

RADIOPROTECTION OF MOUSE LIVER BY PLANT PRODUCTS

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Ionizing radiations cause harmful effects to the body. Because of easy acceptability and low toxicity plants are targeted to be radioprotectors of future. In the present study two plants were tested for radioprotective potential on the basis of LD_{50/30} survival, it was found that *Acorus calamus* (rhizome powder), *Moringa oleifera* (seed powder) and *Moringa oleifera* leaf extract protected mice against radiation induced lethality at related dose levels when given orally one hour prior to irradiation. Then animals were irradiated with Co⁶⁰ gamma rays with and without plant product with each plant separately. Animals were sacrificed at 1/6, 1/4, 1, 2, 4, 7, 10, 14 and 28 days post irradiation. Liver was removed and analysed biochemically. Quantitative estimation of DNA, RNA, total proteins and cholesterol was done in the liver.

Keyword : *Acorus calamus*; Gamma rays; *Moringa oleifera*; Mouse liver; Radioprotection.

Introduction

A large number of compounds from various plant sources have been shown to possess radioprotective properties. In the present study *Acorus calamus* (Linn) and *Moringa oleifera* (Lam.) were tested to find out their radioprotective potential. *Acorus calamus* (AC) family Araceae, known as Bach in Hindi and Sweet flag in English, is a known ayurvedic medicine being used, since thousands of years. It grows in some asian countries including India. The plant is semiaquatic rhizomatous perennial herb, with creeping and branched rhizome. Leaves are ensiform and light brown, flavers are densely packed in simple cylindrical spadix. Fruits are oblong turbinate berries with a pyramidal top and seeds. Rhizome has medicinal and tonic properties. Aromatic vinegar is also prepared from it. It is used to flavour beer. It is mild diuretic and used in urinary stones and dysmenorrhoea. It stimulates uterine contractions. Traditionally it is used in gastro-intestinal disorders such as colic pain and diarrhoea¹. Rhizome is bitter in taste and is laxative, expectorant, carminative, dexteric, emmenagogue antispasmodic, carminative and antihelminthic properties.

Moringa oleifera (MO) is commonly known as "Sahijan" or "Sainjana" in Hindi and Drum stick tree in English. It is common flowering tree in India and various parts of the tree are consumed as food and medicine. It belongs to family moringaceae. *M. oleifera* is native to India, Pakistan, Bangladesh and Afganistan. It is a perennial soft wood tree with traditional medicinal and industrial uses.

All parts of *M. oleifera* tree are edible. It is used as animal forage (leaves and seed cake), biogas (from leaves), domestic cleaning agent, blue dyes, fencing,

fertilizer, juice, green manure, gum, water clarifier, honey, medicine, pesticides and tannin. Its seed oil is also used for various purposes. *M. oleifera* contains cytokinin which is a naturally occurring growth regulator². It has antioxidant effects also³. Ghani *et al.*⁴ reported its hypocholesteral activity while Chumark *et al.*⁵ reported its hypolipidaemic activity. Its leaves are rich in vitamin A and C and fruits are rich in vitamin A. It is widely used for its hepatoprotective and antitubercular medicine induced liver damage⁶. It is a rich source of Lutenin and β -carotene⁷. In the present study Rhizomes of *A. calamus* and leaves and seed of *M. oleifera* were tested separately for their radioprotective activity.

Material and Methods

Animals *Swiss albino* mouse selected from an inbred colony and maintained in the laboratory on standard mice feed (obtained from Hindustan Lever Ltd.) 6-8 week old adult animals weighing 24 \pm 2g were selected and were kept on 12 hr. light and 12 hr dark cycle.

Irradiation - The animals were irradiated with Co⁶⁰ ATC-C9 beam therapy unit in the radiotherapy department of SMS medical college and hospital, Jaipur with 8Gy of Co⁶⁰ gamma rays.

