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Histopathological Effects of Gamma Radiation on *Adhatoda Vasica* Treated Biceps Muscle

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ABSTRACT

The objective of the present study was to evaluate *Adhatoda vasica* extract administered and radiation induced changes in the biceps muscle of Swiss Albino male mice. Animals were divided into four groups: Group I containing normal mice served as control for each experimental stage, mice of Group II received oral administration of *Adhatoda vasica* extract (900mg/kg body weight , mice of group III received oral administration of *Adhatoda* extract and were irradiated with 4Gy γ –rays, mice of group IV were irradiated with 4Gy γ-radiation. *Adhatoda* extract was administered orally to the animals (900mg/kg body weight) for a period of 28 days. After 7 days of extract administration mice were irradiated with 4 Gy γ-rays. 24 hours after irradiation mice were sacrificed by cervical dislocation on days 7, 14, 21 and 28. Excised tissue was fixed in 10% neutral formalin. Sections of 5µm thickness were stained with hematoxylin and eosin for histopathological examination. Histological changes in the mice biceps were observed between 7-28 days of investigation. Extract treated group of mice showed no alterations in the biceps muscle structure. Mice which were pretreated with extract and then irradiated showed compact arrangement of cells but with changes in shape of fibers. In the irradiated group, myonecrosis and splitting of fibers were observed. Interfibrillar spaces were enlarged. Heterogeneous population of muscle cells were seen. Irradiation causes damage to muscle tissue while Adhatoda extract minimized the damage.

KEYWORDS: *Radiation, Adhatoda vasica, Biceps, Swiss Albino Mice, Myonecrosis*

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INTRODUCTION:

During the past few decades developments in the use of radioactive materials have increased amazingly. The threat of accidental or hostile exposure to radiation is of great concern. Ionizing radiation inflicts its adverse effects through the generation of oxidative stress that unleash large-scale destruction or damage of various biomolecules. Plant products appear to have an advantage over synthetic compounds in terms of low/no toxicity at the effective dose. At present, there is hardly any aspect of human welfare in which nuclear radiation does not play an important role. Therefore preventive measures are required to protect not only humans but also for plants and animals are necessary. Plants and herb extracts have served a great deal in this regard (Jagetia, 2007). Some of the chemical components that contribute to the protective mechanisms are flavonoids, phenolic acids, xanthones, flavones etc. These polyphenolic compounds can behave both as pro oxidants and antioxidants depending on their concentration and cytosolic redox status (Girdhani *et al.*, 2005) and can hence act as radiosensitisers or radio protectors.

Recently, interest has been generated to develop the potential drugs of plant origin for the modification of radiation effects.

The herbal drugs have been used by mankind since the time immemorial to treat various ailments and offer an alternative to the synthetic compounds. The studies done on certain plants like Zingerone (Rao *et al.*, 2009), *Saraca indica* (Rao *et al.*, 2010), *Ocimum*

sanctum (Uma devi *et al.*, 1999), *Cynodon dactylon* (Rao, 2006 & 2008), *Cymbopogon citratus* (Rao *et al.*, 2009) &*Ficus racemosa* (Veerapur *et al.*, 2009) displayed reduction in the radiation induced oxidative stress and genotoxicity.

Adhatoda vasica belongs to the family of Acanthaceae. Itis an erect, terrestrial, perennial shrub.

The important active components include alkaloids vasicine and vasicinone. The former is under development as a herbal drug in India, as are the semi-synthetic derivatives of alkaloids, bromhexine and ambroxol. The plant leaf is valued for containing bronchodilator alkaloids, mainly vasicine, quinazoline, vasicinone, deoxyvasicine (Gulfraz, 2006). The crude drug from *Adhatoda vasica* is used in India as a remedy for asthma and the pure alkaloid acts as a bronchodilator (Glasby, 1978). *Adhatoda vasica* have a potent antioxidant and anti-inflammatory effect (Sarwat *et al.,* 2006). Hydroalcoholic extract of *Adhatoda vasica* results in cancer chemoprevention in mice model system (Singh *et al.,* 2010). *Adhatoda vasica* leaf extract pretreatment protects against radiation damage by inhibiting radiation induced GSH depletion, decreasing lipid per oxidation level and by increasing phosphatases activity in mice. Muscloskeletal system is the largest organ system by weight in human body comprising of more than 400 skeletal muscles. Embroyologically skeletal muscle originate from somatic mesoderm, trunk and body muscles originate from the metamerically segmented paraxial myotomes and limb muscles (biceps, triceps and gastrocnemius etc.) from the unsegmented splanchnopleure. Skeletal muscle cells are very large, multinucleated myofibres. Each muscle fiber contains several thousand myofibrils. Each myofibril (actin and myosin) are polymerized protein molecules that are responsible for actual contraction (Guyton and Hall, 2006).

