APPRAISING RADIOPROTECTIVE POTENTIAL OF TINOSPORA CORDIFOLIA (AMRITA) EXTRACT IN SWISS ALBINO MICE EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION

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Summary

The root extract of Tinospora cordifolia (TCE) is a unique drug used in Indian systems of medicine for several diseases and as a vitalizer. Looking at its sundry applications, hydro alcoholic extract was examined for its radio protective potential against gamma radiation in Swiss albino mice. Animals were divided into two groups and irradiated, with or without TCE extracts, given orally at 25, 50, 100, 125, 250, 500, 750, 1000 and 2000 mg.kg.b.wt./day before irradiation. The acute toxicity studies showed that the drug was non-toxic up to a dose of 2500 mg.kg.b.wt./day. Administration of TCE resulted in a dose-dependent decline in radiation induced mortality up to a dose of 75 mg.kg.b.wt./day the dose at which the highest number of survivors (87%) was observed. This was considered optimum dose for radioprotection and used in further radiomodulatory studies exposure to 6, 7, 8, 9, 10 and 11Gy of gamma radiation. The treatment of mice with 75 mg.kg.b.wt./day TCE reduced the severity of symptoms of radiation sickness and mortality at all exposure doses, and a significant increase in survival was observed as compared with the non-treated irradiated group. The TCE treatment effectively protected mice against the gastrointestinal as well as bone marrow related death, as revealed by the increased number of survivors at all irradiation doses. The dose reduction factor was found to be 1.68. To understand the mechanism of action, biochemical parameters as lipid peroxidation (LPx) & Glutathione (GSH) content were evaluated, indicating radioprotection afforded by TCE may be in part due to the scavenging of reactive oxygen species induced by ionizing radiation. Irradiation of animals resulted in an elevation in lipid peroxidation (LPx) and a significant decline in glutathione in liver. Conversely, administration of animals with TCE before irradiation caused a significant decline in LPx accompanied by a significant increase in GSH concentration.

Keywords: Gamma radiation, Tinospora cordifolia, LPx, GSH, Swiss albino mice.

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Introduction

The use of ionizing radiation has become an integral part of modern medicine. It is used for diagnostic as well as therapeutic purposes. Ionizing radiation consists of energetic particles and electromagnetic radiation, which can penetrate living tissues or cells and result in the transfer of radiation energy to the biological material. The energy absorbed by the living matter can break chemical bonds and cause ionization of biologically important macromolecules, such as nucleic acids, membrane lipids, and proteins when radiation passes through matter it deposits some of its energy in the absorbing material by ionization or excitation of the atoms. It is ionization of atoms in tissue, accompanied by chemical changes, that causes the harmful biological effects of radiation. For instance, when ionizing radiation passes through cellular tissue, it produces charged water molecules. These break up into free radicals, which are highly reactive chemically and can alter important molecules such as deoxyribonucleic acid (DNA) in cells. Exposure of cells to ionizing radiation results in immediate and widespread oxidative damage to DNA by both direct and indirect mechanisms. About 60-70% of cellular DNA damage produced by ionizing radiation is estimated to be caused by OH, formed from the hydrolysis of water. Very high doses of radiation can cause serious and immediate effects (acute effects). After whole body exposure to very large doses of radiation, particularly sensitive cells die, resulting in a serious damage to select organs and systems. While, a sufficient radiation induced genetic alteration may lead to cancer, a sufficient cell killing contributes to radiation sickness. Radiation sickness is caused by damage to organs or systems after exposure to very high doses of radiation. The symptoms depend on dose and time of exposure. High doses of radiation can also produce effects long after the exposure (late effects) resulting in adverse health effects within a short time of minutes (CNS Syndrome), days (GI Syndrome) to weeks (Hematopoietic Syndrome or delayed effects observable many months (Birth defects, LD₅₀) or years (Cancer). The manifestation of unfavorable health effects depend on radiation dose, duration of exposure, sensitivity of the tissues and intrinsic antioxidant defense mechanism. Examples are cancers of various types. In some cases, radiation may be the single best treatment of cancer. However, for many solid tumors, a cure with radiation remains elusive. The radiation therapy of cancer depends on achieving a therapeutic differential between the cancer cell cytotoxicity and normal tissue toxicity. The therapeutic differential may be achieved with chemical radiation sensitizers or protectors. The development of radiation protectors is important not only to enhance the effectiveness of cancer treatment, but also for the study of the underlying mechanisms of radiation cytotoxicity. Some radioprotectors are known to offer protection through a direct effect on the cellular targets of radiation; while others enhance the recovery of normal tissues. Researchers are nowadays trying to ferret out radioprotectors in plants & natural products which can be put together as a dietary constituent. Natural preparations were much more accepted in the global village due to their better cultural acceptability, better efficacy, better compatibility with the human body and lesser side effects at their optimum doses. The extracts of Ocimum sanctum, Trigonella foenum graecum, Alstonia scholaris have been reported to protect mice against radiation induced mortality. Certain herbal preparations such as Liv. 52, abana and triphala have also been reported to protect mice against radiation induced sickness, mortality, dermatitis, spleen injury, liver damage, decrease in peripheral blood cell counts and radiation-induced chromosome damage. Tinospora cordifolia, belonging to family Menispermaceae, is considered to be the best herb for clearing the microbiology systems and other bodily channels. It is commonly called Guduchi and is widely used in veterinary folk medicine/ayurvedic system of medicine for its general toxic, anti-periodic, anti-inflammatory, anti-allergic properties. The root of this plant is known for its anti-stress, anti-leprotic activities. The aqueous extract of roots of T. cordifolia has shown the anti-oxidant action in alloxan diabetes rats.
Furthermore, the *T. cordifolia* has been proved to have Hepatoprotective, Immunomodulatory, hypolipidaemic, and antineoplastic properties and scavenges free radicals. The root extract of this plant killed the Hela cells very effectively in vitro and thus it indicates that it needs attention as an anti-neoplastic agent. Therefore looking at its profound medicinal properties, the present study is an effort to identify and evaluate the radioprotective effect of *Tinospora cordifolia* Extract (TCE) in mice.

