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Study on Acetogenin against Radiation-induced Hepatic Biochemical Alterations in Mice

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Abstract : The aim of the present study was to evaluate the radioprotective effect of Acetogenin (AE) 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 (8 Gy). Group III (AE+Irradiated): Mice in this group received AE 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 significantly increased whereas radiation induced elevation of lipid peroxidation level was markedly averted in AE pre-treated animals than those of irradiated group. It showed that AE provides protection against radiation-induced biochemical alterations in liver of Swiss albino mice.

Key words : Acetogenin, liver**,** Antioxidant, Radioprotection

Introduction

Damage to normal tissue by reactive oxygen species (ROS) such occur as hydroxyl (OH*) and peroxyl radicals (ROO*) and the superoxide anion (O_2*) developed 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. Synthetic protectors against oxidative damage to tissue have toxicity. This limits their value in the clinical field. 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 as 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. Antioxidants of plant origin include vitamin E, C, selenium, phenolic compounds, carotenoids and flavonoids (Chandha, 1996). Earlier studies in our laboratory indicated that oral administration of â-carotene (Bhatia *et al*, 1978; Sharma and Sisodia, 2000) and plant extract of Spinach (Bhatia and Jain, 2004), Amaranths (Yadav *et al*, 2004; Verma *et al* , 2002), flaxseed (Bhatia *et al*, 2006) and *Grewia asiatica* (Ahaskar *et al.*, 2007; Ahaskar and Sisodia, 2006) to Swiss albino

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mice protect various tissues against oxidative stress induced by radiation.

India has a rich heritage of medicinal plants, many of which have been explored for the various bioactivities since ages, but the radioprotective potential of *Annona squamosa* has been chosen for evaluating its radioprotective efficacy. *Annona squamosa* L. (Annonaceae), commonly known as Sitaphal and Custard Apple, is a native of West Indies and is cultivated throughout India, mainly for its edible fruit. The young leaves of *A. squamosa* are used extensively for its antidiabetic activity (Atique, 1985). The plant contains aporphine alkaloids (Oliver-Bever, 1986), carvone, linalool, limonene (Ekundayo, 1989), squamosin (Yu *et al.,* 2005) and quercetin (Panda and Kar, 2007). Acetogenins was extracted out from this plant. The acetogenins are a class of compounds endogenous to the Annonaceae. The biological effects of many annonaceous have been related to the ability of Acetogenins to inhibit the NADH: ubiquinone oxiredutase (complex 1) of the mitochondrial electron transport chain (González *et al.,* 1998). This class of molecules has been suggested to be a group of potential anti-neoplastic agents (Alali *et al.,* 1999; Yuan *et al.,* 2003).

The liver of mammals has been reported as highly radiosensitive organ (Bhatia *et al*, 1978). It is the primary organ of drug metabolism. It plays a key role as detoxification agency in the body. Present investigation thus has been undertaken to evaluate the radioprotective efficacy, of a cetogenins which may prove efficient radioprotectant.

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.). The 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 (Acetogenin AE)*:**

Seeds of *A. squamosa* were dried, finely powdered and percolated with 95% ethanol for 12h. The alcoholic extract was filtered and partitioned with dichloromethane and water (1:1). The water fraction was discarded and dichloromethane fraction was further partitioned with hexane and 10% Methanol (1:1). The methanolic fraction was dried *in vacuo*, quantified and referred as crude acetogenin (Li *et al*., 1990).

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

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) was measured by the method of Buege and Aust (1978).

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

Estimation of protein was based on the method proposed by Bradford (1976).

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 > 0.01and p> 0.001 level.

Results

Lipid peroxidation (LPO) : Lipid peroxidation product as reflected by TBARS equivalent content got augmented after radiation exposure (8 Gy) in irradiated mice as shown in Fig. 1. Magnitude of a recovery from oxidative damage in terms of TBARS content with treatment of AE prior to radiation exposure was significantly higher (p>0.001) as compared to irradiated mice. LPO level increased by 34.48%, 38.24%, 33.09% on 1, 3 and 7 day post irradiation in control group when compared to normal level. Whereas, AE pretreatment reduced the LPO level by 7.73%, 18.91% and 22.41% on 1, 3 and 7 days, post irradiation respectively, in comparison to their respective control level.

