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Radioprotective Role of Gymnemic Acid on Mice: Study on Hepatic Biochemical Alterations

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Abstract : The aim of the present study was to evaluate the radioprotective effect of Gymnemic acid (GA) on Swiss albino mice against radiation induced hepatic biochemical alterations. Swiss albino mice (6–8 weeks) were divided into three groups. Group I (Normal) was without any treatment. Group II (Control) was only irradiated group (8Gy). Group III (GA+Irradiated) Mice in this group received GA orally (350 mg / Kg.b.wt / day) one hour before radiation (8 Gy) exposure. Mice were sacrificed on days 1, 3 and 7, post-irradiation. Radiation induced deficit in hepatic GSH and protein levels was significantly increased, whereas radiation induced elevation of lipid peroxidation level was markedly averted in GA pre-treated animals than those of irradiated group. It showed that GA provides protection against radiation-induced biochemical alterations in liver of Swiss albino mice.

Key words : Gymnemic acid, liver**,** Antioxidant, Radioprotection

Introduction

Radiotherapy, which is a chief modality to treat cancer, faces a major drawback because it produces severe side effects developed due to damage to normal tissue by reactive oxygen species (ROS) such as hydroxyl (OH*) and peroxyl radicals (ROO*) and the superoxide anion (O_2*), which develop due to the interaction of radiation with the components of normal living system. Living systems are protected from oxidative damage by these reactive species by enzymes such as superoxide dismutase and glutathione peroxidase and by antioxidant compounds such as ascorbic acid, tocopherols and carotenoids (Sies, 1997). However, when free-radical production exceeds the antioxidant capacity of the organism, these radical species attack lipids, proteins, and DNA, thus damaging structural integrity and function of cell membranes, enzymes, and genetic material (Byers and Perry, 1992). Search for the chemical agents that are able to protect human beings from the ionizing radiation is a key issue in radiation biology. The use of plants and natural products may be beneficial in protecting against the radiation-induced damage, as they are less toxic or practically non-toxic compared to the synthetic compounds at their optimum protective dose levels. Therefore, the interest is generated in development of potential drug of plant origin for the modification of radiation effect. Recent studies have indicated that some commonly used medicinal plants may be good sources of potent but non-toxic radioprotectors. Earlier studies in our laboratory indicated that oral administration of â-carotene (Bhatia *et al*, 1978; Sharma and Sisodia, 2000) and plant extracts (Bhatia and Jain, 2004; Bhojak *et al*, 2006; Kamal *et al*, 2008), Amaranthus (Yadav *et al*, 2004; Verma

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et al, 2002), Flaxseed (Bhatia *et al*, 2006) and *Grewia asiatica* (Ahaskar *et al*., 2007; Ahaskar and Sisodia (2006) are good source of antioxidants.

Gymnema sylvestre R. Br. (Asclepiadaceae) is a slow growing, perennial, climber found in central and peninsular India (Stocklin, 1969). It is commonly called as 'Gurmar', meaning 'sugar-destroying', which is suggestive of the anti-sweet quality experienced after chewing one or two leaves, one is unable to detect a sweet taste. Decoctions of the leaves of this plant are used orally in Ayurvedic medicine for the treatment of diabetes (Nagaraju and Rao, 1990) or are heated with sesame oil to form emulsions, which may be used in drops to treat eye diseases (Dixit and Pandey, 1984). Gymnemic acid was extracted out from this plant. Gymnemic acids are triterpenoid saponins. Exposure of animals to ionizing radiation causes a series of physiological changes known as acute radiation syndrome that is dependent on the exposure dose and may lead to death. Liver, being the primary organ of drug metabolism of mammals, has been reported as highly radiosensitive (Bhatia *et al*, 1978). It plays a key role in detoxification of drugs. Present investigation was undertaken to evaluate the radioprotective efficacy of *G. sylvestre* extract, which may be helpful after radiation therapy as well as for the population residing in areas where they are continuously exposed to radiations.

