

# ANTI-ULCER ACTIVITY OF *ADHATODA VASICA* EXTRACT ON GAMMA RADIATION INDUCED GASTRIC LESIONS IN MICE

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**Abstract:** The role of radiation biology has acquired greater relevance and significance in addressing the health issues. Radiation is often used to treat malignant tumours, or in combination with surgery. Despite the advantage of radiotherapy and improvements in techniques, many patients experience moderate to severe side effects. *Adhatoda vasica*, an evergreen, gregarious, stiff and perennial shrub of family Acanthaceae has been used as herbal medicine in treating a wide variety of diseases in India. The objective of the present study was to evaluate the anti-ulcer effect of *Adhatoda vasica* extract on gamma –radiation induced gastric lesions in mice. Mice showing no signs of morbidity were divided into four groups (i) control (ii) *Adhatoda* extract treated mice (900mg/kg body weight) (iii) Irradiated mice (5gy) (iv) *Adhatoda* extract treated and irradiated mice. *Adhatoda* extract was administered orally to the animals (900mg/kg body weight) for a period of 30 days. After 10 days of extract administration mice were irradiated with 5Gy  $\gamma$ -rays. Mice were sacrificed by cervical dislocation and stomach was excised on 10, 20 and 30 days. Stomach was opened along the greater curvature. It was rinsed with water to remove gastric contents and blood clots. Stomach was then examined by a 10 $\times$ magnifier lens for the assessment of ulcers. Control group and *Adhatoda* extract treated mice stomach revealed normal and healthy gastric architecture. Irradiated group of mice showed multiple haemorrhagic spots and erosions during different stages of investigation. The mice group pretreated with extract and then irradiated showed significant ulceration inhibition providing protection to stomach.

**Keywords:** Gamma radiation, Anti-ulcer effect, Hemorrhagic spots, Ulceration inhibition.

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## 1. INTRODUCTION

In the era of nuclear energy, it is important to understand the basic mechanisms of radiation interaction with living matter. Ionizing radiations are widely used for the treatment of cancer. However, one of the limitations for using radiation is its toxic effects on normal tissues. Radiation induced damage to normal tissues can be partially reduced by the use of radioprotectors that scavenge free radicals produced during irradiation, sparing cancer tissues [1]. Natural products may be beneficial in protecting against the radiation-induced damage, as they are less toxic or practically non-toxic compared to the synthetic compounds at their optimum protective dose levels [2]. The exposure of animals to a single whole-body dose of ionizing radiation results in a complex set of symptoms whose onset, nature and severity depends on both total radiation dose and radiation quality. Radiation symptoms are mainly classified into three syndromes haematopoietic syndrome, the gastrointestinal syndrome and the central nervous system syndrome [3]. Therefore, the interest has been

increased in development of potential drug of plant origin for the modification of radiation induced effects. Recent studies have indicated that some commonly used medicinal plants may be good sources of potent but non-toxic radioprotectors such as *Panax ginseng* [4], *Ocimum* [5] and *Mentha* [6] against radiation induced alterations and mortality. *Adhatoda vasica* is a well known plant drug in Ayurvedic and Unani medicine and is well documented for its therapeutic potential. *Adhatoda vasica*, an evergreen, gregarious stiff and perennial shrub of family Acanthaceae has been used as 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 habitats and types of soil. It has been used as traditional medicine for over 2000 years. It possesses a wide spectrum of medicinal properties. The drug is employed in different forms such as fresh juice, decoction, infusion and powder; also given as alcoholic extract and liquid extract or syrup. The leaf juice is stated to be used for diarrhoea, dysentery and glandular tumor. A number of different principles including alkaloids, vasicine, vasicinone, vasinol, essential oil: betane, vitamins: vitamin C, B-carotene, a non crystalline steroid: vasakin and a mixture of fatty acids have been identified as contributing to the observed medicine effects of the plants. The powder of *A. vasica* is reported to be used as poultice on rheumatic joints as counter irritant on inflammatory swelling, on fresh wounds, urticaria and in neuralgia [7]. In vivo, investigations have revealed that 90% ethanolic extract of *A.vasica* possesses promising teratologic effects in rats [8]. *A. vasica* has antioxidant and hepatoprotective properties [9]. One of the principal mechanisms of the radiation damage is the production of free radicals. Hydroalcoholic extract of *A. vasica* modulate the phases I and II enzyme system and thus results in cancer chemoprevention in mice model system [10]. Gastric ulceration is a benign lesion on the mucosal epithelium upon exposure of the stomach to excess acid and aggressive pepsin activity. It is the most prevalent gastrointestinal disorder ever known, accounting for an estimated 15 mortality out of every 15,000 complications yearly in the world [11],[12]. The main aim of the present study was to evaluate anti ulcer effects induced by *Adhatoda vasica* leaf extract (900mg/kg body weight) on gamma radiation (5 Gy) induced lesions in mice stomach. The present investigation was carried on stomach of Adult Swiss albino mice.