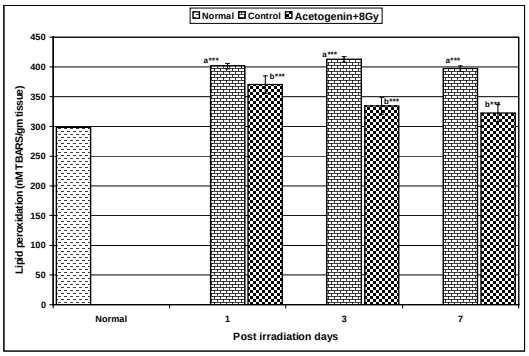

Fig. 1: Graph showing variations lipid peroxidation measured as nm TBARS/gm tissue of mice liver in AE treated-irradiated group and irradiated (8Gy) group in comparison to that of control group. Data have been expressed as mean ± SEM. P values **>0.01, *>0.001; a: Normal v/s Control, b: Control v/s AE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

Reduced glutathione (GSH) : Glutathione (GSH) content was decreased after radiation exposure in the liver of irradiated mice as shown in Fig. 2. Magnitude of protection in terms of GSH content with treatment of AE prior to radiation exposure was significantly higher (p>0.001) as compared to irradiated mice. At day 7 GA pre-treatment showed greater protections than those of AE pre treatment when compare to control group (Bhatia *et al*, 2008), however, both the groups failed to attain the normal levels. 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 that normal group. AE pretreatment provides protection by

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*	35.04 ± 0.81a	48.73 ± 0.48 a	47.33 ± 0.19 a
LPO (n mole MDA/gm)	298.39 ± 3.36	401.28 ± 2.74*	412.52 ± 1.88	397.13 ± 2.12*	370.26 ± 2.36a	334.52 ± 1.69a	308.14. ± 1.39a
Protein (mg/gm)	151.50 ± 1.31	108.92 ± 1.7*	83.92 ± 1.78*	80.35 ± 1.53*	116.29 ± 1.23b	112.47 ± 1.19a	126.24 ± 1.27 a

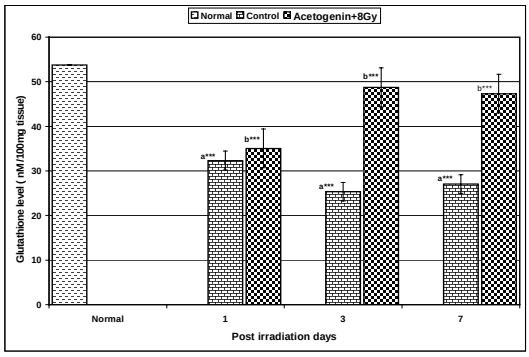

Fig. 2: Graph showing variations glutathione level measured as nm/100mg tissue of mice liver in AE treated- irradiated group and irradiated (8Gy) group in comparison to control group. Data have been expressed as mean ± SEM. P values **>0.01, *>0.001; a: Normal v/s Control, b: Control v/s AE treated +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

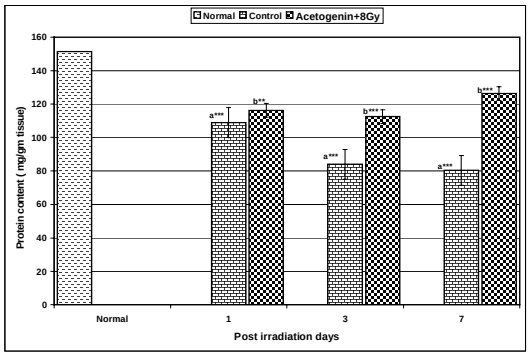

Fig. 3: Graph showing variations protein content measured as mg/gm tissue of mice liver in AE treated- irradiated group and irradiated (8Gy) group in comparison to control group. Data have been expressed as mean ± SEM. P values **>0.01, *>0.001; a: Normal v/s Control, b: Control v/s AE treated + Irradiated(8Gy).**

8.38%, 92% and 74.71% days 1, 3 and 7, post–irradiation, respectively, in comparison to control group.

Protein : Protein estimated in mice liver also showed statistically significant decrease (Fig. 3) after radiation exposure in irradiated group. In AE pre treated-irradiated group, protein content was significantly higher than there corresponding irradiated group at all autopsy interval but as Fig. 3 showed pre treatment with AE provides continuous protection. At day 7th, AE 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. AE pretreatment protect the protein level by 6.77%, 34.02% and 57.11% on days 1, 3 and, 7 post irradiation, respectively, when compared to that of control group.

Discussion

The AE treated animal showed significant radioprotective effect. All the parameters GSH, LPO, and Protein showed remarkable recovery and protective impact due to AE drug administered to the mice. Shirwaikar *et al* (2004) demonstrated improvement in liver glycogen and pancreatic TBARS levels by Aqueous leaf extract of *A. squamosa* on experimental diabetic rat. Pardhasaradhi *et al* (2005) showed that increased levels of ROS and a reduced GSH concomitant with down regulation or loss of Bcl-2 gene expression in MCF-7 and K-562 cells does not occur in COLO-205 cells after treatment with *A. squamosa* extracts. The mode of action of Acetogenin may target mitochondrial electron transport with a specific action at NADH: ubiquinone oxidoreductase (NADH-dehydrogenase, also known as complex I) (Lewis *et al*., 1993; Londerhausen *et al*., 1991). Furthermore, the inhibitory effects of ACGs have been shown to be more potent than those of classical respiratory inhibitors such as rotenone or piericidin A (Degli Esposti *et al.*, 1994). Hence its action as antioxidant is not safely ruled out.

