Modulation of Radiation Induced Biochemical Changes in Testes by *Tinospora Cordifolia* Extract (An Indian Medicinal Plant)

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Increasing use of nuclear radiation in diverse fields necessitates for developing a potential drug of natural origin for the modification of radiation toxicity. In this context, the present study has been undertaken to explore radioprotective potential of the root extract of *Tinospora cordifolia* (an Indian medicinal plant). For this purpose, healthy Swiss albino male mice were selected from an inbred colony and divided into four groups. Group I (normal) received double distilled water (DDW) while Group II was given orally *Tinospora cordifolia* root extract (TCE) at the dose of 75 mg/kg b.wt/day for 5 consecutive days. Group III (irradiated control) received DDW orally and exposed to 2.5 Gy of gamma radiation. Group IV (experimental) was administered orally TCE for 5 consecutive days once daily and exposed to 2.5 Gy of gamma radiation. Mice from all groups were sacrificed at different autopsy intervals viz. 1, 3, 7, 15 and 30 days, and their testes were removed for various biochemical estimations viz. protein, cholesterol and glycogen and sialic acid. A significant decrease (p< 0.01) in total proteins and sialic acid content (p< 0.05), whereas, a significant increase in cholesterol (p< 0.001) and glycogen (p< 0.01) from normal was observed in irradiated control group. On the contrary, TCE administration before administration exhibited a favorable alteration in these biochemical parameters. The results from the present study suggest a radioprotective effect of the *Tinospora cordifolia* extract against radiation induced biochemical alteration in testes of mice.

**Key Words:** gamma radiation, radiation protection, testes, *Tinospora cordifolia* extract.

**Introduction**

All living organism on earth are being perpetually exposed to some amount of radiation originating from a variety of sources. Ionizing radiation are serving the mankind many fold than any other scientific invention in the form of medical, industrial, agriculture application, and scientific solutions. Whole-body exposure to ionizing damages like gamma rays initiates a plethora of cellular and biochemical reactions inside a living cell. As a consequence, it induces a number of lesions such as strand breaks, oxidative damage and also complex interactions like change in gene expression and chromatin remodeling.

Various tissues and organ systems of an individual differ in their response to radiation and as a rule; systems with proliferating cells are most sensitive. Testis is one of the radiosensitive organs due to rapidly dividing cell renewal systems. Ionizing radiation was found to produce marked effects on testes in terms of lethality and impaired spermatogenesis. There is a need to study the protection of male germ cells against deleterious effects of radiation.

Substance capable of inhibiting the formation and spread of free radicals and their damage could protect mammalian organism from deleterious consequences of radiation exposures. Many compounds with antioxidant activities are proved to be effective radio- protectors. These include cysteine\(^1\), cysteamine\(^2\), 2-MPG\(^3\), WR-2721 (gammaphos)\(^4\) and Diltiazem\(^5\) which were tested for their radioprotective capacity in mammals. Owing to their side effects such as nausea, vomiting, hypotension and neurotoxicity, these have not been found successful in the field of clinical radiotherapy\(^6\). Therefore, there remains a need to develop safe, non-toxic yet effective radio-protective formulation.
The study of plants as modifiers of radiation effects is a relatively new area of research. Many photochemicals are known to be antioxidants, and are consumed in the diet and thus may help to protect human from damages induced by irradiation. Most of these plant extracts and photochemicals are nontoxic and inhibit radiation induced deleterious effects.

*Tinospora cordifolia* is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae. It is distributed throughout tropical Indian subcontinent and China, ascending to an altitude of 300 m. The plant is commonly known as Guduchi, Giloy or Amritha, which are Hindu mythological terms that refer to the heavenly elixir that have saved celestial beings from old age and kept them eternally young. Guduchi is widely used in veterinary folk medicine and Ayurvedic system of medicine for the treatment of various ailments like jaundice, gout, rheumatoid arthritis, diabetes, general weakness, skin disease, anemia, emaciation, and infection. The whole plant is used in Ayurvedic “Rasayanas” to improve the immune system and the body resistance against infections and root is known for its anti-stress, anti-leprotic and anti-malarial activities.