## 2. MATERIAL AND METHODS

Oral dose of *Adhatoda* extract (900 mg/kg of body weight) was administered orally for 30 days. After extract administration, mice were irradiated with 5Gy gamma rays and 24 hours after irradiation were sacrificed.

Animals were divided into four groups

1. Normal
2. Extract treated mice
3. Irradiated mice
4. Extract+irradiated mice and were studied for evaluation of gastric architecture status at 10, 20 and 30 days.

### **A. Preparation of leaf extract**

Leaves of *Adhatoda vasica* were collected, washed and dried under shade for one month. Dried leaves were grinded to coarse, green coloured powder. Dried leaf powder was extracted 5 times with 80% ethanolic solution. Extraction was done after every 24 hours.

Collected suspension was concentrated under reduced pressure.

### **B. Source of irradiation**

Mice were irradiated in Gamma chamber-900 with automatic timer having Cobalt -60 as source of gamma rays. Mice were sacrificed by cervical dislocation and stomach was excised on 10, 20 and 30 days. Stomach was opened along the greater curvature. It was rinsed with water to remove gastric contents and blood clots. Stomach was then examined by a 10×magnifier lens for the assessment of ulcers.

### **C. Ulcerative index**

Stomach was opened along the greater curvature. It was rinsed with water to remove gastric contents and blood clots. Stomach was then examined by a 10×magnifier lens for the assessment of ulcers. The number of ulcers were counted. Scoring of ulcer was done as per method adopted by Dashputre and Naikwade (2011). It is followed as:

- Normal coloured stomach( 0),
- Red colouration (0.5),
- Spot ulcer (1),
- Hemorrhagic streak (1.5),
- Deep ulcers ( 2),
- Perforation ( 3).

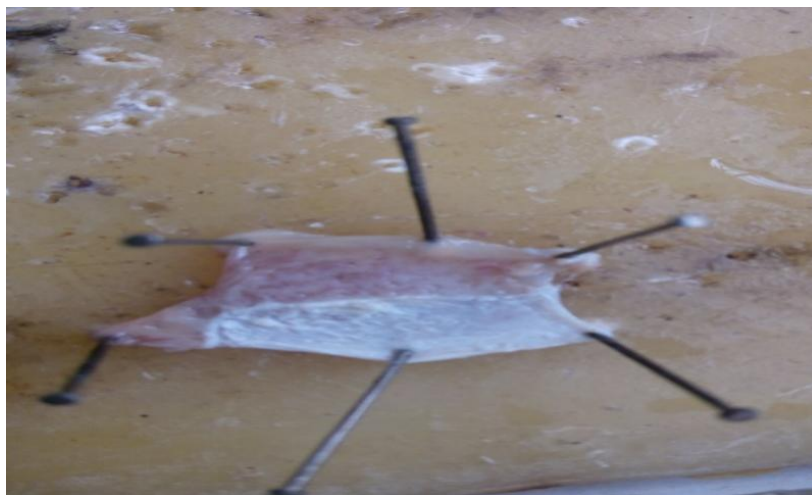
The total ulcerated area and total mucosal area were measured. The ulcer index was then calculated according to the Melese *et al.*,2011 using following equation:

Ulcer index =  $10 \times \frac{\text{total mucosal area}}{\text{ulcerated area}}$

Percentage inhibition of ulceration was calculated as below :

**% inhibition of ulceration** (ulcer index<sub>control group</sub> - ulcer index<sub>test group</sub>) × 100

**ulcer index<sub>control group</sub>**



**Stomach of control mice**



**Stomach of irradiated mice showing hemorrhagic spots and erosions.**



Stomach of extract pretreated and irradiated mice showing healing and depicting normal architecture.

