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Modulation of radiation induced changes in nucleic acid content of liver of *Swiss albino mouse* by *Tinospora cordifolia (Miers)*

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Background: Radiotherapy is the main modality of cancer treatment. There are many chemical radio-protectors which unfortunately have lethal or toxic effect. Therefore the search is on to find out natural plant based radioprotectors. A well known medicinal plant,which is more acceptable to the body, *Tinospora cordifolia*, was tested in animal tissues against gamma radiations. Radioprotective effects of *Tinospora cordifolia (Miers)* extract against radiation induced biochemical changes in liver of *Swiss albino mouse* were investigated. ***Materials and Methods:*** For experimental study, healthy *swiss albino mice* were selected from an inbred colony and divided into six groups and exposed to 6Gy and 8Gygamma radiation (control) or 6Gy and 8Gy gamma radiation with 5mg/ kg body weight of TC extract (experimental), sham irradiated (Normal) and plant extract only. Mice were sacrificed at various post irradiation intervals and liver was removed for quantitative estimation of DNA and RNA. ***Results:*** On the first day post irradiation in control group (6Gy and 8Gy both). DNA content decreased significantly as compared to the sham irradiated controls. Then there was continuous increase uptill 28th day but it remained below the normal .Decrease in DNA content of liver in the experimental group (6Gy) was observed on 1st day but the values were higher than that of the controls. RNA content increased in the control animals treated with 6Gy and 8Gy which was maximum at day 3, followed by a decrease at the subsequent intervals. Increase in the amount of RNA was recorded in the experimental animals also. Then came down to the normal on 10th day in the experimental groups ($P<0.01$). ***Conclusion:*** These results indicate that TC is able to protect nucleic acids the liver of *Swiss albino mouse* against gamma radiation. **Iran. J. Radiat. Res., 2010; 8 (3): 179-185**

Keywords: *Radioprotection, tinospora cordifolia, liver, DNA, RNA.*

INTRODUCTION

Exposure to ionizing radiations arose as a new industrial and medical hazard and also a tool of experiments. The artificial production of radioactive substances has increased tremendously the potentialities of application of ionizing radiations to varied fields of science and industry, but accidental or incidental exposure to large quantities of radiation has also greatly multiplied the hazardous effects. With the increasing use of atomic energy, questions of protection against radiation arises for that few decades intensified searches have been carried out for chemical compounds ,the administration of which before exposure may protect the body from the harmful effects of radiation. The initial interest in the radioprotective compounds was soon followed by disappointment because of the severe toxicity of these drugs in animals and humans. Recently, there has been resurgence of interest in several of the newly developed plant medicines, in particular because they are less toxic, more efficient more stable, orally effective and selectively protect the normal tissues. A large number of compounds from various plant sources have been shown to possess antioxidant properties [1-3]. The naturally occurring compounds of plant origin and extracts of vegetables, fruits and spices which have shown antioxidant activity and free radical scavenging property were tested to find out a new radio-protector, which is more effective than chemical radio-protector and works without causing side effects. In this context radio-protective potential of *Tinospora cordifolia* was studied .It is a native plant of India, which is used in various medicinal preparations since a long time. It is a common climber in India and popularly known as

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"Giloe". It is used as general tonic in treating Kustha (leprosy), Mahajvara (a kind of fever), Asthma and Anorexia [4]. It is also considered in Unani medicine as bitter, appetiser, tonic, antipyretic and expectorant. It is also used to treat jaundice, giddiness, vomiting, piles, anaemia, chronic fever, cough, renews the blood and when mixed with sesame oil, it is useful for massaging the body [5]. Present study was designed to find out the protective effect of *Tinospora cordifolia (Miers)* (aqueous) extract against gamma radiation and to assess the role of the extract against radiation induced changes in the nucleic acid content of mouse liver.

MATERIALS AND METHODS

Source of animals

Experiments were conducted on adult male *Swiss albino mice* obtained from an inbred colony maintained in the laboratory. 6-8 week old mice weighing 32g (±2g) were selected from an inbred colony maintained in the laboratory on standard mice feed obtained from Hindustan Lever Limited, Bombay and water *adlibitum*. Temperature of animal house is maintained at 37±5°C and animals are kept in natural day light and dark night cycles.

