

# Protective Effect of *Adhatoda Vasica* Extract on Gamma Radiation Induced Oxidative Stress in Mice Biceps Muscle

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**Abstract:** In the era of expanding energy program all over the world, the role of radiation biology has acquired greater relevance and significance in addressing the health issues. In present time, nuclear terrorism and weapons related effects are raising much alarm and concern to public health. Radiation is often used to treat malignant tumours, or in combination with surgery. Ionizing radiation is known to produce deleterious effects on living organisms. Despite the advantage of radiotherapy and improvements in techniques, many patients experience moderate to severe side effects including xerostomia, diarrhea, mucositis, dermatitis, ulceration and fibrosis. Reactive oxygen species (ROS) such as hydroxyl radical (OH), superoxide anion radicals (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were produced as a response to ionizing radiation. Mammalian cells are equipped with both enzymatic and non-enzymatic antioxidant mechanisms to minimize cellular damage resulting from interaction between cellular constituents and ROS. Under normal conditions, a delicate balance exists between the generation of ROS and the cellular antioxidant systems. Over production of ROS in both intra and extra-cellular spaces upon exposure of cells to ionizing radiation results in oxidative stress due to the imbalance between pro-oxidants and antioxidants. The objective of the present study was to evaluate the antioxidant enzyme activities (Superoxide dismutase, Catalase and lipid peroxidation) in biceps muscle of mice. Mice were divided into 4 groups. Study was performed at 7, 14, 21 and 28 days stage. Present study convincingly demonstrated that *Adhatoda* extract provides protection against free radical damage.

**Keywords:** Radiation, Antioxidant enzymes, Reactive oxygen species, Superoxide dismutase, Catalase.

## 1. Introduction

In the era of expanding nuclear energy program all over the world, the role of radiation biology has acquired greater relevance and significance in addressing the health issues. In present time, nuclear terrorism and weapons related effects are raising much alarm and concern to public health. Radiation is often used to treat malignant tumours, or in combination with surgery or chemotherapy. Despite the advantage of radiotherapy and the improvements in techniques, many patients experience moderate to severe side effects including xerostomia, diarrhea, mucositis, dermatitis, ulceration and fibrosis (Dormand *et al.*, 2005). Ionizing radiation transmits deleterious effects in biological system. Several chemical and synthetic compounds have been evaluated to meet the need; however, associated toxicity with useful doses has precluded their use. The last one decade has been exhaustively utilized in screening of various plants having radioprotective properties (Arora *et al.*, 2005, Weiss and Landauer, 2009).

Herbal drugs have been used by mankind since time immemorial to treat various ailments and offer an alternative to the synthetic compounds. The development of effective radioprotectors and radiorecovery drugs is of great importance in view of their potential application during both planned (e.g. radiotherapy) and unplanned (e.g. in nuclear industry, natural background radiation emanating from earth and other sources) radiation exposure (Nair *et al.*, 2001). Reports are available for radioactive properties of various plants like *Osmium sanctum* (Devi, 2001) *Adhatoda vasica* (Kumar *et al.*, 2003) *Emblia officinalis* (Singh *et al.*, 2006) *Tinospora Cardifolia* (Sharma and Goyal, 2014).

*Adhatoda vasica* is well known plant drug in Ayurvedic and Unani medicine well documented for therapeutic potential. It is an evergreen, gregarious, stiff and perennial shrub of family Acanthaceae has been used as a herbal medicine in treating a wide variety of diseases in India. It grows throughout the Indian peninsula upto an altitude of 1300 m on wastelands in a variety of habitat and type of soils. It has been used as traditional medicine for over 2000 years and possess a wide spectrum of medicinal properties including positive effect on inflammation. Leaf juice is stated to cure diarrhea, dysentery and glandular tumor.

The powder of *Adhatoda vasica* is reported to be used as poultice on rheumatic joints as counter irritant on inflammatory swelling, on fresh wounds, urticaria and in neuralgia (Claeson *et al.*, 2000). It has antioxidant properties (Dhuley, 1999) and hepatoprotective properties (Kumar *et al.*, 2015). It stimulates contraction of uterine muscle and facilitates child birth. It relieves or eases muscular spasms, cramps or convulsions. *Adhatoda vasica* has been accredited to afford protection against allergen induced bronchial obstruction in guinea pigs (Dorsch and Wagner, 1991). Studies revealed that 50% methanolic extract of *Adhatoda vasica* with low and high drug doses have shown its potentiality as radioprotector against therapeutically induced mutations (Sharma *et al.*, 2009).

