# Radioprotective and antioxidant potential of Tanacetum parthenium extract and synthetic parthenolide in Swiss albino mice exposed to electron beam irradiation

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### Original article

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Revised: March 2020 Accepted: April 2020

Int. J. Radiat. Res., January 2021; 19(1): 145-154

**DOI:** 10.29252/ijrr.19.1.145

## ABSTRACT

Background: The herb feverfew (Tanacetum parthenium L., Asteraceae) has an ancient reputation as an effective anti-inflammatory, analgesic, antipyretic, and anti-asthmatic agent. Parthenolide a gemacranolide-type sesquiterpene lactone is the major constituent of European feverfew. Materials and Methods: The present study was intended to evaluate the in vivo antioxidant potential and radioprotective ability of Tanacetum parthenium leaf extract and synthetic Parthenolide. Male mice were orally administered with Tanacetum parthenium leaf extract and synthetic Parthenolide for 15 days followed by electron beam irradiation exposure. Survival studies in mice exposed to a lethal dose of 10Gy. At 6.0 Gy radioprotective ability was performed in order to find the nature of the compound. Results: The mice liver supernatant was used to measure total antioxidant capacity(TAC), glutathione (GSH) content along with various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) after electron irradiation exposure at 6.0 Gy. Pretreatment with Tanacetum parthenium extract and synthetic Parthenolide, prior to electron beam irradiation resulted in the increased survival rate of the animals as compared to the irradiated group. The treatment groups followed by electron beam irradiation at 6 Gy were significantly higher than the controlled group and the irradiated group, showing immunomodulatory nature. Pre-treatment and post-treatment with Tanacetum parthenium extract and synthetic Parthenolide, also significantly enhanced the activity of antioxidant enzymes and improved hematological parameters. Conclusion: The present study suggests that supplementation with leaf extract of Tanacetum parthenium and synthetic compound parthenolide has potent antioxidant activity and act as a probable radioprotector against electron beam radiation-induced oxidative damage.

*Keywords: Radioprotection, irradiation, antioxidant enzymes, Tanacetum parthenium, synthetic parthenolide.* 

### **INTRODUCTION**

Radiation for the use of therapeutics, scientific research, and energy production among others always raises the problem of radiation hazards to living beings. Ionizing radiation generates various reactive oxygen species in a biological system, by the radiolysis of water, which can damage several cellular components and biomolecules. The major free radicals formed upon aqueous radiolysis are hydroxyl radical ( $OH^\circ$ ), superoxide radical ( $O_2^\circ$ ), hydroperoxy radical  $(HO_2)$ , among others <sup>(1)</sup>. The reactive oxygen species (ROS) cause several oxidative changes in various biomolecules such as lipids, proteins, carbohydrates as well as nucleic acids <sup>(2)</sup>.

Free radicals and reactive oxygen species (ROS) are generated using various endogenous systems or from external sources such as exposure to different physiochemical components. Ionizing radiation damage to the cell can be caused by the direct or indirect effects of radiotherapy processes (3). Hence, there is a great need to protect humans against the deleterious effects of ionizing radiation by pharmacological intervention. The search for new radioprotectors to combat irradiation have often been targeted towards medicinal plants as they contain an abundance of potentially active secondary metabolites with good antioxidant activity.

Moreover, natural radioprotective agents, cysteine, cysteamine, 5such as hydroxytryptamine. hydroxytryptophan, 5 glutathione, and vitamins like A, C, and E, have been extensively studied <sup>(4)</sup>. In addition to the radioprotective agents, a few important synthetic molecules have also been used. However, the inherent toxicity of these agents at the radioprotective concentration warrants further search for new safer and effective radioprotector agents with a new mode of action.

There is an intensive search for а radioprotective drug that meets all the prerequisites of an ideal radioprotector that produces no cumulative or irreversible toxicity, provides effective long-term protection, remains stable for a number of years without losing shelf life, and can be easily administered <sup>(5)</sup>. Herbal formulations which are non-toxic and inexpensive have been evaluated for their radioprotective efficacy <sup>(6)</sup>. WR-2721, also known as amifostine a phosphorothioate is one of the best-known radioprotectors which has undergone extensive preclinical testing and clinical trials for its radioprotective and chemoprotective effects (7). Oxidative stress contributes to normal tissue damage during tumor therapy with irradiation.

