RADIOPROTECTIVE EFFECT OF UNRIPE STONE FRUIT PULP EXTRACT ON SOME LIVER ENZYMES OF *SWISS ALBINO MOUSE.*

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Summary

To find out natural plant based radioprotectors,a well known medicinal plant,which is acceptable to the body, *Aegle marmelos* extract(AME),was tested in animals against gamma radiations.Radioprotective effect of unripe stone fruit pulp extract on some liver enzymes of *swiss albino mouse* were investigated. For experimental study healthy *swiss albino mice* were selected from an inbred colony and divided into two groups and exposed to 8Gy gamma radiation(Control) or 8Gy gamma radiation with 100mg/kg body weight of *Aegle*

marmelos 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 LDH, Acid and Alkaline phosphates activity. It was observed that irradiation increased LDH, Acid and Alkaline phosphate activity in liver which was lowered by AME pretreatment to a significant extent .These results indicate that AME protects damage to the liver of *swiss albino mouse* against lethal dose of gamma radiations.
Keywords: Radioprotection, *Aeglemarmelos*, Liver, Acid phosphatase, Alkaline phosphatase, Lactate dehydrogenase.

Introduction

The twentieth century has seen an increasing use of nuclear energy in industrial, medical, engineering and scientific research that have raised the problem of radiation hazards to living beings. Thus, the development of effective radioprotectors and radio recovery drugs is of great importance in view of their potential application during both planned (i.e., radiotherapy) and unplanned radiation exposure. (i.e. in the nuclear industry, accidents, natural background radiation) .

Extensive work has been carried out in the field of chemical radioprotection during the last few decades but no compound has been found that can provide optimum protection in clinical field without toxicity. Therefore, the search for alternative sources as ideal radioprotective agents, including plants, has been going on .Recently interest has developed in exploring potential drugs of plant origin for modification of radiation effects. Because of their low toxicity, naturally occurring dietary components may offer

opportunities for development as radioprotectors. Plant extract such as that of *Panax ginseng*[1], *Spirulina platenis*[2] , *Podophyllum hexandrumm*[3], *Ocimum sanactum*[4], *Moringa oleifera* [5], *Mentha arvensis*[6], *Adhatoda vasica*[7], *Emblica officinalis*[8] have been found to have an advantage over the synthetic compounds in terms of low / no toxicity at the effective doses with minimum or no side effects. Also, phytochemicals like caffeine and Genistein have multiple physiological effects as well as antioxidant activity that result in radioprotective role [9-10].
A. marmelos known as Beal in Hindi is a spinous tree belonging to family Rutaceae. Its leaf, root, bark, seed and fruits are also valued in Ayurvedic medicine in India[11].Unripe fruits are green and ripe bael fruits are globose, grey or yellowish ,upto 20 cm in diameter, with woody rind, seeds numerous, oblong ,embedded in sacs covered with thick ,orange coloured sweet pulp.The aqueous fruit extract of *A.marmelos* exhibits an antihyperlipidaemic effect in Streptozotocin induced diabetic rats[12].The *A.marmelos* fruit pulp possess antiprotozoal activity in chronic dysentery .The ripe fruit extract used in different formulations for treatment of Chronic diarrhea [13] Its extract has been reported to regenerate damaged pancreatic cells and antilipid peroxidation activity in diabetic rats [14] *A.marmelos* extract at 5µg/ml protected HPBLS against radiation induced DNA damage and genomic instability and its radioprotective activity may be by scavenging of radiation induced free radicals and increased oxidant status[15]

Materials and methods

Animals-Adult male *Swiss albino mice* (6-8 weeks old, weighing 25±2 g) maintained in the animal house as an inbred colony were used .These were given standard mice feed and water *ad-libitum*. The temperature of animal house is maintained at 37 ± 5^0C and animals are kept in 12 hrs natural day light and dark night cycles.

Irradiation –Cobalt teletherapy unit (ATC-CZ) at cancer treatment centre, Radiotherapy Department, SMS Medical College and Hospital, Jaipur was used for irradiation. Whole body of these animals was exposed to gamma radiation at the dose rate of 1.59 Gy /min at a distance (SSD) of 77.5cm from the source to deliver total dose of 8Gy to two different groups in a single exposure.