Source of Irradiation

Animals were irradiated with Co^{60} (ATC-C9) beam therapy unit supplied by Atomic Energy Agency, Canada, in the cancer treatment centre at SMS Medical College and Hospital, Jaipur. Unanaesthetised animals restrained in well ventilated boxes were exposed to whole body gamma radiation with source surface distance (SSD) of 77.5 cms to deliver the dose-rate of 1.59 Gy/min. The dose rate was alibrated throughout the experimental according to decay table of Co^{60}.

Tinospora cordifolia extract

Aqueous extract of plant *Tinospora cordifolia (Miers)* of family – Menispermaceae in dried powder from was obtained from Amsar Private Limited, Indore.

Dose selection

The plant extract was dissolved in double distilled water and the animals were fed by gastric intubation with different doses. Various doses of *Tinospora cordifolia* (5,10, 20mg/kg body wt) were tested against a lethal dose of Co^{60} gamma irradiation (8Gy). The animals which received plant extract one hour before irradiation at the dose rate of 5mg/kg body weight showed significantly higher survival time or the plant extract provided significant protection against the selected lethal dose of gamma radiation.

Design of experiments

Animals were divided into following groups:

Group I: Sham irradiation only

Group II: Animals received plant extract one hour before irradiation at the dose rate of 5mg/kg body weight orally. Animals were exposed to 6Gy of Co^{60} gamma irradiation .

Group III: Irradiated with 6Gy of Co^{60} gamma rays and given equal amount of double distilled water as given with the TC extract.

Group IV: Animals received TC extract one hour before irradiation at the dose rate of 5mg/kg body wt orally and then animals were exposed to 8Gy of Co^{60} gamma radiation.

Group V: Irradiated with 8Gy of Co^{60} gamma rays and given equal amount of DDW as given with the TC extract.

Group VI: Animals received extract of *Tinospora cordifolia* at the dose rate of 5mg/kg body weight orally.

Group II and IV: Served as experimental groups and group III and V as control groups.

The animals from control and experimental groups were sacrificed by cervical dislocation at ¼, 1, 3, 5, 7, 10, 14 and 28 days post irradiation. Six animals were

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sacrificed at each interval. Liver was removed, cleaned and used for biochemical studies.

Biochemical studies

DNA content of the liver was determined by the method of Ceriotti (1952) [6] and RNA content was determined by the method of Ceriotti (1955) [7].

Statistical analysis

All the values are expressed as mean ± standard error (S.E.) in the tables The S.E. was calculated by Fischer's formula (1946) [8]. The data were subjected to students 't' test for comparison between control and experimental group [9] for the assessment of significance of difference. Coefficient of correlation is also calculated by direct method between two series.

RESULTS

DNA content

One day after irradiation to 6Gy in control group DNA content decreased significantly (P<0.001) as compared to the sham irradiated controls. Then there was continuous increase up to 28th day but it remained below the normal value. The same pattern was seen in 8Gy treated control group. A decrease in the DNA content in experimental group (6Gy) was observed on 1st day but the values observed were higher than the controls. Recovery was seen on 3rd day, but the value remained higher in comparison to control till 28th day (P<0.001). Decrease in DNA content in the experimental group 8Gy (P<0.05) was noted till 1st day. Recovery was observed at the subsequent intervals, but did not attain the normal value (table1, figure1).

RNA content

RNA content increased significantly in the control animals treated with 6Gy which was maximum on day 3, followed by a decrease at the subsequent intervals. Significant increase was noticed in the control animals treated with 8Gy also which reached maximum on the 3rd post irradiation day. Then it decreased towards normal. Increase in the amount of RNA was recorded in the experimental animals also

Table 1. Variations in DNA content (mg/gm tissue) in liver of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment.

Treatment	Post-irradiation time (in days)							
	1/4	1	3	5	7	10	14	28
6Gy	0.482±0.023 P<0.01*	0.260±0.023 P<0.01*	0.261±0.012 P<0.001*	0.268±0.021 P<0.001*	0.0270±0.042 P<0.05*	0.283 ±0.082 NS	0.294±0.047 NS	0.311±0.021 NS
6Gy + Plant Extract	0.621±0.015 P<0.001**	0.312±0.013 P<0.01**	0.351±0.021 P<0.05**	0.357±0.020 P<0.05**	0.391±0.031 P<0.05**	0.396±0.066 NS	0.460±0.081 NS	0.483±0.013 P<0.001**
8Gy	0.179±0.012 P<0.001*	0.160±0.012 P<0.001*	0.164±0.015 P<0.001*	0.169±0.013 P<0.001*	0.196±0.012 P<0.001*	0.225±0.023 P<0.001*	ANS	ANS
8Gy + Plant Extract	0.298±0.026 P<0.01**	0.207±0.018 P<0.05**	0.233±0.022 P<0.05**	0.242±0.018 P<0.01**	0.247±0.017 P<0.05**	0.259±0.014 NS	0.261±0.042 -	0.284±0.012 -
Plant Extract Only	0.201±0.013	0.180±0.015	0.292±0.011	0.298±0.083	0.305±0.020	0.347±0.025	0.359±0.049	0.385±0.010