Materials and Methods

The animal care and handling was done according to the guidelines set by World Health Organization, Geneva, Switzerland and INSA (Indian National Science Academy, New Delhi, India). Swiss albino mice, 6–8 weeks old weighing 23±2 gm, from an inbred colony were used for the present study. These animals were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mice feed (procured from Hindustan Levers Ltd., India) and water *ad libitum*. Tetracycline water once a fortnight was given as preventive measures against infections.

Extract preparation **: (Gymnemic acid (GA) :** Fresh leaves of *G. sylvestre* were dried and finely powdered, percolated in ethanol (95%) and refluxed on water bath for 2 hrs. The alcoholic extract was filtered, concentrated and was defatted with cyclohexane. The defatted extract was further partitioned with n-butanol and dried *in vacuo* and quantified. The extract was finally dissolved in Methanol for desired dose. The methanol extract was referred as crude gymnemic acid (Ye *et al*., 2000).

Source of irradiation : The cobalt teletherapy unit (ATC-C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur, Rajasthan, India was used for irradiation. Unanaesthestized animals were restrained in well-ventilated perspex boxes and whole body exposed to 8 Gy gamma radiation.

Dose selection: Single dose at the rate of 350 mg/kg b.wt one hour before the radiation exposure.

Experimental design : Mice selected from an inbred colony were divided into 3 groups (18 animals in each Group).

Group I (Normal): Mice of this group did not receive any treatment.

Group II (Control): Mice received DDW and then exposed to 8 Gy of gamma-radiation.

Group III (GA+Irradiated): In this group oral administration of GAE (350 mg/kg of b.wt./day) was made once daily for 15 consecutive days. One hour after administration of last dose of GAE, mice were whole body exposed to single dose of 8 Gy gamma-radiations as in group second.

Six mice from each groups were necropsied at the same time (10 a.m.) and intervals, i.e. 1, 3 and 7 days *post irradiation*; liver was immediately removed for biochemical assay.

***Lipid peroxidation (LPO) assay* :** LPO was measured by the method of Buege and Aust (1978).

***Reduced glutathione (GSH) assay* :** The reduced glutathione (GSH) content of tissue samples were determined in liver by the method of Moron *et al* (1979).

***Protein Assay* :** Estimation of protein was based on the method proposed by Bradford (1976).

***Statistical analysis* :** The results obtained in the present study were expressed as mean ± SEM. The statistical difference between various groups were analysed by the Student's *t*-test and the significance was observed at the $p > 0.01$and $p > 0.001$ level.

Results

***Lipid peroxidation (LPO)* :** As shown in Fig. 1 the lipid peroxidation product as reflected by TBARS equivalent content **got** augmented after radiation exposure (8 Gy). A significantly higher ($p>0.001$) recovery occurred with treatment of GA prior to radiation exposure as compared to that of irradiated mice. LPO level increased by 34.48%, 38.24%, 33.09% on 1, 3 and 7 days post irradiation respectively, in control group when compared to normal level. Whereas, GA pretreatment reduced the LPO level by 10.56%, 24.38 and 18.8% on 1, 3 and 7 days post irradiation respectively, in comparison to their respective control level.

Glutathione (GSH) : Glutathione (GSH) content decreased after radiation exposure in the liver of irradiated mice as shown in Fig. 2. A significantly higher ($p>0.001$) protection in terms of GSH content with treatment of GA

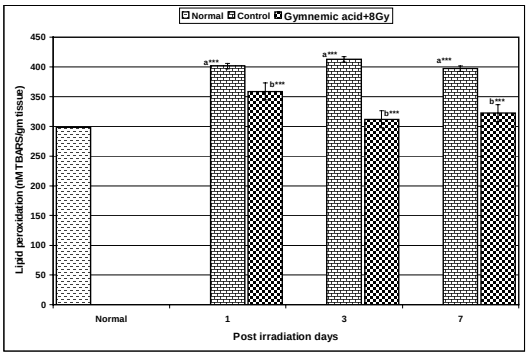

Fig. 1: Graph showing variations lipid peroxidation measured as nm TBARS/gm tissue of mice liver in GA treated-irradiated group and irradiated (8Gy) group in comparison to that of control group. Data have been expressed as mean ± SEM. P values **>0.01, *>0.001; a: Normal v/s Control, b: Control v/s GA treated + irradiated (8Gy).**