**Materials and Methods**

**Animal care and Handling:** The animal care and handling were performed according to the guidelines set by the World Health Organization (Geneva, Switzerland) and the INSA (Indian National Science Academy, New Delhi, India). Swiss albino mice, 6-8 weeks old weighing 22±2 gm from an inbred colony were used. They were maintained under controlled conditions of temperature and light (14 and 10 hr of light and dark, respectively). The animals were provided with standard mice feed (procured from Ashirwad Industries, Chandigarh, India) and water ad libitum. Tetracycline water was also given once a fortnight as a preventive measure against infection. Four to six animals were housed in a polypolypropylene cage containing paddy husk (procured locally) as a bedding throughout the experiment. The Institutional Animal Ethical Committee approved the study.

**Irradiation:** The Cobalt teletherapy unit (ACT- C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical College & Hospital, Jaipur was used for irradiation. Unanasthetized animals were restrained in well ventilated Perspex boxes and exposed whole-body to gamma radiation.

**Preparation of the extract:** *T. cordifolia* was identified by the curator (Voucher No.RUBL 20132), Department of Botany, University of Rajasthan, Jaipur, India. The extract of *T. cordifolia* was prepared according to standard protocols. Briefly, the whole plant was shade-dried, powdered and extracted with 95% ethanol using a Soxhlet apparatus. The total ethanolic extract was then concentrated in-vacuo to a syrupy consistency dried and stored and refrigerated.

**Preparation of the drug solution and mode of administration:** The required amount of the 95% ethanolic extract of *T. cordifolia* (TCE) was dissolved in 200 µl of Double distilled water before administration. The mice were administered with 75 mg.kg.b.wt./day TCE+ 200 µl DDW orally.

