AMELIORATION OF RADIATION MEDIATED BIOCHEMICAL ALTERATIONS IN KIDNEYS OF MICE BY *TINOSPORA CORDIFOLIA* ROOT EXTRACT

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ABSTRACT

The present study was carried out to investigate the radiation induced biochemical alterations in kidney of mice and their possible amelioration by radioprotective effect of *Tinospora cordifolia* root extract (TCE). For this purpose, adult male Swiss albino mice were divided into four groups. Group I (Sham irradiated), Group II (TCE administratered), Group III (5 Gy Irradiated control) and IV (Irradiated experimental). Animals from all the above groups were autopsied after 12 hrs, 24 hrs, 3rd day, 7th day, 15th day and 30th day of treatment. The kidney was taken out and different biochemical parameters such as total proteins, cholesterol, glycogen, lipid per oxidation (LPO), glutathione (GSH) and catalase activity were estimated. Irradiation resulted in a significant decrease in protein, cholesterol, GSH and catalase but an increase in glycogen and lipid per oxidation (LPO) as compared to normal. Conversely, TCE pretreatment reduced the radiation- induced alterations in all such parameters and the recovery was faster as compared to irradiated control. TCE pretreatment resulted in a significant increase in the proteins, cholesterol, GSH and catalase; whereas, glycogen and LPO showed a significant decrease from the respective irradiated control group at the all the autopsy intervals. Hence, the results from the present study suggest that TCE pretreatment provides protection against radiation- induced biochemical dysfunction in kidney.

KEY WORDS: Gamma radiation, Kidney, LPO, GSH, Catlase, *Tinospora cordifolia*, Swiss albino mice.
INTRODUCTION
Radiation is the most deliberated environmental hazard in the world, which exerts its deleterious effects through the generation of chemically active free radicals that in turn can damage the molecular structure resulting in cellular dysfunctions or mutations [1]. The biological effects of radiation exposure are primarily nonspecific and are dependent on the type of exposure (acute or chronic), the level of exposure, and certain biological factors.
Radiation sensitivity of different organ and tissue is directly related to the dividing capability of cells and those that replicate most rapidly are the most sensitive to radiation exposure. Kidney is moderately radio sensitive organ [2], since the epithelium of renal tubule turns over slowly [3] and is capable of regenerating after radiation- induced cytotoxic injuries [4]. Regulatory functions of the kidney maintain the stable environment of the cells necessary for them to perform their various activities and any damage to this organ may cause serious disorders in the form of various diseases. Hence, the protection of such multifunctional organ against deleterious effects of radiation is of prime concern.

Radiation- induced mortality and damage to the normal biological tissues can be partially reduced by the use of radio- protectors like solocoseryl [5] deoxyspergulin [6], lipoic acid [7], vitamins [8], diltiazem [9] and melatonin [10] that may reduce the damaging effects of radiation, including radiation-induced lethality [11]. However, the application of these radio- protective drugs in clinical radiotherapy is limited owing to their inherent toxicity at the effective doses required to obtain significant therapeutic gains and also due to the lack of differential protective activity. Therefore, it is desirable to search other alternatives that are less toxic and can offer high radioprotection.

Therefore, investigators diverted their attention towards the plant and natural products during the last two decades with the hope that one day it would be possible to find a suitable pharmacological agent(s) that could protect humans against the deleterious effects of ionizing radiation and other hazardous conditions. Medicinal plants are widely used by all sections of the society either directly as folk remedies or indirectly as pharmaceuticals preparations of modern medicine. Phytochemicals with biological activity have had great utility as pharmaceuticals and pharmacological actions [12].

One such plant, Tinospora cordifolia (family: Menenspermeaceae), is a popular remedy in India for the treatment of various types of disorders in both the Ayurvedic and folklore systems of medicine. The chemical constituents reported from this shrub belong to different
classes, such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. It is known to have hepatoprotective, hypolipideamic, rejuvenator, astringent, antiastmatic, blood purifier, antioxidants, immunomodulator, antistress, antiulcer, antineoplastic, antifibrotic, skin disease, infections etc. As far as our knowledge goes no attempt has been undertaken to investigate the effect of T. cordifolia root extract on the radiation-induced biochemical alterations in kidney of mammals.

The wide acceptability, common usage, diverse anti-oxidative and pharmacological as well as therapeutic properties of Tinospora cordifolia aroused an interest to obtain insight into its radioprotective potential against radiation induced deleterious effects on kidneys in mice.

MATERIALS & METHODS

Animal care & 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.

