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Research Article

ROLE OF *ADHATODA VASICA* LEAF EXTRACT AGAINST GAMMA RADIATION INDUCED OXIDATIVE STRESS IN MICE PECTORALIS MUSCLE

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ABSTRACT
The science of radiation protection is a fundamental outgrowth of peaceful and military applications of ionizing radiation. The various chemicals that have been used as radio protectors as free radical scavengers are effective if given prior to or during irradiation. Several chemical agents/synthetic radio protectors have been used against the hazardous effects of ionizing radiation in experimental studies with success. Medicinal plants play an important role in pharmacology and medicine for many years. The objective of present study was to evaluate the antioxidant enzyme activities in pectoralis muscle of mice. Mice were divided into four groups i.e. Group (i) containing normal mice served as control; group (ii) mice given 900 mg/kg body wt. of *Adhatoda vasica* extract orally; group (iii) mice were exposed to gamma radiation (6Gy) and group (iv) mice given *Adhatoda vasica* leaf extract plus gamma radiation (6 Gy). Present study demonstrated that *Adhatoda vasica* leaf extract provides protection against free radical damage.

Keywords: *Adhatoda vasica*, gamma radiation, pectoralis muscle, antioxidant enzymes and oral administration.

1. INTRODUCTION
Several chemical agents/synthetic radio protectors have been used against the hazardous effects of ionizing radiation in experimental studies with success. Exposure to whole body irradiation may induce cancer by a number of different mechanisms. Free radicals have been directly associated with biological effects of ionizing radiation such as lethality, physiological disorders, mutation and carcinogenesis. Ionizing radiation is known to affect somatic and germ cells, leading to mutation, cell death, malformation and cancer. Interaction of ionizing radiation with living cells cause a variety of changes, whose damage intensity depend fundamentally on the absorbed dose, type of radiation, conditions of irradiation and intrinsic radio sensitivity of cell [1]. At present, more than 80% cancer patients receive radiation therapy as a part of their treatment [2, 3]. Herbal drugs offer an alternative to synthetic compounds and are considered either non-toxic or less toxic than their synthetic counterparts. Today, it is estimated that about 80% of the world relies on botanical preparations as medicine to meet their health needs [4]. Therefore, they are prone to attack by reactive oxygen species (ROS), which are capable of oxidation of proteins, lipids and DNA leading to cellular damage. Adequate free radicals scavenging enzymes i.e. glutathione (GSH) and lipid per oxidation (LPO) are available in tissues of normal individuals which act directly or indirectly to remove reactive oxygen species, thus elevation of these enzymes suggest an increased need for protection against free radicals. Glutathione (GSH) is referred to as the body's master antioxidant and is found in virtually every cell of the human body. It is also an essential component to the body's natural defense system. Radiation, heavy metal toxicity and even the normal process of aging can cause free-radical damage to healthy cells and deplete glutathione. Oxidative stress occurs as the generation of free radicals exceeds the body's ability to neutralize and eliminate them. A primary function of glutathione is to alleviate this oxidative stress. Present investigation deals with *Adhatoda vasica* which may prove to be efficient antioxidants. It provides complicated, mixed and distinct elements which act as the main basis of drug discovery [5]. The ethanolic extract contains phytochemical constituents for miscellaneous medicinal

activities which are bioactive in nature [6]. The present study focuses on the protective effect of ethanolic extract of *Adhatoda vasica* in oxidative defense. *Adhatoda vasica* used in the present study belongs to the family Acanthaceae and is found throughout India upto the height of 1300 m. The leaves as well as flowers, fruits and roots are extensively used for treating cold, whooping cough and asthma etc. The leaf juice is stated to cure diarrhoea, dysentery and glandular tumor. It has growth inhibitory effects on *Mycobacterium tuberculosis* thereby proving useful in the therapy of tuberculosis [7]. *Adhatoda vasica* a perennial shrub has been accredited to afford protection against allergen induced bronchial obstruction in guinea pigs [8]. The phytoconstituents such as alkaloids, terpenoids, saponins, reducing sugars, tannins, carbonyls, flavonoids, phlobatanins and steroids etc. were present in *Adhatoda vasica*.

2. MATERIAL AND METHODS

Swiss albino mice of Balb-C strain weighing 22-25g were procured from Central Research Institute (CRI) Kasauli, Himachal Pradesh, India. These were maintained in the animal house of Department of Biosciences of Himachal Pradesh University, Shimla under proper hygienic conditions (24+2°C temp. and light). Mice were provided Hindustan lever feed and water *ad libitum*. The entire animal care and experimental procedures were approved by the Institutional animal ethics committee of Himachal Pradesh University, Shimla (IAEC/Bio/12-2009).

2.1. Plant material

Leaves of *Adhatoda vasica* were collected from herbal garden Joginder Nagar, Himachal Pradesh, India.

