RADIOPROTECTION BY FLAXSEED OIL: EFFICACY ON POST-IRRADIATION ADMINISTRATION

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Summary

Hitherto known almost all the studies on radioprotection with synthetic and natural agents have been mainly prophylactic but not therapeutic. To the best my knowledge the present one is the first one which reports the radioprotective efficacy of flaxseed oil in post-irradiation administration as therapeutic agent. Though Swiss albino mice received flaxseed oil in both the cases, prior to irradiation and after the irradiation showed radioprotective effects, the noteworthy feature is the magnitude of effectiveness. Animals exposed to gamma radiation first and then received flaxseed oil for consecutive 15 days were examined for 15 and 30 days intervals after irradiation. The liver showed that glutathione constantly compensated the loss due to radiation to a greater extent, on both the intervals. The value was significantly higher ($P>0.001$) in comparison to their respective controls (irradiated only) and insignificant or less significant ($P>0.05$) as compared to the group where it was administered before irradiation. Augmentation in glutathione content and a check in lipid peroxidation in liver of irradiated group in experimental group at 15 and 30 post treatment days' intervals has demonstrated a profound therapeutic efficacy of flaxseed oil. The paper describes the mechanism of action of flaxseed oil in both the cases, pre- and post irradiation administrations.

Introduction

Flaxseed, also known as flax oil and linseed oil, is derived from the seeds of the plant *Linium usitatissimum.* Flaxseed oil is a very rich source of alpha-linolenic acid. Alpha-linolenic acid concentration in flaxseed oil ranges from approximately 40 to 60%. Lower amounts of linoleic acid and oleic acid (each about 15%) are also present in flaxseed oil. In addition, flaxseed oil contains varying amounts of the lignan, secoisolariciresinol diglycoside (SDG). Plant lignans are a large class of phytochemicals that are formed by the fusion of two coniferyl alcohol residues and are structurally related to the lignins present in plant cell walls. They are dimers of phenylpropane units, present in vacuoles and cell walls and are soluble in organic solvents [1]. They occur throughout the plant kingdom where they exist either as a glycoside or in their free form. Flaxseed is the richest source of the lignan secoisolariciresinol diglucoside (SDG). Flaxseed also has smaller quantities of matairesinol, isolariciresinol, lariciresinol, demethoxysecoisolariciresinol and pinoresinol [2, 3]. The level of SDG in flaxseed varies between 0.6 to 1.8/100g [4, 5] or 1-4% by weight [6, 7], 60-700 times greater than any other edible plant [1, 8] with the variability in components depending on the cultivar [9, 5], the growing location and year [10, 5]. It has been suggested that the lignans within flaxseed are the beneficial components within flax [11].

Hitherto known almost all the studies on radioprotection with synthetic and natural agents have been mainly prophylactic but not therapeutic. Keeping in view the foregoing facts it was thought worthwhile to investigate the therapeutic role of flaxseed oil. After some preliminary investigation, an exhaustive study on brain, liver and other tissues have been made in our laboratory and the results have been quite stimulating. Our previous studies have clearly demonstrated the radioprotective prophylactic effect of flaxseed oil in mice [12, 13, 14]. To the best my knowledge the present one is the first one which reports the radioprotective efficacy of flaxseed oil in post-irradiation administration as therapeutic agent.

Materials & Methods

Swiss albino mice (*Mus musculus*) obtained from All India Institute of Medical Sciences (AIIMS), New Delhi and kept at controlled condition of temperature (25±2 ^{o}C) and light (light : dark, 14 : 10 hrs). They were provided standard mice feed (procured from Hindustan Liver ltd. Mumbai) and *ad libitium*. Flaxseed oil (*linum usittatisimus*) family Lineascey derived through a cold pressure method, procured from commercial shop Mulkusha Natural Oiltak, Ganpati Plaza, Jaipur, India. Mice of each group were administered Flaxseed oil (FO) (4ml/kg /B.wt/day) for 15 consecutive days.

The animals were exposed to whole body to gamma radiation at the source to surface distance (SSD) of 77.5 cm. The dose rate for higher doses was 1.13Gy/min gamma and for sub lethal dose 1.07 Gy/min. The dose rate was calibrated at each exposure using the decay table for Co-60. For Experimentation 5 Gy dose level (sublethal dose) was selected.. Animals were examined on 15th and 30th days after irradiation in all the three cases i.e. in control (irradiatd only, post irradiation administration and pre irradiation administration). Liver was examined for glutathne and its LPO activities.