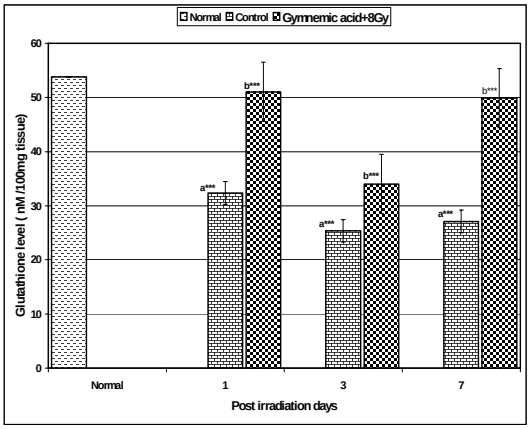

Fig. 2: Graph showing variations glutathione level measured as nm/100mg tissue of mice liver in GA treated- irradiated group and irradiated (8Gy) group in comparison to that of control group. Data have been expressed as mean ± SEM. P values **>0.01, *>0.001; a: Normal v/s Control, b: Control v/s GA treated + Irradiated(8Gy).**

prior to radiation exposure occurred as compared to that of irradiated mice. On day 7, GA pretreatment showed greater protection, but

Table 1. Radiomodulatory influence of *Annona squamosa* plant extract on hepatic GSH , LPO ,and protein ± SEM of swiss albino mice at various post treatment days after 8 Gy radiation exposures.

Parameters	Normal	Irradiated (control)			AE treated + irradiated		
		1 day	3 day	7 day	1 day	3 day	7 day
GSH (n mole/100mg tissue)	53.79 ± 0.79	32.33 ± 0.24*	25.38 ± 0.64*	27.09 ± 0.51*	51.01 ± 0.58a	34.04 ± .0.43a	27.09 ± 0.51*
LPO (n mole MDA/gm)	298.39 ± 3.36	401.28 ± 2.74*	412.52 ± 1.88	397.13 ± 2.12*	358.84 ± 2.15a	311.94 ± 1.21a	322.46 ± 1.47a
Protein (mg/gm)	151.50 ± 1.31	108.92 ± 1.7	83.92 ± 1.78	80.35 ± 1.53*	121.34 ± 1.21a	97.26 ± 0.97a	137.33 ± 1.07a

**p=<0.001 with normal; ᵃp=<0.001 with control*

failed to attain the normal level. Decrease in GSH content by 39.90%, 52.82% and 49.64% was seen on days 1, 3 and 7, post–irradiation, respectively, in control group in comparison to those of normal ones. GA pretreatment provides protection by 57.77%, 34.12% and 84.05% days 1, 3 and 7, post–irradiation, respectively, which is significantly effective in preventive/prophylactic against radiation induced free radical generation and helps sustaining the cellular homeostasis.

Protein estimated in mice liver also showed statistically significant decrease (Fig. 3) after radiation exposure in irradiated group

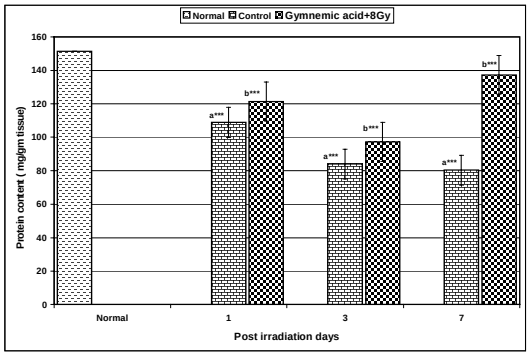

Fig. 3: Graph showing variations protein content measured as mg/gm tissue of mice liver in GA treated- irradiated group and irradiated (8Gy) group in comparison to that of control group. Data have been expressed as mean ± SEM. P values **>0.01, *>0.001; a: Normal v/s Control, b: Control v/s GA treated + Irradiated (8Gy).**

