was then heated for 15 min in a boiling water bath, cooled, and centrifuged at 10,000 rpm for 5 min. The supernatant was taken, and the absorbance of the pink color was read at 535 nm. The concentration of MDA in the sample was obtained by plotting the absorbance against the standard graph. The results were expressed as µg/mg protein.

### *Glutathione*

The GSH levels were measured as total non-protein sulfhydryl groups.[27] As per the protocol, the proteins in the liver homogenate was precipitated with 25% TCA. The supernatants were collected and mixed with 0.2 M sodium phosphate buffer (pH 8) and 0.06 mM 5,5-dithiobis-2-nitrobenzoic acid and incubated at room temperature for 10 min. The absorbance of the sample was read against the blank at 412 nm in the ultraviolet (UV)-visible spectrophotometer (Shimadzu UV-260).The concentration of GSH was calculated from the standard graph and multiplied with the respective dilution factors. The total GSH in the sample was expressed as µg/ml.

### *Total antioxidant capacity*

The method devised by Prieto *et al.*[28] was used to determine the total antioxidant capacity (TAC) in the sample. The liver homogenate was treated with 5% TCA to precipitate the proteins in the sample. The mixture was allowed to stand for 5 min and centrifuged at 3000 rpm for 10 min. The supernatant obtained was mixed with 1 ml of total TAC reagent (0.6 M sulfuric acid, 28 mM sodium dihydrogen orthophosphate, and 4 mM ammonium heptamolybdate) and incubated in a water bath at 90°C for 90 min. The reaction mixture was cooled and the optical density of the greenish-to-bluish color formed was read at 695 nm against the blank. The concentration of the total antioxidants in the sample was obtained by plotting the absorbance of the sample against the standard graph. The total TAC was expressed as µg/ml.

### *Superoxide dismutase*

The estimation of the SOD enzyme was carried out by the method of Beauchamp and Fridovich.[29] The assay mixture of potassium phosphate buffer (50 mM, pH 7.8) contained 45 µM methionine, 84 µM nitro blue tetrazolium (NBT), 5.3 mM riboflavin, and 100 µl of liver homogenate. For each sample analyzed, a corresponding control was maintained without the NBT. A common standard and a blank were kept for each set of the illumination. The samples were subjected to illumination for 10 min in an illumination chamber lined with an aluminum foil fitted with a 15 W fluorescent lamp. Following illumination, the optical density of all the reaction mixtures was immediately read at 560 nm. The units of enzyme present in the sample were calculated and expressed as U/mg protein.

### *Glutathione S-transferase*

The glutathione-S-transferase (GST) activity was measured using 1-chloro-2, 4-dinitrobenzene as the substrate.[30] About 20 µl of the sample was mixed with 0.1 M phosphate buffer (pH 6.5), 20 mM reduced GSH, and 20 mM1-chloro-2, 4 dinitrochlorobenzene (CDNB). The solution was taken in a cuvette and the increase in absorbance was measured for 5 min at 340 nm. The activity of GST was expressed as U/mg protein, i.e., µmol of GSH-CDNB conjugate formed/min/mg protein.

## Statistical analysis

The results obtained were expressed as a mean ± standard deviation. The comparisons between the control and treated groups were done by one-way analysis of variance, followed by Tukey's test using Prism 3.0 (Graphpad Software Inc., San Diego, CA) and SPSS 16 (SPSS Inc., 2000, Chicago, IBM SPSS software) software. $P < 0.05$ was considered statistically significant.

## RESULTS

### Acute toxicity study

The acute toxicity of TME was determined over a 30-day observation period. The intraperitoneal administration of TME did not produce any radiation sickness or mortality until 500 mg/kgbw up to 29 days. However, one animal out of three was found dead at the end of the 30th day. The mortality of mice was observed after 48 h upon administration of TME 1000 mg/kgbw. About 2000 mg/kgbw of TME was found be toxic to the animals as mortality (100%) was observed within 24 h. The $LD_{50}$ of TME was calculated using probit analysis [Figure 1] and was found to be 645.65 mg/kgbw.

### Determination of optimum protective dose against radiation

Within 2 days, animals irradiated with 10 Gy gamma radiation showed radiation-induced sickness such as weight loss, lethargy, and reduction in food and water intake. The administration of 100 mg/kgbw TME increased the 30-day survival of mice exposed to 10 Gy gamma radiation by 30%, indicating protection against radiation-induced toxic effects [Figure 2]. Hence, a dose of 100 mg/kgbw was considered as optimum dose for radioprotection, and further studies were carried out using this dose.