Plant Extract - *Acorus calamus* (Linn) of the family Araceae was obtained and matched with the herbarium. Dried and powdered rhizomes were used for testing. Optimum dose of the plant was selected on the basis of LD_{50/30} survival experiment. Experiment was conducted with different doses of *A. calamus* i.e. 75,100,125,150,175 and 200 mg/kg body weight orally taking 10 animals in each group with and without *A. calamus* pretreatment. The animals which were irradiated without *A. calamus* were

given double distilled water one hour prior to irradiation. The animals were fed with aqueous solution of *A. calamus* orally one hour before irradiation.

Moringa oleifera (Lam.) - This plant belongs to family Moringaceae. Leaves and seeds of *M. oleifera* were tested separately for their radioprotective activity.

Seeds - Seeds were shade dried, powdered, dissolved in distilled water and fed to the animals in desired quantity with the help of a tube. The doses used for the testing were 75, 100, 125, 150, 175 and 200 mg/kg body weight.

Leaves - Leaves of *M. oleifera* were collected shade dried, and powdered. Extract of leaf powder was prepared in 40% distilled water and 60% Ethanol by Soxhlet apparatus. Extract was dried and then dissolved in the distilled water. Aqueous solution of it was given to different groups of animals at the dose rate of 75, 100, 125, 150, 175 and 200 mg/kg body weight orally one hr. before irradiation.

The animals were treated in the following way

Group I : Without any treatment (normal)

Group II : Plant extract one hr. before irradiation to 8 Gy of Co⁶⁰ gamma rays (experimental)

Group III : Distilled water one hr. before irradiation to 8 Gy of Co⁶⁰ gamma rays (control).

Separate experiments were conducted with different doses of *A. calamus* rhizome powder / *M. oleifera* leaf extract / *M. oleifera* seed powder.

Animals from all the groups were sacrificed at 1/6, 1/4, 1, 2, 4, 7, 10, 14 and 28 days after treatment. Liver was removed and processed for biochemical studies. Quantitative estimation of DNA was done by the method of Ceriotti⁸. RNA was estimated by the method of Ceriotti⁹. Total protein content and cholesterol content in the liver was estimated by the methods of Lowry *et al.*¹⁰ and Zlatkins¹¹, respectively. Data obtained are expressed as Mean \pm standard error in the tables. Statistical analysis was done by Banrke's method¹². Control and experimental group were compared by using student's 't' test.

Results and Discussion

DNA content decreased in control animals till 4th day and increased till 10th day. Then animals died. In all the plant extract treated groups animals survived till 30th day. In *A. calamus* pretreated experimental group DNA content was near to the normal but higher than their respective controls at approximately all the post irradiation intervals. In *M. oleifera* seed powder and *M. oleifera* leaf extract treated animals also DNA content of the liver was higher than their respective controls. DNA content in experimental animals was in the following order AC > MOS > MOL (Table 1). RNA content in the animals irradiated without plant extract (control) group was lesser as compared to normal except on 7th day. It was a little bit higher on 7th day. In *A. calamus* pretreated and then irradiated animals

it was higher than the control at all the intervals but was significantly below to the normal level on 14th day and remained low till 28th day (Table 2). Protein content in control animal decreased after irradiation till 10th day. In *A. calamus* pretreated animals also protein content was decreased. It started to recover after 10th day but could not attain normal values till 28th day. In *M. oleifera* leaf extract and *M. oleifera* seed powder pretreated animals also it decreased till 10th day and increased a little on 28th day. Protein content was in the following order in the experimental groups MOS>MOL>AC (Table 3).

In the control animals cholesterol content was lower than normal at all the autopsy intervals which was maximum on 7th day. Recovery was visible on 10th day but till then it was below normal. In *A. calamus*, *M. oleifera* leaf extract and *M. oleifera* seed powder pretreated groups also the same trend was observed but on 28th day it was approximately near to the normal. Cholesterol content of experimental groups was in the following order MOL>MOS>AC (Table 4).

