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Research Article

MODULATION OF BIOCHEMICAL AND ANTIOXIDANT ENZYMES IN BLOOD BY *TINOSPORA CORDIFOLIA* AGAINST GAMMA RADIATION MEDIATED DAMAGE IN MICE

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ABSTRACT

Objective: The present investigation has been carried out to evaluate the possible radio- protective potential of root extract of *Tinospora cordifolia* (TCE) against 2.5 Gy gamma radiations in adult Swiss albino mice.

Methods: For this purpose, healthy Swiss albino male mice were selected from an inbred colony and divided into four groups. Group I (normal) was administered double distilled water (DDW) volume equal to TCE (75 mg/kg body weight [b.wt]/animal) by oral gavage. Group II was orally supplemented TCE as 75 mg/kg b.wt once daily for 5 consecutive days. Group III (irradiated control) received DDW orally equivalent to TCE for 5 days then exposed to 2.5 Gy gamma radiation. Group IV (experimental) was administered TCE as in Group II and exposed to radiation (as in Group III). Biochemical alterations were observed in the blood of mice at various post-irradiation intervals between 12 hrs and 30 days.

Results: The irradiation of mice caused considerable decrease in the level of total proteins, glutathione, catalase, and superoxide dismutase activity along with significant increase in cholesterol, lipid peroxidation (LPO). Whereas, administration of TCE prior to irradiation enhanced the activity of various antioxidant enzymes and reduced the radiation- induced variations in total proteins, cholesterol and LPO levels in the blood serum.

Conclusions: Hence, the data of present investigation indicate that *T. cordifolia* root extract reduce the bioeffects of gamma radiation in mammals.

Keywords: Gamma radiation, *Tinospora cordifolia*, Swiss albino mice, Blood, Antioxidant enzymes.

INTRODUCTION

The global environmental contamination is accountable for the exposure of living beings to the influence of a variety of technogenic factors, together with ionizing radiation. The inadvertent exposure of human to radiation causes ionization of molecules, the explosion of potentially damaging reactions by means of free radical production [1]. Whole - body exposure to ionizing radiation damages multiple tissues concurrently leading to the manifestation of multi organ syndrome or radiation syndrome whose onset, nature, and severity are a function of both total radiation dose and radiation quality. To combat such a syndrome we need to develop a protective formulation that is effective in multidirectional manner to living cells.

In spite of intense global research in the field of radioprotection, no molecular or synthetic drugs of present day (comprising of single molecule or a small group of molecules) has been able to meet out the criteria of a clinically acceptable radio protector because of accompanying toxic effects to one or more vital body systems at the effective concentration [2-4]. This has shifted the focus of researchers towards natural compounds as radio protectors. A phyto therapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants [5].

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society either directly as folk remedies or indirectly as pharmaceutics preparation of modern medicine [6]. Several herbals and their formulations have been screened for their radio protecting properties, mainly due to their antioxidant activity by which they effectively scavenge the highly reactive and dangerous molecular species called free radicals in cells and tissue generated during radiation exposure [7-9].

In this context, *Tinospora cordifolia* (Family: Menisspermaceae) is a large climbing shrub that has a long history of medicinal values in Ayurvedic system of medicine as it has been used for centuries for wide range of diseases including diabetes mellitus, rheumatoid arthritis, general weakness, anemia, and infections [10]. This plant is commonly known as guduchi, giloy or amrita, which are Hindu mythological terms that refer to the heavenly elixir that have saved celestial beings from old age and kept them eternally young [11].

A variety of constituents have been isolated from *T. cordifolia* that belonging to different classess such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpnoides, phenolics, aliphatic compounds and polysaccharides [12]. The literature survey revealed that *Tinospora* possess a plethora of benefits under various experimental conditions. The bitter principle shows adaptogenic, antispasmodic, anti-inflammatory, anti- pyretic, anti- neoplastic, hypolipidemic, hypoglycemic, antioxidant, immunopotentiating, and hepatoprotective properties [13]. It is also used in digestive disturbance, loss of appetite and fever in children [14], and root is known for its anti- stress, anti- leprotic and anti- malarial activities [15,16].

Therefore, based on the above pharmacological and therapeutic properties, present study was intended to investigate radio modulatory potential of the *Tinospora cordifolia* extract (TCE) in terms of radiation-induced biochemical lesions in blood of mice.