DNA content of normal *Swiss albino mouse* without any treatment is = 0.398±0.015 (mg/gm of tissue).
P value = Control Vs Normal* Control Vs Experimental** NS= Not Significant
Experimental = The plant extract was given 1 hr before irradiation at the dose rate of 5mg/kg body weight.
Control = Irradiated only

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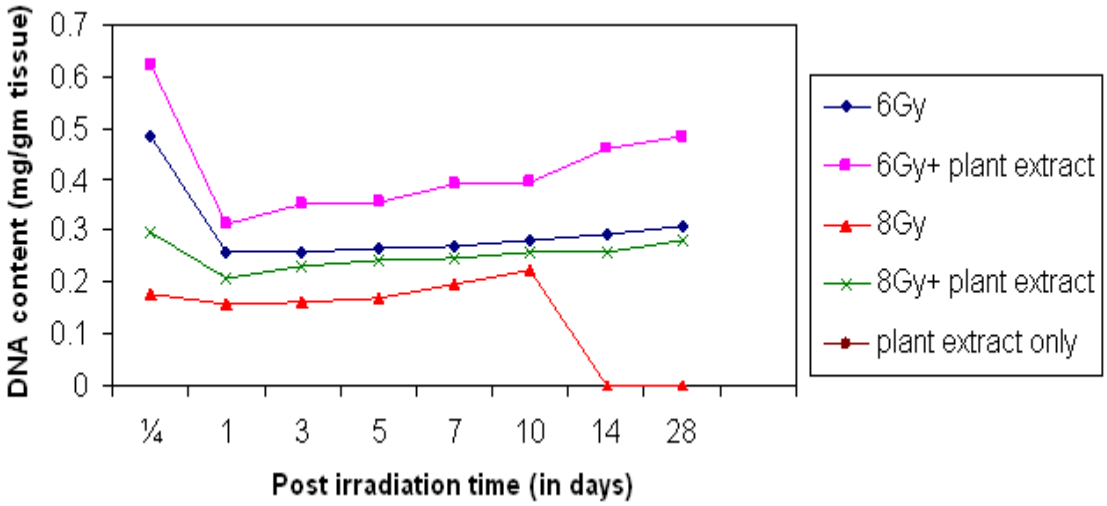


Figure 1. Variations in DNA content (mg/gm tissue) in liver of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia pretreatment*.

(6Gy) on 3rd post irradiation day. Then it came down to the normal on 10th day in the experimental group (P<0.01).Increase in the RNA content was observed in the experimental animals exposed to 8Gy from the very first interval (6hrs.). Maximum increase was observed on the 3rd post irradiation day. It was always lesser than the control (P<0.001). Recovery started by the 5th day which continued till 28th day (table 2, figure 2).

DISCUSSION

Changes in nucleic acid contents were altered in the liver of TC pretreated *Swiss albino mice* after exposure to various doses of gamma rays at different post irradiation intervals. DNA has unique biological activities. The loss of such biological functions as a result of irradiation of these molecules is of prime importance to some radiobiological effects. Semenov *et al.* [10]1994 reported that

Table 2. Variations in RNA content (mg/gm tissue) in liver of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment.