Fig.1

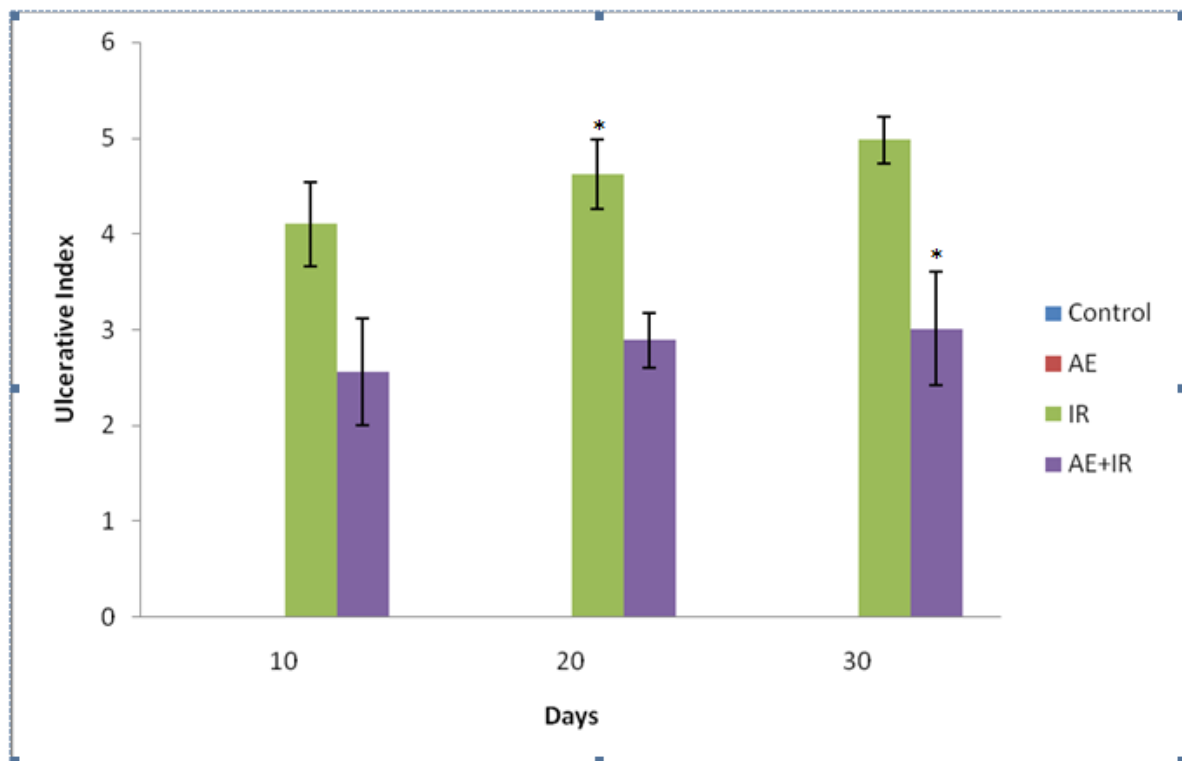


Fig. 1: Ulcerated area in mice stomach were observed at different stages of investigation (10-30 days) of experiment in control, Adhatoda Extract treated (AE), Irradiated (IR) and Adhatoda extract treated + Irradiated (AE+IR) group of mice. Values are mean  $\pm$  SD (n=6). p value : \*p < 0.05, \*\*p < 0.01.