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 g from an inbred colony, were used in the present study. They were maintained under controlled conditions of temperature and light (14 and 10 hrs 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. 4-6 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-60 source in the cobalt teletherapy unit at Cancer Treatment Center, Department of Radiotherapy, SMS Medical College and Hospital, Jaipur, India. Unanaesthestized mice were restrained in well ventilated boxes and exposed whole-body to gamma radiation (2.5 Gy) at the dose- rate of 221 c Gy/min from the source to surface distance i.e., 80 cm.

Preparation of the plant extract

T. cordifolia was identified by a competent Botanist in Herbarium of Botany Department, University of Rajasthan, Jaipur (RUBL No. 20132). Root of the *T. cordifolia* was collected, cleaned, shade dried, powdered and extracted. The extract was prepared by refluxing with double-distilled water (DDW) for 36 (12 × 3) hrs. 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 *T. cordifolia* root extract will be called as TCE.

Experimental Design

Dose selection of TCE

Dose selection of *T. cordifolia* was done in our previous study on the basis of drug tolerance survival experiment [17].

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 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.wt/day of TCE dissolved in DDW through oral gavage for 5 consecutive days once daily. In group III (Irradiated Control), DDW volume equal to TCE was administered for 5 consecutive days (as in Group-I) and then exposed to 2.5 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 minutes of the last dose administration on day 5th such animals were exposed to 2.5 Gy gamma radiation.

Autopsy schedule

Animals from all the above treated Groups (I-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 the biochemical parameters.

Biochemical study

Biochemical alterations were studied in animals of all the groups at 1 hr post-exposure to gamma radiation. The level of total proteins and cholesterol were evaluated by Lowry *et al*., (1951) [18] and Rudel and Morris (1973) [19], respectively. The level of LPO, glutathione (GSH), catalase and superoxide dismutase (SOD) in blood was determined by methods of Ohkhawa *et al*., (1979) [20], of Beutler *et al*., (1963) [21], Abei (1984) [22], Marklund *et al*., (1974) [23], respectively.

Statistical analysis

The result for all the groups at various necropsy intervals were expressed as mean ± standard error (SE). 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 by the method of Bourke *et al*., (1985) [24]. The significance level was set at different levels as $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

TCE alone treated mice exhibited an insignificant variation in biochemical parameters (*viz*. Total proteins, cholesterol) and anti-oxidative parameters (lipid peroxidation [LPO], GSH, SOD and catalase) than the normal/sham irradiated ones, from 1 to 30 days of post-treatment time. It indicates that *T. cordifolia* treatment did not bring any significant alterations in all such parameters throughout the experimental period.

A non-significant decrease in total proteins level was observed on the first autopsy interval (i.e., 12 hrs) post-irradiation but a significant decline ($p\leq0.001$) was observed at 24 hrs and day 3rd post-treatments with its minimum value on 3rd day (4210±16.81). After this, the protein level elevated and but failed to maintain the normal level even till the last autopsy day. In TCE pretreated irradiated mice, a gradual recovery in total proteins was observed at all the treatment intervals and a value extremely close to normal was restored by day 30th (4718±19.42; 99.49%) post-exposure (Fig. 1).

Cholesterol content in blood plasma irradiated control animals, showed a considerable augmentation after irradiation up to day 3rd (134.04±1.12; $p<0.001$) followed by a significant depletion, however the values were fairly higher than the normal (135.11%) 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, the recovery process was recorded 3rd day onwards, and almost normal level (72.64±1.02) was recovered at the last autopsy day (i.e., 30th day) (Fig. 2).

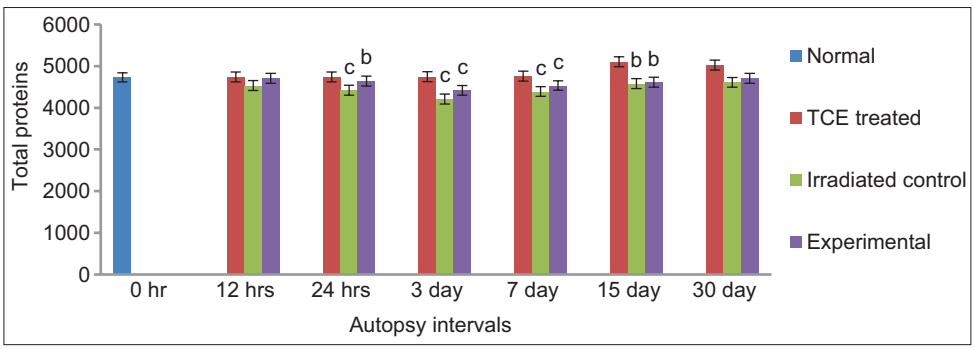

Fig. 1: Variations (mean ± standard error) in total proteins levels in the blood of mice after the exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) extract of *Tinospora cordifolia*, respectively. Statistical analysis: Control versus normal; experimental versus control; significance levels: $^{a}p\leq0.05$, $^{b}p\leq0.01$, $^{c}p\leq0.001$