One of the major reasons for cellular injury after radiation exposure is the generation of reactive oxygen species (ROS) <sup>(8)</sup>. Radiation attenuates the endogenous antioxidant enzymes, which are considered as the first-line defense mechanism in the maintenance of redox balance and normal biochemical processes <sup>(9)</sup>. The development of radioprotective agents has focused primarily on cytoprotection from relatively higher doses of therapeutic radiation and nuclear disasters. Epidemiological studies and radiobiological models report the potential for stochastic effects from relatively low-dose radiation exposure. When targeting multiple mechanisms an enhanced efficacy is achieved for protection which include free radical scavenging, caloric restriction, non-steroidal anti -inflammatory agents, humoral factors, and an oxidative agent (10).

The data presented provides the scientific foundation for the future development of a radioprotectant that may reduce the risk of carcinogenesis from low-dose exposure hence we have studied on the herb feverfew (Tanacetum parthenium L., Asteraceae) has an ancient reputation as an effective antiinflammatory, analgesic, antipyretic, and antiasthmatic agent (11). After its reemergence over the last 2 decades, it is recommended and migraine prophylaxis<sup>(12)</sup>. accepted for Parthenolide gemacranolide-type а sesquiterpene lactone is the major constituent of European feverfew, (Tanacetum parthenium L.), and several other members of the Asteraceae and Magnoliaceae families (13).

Parthenolide is available in an ample amount of the traditional medical plant feverfew (*Tanacetum parthenium*) acting as a covalently reactive compound, it displays antiinflammatory, redox-modulating, and epigenetic activities, as well as selective cytotoxicity towards cancer stem and progenitor cells <sup>(14)</sup>.

### **MATERIALS AND METHODS**

#### Animals

Female Swiss albino mice (*Mus musculus*) 6–8 weeks old, weighing 25–30 g were used for

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this study. The guidelines set by the WHO Health Organization, (World Geneva. Switzerland) was followed towards animal care and handling. The animals were housed for a minimum of one week in the laboratory animal room prior to testing in standard polypropylene cages at room temperature of  $34 \pm 20$  °C and at 60- 65% relative humidity. They were housed under standard animal house conditions and fed with standard laboratory pellets and water ad libitum. All experimental protocols were reviewed and approved by the Animal Ethics Committee of Nitte University, Ref. KSHEMA/ IAEC/24/2014 dated 07-11-2014. India.

#### Chemicals

The entire experiment was carried out at the Central Research Laboratory (CRL), Nitte University, Mangalore, India. The ethanolic and aqueous leaves extract of Tanacetum parthenium were obtained from Organic Inc China. The leaf extracts were stored in airtight containers. The synthetic compound parthenolide (98% min; HPLC grade) was obtained from Shanghai Better Biochem Co Limited China and all the other chemicals required were purchased from Merck and Hi-media from India.

#### Acute toxicity studies

Acute oral toxicity study was conducted following the guidelines of the Organization for Co-operation Economic and Development (OECD, 425). The animals were randomly allocated into seven groups of six animals each. Group, I Control: animals were administered orally with vehicle normal saline. Group II, III, IV, V administered orally with 1000 mg/ kg and 2000mg/kg body weight of aqueous extract of Tanacetum parthenium (ATP) and ethanolic extract of Tanacetum parthenium (ETP), prepared using distilled water and the dose-volume was not more than 1 ml/100g body weight. Group, VII 100 mg/ kg and 200mg/kg bodyweight of standard compound synthetic parthenolide (SP), prepared using 3% ethanol and dose-volume was not more than 1 ml/100g body weight according to the method of <sup>(15)</sup>, the animals were observed continuously for the first

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4 hours and then once every hour for the next 24 hours and thereafter once every 6 hours for the 48 hours after administering the drug, to observe any death or changes in general behavior and other physiological activities.