### Determination of dose reduction factor

The DRF was determined in mice pretreated with 100 mg/kgbw and exposed to 7–11 Gy gamma radiation. A dose-dependent increase in survival was observed in mice pretreated with TME compared with the radiation control [Figure 3]. The lethal dose 50%/30% ($LD_{50/30}$) was found to be 9 Gy for radiation alone. The value was increased by 1.35 Gy with the $LD_{50/30}$ of 10.35 upon TME pretreatment, indicating a DRF of 1.15.

### Hematological parameters of whole blood sample

The hematology results of whole blood sample are represented in Table 1 along with their mean and standard deviation. Gamma irradiated groups showed a decrease in white blood cell (WBC) ($P < 0.001$), red blood cell (RBC) ($P < 0.001$), hemoglobin (Hb) ($P < 0.001$), percent lymphocytes (LYs) ($P < 0.001$), monocytes (MOs) ($P > 0.05$), platelet (PLT) ($P < 0.001$), and hematocrit (HCT) ($P < 0.001$) values, when compared with the normal control (untreated). However, there was an increase in the granulocytes percent ($P < 0.001$). Animals pretreated with 100 mg/kgbw TME and exposed to a sublethal dose of 6 Gy gamma radiation showed significant increase in RBC count compared with the radiation control ($P < 0.001$). There was an increase in WBC ($P < 0.001$), Hb ($P < 0.001$), percent LYs ($P < 0.01$), PLT ($P < 0.001$), and HCT ($P < 0.001$) values compared to radiation control. However, a decrease in the percent MOs ($P > 0.05$) and granulocytes ($P < 0.001$) were noted in comparison with radiation control. Test group (TME + irradiation) when compared to its respective control (Tragia methanolic extract control) showed a significant decrease ($P < 0.001$) in all the parameters except a nonsignificant increase in MO percent ($P < 0.05$) and a significant increase in granulocytes ($P < 0.001$).

### Effect of preadministration of *Tragia involucrata* methanolic extract on antioxidant status of mice liver

The antioxidant study of mice liver homogenate pretreated with 100 mg/kgbw TME and irradiated with 6 Gy gamma radiation resulted in statistically significant and nonsignificant values between the experimental groups.

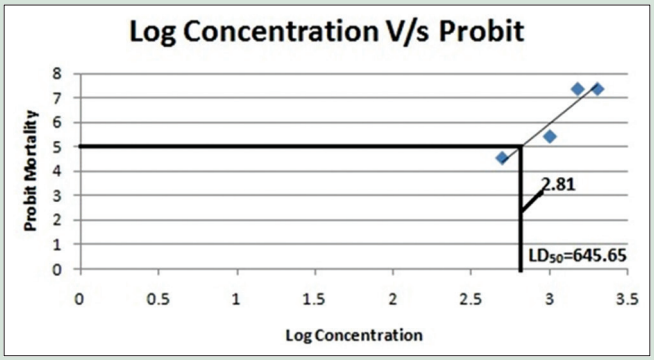


**Figure 1:** Probit analysis for the determination of lethal dose 50% of *Tragia involucrata* methanolic extract. (Lethal dose 50%) $m = 2.81$; lethal dose 50% = Log 10 of 2.81 = 645.65

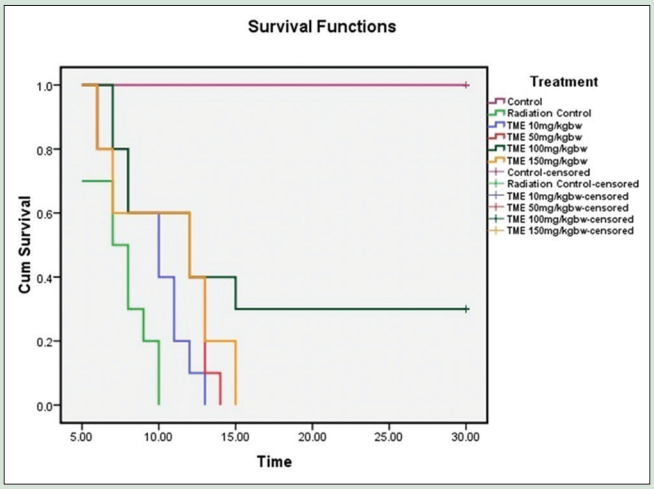


**Figure 2:** Kaplan–Meier survival curve of mice treated with different doses of *Tragia involucrata* methanolic extract (10–150 mg/kgbw) intraperitoneally for 5 days and irradiated with 10 Gy gamma radiation 1 h after the last dose on the 5th day. Values expressed as mean ± standard deviation