Treatment	Post-irradiation time(in days)							
	1/4	1	3	5	7	10	14	28
6Gy	0.936±0.014 P<0.05**	1.001±0.019 P<0.01**	1.181±0.022 P<0.05**	1.124±0.031 P<0.01**	0.925±0.014 P<0.05**	0.801±0.031 P<0.01**	0.800±0.012 P<0.01* *	0.783±0.011 P<0.01**
6Gy + Plant Extract	0.982±0.015 P<0.01*	1.131±0.022 P<0.001*	1.322±0.043 P<0.001*	1.260±0.023 P<0.001*	0.984±0.013 P<0.01*	0.950±0.013 NS	0.920±0.015 NS	0.915±0.021 P<0.001*
8Gy	1.023±0.040 P<0.001**	1.232±0.023 P<0.01**	1.352±0.025 P<0.001**	1.150±0.030 P<0.001**	0.985±0.027 P<0.001**	0.953±0.019 P<0.001**	Animal not survived	Animal not survived
8Gy + Plant extract	1.272±0.017 P<0.001*	1.450±0.043 P<0.001*	1.615±0.021 P<0.001*	1.490±0.035 P<0.001*	1.250±0.039 P<0.001*	1.230±0.018 P<0.001*	0.931±0.031 -	0.925±0.014 -
Plant Extract Only	0.901±0.041	0.912±0.061	0.928±0.067	0.933±0.016	0.931±0.021	0.929±0.063	0.914±0.017	0.912±0.040

RNA content of normal *Swiss albino mouse* without any treatment is =0.915±0.013 (mg/gm tissue).
P value = Control Vs Normal* Control Vs Experimental**
NS= Not Significant
Experimental = The plant extract was given 1 hr before irradiation at the dose rate of 5mg/kg body weight.
Control = Irradiated only

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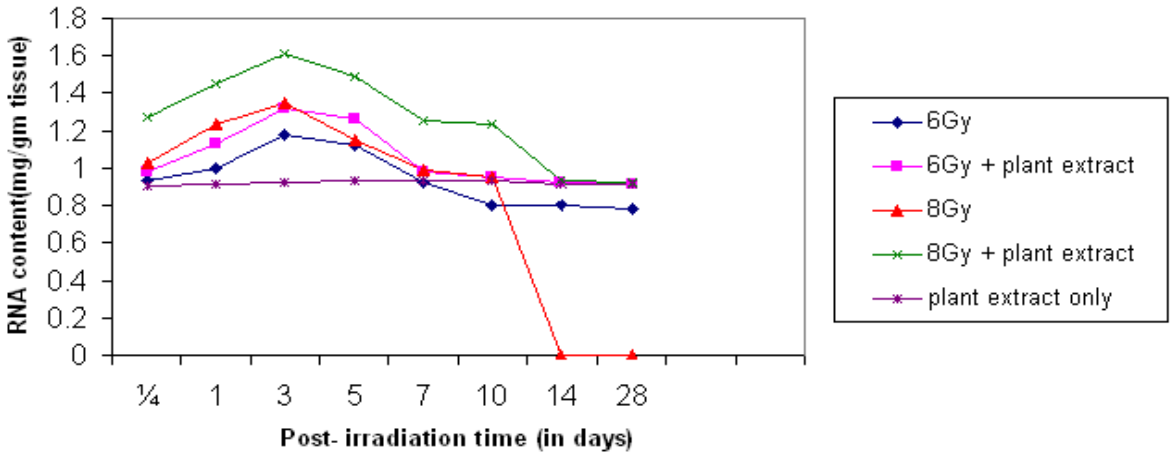


Figure 2. Variations in RNA content (mg/gm tissue) in liver of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia pretreatment.*

the modification of primary and secondary DNA structure in irradiated animals demonstrate serious disturbance of hepatocyte genetic apparatus. This is responsible for morphologic and ultra structural changes in rat liver tissue after exposure to ionizing radiation. DNA is considered to be the primary target for cell killing by ionizing radiation. Radiation produces a spectrum of lesions, including DNA single and double strand breaks, interstrand and protein cross links, and damage to the DNA bases and sugars. While several of these lesions may be involved in cell killing by radiation, for example unrepaired double strand breaks which are likely to lead to cell death. Irradiation causes a transitory mitotic arrest. This leads to a decreased number of cells passing through mitosis. The relative incorporation of DNA precursors into a given tissue is depressed because fewer cells are in S phase. Although the amount of DNA synthesized per cell in the 'S' period is normal, the total amount of DNA synthesis is depressed. In the present study the amount of DNA in 6Gy and 8Gy irradiated control groups maximum decreased 24 hours after irradiation and recovery (normal value) was obtained on 10^{th} day in the group exposed to 6Gy while normal value was never attained in the group exposed to 8Gy. On 10^{th} day the difference between control and experimental values was statistically non significant. Significantly higher value of DNA was obtained 6 hours after exposure to 6Gy in control and experimental groups. It may be attributable to unscheduled DNA synthesis after exposure to a sublethal dose. Bhatavadekar *et al.*[11] (1977) carried out cytochemical and biochemical studies on nucleic acids in the liver, kidney and pectoral muscle of guinea pig, rat and mouse after 240 X irradiation and observed significant depletion in the DNA content of mouse tissues.