#### In vivo radioprotective studies Irradiation procedure

The present study was carried out at Oncology Department, Nitte Leela Narayan Shetty Memorial Cancer Institute using the linear accelerator. The animals were exposed to whole-body electron beam radiation (EBR) source at a distance of 100 cm from the beam exit point of the linear accelerator and at a dose rate of 1 Gy/min and15MeV energy by placing in a well-ventilated perspex box.

### Experimental design Survival studies

According to this study Lethal Dose (LD), 50 of *Tanacetum parthenium* in mice is >2000mg/ Kg body weight, so 2.5, 5, 12.5% dose of LD 50 for aqueous and ethanolic *Tanacetum parthenium* was selected. The LD 94 of synthetic parthenolide in mice is >200mg/kg body weight therefore 1, 2, 3% dose of LD 50 of synthetic parthenolide was selected to find an effective dose.

11 groups of 10 mice each(total 110 mice) were used for the present study. Group 1 served as positive control (Radiation Control), Group 2 served as Normal control (Control), Group 3,4, and 5 were orally administered with SP, Group 6 ,7 ,8 ,9 ,10 and 11were orally administered with ATP, ETP extracts 250,100 and 50 mg/Kg body weight for 15 consecutive days and the animals were exposed to lethal dose of 10Gy (challenging dose of 1.09Gy) of electron beam radiation on the 15<sup>th</sup> day. After irradiation, the animals were observed for survival and radiation symptoms for the next 30 days. The dose which provided maximum survival according to Kaplan- Meier analysis was selected for further studies. Kaplan-Meier analysis is one of the versatile methods basically done to know the trend of drug action (usually based on mortality). Based on these results, the optimum dose of the compound for radioprotection was

obtained.

#### Grouping of animals

In-vivo study swiss albino mice were divided into four groups. Group 1 (control) normal mice and Group 2 (radiation control) oral administration of saline for 15 days. The treatment groups including a daily dose of 100 mg/kg Tanacetum parthenium leaf aqueous extract (ATP), Tanacetum parthenium leaf ethanolic extract (ETP) and 4mg/kg synthetic parthenolide (SP) for fifteen consecutive days before Group 3 (pre-treatment) and after Group 4(post-treatment).On the 15<sup>th</sup> day, all were irradiated with 6Gy whole body irradiation for pre and post-irradiation treatments. A sub-lethal dose of radiation was given to the groups to study the hematological and antioxidant studies.

# Collection and treatment of the biological samples

The mice were gently euthanized under anesthesia. The blood was collected in tubes containing 3.8% sodium citrate by the cardiac puncture. A part of the blood was used for hematological examination. The liver was excised immediately and washed several times with ice-cold 0.1 M phosphate-buffered saline (PBS, 1:9), pH 7.4. The tissue was blotted dry, weighed and minced with stainless steel scissors to prepare tissue homogenate in PBS for antioxidant estimations.

#### Preparation of tissue homogenate

A 10% tissue homogenate was prepared in 0.1 M PBS (1:9), pH 7.4 using a Remi (RQ-127A) type homogenizer for antioxidant enzymes assay. The homogenate was centrifuged at 10,000 rpm for 20 min at 4 °C in Remi cooling centrifuge (C24BL). The supernatant was separated and used for all the estimations.

#### Hematological Studies

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

#### Antioxidant Studies

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Spectrophotometric methods were used for

the measurements which were recorded in Systronics PC-based double-beam ultraviolet spectrophotometer 2202.

#### Assay for Total Antioxidant Capacity

The total antioxidant capacity was evaluated by the phosphomolybdenum assay<sup>(16)</sup> The total antioxidant capacity assay is a nonspecific assay that measures the level of total antioxidants present in the system that scavenge the free radicals. 100  $\mu$ l of the sample was treated with 100 µl of trichloroacetic acid (TCA). The mixture was allowed to stand for 5 min and centrifuged at 3000 rpm and the supernatant was separated. 100 µl of supernatant was taken and 1 ml of molybdic acid reagent was added. The mixture was kept in a boiling water bath for 90 min. The absorbance was read at 695 nm. The molvbdic acid reagent contained 0.6 M sulfuric acid, 28 mM sodium dihydrogen orthophosphate, and 4 mM ammonium heptamolybdate.