The early effects of radiations are anorexia, nausea, diarrhea, hypertension, tachycardia, leucopenia, cataract, sterility, shortening of life span, neoplasms, developmental anomalies and mutations.

Radiation is one of the physical agents that induce oxidative stress which is defined as an increase in reactive oxygen

species or a decrease in antioxidant defence mechanisms (Hazra *et al.*, 2008). Reactive oxygen species are involved in pathogenesis of many diseases (Wilcox and Gutterman, 2005). To maintain redox balance and to protect organs from free radicals action the living cells have evolved an endogenous antioxidant defence mechanism which includes enzymes like superoxide dismutase, catalase, glutathione peroxidase reduced glutathione and metallothioneins. Glutathione (GSH) a free radical scavenger is believed to be one of the major cellular constituents involved in defense against lipid peroxidation. GSH amounts to about 90 % of the non protein thiol in cell (Kosower and Kosower, 1976) and is involved in number of reductive reactions in the cell and acts as substrate or cofactor for antioxidant enzymes (GSH peroxidase, GSH transferase and reductase that are involved in termination of peroxidation. SOD and CAT constitute a mutually supportive team of defence against ROS. Hydrogen superoxide, a product of SOD activity, is also a strong inhibitor of this enzyme. That is why the effective detoxification of active oxygen forms takes place with concordant SOD and catalase action. Catalase is a common enzyme found in nearly all living organisms which are exposed to oxygen, where it functions to catalyse the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al.*, 2004). Lipid peroxidation has been suggested as one of the main causes of radiation induced membrane damage (Purohit *et al.*, 1966). Singh *et al.* (2000) showed that hydroalcoholic extract of *Adhatoda vasica* modulate the Phase I and Phase II enzyme system and thus result in chemoprevention in mice. The present study was undertaken for the biochemical quantification of SOD, catalase and lipid peroxidation in biceps of mice at 7, 14, 21, 28 days of investigation.

## 2. Materials and Methods

### Animals

The present investigation was carried out on biceps muscle of mice. Adult sexually mature Swiss albino mice weighing between 20 – 30 g were procured from Central Research Institute (CRI), Kasauli (H.P.). They were maintained in polypropylene cages in the animal house of Department of Biosciences, H.P. University, under hygienic conditions with proper temperature and light (24±2°C, 12:12 hours light dark cycle). Mice were fed upon Hindustan lever pellets diet and water *ad libitum*. All experimental procedures were conducted after Institutional Animals Ethics Committee Approval (IAEC/Bio/2009/11) of H.P. University, Shimla.

### Preparation of *Adhatoda Vasica* leaf extract

Leaves of *Adhatoda vasica* were collected from herbal garden Joginder Nagar, H.P. and were properly identified by the taxonomist of Biosciences, H.P. University, Shimla. Leaves were washed thoroughly and dried under shade for one month. Dried leaves were grinded to a coarse, green coloured powder.

### Extraction

Dried leaf powder was extracted 5 times with 80% ethanolic solution. Extraction was done after every twenty four hours. Collected suspension was concentrated under reduced pressure.

### Source of irradiation

20-30 g mice were irradiated in “Gamma Chamber-900” (BARC) with automatic timer having Cobalt 60 as the source of gamma rays.

### Grouping of animals

Mice were randomly divided into 4 groups of 8 animals each.

- (i) First group i.e. designated as control: Group I containing normal mice served as control for each experimental stage.
- (ii) Second group i.e. designated as treated: Mice of Group II were maintained under identical conditions and received oral administration of *Adhatoda vasica* extract (900mg/kg body weight).
- (iii) Third group- i.e. designated as irradiated only:- Mice of group III were maintained under identical conditions & were irradiated with  $\gamma$ -radiation.
- (iv) Fourth group i.e. designated as extract treated and irradiated: Mice of group IV were maintained under identical conditions and received oral administration of *Adhatoda* extract and were irradiated with  $\gamma$ -rays.