**Determination of acute drug toxicity:** The acute toxicity of TCE was determined according to standard protocol of Prieur *et al* and Ghosh *et al* briefly; the mice were fasted by withdrawing food and water for 18 h. They were then divided into several groups of 10 mice each. Each group was injected orally with various doses 25, 50, 75 100, 125, 250, 500, 750, 1000 and 2000 mg.kg.b.wt./day of the freshly prepared TCE solution. The mice were provided with food and water immediately after the drug administration. Mortality was observed up to 30 days after drug treatment. The treatment of mice with the various doses of TCE did not induce death during the period of the study and hence it was considered safe for administration and was whole-body exposed to 10Gy of Co60 gamma radiation (Theratron, Atomic Energy Agency, Canada) in a specially designed well-ventilated acrylic box. A batch of 10 mice was irradiated each time at a rate of 1.36 GYmin-1 at a source to animal distance (surface) of 84.9 cm. The following experiments were then carried out.
Selection of the optimum dose of TCE: To select the optimum dose of TCE for radioprotection, the mice were divided into two groups as described above. The mice in the DDW +irradiation group received DDW, while those in the TCE +irradiation group were administered with 25, 50, 75, 100,150 and 200, mg.kg.b.wt./day of TCE orally, before exposure to 10Gy of gamma radiation. A dose of 75 mg.kg.b.wt./day TCE was found to be the optimum radioprotective dose and therefore further experiments were carried out using this dose.

Radioprotective effect of TCE: To determine the radioprotective effect of TCE, the mice were divided into two groups as described above. One group of mice received double distilled water (DDW), while the other group was injected with 75 mg.kg.b.wt./day TCE before exposure to 6, 7, 8, 9, 10 and 11 Gy of gamma radiation. The mice in both groups were monitored daily for the development of symptoms of radiation sickness, and mortality for a period of 30 days after irradiation. The death of animals between 3 and 10 days after irradiation was considered to be due to gastrointestinal damage, while death between 11 and 30 days after irradiation was due to damage to hematopoietic organs. Percentage survival was calculated and plotted against the radiation dose. The dose reduction factor was calculated by the method of Miller & Tainter et al 28, as follows: dose reduction factor =LD50/30 of the TCE+ irradiation group/LD50/30 of DDW+ irradiation group.

Endogenous spleen colony forming unit (CFU) Assay: The mice were sacrificed by cervical dislocation on the 10th post-irradiation day in all groups. For endogenous CFU assay29 spleens were dissected out and fixed in Bouin’s solution for 24 h. macroscopic colonies (CFU) visible to naked eyes scored from each spleen.

Preparation of homogenates: The liver was perfused in situ immediately with cold 0.9% NaCl and thereafter removed, and was rinsed in chilled 0.15 M Tris-KCl (pH 7.4). The liver was then blotted dry, weighed and homogenized in cold 0.15 M Tris-KCl buffer (pH 7.4) to yield a 10% w/v homogenate. Aliquots (1 ml) of this homogenate were used for assaying various parameters. Blood was collected from orbital sinus (mice) into vial containing EDTA. Plasma was obtained by low-speed centrifugation of whole blood.

Estimation of Glutathione and Lipid Peroxidation: The Animals for this experiment were divided into following four groups for estimation of Lipid Peroxidation (LPx) and Glutathione (GSH). Animals were autopsied 24 h post-irradiation.
Group I: These animals were given orally DDW equivalent to the dose of TCE (i.e. 75 mg.kg.b.wt./day) for five consecutive days and were considered as vehicle treated control.
Group II: Animals of this group received only TCE (75) mg.kg.b.wt./day for five consecutive days and were considered as Drug treated control.
Group III: These animals were given orally DDW equivalent to the dose of TCE (i.e.75 mg.kg.b.wt./day for 5 consecutive days. 30 minutes after the last administration, these were exposed whole-body to 8 Gy gamma radiations.
Group IV: Animals of this group received only TCE (75 mg.kg.b.wt./day) for 5 consecutive days and were exposed on day 5th to the similar dose of radiation as in Group II

Glutathione (GSH) assay: The hepatic level of glutathione (GSH) was determined by the method of Moron et al30. The absorbance was read at 412 nm using a UV-VIS Systronic Spectrophotometer. Blood GSH levels measured as per standard protocol of Beutelers et al31
Lipid peroxidation (LPx) assay: The lipid peroxidation (LPx) level in liver was measured by the assay of thiobarbituric acid reactive substances (TBARS) using the method of Okhawa et al.\textsuperscript{32}, in which the absorbance was read at 532 nm using a UV-VIS Systronic Spectrophotometer.

