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Original Article

PROTECTIVE ACTION OF *TINOSPORA CORDIFOLIA* EXTRACT AGAINST RADIATION INDUCED BIOCHEMICAL ALTERATIONS IN LIVER

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ABSTRACT

Objective: The present investigation has been carried out to evaluate the possible radio protective potential of *Tinospora cordifolia* root extract (TCE) against 2.5 Gy gamma radiations induced biochemical alterations in the liver of 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 (75mg/ kg.b.wt/ animal) by oral gavage. Group II was orally supplemented TCE as 75mg/ 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).at various post-irradiation intervals between 12 hrs and 30 days.

Results: The irradiation of mice caused a considerable elevation in glycogen, total proteins, acid phosphatase and LPO along with a significant decrease in alkaline phosphatase, GSH, catalase and SOD activities. On the contrary, oral administration of TCE before irradiation reduced the radiation- induced variations in all such parameters and the recovery and regeneration was faster as compare to irradiated control group.

Conclusion: The present investigation indicates that *Tinospora cordifolia* has the potential to alleviate the radiation mediated adverse effects in liver and it could be exploited as a protector against planned and unplanned radiation exposure .

Keywords: Gamma Radiation, Radioprotection, *Tinospora Cordifolia,* Liver, Swiss Albino Mice, Oxidative Stress, Antioxidant.

INTRODUCTION

Revelation to ionizing radiation generates momentous alterations in the oxidant activity in different tissues, and causes over production of ROS and oxidative stress leading to oxidative damage of the lipids, proteins and DNA in the biological system. Oxidative stress is described generally as a condition under which elevated production of free radicals, reactive species (including singlet oxygen and reactive lipid per oxidation products, such as reactive aldehydes and peroxides), and oxidant-related reactions occur that result in deleterious consequences on different organ systems [1].

Liver of mammals has been reported as highly radiosensitive organ [2]. Since liver has a pivotal role in regulation of various physiological processes and involved in several vital functions such as metabolism, secretion and storage, and most importantly in detoxification of a variety of drugs and xenobiotics. Therefore, any harm to such a versatile organ may lead to serious disorders in the form of alterations in various biochemical end points as well as inactivation of endogenous enzymatic and non enzymatic antioxidant systems. A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage mainly by inducing lipid per oxidation and other oxidative damages [3]. As the oxidative stress plays a central role in liver pathogenesis and progression, the use of antioxidants has been proposed as therapeutic as well as drug coadjutants to counteract liver damage and protects the cellular machinery from per-oxidative injury inflicted by ROS [4, 5]. Therefore, antioxidant defense system is an important area which needs to be considered for exploring the effect of radiation on hepatic cells.

A number of natural and synthetic compounds of diverse structure and presumed mechanism of action has displayed significant protection against irradiation of mammalian organ system, which include thiol, WR- 2721 (Amifostine), natural antioxidant such as GSH, biological response modifiers such as cytokine (interleukin 1), immune modulators, some vitamins and pro- vitamins [6-10]. However, the toxicity incurred after repeated administration limits their clinical use. Therefore, the search for effective, inexpensive and non- toxic radio- protective drugs for limiting hepatic injury has been of interest recently [11]. The traditional systems of medicine like Ayurveda, Siddha, Unani have a major role in the treatment of liver ailments [12]. Numerous plants and polyherbal formulations are used for the treatment of liver diseases. For developing satisfactory herbal combinations to treat severe liver diseases, plants have to be evaluated systematically for properties such as antiviral activity, anti- hepatotoxicity and stimulation of liver regeneration [13].

In this context, *Tinospora cordifolia* (Family: Menispermaceae) a well known plant of Indian medicinal system, is gaining more attention for electing a wide spectrum of pharmacological activities. It is known for its general tonic, anti- cancer, anti- leprotic, anti-hyperglycemic, anti-allergic and anti- diabetic properties [14-16]. It improves the phagocytic and bactericidal capacity of polymorphs, protects against gastric mucosal damage and scavenges free radicals [17]. Since this plant has also been reported to possess anti- fibrotic, anti- oxidant, anti- inflammatory, immune modulatory, radio protective and activator of phagocytic and killing activity of macrophages [18- 21], hence the following study was undertaken to determine whether it can modulate the radiation induced hepatic injury in terms of various biochemical and anti-oxidative parameters in experimental animal model.