Glutathione(GSH)

GSH content was measured by the method of Moron *et al* (1979). Briefly, proteins were precipitated by 25% TCA, centrifuged and the supernatant was collected. The supernatant was mixed with 0.2 M sodium phosphate buffer (pH 8.0) and 0.06 mM DTNB (5,5'di nitro bis nitro benzoic acid) and incubated for 10 min at room temperature. The absorbance of the sample/s was read against the blank at 412 nm in an ultraviolet-visible light (UV-Vis) double beam spectrophotometer, and the GSH concentration was calculated from the standard curve.

Lipidperoxidation(LPx)

LPx was measured by the method of Buege and Aust [16]. Briefly, tissue homogenate was mixed with TCA-TBA-HCl and was heated for 15 min in a boiling water bath. After centrifugation the absorbance was recorded at 535 nm using a UV-Vis double beam spectrophotometer. The LPx has been expressed as MDA in n mole/ gm tissue.

Animals were exposed to gamma radiation first and then received flaxseed oil for consecutive 15 days were examined for 15 and 30 days intervals after irradiation. Animals of each were humanly sacrificed by cervical dislocation at 15, and 30 days after irradiation, in both the cases *i.e.* administration of flaxseed oil pre- and post irradiation. A minimum of 6 animals from each group were sacrificed at each autopsy interval.

Results and Discussion

The glutathione values reached to almost normal level in 30 days. Though Swiss albino mice received flaxseed oil in both the cases, prior to irradiation and after the irradiation showed radioprotective effects, the noteworthy feature is the magnitude of effectiveness. Animals exposed to gamma radiation first and then received flaxseed oil for consecutive 15 days were examined for 15 and 30 days intervals after irradiation. The liver showed that glutathione constantly compensated the loss due to radiation to a greater extent, on both the intervals. The value was significantly higher ($P>0.001$) in comparison to their respective controls (irradiated only) and insignificant or less significant ($P>0.05$) as compared to the group where it was administered before irradiation.

Augmentation in glutathione content and a check in lipid peroxidation in liver of irradiated group in experimental group at 15 and 30 post treatment days' intervals has demonstrated a profound therapeutic efficacy of flaxseed oil.

Lipid peroxidation: - Results show that radiation induced the lipid peroxidation as reflected by the TBARS equivalents content which increases in liver on days 15 and 30, post irradiation. TBARS equivalents content decreased at day 30 post irradiation in pre irradiated administration. The oil supplementation significantly prevents the radiation-induced lipid peroxidation in liver, as statistically there is a significant difference between irradiated and experimental animal. Flaxseed oil pre-treatment as well as post treatment significantly lower the radiation induced LPO in terms of malondialdehyde.

Inhibition of LPO in cell membrane can be caused by antioxidants [17]. Shimoi *et al.*, [18] concluded that plant flavonoides, which show antioxidants activity *in vitro* also, function as antioxidants in vivo, and their radioprotective effect may be attributed to their radical

scavenging activity. Flaxseed oil has very high content of alpha linolenic acid (c 18: 3n-3 omega-3 (n-3) fatty acid) together with small amount of phytoestrogen /lignan. Lignan are platelets activating factor receptor antagonists [19] and have antioxidant activity [4]. Due to antioxidant property of lignan, it is suggested that hepatic cells can be protected from radiation induced free radical damage by flaxseed oil. In present study the reduction in MDA level in the oil treated animals suggests that flaxseed oil has the potential to scavenge the free radical formed during oxidative stress.

Table: The degree of differences in percentage with respect to normal amongst Control (Irradiated Group), Experimental = FO+IR Group and Experimental = IR+FO Group at 15th and 30th Post Irradiation Days

Parameter studied	Normal	Control (Irradiated only)		Experimental-1 (FO+IR) Pre-irradiated Administration		Experimental-2 (IR+FO) Post-irradiated Administration	
		15 day	30 day	15 day	30 day	15 day	30 day
LPO (nmole/gm)	266.76 ± 0.291 (100%)	357.28 ± 2.81 (133.91%)	326.56 ± 1.19 (122.41%)	311.46 ± 1.0 (116.75%)	291.76 ± 2.06 (109.37%)	319.01 ± 5.19 (119.58%)	301.24 ± 3.85 (112.92%)
GSH (nmole/gm)	7.012 ± 0.028 (100%)	3.79 ± 0.85 (54.04%)	4.30 ± 0.18 (61.38%)	5.97 ± 0.12 (85.26%)	6.69 ± 0.26 (95.50%)	5.56 ± 0.08 (79.32%)	6.18 ± 0.08 (88.24%)