Hence, the wide acceptability, common usage, diverse anti-oxidative and pharmacological properties of *Tinospora cordifolia* aroused an interest to obtain insight into its radioprotective potential against testicular lesions in mice.

**Materials and Methods**

**Animal care and Handling**

The animal care and handling were performed according to the guidelines set by the WHO (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 in the present study. 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 polypropylene 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. Unanesthetized animals were restrained in well-ventilated perspex boxes and exposed whole-body to gamma radiation.

**Preparation of the Plant Extract**

*Tinospora cordifolia* was identified by a competent Botanist in Herbarium of Botany Department, University of Rajasthan, Jaipur (RUBL No. 20132). Shed dried root of the *Tinospora cordifolia* was collected, cleaned, shade dried, powdered and extracted. The extract was prepared by refluxing with double-distilled water (DDW) for 36 (12x3) hours. The cooled liquid extract was concentrated by evaporating its liquid contents to render it in powder form. An approximate yield of 22 % extract was obtained. The extract was re-dissolved in DDW just before oral administration in mice. Henceforth in this article, the extract of *Tinospora cordifolia* root extract will be called as TCE.
Source of radiation

The Cobalt teletherapy unit (ATC-C9) at Cancer Treatment Center, Department of Radiotherapy, SMS Medical College and Hospital, Jaipur was used for irradiation. Unanaesthetized mice were restrained in well ventilated boxes and exposed whole-body to gamma radiation (2.5 Gy) at the dose-rate of 117 c Gy/min from the source to surface distance (SSD) i.e. 80 cm.

Dose selection of TCE

Dose selection of *Tinospora cordifolia* was done on the basis of drug tolerance study. For this purpose various doses of TCE (25, 50, 75, 100, 150 and 200 mg/animal/day) for 5 consecutive days were tested against gamma irradiation (10 Gy) from which 75 mg/kg b.wt./day was found as an optimum dose on the basis of survival data and the same was used for the further experimentation.

Experimental design

Male mice selected from an inbreed colony were randomly divided into following groups:

*Group I* (Normal/Sham-irradiated): Mice (n=7) of this group were given double distilled water (DDW) through oral gavages once in a day for 5 consecutive days (dose equivalent to TCE).

*Group II* (TCE alone treated): Mice (n=48) of this group were treated with 75 mg/ kg b. w.t/ day of TCE dissolved in distilled water through oral gavage for 5 consecutive days once daily.

*Group III* (Irradiated Control): Mice (n=48) of this group were given distilled water for 5 days and then exposed to 2.5 Gy dose of gamma radiation. This group served as positive control.

*Group IV* (experimental): (n=48) Extract of TCE was given at the dose of 75 mg/ kg b.wt / day to mice orally for 5 consecutive days; once daily and after 30 min of last dose administration, they were exposed to 2.5 Gy gamma radiation.

Autopsy schedule

Male mice from each group (I, II, III & IV) were sacrificed by cervical dislocation at 12 hrs, and 1, 3, 7, 15 and 30 days post-treatment and their testes were excised at each autopsy interval from the sacrificed animals of each group and homogenates were prepared and estimated for various biochemical changes viz. proteins, glycogen, cholesterol and sialic acid.

Spectrophotometer (Systronics-119) was used to measure the optical densities.

Statistical analysis

The result obtained in the present study were expressed as the mean ± SE. Statistical analysis (Student’s ‘t’-test) was applied to find significant difference between values of various parameters recorded for control and treated animals. The significance levels were obtained from the table of significance of limit for the Student’s test. Distribution of the values between p< 0.001 and 0.01 were considered to be highly significant and the values between p< 0.01 and p< 0.05 were considered to be significant.
Results

**Protein:** No significant difference in the total proteins contents in testes was observed between normal and TCE treated animals throughout the experiment. However, in irradiated control animals, a statistically significant (p<0.05) decrease in proteins level was evident up to day 7th as compared to normal, and afterwards a slight increase was observed till the end of experiment. TCE treated irradiated animals also showed a similar mode of variation throughout the experiment with significant (p<0.05) increase in total proteins level with respect to irradiated control on day 3rd, 7th and 30th of post treatment, but the value remained below normal even until the end of experiment (Fig. 1).