Radiation exposure resulted in a considerable rise in LPO level in blood, which increased up to day 7 in both experimental and control groups (5.94±0.21 and 4.87±0.12, respectively) being significantly higher ($p<0.001$) in control group than the respective experimental group. Afterward, these values started to decrease in both the groups and tend to be normalized. Although the values were significantly lower ($p<0.01$) in experimental animals than irradiated controls, but the normal level could not be recovered even by the end of experimentation and found 109.56% higher than the normal (Fig. 3).

GSH level in blood was found to be significantly decreased up to day 7^{th} (2.40±0.10; $p<0.01$) in irradiated control followed by a significant increase till the last autopsy day. While, TCE pretreated animals exhibited a statistically significant elevation in glutathione as compared to irradiated controls, and almost normal value was regained at the end of the experiment i.e., day 30^{th} (4.24±0.12 mg/100 ml) (Fig. 4).

A continuous decrease in SOD level was recorded up to day 7^{th} in both irradiated control (1.45±0.03; $p<0.001$), as well as experimental group

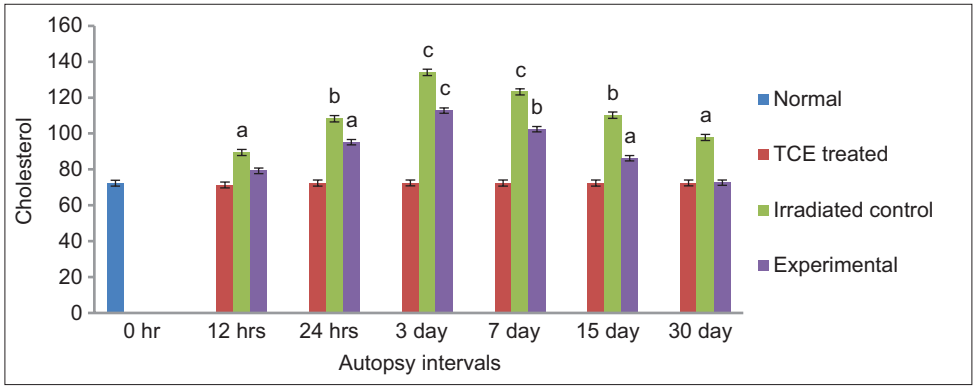

Fig. 2: Variations (mean ± standard error) in cholesterol levels in the blood of mice after the exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) extract of *Tinospora cordifolia*, respectively. Statistical analysis: Control versus normal; experimental versus control; Significance levels: [a]p≤0.05, [b]p≤0.01, [c]p≤0.001

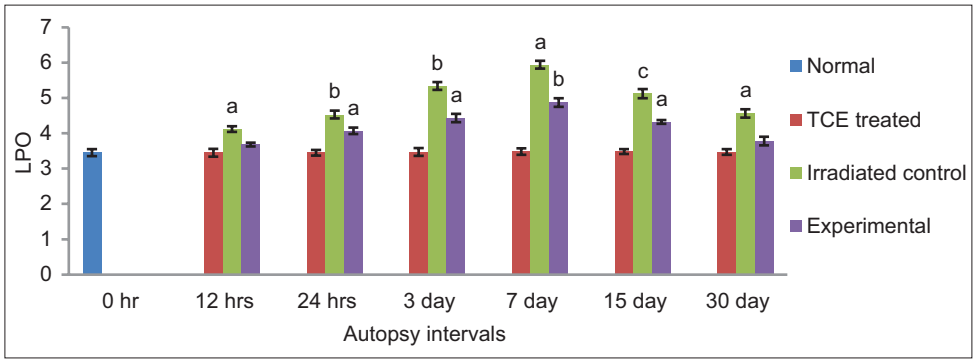

Fig. 3: Variations (mean ± standard error) in lipid peroxidation levels in the blood of mice after the exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) extract of *Tinospora cordifolia*, respectively. Statistical analysis: Control versus normal; experimental versus control; Significance levels: [a]p≤0.05, [b]p≤0.01, [c]p≤0.001