Oxidative stress leads to lipid peroxidation, protein and carbohydrate oxidation and metabolic disorders [20, 21 and 22]. The measurement of lipid peroxidation is thus a convenient method to monitor oxidative cell damage [23]. Radiation induced lipid peroxidation is a free radical process which brings several changes in biological membrane. The products of lipid peroxidation and 4-hydroxynoneal are toxic to cell [24, 25]. Lipid peroxidation within the membrane has a devastating effect on the functional state of the membrane because it alters membrane fluidity, typically decreasing it and thereby allowing ions such as Ca++ to leak in to the cell. The peroxyl radical formed from lipid peroxidation attacks membrane protein and enzymes and reinitiates lipid peroxidation. In lipid peroxidation, a hydrogen atom is liberated

from the fatty acid a reactive free radical and there is formation of lipid radical [26], which on attack by molecular oxygen produces a lipid peroxy radical which can either from a lipid hydroperoxide or endoperoxide. The formation of lipid endoperoxide in unsaturated fatty acids leads to formation of MDA as breakdown product. This MDA interacts with DNA and other cell materials leading to chronic occurrence of mutagenesis and carcinogenesis [27].

The lower value of TBARS in post irradiation flaxseed administered group as compared to control and flaxseed oil pre irradiation group exhibited the radioprotective potential of flaxseed oil. It showed greater effectiveness than those of post-irradiation administration as comparison to those of pre-irradiation administration. Nevertheless, this is great unravel that there should be a drug that can be used after sudden radiation exposure. So this preliminary study shows that flaxseed oil can be used after radiation exposure also.

Glutathione: -

The liver showed that glutathione constantly compensated the loss due to radiation to a greater extent, on both the intervals (Table 1). The value was significantly higher ($P>0.001$) in comparison to their respective controls (irradiated only). When comparing the results between the 2 different modes of administration, the difference was found insignificant or less significant ($P>0.05$). Augmentation in glutathione content and a check in lipid peroxidation in liver of irradiated group in experimental group at 15 and 30 post treatment days' intervals has demonstrated a profound therapeutic efficacy of flaxseed oil.

Mechanism of Action

As shown in Figure, when flaxseed oil is administered for a 15 days period of time just after irradiation it combats the oxidative load already generated by irradiation. SDG, following ingestion, is transported to the large intestine, where it is hydrolyzed by bacteria to the aglycone secoisolariciresinol. Secoisolariciresinol, in turn, is metabolized by bacteria in the large intestine to the mammalian lignans enterolactone (EL) and entero dextone (ED). EL and ED are absorbed from the large intestine. Little is known about the distribution of EL and ED to the various tissues of the body. There appears to be considerable individual variation in the absorption and metabolism of the SDG metabolites ED and EL. It is combated and balanced

by lignan SDG as well as ALA present in flaxseed oil in the form of a triglyceride. ALA is metabolized to eicosopentaenoic acid (EPA), a precursor of the series-3 prostaglandins, the series-5 leukotrienes and the series-3 thromboxanes. Incorporation of ALA and its metabolites in cell membranes can affect membrane fluidity and may play a role in anti-inflammatory activity, inhibition of platelet aggregation and possibly in anti-proliferative actions of ALA. Hence, 3 omega fatty acids both; lignan SDG on one hand not only quench and scavenge the free radicals it prevents its entry in other compartments. 3 omega fatty acids help repair the newly generated membrane rapidly and ultimate the mode of action becomes ameliorative