Source of irradiation

Animals were irradiated by a Co\textsuperscript{60} source in the cobalt therapy unit at Cancer Treatment Center, Department of Radiotherapy, SMS Medical College and Hospital, Jaipur, India. Unanaesthetized mice were restrained in well ventilated boxes and exposed whole-body to gamma radiation (5.0 Gy) at the dose-rate of 119 c Gy/min from the source to surface distance (SSD) i.e. 80 cm.

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). Root of the Tinospora
*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.

**Experimental design**

1. **Dose selection of TCE**

Dose selection of *Tinospora cordifolia* was done in our previous study on the basis of drug tolerance survival experiment [23].

2. **Modification of radiation response**

To evaluate the adverse effects of gamma rays and the possible radio-protective efficacy of TCE extract, a total of 48 animals were selected from an inbreed colony and randomly assorted into four groups of 12 mice each. Animals in group I (Normal/Sham-irradiated) were administered with double distilled water (DDW), volume equal to TCE as vehicle through oral gavage once in a day for 5 consecutive days to serve as normal. Mice in group II (Negative control) were administered with 75 mg/ kg b. w.t/ day of TCE dissolved in double distilled water through oral gavage for 5 consecutive days once daily. In group III (Irradiated Control), double distilled water volume equal to TCE was administered for 5 consecutive days (as in Group-I) and then exposed to 5.0 Gy dose of gamma radiation. This group served as irradiated positive control. Mice in Group IV (Experimental) were treated with TCE, orally for 5 consecutive days (as in Group-II) and after 30 min of the last dose administration on day 5th such animals were exposed to 5.0 Gy gamma radiation.

3. **Autopsy schedule**

Animals from all the above treated groups (I, II, III & IV) were regularly observed till 30 days for their weight change, any sign of sickness, morbidity, fur and skin changes, behavioral toxicity, any visible abnormalities and mortality. A minimum of 6 animals from each group were necropsied at 12 hrs, 1, 3, 7, 15 and 30 days post-treatment to evaluate various biochemical parameters.

4. **Biochemical study**: Both (Right and left) kidneys were collected and polled from each necropsied animals at each autopsy interval, homogenate was prepared, and glycogen, protein
and cholesterol content were measured using Montogomery (1957) [24], Lowery et al (1951) [25] and Burchard (1959) [26] methods, respectively. The level of LPO, glutathione (GSH), catalase and SOD in kidney was determined by methods of Ohkawa et al (1979) [27], Moron et al (1979) [28], Abei (1984) [29], Marklund et al (1974) [30], respectively.

Statistical analysis

The result for all the groups at various necropsy intervals were expressed as mean ± Standard error (S.E.). To find out whether mean of sample drawn from experimental (group IV) deviates significantly from respective control (group III), Student's ‘t’ test was used. The significance level was set at different levels as P < 0.05, P < 0.01 and P < 0.001.

RESULTS

In the current study, TCE alone treated mice showed an insignificant variation in various biochemicals (glycogen, proteins, cholesterol) as well as in anti-oxidative parameters (LPO, GSH and catalase) as compared to normal/Sham irradiated animals throughout the experiment. (Fig. 1-7).

Fig. 1   Variations (mean ± S.E.) in the levels of testicular glycogen (mg/gm) of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively. Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: a p ≤ 0.05, b p ≤ 0.01, c p ≤ 0.001.

Renal glycogen content exhibited a similar mode of variation in both irradiated control as well as in experimental group but the magnitude of alterations was fairly lesser in experimental group. A gradual and incessant elevation in glycogen content was recorded up
to day 7\textsuperscript{th} of post-exposure in irradiated control animals, being significantly (P<0.001) higher (116.16\%) than the respective experimental group. Afterwards, a noticeable depletion in glycogen was recorded at later intervals and the observed values were 212.73 \% and 106.13\% higher on the last autopsy interval (Fig. 1).

Radiation exposure resulted in a noticeable increase in proteins content in kidneys, which increased up to day 7\textsuperscript{th} of post exposure in both experimental and irradiated control groups (158.23±9.11 & 137.23±8.01 respectively) being significantly higher (P<0.01) in control group than the respective experimental group. Afterward, these values started to decrease in both the groups and tended to be normalized. Although the values were significantly lesser (P<0.001) in experimental animals than the irradiated controls, but the normal level could not be restored even till the end of study and observed as 101.88 \% higher than the normal (Fig. 2).

![Fig. 2 Variations (mean ± S.E.) in the levels of testicular total proteins (mg/gm) of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively. Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: a p ≤ 0.05, b p ≤ 0.01, c p ≤ 0.001.](image)

Renal cholesterol content, in irradiated control animals, showed a considerable decreases after irradiation up to day 7\textsuperscript{th} (1.19±0.008; p<0.01) followed by a significant increase, however, the values were fairly lesser than the normal (26.66 \%) even till the last autopsy interval. TCE treated irradiated animals also experienced the similar mode of alterations, but the magnitude of reduction was rather lower than the respective irradiated control throughout the experiment. In experimental animals, recovery process was recorded from day 7\textsuperscript{th} day
onwards, and almost normal level (3.67±0.20) was started at the last autopsy day (i.e. 30th day) (Fig. 3).