#### Assay for reduced glutathione

The reduced GSH was estimated by the glutathione assay (17) when glutathione reacts with 5, 5'-dithiobis (2-nitro benzoic acid (DTNB) to form a stable yellow color complex. By treating 0.1 ml of the sample with 1.5 ml of precipitating solution containing metaphosphoric acid and sodium chloride. The mixture was allowed to stand for 10 min and centrifuged. 0.5 ml of this supernatant was treated with 2 ml of 0.3M phosphate solution and 0.25 ml of 5,5'- dithio-bis-(2-nitrobenzoic acid). The absorbance was read at 412 nm within 10 mins and calculated using a GSH standard curve.

#### Assay for superoxide dismutase

The superoxide dismutase (SOD) activity was determined <sup>(18)</sup>, when nitro blue tetrazolium chloride (NBT) which reacts with superoxide anions produced upon illumination of riboflavin in the presence of methionine as an electron donor, to produce formazan which is a blue-green colored complex. By treating 0.1 ml of the sample with a mixture of 2.5ml of methionine, 0.3ml of riboflavin and 0.1ml of nitro blue tetrazolium chloride was prepared

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using the 0.05M phosphate buffer. This mixture was allowed to stand 10 minutes under bright light. The blue-green colored solution was read at 560 nm. The activity of SOD was expressed in units/mg of protein for homogenates.

#### Assay for catalase

The catalase activity was performed<sup>(19)</sup> when decomposition of  $H_2O_2$  in the presence of CAT was determined by treating 10 µl of the sample with 3 ml of 60 mM hydrogen peroxide. The kinetic measurement was taken at 240 nm with a 30-second delay for 2 minutes. The activity was expressed in units/mg of protein for homogenates.

#### Statistical analysis

The data obtained were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post hoc* test, the procedure to calculate interrelation between the groups. Differences with p values  $\leq 0.05$  were regarded as statistically significant. The data are expressed as the mean  $\pm$  S.E. The dose optimization was done using Kaplan-Meir survival analysis. P-value was calculated by statistical software program "SPSS evaluation version 16.0".

### RESULTS

#### In vivo radioprotection study Survival studies

Exposure of animals to 10.0 Gy electron beam irradiation-induced the symptoms of severe radiation sickness like irritability, lethargy, watering of eyes, ruffling of hairs, reduced food, and water intake, diarrhea, and facial edema. The first mortality in the irradiation group was observed on day 6 and subsequent mortality at various post-irradiation days. All electron beam irradiated animals without any pretreatment died within 13th-days post-irradiation. The pretreatment of mice with survival studies. 100 mg/Kg body weight of Tanacetum parthenium leaf aqueous extract (ATP), Tanacetum parthenium leaf ethanolic extract (ETP) and

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4mg/kg synthetic parthenolide (SP) prior to electron beam irradiation increased 30 days survival of the animals shown in figure. 1,2,3. The pretreatment of animals delayed or reduced the severity of irradiation sickness and also delayed the onset of irradiation-induced mortality when compared with the concurrent irradiation group.

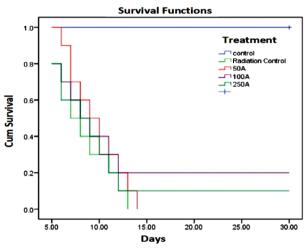


Figure 1. Survival function graph of aqueous leaf extract of Tanacetum parthenium.

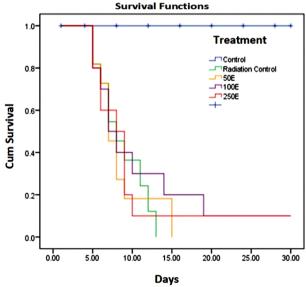
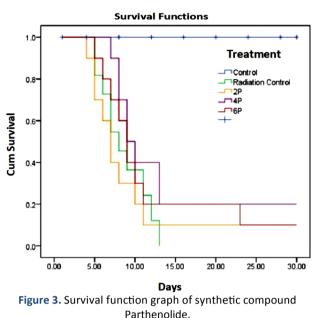


Figure 2. Survival function graph of ethanolic leaf extract of Tanacetum parthenium.