MATERIALS & METHODS

Animals 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 the 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 has approved the study.

Source of irradiation

Animals were irradiated by a Co^{60} source in the cobalt therapy unit at Cancer Treatment Center, Department of Radiotherapy, SMS Medical College & Hospital, Jaipur, India. Un anaesthestized 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 (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). Roots of the *Tinospora cordifolia* were collected, cleaned, shade dried, powdered and extracted. The extract was prepared by refluxing with double-distilled water (DDW) for 36 (12x3) 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 the mice. Henceforth in this article, the extract of *Tinospora cordifolia* root extract will be called as TCE.

Experimental design

Dose selection of TCE

Dose selection of *Tinospora cordifolia* was done in our previous study on the basis of drug tolerance survival experiment [22].

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 the 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 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 min of the last dose administration on day 5^{th} such animals were exposed to 2.5 Gy gamma radiation.

Necropsy 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.

Biochemical study

The liver was collected from each nacropsied animals at each autopsy interval and homogenate of liver was prepared to measure glycogen, proteins and cholesterol contents by using Montogomery (1957) [23], Lowery *et al* (1951) [24] and Burchard (1959) [25] methods, respectively. Activities of acid and alkaline phosphatase were also assayed using protocol given by Fiske and Subbarow (1925) [26]. The level of LPO, glutathione (GSH), catalase and SOD in liver was determined by the methods of Ohkhawa *et al* (1979) [27], Moron *et al* (1979) [28], Abei (1984) [29], Marklund *et al* (1974) [30], respectively.

Statistical analysis

The results 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 by the method of Bourke et al (1985) [31]. 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 various biochemical parameters (viz. glycogen, protein, cholesterol, ACP & ALP) and anti- oxidative parameters (LPO, GSH, catalase and SOD) 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.

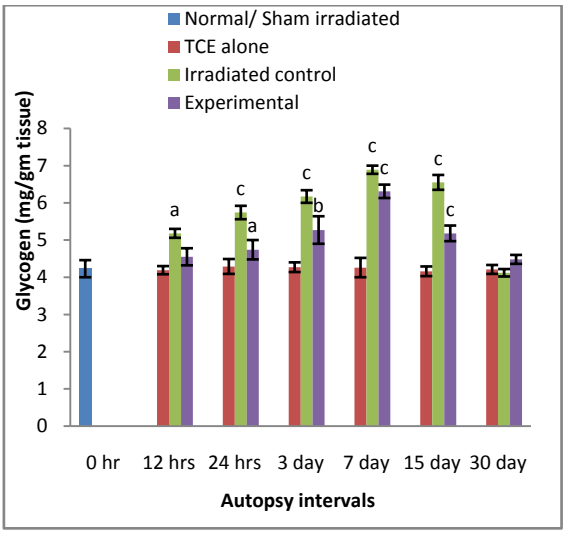


Fig. 1: Variations (mean ± S.E.) in glycogen levels of mice exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively.

Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: ^{a} $p \leq 0.05$, ^{b} $p \leq 0.01$, $^{c}p \leq 0.001$.

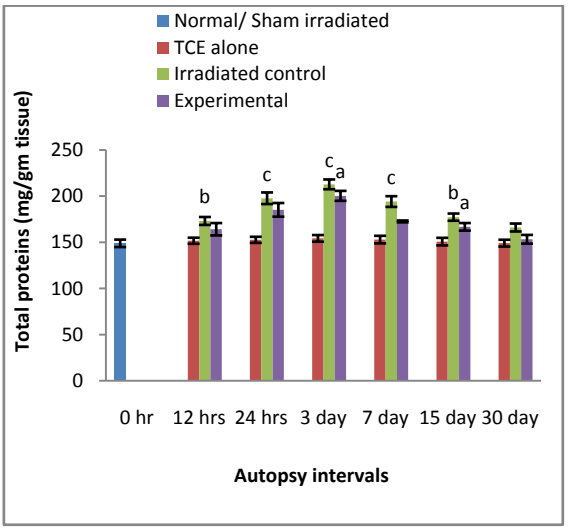


Fig. 2: Variations (mean ± S.E.) in total proteins of mice exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively.

Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: ^{a} $p \leq 0.05$, ^{b} $p \leq 0.01$, $^{c}p \leq 0.001$..

In the present study, a gradual and continuous augmentation of hepatic glycogen content in irradiated control group was evident up to 7^{th} post- exposure day (6.89±0.11), being significantly ($P<0.05$) higher (162.88 %) than the respective experimental group (6.31±0.18). Thereafter, the values were started to decrease at remaining intervals, but normal value could not be restored even till the end of study. However, the values in experimental mice were

always found to be lesser than the irradiated control at all the autopsy intervals. (Fig. 1).

Radiation exposure resulted in a considerable rise in the total protein contents in the liver which increased up to day 7th in both the experimental and the control groups (194.15±5.82 & 172.62±1.02, respectively) being significantly higher (P<0.001) in the 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<0.001) in experimental animals than irradiated controls, but the normal level could not be recovered even till the end of experimentation and found 100.75 % higher than the normal (Fig. 2)

In irradiated control animals, hepatic cholesterol content showed a considerable decrease after irradiation up to day 7th (4.28±0.11) followed by a noticeable increase, however the values were fairly lesser than the normal even till the last autopsy interval. TCE treated irradiated animals also experienced the similar mode of alterations, but the magnitude was rather lower than the respective irradiated control throughout the study period. In experimental animals recovery process was started from day 7th to last day of study, where the observed values were found to be 105.12 % higher than the normal (Fig. 3).

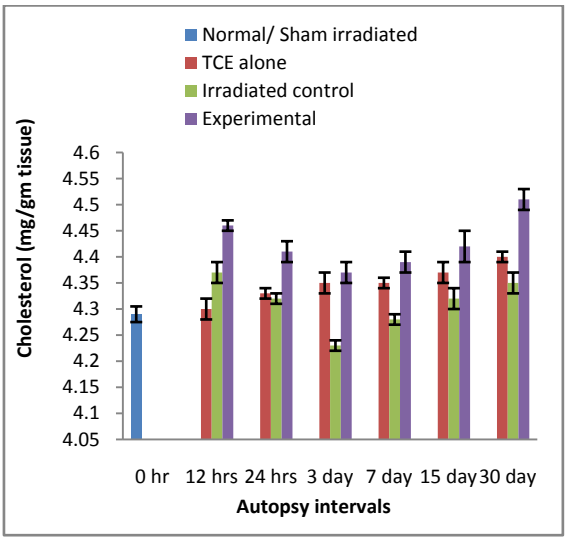


Fig. 3: Variations (mean ± S.E.) in cholesterol levels of mice exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively.

Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: ^{a} p ≤ 0.05, ^{b} p ≤ 0.01, ^{c}p ≤ 0.001.

Alkaline phosphatase activity showed a significant elevation up to day 7th (3.87±0.12) followed by a gradual decline till the last autopsy interval in irradiated control animals as compared to normal but the values were observed as 123.62% & 105.51 % higher than the normal at day 15th and 30th post- exposure respectively. At the same time, TCE treated animals also exhibited essentially similar mode of variation, but the values were found considerably lower than the respective control throughout the experiment (Fig.4). On the contrast, radiation exposure resulted in considerable decrease in acid phosphatase activity up to day 7th (2.46±0.12**;** p<0.001) post exposure. Thereafter, the values started to increase till the last autopsy interval but remained lesser than the normal. TCE treated group showed similar mode of variation but the values were lesser as compared to irradiated control at all autopsy intervals, however, the normal level could not be restored even by the end of experiment (Fig. 5).