### Biochemical Analysis

Tissues were employed for biochemical studies at each stage of investigation. The parameters included were estimation of total proteins, lipid peroxidation, reduced enzymes SOD and catalase.

**Estimation of proteins** Tissue homogenate (10%) was homogenized in 0.15 tris buffer. The homogenate was centrifuged at 5000 rpm for 10 minutes at 4 °C and then the supernatant was collected. Total protein in the supernatant homogenate was measured as per method of Lowry *et al.* (1951)

### Superoxide dismutase (SOD) activity

Superoxide dismutase activity was measured as per the method of Mishra and Fridovich (1972)

### Catalase activity (CAT)

This enzyme assay was done as per the method of Aebi (1984).

### Lipid peroxidation

Levels of malonaldehyde index of lipid peroxidation was measured according to Dhindsa *et al.*, 1981 using barbituric acid.

## 3. Results and Discussion

### SOD activity in biceps (Fig- I)

Determination of SOD enzyme activity was done in normal, *Adhatoda* extract treated, irradiated and extract + radiation treated biceps muscle. The control mice biceps muscle shows  $9.29 \pm 1.72$  to  $9.90 \pm 0.68$  units /mg protein enzyme activity. SOD activity increased to  $10.15 \pm 1.52$  units after extract treatment at 7 days stage. The increase in activity was calculated to be 9.34%. At day 14, further 10.53% increase of  $10.39 \pm 0.10$  units /mg protein in extract treated mice. SOD activity enhanced at day 21 and 28 with increase of 11.05 % and 11.11 % and recorded enzyme activity of

10.55 ± 0.90 and 11.00 ± 0.40 respectively. SOD activity was 5.10 ± 0.22 and 5.30 ± 0.15 units /mg protein at 7 and 14 days after irradiation. The decrease in enzymatic activity was 45.10% and 43.6% respectively. At 21 and 28 days, further 41.47% and 41.51% decrease of 5.56±1.06 and 5.79±1.56 units/mg protein was noticed as compared to normal. SOD activity increased from 7.01 ± 1.05 to 7.45 ± 3.15 units after extract + radiation treatment at 7 and 14 days stage. The increase in enzymatic activity was calculated to be 37.345% and 40.56% respectively. At days 21 and 28, further 44.18% and 44.91 % increase of 7.79 ± 3.26 and 8.35 ± 2.16 units /mg protein respectively was noticed.

#### Catalase activity in biceps muscle (Fig-II)

The specific activity of catalase was 8.29 ± 1.55 units /mg protein in biceps muscle at 7 days stage. The enzyme activity was almost same at 14, 21 and 28 days stage in normal mice biceps muscle. Catalase activity was 9.05 ± 0.81 units /mg protein after extract administration at 7 days stage. The percentage increase in enzyme activity was calculated to be 9.26 % at 7 days stage, which further increased the enzyme activity to 9.30 ± 0.84, 9.89 ± 1.78 units /mg protein and percentage increase was calculated to be 10.96 % and 14.76% at 14 and 21 days stage respectively. At 28 days stage, the enzymatic activity was 10.27± 1.99 units /mg protein with percentage increase of 14.94%. Catalase activity was recorded as 5.95 ± 2.60, 6.18 ± 1.30 and 6.94 ± 0.84 showing a percentage decline of 22.52%, 36.22%, 24.34% at 7,14,21 days stage as compared to the normal. At 28 days stage, the enzyme activity was 7.01 ± 1.91 with a percentage increase of 27.57%. Extract + Radiation treatment showed increase in enzyme activity (7.09 ± 3.76, 7.75 ± 0.09 unit/mg protein, at 7 and 14 days of investigation. The percentage increase was 19.32% and

25.73% as compared to the irradiated group. The percentage decrease in enzyme activity was calculated to be 19.27% and 27.28% at day 21 and 28. The specific activity was found to be 8.27 ± 1.51 and 8.92 ± 1.78 units /mg protein respectively.