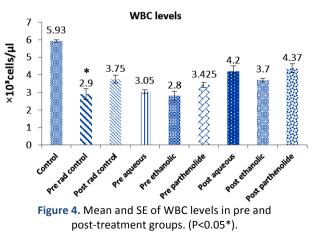
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## Faithenolide.

# Effect of leaf extracts and SP on hematological parameters

The hematological parameters of mice against electron beam irradiation damage are depicted in the radiation control group in the WBC, RBC and hemoglobin content decreased significantly in electron beam irradiated mice as compared to the controlled group. There was significant change observed in pre radiated control in WBC when compared with the pre-treatment groups. RBC level in aqueous treated showed significant change compared to the radiated control groups, there was an increase in RBC level the post-treated groups. However, hemoglobin content of pre and post-treated ATP, ETP and SP groups showed a significant increase compared to radiated control of electron beam irradiation.



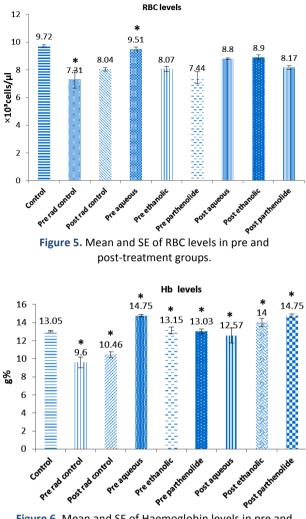


Figure 6. Mean and SE of Haemoglobin levels in pre and post-treatment groups. (P<0.05\*).

# *Effect of leaf extracts and SP on total antioxidant capacity in irradiated mice*

The Total Antioxidant Capacity of electron beam irradiated mice was found to be decreased when compared to the controlled group is represented in figure 7. The pre and post-treated ATP, ETP, and SP showed no significant increase when compared to the controlled group. The pre and post-treated groups showed a slight decrease as compared to the irradiated control groups.

# Effect of leaf extracts and SP on GSH activity of irradiated mice

The effect of pre and post-treatment on GSH content is presented in figure 8. The GSH

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content showed a significant change in electron irradiated group as compared to the controlled group. The ATP and SP pre-treatment groups with irradiation exposure showed a significant change in GSH content compared to electron beam irradiated mice. However, post-treated groups showed no significant increase in GSH content when compared to electron beam irradiated mice.

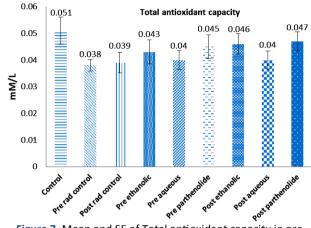


Figure 7. Mean and SE of Total antioxidant capacity in pre and post-treatment groups.

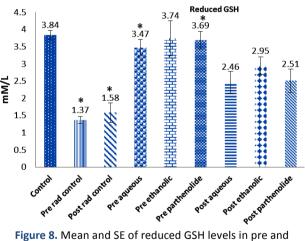


Figure 8. Mean and SE of reduced GSH levels in pre and post- treatment groups. (P<0.05\*).

# *Effect of ATP, ETP and SP on SOD activity in irradiated mice.*

The SOD activity in mice exposed with electron beam irradiation is presented in Figure 9, showed a significant decrease in pre and post irradiated mice when compared to the controlled group. The pre and post-treated ATP,

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ETP, and SP showed a significant increase when compared electron beam irradiated mice.

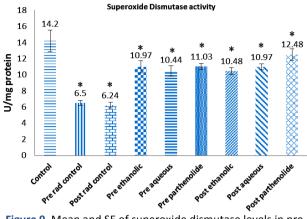
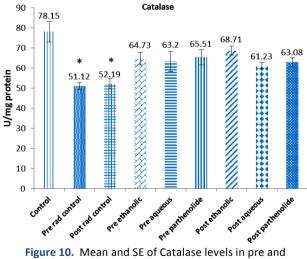


Figure 9. Mean and SE of superoxide dismutase levels in pre and post-treatment groups. (P<0.05\*).