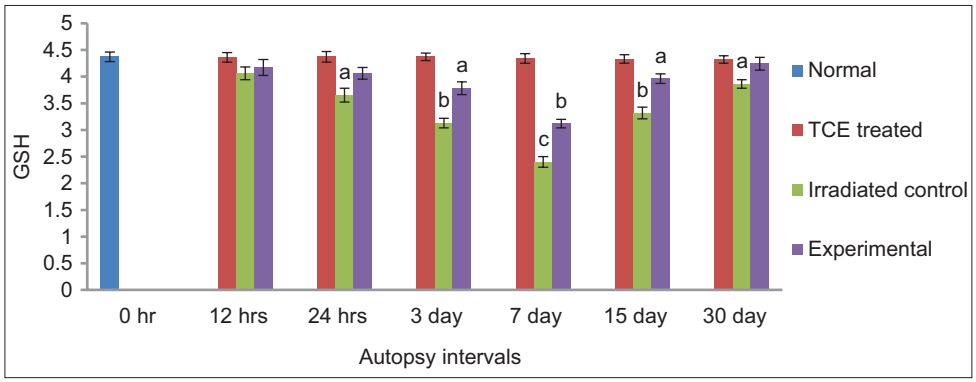

Fig. 4: Variations (mean ± standard error) in glutathione levels in the blood of mice after the exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) extract of *Tinospora cordifolia*, respectively. Statistical analysis: Control versus normal; experimental versus control; significance levels: [a]p≤0.05, [b]p≤0.01, [c]p≤0.001

(2.64±0.04; p<0.01), and the observed values were found 64.80% and 35.92% lower than the normal, respectively. However, the extent of reduction was comparatively lower in the experimental group than the respective irradiated controls at all autopsy intervals. Thereafter, a significant elevation was recorded at the remaining intervals but the values were quite below (2.18%) than the normal (Fig. 5).

Catalase activity in irradiated control animals, showed a considerable decreases after irradiation up to day 7 (1.63±0.02; p<0.001) followed by a significant increase, however the values were fairly lesser than the normal (17.72%) 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, the recovery process was recorded 7th day onward, and almost normal level (3.68±0.10) was recovered at the last autopsy day i.e., 30th day (Fig. 6).

DISCUSSION

Exposure of the living organisms to radiation produces ionization in the tissues and damages the cell membranes in such a manner that these not only loose certain compounds but also are unable to take them from surrounding extra cellular medium causing biochemical changes. The biochemistry of the blood is directly associated to the functional capacity of the blood which is linked with the status of the blood components.

In the current study, the values of total plasma proteins were not much altered after 2.5 Gy irradiation as compared to normal/sham irradiated animals. This is in close agreement with the findings of Singh *et al*., (2005) [9] and Chaudhary (2009) [25] who also did not observe any significant alterations in plasma protein level after the similar radiation dose range. A depression in number of ribosome after irradiation due to decrease in RNA synthesis and loss of proteins from gastro-intestinal tract, kidney, liver or from thermal injury to skin can be attributed to decreased plasma protein content in the present investigation as also suggested by others also Keren (1994), Hampton (1966), Kumar (1981) [26-28].

Contrary to proteins, plasma cholesterol exhibited a continuous augmentation up to day 7th which may be due to the increased ability of the liver to bio-synthesize cholesterol [29] as well as to the decreased activity of cholesterol 7-hydroxylase, the key enzyme involved in degradation of cholesterol in the liver, as mentioned by Chupukcharoen *et al*. (1985) [30]. These observations substantiate with early findings of Edrees *et al*. (2008) [31], Gupta (2009) [32] where an increased plasma cholesterol level was noted in mice after irradiation.

TCE mediated improvement in plasma proteins as compared to irradiated control, could be due to the physiological role of its antioxidants in minimizing radiation-induced injuries, particularly to cell membrane and increased ribosomal activities, which enhanced the protein synthesis and this explains the radio protective effect of TCE to some extent. Increase in protein concentration with supplementation of various medicinal plants extract after gamma irradiation was reported earlier also by some investigators [33]; Bhatia *et al*., 2008 [34] whereas, decreased plasma cholesterol concentration, as compared to irradiated control, recorded in the present study in TCE supplemented irradiated mice indicates a radio-protective effect that possible could be due to the anti- oxidative properties of alkaloids present in its root extract such as

