

Alteration of Acid Phosphatase Activity in the Liver of Gamma Irradiated Mouse by *Centella asiatica*

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Acid phosphatase activity increases above normal in the liver of whole body irradiated animals. It is considered to be one of the adverse radiation effects. Increase in the acid phosphatase activity was dose dependent. *Centella asiatica* extract, which increased the survival of gamma irradiated Swiss albino mouse when given prior to irradiation was tested to observe changes in the acid phosphatase activity in the liver of irradiated mouse. It was observed that *Centella asiatica* extract when given orally one hour prior to irradiation, significantly decreased acid phosphatase activity towards normal in the liver of irradiated mouse. This shows sign of protection offered by the plant extract.

Key Words : Gamma Radiation, *Centella asiatica*, Liver, Acid Phosphatase.

Introduction :

Background radiation is a phenomenon always existing in nature and all the organisms living on the earth are adapted to it. Excessive doses of ionizing radiation are harmful to organisms and the damage depends upon the dose of radiation. Other factors on which the response may depend are dose rate, quality, geometry of exposure and portion of the body exposed, species, age, sex, and metabolic status of the organisms. Exposure to the gamma radiation causes pathological and biochemically important changes in the body tissues resulting in metabolic dearrangement which in due course of time may lead to cellular damage or death of the cell. It has been suggested that radiation induces physical or functional changes in the membrane permitting the release of the hydrolytic enzymes and indirectly causing death by this mechanism.

Liver is a vital organ of the body which also plays an important role in the expression of radiation induced changes and then in the recovery process. It is very sensitive to radiation and is affected adversely by exposure to gamma rays.

To protect normal tissues a large number of synthetic chemicals and some plants originated agents have been tested to find out a nontoxic radioprotectors. Most of them were found to be toxic at their optimum dose levels and no perfect radioprotector has been reported till date. Hence, the hunt is going on to find a suitable radioprotective drug in experimental models. Therefore, the present study was planned to assess the role of *Centella asiatica* in modifying the radiation-induced damage on the phosphatase activity of liver.

Centella asiatica is a medicinal plant, which belongs to the family Apiaceae. It is commonly known as Gotukala and Marsh Pennywort (USA). *Centella asiatica* has been used for centuries as a medicinal herb and was referred to in the French Pharmacopoeia in 1884, as well as the ancient traditional Chinese Shennong Herbal some 2000 years ago, well as in Indian Ayurvedic Medicine some 3,000 years ago.

It has been used for wound healing, better circulation, memory enhancement, cancer vitality, general tonic, respiratory ailments, detoxifying agent, treatment of skin disorders (such as psoriasis and eczema), revitalizing connective tissue, burns and scar treatment, clearing up skin infections, slimming and edema, arthritis rheumatism, treatment of liver and kidneys disorders, periodontal disease, strengthening of veins (varicose veins), blood purifier, high blood pressure, sedative, anti-stress, anti-anxiety, an aphrodisiac, immune booster, anabolic, adaptogen and radioprotector (Rastogi *et al.*, 1960; Diwan *et al.* 1991; Nalini *et al.*, 1992; Srivastava and Shukla, 1997; Sharma and Jaimala, 2001).

Materials and Methods :

Animals : The experiments were conducted on an animal model Swiss albino mice, 6-8 week old, weighing 25 ± 2 gm. These animals were selected from an inbred colony maintained in the animal house on standard mice feed and water *ad libitum*.

Plant extract : The whole plant extract of *Centella asiatica* was obtained from Amsar Pvt. Ltd., Indore. The extract was dissolved in distilled water and the animals were fed by gastric **intubation** at the dose rate of 100 mg/kg b.w. one hour before irradiation.