# Effect of ATP, ETP and SP on Catalase activity in irradiated mice.

Catalase activity was significantly decreased in electron beam irradiated mice as compared to the controlled group is presented in figure 10. Catalase activity in mice pre and post-treated ATP, ETP, and SP showed an increase when compared to electron beam irradiated group. However, no significant change was observed in the catalase activity as compared to electron beam irradiated mice.



igure 10. Mean and SE of Catalase levels in pre and post-treatment groups. (P<0.05\*).

### DISCUSSION

The present study was to evaluate the effects of electron beam irradiation on the antioxidant

defense system and the radioprotection of aqueous and ethanolic *Tanacetum Parthenium* leaf extract and Synthetic Parthenolide. Parthenolide is one of the major components found in *Tanacetum parthenium*. The study reveals that parthenolide, an ethanolic and aqueous extract of *T. parthenium* showed effective free radical scavenging activity. Thus the results support its traditional use in ailments and as a source of natural antioxidants that protect cells against oxidative stress <sup>(20)</sup>.

There is a continued interest in and need for the identification and development of non-toxic and effective radioprotective compounds that can reduce the effect of radiation. Such compounds could potentially protect humans against genetic damage, mutation, alteration in the immune system and teratogenic effects of toxic agents including radiation, which act through the generation of free radicals. The reactive oxygen species (ROS) produced by ionization radiation are tightly controlled by systems, antioxidant defense including non-enzymatic radical scavengers and enzymes that can either directly detoxify ROS or indirectly regulate their levels. Antioxidant enzymes such as SOD, CAT, and GPx are important in providing protection from radiation exposure to the proper balance of the enzymes in specific cells and in the whole required maximum organism for radioprotection (21). The mortality of animals following radiation may be due to several factors like damages to the hematopoietic system and gastrointestinal system, which may ultimately lead to immune suppression (22). In the present study, after a lethal acute dose of 10 Gy radiation, 100% mortality was seen within the 15th day in the electron beam irradiated group, 50 mg/kg body weight of aqueous and ethanolic Tanacetum Parthenium and 2 mg/kg body weight Synthetic Parthenolide.

Based on survival studies, dose selection was done using Kaplan- Meier analysis. 100 mg/kg body weight of aqueous and ethanolic *Tanacetum Parthenium* and 8mg/kg bodyweight of Synthetic Parthenolide were selected for further studies. This would suggest that the administration of extract bestowed survival advantage to the animals following exposure to the lethal dose irradiation, as the lifespan of the irradiated group was extended for more than 3 weeks compared to the control irradiated group. This would suggest the protective effect of aqueous and ethanolic extract of *Tanacetum Parthenium* and Synthetic Parthenolide against the lethal dose (10 Gy) radiation exposure.

Exposure to a lethal dose of ionizing radiation severely increases the oxidative burden on the body and the endogenous antioxidant defense mechanism cannot cope with this increased stress (23). It is a well-established fact that ionizing radiation at a cellular level can induce the biologically important damage in macromolecules such as DNA, proteins, lipids, and carbohydrates in the various organs. In some organs, the damage is expressed early while in others, it may be expressed over a period of time depending upon the cell kinetics and the radiation tolerance of the tissues <sup>(24)</sup>.

The induction of symptoms of radiation sickness like reduction in food and water intake, irritability, epilation, weight loss, emaciation, lethargy and ruffling of hairs within 3–5 days by 10.0 Gy of gamma irradiation is in agreement with the earlier studies (24,25). The death due to irradiation from 8 to 13 days is due to the hemopoietic damage inflicted by irradiation. The survival after exposure to high doses of irradiation, i.e., 10.0 Gy depends on the survival of a critical number of hemopoietic stem cells (HSC) and the ability of these cells to generate an effective level of mature cells of multiple repopulate the depleted lineages to hematopoietic compartment (26).