***Aegle marmelos* extract (AME)** –Extract of AME unripe fruits (cut into pieces and shade dried) (60 ethanol + 40 distilled water) obtained from Amsar Pharmaceutical Private Limited, Indore was used. The extract was dissolved in double distilled water and given at the dose rate of 100 mg/kg body weigh one hr. before irradiation. The dose of AME was selected on the basis of experiment conduced with various doses of plant extract for various treatment periods.

Experimental design-

Healthy animals were selected and divided into four groups.

Group I (normal) –Mice of this group were kept without any treatment.

Group II (Drug) - Mice of this group were administered with *Aegle marmelos* (unripe fruit pulp) extract (100 mg/kg b.wt/day) only at the same dose rate.

Group III (Control) –Mice of this group was irradiated only, exposed to 8Gy of gamma radiation.

Group IV (Experimental) –Mice of this group received AME extract one hour before irradiation.

The animals were autopsied at 1,3,5,7 and 28 days after irradiation and six animals were sacrificed at each intervals, and their liver was removed and Lactate dehydrogenase , Alkaline and Acid Phosphate activity were measured quantitatively.

Statistical Analysis: The data were subjected to students't' test for comparison between the groups. The results obtained in the present study were expressed as mean ±SE. Significance level was $P<0.05$, $P<0.01$,$P<0.001$.

Results

Acid phosphatase activity significant increase in the acid phosphatase activity was observed after 1, 3, 5 and 7 days post irradiation in the control group.

In the plant extract pretreated group an increase in the acid phosphatase activity was observed uptill 7th post irradiation day. Then it started to decline on 28th day. It was always lesser than control group at all the autopsy intervals.

In the animals treated with plant extract only, the values were higher at 1st, 2nd, and 5th post irradiation day in comparison to normal (without any treatment) animals. On day 7th it started to decline and continued to decline till 28th day.(Table:1 and Fig:1)

Table 1: Variations in the Acid Phosphate (mg pi/gm/hr) of liver of Co^{60} Gamma ray irradiated *Swiss albino mouse* with and without *Aegle marmelos* pretreatment.

The healthy normal *Swiss albino mouse* without any treatment is

Treatment	Post Irradiation Time (in days)				
	1day	3 days	5 days	7 days	28days
8Gy	2.521±0.011	2.568±0.007	2.643±0.078	2.931±0.023	Animals not survived
8Gy + Plant Extract	2.475±0.003 P<0.01	2.490±0.0052 P<0.001**	2.616±0.022 NS	2.675±0.006 P<0.001**	2.173±0.026
Plant Extract Only	2.46± 0.027	2.47±0.007	2.48±0.092	2.17±0.004	2.15± 0.017

= 2.16± 0.044

P value = Control Vs Normal* Control Vs Experimental**

NS= Not Significant

Experimental = The plant extract was given 1hr before irradiation at the dose rate of 100mg/kg body weight.

Control = Irradiated only

Alkaline phosphatase activity significant increase in alkaline phosphatase activity was observed after 1,3,5 and 7 days post irradiation in the control group.
In the plant extract pretreated group, an increase in the alkaline phosphatase activity was observed till 7th post irradiation day. Then it started to decrease on 28th day.It was always lesser than the control group at all the autopsy intervals.
Animals treated with plant extract only, alkaline phosphatase activity was higher at 1st 3rd and 5th post irradiation day, on day 7th it started to decline and continued till day 28. (Table:2 and Fig:2)

Table 2: Variations in the Alkaline Phosphate(mg pi/gm/hr) of liver of Co^{60} Gamma ray irradiated Swiss albino mouse with

Treatment	Post Irradiation Time (in days)				
	1day	3 days	5 days	7 days	28days
8Gy	2.895±0.045	2.961±0.015	3.235±0.012	3.328±0.046	Animals not survived
8Gy + Plant Extract	2.735±0.114 P<0.014	2.795±0.067 P<0.05**	2.815±0.069 P<0.001**	2.946±0.116 P<0.05**	2.583±0.038 -
Plant Extract Only	2.441±0.005	2.613±0.026	2.615±0.049	2.513±0.029	2.302±0.018

and without *Aegle marmelos* pretreatment.