#### Lipid peroxidation in biceps muscle (Fig-III)

It has been found that MDA production at 7 days stage in normal mice biceps muscle was 21.60 ± 0.40 n moles/g of fresh tissue weight. At days 14 and 21, the MDA production in control mice was 21.60 ± 0.28 and 22.70 ± 0.11 n moles/g of fresh tissue weight. The MDA production was 22.70 ± 0.11 n moles/g of fresh tissue weight at 28 days stage. The extract treated muscle recorded MDA value of 22.00 ± 0.34 n moles/ g of fresh tissue weight at 7 days stage with percentage increase of 1.90%. At 14, 21, 28 days stage, the peroxidation level risen to 22.75 ± 0.06, 23.30 ± 0.10, 23.90 ± 0.10 n moles /g of fresh tissue weight showing a increase of 5.32%, 2.64% and 5.63%. The irradiated mice muscle witnesses increase in MDA value. MDA value is 26.81 ± 0.06 and 27.19 ± 0.06 n moles/g of fresh tissue weight at 7 and 14 days stage with percentage increase of 24.80% and 25.87% as compared to control. At 21 and 28 days stage, the MDA production was 28.01 ± 0.03 and 28.49 ± 0.03 n moles/g of fresh tissue weight resulting in an increase of 23.39% and 25.50%. The extract + irradiated mice muscle recorded MDA value of 24.01 ± 0.02 and 24.37 ± 0.06 n moles/g of fresh tissue weight. The decrease was 10.76% and 10.40% as compared to irradiated mice. At 21 and 28 days stage, the peroxidation level was 24.90 ± 0.03 and 25.00 ± 0.03 n moles /g of fresh tissue weight showing decline of 11.10% and 12.28% respectively.

