

**EVALUATION OF RADIOMODULATORY INFLUENCE OF *TRIBULUS TERRESTRIS*
ROOT EXTRACT AGAINST GAMMA RADIATION: HEMATOLOGICAL,
BIOCHEMICAL AND CYTOGENETIC ALTERATIONS IN SWISS ALBINO MICE**

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Summary

The radiomodulatory influence of *Tribulus terrestris* root extract against radiation induced alterations Swiss albino mice was studied at 6 hr and 24 hr post-irradiation intervals. The oral administration of *Tribulus terrestris* root extract (800 mg/kg body weight) prior to whole body irradiation showed a significant protection in terms of hematological, biochemical and cytogenetic parameters. Mice exposed to radiation (8.0 Gy) without *Tribulus terrestris* root extract pre-treatment showed a significant decline in hematological constituents (RBCs, WBCs, Hb and Hct) at 6 hr and 24 hr autopsy intervals. Conversely, animals pre-treated with *Tribulus terrestris* root extract showed a significant recovery in the hematological values. A significant decrease in hepatic reduced glutathione (GSH) content and increase in lipid peroxidation (LPO) level was observed in control animals (radiation alone). However, *Tribulus terrestris* root extract pretreated irradiated animals exhibited a significant increase in GSH content and decrease in LPO level. A significant decrease in chromosomal aberrations and micronucleus frequencies was also observed in experimental group as compared to radiation alone group. The results from the present study suggest that hematopoietic stem cells can be protected from radiation induced free radical damage by *Tribulus terrestris* root extract, which was evident in hematological constituents in peripheral blood and cytogenetic parameters in bone marrow cells of mice.

Key words: Radiomodulatory influence, *Tribulus terrestris*, hematological constituents, reduced glutathione, lipid peroxidation, chromosomal aberrations and micronucleus frequencies.

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INTRODUCTION

The medicinal herb *Tribulus terrestris* Linn. (Family : Zygophillaceae) is native to the mediterranean, tropics, subtropics and temperate regions of the world has been subjected to long term clinical trails in "AYURVEDA". The fruits of *Tribulus terrestris* are credited with aphrodisiac, diuretic, cooling, demulcent and tonic properties, which are used to treat kidney stones, painful urination, genito-urinary disorders, diabetes, piles, rheumatism, dropsy, breathing difficulties, heart disease and impotence. The root is considered to have tonic properties, and is a constituent of the Ayurvedic preparation Dasamula (1, 2). *Tribulus terrestris* possesses anti-inflammatory, smooth muscle relaxation and diuretic actions, which are useful in genitourinary infections, painful micturation, hematuria, dysuria and benign prostatic hyperplasia (3, 4). It is an herbal nutritional supplement that is promoted to produce large gains in strength and lean muscle mass (5). It has been reported that *Tribulus terrestris* contains saponins, quercetin, kaempferol and rutin which are known to have antioxidant and anticancer properties (6). Two new steroid saponins named terrestrinins A(1) and B(2), along with furostanol, gigenin, hecogenin, ruscogenin, gitogenin and tigonin were isolated from the *Tribulus terrestris* (7, 8). Crude extract having phenolic content from *Tribulus terrestris* was screened for its *in vitro* antioxidant and antimicrobial properties (9). The diuretic activity of *Tribulus terrestris* helps relieve associated sub clinical urinary insufficiency and also helps to reduce post-void urine. Also, it possesses antibacterial activity against susceptible microorganisms and helps prevent symptoms of urethritis and prostatitis (10). *Tribulus terrestris* L. saponin (TTLS) can decrease the apoptosis in cortical neurons induced by hypoxia and reoxygenation (11). Anticancer properties of *Tribulus terrestris* have been reported on various cell lines *i.e.* mouse sarcoma 180 (ASC), Bcap-37 breast cancer cell line, BEL- 7402 liver cancer cell line, SK- MEL, KB, BT- 549 and SK- OV-3 (12-15). The purpose of cancer prevention is to cause delay in onset of cancer, progression from precancerous lesion or recurrence after treatment, as an alternative to treatment of cancer cases after clinical symptoms have appeared (16).