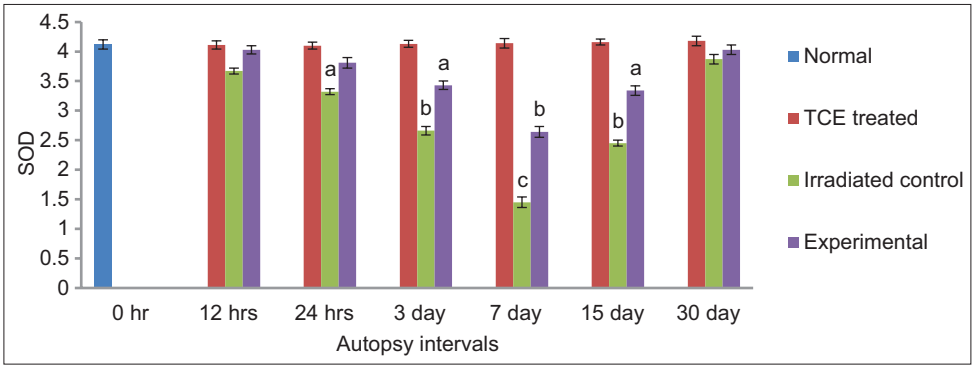

Fig. 5: Variations (mean ± standard error) in superoxide dismutase activity in the blood of mice after the exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) extract of *Tinospora cordifolia*, respectively. Statistical analysis: Control versus normal; experimental versus control; significance levels: [a]p≤0.05, [b]p≤0.01, [c]p≤0.001

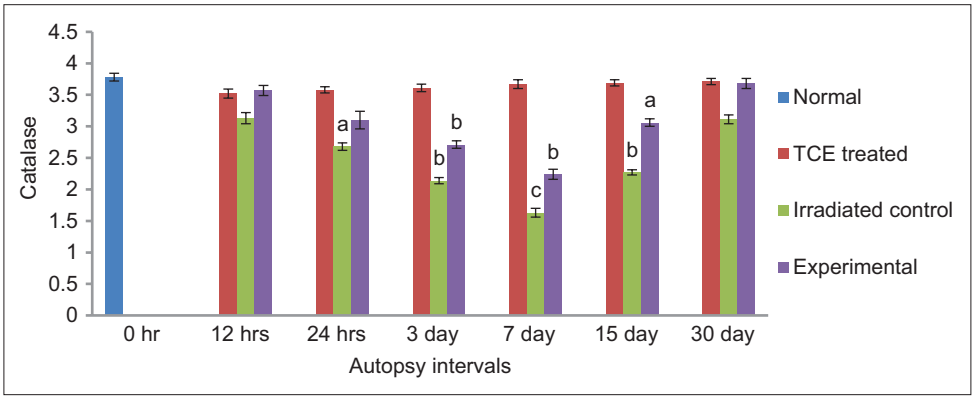

Fig. 6: Variations (mean ± standard error) in catalase activity in the blood of mice after the exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) extract of *Tinospora cordifolia*, respectively. Statistical analysis: Control versus normal; experimental versus control; significance levels: [a]p≤0.05, [b]p≤0.01, [c]p≤0.001

choline, tinosporin, isocolumbin, palmatine, tetrahydropalmatine, and mgnoflorine [35]. These finding are in close proximity with Baliga *et al*. (2004) [36] and Gupta (2009) [32], who also reported that the alkaloid lower down the level of plasma cholesterol. In spite of this they also suggest that alkaloid exhibited a dose and time dependent cholesterol anti-peroxidative effect.

Ionizing radiations are identified to damage the biological system by producing reactive oxygen and an imbalance between pro-oxidants and endogenous antioxidants in the cellular milieu as reported by Faidan *et al*., 2008 [37]. Whole-body radiation exposure resulted in degradation of bio- molecules, irreversible cellular dysfunctions and ultimately cell death. The quantum of antioxidants in the biological system presented at the time of exposure can be determined the extant of such damages as cited by Hawlliwell and Gutteridge (1989) [38]. Thus the availability of endogenous enzymatic antioxidants and non- enzymatic entities along with exogenous agents that can retard reactive oxygen species (ROS) generation or scavenge them to restore normal redox state, may have the potentiality to mitigate radiation mediated such lethal damages.