Lipid peroxidation is a highly destructive process and cellular organelles and whole organism, lose biochemical function and/or structural and architecture (Kale and Sitaswad, 1990) which may lead to damage or death of

cell. 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, however, AE 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).

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 (Bump and Brown, 1990). 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 AE 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 AE 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). The decrease of protein noted may be due to its lyses, by X-irradiation or may be at the synthesis level, also may be the depression of enzyme involved in the activation of amino acid and transferring to t-RNA (Wender, 1970), or by the inhibition of release of synthesized polypeptides from polysomes (Kim *et al*. 1970). Increased protein concentration recorded in our study, shows that AE supplemented irradiated mice are a beneficial effect. This proves an improvement in ribosomal activities, which enhance the protein synthesis, can be treated as antiradiation effect. Fresh leaves mixed with food oil are used to cure scorpion bite (Jagtap *et al.*, 2006) and to kill germs, worms and insects (Acharya and Pokhrel, 2006).

Results obtained from the present study indicate that the natural medicines found in AE substantially protect the liver from radiation damage.

References

Acharya E. (Siwakoti) and Pokhrel B. (2006): Ethno-Medicinal Plants Used by Bantar of Bhaudaha, Morang, Nepal. *Our Nature: An International Biological Journal*, **4 (1)**, 96-103

Ahaskar M., Sharma KV, Singh S. and Sisodia R. (2007): Radioprotective effect of the fruit extract of *Grewia asiatica* in Swiss albino mice against lethal dose of γ-irradiation. *Asian J Exp Sci.*, **21(2)**, 295-308.

Ahaskar M., Sisodia R. (2006): Modulation of Radiation induced Biochemical Changes in brain of Swiss Albino Mice by *Grewia asiatica*. *Asian J Exp Sci.*. **20**(2): 399-404.

Alali FQ., Liu XX., and Mclaughli, JL., (1999): Annonaceous acetogenins: recent progress. *J. Nat. Prod.*, **62(3)**, 504-540.

Atique A., Iqbal M. and Ghouse A.K.M. (1985): Use of *Annona squamosa* and *Piper nigrum* against diabetes. *Fitoterapia.*, **56**, 190–192.

Bacq Z.M. and Alexander P. (1961): Fundamentals of Radiobiology, English Language Book Co. and Pergamon Press. New York.

Beutler E., Duron O., Kellin B.M. (1963): Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.*, **61**, 882–888.

Bhatia A.L. and Jain M. (2004): *Spinacea oleracea* L. protects against gamma radiations: A study on glutathione and lipid peroxidation in mouse liver. *Phytomedicine*.,**11**, 607-615.

Bhatia A.L., Gupta M.L. and Singh R.P. (1978): Response of mice liver to continuous beta irradiation from tritiated water. *J. Radiat.Res*., **19**, 197- 204.

Bhatia A.L., Manda K.,Patni S. and Sharma A.L.(2006): Prophylactic action of Linseed (*Linum usitatissimum*) oil against cyclophosphamide induced oxidative stress in mouse brain. *J.Med. Food*., **9(2)**, 261-264.

Biaglow J.E., Varnes M.E., Epp E.R. and Clark E.P. (1987): In: Anticarcinogenesis and Radiation Protection, Eds. P. A. Cerrutti, O. F. Nygaard and M.G. Simic, Plennum Press, New York 387.

Bradford M.M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principal of protein-By binding. *Anal Biochem.*, **72**, 248-254.

Buege J.A. and Aust S.D. (1978): In: Methods in Enzymology, Academic Press, New York 52: 302–314.

Bump E.A. and Brown J.M. (1990): Role of glutathione in the radiation response of mammalian cells *in vitro* and *in vivo*. *Pharmacol. Ther*, **47**, 117-136

Byers T. and Perry G. (1992): Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers. *Annu Rev Nutr*, **12**, 139-159.

Chandha S.L. (1996): Natural sources of antioxidants and their adequacy in diet to prevent atherosclerosis. M*ediquest,* **143**, 337-351.

Degli Esposti M., Ghelli A., Ratta M., Cortes D. and Estornell E. (1994): Natural substances (acetogenins) from the family Annonaceae are powerful inhibitors of mitochondrial NADH dehydroganse (complex I). *Biochem. J*., **301**, 161-167.

Ekundayo O. (1989): A review of the volatiles of the Annonaceae. *J Essent Oil Res*., **1**, 223.