One of the principle mechanisms of the radiation damage is the production of free radicals which leads to lipid peroxidation and decrease in antioxidant and conjugating phase II enzymes, the scavenging of these free radicals by conjugation Phase II enzymes results in radioprotection. Recently, we reported that aqueous extract of *Tribulus terrestris* modulate the reduced glutathione and lipid peroxidation level in liver of Swiss albino mice and showed cancer chemoprevention in skin papillomagenesis model (17). Therefore, the present study has been undertaken to investigate the radioprotective activity of *Tribulus terrestris* root extract in terms of hematological, biochemical and cytogenetic parameters in Swiss albino mice.

Materials and methods

Animals

Male Swiss albino mice, 6-8 weeks old with 25 ± 2 gm body weight, from an inbred colony (obtained from Hamadard University, Delhi) were used for the present study. Animals were maintained under controlled conditions of temperature and light in an animal house, and were provided standard mice feed (Procured from Hindustan Lever's Ltd. Delhi) and water *ad libitum*.

Irradiation

Cobalt Teletherapy Unit (ATC-C9) at the Cancer treatment centre, Radiotherapy Department, SMS Medical College & Hospital, Jaipur was used for irradiation. Unanaesthetised animals were restrained in well ventilated Perspex boxes and exposed whole-body to gamma radiation (with the source to surface distance, SSD of 77.5 cm and 1.02 Gy/min of dose-rate).

Preparation of *Tribulus terrestris* extract

Plant material (*Tribulus terrestris* Linn.) was collected locally and identified and the specimen was placed at Herbarium, Department of Botany, University of Rajasthan, Jaipur. The voucher number is RUBL - 19900. The roots were washed, air dried, powdered and extracted with double distilled water (DDW) by refluxing for 36 hr (12 x 3) at 40⁰C. The extract thus obtained was vacuum evaporated to make it in powder form. The extract was redissolved in DDW just before oral administration.

Experimental design

Determination of optimum dose of *Tribulus terrestris* root extract against radiation

Mice were divided into six groups of ten animals each and were given *Tribulus terrestris* root extract orally (200, 400, 800, 1000 and 1200 mg/kg body weight/day) for seven consecutive days. Thirty minutes after the last administration, animals were exposed whole body to 8.0 Gy gamma radiation. All such animals were observed till 30 days for any sign of radiation sickness, morbidity, behavioral toxicity and mortality. The optimum dose thus obtained was used for further investigation.

Modification of radiation response

Animals selected for this study were divided into two groups. Animals of one group were administered *Tribulus terrestris* root extract orally (800 mg/kg body weight/day) for seven consecutive days to serve as an experimental while the other group received DDW (volume equal to *Tribulus terrestris* root extract) to serve as control. After 30 min. of above treatments on 7th day, animals of both the groups were exposed to gamma radiation (8.0 Gy).

Hematological study

Blood was collected from tail vein in a vial containing 0.5 M EDTA. Peripheral blood counts (RBC/WBC), hemoglobin (Hb) concentration and hematocrit (Hct) percentage were determined at 6 and 24 hr post-irradiation by adopting standard procedures.

Biochemical study

Preparation of Homogenate for Biochemical Studies

Animals were killed by cervical dislocation and the entire liver was then perfused immediately with cold 0.9% NaCl and thereafter carefully removed, trimmed free of extraneous tissue. It was then weighed and blotted dry. For assaying reduced glutathione it was homogenized in ice-cold Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. A 0.5 ml aliquot of this homogenate was used for assaying reduced glutathione. For assaying lipid peroxidation this tissue was homogenized in ice-cold 1.15% KCl to yield a 10% (w/v) homogenate. A 0.8 ml aliquot of this homogenate was used for assaying lipid peroxidation.

Reduced glutathione

Reduced glutathione was estimated as total nonprotein sulphhydryl group by the method as described by Moron *et al.* (18). Homogenates were immediately precipitated with 0.1 ml of 25% trichloroacetic acid and the precipitate was removed after centrifugation. Free SH groups were assayed in a total 3 ml volume by adding 2 ml of 0.6 mM DTNB prepared in 0.2 M Sodium phosphate buffer (pH8.0), to 0.1 ml of the supernatant and absorbance was read at 412 nm using a UV-VIS Systronics spectrophotometer. GSH was used as a standard to calculate n mole of - SH content / gm tissue.