In the present study, radiation exposure resulted in continuous and significant reduction in the GSH level as well as in SOD and CAT activities in blood as compared to normal/sham irradiated animals. GSH with its sulfhydryl group functions in the maintenance of sulfhydryl groups of other molecules (especially proteins), as a catalyst for disulfide exchange reactions, and in the detoxification of foreign compounds, hydrogen peroxide and free radicals [39]. Similarly, SOD is an antioxidant enzyme which mops up free-radicals and protects oxygen-metabolizing cells against harmful effects of free-radicals [15]. In normal conditions, the cells are intact and healthy, and GSH is restored by synthesis, but following irradiation normal synthesis/repair is disrupted and reduced the GSH activity. Radiation induced depletion in GSH level has been reported to promote ROS generation that promptly affect the functional and structural integrity of cells as also observed by other researchers also [40-42]. A major cellular defense against ROS is provided by SOD and catalase, which together convert O_2– first to H_2O_2, and then to water and molecular oxygen [43].

Pre-treatment with TCE provided noticeable recovery in radiation induced elevation in SOD and catalase activity in plasma which provide reasonable radioprotection to the organism and increase the tolerance of animals through the detoxification of ROS. Pretreatment of TCE did not significantly influence the endogenous GSH levels in blood, but its mere presence protects the radiation induced endogenous GSH depletion that could be due to higher availability of GSH, which increases the ability to cope with the free radicals produced by radiation or it might be due to less utilization of GSH in TCE pretreated animals.

Due to depletion in antioxidant levels, the free radicals are not neutralized and lipid content of irradiated organs show enhanced susceptibility to oxidation and as a result lipid peroxides formed that can reduce membrane fluidity, inactivation of membrane-bound proteins and decompose into cytotoxic aldehyde like melondialdehyde [44]. The increase in LPO may also cause an increase in the antioxidant defense status to regulate the cellular homeostasis. TCE pretreatment resulted in a significantly reduced level of LPO level as compared to respective irradiated controls at all the autopsy intervals that may be due to the anti- oxidative as well as O_2– andsinglet oxygen quenching ability of polyphenol and flavonoids contents which present in its root extract. Some earlier studies from our laboratory also reported that antioxidants present in other synthetic and natural products have been found to reduce the increased level of LPO [45-48].

The antioxidant activity of a compound has been attributed to various mechanisms among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [49]. Although the exact mechanism of action of TCE is not known but it can be said that the above radio protective action against radiation induced hematopoietic injury cannot be attributed to a single mechanism but several mechanisms may be operational simultaneously for effective results. Since *T. cordifolia* has been reported to possess several medicinal properties some of them are anti- fibrotic, anti- oxidant, anti- inflammatory, immunomodulatory, radio protective, activator of phagocytic and killing activity of macrophages, anti-diabetic, anti- pyretic, anti-spasmodic, anti-arthritic, anti-allergic, anti-stress, anti-leprotic, anti- malarial, hepato-protective, anti-neoplastic, hypolipidemic, rejuvenator, astringent, anti-asthmatic, bitter tonic, blood purifier, anti- ulcer, memory booster etc. [35,50-55].

Most of the bioactive constituents which were present in the TCE have been reported to provoke free radical scavenging enzymes system [56,57]. This contention further confirmed the observation of Goel *et al*., 2004 [58] who carried out the experiments on free radical scavenging, where *T. cordifolia* has been found to scavenge radiation mediated OH, and O_2– radicals. *T. cordifolia* contain high concentration of polyphenols, chiefly flavonol-3 glucosides [59], along with gallic acid and tannins which provides maximum conjugation with ROS, RNS, and inhibiting the activity of many enzymes and nuclear transcription factor, thus reducing the number of free radicals available and extent of cellular damages produced in the body by radiation [60]. Furthermore, quercetin (flavonoid) is also very effective scavengers of hydroxyl and peroxyl radicals raises the possibility that TCE extract may act as protective factors against radiation mediated DNA damage [61]. *Podophyllum hexandrum, Ocimum sanctum, Ginko biloba, and Mentha piperita* are among some herbal plants which execute their radioprotective properties mainly due to the presence of polyphenols and flavonoids [62-65]. Radioprotective and antioxidantive effects of various other medicinal plant and related natural product have also been reported [66]. Looking towards these promising results it can be said that guduchi is a potent drug entity which should enter the world market by evidenced based research for therapeutics.

CONCLUSIONS

Therefore, the present study suggested that deleterious effects of radiation on biochemical section of blood may be reduced by synergetic action of bioactive constituents present in the *T. cordifolia* root extract such as quercetin, gallic acid, flavonoids and alkaloids, which shows strong free radical and antioxidant activities.

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