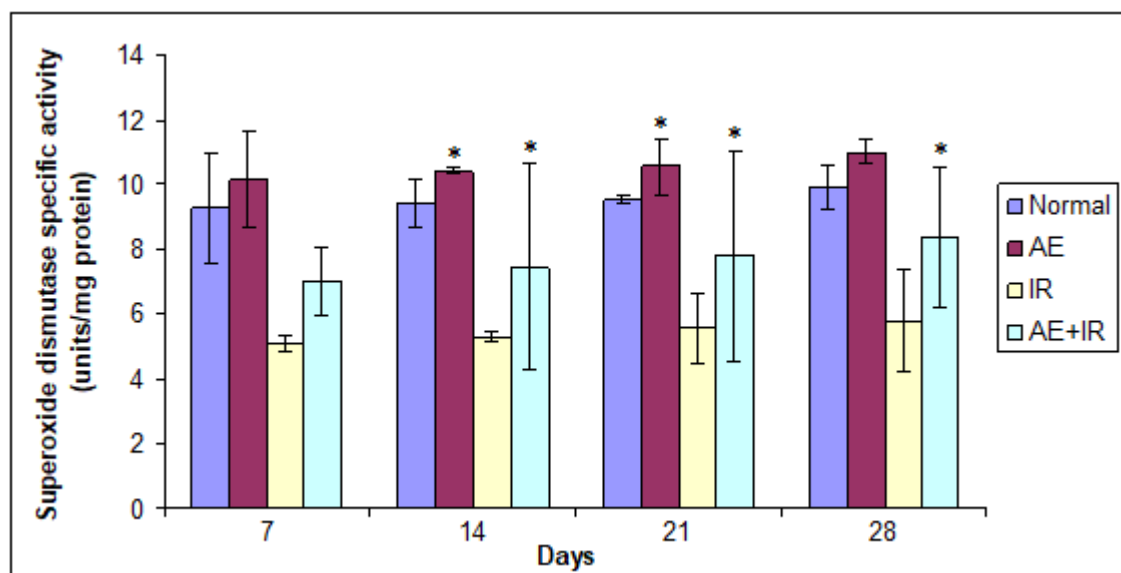


Figure I

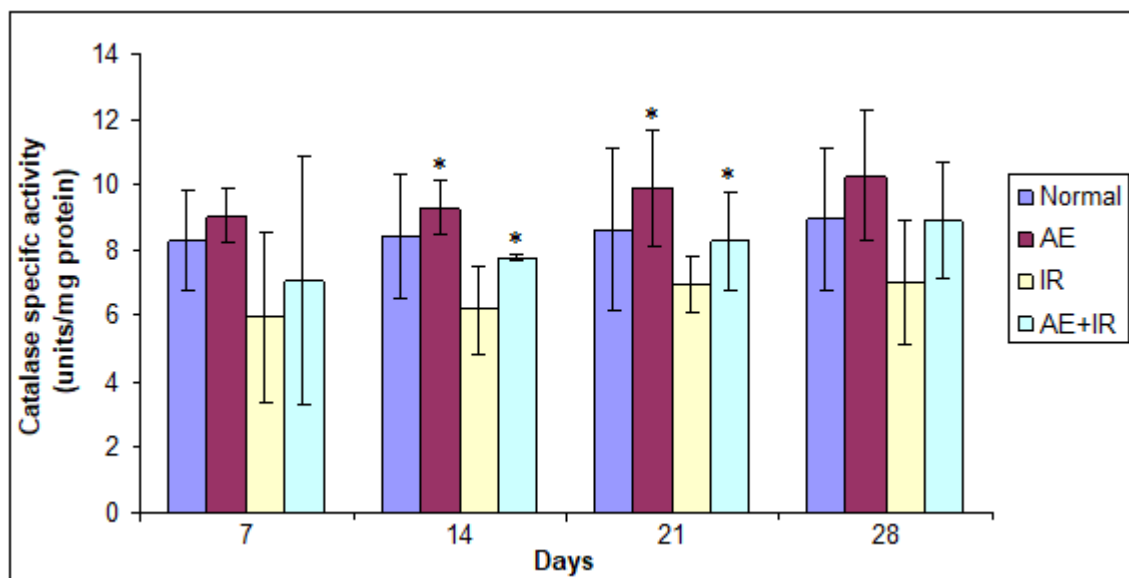


Figure II

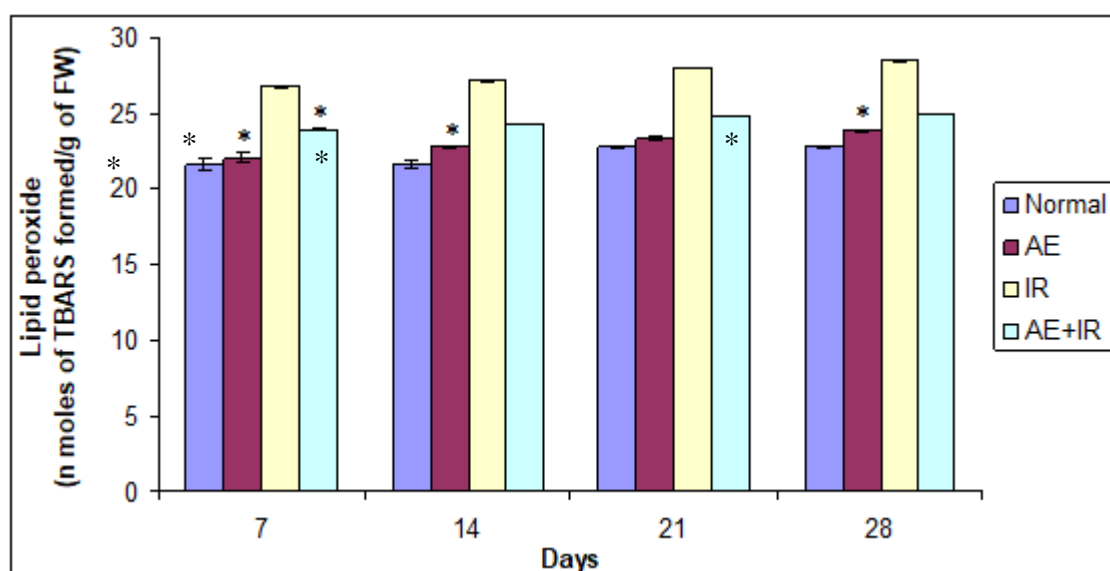


Figure III

*Adhatoda vasica* extract and gamma radiations are studied on biceps muscle by the biochemical quantification of SOD, catalase and malondialdehyde (MDA) levels. SOD is an endogenously produced intracellular enzyme which catalyses the dismutation of superoxide into oxygen and hydrogen peroxide which are produced by polymorphonuclear leukocytes when they ingest bacteria or immune complexes (Ismail *et al.*, 2008). These radicals cause the membrane damage alteration in protein structure and destruction of antioxidants with the synovial fluid (Gutteridge, 1986). An increased rate of free radical production may exceed the capacity of cellular defence systems. These radicals are capable of damaging virtually any biomolecule, sugars, fatty acids and nucleic acids. SOD levels show little variation in *Adhatoda* extract treated animals biceps muscle. A significant reduction in SOD activity is observed in irradiated animals. This could be due to the enhanced utilization of antioxidant system as an attempt to detoxify the free radicals generated by radiation.