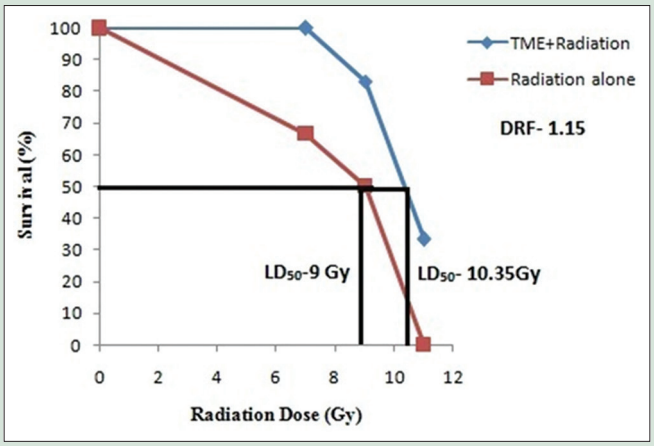


**Figure 3:** Dose–response curve of mice showing 30 days survival pretreated with or without *Tragia involucrata* methanolic extract (100mg/kgbw) exposed to whole-body gamma radiation (7–11 Gy). Lethal dose $_{50\%/30\%}$ of radiation alone was 9 Gy and of treatment group (*Tragia involucrata* methanolic extract + Radiation) was 10.35 Gy. Dose reduction factor was 1.15. Values expressed as mean ± standard deviation

## Catalase activity

The CAT activity in liver homogenate was significantly depleted in the radiation control group compared to the normal control ($P < 0.001$). However, the CAT levels were restored in the test group, pretreated with TME compared with the radiation control group ($P < 0.001$) being statistically significant. There was a significant increase in activity of the TME control, compared with the test group ($P < 0.001$). The results are depicted in Figure 4a.

## Lipid peroxidation

The irradiation of mice with 6 Gy gamma radiation resulted in increase in MDA level, indicating an increase in LPO compared to the normal control ($P < 0.001$). A significant decrease in MDA levels was observed in the test group, TME control, and the untreated group [Figure 4b] compared with the radiation controls ($P < 0.001$).

## Glutathione

A significant decrease in the reduced GSH levels was observed in the radiation control groups compared with the other experimental groups. The TME treatment prior to irradiation restored the reduced GSH levels to normal with the $P < 0.001$, as indicated in Figure 4c.

## Total antioxidant capacity

The total TAC of the liver homogenate was found to be statistically significant within the groups ($P < 0.001$). There was an increase in the TAC in the test group compared to radiation control ($P < 0.001$), whereas the test group differed nonsignificantly compared to the TME control ($P > 0.05$). Hence, the irradiated group which showed a decrease in value was restored to normal upon TME pretreatment [Figure 5a].

## Superoxide dismutase

The results of the SOD are represented in Figure 5b. The test group under study showed significant increase in SOD levels in comparison with the radiation control ($P < 0.01$), whereas the $P < 0.05$ in the test group compared with the TME control. However, irradiation significantly ($P < 0.001$) decreased the SOD levels compared to normal control.

**Table 1:** Hematological parameters of Swiss albino mice after a pretreatment with *Tragia involucrata* methanolic extract intraperitoneally (100 mg/kgbw) and exposed to 6 Gy gamma radiation

| Groups | RBC (×10⁶/µl) | WBC (×10³/µl) | Hb (g/dl) | LY (%) | MO (%) | GR (%) | PLT (×10³/µl) | HCT (%) |
|---|---|---|---|---|---|---|---|---|
| NC | 10.75±0.11 | 11.23±0.61 | 14.07±0.15 | 78.87±0.01 | 8.70±0.00 | 12.77±0.00 | 803±0.58 | 63.2±0.03 |
| RC | 2.62±0.50*** | 2.83±0.76*** | 7.27±0.25*** | 67.57±0.01*** | 8.27±0.00$^{NS}$ | 24.17±0.00*** | 224±0.60*** | 25.1±0.02*** |
| TMEC | 9.95±0.73 | 10.8±0.78 | 13.3±0.30 | 80.93±0.01 | 7.37±0.00 | 11.70±0.00 | 706±0.60 | 48.8±0.01 |
| TG | 7.7±0.08*** | 9.6±0.53*** | 10.13±0.15*** | 71.20±0.01*** | 8.13±0.00$^{NS}$ | 20.67±0.00*** | 695±0.57*** | 37.8±0.02*** |