2.2. Extraction of plant materials

The collected leaves were washed thoroughly and dried under shade for one month. Dried leaves were ground to a coarse, green colored power. Dried leaves powder was extracted with 80% ethanolic solution (1litre) at room temperature for 24 h. The ethanolic extract was percolated and fresh 80% ethanolic solution was added to the powder and was left for 24 h at room temperature. The ethanolic extract was again percolated and the process was repeated five times. The combined ethanolic extract was concentrated under reduced pressure and a gummy residue was obtained. The gummy extract was acidified with 2% aqueous organic acid (acetic acid) and continuously stirred for 24 h at room temperature. The acidic solution obtained was fractionated with $CHCl_3$ (50 ml × 4). Aqueous acidic layer thus obtained after extraction with chloroform was basified with ammonia (pH=9). The basic layer was again extracted with $CHCl_3$ and the organic layer obtained was concentrated under reduced pressure to give amorphous residue. The amorphous residue was tested for various components on TLC.

2.3. Column Chromatography

Different components were separated by column chromatography. The column was packed with Silica gel by wet method using ethyl acetate/hexane as solvent. The amorphous residue obtained above was added at the top of the column. The column was run by gradient elution method using mixture of solvents with varying polarity (Hexane: Ethyl acetate) in different proportions starting with 90:10 v/v and continued till 45:55 v/v mixture. The different elutes were tested on TLC which indicated the presence of number of constituents.

2.4. Phytochemical Analysis

As the amorphous residue obtained was found to show the presence of many components when run on the TLC. Phyto-chemical investigations were done to determine the chemical nature of the constituents such as alkaloids, terpenoids, saponins, reducing sugars, tannins, carbonyls, flavonoids, phlobatanins and steroids by simple chemical reactions [9].

2.5. Source of Irradiation

About 6-8 weeks old male mice were irradiated in "Gamma chamber-900" (BARC) with automatic timer having cobalt- 60 as the source of gamma rays.

2.6. Experimental design

Normal healthy animals showing no sign of morbidity were divided into following groups: (i) Mice in first group served as control (ii) Mice of second group were administered *Adhatoda vasica* extract (900mg/kg body wt.) for 15 days. (iii) Mice of third group were exposed to gamma radiation (6Gy). (iv) Mice of fourth group were administered *Adhatoda vasica* extract (900mg/kg body wt.) and then exposed to gamma radiation (6Gy).

2.7. Extract and Radiation Administration

The mice were given *Adhotoda vasica* extract orally (900 mg/kg body wt.) for 15 days and after 30 min. of last dose; they were exposed to 6 Gy dose of gamma radiation. After that mice were autopsied by cervical dislocation on day 1, 5, 15 and 30. The pectoralis

muscle of normal, extract treated, irradiated and extract treated plus irradiated mice were excised and investigated for further studies.

2.8. Biochemical Assay

2.8.1. Estimation of Lipid peroxidation

Levels of malondialdehyde, index of lipid peroxidation were estimated according to reported method [10] using thiobarbituric acid (TBA). Pectoralis muscles were homogenized in 2 ml of 0.1% trichloroacetic acid (TCA) in pestle and mortar. Homogenate was then centrifuged at 6000 rpm for 15 minutes. To 1ml of supernatant, 2 ml of 0.5% TBA prepared in 10% TCA was added. The test tubes containing above solution were kept in boiling water bath for 30 minutes. Tubes were then ice cooled in cold water and centrifuged again. Absorbance of supernatant was taken at 532 nm and 600 nm. Difference of two absorbance values was taken as actual value and used for calculating TBA reactive substance malondialdehyde formed. The MDA contents were calculated in n moles/g of fresh tissue weight.

2.8.2. Estimation of Glutathione assay

Glutathione (GSH) activity was estimated as per reported method [11]. GSH was measured by its reaction with Di-thio-bis-nitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. Pectoralis muscles were homogenized in 100 ml phosphate saline buffer (PBS). A portion of muscle homogenate (0.5 ml) was precipitated by adding 125 µl of 25% TCA (trichloroacetic acid) and the tubes were cooled for 5 minutes on ice. The mixture was further diluted with 0.6 ml of 5% TCA, centrifuged at 1000 rpm for 10 minutes and 0.1 ml supernatant was made up to 1ml with 0.2 M sodium phosphate buffer (pH 8.0). Freshly prepared DTNB solution (2 ml) in 0.2 M sodium phosphate buffer was added to the tubes and yellow colour formed after 10 minutes was spectrophotometrically measured.