The healthy normal *Swiss albino mouse* without any treatment is = 2.525±0.049
P value = Control Vs Normal* Control Vs Experimental**
NS= Not Significant
Experimental = The plant extract was given 1hr before irradiation at the dose rate of 100mg/kg body weight .
Control = Irradiated only

Lactate dehydrogenase activity (LDH) significant increase in LDH activity was observed in control group (8Gy).In the plant extract pretreated group, an increase in the LDH activity was observed till 7th post irradiation day. Then it started to decline on day 28.It was always lesser than the control groups at all the autopsy intervals. In the animals treated with plant extract only, LDH activity is near to the normal.(Table:3 and Fig:3)

Table 3: Variations in the LDH of liver of Co^{60} Gamma ray irradiated *Swiss albino mouse* with and without *Aegle marmelos* pretreatment.

Treatment	Post Irradiation Time (in days)				
	1day	3 days	5 days	7 days	28days
8Gy	3.91±0.200	4.16±0.033	4.48±0.047	4.58±0.024	Animals not survived
8Gy + Plant Extract	1.98±0.157 P<0.001**	2.38±0.060 P<0.001**	2.56±0.09 P<0.001**	2.73±0.029 P<0.001**	2.58±0.003
Plant Extract Only	2.44±0.104	2.50±0.015	2.51±0.004	2.54±0.006	2.52±0.007

The healthy normal *Swiss albino mouse* without any treatment is = 2.56±0.030
P value = Control Vs Normal* Control Vs Experimental**
NS= Not Significant
Experimental = The plant extract was given 1hr before irradiation at the dose rate of 100mg/kg body weight.
Control = Irradiated only

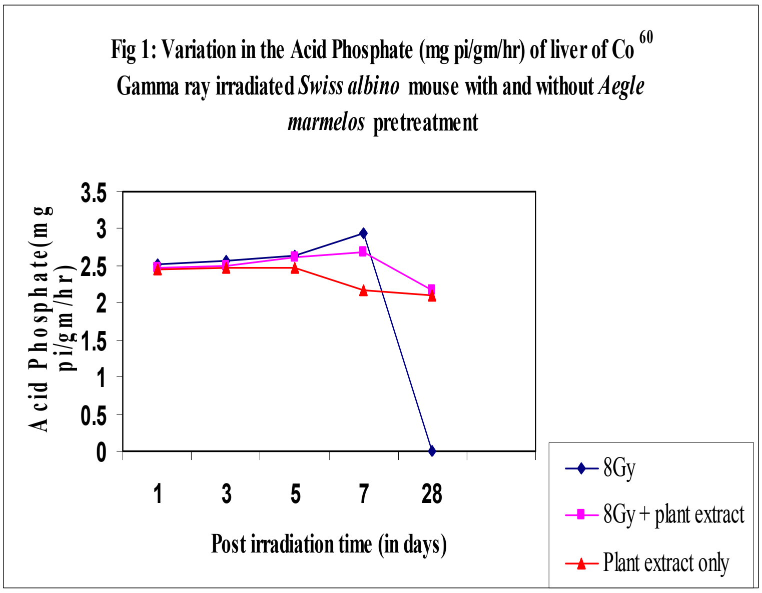


Fig 1: Variation in the Acid Phosphate (mg pi/gm/hr) of liver of Co 60
Gamma ray irradiated *Swiss albino* mouse with and without *Aegle marmelos* pretreatment
Acid Phosphate(mg pi/gm/hr)
3.5
3
2.5
2
1.5
1
0.5
0
1
3
5
7
28
Post irradiation time (in days)
8Gy
8Gy + plant extract
Plant extract only

