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Wound Healing Profile of *Asparagus Racemosus (Liliaceae) Wild.*

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Abstract
Asparagus racemosus is a well known plant of medicinal value, with proved antioxidant, anti-inflammatory and immunomodulatory properties. Antioxidants are reported to enhance wound healing. The present study is aimed to investigate the wound healing profile of *Asparagus racemosus* on incision and excision wound models in rats. The aqueous extract of the roots of *Asparagus* racemosus is made use of to study the effect on wound healing in albino rats using incision and excision wound models in 200mg/kg and 400mg/kg orally for 10 to22 days. The skin breaking strength, epithelialisation period, wound contraction rate were estimated. The extract showed significant improvement in the epithelialisation period, remarkable enhancement of wound contraction rate, increased skin breaking strength in the test groups suggesting the possible utility of this plant to enhance wound healing.

Key Words
Asparagus racemosus, wound healing, incision wound, excision wound.

Introduction
The search for a drug that enhances the wound healing, other than antibiotics, is on for quite a long time. A drug which aids in wound healing, with minimal side effects and economical is the one which is always looked for. Asparagus racemosus (AR) is the important medicinal plant in Indian medicine[2], with proved antioxytocic, antidiarrhoeal, antidysentric, antispasmodic, diuretic, antioxidant[3], anti-inflammatory[4], immunomodulatory[5] properties. It is widely used in Ayurvedic medicine in treating the problems of female reproductive system, abdominal pains, and generalized weakness. It has been reported that antioxidants facilitate wound healing[6]. So the present study is planned with an objective to investigate the effect of aqueous extract of *Asparagus racemosus*, administered orally, on wound healing in albino rats on incision and excision wound models.

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 150-250 g. were selected for the study. They were housed under controlled conditions of temperature (23±2^{o}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 were identified by the faculty members of 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).

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Acute toxicity studies

The extract was administered orally, one dose, to 5 groups of six rats each in the dose of 2000mg., 4000mg., 8000mg/kg., were observed initially for 24 hrs for general behavior, central nervous system, cardiovascular system, respiratory, gastrointestinal tract activities and thereafter daily up to 14 days.

Treatment schedule: Six groups (n=6) of albino rats of either sex were taken, three groups each for incision [I,II,III] and excision wound [IV,V,VI]models. In all wound models Asparagus extract was given orally. Groups I, II, III the extract was administered orally ,daily, for 10 days, then groups in IV, V, VI the extract was given orally, daily, for the maximum period of 22 days. The extract and the vehicle were given in 2ml volume per animal.

I group received tap water

II group received Asparagus extract [AR extract] 200 mg /kg., oral

III group received Asparagus extract 400 mg /kg., (oral)

IV group received tap water.

V group received Asparagus extract 200 mg /kg., oral

VI group received Asparagus extract 400 mg/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.

Incision wound

On the depilated back of the animals, two paravertebral incisions, 6 cm in length, one on either side were made, cutting through the full thickness of the skin. Interrupted sutures at 1 cm apart, were placed to approximate the cut edges of the skin[7]. The sutures were removed on the 7^{th} post wounding day and breaking strength was measured on the 10^{th} day by continuous water flow technique of Lee [8.]

Determination of wound breaking strength

Rats were secured to the operation table and a line was drawn on either side of the wound 3mm.away from the wound. Two allice forceps were firmly applied on to the line facing each other. 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 wound site pulling apart the wound edges .Water flow was arrested when the wound was just opened and the volume of water collected in the bottle (approximately equal to its weight) was noted. Three readings were recorded for a given incision wound and the procedure was repeated on the incision wound on the contra lateral side. The average of six 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 of a given group.

Excision wound

An excision wound was inflicted by cutting away a circular piece of 500 mm^2 full thickness of skin on a pre-determined area on the depilated back of the rat. Epithelization period was noted as the number of days after wounding, required for the eschar to fall off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of the wound area on alternate days. This was achieved by tracing the wound on a $1mm^2$ graph paper on alternate days. Reduction in the wound was expressed as percentage of the original wound size[9].

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 central nervous system, cardiovascular system, gastrointestinal tract and on general behavior even in the dose of 8000mg/kg. So one tenth of smaller doses (i.e. 200mg / kg & 400 mg /kg) were selected for further studies on wound healing.

Incision wound model: The wound breaking strength in control group I was 241.03±18.73gm and groups II was 324.33±6.93gm and in group III, where animals received Asparagus extract in the dose of 400 mg/kg p.o. was 360.83±5.83gm. The wound breaking strength was significantly ($p<0.001$)

increased in group II & III as compared to the control in group I (Fig 1).There was no significant difference in the wound breaking strength in between the two doses used.