Data expressed as mean±SD, $n$=6. Statistical comparisons are made with their respective controls. ***$P$<0.001. RBC: Red blood cells; WBC: White blood cells; Hb: Hemoglobin; LY: Lymphocyte; MO: Monocyte; GR: Granulocyte; PLT: Platelet; HCT: Hematocrit; NC: Normal control; RC: Radiation control; TMEC: *Tragia* methanolic extract control; TME: *Tragia involucrata* methanolic extract; TG: Test group (TME + radiation); NS: Nonsignificant; SD: Standard deviation

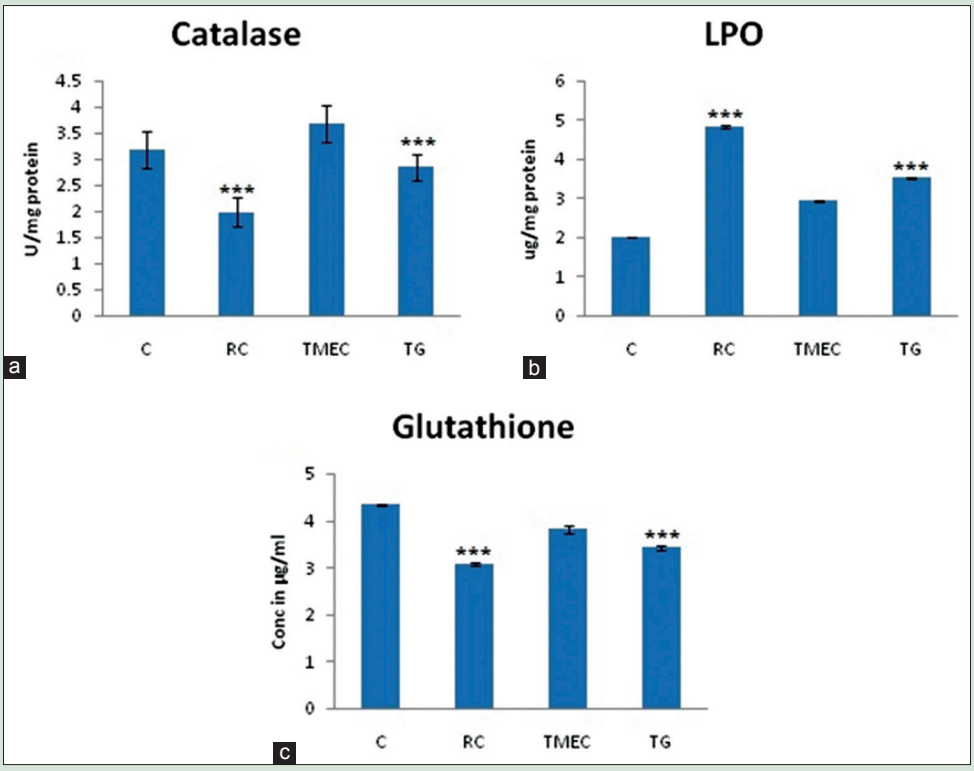


**Figure 4:** Changes in the activities of catalase (a), lipid peroxidation (b) and glutathione (c) levels in mice liver homogenate after an exposure to 6 Gy gamma radiation with or without *Tragia involucrata* methanolic extract (100 mg/kgbw) pretreated intraperitoneally for 5 days. Values expressed as mean ± standard deviation; $n = 6$. (NC: Normal control; RC: Radiation control; TMEC: Tragia methanolic extract control; TG: Test group [TME + Radiation]) Statistical comparisons are made with their respective controls. (***$P < 0.001$)