After radiation exposure, a significantly (p<0.001) increased level of lipid per oxidation was evident in the liver up to day 15 (6.12±0.2) of control and up to day 7 (3.21±0.11) in experimental mice. Thereafter, a noticeable depletion in LPO was recorded at the remaining intervals in irradiated control as well as in experimental group. However, the observed values were higher in irradiated control as compared to experimental and the last autopsy interval these were found as 215.80 %, 100.93 % higher than the normal respectively (Fig. 6).

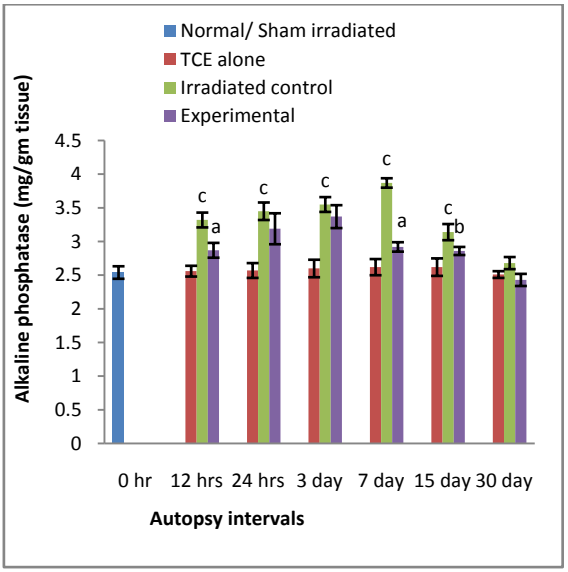


Fig. 4: Variations (mean ± S.E.) in alkaline phosphatase levels of mice exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively.

Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: ^{a} p ≤ 0.05, ^{b} p ≤ 0.01, ^{c}p ≤ 0.001.

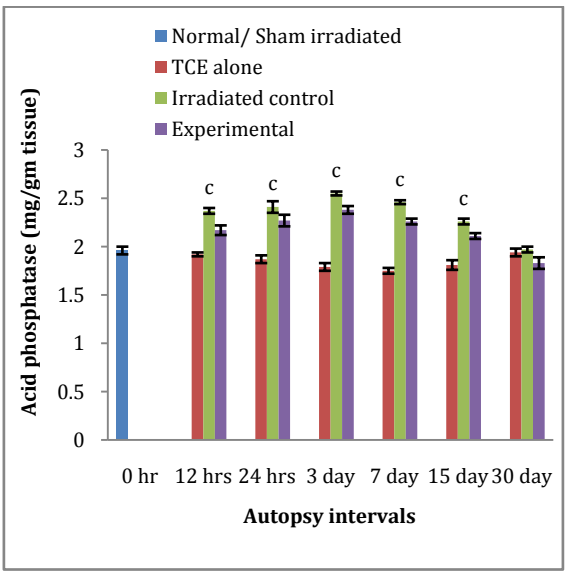


Fig. 5: Variations (mean ± S.E.) in acid phosphatase levels of mice exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively.

Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: ^{a} p ≤ 0.05, ^{b} p ≤ 0.01, ^{c}p ≤ 0.001.

A continuous decreasing pattern was followed by hepatic glutathione level up to day 15th in both irradiated control as well as experimental groups (41.86±1.12**;** p< 0.001&46.53±1.21; p< 0.00, respectively), where the observed values were 28.41%, 20.42% lower than the normal, respectively. However, such extent of decrease the extent was comparatively lower in the experimental group than the respective irradiated control at all autopsy intervals. Thereafter, a significant elevation was recorded at the consecutive interval but the level was significantly (p<0.01) lower in experimental group (Fig.7).

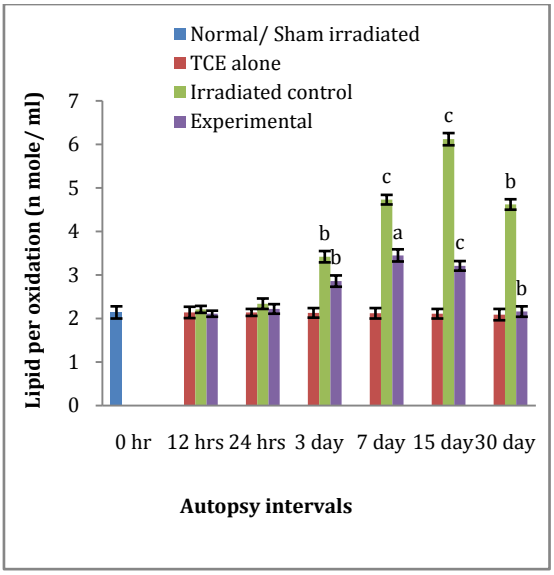


Fig. 6: Variations (mean ± S.E.) in the levels of lipid per oxidation of mice exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively.

Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: $^{a}p \leq 0.05$, $^{b}p \leq 0.01$, $^{c}p \leq 0.001$.

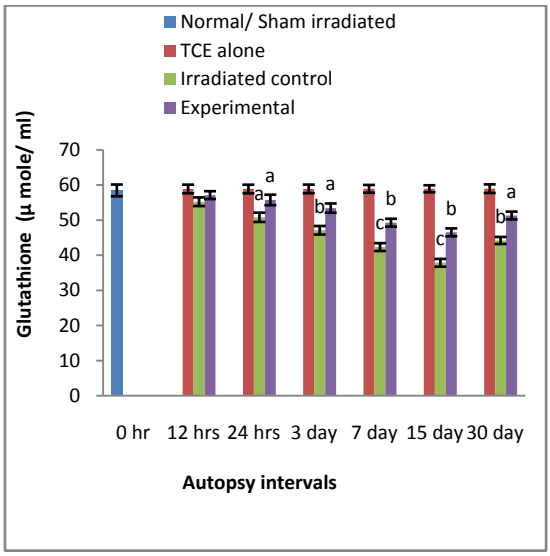


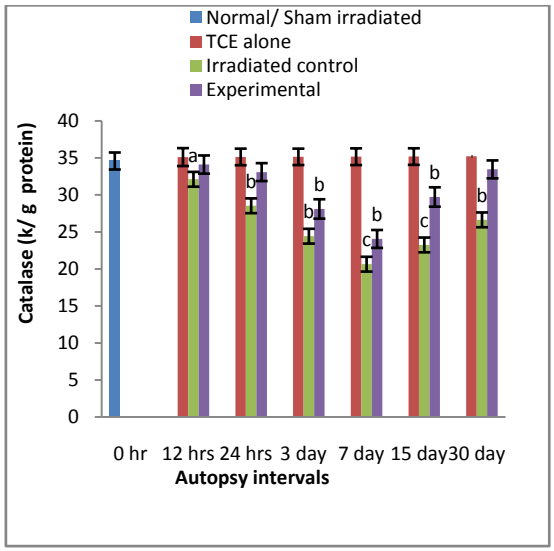
Fig. 7: Variations (mean ± S.E.) in glutathione levels of mice exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively.

Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: $^{a}p \leq 0.05$, $^{b}p \leq 0.01$, $^{c}p \leq 0.001$.

Similarly, SOD and catalase activities were also found to decrease up to day 7th of post- exposure in the irradiated control animals, where the observed values were recorded as 40.31% and 57.57% lesser than the normal, respectively. Similarly, TCE treated irradiated animals also exhibited similar pattern of alterations, and 7th day onwards showed a significant elevation, but the normal values could not be recovered even by the end of experiment and found to be 16.74 % 15.10 % lesser than the normal (Fig.8 & 9).

DISCUSSION

In the present study, primary consequences of radiation exposure resulted in the tissue breakdown and altered enzymatic activity. Elevation in hepatic glycogen concentration after irradiation possibly due to increased substrate availability after stimulation of the pituitary and adrenal systems for an increasing energy necessity of degenerating and aberrant hepatic cells as also suggested by Soyal *et al* (2007) [32]. Furthermore, a significant increased level of total proteins in hepaitic tissue of irradiated animals was evident up to day 3 which may be attributed to higher amino acid precursor pool in the liver of γ- irradiated mice, as result of permeability change in irradiated cell membrane and increase in the number of ribosomes on account of increased mobilization from endoplasmic reticulum [33]. Furthermore, excessive protein loss through injury of kidney or gastro- intestinal tract or from thermal injury to skin [34] could also be added to radiation- induced reduction in hepatocytic total proteins at the later intervals.