Experimental Design : Animals were divided in following groups :

- Group I – Animals of this group were Sham. irradiated with equal amount of double distilled water as given with the plant extract.
- Group II – Animals of this group were exposed to 6 Gy of ⁶⁰Co gamma rays with pretreatment of double distilled water (volume equal to plant extract).
- Group III – Animals of this group were exposed to 8 Gy of ⁶⁰Co gamma rays with pretreatment of double distilled water (volume equal to plant extract).
- Group II and III served as control groups.
- Group IV – Animals of this group were exposed to 6 Gy of gamma rays and were treated with an oral dose of the plant extract at the dose rate of 100 mg/kg b.w. one hour before irradiation.
- Group V – Animals of this group were exposed to 8 Gy of gamma radiation and were treated with an oral dose of the plant extract at the dose rate of 100 mg/kg b.w. one hour before irradiation.
- Group IV and V served as experimental groups.
- Group VI – Plant extract (*Centella asiatica*, 100 mg/kg b.w.) only.

The animals were sacrificed at 1, 2, 4 and 7 days of post-treatment intervals. At least six animals were used at each interval from all the groups.

The whole liver was removed, weighted and processed for the estimation of acid phosphatase activity by using Fiske and Subbarow (1965) method.

The data were subjected to Student's 't' test for comparison between the groups. The values are expressed as mean \pm SEM.

Results :

Significant increase in the acid phosphatase activity was observed after 1, 2, 4 and 7 days *post-irradiation* in both the control groups. This increase was dose dependent. In the animals irradiated with 8 Gy, this increase was lesser in comparison to 6 Gy irradiated animals. On day 7, slight recovery was noticed in the control groups.

In the plant extract pretreated groups, an increase in the value of acid phosphatase was also observed upto 4th post irradiation day. Then it started to decline on day 7. It was always lesser than control groups at all the autopsy intervals.

In the animals treated with plant extract only (Group-VI), the values were higher at 1st and 2nd post-irradiation day in comparison to normal (without any treatment) animals. On day 4th it started to decline and remained continue even as day 7.

Discussion :

In 1942, Dale was the first to postulate that 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 enzyme or on its prosthetic groups, when acting on the protein moiety they may destroy selectively certain groups in the side chain of the molecules 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 these may result in a number of oxidation by the products of ionization of water, such as oxidation of sulfhydryl groups, among the enzymes that requires their presence for activity. It is, therefore, reasonable to assume that these enzymes may be preferentially inhibited on irradiation through oxidation of their sulfhydryl groups to the disulfide. This inhibition would be reversible, if the irradiation dose were increased other groups on the protein might be attacked.

In the present study, increase in acid phosphatase activity was found to be dose dependent. At 6 Gy, less increase was there while at 8 Gy higher increase in acid-phosphatase activity was observed. Ionizing radiation causes

disruption of many powerful hydrolytic enzymes such as cathepsin, phosphatases and nucleases which upon release, causes great damage. Liberation of hydrolytic enzymes from the lysosomes causes extensive damage to proteins, nucleic acids and other molecules in the cells. Release of enzymes from lysosomes by irradiation may be either a result of a direct effect of irradiation on the lysosomal membrane or by some indirect effect such as the liberation of hormones. Enzyme release from the lysosomes is an indirect effect and is a consequence of thyroid-hormone action (Rehman, 1964). Many membranes are known to contain polyunsaturated acids as part of their structure. Lysosomal membrane is similar in this respect. Formation of peroxides of unsaturated fatty acids in the phospholipids of lysosomal membrane may result in membrane damage. Radiation damage to lysosomes is strictly time and dose dependent and very little enzyme release occurs immediately after the irradiation. Thus, it appears that radiation causes some change in the membrane that subsequently leads to its rupture and oozing out of the enzyme resulted to an increased acid-phosphatase activity (Wills and Wilkinson, 1966). Lysosomes from different cell types or even from the same tissue vary greatly in their susceptibility to damage by irradiation. (Beck *et al.*, 1964).

In the plant extract treated group, highly significant protection was observed against the radiation induced increase in the activity of acid phosphatase at both the dose levels (6 and 8 Gy). This increase was lesser in comparison to their respective controls.