II. In GA pre treated+irradiated (group III), protein content was significantly higher than there corresponding irradiated group at all autopsy interval .At day 7th, both group i.e. GA pretreated- irradiated group unable to achieve the normal levels. Reduction in protein content by 28.11%, 44.61% and 46.97% was seen on days 1, 3 and 7, post irradiation respectively in control group in comparison to normal level. GA pretreatment protect the protein level by 11.4%, 15.89% and 70.91% on days 1, 3 and 7, post irradiation respectively in comparison to that of control group

Discussion

Ohmori and coworkers (2005) assessed the antioxidant activity of six teas, including the aqueous extracts of green tea and oolong tea (*Camellia sinensis*), tochu (*Eucommia ulmoides*), *Gymnema sylvestre*, Japanese mugwort (*Artemisia princeps*), and barley (*Hordeum vulgare*), against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. *In vitro*, the inhibitory effects of DPPH radicals and LDL oxidation were found to be strongest in the extract of green tea and weakest in that of barley. Results obtained from the present study indicate that the natural bioactive molecules gymnemic acid in GA are able to substantially protect the liver from radiation damage. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions. The presence of antioxidants in the plants

suppresses the formation of free lipid radical and thus prevents the formation of endoperoxidation. In the present study, GA pre-treatment significantly lowered the radiation-induced lipid peroxidation in terms of malondialdehyde. The inhibition of lipid peroxidation in biomembranes can be caused by antioxidants (Konings and Drijver, 1979; Konings and Osterloo, 1979). Under normal conditions, the inherent defense system including glutathione and antioxidant enzymes protects against the oxidative damage. The present study demonstrates a significant reduction in hepatic GSH following 8 Gy 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. Oral administration of GA protects the endogenous GSH depletion due to irradiation may be due to the higher availability of GSH, which increases the ability to cope up with the free radicals produced by irradiation. The increased GSH level suggests that protection by GA may be mediated through the modulation of cellular antioxidant levels.

Reduction in rate of the protein synthesis may be due to unfavorable conditions like unavailability of one or more essential enzymes and/or reduction in sites of protein synthesis (Bacq and Alexander, 1961). Increased protein concentration recorded in our study shows that GA supplemented irradiated mice exert prophylactic or/and ameliorative effects against radiation action. This proves an improvement in ribosomal activities, which enhance the protein synthesis and can be treated as antiradiation effect.

Ananthan *et al* (2003a) worked on *Gymnema montanum* Hook and reported a modulatory effect on rate limiting enzymes of glycolysis, gluconeogenesis as well as antihyperlipidemic effect in the liver of diabetic rats (Ananthan *et al*, 2003b) and also antiperoxidative effect in alloxan induced diabetic rats (Ananthan *et al*, 2003c). In induced diabetes, decrease in lipid peroxides and increase in reduced glutathione (GSH), ascorbic acid and α-tocopherol (Vitamin C & E) clearly show the antioxidant properties of ethanolic extract of *G. montanum.* Ramkumar *et al* (2004) showed regulatory effect of *G. montanum* leaf extract on brain antioxidant status and also lipid peroxidation in diabetic rats. They found that treated group showed significant decrease in formation of TBARS and hydroperoxides in brain, which suggests a role in protective action, against lipid peroxidation-mediated membrane damage.

In the present investigation *G. sylvestre* extract showed significant hepato-protective effect against irradiated mice as compared to normal and control groups. It also suggests that the herbal preparations can have modulatory as well as protective efficacy against the radiation hazards during the radiation therapies or against high background radiation helping population living in teh vicinity of nuclear reactiors.

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