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Effect of *Asparagus racemosus* (Liliaceae) willd on dead space wound healing

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ABSTRACT

Objective of the study: *Asparagus racemosus* is a well known plant of medicinal value, with proven antioxidant, anti-inflammatory and immunomodulatory properties. Antioxidants are known to enhance wound healing. So the present study is aimed to investigate the wound healing profile of *Asparagus racemosus* on dead space wound model. **Materials and methods:** The aqueous extract of the roots of *Asparagus racemosus* was used to study the effect on wound healing in albino rats using a dead space wound model. The extract was administered orally in the dose of 200mg/kg or 400mg/kg for 10 days. The granulation tissue breaking strength and hydroxyproline, hexosamine and hexuronic acid content of granulation tissue were measured. **Results & Discussion:** The extract showed a significant increase in granulation tissue breaking strength, and a significant increase in hydroxyproline, hexosamine and hexuronic acid in granulation tissue. The observed effects in the test groups suggest the facilitatory effect of Asparagus racemosus on wound healing and the possible utility of this plant to enhance wound healing.

Key words: *Asparagus racemosus*, anti-inflammatory, wound healing, dead space wound.

INTRODUCTION

The search for drug that enhances wound healing, other than antibiotics, has been an active area of research for some time. Identifying a drug which aids in wound healing, with minimal side effects and is economical is the primary objective of this search. *Asparagus racemosus* (AR) is an important medicinal plant in Indian medicine[1,2], with proven antioxytocic, antidiarrhoeal, antidysentric, antispasmodic, diuretic, antioxidant[3], anti-inflammatory[4]and immunomodulatory[5] properties. It has been widely used in treating the problems of female reproductive system, abdominal pains and generalized weakness in Ayurvedic medicine. It has been reported that antioxidants promote wound healing[6]. Present study is planned with the objective to investigate the effects of aqueous extract of roots of *Asparagus racemosus*, administered orally on wound healing in albino rats on dead space wound model.

MATERIALS AND METHODS

Animal care and handling: The animal care and handling was done according to the guidelines set by the Indian National Science Academy, New Delhi, India. Twelve-week-old healthy male Wistar rats, bred locally in the animal house of Kasturba Medical College Manipal, weighing 150-250g. were selected for the study. They were housed under controlled conditions of temperature (23±2 °C), humidity (50 ±5%) and 10-14hr of light and dark cycles respectively. The Animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food (animal chow supplied by Hindustan Lever Ltd.) and water *ad libitum*. The study was undertaken after obtaining the approval of institutional animal ethical committee. (Ref no: IAEC /KMC/06 / 2006-07)

Preparation of Asparagus route extract:

The Asparagus roots were collected locally, between September to January and was identified by the faculty members from the department of botany, Sri Poornaprajna College, Udupi. The roots were crushed and boiled in distilled water for two hours. The extract was dried in a water bath (yield=10%; 1 kg root = 100 gm. of extract).

Acute toxicity studies:

The extract was administered orally, one dose, to 3 groups of six rats each with the dose of 2000mg. 4000mg. 8000mg/kg. The rats were observed initially for 24 hrs for general behavior, central nervous , cardiovascular , respiratory system, gastrointestinal tract activities and thereafter daily up to 14 days.

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Treatment schedule:

Three groups (I, II& III, n=6) albino rats of either sex were used for the dead space wound models. The aqueous extract of *Asparagus racemosus* was given orally, once daily, for 10 days. The extract[AR] and the vehicle were given in the volume of 2m per animal.
I.Group acts as a control and received 2ml tap water.
II. Group received *Asparagus* extract 200 mg/kg., oral
II.III. Group received *Asparagus* extract 400mg/kg., oral

Wound models:

All wounding procedures were carried out under light ether anesthesia. In the present study no animal showed visible signs of infection.

Dead space wound:

Dead space wounds were created through a small transverse incision made on either side of the lumbar region[7]. A polypropylene tube (2.5x0.5 cm) was implanted subcutaneously beneath the dorsal paravertebral lumbar skin. The day of the wounding was considered as day zero. Granulation tissue formed on the polypropylene tube was harvested by careful dissection on day 10 and the tensile strength of the granulation tissue was measured. A piece of this tissue was preserved in 10% formaldehyde and used for histopathological examination. Another piece of granulation tissue was kept in normal saline and used for measurement of hexosamine and hexuronic acid[8]. The remaining granulation tissue was dried in an oven at 60°C overnight and the dry weight was noted. Acid hydrosylate of the dry tissue was used for the determination of the hydroxyproline content. The standardization was done for hydroxyproline using the Neumann and Logan method [9.10].