Lipid peroxidation

Lipid peroxidation in the liver was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method, as described by Ohkhawa *et al.* (19) and is expressed in terms of malondialdehyde (MDA) formed per mg tissue. In brief, 0.8 ml of homogenates was mixed with 0.2 ml of 8.1% Sodium dodesylsulphate (SDS) to which 1.5 ml of 20% acetic acid was added. Then 1.5 ml of 0.6% TBA was added and placed in a water bath for 1 hr at 80⁰C, cooled in ice and mixed with 5 ml mixture of n-butanol and pyridine (15:1). Then, centrifuged at room temperature for 10 min at 3,000 rpm. The absorbance of the clear supernatant was measured against blank of distilled water at 532 nm.

Cytogenetic study

Chromosomal Aberration Analysis

Cytogenetic damage in the bone marrow cells was studied by chromosomal aberration analysis at the end of the experiments. All the animals were injected i.p. with 0.025% colchicine and sacrificed 2 hr later by cervical dislocation. Both femurs were dissected out. Metaphase plates were prepared by the air drying method (20, 21). Bone marrow from the femur was aspirated, washed in saline, treated hypotonically (0.6% sodium citrate), fixed in 3 methanol: 1 acetic acid, dried and stained with 4% Giemsa (Sigma, U.S.A.). Different types of aberration like chromatid breaks, chromosome breaks, fragments, rings, exchanges and dicentrics were scored. Chromosomal aberrations were scored under a light microscope. A total of 400 metaphase plates were scored per animal and each aberration type was calculated in percentage.

Micronuclei Assay

The method of Schmid (22) was employed for the micronucleus assay. The femurs were dissected out and the bone marrow was flushed out, vortexed and centrifuged. The pellet was resuspended in a few drops of fetal calf serum. Smears were made on pre-cleaned, dry slides, air dried and fixed in absolute methanol. The slides were stained with May-Grunwald's and Giemsa stain. At least 2000 erythrocytes were observed and the number of polychromatic erythrocytes and normochromatic erythrocytes were counted. The micronuclei in them were recorded and micronuclei per 1000 cells were calculated.

Statistical analysis

The results obtained were expressed as mean \pm SE. Student's 't' test was used to make a statistical comparison between the groups. Significance levels were set at $P < 0.05$, $P < 0.005$ and $P < 0.001$.

Results

In control group (radiation alone), maximum decline in total RBC count was noted as early as at 6 hr ($4.57 \pm 0.32 \times 10^6/\text{mm}^3$) post irradiation interval. It was observed significantly below the normal value, at 24 hr post irradiation ($5.72 \pm 0.68 \times 10^6/\text{mm}^3$) interval. WBC count was declined significantly, on 24 hr ($3.92 \pm 0.67 \times 10^3/\text{mm}^3$) post irradiation. Hemoglobin level was also declined significantly in comparison to normal ($15.24 \pm 0.98 \text{ gm}/100\text{ ml}$), however, maximum decline was noted on 24 hr ($9.44 \pm 0.91 \text{ gm}/100\text{ ml}$) interval. The values of hemoglobin showed continuous decrease from 6 hr ($9.49 \pm 0.70 \text{ gm}/100\text{ ml}$), interval after exposure to 8 Gy gamma radiation. Hematocrit value gradually decreased from 6 hr ($30.51 \pm 1.84\%$) to 24 hr ($30.07 \pm 2.08\%$) of autopsys intervals (Table 1).

In *Tribulus terrestris* extract pretreated and irradiated animals, maximum decline in total R.B.C. count ($7.71 \pm 0.87 \times 10^6 / \text{mm}^3$) was observed at 6 hr autopsy interval. WBC counts were observed as significantly higher than control group of animals. Maximum decline in WBC count was observed on 6 hr autopsy interval ($5.16 \pm 0.27 \times 10^3 / \text{mm}^3$) this value increased and reached to normal value till 24 hr autopsy interval ($5.25 \pm 0.57 \times 10^3 / \text{mm}^3$). Hemoglobin level was declined to maximum at 6 hr ($13.24 \pm 0.63 \text{ gm}/100 \text{ ml}$) autopsy interval however, minimum decline was observed at 24 hr ($14.15 \pm 1.30 \text{ gm}/100 \text{ ml}$). A significant increase was observed in hematocrit value as compared to control group. However, maximum decline ($33.56 \pm 4.04\%$) was observed on 24 hr, whereas minimum decline ($34.52 \pm 1.94\%$) was observed on 6 hr (Table 1).