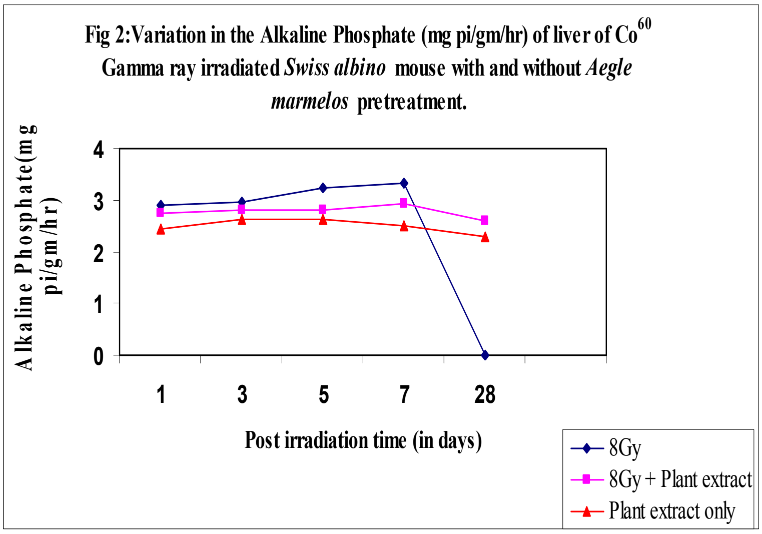
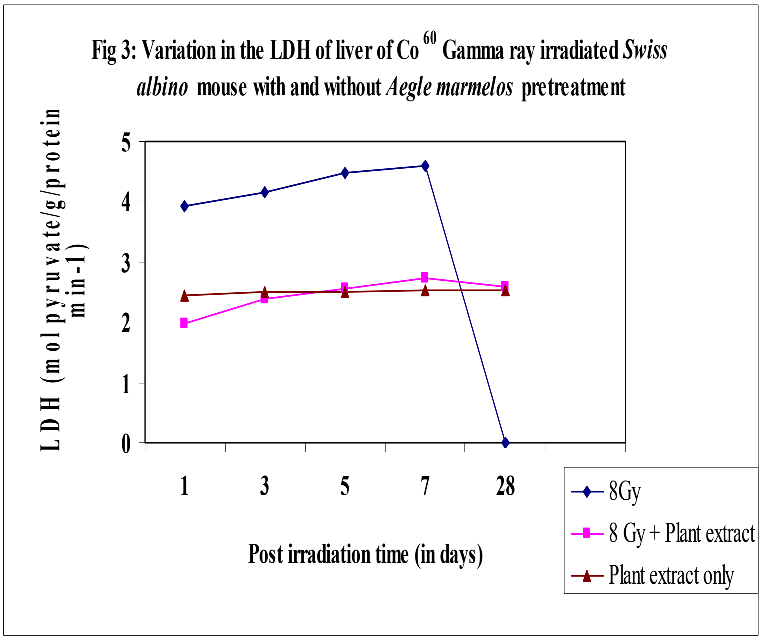


Fig 2:Variation in the Alkaline Phosphate (mg pi/gm/hr) of liver of Co 60
Gamma ray irradiated *Swiss albino* mouse with and without *Aegle marmelos* pretreatment.
Alkaline Phosphate(mg pi/gm/hr)
4
3
2
1
0
1
3
5
7
28
Post irradiation time (in days)
8Gy
8Gy + Plant extract
Plant extract only



Discussion

Most toxic effects from acute ionizing radiation are due to an increased flux of free radicals. An ideal radioprotector is, therefore, expected to prevent accumulation of free radicals and decrease the load of reactive oxygen species (ROS) to a level manageable by the inherent antioxidant system. The significantly reduced ferric reducing ability of plasma after irradiation induced a decrease in the inherent antioxidants in blood plasma. Increased lipid peroxidation and higher alkaline phosphatase activity in liver in irradiated controls as compared with non-irradiated animals indicated tissue damage due to radiation-induced free radicals and ROS.

The ROS and free radicals are known to react with unsaturated lipids, produce hydroperoxides, induce changes in the lipid bi-layer, alter membrane permeability, and cause lipid peroxidation .The alkaline phosphatase is important for maintaining cell membrane permeability and transport of molecules is known to have increased activity under diseased conditions, such as cirrhosis, hepatitis ,fatty liver, drug intoxication and radiation exposure[16].