![Graph showing variation in total proteins in testes of mice after exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE. The values represent mean ± S.E. The statistical significance was obtained between normal v/s irradiated control and irradiated control v/s experimental (p< a=0.05, b=0.01, c=0.001)](image)

**Sialic acid:** Radiation treated control group showed a considerable decline in testicular sialic acid content at all the autopsy intervals as compared to DDW and TCE alone treated groups. In TCE pre-treated irradiated mice, a recovery in sialic acid was observed up to day 7th and almost normal level (p<0.05) was noted on same autopsy interval. Thereafter, a significant decrease in sialic acid content was observed on remaining intervals (Fig. 2).
Fig 2. Variation in sialic acid in the testes of mice after exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE. The values represent mean ± S.E. The statistical significance was obtained between normal v/s irradiated control and irradiated control v/s experimental (p<α=0.05)

Cholesterol: Cholesterol level was found to be significantly higher in irradiated control animals (Group III) as compared to distilled water treated ones (Group I). It increased up to day 7th and with level of significance p<0.001, p<0.01, p<0.05 at 12 hrs, 24 hrs and 3rd day of post-treatment respectively. Afterwards, cholesterol content decreased gradually and significantly (p<0.001) till the end of experiment. Similar trend of variation in cholesterol was evident in TCE treated irradiated group and by the end of experiment the value of it was found below the normal with significant (p<0.001) decrease than the respective control group (Fig.3).

Fig 3. Variation in cholesterol in the testes of mice after exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE. The values represent mean ± S.E. The statistical significance was obtained between normal v/s irradiated control and irradiated control v/s experimental (p<α=0.05, β=0.01, γ=0.001)
Glycogen

A highly significant ($p<0.001$) elevation in testicular glycogen content was observed at all autopsy intervals in mice belonging to irradiated control group as compared to DDW and TCE alone treated groups. On the contrary, administration of *Tinospora cordifolia* extract (TCE) significantly ($p<0.05$) reduced the level glycogen in experimental mice (Group- IV) up to day 15th of post-treatment (except day 7th) in comparison to irradiated control (Group-III) but it was found above the normal glycogen value even on the 30th day of irradiation (Fig. 4).

![Fig 4. Variation in total glycogen in the testes of mice after exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE. The values represent mean ± S.E. The statistical significance was obtained between normal v/s irradiated control and irradiated control v/s experimental ($p< a=0.05$, $c=0.001$)](image)

**Discussion & Conclusions**

Ionizing radiation is one of the most horrible ecological crisis to which living beings are subjected. Thus, it is considered important to explore radio-protective compounds that would be effective, safe, and non toxic to human beings. Now-a-days, the world is looking towards radiation countermeasure agents of natural origin with a lot of hope since the synthetic compounds have not yielded desirable results at the optimum dose level. Several botanicals have been screened for their radioprotective property, mainly due to their antioxidant activity by which they effectively scavenge the free radicals generated during radiation exposure 18, 19. Therefore, an attempt has been made in the present study to screen the radioprotective activity of *Tinospora cordifolia* using various biochemical indices viz. total proteins, glycogen, cholesterol and sialic acid in testes at different intervals.
Proteins are supposed to have –SH groups at their active sites and these –SH groups are oxidized by gamma radiation causing deterioration of the protein and loss of their active sites. Administration of TCE prior to irradiation had the pattern of alterations in the total proteins concentration essentially similar to that of irradiated control. An increase in total proteins noticed on 3rd day of irradiation (119.35%) may be due to the loss of spermatogenic germ cells in testes. Thereafter, it decreased significantly on day 7th (111.60%) of irradiation. A highly significant increase in protein concentration recorded in our study at day 30th which showed an improvement in ribosomal activities that in turn is responsible for the enhanced protein synthesis in testes. This finding is in accordance with the others who have also noted a similar time-dependant decline in the total protein concentration in the testes of mice after gamma irradiation.