Table 1: Hematological alterations in peripheral blood of Swiss albino mice with or without *Tribulus terrestris* extract (TE) treatment following 8 Gy gamma radiation

Post-irradiation Time	Group	Haematological Parameters			
		RBC x $10^6 / \text{mm}^3$	WBC x $10^3 / \text{mm}^3$	Hb gm/100 ml	Hct %
6 hr	Con	4.57 ± 0.32^b	4.19 ± 0.24^b	9.49 ± 0.70^b	30.51 ± 1.84^c
	Exp	7.71 ± 0.87^b	5.16 ± 0.27^a	13.24 ± 0.63^b	34.52 ± 1.94
24 hr	Con	5.72 ± 0.68^a	3.92 ± 0.67^a	9.44 ± 0.91^b	30.07 ± 2.08^c
	Exp	7.73 ± 0.74^a	5.25 ± 0.57	14.15 ± 1.30^a	33.56 ± 4.04
Normal		8.63 ± 0.93	6.34 ± 0.46	15.24 ± 0.98	44.43 ± 1.80
TE alone		9.31 ± 0.60	6.17 ± 0.07	15.26 ± 0.95	43.74 ± 0.76

Each value represents Mean \pm SDEV.

Con = 8.0 Gy gamma rays

Exp = TE + 8.0 Gy gamma rays

Normal = No treatment

TE alone = 800mg/kg/day TE

Statistical comparison

Control V/s Normal

Experimental V/s Control

Significance Levels:

^a $p < 0.05$, ^b $p < 0.005$ and ^c $p < 0.001$.

Hepatic GSH level showed significant variation in *Tribulus terrestris* extract (TE) treated animals. The GSH level observed in *Tribulus terrestris* extract (TE) treated animal group was (0.1736 ± 0.0049 μ mole/gm tissue). The maximum decrease in hepatic GSH was observed in animals exposed to 8 Gy gamma radiation (0.0506 ± 0.0017 μ mole/gm tissue) on 24 hr. *Tribulus terrestris* extract (TE) treated and irradiated animals showed a significant increase in GSH level over the control group of animals but this value remain below TE alone group (Table-2). The significant rise in lipid peroxidation (LPO) level was observed in control group of animals. The LPO level in *Tribulus terrestris* extract treated and irradiated group of animals was significantly below than the respective control group (Table-2).

Table 2: Biochemical changes in liver of Swiss albino mice with or without *Tribulus terrestris* extract (TE) treatment following 8 Gy gamma radiation

Post-irradiation Time	Group	GSH (μ mole/gm tissue)	LPO (n mole/mg tissue)
6 hr	Con	0.0532 ± 0.0024^c	5.9242 ± 0.0358^c
	Exp	0.1352 ± 0.0032^c	2.2348 ± 0.0276^c
24 hr	Con	0.0506 ± 0.0017^c	5.1818 ± 0.0238^c
	Exp	0.1535 ± 0.0037^c	1.9848 ± 0.0358^c
	Normal	0.0802 ± 0.0038	3.2803 ± 0.0434
	TE alone	0.1736 ± 0.0049^c	1.0151 ± 0.0653^c

Each value represents Mean \pm SDEV.

Con = 8.0 Gy gamma rays

Exp = TE + 8.0 Gy gamma rays

Normal = No treatment

TE alone = 800mg/kg/day TE

Statistical comparison

Control V/s Normal

Experimental V/s Control

Significance Levels:

^a $p < 0.05$, ^b $p < 0.005$ and ^c $p < 0.001$.

The exposure of 8 Gy gamma radiation to Swiss albino mice resulted in significantly increased chromosomal anomalies and micronuclei frequency in bone marrow cells. The frequency of micronuclei/1000 cells in the irradiated animals were 18.52±1.31 and 20.21±1.25 respectively on 6 and 24 hr (compared with a frequency of 0.28±0.04 in the normal; Table 3; Fig. 1). In irradiated groups, significant increases were observed for chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments (Table -4; Fig. 2 b). Significant increases in the percentage of pulverized cells, polyploids, total aberrations and aberrations/damaged cells were also observed (Table 5; Fig. 2 c, d). However, *Tribulus terrestris* extract treated and irradiated group of animals showed a significant decrease in chromosomal aberrations and micronucleus frequencies compared with those found in irradiated alone group.