Determination of granulation tissue breaking strength [11]:

The granulation tissue harvested from the inserted polypropylene tube was cut into two pieces. Each piece of granulation tissue was secured /placed to the operation table and was held by two Babcock's tissue holding forceps. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated bottle through a string run over the pulley. Water was allowed to flow from the reservoir slowly and steadily into the bottle. As the water level rose in the graduated bottle, the increasing weight of the bottle was transmitted to the tissue site ,pulling apart the tissue edges .Water flow was arrested when the tissue shows the signs of just breaking and the volume of water collected in the bottle (approximately equal to its weight) was noted. Two readings were recorded for a given granulation tissue and the procedure was repeated on the granulation tissue on the contra lateral side. The average of four readings in one animal was taken as an individual value of breaking strength in that animal. Mean value of breaking strength of six animals gives the breaking strength for a given group.

Statistical analysis:
Statistical analysis was done by using one way analysis of variance (ANOVA) followed by Post Hoc test wherever required using SPSS package. Significance was noted at p value < 0.05.

RESULTS:
Toxicity study:

The animals did not show any toxic symptoms on the central nervous system, cardiovascular system, gastrointestinal tract or on general behavior even with the dose of 8000mg/kg. For the subsequent studies one tenth of smaller doses (i.e. 200mg / kg & 400 mg /kg) were selected for studies on wound healing.

Dead space wound model.
Breaking strength:
There was a significant ($p < 0.001$) increase in the breaking strength of granulation tissue in groups II & III when compared to the control (228.18±12.12g) in group I. (Fig.1a).

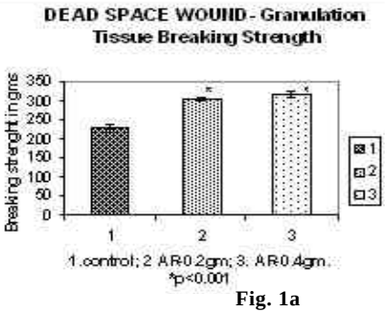

Fig. 1a

Biochemical parameters:
There was a significant increase (p,0.001) in the hydroxyproline content of granulation tissue in group II & III (28.7±1.71 & 34.19 ± 1.51) as compared to the control group I (12.43±1.11). There was no significant difference in the hydroxyproline contents in between the tissues of two doses of test drugs (Fig.1b).The hexoseamine and hexuronic acid contents (20.22± 0.78; 21.45+±0.71 & 33.18±1.16; 37.7± 1.97) in the granulation tissue in group II & III were significantly increased ($p<0.001$) when compared to the control group (11.9±0.64 & 19.19 ± 1.9). (Fig.1c&1d).

Histopathalogical study :
There was a good connective tissue response observed in group II & III which was accompanied by a decrease in inflammation. Histopathological study showed that there was a significant increase in the fibroblasts in group II & III and a significant increase in the connective tissues (Fig 2a & 2b).