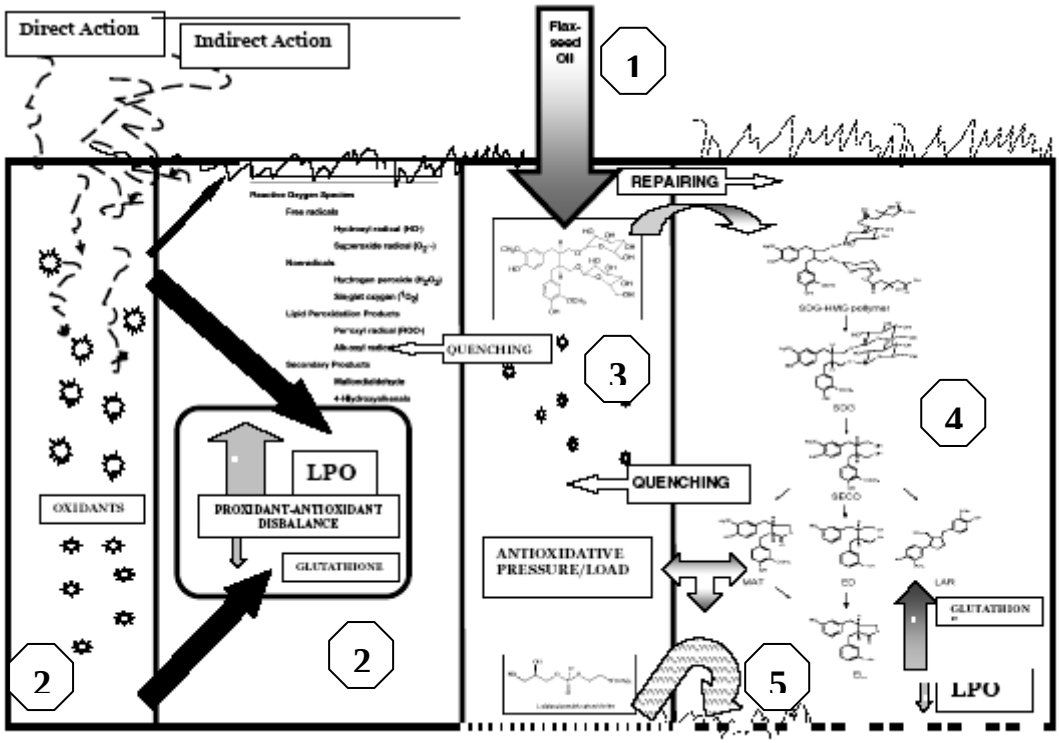

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Figure 1: Administration of flaxseed oil for a 15 days period of time just after irradiation (1) combats the oxidative load (2) already generated by irradiation. SDG, following ingestion, is hydrolyzed by bacteria to the aglycone secoisolariciresinol. Secoisolariciresinol (3). In turn, it is metabolized by bacteria in the large intestine to the mammalian lignans EL and ED (4). The oxidative load is combated and balanced by lignan SDG and ALA present in flaxseed oil in the form of a triglyceride. ALA is metabolized to eicosopentaenoic acid (EPA), a precursor of the series-3 prostaglandins, the series-5 leukotrienes and the series-3 thromboxanes. Incorporation of ALA and its metabolites in cell membranes (5) can affect membrane fluidity and may play a role in anti-inflammatory activity. 3 omega fatty acids both; lignan SDG on one hand not only quench and scavenge the free radicals, it prevents its entry in other compartments. 3 omega fatty acids help repair the newly generated membrane rapidly and ultimate the mode of action becomes ameliorative.