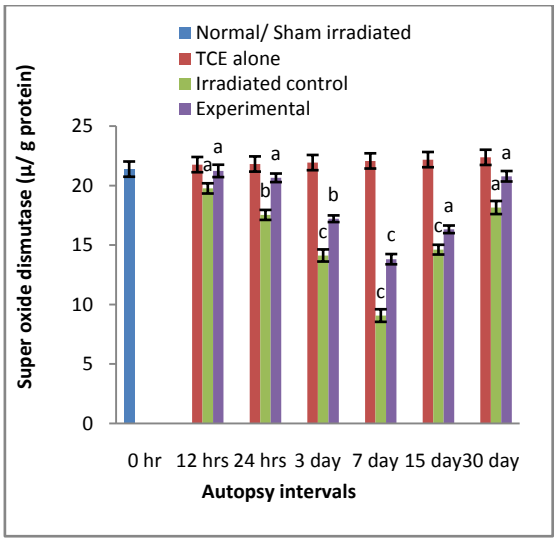


Fig. 8&9: Variations (mean ± S.E.) in Catalase & SOD activity of mice exposure to 2.5 Gy gamma radiation with (experimental) or without (irradiated control) TCE, respectively.

Statistical analysis: Control v/s Normal; Experimental v/s Control; Significance levels: $^{a}p \leq 0.05$, $^{b}p \leq 0.01$, $^{c}p \leq 0.001$.

Liver is the chief organ concerned with regulation of total body contents of cholesterol and plasma cholesterol. In this study, irradiation caused a considerable decrease in cholesterol up to day 3rd, indicating its increased catabolism due to radiation- induced stress response and consequently augmented utilization which exerts positive feedback action on the rate of steroid hormones synthesis.

These explanations also confirm the earlier findings of Sharma & Goyal, (2005), who also studied the radiation effects on hepatic tissue [35]. Experimental group showed a significant compensatory concentration of cholesterol than their corresponding control groups.

Furthermore, radiation exposure resulted in elevation of liver phosphatase activity which may be attributable to the tissue impairment and per- oxidation of membrane lipids leading to activation of suppressed acid hydrolyases [36]. An increase in acid phosphatases activity after radiation exposure in the present experiment could be ascribed either to a direct effect of radiation which results in enhanced Golgi activity [37] or per- oxidation of lysosomal membranes causing lysis of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the ACP activities in liver as also described by Ramadan *et al* (2002) [38]. ALP has been reported to be the marker enzyme for the plasma membrane [39] and is required in certain amounts for proper functioning of organs [40] and dissolution of dead cells of the body. Therefore, ALP activity is correlated with radiation- induced deletion in heapocytes and peroxidation of membrane. Similar elevating pattern in hepatic phosphatase activity was also observed also by others [41 & 42] in lethally irradiated mice.

Post- irradiation repair mechanisms depend upon the status of endogenous antioxidant enzymes during and after the radiation period. Antioxidants such as superoxide dismutase (SOD), reduced glutathione and catalase (CAT) are part of the primary cellular defense and act cooperatively at different sites in the metabolic pathway against reactive oxygen species generated in vivo during oxidative stress. The level of such enzymes may not be sufficient to cope with the level of oxidant influx caused by the radiation which may overload the endogenous detoxification mechanism of the cells. By the radiation exposure, the loss of enzyme activities from tissues and sometimes the elevated activities of tissue enzymes as observed with the enzymes studied may be attributed to membrane lipid-peroxidation and direct free radical damage to membrane proteins which directly proportional to oxidative stress [43]. Lipid-peroxidation results in the loss of polyunsaturated fatty acids, decreased membrane fluidity and severe structural changes leading to loss of enzymes and in other cases receptor activity [44].