### 3. DISCUSSION

Ulcers are defined histologically as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa into the submucosa or deeper. Ulcer occurs due to imbalance between aggressive (acid, pepsin) and defensive (mucus gastric mucosal barrier) factors of gastric mucosa. Local mechanisms implicated in mucosal defense are mucus-alkaline secretion, mucosal hydrophobicity, rapid epithelial cell renewal and rich mucosal blood flow. Ulcerative lesions of GI tract are one of major side effects associated with the use of NSAIDS, alcohol, stress and ischemic reperfusion. Gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation,

which causes damage to cell and cell membrane [13]. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms [14]. The normal mice stomach revealed normal and healthy gastric architecture throughout the period of investigation. Mucosa was healthy with no visible erosions, red spots or hemorrhagic streaks. The ulcer index was recorded to be nil at 10, 20 and 30 days of study. Similarly in the Adhatoda treated mice stomach there were no alterations seen during 30 days period of investigation. The stomach was healthy with well-preserved mucosal structure. No signs of injuries were observed. Mucosa revealed no erosions or streaks at 10, 20 and 30 days stage respectively. Ulcerative index was recorded to be nil. Irradiated group of mice stomach at 10 days stage revealed initiation of ulcers. Gastric mucosa showed the appearance of red spots and erosions at places. The ulcerative index was calculated to be  $4.10 \pm 0.44$ . Further, after 20 days the mucosa showed increased ulceration. The appearance of red spots and erosions of mucosa were more prominent. The mucosal architecture was distorted as compared to the normal and extract treated groups of mice. The ulcerative index was calculated to be  $4.62 \pm 0.36$ . At 30 days stage, mucosal injuries were increased, Spots, erosions and hemorrhagic streaks were observed. The ulcerative index was calculated to be  $4.98 \pm 0.24$ . Extract+irradiated group at 10 days stage showed nearly normal gastric architecture. The presence of spots or lesions was reduced. The ulcerative index was calculated to be  $2.56 \pm 0.56$ . After 20 days, the mucosa was found to be more healed and the ulcerative index was found to be  $2.89 \pm 0.29$ . Finally at 30 days stage, the mucosa was in healthy condition as compared to the irradiated ones. Lesions were very mild. Spots and haemorrhagic streaks were reduced to a greater extent. The ulcer index in this group was found to be  $3.01 \pm 0.59$ . Total inhibition of ulcer in this group as compared to the irradiated one was recorded to be 68% (Fig.1). Thus ulceration was found to be greatly reduced in animals which were pretreated with Adhatoda extract as compared to the irradiated ones only providing protection against gastric injury.

Irradiation led to the increase of ulcers during 10-30 days of investigation as assessed by the appearance of red spots, erosions and streaks in stomach as compared to all other groups. The normal architecture of stomach was preserved by reduction in the ulcerative index in Adhatoda extract treated and irradiated group of mice. Anti-ulcer activity of Adhatoda *vasica* was reported by Shrivastava, Vinothapooshan and Sunder [15],[16]. *Adhatoda vasica* leaf powder showed a considerable degree i.e. around 80 per cent, of anti-ulcer activity in rats. The pathogenesis of mucosal damage in the stomach includes the generation of ROS that seem to play a vital role in the formation of lipid peroxides, accompanied by impairment of anti-oxidative enzyme activity of cells [17]. Antioxidants consist of vitamins, polyphenols, flavonoids, minerals and endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase that have the capability to neutralize unstable molecules called free radicals. Vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (tocopherol) and selenium are valuable antioxidants. Antioxidants disrupt the chain reaction in which free radicals turn other molecules into free radicals like themselves, a process of chain breaking or stabilization. Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, *H.pylori* and non-steroidal anti-inflammatory agents) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). Oxidative stress-induced tissue damage with reactive oxygen species (ROS) is implicated as a cause and consequence of a variety of disorders, including coronary heart disease, neurodegenerative disorders, autoimmune pathologies, cancer, apoptosis etc [18]. Exposure of gastric mucosa to damaging factors such as ethanol, thermal stress or various irritants that are commonly named 'breakers' of gastric mucosal barrier produces pathological changes [19]. Extract of Adhatoda has significantly protected the mice stomach against irradiation induced damage as shown by reduced value of lesion index as compared to the irradiated ones only suggesting its protective effect.

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