González M.C., Lavaud C., Gallardo T., Zafra-pollo M.C. and Cortes D. (1998): New Method for the determination of the absolute Stereochemistry in Antitumoral Annonaceous Acetogenins. *Tetrahedron*, **54(22)**, 6079-6088.

Jagtap S.D., Deokule S.S. and Bhosle S.V. (2006): Some unique ethnomedicinal uses of plants used by the Korku tribe of Amravati district of Maharashtra, India. *J ethnopharmacol*., **107(3)**, 463-469.

Kale R.K. and Sitaswad S.L. (1990): Radiation induced lipid Peroxidation in liposomes. *Radiat Phys Chem*, **36**, 361-364.

Kim S.H., Kim J.H. and Djorddevic D. (1970) : Effects of x-irradiation on RNA and Protein Synthesis inHela Cells. *Radiat. Res.*, **42**, 577-589.

Konings A.W.T. and Drijver E.B. (1979): Radiation effect on membranes. I. Vitamin E deficiency and lipid peroxidation. *Radia Res.*, **80**, 494.

Konings A.W.T. and Osterloo S.K. (1979): Radiation effect on membranes. II. A comparison of the effect of X- irradiation and ozone exposure with respect to the relation of antioxidant concentration and the capacity for lipid peroxidation. *Radiat Res.*, **81**, 200.

Lewis M. A., Arnason J. T., Philogene B. J. R., Rupprecht J. K. and McLaughlin J. L. (1993): Inhibition ofrespiration at site I by asimicin, an insecticidal aceto-genin of the Pawpaw, Asimina triloba (Annonaceae). *Pest. Biochem. Physiol*., **45**, 15-23.

Li X.H., Hui Y.H., Rupprecht J.K., Lui Y.M., Smith D.L., Chan C.J. and Mc Laughlin J.L. (1990): Bullatacin, bullatacinone, and squamone, a new bioactive acetogenin from bark of Annona squamosa. *J. Natural Products*., **53**, 81-86.

Londerhausen M., Leicht W., Lieb F., Moeschler H. and Weiss H. (1991) : Molecular mode of action of annonins. *Pestic. Sci*., **33**, 427-438.

Moron M.S., Depierre J.W. and Mannervik B. (1979): Levels of GSH,GR and GST activites in rat lung and liver.*biochem.biophys.acta*., **582**, 67-78.

Oliver-bever B**.** (1986): Medicinal plants in tropical West Africa. Cambridge University Press,Cambridge, UK.

Panda S and Kar A. (2007): *Annona squamosa* seed extract in the regulation of hyperthyroidism and lipid-peroxidation in mice: *Possible involvement of quercetin*., **14(12)**, 799-805.

Pardhasaradhi B.V.V., Redy M., Mubarak Ali A., Leela Kumari A. and Khar A. (2005): Differential cytotoxic effect of Annona Squamosa seed extract on human tumour cell lines: role of Reactive Oxygen Species and Glutathione. *J. Biosci*., **30(2)**, 237-244.

Sharama M.K. and Sisodia R.(2000): â-carotene against Radiation-induced oxidative stress in mice brain. *Asian J. of Exp. Sci*, **14**, 43-44.

Shirwaikar A., Rajendran K., Kumar C.D. and Bodla R. (2004): Antidiabetic activity of aqueous leaf extratct of Annona squamosa in streptozotocin-nicotinamide type 2 diabetic rats. *J. Ethnopharmacol*., **91(1)**, 171-175.

Sies H. (1997): Oxidative stress: oxidants and antioxidants. *Exp Physiol.*, **82**, 291-295.

Verma R.K., Sisodia R. and Bhatia A.L. (2002): Radioprotective role of *Amaranthus gangeticus* Linn.: A biochemical study on mouse brain. *J. med food*., **5**, 189-195.

Wender M. and Zgorzalewiez B. (1970): Activation of amino acids. following parental x-irradiation in the developing rabbit brain. *Folia Biol.*, **18**, 343

Yadav R.K., Bhatia A.L. and Sisodia R. (2004) : Modulation of radiation induced biochemical changes in testis of swiss albino mice by *Amaranthus paniculates* Linn. *Asian J. Exp. Sci*., **18**, 63-74.

Yu J.G., Luo X.Z., Sun L., Li D.Y., Huang W.H. and Liu C.Y. (2005): Chemical constituents from the seeds of *Annona squamosa*. Yao Xue Xue Bao., **40(2)**, 153-158.

Yuan S.S.F., Chang H.L., Chen H.W., Yeh Y.T., Kao Y.H., Lin K.H., Wu Y.C. and Su J.H. (2003): Annonacin, a mono-tetrahydrofuran acetogenin, arrests cancer cells at the G1 phase and causes cytotoxicity in a Bax- and caspase-3-related pathway. *Life Sci.*, **72(25)**, 2853-2861.