Sialic acid is a secretory product of epididymes in male reproductive system. After gamma irradiation, the value of sialic acid declined significantly in irradiated control and experimental animals (Groups III & IV), and maximum decreased was noted at 7th day of irradiation. The depletion observed in sialic acid may be due to radiation-induced degenerative changes in the testes as reported by others also. TCE pre treated mice showed a significant rise in sialic acid than the irradiated control which might be due to prevention of degeneration of spermatogenic germ cells.

Glycogen is a polymer of glucose residues that works as main energy source of the animal reproductive system. A highly significant (p< 0.001) increase, almost 3 times, in the amount of glycogen as compared to the normal is observed in the irradiated control group initially at 12 hrs and 24 hrs of irradiation. Thereafter, a significant decrease was noted continuously up to 15 days post-exposure possibly could be due to inhibition of glycolysis. Afterwards, it failed to sustain on later intervals and again resulted in significant increase till the end of experiment that may be due to the inhibition of glucose 6-phosphatase and phosphorylase enzymes take part in glycolysis.

Cholesterol is present in sertoli cells, spermatogonia, spermatocytes, and it works as a precursor for steroid synthesis thus maintaining normal testicular activity. In TCE pre-treated irradiated group, the value of cholesterol was found to be raised above normal at 12 hrs. and thereafter it decreased till day 3rd where the values were recorded below the normal. On 7th and 15th day of post-irradiation, a slight increase in cholesterol was noted but afterwards it again decreased significantly (p< 0.001) till day 30th and found below the normal. The reduction in testicular cholesterol content after irradiation may be linked to altered androgen synthesis. The increase in level of cholesterol may be attributed to its decreased utilization of steroidogenesis which may be due to the inhibition of glucose 6-phosphatase and phosphorylase enzymes take part in glycolysis.

The mechanism of action of herbal drugs and their extract preparation differ in many ways from those of the synthetic drugs or single substance. For radioprotection, various mechanisms such as free radical scavenging, calcium channel blocking, inhibition of lipid peroxidation, enhancement of DNA repair, and stimulation of stem cell proliferation are considered important. *Tinospora cordifolia* has some of the above mentioned properties under different experimental conditions. *T. cordifolia* is appreciated as a medicinal plant for its several biological properties like antioxidant, anti-allergic, anti-cancer, immunomodulatory, anti-neoplastic, anti-diabetic, and radio-protective properties.
The radioprotective action of TCE could be attributed to the presence of alkaloids, diterpenoid lactones, glycosides, steroids, polysaccharides, glucoside, bitter principle crystalline compounds, polyphenols which elicit protection against several stress and pathological conditions by acting through different mechanisms such as antioxidant defense system, stimulation of cell proliferation immunomodulation, anti-inflammatory activity and free radical scavenging activity. TCE has been found to inhibit the radiation-induced depletion in glutathione (GSH) and elevation in lipid per oxidation (LPO) in blood and testes of mice (unpublished data). Thus, the available antioxidants in the *Tinospora cordifolia* extract are able to cope up with the radiation-induced oxidative stress to an extant. This may be due to the synergistic effect of the available bioactive constituents present in this herb.

The above results clearly point out that *T. cordifolia* efficiently prevents radiation-induced injury in male germ cells by inhibiting the generation of ROS. Supplementation of TCE might have increased the concentration of antioxidants, which participated in scavenging of free radicals, and resulting in the protection against the radiation induced biochemical damage in the present study. The protection afforded with TCE in biochemical constituents of testis in the present study may prove to be beneficial for its clinical use as a radio protector.

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