Enzyme molecules are not directly affected by the ionizing radiation, but indirectly through collision with a labile product resulting from the ionization of water. Ionizing radiation may act on the protein moiety of the enzymes or on its prosthetic groups, when acting on the protein moiety they may destroy selectively certain groups in the side chain of the molecule selectivity that are essential for enzymatic activity or they may act by breaking hydrogen bonds with production of denaturation or precipitation. When ionizing radiation act on solutes dissolved in water, it may increase oxidation by the products of ionization of water. In the present study, increase in acid or alkaline phosphatase activity was observed in control group. Ionizing radiation causes disruption of many powerful hydrolytic enzymes such as cathepsin, phosphatase and nucleases which cause damage upon release, acid phosphatase activity in animals of the AME and radiation combined group was found to be significantly lower than normal in the animals of the irradiation alone group and attained the normal value on 28th day .

This suggests that AME may help in early recovery by the rapid removal of cellular debris from the tissue. Acid phosphatase is a lysosomal enzyme that hydrolyses the ester linkage of phosphate ester and helps in the autolysis of the degenerated cells. On the other hand, alkaline phosphatase, a brush border enzyme, splits various phosphate esters in an alkaline medium and mediates membrane transport[17] .Thus an increase in these enzymes suggests that acid phosphatase helps in removal of debris, and alkaline phosphatase helps in stabilizing the membrane for early recovery.

LDH is an enzyme which is present in various components of the cell. It is also released when cells are damaged and amount LDH released is proportional in the liver also amount of LDH is linked to cellular damage. After irradiation it was found that amount of LDH increased one hour after irradiation. LDH is very important measure to check for tissue damage. As the cells die, their LDH is released and find its way into the blood. LDH levels increased in condition of cerebrovascular accidents, heart attack, hematolytic anemia, low blood pressure, mononucleosis, blood deficiency, liver disease, muscular dystrophy, pancreatitis and tissue death [18].

For a long time, LDH is considered a by product of anaerobic glycolysis whose accumulation is linked to a variety of biochemical abnormalities[19-20]. Presence of LDH was recently confirmed in liver peroxisomes[21-22]. Radiation may induce a metabolic alteration in cells resulting in the increased level of LDH enzyme. It is assumed that these changes are related closely to alteration of membrane permeability and cell disintegration. In the present study it was observed that pretreatment of AME decreased radiation induced changes in the LDH activity which reveals that the plant extract have provided protection against gamma radiation.

In plant extract treated group, highly significant protection was observed against the radiation induced increase in the activity of acid and alkaline phosphatase. This increase was lesser in comparison to the respective control.

Pre-treatment of AME increases survival time of *Swiss albino mouse* to a significant extent[23]. Other vital organs are also protected by *Aegle marmelos* pre-treatment for example intestine, liver and spleen.

Aegle marmelos is a cosmopolitan in distribution and has rich medicinal properties[24]. Fruits, stem and roots of this tree at all stages of maturity are used as medicine against various human ailments[25]. *Aegle marmelos* contains many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids and terpenoids and has innumerable traditional medicinal uses[26].

Various crude extracts of this plant have shown activities including antiulcer, antidiabetic, antihyperlipidemic, antioxidant, anticancer, antimicrobial, radioprotective, anti-inflammatory, antipyretic, analgesic and antispermatogenic effects on various animal models[27-30].

Aegle marmelos is known to enhance glutathione level and glutathione-s-transferase hence improving the natural defense of the body[23,31].

The bioactive compounds isolated from *A. marmelos* are marmelosin , luvangetin , aurapten, proralen , marmelide and tannin [32-38]. Marmelosin ($C_{16}H_{14}O_4$) has shown antihelmintic as well as antibacterial activities [35-37]. Luvangetin ($C_{15}H_{14}O_4$), a pyranocoumarin isolated from the seeds of beal fruit protects against multiple models of gastric ulceration in rodents[33,35]. Aurapten ($C_{19}H_{22}O_3$) inhibits the chromotrophic effects on cardiac tissue and thus may be useful in treatment of hypertension [34]. Psoralen ($C_{11}H_6O_3$) shows various activities such as antispasmodic[39] artemicide and cytotoxic[38]. Marmelide ($C_{16}H_{14}O_4$) is very effective against viruses and is found to influence the early stages of replicative cycle such as adsorption, penetration, etc[32] . Tanin, present in the unripe fruit of this plant, has astringent property and is an excellent remedy for diarrhea [37].

Hence, it can be concluded that *Aegle marmelos* works in multifarious ways to protect radiation induced injury and it protects the whole bodily irradiated animals, thus reflecting the action in the liver.

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