A lower depletion of SOD in the *A vasica* leaf extract pretreated animals could be due to the higher availability of

SOD, which increases the ability to cope up with the free radicals production by radiation. Increased SOD level suggests that protection by *Adhatoda vasica* leaf extract may be mediated through modulation of cellular antioxidant level. An increase in rate of free radical production may exceed the capacity of cellular defence system (Hopper, 1989). The concerted action of various antioxygenic enzymes keep concentration of free radicals in cells relatively low.

The activity of catalase in *Adhatoda* extract treated mice stomach and muscle did not show much variation from 7-28 days of treatment. But in the animals exposed to gamma radiation a continuous decrease in activity of catalase in biceps muscle. Increase in catalase activity in *A vasica* extract pretreated irradiated animals were observed. As the principle mechanism of radiation damage is the production of free radicals by conjugation Phase II enzymes results in radioprotection. Singh *et al.*, (2000) studied the modulatory effect of the *Adhatoda* extract on extra hepatic organs like lung, kidney and forestomach for activities of SOD, catalase and GSH. In this study the extract was effective in



stimulating these organs to increase the potential of machinery associated with detoxification of xenobiotic compounds. Thus our findings are in agreement to these studies suggesting possible chemopreventive role of *Adhatoda* leaf extract. Shimoi *et al.*, (1996) concluded that plant flavonoids which show antioxidant activity in vitro also function as antioxidants in vivo and their radioprotective effect may be attributed to their radical scavenging activity. An extract of *Phyllanthus amarus* has been reported to protect against radiation induced decline in SOD, catalase and glutathione -S- transferase (Kumar and Kuttan, 2004).

*Aegle marmelos* (AME) hydroalcoholic extract was also reported to scavenge OH, O<sub>2</sub><sup>-</sup>, DPPH, ABTS + and NO (nitric oxide) radicals in vitro in concentration dependent manner. *Podophyllum hexandrum* has also been reported to protect against radiation induced mortality, gastrointestinal damage and embryonic nervous system of developing mice. It also protected against radiation induced decline in SOD in liver and intestine of irradiated mice (Mittal *et al.*, 2001). Thus, studies show that various plant and herbs protect against radiation induced damage by scavenging free radicals and increasing antioxidant status. Role of catalase is the removal of toxic hydroxyl radicals or H<sub>2</sub>O<sub>2</sub> mediated damage which is produced through the reduction of oxygen by  $\gamma$ -irradiation induced radicals (McLennan *et al.*, 1980). The major danger of H<sub>2</sub>O<sub>2</sub> accumulation is the production of highly reactive hydroxyl radical for which no physiological defense system occur. As a result, catalase become most crucial enzyme to detoxify H<sub>2</sub>O<sub>2</sub>. A sufficient catalase activity is essential for removal of H<sub>2</sub>O<sub>2</sub>.

MDA is a breakdown product of unsaturated fatty acids and significant increase in levels of lipid peroxide indicates enhanced lipid peroxidation by free radicals. The end products of lipid peroxidation may be mutagenic and carcinogenic (Marnett, 1999). Expression of radiation induced normal tissue injury is not solely a result of loss of a particular cell population, rather it involves a complex and dynamic interaction between several cell types in an organ (Robbins, 1995).

The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. LPO can be initiated by radiolytic products, including hydroxyl and hydroperoxyl radicals (Rayleigh, 1987). It was observed that pretreatment of mice with *Adhatoda* extract did not cause any significant changes in LPO level but *A.vasica* leaf extract treatment significantly lower the radiation induced LPO in terms of malondialdehyde. LPO inhibition in biomembranes can be caused by antioxidants (Konings and Osterloo, 1980). This view is supported by similar studies on the anti lipoperoxidant activities of the young sprouts of *Rosmarinus officinalis* that has shown to reduce the formation of malondialdehyde significantly in rat hepatocytes (Joyeux *et al.*, 1990). Our results are also in accordance with Sotelo- Felix *et al.*, (2002) who proposed that carnosol could scavenge free radicals, consequently avoiding the propagation of lipid peroxides in liver of mice.