Liver is a moderately sensitive organ when damaged it can regenerate itself. Irradiation to gamma radiations produces reactive oxygen species (ROS), which cause most of the damage. Biological macromolecules like DNA and RNA suffer from the direct damage also. DNA may have double or single strand breaks besides other structural changes. Proteins and cholesterol are structural components of the cell also. Proteins get denaturated after irradiation. Denaturation of enzymatic proteins makes them inactive. Liver pathology shows that irradiation to 8Gy of Co⁶⁰ gamma rays causes derangement of its, cellular tiers, cell death, karyolysis, necrosis, pycnosis, haemorrhage, cytoplasmic degranulation and cytoplasmic vacuolation. All the pathological symptoms recover within 14 days after irradiation. Decrease in DNA, RNA, total protein and cholesterol contents at early intervals is due to direct damage to these molecules. Then cell death and removal of cellular debris adds to this decrease. When recovery begins, increase in quantity of biological macromole is observed, because a large number of cells die after radiation exposure and finally they get lost from the tissue. Then mitotic activity begins and new cells take over. The liver regenerates after 7th day. In many parts of the tissue complete recovery is observed on 14th day. Regenerating liver synthesizes sufficient amount of DNA, RNA, proteins and cholesterol. According to Foca Nici *et al.*¹³ it is the water content of the tissue which is mainly responsible for the degree of nucleic acid damage. Liver contains good amount of water.

In the present study *A. calamus* was least effective in protecting DNA damage while *M. oleifera* leaf extract was moderately effective. Highest protection and

Table 1. Variation in DNA content (mg/g) in liver of irradiated (8 Gy) Swiss albino mouse with and without plant powder / extract pretreatment.

Treatment	Post irradiation time										
	4 hrs.	6 hrs.	1 day	2 days	4 days	7 days	10 days	14 days	28 days		
Irradiation (8 Gy)	0.323±0.008	0.290±0.011	0.293±0.012	0.283±0.011	0.269±0.009	0.276±0.010	0.323±0.615	**	**		
AC + 8 Gy	0.364±0.008 P<0.005	0.354±0.009 P<0.001	0.363±0.006 P<0.01	0.319±0.009 P<0.001	0.297±0.016 P<0.005	0.309±0.011 P<0.001	0.340±0.008 P<0.005	0.349±0.012	0.368±0.010		
MOL + 8 Gy	0.325±0.001 P<0.01	0.311±0.001 P<0.01	0.299±0.002 P<0.01	0.304±0.001 P<0.01	0.280±0.003 P<0.01	0.231±0.004 P<0.05	0.340±0.012 P<0.001	0.358±0.011 P<0.001	0.368±0.013		
MOS + 8 Gy	0.361±0.0613 P<0.001	0.327±0.0012 P<0.001	0.343±0.013 P<0.001	0.365±0.009 P<0.05	0.271±0.052 P<0.001	0.276±0.002 P<0.001	0.336±0.0621 P<0.05	0.349±0.023 P<0.08	0.362±0.013 P<0.001		

* DNA content in liver of untreated healthy mouse 0.374±0.06 mg/g

AC = *A. calamus* rhizome powder

** Animals not survived

MOL = *M. oleifera* leaf extract

NS = Non significant

MOS = *M. oleifera* seed powder

Table 2. Variation in RNA content (mg/g) in liver of irradiated (8 Gy) Swiss albino mouse with and without plant powder / extract pretreatment.

Treatment	Post irradiation time										
	4 hrs.	6 hrs.	1 day	2 days	4 days	7 days	10 days	14 days	28 days		
Irradiation (8 Gy)	1.300±0.012	1.340±0.012	1.365±0.013	1.401±0.007	1.420±0.058	1.463±0.008	1.37±0.027	**	**		
AC + 8 Gy	1.321±0.009	1.350±0.016	1.37±0.01	1.405±0.007	1.446±0.012	1.495±0.007	1.450±0.092	1.40±0.09	1.208±0.017		
MOL + 8 Gy	1.365±0.002	1.82±0.026	1.56±0.012	1.57±0.019	1.55±0.024	1.64±0.012	1.48±0.012	1.58±0.013	1.78±0.017		
MOS + 8 Gy	1.39±0.011	1.361±0.016	1.379±0.017	1.43±0.029	1.45±0.012	1.46±0.012	1.39±0.012	1.37±0.014	1.212±0.016		

* RNA content in liver of untreated healthy mouse 1.45±0.08 mg/g

AC = *A. calamus* rhizome powder

** Animals not survived

MOL = *M. oleifera* leaf extract

NS = Non significant

MOS = *M. oleifera* seed powder

Table 3. Variation in Protein content (mg/g) in liver of irradiated (8 Gy) *Swiss albino mouse* with and without plant powder / extract pretreatment.