Materials and methods: Present study was conducted on biceps muscle of adult sexually mature Swiss albino mice weighing between 20–30g. They were maintained in polypropylene cages under hygienic conditions with proper temperature and light (24±2˚C, 12:12 hours light dark cycle) and were allowed to acclimatize to the laboratory conditions. 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, India.

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

2-3 weeks old mice were irradiated in "Gamma Chamber-900" (BARC) with automatic timer having Cobalt 60 as the source of gamma rays.

Grouping of animals:

Normal and healthy mice were randomly divided into 4 groups of 8 animals each.

- First group i.e. designated as control: Group I containing normal mice served as control for each experimental stage.
- 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).
- Third group- i.e. designated as irradiated only: Mice of group III were maintained under identical conditions & were irradiated with γ-radiation.
- 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 γ –ray.

Dose determination of *ADHATODA VASICA* extract:

Experiment 1:

Mice were given *Adhatoda* extract orally (600 mg/kg body weight) for 7 consecutive days. One hour after last administration animals were exposed whole body to 6.0Gy gamma radiation. Only the mortality rate was studied in this group.

Experiment 2:

Mice were given *Adhatoda* extract orally (900mg/kg body weight) for 7 consecutive days and one hour after last administration. Animals were exposed whole body to 6.0Gy gamma radiation. Only the mortality rate was studied in this group.

Modification of radiation response:

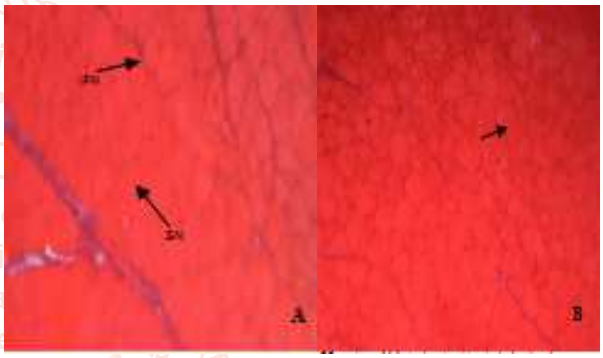
Adhatoda extract was administered orally to the animals (900mg/kg body weight) for a period of 28 days. After 7 days of extract administration mice were irradiated with 4 Gy γ-rays. 24 hours after irradiation mice were sacrificed by cervical dislocation. Similarly, after each week mice were sacrificed by cervical dislocation on days 7, 14, 21, 28. Muscle of normal, treated and radiated mice were excised, kept in refrigerator and then processed for histopathological investigation.

Histological study:

The biceps muscle were excised immediately after sacrificing the animals. Tissue was fixed in aqueous bouin's fixative for 24 hours. Tissue was then washed thoroughly in running tap water till excess of fixative got washed away. Tissues were dehydrated finally in different grades of alcohol. (30%, 50%, 70%, 90%, 100% for 30 min each) cleared in xylene (15min.) and lastly embedded in paraffin wax (58-60˚C). Thin sections (5-6µ) were cut on a rotary microtome and subjected to haematoxylin eosin staining. The permanent slides were dried in oven, examined and photographed for further studies.

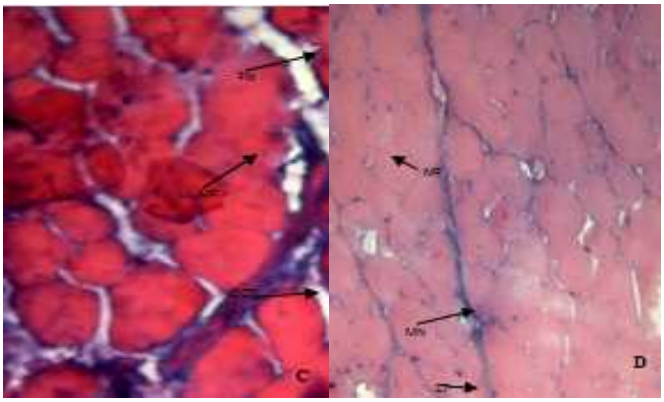
Results:

Animals treated with extract showed no effects such as sickness, body weight change, mortality and visible abnormality during experimentation. Animals exposed to radiation become lethargic, resulting in reduced food and water intake. Histological examination of control mice biceps muscle revealed circular, oval or polygonal cells with subsarcolemmal disposition of nuclei. Innumerable interfibrillar nuclei in muscle fibers were seen along with compact fiber arrangement. Animals which were extract treated also demonstrated tightly packed muscle cells. Nuclei are rounded to oval shaped. There were no visible changes in muscle sections. Mice which were irradiated with 4Gy rays at 7days stage, merged fibers were seen loosing their boundaries and increasing the connective tissue area. Some of the muscle cells demonstrate transverse breaks and fibers are likely to undergo splitting. Fiber necrosis was also visualized. After 14 days some spindle shaped muscle fibers were observed. Some of atrophied fibers were also seen in the muscle sections. Constituent muscle fibers point towards heterogeneous population of cells. Some of fibers were small in size while some of them were large. At 21days stage, some of subsarcolemmal nuclei shift their positions to interfibrillar regions. Muscle sections show different muscle fibers exhibiting necrosis. Fibers were apart from each other with large interfibrillar spaces. At 28 days stage, fibrolysis and fiber necrosis were observed. Nuclei protrude out from the muscle fibers and are arranged in interfibrillar spaces. In animals given Adhatoda extract and then irradiated, merging of muscle fibers was seen. Migration of nuclei from subsarcolemmal position to center of fiber was noticed. Interfibrillar spaces are occupied by connective tissue. At 14 days stage, nuclei shape and size changed to a large extent associated with disfigured fibers. Small nuclear streaks were observed among interfibrillar spaces. Some elongated fibers and merged fibers losing their boundaries were noticed. At 21days stage, myofibrillar degeneration can be noticed in the form of foci of degeneration. An extreme variability in fiber size among different population was observed. Fibers of various shapes like triangular, rectangular and polygonal were visible. Fibers were more appressed with each other leaving no interfibrillar spaces. Nuclear streaks among interfibrillar spaces were observed. Some Atrophied fibers were commonly seen. Individual fibers started merging.



(A) Normal biceps muscle section showing sarcolemmal disposition of nuclei(SN) and many interfibrillar nuclei (IFN) with circular shaped fibers.

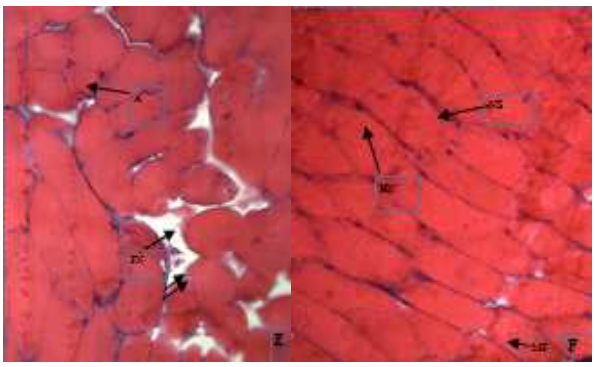
(B) Adhatoda extract treated muscle section depicting compactly arranged muscle fibers (single arrow).



(C) 7days irradiated muscle section demonstrating merged fibers (MF) and fiber necrosis (FN) and degenerating connective tissue (DCT) and splitting of fibers is observed.

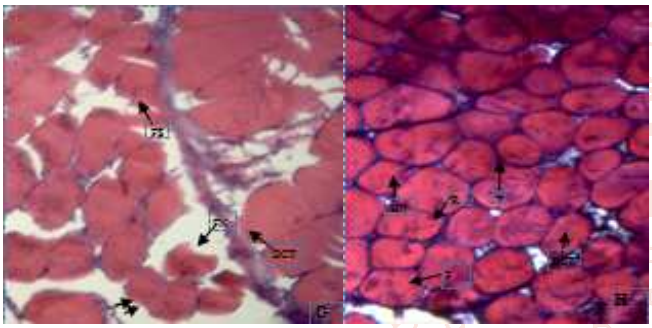
(D) 7days Adhatoda extract treated + Irradiated muscle depicting merging of fibers (MF). Thin sheet

of connective tissue (CT) is also seen. Migration of nuclei towards the centre of fiber (MF) is observed.



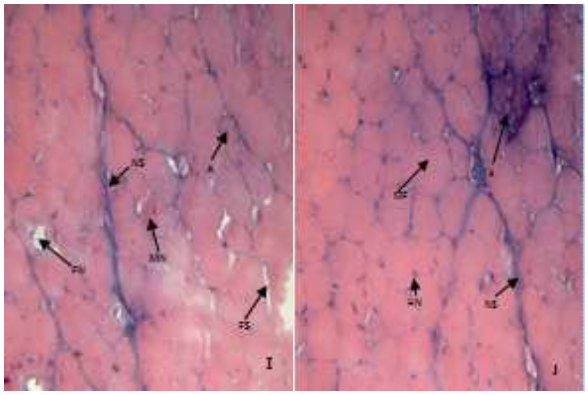
(E) 14days Irradiated muscle section showing fiber atrophy (A), merging fiber (MF) and large interafibrillar spaces (Double arrow) are observed.