Flavonoids, tannins and microelements have been suggested to act as antioxidants and exerts their antioxidant activity by

scavenging the lipid peroxidation. Treatment with *Phyllanthus emblica* also lowered the elevated levels of lipid peroxides in the serum (Hari Kumar *et al.*, 2004).

The findings of above studies indicate that *Adhatoda* extract protect biceps muscle against radiation induced damage. Thus present results suggest that free radical damage induced by the radiation can be lowered by *Adhatoda vasica* leaf extract. The radioprotective effects of *Adhatoda* extract observed in present study can be attributed to the antioxidant properties of this plant due to the chemical constituents present in it.

## References

- [1] Arora, R., Gupta, D., Chawla, R., Sagar, R., Sharma, A., Kumar, R., Prasad, J., Singh, S., Samanta, N. and Sharma, R.K. (2005). Radioprotection by plant products: Present Status and Future Prospects. *Phytother. Res.*, 19: 1-22.
- [2] Claeson, U.P., Malmfors, T., Wikman, G. and Bruhn, J.G. (2000). *Adhatoda vasica*: a critical review of ethnopharmacological and toxicological data, *J. Ethnopharmacol.*, 72: 1-20.
- [3] Chelikani, P., Fita, T. and Loewen, P.C. (2004). Diversity of structures and properties among catalases. *Cell. Mol. Life Sci.*, 61(2):192-208.
- [4] Dormand, E.L., Banwell, P.E. and Goodacre, T.E. (2005). Radiotherapy and Wound Healing. *Int. Wound J.*, 2 : 112-127.
- [5] Devi, P.U. (2001). Radioprotective anticarcinogenic and antioxidant properties of Indian holy basil, *Ocimum sanctum* (tulsi). *Ind. J. Exp. Biol.*, 39:189-190.
- [6] Dhuley, J.N. (1999). Antitussive effect of *Adhatoda vasica* extract on mechanical or chemical stimulation induced coughing in animals. *J. Ethnopharmacol.*, 67: 361-365.
- [7] Dorsh, W. and Wagner, H. (1991). New antiasthmatic drug from traditional medicine. *Int. Arch. Allergy Appl. Immunol.*, 94:262-265
- [8] Gutteridge, J.M.C. (1986). Antioxidant properties of the protein ceruloplasmin, albumin and transferrin: A study of the activity in serum and synovial fluid from patients with rheumatoid arthritis. *Biochem. Biophys. Acta.*, 869: 119-127.
- [9] Hari Kumar, K.B. (2004). Modulation of hematopoietic system and antioxidant enzymes by *Embllica officinalis* gaertn and its protective role against gamma-radiation induced damages in mice. *J. Radiat. Res.*, 45 (4): 549-555.
- [10] Hazra B., Santana, B., Nripendranath, M. (2008) Antioxidant and free radicals scavenging activity of *Spondias Pinnata*. *Complementary Alternative Medicine* 2008, 8:63.
- [11] Hooper, C. (1989): Full radicals: Research on Biomedical Bad boys comes of age. *J.N.T.H. Res.*, 1: 101-106.
- [12] Ismail, M.F., Shohda, A., Maraghy, E.L. and Saaik, N.A.H. (2008). Study of the immunomodulatory and inflammatory effects of evening primrose oil in adjuvant arthritis. *Afr. J. Biochem. Res.*, 2 (3): 74-84.
- [13] Joyeux, M., Rolland, A., Fleurentin, J., Mortier, F. and Dorfman, P. (1990). Tert-butyl hydroperoxide induced