The main cause of bone marrow syndrome is the severe depletion of the HSCs since these are more sensitive to radiation than the committed and mature peripheral blood cells. Thus 30 day time period after lethal whole-body irradiation for survival studies indicates the capacity of aqueous and ethanolic extract of Tanacetum Parthenium and Synthetic Parthenolide to modulate the recovery and regeneration of the gastrointestinal epithelium (GI) and the hemopoietic progenitor cells in the bone marrow, the two most radiosensitive organs that are essential for sustaining the life. The

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pretreatment of mice resulted in a reduction of radiation-induced mortality as compared to irradiated animals. It was reported that the gastrointestinal syndrome occurs between doses 5.0 and 12.0 (primarily >10.0) Gy of irradiation exposure and death occurs within 3-10 days, while bone marrow syndrome occurs between dose 2.5 and 8.0 Gy and death occurs within 1-2 months <sup>(27)</sup>. The 30 days survival of the mice following whole-body lethal irradiation can be correlated with hemopoietic recovery and regeneration.

Animal survival in the presence of a high dose of ionizing radiation suggests the occurrence of physiological adaptive mechanisms, supported pretreatment, which protects bv against excessive radiation damage. The percent of survival indicates the effectiveness of the antioxidant in arresting gastrointestinal death. This reduction in gastrointestinal death may be due to the protection of intestinal epithelium, which would allow the proper absorption of the antioxidants. There are reports that oral administration isoflavone of genistein significantly enhanced protection against radiation-induced lethality (28). Thus the present work could be a promising therapeutic adjunct in radiation exposure.

Antioxidant enzymes, i.e., SOD, CAT activity in the liver showed a significant decrease with electron beam irradiation exposure, probably due to increased oxidative stress. A decrease in the TAC and CAT levels were seen with irradiated groups when compared to the non-irradiated groups. The superoxide anions are generally dis-mutated by SOD to form  $H_2O_2$ , which is decomposed by CAT <sup>(29)</sup>. It is suggested that a decrease in liver antioxidant enzymes activity of electron beam irradiated animals may be due to the denaturation of enzymes structure irradiation by electron beam exposure. Moreover, a decrease in CAT activity in animals exposed with electron beam irradiation was probably due to the inactivation of CAT, as the flux of superoxide anions have been shown to reduce CAT activity (30).

Electron beam irradiation also resulted in a significant decrease in GSH content in the liver. The efflux of GSH from the liver is the source of

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GSH to other organs (31). Similarly, the extracts of dark tea, with antioxidative activity, could greatly mitigate the hematopoietic injury caused by ionizing radiation, by decreasing the levels of ROS and increasing the activities of antioxidant enzymes including SOD, CAT, and GSH-Px in the livers<sup>(32)</sup>. The results showed that electron beam irradiation exposure resulted in a significant decrease in the number of WBC, RBC and platelet count, which may be due to alteration in bone marrow as well as the hemopoietic system of the animals. Studies have shown that radiation reduces the hematological parameters like hemoglobin, red blood cell count, white blood cell count, platelet count, and inhibits hematopoiesis (33).

Thus aqueous and ethanolic *Tanacetum Parthenium* leaf extract and Synthetic Parthenolide have shown to counteract electron beam irradiation-induced oxidative stress due to their antioxidant properties.

#### CONCLUSION

The search for radioprotective agents to counteract radiation damage is of immense use in radiotherapy. Radioprotective agents minimize or prevent the damage from radiation exposure. Thus it can be concluded that aqueous and ethanolic *Tanacetum Parthenium* leaf extract and synthetic parthenolide scavenge free radicals produced by radiation exposure and thus inhibit radiation-induced oxidative stress, act as a probable radioprotector having potent antioxidant properties.

#### ACKNOWLEDGMENTS

The authors are thankful to the Board of Research in Nuclear Sciences (BRNS) Sanction Number BRNS/2013/34/2 for constantly supporting the work and also express their sense of gratitude to the Central research laboratory, KS Hegde Medical Academy, Nitte University for providing the instrumentation facility and the technical support.

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Conflicts of interest: Declared none.

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Int. J. Radiat. Res., Vol. 19 No. 1, January 2021