Ionizing radiation induces reactive oxygen species in the form of hydroxyl ion, hydrogen ion, singlet oxygen and peroxyl radicals that fallow as cascade of events leading to DNA damage such as single or double strand breaks (DSB), base damage, and DNA-DNA or DNA- protein cross-links, and these lesions cluster as complex local multiply damaged sites. The DNA-DSBs are considered the most lethal events following ionizing radiation. The radioprotective activity of plants and herbs may mediate through several mechanisms, since they are complex mixtures of many chemicals. The majority of plants and herbs contain polyherbs, scavenging of radiation-induced free radicals and elevation of cellular antioxidants by plants in irradiated systems could be leading mechanisms for radioprotection. Upregulation of DNA repair genes may also

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protect against radiation induced damage by bringing error free repair of DNA damage.

Tinospora cordifolia provided significant protection against the radiation induced depletion of DNA content. The radiation induced decrease in the DNA content in control animals was more as compared to the experiment group. An early recovery to normal values was also recorded in the plant extract pretreated groups. Partial hepatectomy (PH) of rats resulted in acceleration of DNA synthesis in liver which reached maximum at 36 hours after PH. When whole body was exposed to 10Gy radiation after PH, it completely arrested this stimulation in DNA synthesis. DNA polymerase in nuclei and nuclear matrices, with and without exogenous DNA template revealed the whole body irradiation blocked induction of DNA polymerase alpha activity [12]. Nitroxides also protect radiation induced DNA damage [13]. An increase in protease activity was observed in nuclei of rats exposed to gamma radiation. Nuclear protease tightly bound to histones and specifically cleaving histones were observed and activated by apoptogenic factors of the mitochondrial intermembrane fraction. The apoptogenic action of gamma radiation involves only a direct DNA damage that induces activation of DNA dependent proteases but also as indirect component [14]. Choline chloride (200 mg/kg) given to rats 15 min before 6Gy irradiation increases the survival rate to prolong their average life, and to complete restoration of DNA supra molecular complexes in liver. When administered immediately after irradiation the drug increased the survival rate of rats [15]. Effect of Silymarin on nucleic acid was investigated in rats after total body gamma irradiation with a dose of 0.6Gy. Silymarin treatment altered radiation induced changes in the nucleic acid content of liver. It may be caused by activation of cellular metabolism including the metabolism of nucleic acids [16].

A significant increase in RNA content was observed in the present study at both the doses. This increase was maximum on the fifth day in 6Gy control and on third day with 8Gy control group. Synthesis of RNA is correlated to DNA. The correlation coefficient of 6Gy control is 0.95 and in 8Gy control it is 0.983. In the experimental animals also, the coefficient of correlation is very high i.e. 0.95 for 6Gy and 0.97 for 8Gy. In the only plant extract treated group it is 0.98.

Liver RNA, an interferon inducer, is able to offer significant cytogenetic protection from radiation, implying indirectly that the induction of interferon by low dose radiation may also play a protective role as one of the mechanism in the induction of the cytogenetic adaptive response [17]. Mentat, herbal formulation inhibited radiation induced damage by free radical scavenging [18].

In the present study, increased RNA content were noted at all the dose levels in *Tinospora cordifolia* treated groups also. But this increase was significantly lesser than that of the control groups. An earlier recovery to normal values was also observed in the TC treated animals. This suggests significant protective effect of the *Tinospora cordifolia* against radiation induced alteration in RNA content of mouse liver.

Present study reveals that *Tinospora cordifolia* is effective against radiation induced oxidative stress. Oxidative stress refer to the cytotoxic consequence of free oxygen radicals, superoxide anions, hydroxyl radicals and hydrogen peroxide, which are generated as by products of normal and aberrant metabolic processes that utilize molecular oxygen [19]. The oxidative stress may lead to DNA damage and mutagenesis, protein and carbohydrate oxidation and metabolic disorders [20-21].

CONCLUSION

Thus, the results from the present study suggest that pre treatment of *Tinospora cordifolia* extract protects mouse liver

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against the radiation. It works at a very low dose without causing side effects. Hence, it can be used as a radioprotector clinically.

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