2.9. Statistical Analysis

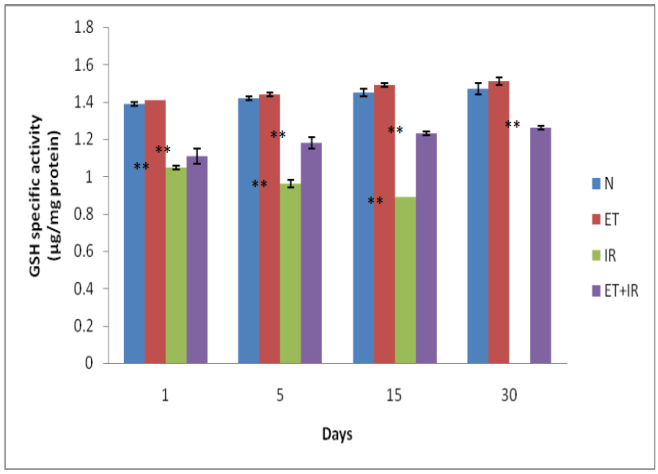
Data were presented as mean ± SEM. Statistical significance was determined by one way ANOVA with post-hoc Tukey HSD to find out the mean differences between groups. Differences were considered to be significant at * $P < 0.05$ and ** $P < 0.01$.

3. RESULTS

The results obtained for GSH specific activity and lipid peroxides in pectoralis muscle of normal, extract treated, irradiated and extract treated plus irradiated mice were presented in figs. 1 & 2.

3.1. GSH

Glutathione levels in pectoralis muscle of normal mice muscle were recorded between 1.39±0.01 and 1.47±0.03 µg/mg protein. *Adhatoda vasica* extract treated mice muscle exhibited increase in levels of GSH. GSH levels at 1, 5, 15 and 30 days stage in extract treated muscle were 1.41±0.00, 1.44±0.01, 1.49±0.01 and 1.51±0.02 µg/mg protein. Irradiated mice pectoralis muscle observed decline in levels of GSH. At 1, 5 and 15 days stage glutathione levels were 1.05±0.01, 0.96±0.02 and 0.89±0.00 µg/mg protein. At 30 days stage no survival of mice was seen in irradiated group. *Adhatoda vasica* extract treated plus irradiated mice pectoralis muscle showed increase in levels in comparison to irradiated mice pectoralis muscle. At 1, 5, 15 and 30 days stage the levels of glutathione were 1.11±0.04, 1.18±0.03, 1.23±0.01 and 1.26±0.01 µg/mg protein.



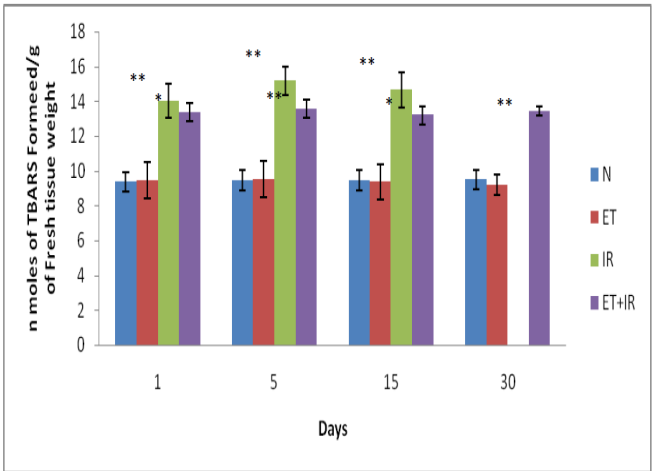
*N = Normal; ET = Extract treated; IR = Irradiated; ET+IR = Extract treated + Irradiated; NS = No survival. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).*

Fig. 1: GSH specific activity (µg/mg protein) in pectoralis muscle of mice from 1-30 days period.

3.2. Lipid peroxidation

Indirect determination of lipid per oxidation (LPO) was done by estimating the amount of thiobarbituric acid reactive substances (TBARS) formed in the tissues. Extent of lipid peroxidation was considered as a tool to indirectly measure the involvement of free radicals in inducing the oxidative stress. MDA production in normal mice pectoralis muscle was 9.36±0.56,

9.44±0.58, 9.47±0.59 and 9.51±0.56 n moles/g of fresh tissue weight respectively. *Adhatoda vasica* extract treatment showed significant variation in LPO level. The extract treated pectoralis muscle recorded MDA value of 9.45±1.05, 9.54±1.06, 9.37±0.99 and 9.22±0.59 n moles/g of fresh tissue weight at 1, 5, 15 and 30 days stage. An increase in MDA production was observed up to 5 days stage in irradiated mice. Irradiated mice pectoralis muscle MDA value was 14.01±0.98, 15.17±0.79 and 14.67±1.02 n moles/g of fresh tissue weight at 1, 5 and 15 days, thereby recording an increase of 48.25%, 59.01% and 56.56% respectively in comparison to extract treated mice. At 30 days stage no comparison could be made as there was no survival of mice in irradiated group. MDA production in *Adhatoda vasica* extract treated plus irradiated mice pectoralis muscle was significantly low than irradiated mice throughout the period of investigation. At day 1, 5, 15 and 30 the MDA production was 13.39±0.53, 13.55±0.53, 13.21±0.52 and 13.43±0.25 n moles/g of fresh tissue weight showing decrease of 4.42%, 10.67% and 9.95% in comparison to irradiated mice. A comparison could not be made as no survival of mice was there at 30 days stage in irradiated group.