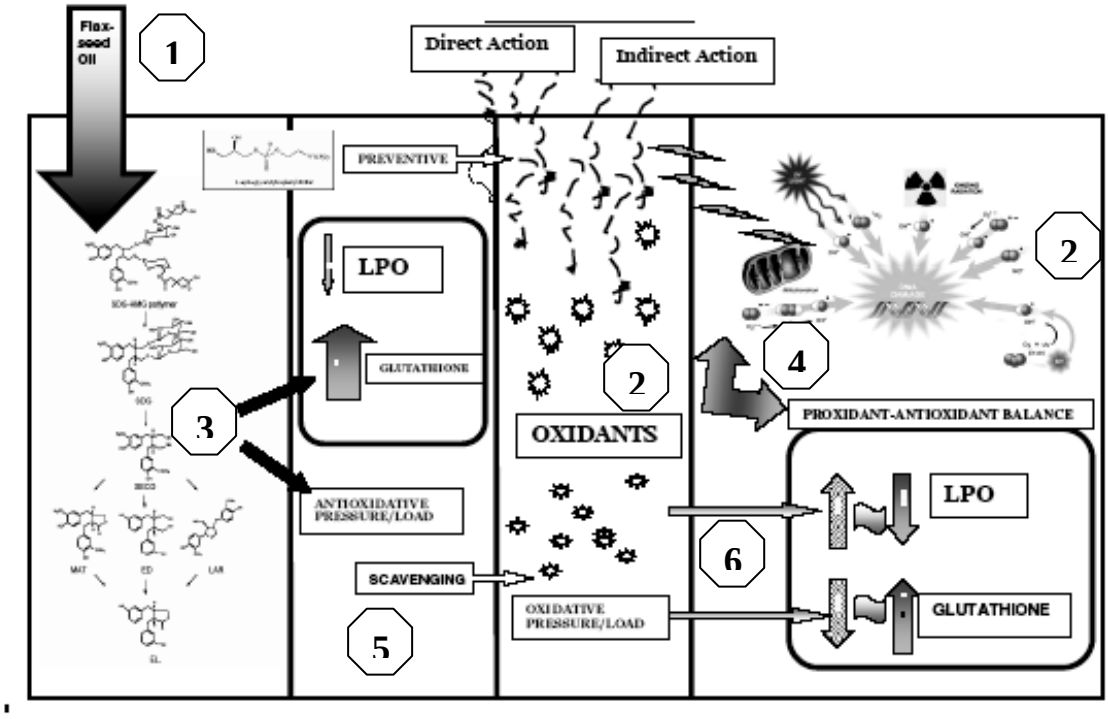

Figure 2: When flaxseed oil is administered for a 15 days period of time just before irradiation (1), the oxidative load generated by irradiation (2) is prevented by the pre-existing antioxidant load (3). It combats and balances by lignan SDG and 3 omega fatty acids (4). SDG, EL and ED have a number of antioxidant activities (3), including inhibition of lipid peroxidation and scavenging of hydroxy radicals (5). The possible antioxidative activities of SDG are thought to be due, in large part, to the antioxidant activities of its metabolites EL and ED and in this manner the free radicals are not only quenched and scavenged by SDG (6) but also subsided the oxidant load and ultimate mode of action is prophylactic too.

As shown in Figure 2, when flaxseed oil is administered for a period of 15 days of time just before irradiation it confronts the oxidative load generated by irradiation. This is prevented by the pre-existing load of antioxidants. The antioxidative load combats and lignan SDG and 3 omega fatty acids balances. SDG, EL and ED have a number of antioxidant activities, including inhibition of lipid peroxidation and scavenging of hydroxy radicals. SDG, via its metabolite EL stimulate the synthesis of sex hormone binding globulin (SHBG). Both of these actions could account for the possible anti-estrogen activity of SDG. The possible antioxidative activities of SDG are thought to be due, in large part, to the antioxidant activities of its metabolites EL and ED. The free radicals are not only quenched and scavenged by SDG but also suppresses the oxidant load. In this manner the ultimate mode of action becomes prophylactic too.

The lower depletion of GSH in liver in FO (flaxseed oil) pre- irradiated administration as well as in post-irradiated treatment could be due to higher availability of GSH, which increase the ability to cope up with the free radicals produced by radiation [28]. The present difference in both the administrations suggests that post irradiation administration of FO is more effective in combating the free radicals generated due to action of radiation. The present study demonstrates a significant reduction in 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. 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 [29].

GSH acts as a reducing agent, its SH becomes oxidized and forms a disulfide link with other molecules of GSH, oxidizes glutathione (GSSG), in turn, can be reduced to GSH by the action of GSSG reductase enzyme, in a reaction using NADPH. NADPH is recycled by glucose 6–phosphate dehydrogenase via the pentose phosphate pathway [30] Radiation exposure as well as a number of natural or synthetic radio protectors can alter the balance of endogenous protective systems, such as glutathione and antioxidant enzyme systems [31]. This could be due to the enhanced utilization of the antioxidant system in an attempt to detoxify the free radicals generated by radiation. GSH offers protection against oxygen-derived free radicals and cellular lethality following exposure to ionizing radiation [32]. The GSH/GST detoxification system is an important part of cellular defence against a large array of injurious agents. Under normal conditions the inherent defence system including glutathione and antioxidant enzymes protects against the oxidative damage. Glutathione with its sulfhydryl group functions in the maintenance of sulfhydryl groups of other molecules (especially proteins) and in the detoxification of foreign compounds, hydrogen peroxide and free radicals [33]. The depletion of GSH promotes generation of reactive oxygen species and oxidative stress with a cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes [34]. The increased GSH level suggests that protection by the flaxseed oil may be mediated through the modulation of cellular antioxidant levels as revealed in glutathione peroxidase, glutathione reductase, and superoxide dismutase and catalase activities.