Statistical analysis: The statistical significance between various groups was determined using student’s t-test. Figures have been drawn using data expressed as mean (±) standard deviation and comparing it with respect to the control, taken as one, for observations at a particular time interval.

Results

1. Determination of TCE tolerance: No adverse effects were observed in terms of sickness, body weight changes, mortality and visible abnormality throughout the study in animals treated with different drug doses (125, 250, 500, 750, 1000 & 2000 mg.kg.b.wt./day) of TCE for 5 consecutive days. These mice were observed first for 12 hrs and then for 30 days post-treatment. However, after the termination of the experiment, no sickness and mortality were observed in any of the above group, which indicates that even the high dose of TCE (i.e. 2000 mg.kg.b.wt./day) is well tolerable in Swiss albino mice (Fig.1,2).

2. Selection of optimum dose of TCE against irradiation: The optimum dose of TCE against lethal gamma radiation (i.e. 10 Gy) in Swiss albino mice was selected on the basis of survival experiment, where number of deaths and surviving animals were recorded up to 30 days of irradiation. Mice treated with TCE at doses of 25, 50, 75, 100, 150, 200\textsuperscript{f} mg.kg.b.wt./day or 5 consecutive days prior to irradiation exhibited 28, 43, 60, 88, 50 and 48 per cent survival respectively (Fig.1,2). The dose 75 mg.kg.b.wt./day was found to be the optimum dose based on the above data, and the further studies were carried out using this dose of TCE.
3. Calculation of Dose Reduction Factor (DRF): In order to establish the survival-dose-response of Swiss albino mice to radiation in the presence or absence of TCE, two groups of animals were used. The mice of one group were given orally double-distilled water (DDW), equal to volume of TCE and were exposed to different doses (6, 7, 8, 9, 10, 11 Gy) of gamma radiation, while the animals of other group were given orally, the optimum dose of TCE for five consecutive days once daily, and were exposed on 5th day to similar doses of gamma radiation (as in Group-I). On the basis of survival data in different groups, with or without TCE, DRF was calculated as 1.80. (Fig.3)
4. Colony forming unit (CFU) assay: The effect of various doses of radiation on endogenous CFU and its modulation by a pre-irradiated administration of TCE (mg.kg.b.wt./day) are depicted in (Fig.4). CFU counts in spleen decreased with increasing radiation doses (6, 7, 10 Gy). Animals given TCE with no other treatment rendered significant change in CFU counts. Pre-irradiation treatment with TCE rendered significantly higher CFU counts in comparison to corresponding irradiated groups.

5. Lipid Peroxidation (LPx): Administration of TCE when compared with DDW treatment did alter the lipid peroxidation (LPx) in Groups II and IV. TCE pretreatment significantly reduced LPx induction in the TCE + irradiation group, thereby protecting liver and mice against radiation induced LPx (Figure5)
6. Glutathione (GSH) assay: No significant difference of the hepatic and blood GSH contents was observed between normal and TCE treated animals. However, a statistically significant (p<0.001) decreased in GSH was evident in radiation treated experimental animals. TCE treated irradiated animals showed a significant increase in GSH content (blood & liver) with respect to irradiated control, but the value remained below normal. (Figure 6)

Fig 6: Reduced Glutathione (GSH) levels in blood and liver of Swiss albino mice after exposure to 8 Gy gamma rays with or without *Tinospora cordifolia* extract (TCE)

![Graph showing reduced GSH levels](image)

Discussion

Since the discovery of the deleterious effects of radiation, efforts have been directed to alleviate radiation-induced damage. Synthetic thiol compounds used as radioprotectors have limited practical applicability owing to their inherent toxicity at optimum doses. Despite screening of several synthetic compounds for radioprotective activity no single compound has emerged as a good radioprotector. Dietary ingredient may be very useful if they protect against deleterious effects of ionizing radiation. *T. cordifolia* (Family Meninspermaceae), known as Amrita (Guduchi) possesses several medicinal properties.