Treatment	Post irradiation time									
	4 hrs.	6 hrs.	1 day	2 days	4 days	7 days	10 days	14 days	28 days	
Irradiation (8 Gy)	166±4.46	163±3.18	151±3.54	137±2.17	132±2.99	127±3.38	118±3.21	**	**	
AC + 8 Gy	167±1.71 P<0.05	165±1.16 P<0.001	159±1.68 P<0.05	142±4.02 P<0.03	139±2.47 P<0.2	132±2.45 P<0.2	121±2.61 NS	134±4.08	145±3.16	
MOL + 8 Gy	171.29±0.61 P<0.001	169.61±0.46 P<0.001	156.17±0.41 P<0.001	148.19±1.29 P<0.001	138.20±0.71 P<0.001	134±0.52 P<0.001	114.20±0.31 P<0.01	117±0.37	145±0.52	
MOS + 8 Gy	166±2.48 P<0.05	170±1.18 P<0.001	152±2.54 P<0.05	139±2.17 P<0.05	153±2.91 P<0.001	131±1.38 P<0.001	157±2.21 P<0.001	171±1.4	141±2.34	

* Protein content in liver of untreated healthy mouse 165±3.09 mg/g

** Animals not survived

NS = Non significant

AC = *A. calamus* rhizome powderMOL = *M. oleifera* leaf extractMOS = *M. oleifera* seed powder**Table 4.** Variation in Cholesterol content (mg/g) in liver of irradiated (8 Gy) *Swiss albino mouse* with and without plant powder / extract pretreatment.

Treatment	Post irradiation time									
	4 hrs.	6 hrs.	1 day	2 days	4 days	7 days	10 days	14 days	28 days	
Irradiation (8 Gy)	2.97±0.39	2.95±0.037	2.93±0.036	2.81±0.038	2.73±0.046	2.43±0.031	2.57±0.021	**	**	
AC + 8 Gy	3.32±0.061 P<0.001	3.30±0.052 P<0.001	3.29±0.057 P<0.001	3.21±0.029 P<0.001	310±6.018 P<0.001	254±0.014 P<0.05	3.02±0.035 P<0.001	3.51±0.032	4.35±0.044	
MOL + 8 Gy	4.11±0.31 P<0.05	3.70±6.32 P<0.01	4.14±0.37 P<0.01	3.24±0.21 P<0.05	3.71±0.24 P<0.05	3.54±0.21 P<0.01	3.66±0.14 P<0.01	3.71±0.70	4.94±0.37	
MOS + 8 Gy	4.25±0.39 P<0.05	4.75±0.36 P<0.05	4.25±0.31 P<0.01	3.91±0.42 P<0.05	3.55±0.21 P<0.01	3.48±0.24 P<0.01	3.34±0.14 P<0.01	3.29±0.63	4.31±0.41	

* Cholesterol content in liver of untreated healthy mouse 4.68±0.029 mg/g

** Animals not survived

NS = Non significant

AC = *A. calamus* rhizome powderMOL = *M. oleifera* leaf extractMOS = *M. oleifera* seed powder

fastest recovery was provided by *M. oleifera* seed powder. It appears that ROS generated by irradiation are more rapidly removed by *M. oleifera* seed powder in comparison to *M. oleifera* leaf extract or *A. calamus* rhizome powder.

Irradiation induces unscheduled DNA synthesis in the cells which appears to be a step towards recovery. Mitochondrial DNA is protected by its associated proteins against radiation induced damage as they protect it against attack of ROS, thus significantly decreasing the level of the oxidative damage to it.