*N = Normal; ET = Extract treated; IR = Irradiated; ET+IR = Extract treated + Irradiated; NS = No survival. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).*

Fig. 2: Lipid peroxides (N moles of TBARS formed/g of fresh tissue weight) in pectoralis muscle of mice from 1-30 days period

4. DISCUSSION

The herbal drugs occupied a distinct place in the life right from the primitive period till today. Radiation injuries are manifested as a result of enhanced production of free radicals due to oxidative stress. The World Health Organization estimated that about 80 percent of the world's population still relies on plant based medicines for their primary health care [12]. The protective effect of *Adhatoda vasica* leaf extract and gamma radiation on pectoralis muscle of mice was noticed by studying the biochemical quantification of GSH and malondialdehyde (MDA) levels. The interaction of radiation with the components of living system results in the generation of free radicals. Over-production of such free radicals cause oxidative damage to biomolecules leading to many chronic diseases [13]. Free radicals attack virtually all components including DNA, protein and cause lipid peroxidation. This results into oxidative stress in the cell or tissue if the concentration of reactive oxygen species (ROS) generated exceeds the antioxidant capability of that cell [14]. An increased rate of free radical production may exceed the capacity of cellular defense system which surpass the body's immune system to combat oxidative stress. Consequently cell becomes incapable of defending itself against the oxygen derived species resulting in oxidative injury [15]. Glutathione is an essential bio-factor and is synthesized in all living cells. It functions mainly as an effective intracellular reductant [16]. It can act as a non-enzymatic antioxidant by direct interaction of the SH group with ROS or it can be involved in the enzymatic detoxification reactions for ROS as a cofactor [17]. Resistance of many cells against oxidative stress is associated with high intracellular levels of GSH [18, 19]. GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation [20].

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. The depletion of GSH promotes generation of reactive oxygen species and oxidative stress with cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes [21]. The lower depletion of GSH in the *Adhatoda vasica* leaf extract treated plus irradiated mice pectoralis muscle could be due to the higher availability of GSH, which increases the ability to cope up with the free radicals produced by radiation. The increased GSH level suggests that protection by *Adhatoda vasica* leaf extract may be mediated through the modulation of cellular antioxidant levels. Singh et al. [22] showed that ethanolic extract of *Adhatoda vasica* modulate the phase I

and II enzyme system and thus result in cancer chemoprevention in mice. In the present study, it was observed that *Adhatoda vasica* extract treated plus irradiated mice pectoralis muscle exhibited a significant increase in GSH in comparison to irradiated mice muscle. Lipid per oxidation (LPO) has been established as a major mechanism of cellular injury in many biological systems of plant and animal origin and is measured by determining the concentration of MDA in the blood [23]. The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. It was observed that, although *Adhatoda vasica* leaf extract treatment plus irradiation did not significantly alter the LPO level in mice pectoralis muscle. However, *Adhatoda vasica* leaf extract treated mice pectoralis muscle significantly lower the radiation induced LPO in terms of malondialdehyde. Inhibition of LPO in bio membranes can be caused by antioxidants [24, 25]. The elevated levels of lipid peroxidation in irradiated mice pectoralis muscle in the present investigation were indicative of the oxidative damage caused by gamma radiation. In the present study, it was also observed that *Adhatoda vasica* extract treated plus irradiated mice pectoralis muscle exhibited a decrease in lipid peroxidation level in comparison to irradiated mice muscle. Our results confirm the earlier reports that irradiation induced cell death may be a result of accumulation in the membranes including cellular, nuclear and organelle changes.

5. CONCLUSION

The oral administration of *Adhatoda vasica* leaf extract in the present study has shown that it provides protection against free radical damage. Thus *Adhatoda vasica* leaf extract may be used as a cancer chemopreventive agent which may help enhance the cells detoxification reaction. In the present study, it was observed that *Adhatoda vasica* extract treatment reduce the gamma radiation effect on mice muscle. *Adhatoda vasica* is a chief source of many pharmacologically and medicinally important chemicals such as vasicine, vasicinone, vasicolone and other useful minor alkaloids. Therefore, further research should be conducted on these chemicals.

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Conflict of interest

The authors confirm that there is no conflict of interest.

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None

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