(F) 14days Adhatoda extract+ Irradiated muscle section revealed hypertrophied nuclei (HN) with merging fibers (MF). Cell arrangement is compact.



(G) 21daysIrradiated muscle showing fibers apart from each other having large interfibrillar spaces (Double arrow). Splitting of fibers (FS) and Fiber necrosis (FN) are observed.

(H) 21days Adhatoda extract treated + Irradiated muscle showing hypertrophied nuclei (HN), different shapes of muscle fibers like rectangular (R), triangular (T) and polygonal (P) are clearly seen. Fibers are compactly arranged.



(I) 28 days irradiated biceps muscle showing fiber necrosis (FN), migration of nuclei (MN) from subsarcolemmal position to center of nuclei and some atrophied fibers are also seen.

(J) 28days Adhatoda extract treated + irradiated muscle showing atrophied fibers (A), merging of fibers (MF) and nuclei forming long streaks in interfibrillar spaces (NS) are observed along with some hypertrophied nuclei(HN).

Discussion:

Radiation is the most deliberated environmental hazard in the world. It exerts its deleterious effects through generation of chemically active free radicals that in turn can damage the molecular structure resulting in cellular dysfunctions or mutations (Masumura *et al.,* 2002). Radiation sensitivity of different organ and tissue is directly related to the dividing capacity of cells. The cells which replicate most rapidly are the most sensitive to radiation exposure. It is a well known fact that the demand for herbal drug treatment of various ailments is increasing and plant drugs from the Ayurvedic system are being explored more, not only in India but globally also. Herbal medicines have been used in medical practice for thousand of years and have made a great contribution to maintain mental health (Emeka and Elizabeth, 2009). Skeletal myogenesis follows an ordered set of cellular events involving cell cycle of myoblasts, their subsequent differentiation, and fusion to form multinucleated myofibers in vivo or myotubes in vitro. In most of the cases, mammalian muscle growth requires the fusion of differentiated muscle cells with growing multinucleated muscle cell(Barton Davis *et al*.,1991; Horsley *et al*.,2001; Mitchell and Pavlath, 2001).By adding additional nuclei to muscle cells during growth, an increased number of nuclei are contained within one cytoplasm, allowing each nucleus to regulate more cytoplasm(Allen *et al.,*1999).The present histopathological findings have demonstrated that gamma radiation induces anatomical changes in biceps muscle section. *Adhatoda vasica* treated muscle displayed compact arrangement of muscle fibres. Understanding the molecular pathways that regulate muscle growth are important for treating muscle disorders and loss of muscle mass during aging. However few molecules such as prostaglandins (PGs) are known to stimulate increased muscle cell fusion and skeletal muscle growth. Prostaglandins (PGs) are paracrine signaling molecules that are synthesized from arachidonic acid in response to cytokines and growth factors (Funk, 2001). The synthesis of PGs involves the metabolism of arachidonic acid by cyclooxygenase enzyme into intermediate PG and this intermediate is converted by specific synthases into primary PG molecules (PGE2, PGFα, PG12 and PGD2). *Adhatoda vasica* contains alkaloids, tannins, flavonoids, terpenes, sugars and glucosides. Flavonoids have been shown to possess various biological properties related to antioxidant, antiociceptive and anti-inflammatory mechanisms by targeting ROS and prostaglandins (Di Carto *et al.,*

1999; Hesham *et al.,* 2007). A.vasica exerts an analgesic effect (Wahid *et al.,* 2010). The data suggest that PGs regulate muscle cell growth by influencing myogenesis but extended use of drugs that inhibit PG production may be deleterious for skeletal muscle growth. A unique observation in the histopathological preparation of irradiated mice muscle is myonecrosis which is characterized by anatomical changes in nuclear morphology. Splitting of fibers and enlarged interfibrillar spaces are observed. A combination of fiber necrosis and insufficiency of regeneration of affected muscle fibres is thought to be responsible for replacement of muscle tissue with connective tissue (Stedman *et al.,* 1991). Rabbit muscles exposed to 72Kr exhibited a gradually progressing impairment of function was reached within 24 hours following irradiation and was accompanied by severe histopathological alterations (Herbert *et al.,* 1954). As the effects of gamma rays are suppressed when Adhatoda extract administered orally before irradiation therefore it can be concluded that gamma radiation induced cell death is an active process which is associated with protein. Similar reduction in number of dead cells induced by radiation was observed in skeletal muscle following extract after irradiation (Ferrer, 1992).

Conclusion:

The present studies suggested that *Adhatoda vasica* extract provided protection against radiation induced damage to muscle cells.

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