Pre-treatment of *Centella asiatica* increases survival time of Swiss albino mouse to a significant extent (Sharma and Sharma, 2002). Other vital organs are also protected by *Centella asiatica* pre-treatment, of which radioprotection of intestine is very important as it takes care of nutritional status, fluid and electrolyte balance of body (Unpublished data).

Plant extract contains several compounds, which may act on the animal physiology in their own specific way. They are rich in vitamins, minerals and nutrients which are generally nontoxic to the body. *Centella asiatica* is also a known rasayana in Ayurveda used as a brain tonic and wound healer. Its aqueous extract contains asiaticoside, asiatic acid, triterpenes, centoic acid, centellic acid and their esters. Besides this, many other compounds are there, including ascorbic acid.

Acid phosphatase activity in the experimental animals was significantly lower in comparison to their respective controls, thus illustrating the protective potential of *Centella asiatica* against radiation induced damage, as lesser the damage, lower the enzymatic activity.

Centella asiatica is known to enhance glutathione level and glutathione-S-transferase hence improving the natural defense of the body (Chandraprabha *et al.*, 1996a; Verendra and Gupta, 2001).

This plant contains several compounds, which are free radical scavenger and have shown antioxidant activity (Rouillard 1997, Padma *et al.*, 1998, Rekha 2000, 2001). Chandraprabha *et al.* (1996c) reported that *Centella asiatica* inhibited lipid peroxidation in the liver, kidney, lungs, heart, brain, spleen and serum. In their another report they observed that *Centella asiatica* decreases the level of total ATPase, Mg⁺² ATPase, Na⁺² and K⁺ ATPase and increases level of Ca⁺² ATPase to protect the tissues against peroxidation reaction thereby protecting against the cell damage (Chandraprabha *et al.*, 1996b). According to Uma devi (2002) antioxidant activity has prominent role in the radioprotective effects of these compounds. Sulochana *et al.* (2002) and Shobi and Goel (2000) found that behavioral alterations and growth retardation induced by irradiation are prevented by *Centella asiatica* pre-treatment. According to Chen *et al.*, (1999) extracts of *Centella asiatica* used with tetrandrine are able to reduce acute radiation dermatitis in rats.

Hence, it can be concluded that *Centella asiatica* works in multifarious ways to protect radiation induced injury and it protects the whole bodily irradiated animals, thus reflecting the action in the liver. Reduced activity of acid and alkaline phosphatase in the experimental animals is an indication of it, which moves towards normally as debris is removed and regeneration begins.

Table : Variations in the acid-phosphatase activity (mg pi/gms/hr) in the liver of control and experimental mice

Treatment	Post Irradiation Time (in days)			
	1	2	4	7
8 Gy	2.892 ± 0.05 p < 0.001*	4.000 ± 0.064 p < 0.001*	4.985 ± 0.021 p < 0.001*	1.784 ± 0.091 p < 0.001*
8 Gy + Plant extract	1.785 ± 0.008 p < 0.001**	2.400 ± 0.028 p < 0.001**	4.161 ± 0.093 p < 0.001**	1.600 ± 0.038 NS**
6 Gy	2.123 ± 0.046 p < 0.001*	2.985 ± 0.036 p < 0.001*	3.877 ± 0.087 p < 0.001*	1.880 ± 0.040 NS*
6 Gy + Plant extract	1.446 ± 0.058 p < 0.001**	1.452 ± 0.010 p < 0.001**	3.338 ± 0.009 p < 0.001**	1.830 ± 0.079 NS**
Plant Extract Only	1.862 p ± 0.380	2.692 p ± 0.312	1.723 p ± 0.332	1.046 p ± 0.123

Acid phosphates activity of normal mice without any treatment = 1.766 ± 0.031

p value = Control Vs Normal*

Control Vs Experimental**

NS = Not Significant

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

Control = Irradiated only

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