It is therefore concluded that the content of flaxseed oil (omega 3- fatty acid, lignan) contribute to the synergistic efficacy of the extracts as powerful antioxidants in protecting against radiation induced damage in liver of mice. The protection afforded by flaxseed oil might be due to the antioxidative action of its important constituents, the lignans (a phytoestrogen. The present study may be corroborated with the finding of Endoh *et al* [35] who has demonstrated that flaxseed extract inhibit the CCl4 induced decreased in the level of reduced glutathione in rat liver.

The present study shows that FO exerts its radioprotective effect in two ways:

- It is able to curb the initial damage caused due to radiation (by antioxidant activity).
- It stimulates the cellular regeneration in the post-irradiation period (particularly haematopoietic regeneration, liver recovery, and gastrointestinal system recovery).
- FO extract may offer radioprotection EVEN when administered after irradiatioin, and it is carried out by the following mechanism:
    - By decreasing radiation induced lipid peroxidation level in unirradiated animals and by subsiding the generation of the radiation induced lipid peroxidation in terms of MDA.
    - By checking or preventing/controlling the depletion of endogenous glutathione.
    - By decreasing acid phosphatase activity.

References

1. Axelson, M., J. Sjovall, B.E. Gustafssen and K.D. Setchell., Origins of Lignans in Mammals and Identification of a Precursor from Plants. *Nature.,* 1982, **298**, 659-660.
2. Meagher, L.P., Beecher, G.R., Flanagan, V.P and Li, B.W., Isolation and characterization of the lignans, isolariciresinol and pinoresinol, in flaxseed meal. *J. Agric. Food Chem.,* 1999, **47**, 3173-3180.
3. Sicilia, T., Niemeyer, H.B., Honig, D.M., Metzler, M., Identification and stereochemical characterization of lignans in flaxseed and pumpkin seeds. *J. Agric. Food Chem.* 2003, **51,** 1181–1188.
4. Prasad, K., Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed., *Circulation*, 1999, **99**, 1355-1362.

5. Westcott, N. D. and Muir, A. D., Variation in flax seed lignan concentration with variety, location and year. Proceedings of the 56th flax institute of the United States, 1996, March 20-22, 77-85.
6. Johnsson, P., Kamal-Eldin, A., Lundgren, L. N. and Aman, P., HPLC method for analysis of secoisolariciresinol diglucoside in flaxseeds. *J. Agric. Food Chem*., 2000, **48**, 5216-5219.
7. Eliasson, C., Kamal-Eldin, A., Andersson, R. and Aman, P., High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction. *J. Chromatogr. A*, 2003, **1012,** 151-159.
8. Thompson, L.U., Robb, P., Serrainio, M. and Cheung, E., Mammalian Lignan Production from Various Foods. *Nutr. Cancer.*, 1991, **16**: 43-52.
9. Ford, J.D., Huang, K.S., Wang, H.B., Davin, L.B. and Lewis, N.G., Biosynthetic pathway to the cancer chemopreventive secoisolariciresinol diglucoside-hydroxymethyl glutaryl ester-linked lignan oligomers in flax (*Linum usitatissimum*) seed. *J. Nat. Prod*., 2001, **64**, 1388-1397.
10. Thompson, L.U., Rickard, S.E., Cheung, F., Kenaschuk, E.O,. Obermeyer WR. Variability in anticancer lignan levels in. flaxseed. *Nutrition and Cancer*, 1997, **27**: 26-30
11. Hemmings, S. J. and Barker, L., The effects of dietary flaxseed on the Fischer 344 rat: I. Development, behaviour, toxicity and the activity of liver gamma-glutamyltranspeptidase., *Cell Biochem. Funct.*, 2004, **22**, 113-121.
12. Bhatia, A.L., Manda, K., Patni, S., Sharma, A.L., Prophylactic Action of flaxseed (*Linum usitatissimum*) oil against cyclophosphamide-induced oxidative stress in mouse brain. *J. Med. Food*, 2006, **9 (2),** 261-264.
13. Bhatia, A.L., Sharma, A., Patni, S., Sharma, A.L., Prophylactic Effect of Flaxseed Oil against Radiation-induced Hepatotoxicity in Mice.. *Phytotherapy Res*., 2007a. (In Press).
14. Bhatia, A.L., Patni, S., Sharma, A.L, Sharma, A., Prophylactic Effect of Flaxseed and Sesame Oil against Radiotoxicity. *Nutrition Research*, 2007b (communicated).
15. Moron, M.S., Depierse, J.W., and Manrerirk, B., Levels of GSH, GR and GST activities in rat lung and liver. *Biochem. Biophysics. Aceta*., 1979, **582**, 67-78.
16. Buege, J.A., Aust, S.D, Biological Oxidations; Microsomal Electron Transport and Cytochrome P-450 Systems in: Methods in Enzymology, *Biomembranes*, Part C: (Packer L, Fleischer S, eds.), Academic Press, New York, , 1978, **52 ,** 302–314.
17. Konings, A.W.T.and Osterloo, S.K., Radiation effects on membranes. a comparison of the effects of X- irradiation and ozone exposure with respect to the relation of antioxidant concentration and the capacity for lipid peroxidation. *Radiat. Res.*, 1980: **81,** 200-207.
18. Shimoi, K., Masuda, S., Shen, B., Furugori, B., Kinae, N., Radioprotective effect of antioxidative plant flavonoids in mice. *Mutation Research*, 1996, **350**, 153-61.
19. Cox and Wood…?
20. Sies, H., Oxidative stress: introductory remarks. In: *Oxidative stress* (Sies H, ed.), Academic Press, London, 1985, 1–8.
21. Sies, H., Biochemistry of oxidative stress. *Angew. Chem. Int. Ed. Engl.*, 1986, **25**, 1058–1071.