Fig. 3 Variations (mean ± S.E.) in the levels of testicular cholesterol (mg/gm) of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively. Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: a p ≤ 0.05, b p ≤ 0.01, c p ≤ 0.001.

Fig. 4 Variations (mean ± S.E.) in the levels of testicular lipid per oxidation (nmole/mg) of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively. Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: a p ≤ 0.05, b p ≤ 0.01, c p ≤ 0.001.
Radiation exposure resulted in a considerable increase in kidney LPO level up to day 15th and 7th of exposure in irradiated control (5.87±0.13; p<0.001) and experimental animals (4.5±0.12; p<0.05), respectively. Thereafter, the values started to increase in both the groups (III & IV), but the normal level could not be restored even till the end of experimentation and the same were found to be 157.43 % and 116.11% higher than the normal, respectively (Fig.4).

A continuous decrease in glutathione level was recorded up to day 7th in both irradiated control (1.78±0.12; p<0.05) as well as experimental group (2.56±0.10; p<0.01), and the observed values were found 51.63 % and 30.43% lower than the normal, respectively. However, the extent of reduction was comparatively lower in experimental group than the respective irradiated control at all autopsy intervals. After day 7th, a significant elevation in GSH was recorded at the remaining intervals but the values were measured quite below from the normal (Fig. 5).

Super oxide dismutase (SOD) and catalase activities were also found to be decreased up to day 7th of post- exposure in irradiated control animals, and were recorded as 1.18±0.09 (p<0.001) and 1.23±0.07 (p<0.05), respectively. Correspondingly, TCE pretreatment also resulted in the decreasing pattern similar to irradiate controls and 7th day onwards a significant elevation in both the parameters was recorded, and almost normal levels were
recovered by the end of experiment, where the observed values were 0.64% and 2.26 % lesser from the normal (Fig.6 & 7).

Fig. 6   Variations (mean ± S.E.) in the activity of testicular superoxide dismutase (mole/g tissue) of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively. Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: $^a$p ≤ 0.05, $^b$p ≤ 0.01, $^c$p ≤ 0.001.

Fig. 7   Variations (mean ± S.E.) in the activity of testicular catalase (U/mg tissue) of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively. Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: $^a$p ≤ 0.05, $^b$p ≤ 0.01, $^c$p ≤ 0.001.

**DISCUSSION**

The results of present investigation clearly revealed that radiation exposure causes considerable increase in lipid per oxidation level with subsequent decrease in redox agents
GSH, SOD and catalase in kidneys. ROS such as hydrogen peroxide, the superoxide anion, and hydroxyl radicals are generated under normal cellular conditions and are immediately detoxified by endogenous antioxidants like GSH, catalase and superoxide dismutase, but excessive ROS accumulation by irradiation causes an antioxidant status imbalance and leads to lipid peroxidation and GSH depletion\[^{31}\].

Decreased glutathione level has generally been considered as an index of increased formation of ROS that might be due to an increase in expression of mRNA for \(\gamma\)-glutamylcysteine synthetase, a rate limiting enzyme in GSH synthesis \[^{32, 33}\]. Since, GSH acts as potential free radical scavenger, therefore immediate depletion after radiation exposure reflects that it acts as first line of defense to protect the cell against the increased oxidative stress. Radiation induced oxidation of lipids can reduce membrane fluidity; inactivation membrane bound proteins loss of polyunsaturated fatty acids and enzymatic activities that ultimately leads to cell death. This increase in LPO with following decrease in GSH level in kidney after radiation exposure is in agreement with the findings of Bhatiya and Manda \[^{34}\] and Pathak et al \[^{35}\].

Moreover, lowered activities of SOD and CAT at all the autopsy intervals in irradiated control animals as compared to normal may result in the accumulation of these highly reactive free radicals leading to deleterious effects such as loss of membrane integrity and membrane function (Reedy & Lokesh, \[^{36}\]). Catalase has been reported to be responsible for the detoxification of \(H_2O_2\), which is an effective inhibitor of SOD, therefore, decreased SOD activity may be related with CAT inactivation \[^{37}\].