Decrease in RNA concentration is in correlation to the DNA concentration. The reason of decrease at initial levels is direct damage and ROS activity. Later on, it is one of the consequences of decreased DNA levels. Cong *et al.*¹⁴ found that interferon inducer provides significant protection against radiation to liver RNA content and induces cytogenetic adaptive response. Markov *et al.*¹⁵ found that irradiation causes inhibition of RNA synthesis in the regenerating liver. El-missiry *et al.*¹⁶ also observed decreased hepatic DNA and RNA after irradiation to gamma rays. According to them melatonin protects radiation induced damage to RNA and DNA by antioxidant activity. Protein content of the liver also decreased after irradiation in control and experimental mice till 10th day. Protein content also decreases in correlation with DNA and RNA contents. Cholesterol is also decreased. Cholesterol is required for steroidogenesis also. Decrease in cholesterol content is different from that of other three molecules. The decreased concentration of cholesterol might also be due to increased demand for cortical secretion or increased ACTH secretion by pituitary leading to decreased cholesterol concentration. Regulation of cholesterol homeostasis by variation in the rate of synthesis which is one of the primary functions of the liver. Decrease observed in liver cholesterol seems to be due to stress response caused by irradiation and stimulated synthesis of steroid hormones via hypothalamic pituitary system. Stroudemire *et al.*¹⁷ also observed decrease in liver cholesterol after irradiation. It is also possible that more than usual amount of cholesterol is released in the blood, as an increase in the blood cholesterol is observed after irradiation by Feurgard *et al.*¹⁸. Cholesterol is also a detector of singlet oxygen in biological system^{19,20}.

In all the experimental groups all the plant products (AC, MOS and MOL) have protected these molecules. According to Jagetia²¹ plants contain several constituents, vitamins and minerals which are responsible for radioprotection offered by the plants. *M. oleifera* seed powder and *M. oleifera* leaf extract are rich in vitamin A and C, both of which are radioprotective in nature. *A. calamus* rhizome powder is a nerve tonic, antioxidant and free radical scavenger. *M. oleifera* seed powder and *M.*

oleifera leaf extract also have antioxidant and free radical scavenging activity. Because of these properties, these plants would have prevented radiation damage to the cell membrane, thus preventing their integrity and permeability. Manikandan and Devi²² have reported free radical scavenging and antilipid peroxidation activity of *A. calamus*. Plasma corticosterone levels are also reported to be lowered by *A. calamus* treatment which might have added to its radioprotective activity. It also appears that *A. calamus* might have protected radiation induced inhibition of DNA synthetic activity and oxidative damage. *A. calamus* is also known to increase the activity of Glutathione-S-transferase²³.

Calcium channel blockage also exerts protective effect against ionizing radiation Gilani *et al.*¹ observed that n-Hexane fraction of *Acorus calamus* works as a calcium channel blocker and shows antispasmodic effect also.

M. oleifera leaf extract is known to protect effect of iron deficiency in the rat liver. Ndong *et al.*²⁴ found that *M. oleifera* leaf extract prevents hyperlipidaemia and changes in hepatocyte structure due to presence of taxifolin, a plant flavonoid. Ghasi *et al.*⁴ found its crude extract as hypercholestromaemic. According to Chumark *et al.*⁵ *M. oleifera* has antioxidant, hypolipidaemic, antiatherosclerotic activity and therapeutic potential for the prevention of cardiovascular diseases. *M. oleifera* also have a modifying effect on tumour related enzymes in the liver²⁵. Serotonin which is a natural radioprotector is reported to increase in mice after *M. oleifera* treatment²⁶. Rao *et al.*²⁷ studied radioprotective activity of methanolic extract of *M. oleifera* leaf extract. They found that they confer significant protection to the bone marrow chromosomes.

In the present study it appears that active component of *A. calamus* is Asarone and in the case of *M. oleifera* flavonoides and vitamins present in them. The mechanism of radioprotection appears to be

- (i) Protection of general health of the animal.
- (ii) activation of natural defence mechanisms of the body.
- (iii) DNA binding activity.
- (iv) Free radical scavenging activity
- (v) Antioxidant and anti-lipid peroxidation activity.

These plants have induced faster recovery also and hence liver was in better condition in plant extract treated animals.

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