The aim of the present study was to evaluate the radioprotective effects of Guduchi in mice exposed to whole body γ radiation. The exposure of animals to γ radiation resulted in radiation-induced sickness and mortality; the higher doses killed all animals within 10-15 days, in agreement with earlier reports. In mice, death within 10 days postirradiation is due to gastrointestinal damage. The bone marrow stem cells are more sensitive to radiation damage than liver hepatocytes, but the peripheral blood cells have a longer transit time than the liver cells, Hence the gastrointestinal syndrome appears earlier than the bone marrow syndrome. In mice, death from 11 to 30 days is due to the hematopoietic damage.

The radioprotective effect of TCE increased in a dose dependent manner up to 75mg/kg.b.wt./day (once daily for 5 consecutive days); above that dose, the radioprotective effect declined. As above a particular concentration, a compound may start manifesting its toxic effects. The increased survival of mice after 75mg/kg.b.wt./day, *T. cordifolia* have been reported to increase survival percentage, prevented body weight loss, increased the reduced number of endogenous colony forming unit (CFU) counts, increased the impaired S-phase cell population and decreased irradiation-induced micronuclei.
TCE after various radiation doses indicates effectiveness of *T. cordifolia* in arresting deaths from both the GI & bone marrow syndromes. This reduction in death from the GI syndrome may be due to protection of liver. *T.cordifolia* is traditionally used in the treatment of jaundice and liver diseases. Administration of *T. cordifolia* to CCL4 intoxicated rats found to reduce elevated serum enzyme levels down near control values. An arabinogalactan has been isolated from the dried stems of *T.cordifolia*. The roots of *T. cordifolia* contain isocolubin, palmatine, tetrahydropalmatine, magnoflorine and jatroechizine reported to have antiulcerative activities. In Ayurvedic literature, Guduchi has been reported to be a blood purifier that possibly acts by stimulating liver and spleen, which remove defective and damaged RBC’s from peripheral blood circulation. The hamatopoietic syndrome is induced at low doses of radiation and is manifested by hamatopoietic stem cell depletion and ultimately by the depletion of mature hamatopoietic and immune cells. The reduction in deaths from bone marrow syndrome by TCE may be the resulted protection of stem cell compartment of bone marrow. The LD<sub>50/30</sub> was increased to 10 Gy, with a DRF of 1.68. Other plants including *Panax ginseng*, *Ocimum sanctum*, *Trigonella foenum*, *Rosemary* reported to protect mice against radiation-induced mortality. A direct comparison of the present findings with polyherbal preparations like Amritarishtam, Dhanvataram tailum, Cheriya rasnadi kashayam and Valiya marmagulika is not possible because of the presence of several plant ingredients, including, *T.cordifolia* in these drugs. The mechanism of action of TCE is not known. Free radical scavenging is a common mechanism of radioprotection. *T.cordifolia* have been reported to scavenge free radicals like Superoxide, H2O2, NO2 in vivo. Unlike most antioxidants and free radical scavengers, *T.cordifolia* has been found to be anti-inflammatory. Ionizing radiation induces lipid peroxidation, which can lead to DNA damage and cell death. Therefore, an agent that protects against such damage can provide protection against radiation damage. The administration of TCE significantly reduced the amount of lipid peroxidation compared to concurrent control groups. This inhibition of lipid peroxidation by TCE extract may also have been responsible for the observed radioprotection.

The significant elevation in the GSH level by TCE at all radiation doses may be responsible for the scavenging of radiation-induced free radicals, including lipid peroxidation, and thereby protecting against radiation-induced mortality. *T.cordifolia* has been reported to elevate GSH levels in mice and rats and to reduce lipid peroxidation. It has also found to increase Superoxide dismutase, Catalase and glutathione peroxidase in experimental animals. Several investigations have reported that lipid peroxidation starts as soon as the endogenous GSH is exhausted, and that the addition of GSH promptly stops further peroxidation.

**Conclusions**

In conclusion, *T.cordifolia* provided protection against radiation-induced mortality in mice by protecting against GI and bone marrow syndromes. The free radical scavenging, elevation in antioxidant status, and reduction in lipid peroxidation appear to be the important mechanisms of radioprotective action by *T.cordifolia*.

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