Table 3: Micronucleus frequency in bone marrow cells of Swiss albino mice with or without *Tribulus terrestris* extract (TE) treatment following 8 Gy gamma radiation

Post-irradiation Time	Group	Number of Mn/1000 Cells
6 hr	Con	18.52±1.31 ^c
	Exp	4.28±0.80 ^c
24 hr	Con	20.21±1.25 ^c
	Exp	5.52±0.62 ^c
	Normal	0.28±0.04
	TE alone	0.30±0.01

Each value represents Mean ± SDEV

Con = 8.0 Gy gamma rays

Exp = TE + 8.0 Gy gamma rays

Normal = No treatment

TE alone =800mg/kg/day TE

Statistical comparison

Control V/s Normal

Experimental V/s Control

Significance Levels:

^a p<0.05, ^b p<0.005 and ^c p<0.001.

Figure 1: Radiation-induced micronuclei in bone marrow cells of mice, micro nucleated polychromatic erythrocytes (arrows).

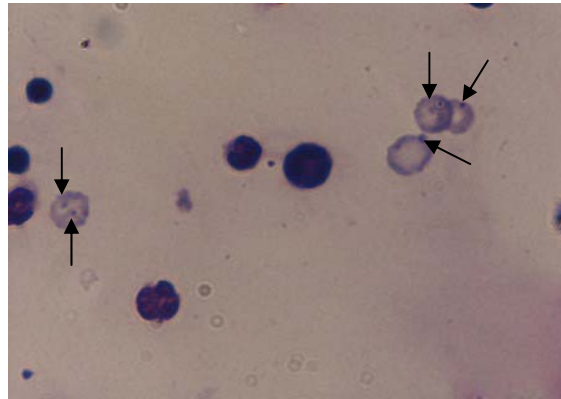


Figure 2: Radiation-induced chromosomal aberrations in bone marrow cells of mice. (a) Normal metaphase showing 40 chromosomes in animals (b) Radiation-induced chromatid breaks, exchange and ring (arrows). (c) Pulverization and (d) Polyploidy.

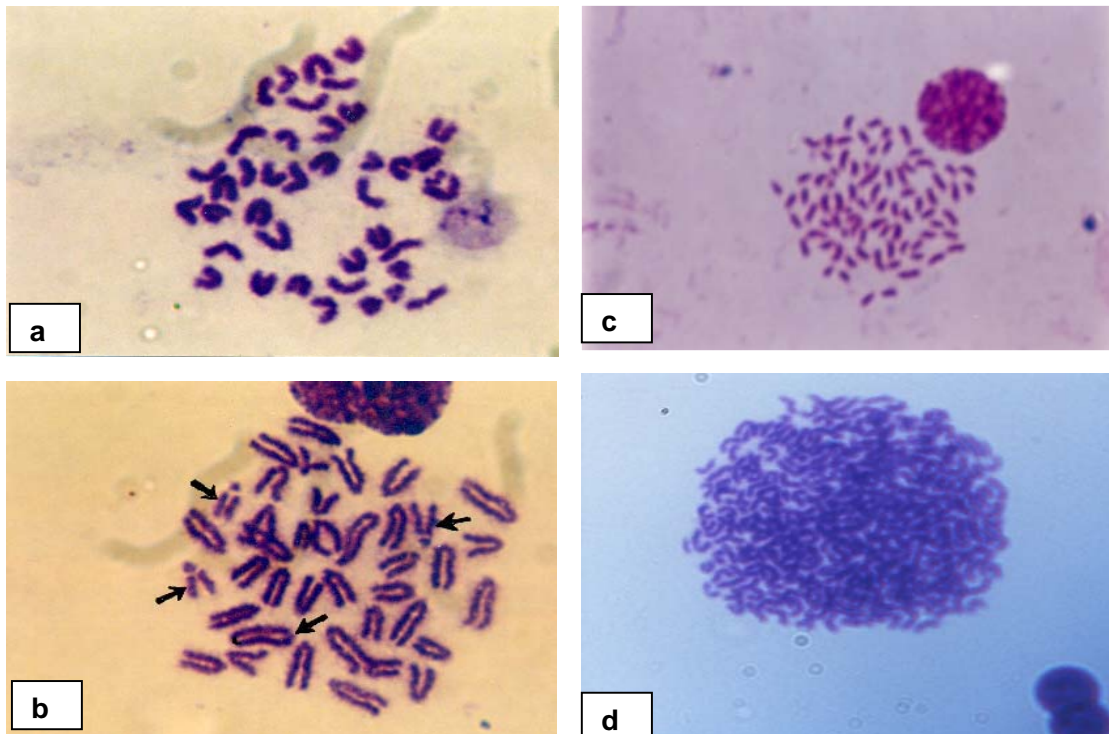


Table 4: Frequencies of chromosomal aberrations in Swiss albino mice with or without *Tribulus terrestris* extract (TE) treatment following 8 Gy gamma radiation