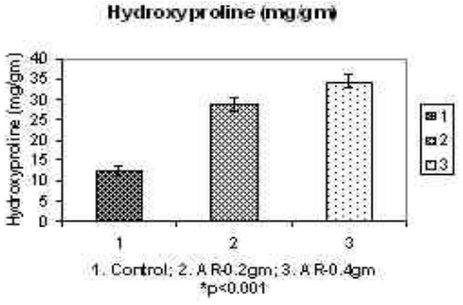

Fig. 1b

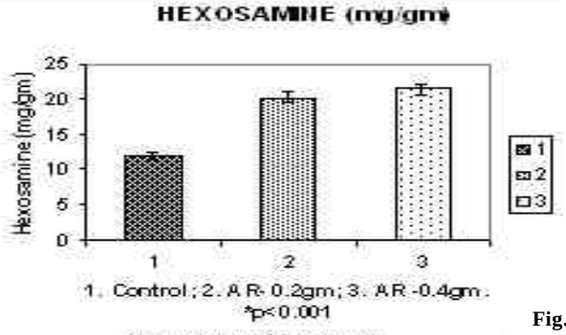

Fig. 1c

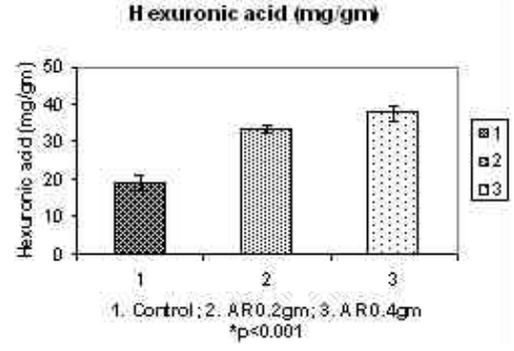

Fig. 1d

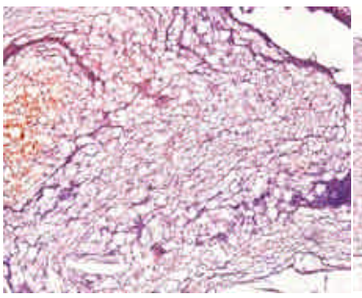

Fig. 2a Control shows normal microscopic Picture

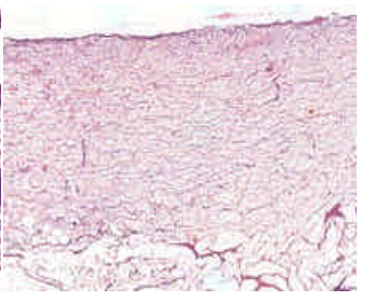

Fig. 2b AR treated group shows increased fibroblasts

DISCUSSION:
It is a well accepted fact that wounds in most tissues heal by repair, through a process of laying down nonspecific connective tissues[12]. Granulation tissue formation, wound contraction and scar formation are some of the many phases of wound healing, which run concurrently, but independently of each other. The use of a single wound model in wound healing studies is inadequate and no reference standard exists that can collectively represent the various phases of wound healing. Amidst the three different models, deadspace wound model has been used in this study to assess the effect of *Asparagus racemosus* extract on the various phases of wound healing.

In dead space wound, the increase in tensile strength of granulation tissue may be due to increase in collagen concentration and stabilization of fibers.[13] The root extracts of *Asparagus racemosus* is known to increase the levels of IL-1 and TNF which in turn stimulate the fibroblast activity and increases the collagenase activity[14] .Thus *Asparagus racemosus* not only aids in wound healing but also helps in wound remodeling. Increase in wound breaking strength (WBS) and role of antioxidants were experimentally proved by Michel and Fredrickson[15]. The faster wound healing and remodeling observed with *Asparagus racemosus* extract may be due to stimulation of interleukin -8, an inflammatory alfa –chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It is proposed to increase the gap junctional intracellular communication in cultured fibroblasts and to induce a more rapid maturation of granulation tissue [16, 17]. In our current study the aqueous extract of *Asparagus racemosus* increased the cellular proliferation and collagen synthesis at the wound site.This conclusion is supported by the observed increases in total protein, total collagen content and increase in increase in hydroxyproline content of granulation tissues. The glycosaminoglycans are a major component of the extracellular matrix of skin, joints, eyes, and many other tissues and organs. In spite of their simple structure, they demonstrate remarkable viscoelastic and hygroscopic properties which are relevant for dermal tissue function. Biological activities in skin are due to their interaction with various extracellular binding proteins. Due to an influence on signaling pathways, hyaluronic acid is involved in the wound healing process and scar less fetal healing. In clinical trials, topical application of hyaluronic acid has improved the healing of wounds [18]. In addition the mucopolysaccharide hyaluronic acid protects granulation tissues from oxygen free radical damage and thereby stimulates wound healing. [17]

Among the glycosaminoglycans, dermatan sulfate and dermatan have also been implicated in wound repair and fibrosis. Their ability to bind and alter protein – protein interactions has identified them as important determinants of cellular responsiveness in development, homeostasis and disease[19,20]. In our study, hexuronic acid and hexosamine concentrations which are the components of glycosaminoglycans were significantly increased with aqueous extract of Asparagus racemosus as compared to the control. The glycosaminglycans are known to stabilize collagen fibres by enhancing electrostatic and ionic interactions with it and possibly control their ultimate alignment and characteristic size. In our current study Asparagus extract in both the doses increased the levels of these compounds considerably. It is therefore likely that the observed increase in tensile strength was not only due to increased collagen synthesis, but also due to its interaction with glycosaminoglycans leading to its proper deposition and alignment in the extracellular matrix.

Applications:
Chronic non healing ulcers are a recurrent concerns for most of the clinicians in their clinical practice. Our study has demonstrated the beneficial effects of *Asparagus racemosus* on wound healing. This extract availability and cost makes it an useful adjunct in wound healing therapy. In addition *Asparagus racemosus* extract has proven antioxidant, anti-inflammatory, immunomodulatory properties, with minimal side effects. Therefore it could also have some use in the treatment of ulcers in diabetic patients.

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