In present study, a gradual and continuous augmentation in the level of TBARS contents in test organ was observed till 7th day of post-irradiation, which may be due to increased oxidative stress and decrease in body weight, organ weight and protein value after radiation exposure as also suggested by Yadav *et al* [45]. Along with this outcome, 2.5 Gy γ- irradiation induces changes in antioxidant activities expressed as a progressive decrease in GSH and SOD activities up to day 7th post- irradiation which leads to increase in the formation of O_2 and H_2O_2. But no statically significant difference was found in catalase activity after irradiation when compared with normal ones suggesting that it was not induced by 2.5 Gy irradiation under these experimental conditions. This observed considerable inhibition in the all such enzymatic activity further stipulates radiation effects on inherent defense system that could possibly be due to utilization of these enzymes in converting superoxide radicals to H_2O_2 and H_2O and in protecting the oxygen- metabolizing cells against oxidative stress through moping up free-radicals. These findings are in close agreement with the earlier report of Kumar and Kuttan (2004) [46] which documented the degenerative effects of gamma rays on antioxidant system of liver in lethally irradiated mice.

It has been observed that pre- treatment with *T*inospora extract protected radiation- induced hepatic injuries effectively by exhibiting a significant decrease in protein, glycogen, ACP, ALP activity and LPO level, and significant increase in body weight, liver weight, cholesterol, GSH content, SOD and catalase activity in liver as compared with the irradiated control mice. In addition, some studies from our laboratory have also reported that commonly used medicinal plants such as *Aloe vera* [47], *Alstoni scholaris* [48] *Trigonella foenum* [49] and *Rossmary officinalis* [32] are ideal source of radioprotection against hepatic injury in experimental model chiefly due to the inhibition of free radical- induced chain reactions and the resultant prevention of peroxidative deterioration of structural lipid in membranous organelles. Furthermore, some other herbal plants and herbal preparations [50-52] suggests that circulating enzymatic and non- enzymic antioxidants such as SOD, catalase and reduced glutathione play an important role in alleviating tissue damage due to the formation of free radicals. Thus, these findings, suggest that TCE may also exerts its radio- protective effect due to the ability to limit the initial damage, caused during irradiation by detoxifying radiation induced oxygen species, scavenging of free radicals and increased concentration of endogenous antioxidant system and other possible antioxidant activity along with stimulation of cellular regeneration in the post-irradiation period (particularly hematopoietic regeneration, liver recovery, gastrointestinal system recovery) and up regulating the activity of early response genes. Some other investigators also confirm this contention by the experiments on free radical scavenging, where TCE has been found to scavenge OH and O_2^- radicals under radiation induced oxidative stress [53 & 54].

The hepato-protective role of TCE in the current study may be due to the synergistic effects of various bioactive constituents that present in its root extract like polyphenols (3- glucosides, gallic acid, tannins), flanonoids (quercetin), alkaloids (berberine), and triterpenoids compounds which have been reported to possess strong antioxidant activity and provokes free radical scavenging enzymes system against radiation and other pathological conditions [55- 58]. These all are responsible for the reversal of antioxidant level in tissue of irradiated mice. Furthermore, it has been reported that *T. cordifolia* exerts its hepato-protection by the means of the antifibrotic action and activation of kuffer cells [59]. Antioxidant such as gallic acid has been reported to protect liver injury and fibrosis induced by hepatoxins. In addition, radio-protectiove action of TCE may also be due to the presence of known anti- inflammatory flavonoid and glycoside derivatives, which provide maximum conjugation with free radicals species, thus reducing the number of free radicals available and extent of cellular damages [60]. In a structure dependent manner, flavonoids and polyphenols are capable of scavenging ROS, RNS and inhibiting the activity of many enzymes and nuclear transcription factor [61& 62]. Thus, it can be concluded that the prophylactic, curative and restorative effects of the TCE in the present study were thought to be mainly due to all these factors chiefly by scavenging the free radical and suppressing the formation of ROS.

CONCLUSION

Based on the promising results obtained from the above study, it can be concluded that scavenging of free radicals and elevated concentration of endogenous antioxidant system are considered important in inhibiting the radiation- induced biochemical alterations in liver of mammals on account of the synergistic impact of various bioactive constituents present in *T. cordifolia* root extract.

CONFLICT OF INTERESTS

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

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