Post-irradiation Time	Group	Chromatid breaks (%)	Chromosome breaks (%)	Centric rings (%)	Dicentrics (%)	Exchanges (%)	Fragments (%)
6 hr	Con	5.80±1.05 ^c	2.09±0.33 ^c	1.72±0.27 ^c	1.82±0.35 ^c	1.80±0.43 ^b	90.6±5.19 ^c
	Exp	4.08±0.54	1.14±0.23 ^a	1.16±0.28	1.00±0.13 ^a	1.16±0.33	18.6±3.28 ^c
24 hr	Con	8.2±1.22 ^c	4.27±0.42 ^c	2.56±0.29 ^c	1.25±0.30 ^b	0.62±0.10 ^c	102.20±7.62 ^c
	Exp	2.46±0.63 ^b	2.46±0.52 ^a	1.91±0.14 ^a	0.29±0.10 ^a	0.32±0.06 ^a	20±3.93 ^c
	Normal	0.16±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.10±0.05
	TE alone	0.18±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.15±0.05 ^c

Each value represents Mean ± SDEV. 400 metaphases were scored/animal.

Con = 8.0 Gy gamma rays

Exp = TE + 8.0 Gy gamma rays

Normal = No treatment

TE alone =800mg/kg/day TE

Statistical comparison

Control V/s Normal

Experimental V/s Control

Significance Levels:

^a p<0.05, ^b p<0.005 and ^c p<0.001.

Table 5: Frequencies of chromosomal aberrations in Swiss albino mice with or without *Tribulus terrestris* extract (TE) treatment following 8 Gy gamma radiation

Post-irradiation Time	Group	Pulverized cells (%)	Polyploidy (%)	Aberrant cells (%)	Total aberrations (%)	Aberrations per damaged cell
6 hr	Con	5.00±0.06 ^c	3.20±0.06 ^c	50.54±2.10 ^c	156.86±8.26 ^c	2.50±0.22 ^c
	Exp	0.28±0.02 ^c	0.30±0.01 ^c	7.80±0.80 ^c	35.14±3.62 ^c	2.00±0.20 ^a
24 hr	Con	5.20±0.08 ^c	3.80±0.07 ^c	48.25±1.20 ^c	176.11±8.08 ^c	3.00±0.40 ^b
	Exp	0.30±0.03 ^c	0.28±0.02 ^c	8.20±1.00 ^c	40.45±3.60 ^c	2.20±0.20 ^a
	Normal	0.00±0.00	0.12±0.02	0.52±0.03	0.72±0.05	1.22±0.04
	TE alone	0.00±0.00	0.18±0.02	0.61±0.04	0.81±0.02	1.51±0.03

Each value represents Mean ± SDEV. 400 metaphases were scored/animal.

Con = 8.0 Gy gamma rays

Exp = TE + 8.0 Gy gamma rays

Normal = No treatment

TE alone =800mg/kg/day TE

Statistical comparison

Control V/s Normal

Experimental V/s Control

Significance Levels:

^a p<0.05, ^b p<0.005 and ^c p<0.001.

Discussion

A significant radioprotection was achieved when *Tribulus terrestris* root extract was given orally (800 mg/kg body weight/day) for seven consecutive days prior to irradiation. In the present study, a significant deficit in hematological constituents of peripheral blood of control animals (radiation alone) was observed. The decrease in hematological constituents may be attributed to a direct damage by radiation dose (23). Although 3 Gy total body dose is required to produce a detectable depression in total red blood cells, whole body irradiation of moderate dose range (5-10 Gy) leads to a decreased concentration of all the cellular elements in blood. This can be due to direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage or leakage through capillary walls and loss of production of cells (24). The results of the present study indicate that *Tribulus terrestris* root extract pretreatment has provided protection against the hematopoietic damage.