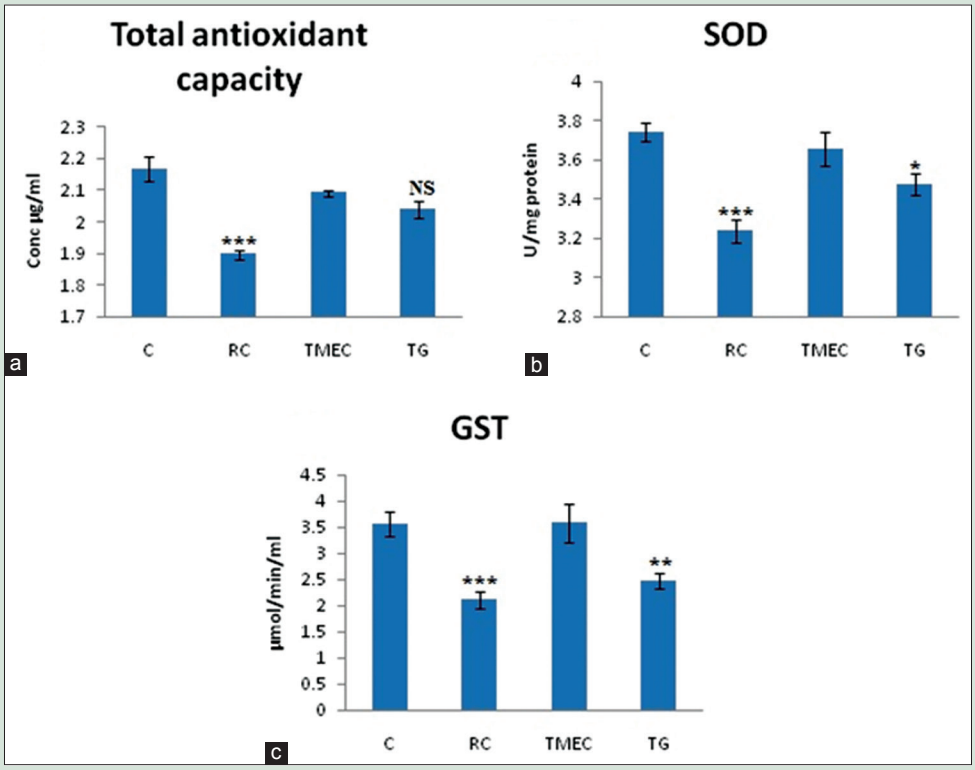


**Figure 5:** Changes in the activities of total antioxidant capacity (a), superoxidedismutase (b) and glutathione s transferase (c) activities in mice liver homogenate after an exposure to 6 Gy gamma radiation with or without *Tragia involucrata* methanolic extract (100mg/kgbw) pretreated intraperitoneally for 5 days. Values expressed as mean ± standard deviation; $n = 6$. (NC: Normal control; RC: Radiation control; TMEC: Tragia methanolic extract Control; TG: Test group [TME + Radiation]). Statistical comparisons are made with their respective controls. (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ and NS: Nonsignificant)

### Glutathione S-transferase

The GST activity in the liver homogenate is depicted in Figure 5c. The level of GST decreased in the radiation control group when compared to normal control ($P < 0.001$). Upon pretreatment with TME, the enzyme levels were normalized. However, the results differed statistically ($P > 0.05$) compared to the radiation control. Similarly, the GST levels in the TME control were statistically increased be compared with the test group ($P < 0.01$).

## DISCUSSION

The current study uncovered the effects of TME pretreatment on mice irradiated with a lethal dose of radiation. The first phase of the study was to determine the acute toxicity of TME. The study revealed the nontoxic effect of TME up to 500 mg/kgbw with a calculated $LD_{50}$ of 645.65 mg/kgbw by probit and regression analysis. At doses of 1000 mg/kgbw, some degree of mortality (66.67%) was observed. This could be due to a higher amount of phenolics and flavonoids present in the TME.[21,22] In the second phase of the study, the optimization of the TME dose was carried out against the lethal dose (10 Gy) of gamma radiation. In general, mortality of animals observed within the initial 10–15 days is primarily due to gastrointestinal syndrome with diarrhea and apparent physical radiation sickness.[31] Further deaths observed after 15$^{th}$ day and up to 30 days are essentially due to hematopoietic syndrome and other secondary infections causing damage to the intestinal epithelium.[32] The administration of TME (100 mg/kgbw i.p. for 5 days) prior irradiation substantially reduced the mortality and prolonged the 30-day survival of mice with an increase in survival rate by 30%. This can be attributed to an overall protection offered by TME at the biochemical, antioxidant, and cytogenetic level. A similar protective effect of *Emblica officinalis* fruit pulp extract was reported at 100 mg/kgbw against sublethal doses of radiation.[33]