- injury in isolated rat hepatocytes. A model for studying anti-hepatotoxic crude drugs. *Planta Med.*, 56:171-174.
- [14] Kumar, K.B. and Kuttan, R. (2004). Protective effect of an extract of *Phyllanthus amarus* against radiation induced damage in mice, *J. Radiat. Res.* (Tokyo), 45: 133.
- [15] Konings, A.W.T. and Osterloo, S.K. (1980). Radiation effects on membranes. II. 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.*, 81: 200-207.
- [16] Kosower, N.S. and Kosower, E.M. (1976). In "free radicals in biology" Vol.II (Ed) Pryor, W.A. New York Academic Inc. PP.55.
- [17] Kumar, K.B., Ram, J., Samarth, R.M. and Kumar, M. (2003). Modulatory influence of *Adhatoda vasica* leaf extract against gamma irradiation in Swiss albino mice. *Phytomed.*, 12:285-293.
- [18] Kumar, M., Dandapat, S. and Sinha, M.S. (2015). Hepatoprotective activity of *Adhatoda vasica* and *Vitex Negundo* leaf extract against CCl<sub>4</sub> induced hepatotoxicity. *Advances in Biological Research* 9(4):242-246.
- [19] Lowry, O.H., Rosenbrough, N.J., Fars, A.L. and Randall, R.J. (1951). Protein measurements with the Folin-Phenol reagent. *J. Biol. Chem.*, 193:265-275.
- [20] Marnett, L.J. (1999). Lipid Peroxidation –DNA damage by malondialdehyde. *Mutat. Res.*, 424 (1-2): 83-95.
- [21] McLennan, G., Oberly, L.W. and Autor, A.P. (1980). The role of oxygen derived free radicals in radiation induced damage and death of non-dividing eukaryotic cells. *Radiat. Res.*, 84, 122.
- [22] Mittal, A., Pathania, V., Agrawala, P.K., Prasad, J., Singh, S. and Goel, H.C. (2001). Influence of *Podophyllum hexandrum* on endogenous antioxidant defence system in mice: Possible role in radioprotection. *J. Ethnopharmacol.*, 76: 253.
- [23] Mishra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, 247:3170-3175.
- [24] Nair, C.K.K., Parida, D.K. and Nomura, T. (2001) Radioprotectors in radiotherapy. *J. Radiat. Res.* 159:812-834.
- [25] Purohit, R.K. (1996). Modification of radiation induced changes in mammalian skin and liver by liv.52, A Ph.D thesis, University of Rajasthan, Jaipur.
- [26] Rayleigh, J.A. (1987). Prostaglandin and Lipid Metabolism in Radiation Injury (Eds. Jr. T.C. Walden, H.N. Huges), Vol. 3. Plenum Press, New York.
- [27] Robbins, M.E.C. (1995): Amelioration of late normal tissue injury by pharmacological intervention after radiotherapy. *Abs. In Proc. 10<sup>th</sup> Intern. Congress Radiat. Res.*, P.30.
- [28] Sharma, P. and Goyal, P.K. (2014). Protective action of *tinospora cordifolia* extract against radiation induced biochemical alterations in liver. *Int.J. of Pharma and Pharma .Sci.* Vol 6:6.
- [29] Singh, R.P., Padmavathi, B., Rao, A.R., (2000). Modulatory influence of *Adhatoda vasica* (*Justicia Adhatoda*) leaf extract on the enzymes of xenobiotic metabolism, antioxidant status and lipid peroxidation in mice. *Mol. Cell. Bio. Chem.*, 213, 99-109.
- [30] Singh, I., Sharma, A., Jindal, A., Soyad, D., Goyal, P.K. (2006). Fruit extract of *emblica officinalis* (Amla) protects radiation induced biochemical lesions in the brain of Swiss albino mice. *Annals of Neurosciences*, Vol 13:3.
- [31] Shimoi, K., Masuda, S., Shen, B., Furugori, B. and Kinae, N. (1996). Radioprotective effect of antioxidative plant flavonoids in mice. *Mutat. Res.*, 350: 153-161.
- [32] Sotelo- Felix, J.I., Martinez- Fong, D., Muriel and De la Torre, P. (2002). Protective effect of carnosol on CCl<sub>4</sub>-induced acute liver damage in rats. *Eur. J. Gastroenterol. Hepatol.*, 14 (9): 1001-1006.
- [33] Sharma, P., Rajesh, S.J, Singh, D. and Ganesh, N. (2009). Radiation protective potential of *Adhatoda vasica*. *Int. J. Phytomed.*, 1: 39
- [34] Weiss, J.F. and Landaver, M.R. (2003). Protection against ionizing radiation by antioxidant nutrients and phytochemicals. *Toxicol.*, 189: 1-20.
- [35] Wilcox, C.S. and Gutterman, D. (2005). Focus on oxidative stress in cardiovascular and renal systems. *Am.J. Physiol. Heart Circ Physiol.*, 288:3-6.