It is well known that free radicals generated during radiolysis of water play the most significant role in the indirect biological damage induced by ionizing radiation (25). GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation (26). The present study demonstrates a significant reduction in hepatic GSH, following radiation exposure. This could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. The depletion of GSH promotes generation of reactive oxygen species and oxidative stress with cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes (27). The lower depletion of hepatic GSH in the *Tribulus terrestris* root extract pretreated irradiated animals could be due to the higher availability of GSH, which increases the ability to cope up with the free radicals produced by radiation. The increased GSH level suggests that protection by *Tribulus terrestris* root extract may be mediated through the modulation of cellular antioxidant levels. GSH is a versatile protector and executes its radioprotective function through free radical scavenging, restoration of the damaged molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state (28).

The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. Lipid peroxidation can be initiated by radiolytic products, including hydroxyl and hydroperoxyl radicals (29). It was observed that, although *Tribulus terrestris* root extract treatment did not significantly alter the lipid peroxidation level in unirradiated animals but, *Tribulus terrestris* root extract pretreatment significantly lower the radiation induced lipid peroxidation in terms of malondialdehyde. Inhibition of lipid peroxidation in biomembranes can be caused by antioxidants (30, 31). The results from the present investigation indicate that the *Tribulus terrestris* root extract pretreatment protects against radiation damage by inhibiting radiation induced GSH depletion and decreasing LPO level in liver of mice.

In the present study, the frequency of aberrant cells; chromosome breaks, chromatid breaks, centric rings, dicentrics, exchanges and acentric fragments significantly increased in bone marrow cells of animals exposed to 8 Gy gamma radiations. An exposure to radiation is known to produce significant increases in the per cent aberrant metaphases as well as in the different aberrations. Damage to the chromosomes is manifested as breaks and fragments, which appear as micronuclei in the rapidly proliferating cells (32).

Enhancement in the frequency of micronuclei and chromosomal aberrations has been reported earlier in the bone marrow of irradiated mice (33-35). DNA is the critical target for cell killing by ionizing radiation, and there is growing evidence that the particular damage responsible are DNA double strand lesions, such as DSB (36), while damage to other biological molecules does occur and is potentially cytotoxic. It has been recognized that structural aberrations can be induced in chromosomes by radiation at any stage of mitotic cycle. When cells are irradiated just as they enter division, there is apparently some change in the surface properties of the chromosomes, which cause them to adhere to each other when they happen to touch. This stickiness has been attributed to a partial dissociation of the nucleoproteins and an alteration in their pattern of organization (24). Natarajan *et al.* (37) and Bryant (38) suggested that double-strand breaks are mainly responsible for the formation of chromosomal aberrations.

As the principal mechanism of the radiation damage is the production of free radicals and the scavenging of these free radicals by conjugation Phase II enzymes results in radioprotection. Shimo *et al.* (39) concluded that plant flavonoids which show antioxidant activity *in vitro* also function as antioxidants *in vivo*, and their radioprotective effect may be attributed to their radical scavenging activity. The mechanisms implicated in the protection of cells by radioprotectors include free radical scavenging that protects against reactive oxygen species (ROS) generated by ionizing radiation or chemotherapeutic agents, and hydrogen atom donation to facilitate direct chemical repair at sites of DNA damage (40). ROS generated by ionizing radiation are scavenged by radioprotectors before they can interact with biochemical molecules, thus reducing the harmful effects of radiation. The antioxidant mechanism of radioprotection and free radical scavenging to be a likely mechanism of radiation protection by plant extracts has been suggested (41, 42).

Tribulus terrestris possesses tonic properties, antibacterial, anti-inflammatory, smooth muscle relaxation and diuretic actions, which are useful in genitourinary infections, painful micturation, hematuria, dysuria, benign prostatic hyperplasia, urethritis and prostatitis (1-4, 10). It has been reported that *Tribulus terrestris* contains saponins, quercetin, kaempferol, rutin, furostanol, gigenin, hecogenin, ruscogenin, gitogenin, tigonin, terrestrinins A(1) and B(2), which are known to have antioxidant and anticancer properties (6-8). The results from the present study suggest that hematopoietic stem cells can be protected from radiation induced free radical damage by *Tribulus terrestris* root extract, which was evident in hematological constituents in peripheral blood and cytogenetic parameters in bone marrow cells of mice. Thus the radioprotective effects of *Tribulus terrestris* root extract observed in the present study can be attributed to the antioxidant properties of *Tribulus terrestris* due to its chemical constituents.

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