Excision wound model: In the excision wound model, the percent of wound contraction was measured on alternate days. The percent of wound contraction was nearly 100% for both doses of Asparagus extract in 16 days, while for the control it was 22 days($p<0.001$). The period of epithelization in group IV (control group) was 22.66±0.2 days. In group V (AR extracts 200 mg/ kg. p.o.), group VI (AR extract 400mg/kg. p.o.), the epithelisation period was 15.66±0.33 days and 15.33±0.4 days respectively. Epithelization period was significantly ($p<0.001$) reduced in drug treated groups as compared to the control, group IV (Fig 2a).

Percentage of wound contraction: The percentage of wound contraction in the test groups V & VI were significantly enhanced compared to the control in group IV. On the 4^{th} day the percentage of the wound contraction was ranged from 24.7±3.35 in control to 29.9±5.5 in the extract treated groups. There was no statistical significance in the percentage of wound contraction in drug treated in groups V & VI group but on the 6^{th} day there was statistically significant ($p<0.05$) difference in percentage of wound contraction between control and test groups. The wound contraction was significantly enhanced from 6^{th} day onwards and epithelisation and wound contraction was complete by 16^{th} day ($p< 0.001$) in both the test groups. (Fig. 2b).

Discussion

It is a well accepted fact that wounds in most tissues heal by repair, by laying down nonspecific connective tissues[10]. Granulation tissue formation, wound contraction and scar formation are some of the many phases of wound healing, which run concurrently, but independent 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. Hence two different models have been used in this study to assess the effect of *Asparagus racemosus* on the various phases of wound healing. In incision wound, the increase in tensile strength of skin in treated wounds may be due to increase in collagen concentration and stabilization of fibers[11]. 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[12] .Thus *Asparagus racemosus* not only aids in wound healing but also helps in wound remodeling. Increase in skin wound breaking strength (WBS) and role of antioxidants were experimentally proved by Michel and Fredrickson[13]. In excision wound, the *Asparagus racemosus* extract showed faster healing compared with control group. The faster wound contraction rate 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 may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue[14]. The aqueous extract of *Asparagus racemosus* increased the cellular proliferation and collagen synthesis at the wound site as evidenced by increase in wound breaking strength in incision wound model.

So it is concluded that *Asparagus racemosus* promote wound healing in excision and incision wound models. Chronic non healing ulcers are recurrent problems faced by most of the clinicians in their clinical practice. Since our study has proved the beneficiary effects of *Asparagus racemosus* on wound healing and it is economical, the same can be considered as adjuvant in the wound healing therapy. Since AR has proven antioxidant, immunomodulatory properties, with least known side effects, the usage of the same can be considered in preventing or in treating ulcers in diabetic patients.

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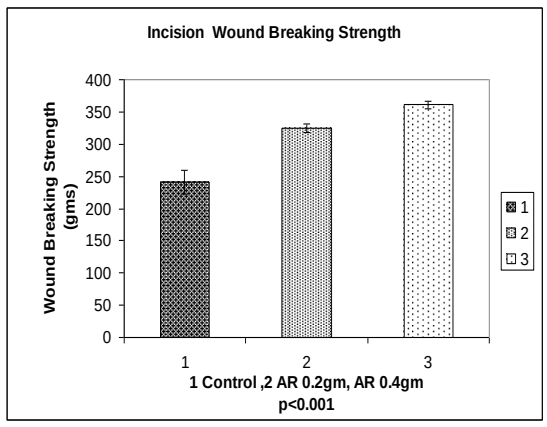

Figure 1

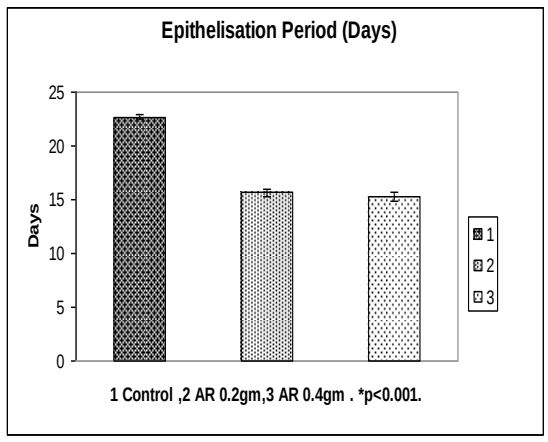

Figure 2

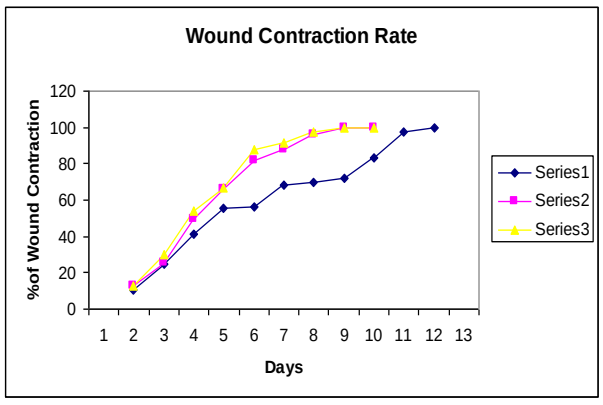

Figure 3

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