Irradiation of mice at different doses of gamma radiation (7, 9, and 11 Gy) resulted in a dose-dependent decrease in the survival rate. The TME (100 mg/kgbw) treatment before the gamma radiation showed an increase in survival of 100%, 83.33%, and 33.33%, respectively. Earlier studies on pretreatment before irradiation of plant extracts of *Nigella sativa*,[34] *Aegle marmelos*,[32] *Zingiber officinale*,[35] and *Syzygium cumini*[36] reported a comparable increase in the survival of mice.

The DRF is the measure of effectiveness of the compound or drug which indicates the ability of a radioprotectant to enhance the tolerance of tissues in order to decrease radiation sickness and mortality.[37] In the present investigation, the $LD_{50/30}$ of radiation on mice was found to be 9 Gy. The pretreatment of TME increased the value to 10.35 Gy with DRF of 1.15. Similar results were obtained with the studies on *Z. officinale*[35] hydroalcoholic extracts, *Pilea microphylla*,[38] and *A. marmelos*.[32] However, the DRF values of different plant extracts varied, *E. officinalis*,[33] *N. sativa,*[34] and *Ageratum conyzoides*[39] with a DRF of 1.9, 1.2, and 1.3, respectively.

The third phase of the study involved irradiating the mice with a sublethal dose (6 Gy) gamma radiation with prior treatment of TME. This was followed by assessment of radiation damage using hematological and antioxidant parameters. Radiation majorly caused a considerable decrease in many of the hematological parameters such as RBC, WBC, Hb, LY, MO, PLT, and HCT levels. At sublethal doses, radiation causes formation of free radicals which initiate a chain of events leading to cytotoxicity and implicated for the decline in the levels of hematological parameters.[40] There is an apparent elevation in the levels of granulocytes in the radiation as well as the TME-treated groups. This could be a

counter mechanism to the depleting LYs which are more sensitive to radiation. The TME in the present study offered a significant protection to the hematological system. This can be observed with a normalized level of RBCs and Hb in the pretreated group. As WBC's are sensitive to radiation, TME might have maintained its levels by reducing the intracellular effects of radiation. Too stabilize the cell number, some amount of TME must be present in circulation which can prevent it from radiation-mediated cytotoxicity. Equivalent results were obtained in the aqueous extracts of *Mentha piperita*.[41]

Numerous antioxidants are known to protect the cells from reactive oxygen and nitrogen species by free radical scavenging activity in the cellular milieu.[42] Endogenous antioxidant enzymes such as CAT, GSH, SOD, and GST provide the first line of defense against any radiation effects. The homeostasis of these enzymes in the cells is crucial for maximum radioprotection.[41] Mice irradiated with a sublethal dose of 6 Gy depleted antioxidant enzyme levels in the liver homogenate observed with decreasing TAC, CAT, reduced GSH, SOD, and GST activity. These intracellular antioxidants play a critical role in scavenging the free radicals.[4] The presence of polyphenols and flavonoids in the methanolic extracts of *T. involucrata* might be responsible for maintaining a balanced antioxidant enzyme levels. Previously, *Phyllanthus amarus* has been reported to restore the depleted levels of enzymes to normal.[43] The current study of TME at 100 mg/kgbw ameliorated the gamma radiation-induced morbidity and mortality in mice. The mechanism of radioprotection may be likely due to the antioxidants, especially the polyphenols present in them scavenging the free radicals. The structure of the bioactive compound in TM, which offers a possible protection, needs to be explored and identified.

## CONCLUSION

The present study provides valuable data regarding acute toxicity profile and the radioprotective effects of TME against radiation-induced mortality and biochemical changes. The intraperitoneal administration of TME protected the mice against hematological alterations and change in antioxidant enzyme levels induced by radiation. The TME administration thereby increased radiation tolerance. TME hence acts as an antioxidant. The present study suggests the use of TME as an effective radioprotective agent. So far as it is known, this is the first study on the radioprotective potential of TME. Further studies at the molecular level will unravel the mechanism of radioprotection by TME.

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### Conflicts of interest

There are no conflicts of interest.

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