Preservation of such antioxidative alterations and cellular membrane integrity depend on protection or repair mechanisms capable of neutralizing oxidation reactions. It was observed that when TCE given in combination with radiation significantly prevents the radiation induced elevation in lipid peroxidation in renal tubular membranes, as there is statistically significant difference between irradiated control and experimental animals. Quercitin and gallic acid have been reported in \(T. cordifolia\), whose anti-oxidative mechanism has been suggested to be singlet oxygen quenching, free radical scavenging and chain breaking during lipid per oxidation \[^{38, 39}\]. Contrary the observed restoration of SOD and catalase activities in TCE pretreated irradiated animals may be due to direct stimulatory effects of TCE on SOD and CAT. Furthermore, the decreased in tissue LPO level after TCE pretreatment can also be correlated with the elevated SOD and CAT activities. Besides this lower depletion of kidney
GSH in TCE pretreated group could be due to higher availability of GSH and modulation of cellular antioxidant levels, which increase the ability to cope with the free radicals produced by radiation. Similarly, TCE pretreatment exhibited protection against radiation induced alterations in all such anti-oxidative parameters in testes \[^{[40]}\], liver, intestine and blood as well (unpublished data).

The elevated level of glycogen content in kidney at early intervals may be owing to destructive action of radiation on tissue and enzymatic activities which leads to higher substrate availability for the synthesis of kidney glycogen \[^{[41]}\]. The elevation in glycogen concentration decreased at later autopsy interval which may be due to the recovery in renal tubular architecture at such post-irradiation intervals. Furthermore, hepatic glycogen content was also found elevated after exposure with similar dose (5.0 Gy) of gamma radiation, which might be due to increased gluconeogenesis (unpublished data). Since cholesterol is an important constituent of the cell membrane and a precursor for the steroid hormone, radiation induced depletion up to day 7\(^{th}\) may be related to either a disruption of plasma membrane and/or altered steroidogenesis \[^{[42]}\]. These findings confirm the observations of Purohit et al \[^{[43]}\] and Chakrawarti et al \[^{[44]}\]. The present study revealed that irradiation resulted in continuous augmentation in total proteins in renal tissue up to day 7\(^{th}\) that probably as a result of an increased transport of amino acid through plasma membrane as a consequence of permeability changes in irradiated cell membrane \[^{[45]}\]. In addition, increased synthesis of m-RNA and ribonucleoprotein could also be added to the radiation induced increased level in proteins as documented by others also \[^{[43, 44]}\].

Simultaneously, TCE pretreatment exhibited a noticeable recovery against radiation induced elevated level of glycogen, cholesterol and proteins throughout the experiment, that indicating anti-radiation and anti-oxidative property which may be ascribed to the combined protective effect of different phytochemical constituents which are present in its root extract. \(T. \ cordifolia\) has been reported to contain alkaloids (choline, tinosporin, isocolumbin, palmatine, tetrahydropalmatine, magnoflorine) \[^{[46]}\], diterpenoid lactones (furanoalclone, tinosporon, tinosporides) \[^{[47]}\], glycosides \[^{[48]}\], steroids \[^{[49]}\], polysaccharides \[^{[50]}\], bitter principle crystalline compounds \[^{[51]}\], polyphenols (3-glucosides, gallic acid, tannins), flanonoids (quercetin) \[^{[52]}\] and triterpenoids compounds. Most of these bioactive compounds have been reported to possess strong antioxidants, immunomodulatory activity and provokes free radical scavenging enzymes system \[^{[53, 54]}\]. This contention is further supported by the
experiments on free radical scavenging, where *T. cordifolia* has been found to scavenge radiation mediated OH, and O$_2^-$ radicals (Goel *et al.* [55] and Tyagi *et al.* [52].

Antioxidants are bioactive compounds that can delay or inhibit oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Velioglu *et al*., 1998) [56]. Plant phenolics such as flavonoids constitute one of the most diverse and widespread groups of natural compounds acting as primary antioxidant free radical terminator (Agrawal, 1989 [57], Shimoi, [58]. A strong relationship between total phenolic content and antioxidant activity in some other herbal plants has been reported by some other investigators also [59–62]. Radioprotective and antioxidantive effects of various other medicinal plants and related natural products have also been reported (Tripathi, Mohan & Kamat, [63]. Furthermore, *T. cordifolia* has also been reported to have diuretic effects [64] and effective in modulation of morphology and some gluconeogenic enzymes activity in diabetic rat kidney [65].

**CONCLUSIONS**

From the present study, it is obvious that scavenging of free radicals and increased concentration of endogenous antioxidant system along with other different mechanisms such as anti-oxidation, free radical scavenging and inhibition of lipid peroxidation are considered important to ameliorate radiation-induced deleterious effects in terms of different biochemical alterations in kidneys on account of the synergistic impact of various bioactive constituents present in *T. cordifolia* root extract.

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**CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interest regarding the publication of this paper.
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