22. Pryor, W. A. and Godber, S. S., Noninvasive measures of oxidative stress status in humans. *Free Radic. Biol. Med.*, 1991, **10**, 177–184.
23. Girotti AW J Mechanisms of lipid peroxidation Free Radic Biol Med. 1985;1(2):87-95.
24. Raleigh, J.A., Radioprotection of membranes. *Pharmacology and Therapeutics.*, 1985, **39**, 109-113.
25. Esterbauer, H., Zollner, H., Schaur, R.J., Hydroxyalenals: Cytotoxic products of lipid peroxidation . *ISI Atlas, Sci. Biochem.*, 1988, **1**, 311-317.
26. Pryor, W. A., Stanley, J. P. and Blair, E., Autoxidation of polyunsaturated fatty acids . II . A suggested mechanism for the formation of TBA-reactive material from prostaglandin-like endoperoxides . *Lipid*, 1976, **11**, 370-379.
27. Pryor WA, The formation of free radicals and the consequences of their reactions in vivo. *Photochem Photobiol.* 1978, **28(4-5),** 787-801.
28. Bhatia, A.L. and Jain, M., *Spinacia oleracea* L. Protects against gamma radiations: a study on glutathione and lipid peroxidation in mouse liver. *Phytomedicine*, 2004, **11**, 607-615.
29. Hall, E. J., In: Radiobiology for the Radiologist, second ed. Harper & Row publishers, Philadelphia, 1978.
30. Gul, M., Kuta, F. Z., Temocin, S., Hanninen, O., Cellular and clinical implication of glutathione. Ind. *J. Exp. Biol*., 2000, **38**, 625.
31. Weiss, J. F. and Kumar, K. S., Antioxidant mechanisms in radiation injury and radioprotection. "Cellular Antioxidant Defense Mechanisms", C. Chow, (Ed), CRC Press, Boca Raton, FL, pp., 1988, 163-189.
32. Biaglow, J. E., Varnes, M. E., Epp, E. R. and Clark, E. P., Anticarcinogenesis and Radiation Protection, (Cerrutti, P.A., Nygaard, O.F., Simic, M.G., Eds), 1987, **387**, Plennum Press, New York.
33. Meister, A., Glutathione and the gamma glutamyl cycle, in Glutathione: metabolism and function, edited by I M Arias and W B Jakoby (Reven press, New York), 1976, **35.**
34. De Leve, L. D., Wang, X., Kuhlenkamp, J. F. and Kaplowitz, N., Toxicity of azathioprine and monocrotaline in murine sinusoidal endothelial cells and hepatocytes: the role of glutathione and relevance to hepatic venoocclussive disease. *Hepatology*, 1996, **23**, 589 – 599.
35. Endoh, D., Okui, T., Ozawa, S., Yamato, O., Kon, Y., Arikawa, J., Hayashi, M., Protective effect of a lignan-containing flaxseed extract against CCl(4)-induced hepatic injury. *J. Vet. Med. Sci.* **2002,** 64: 761-765.