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SHORT COMMUNICATION

INFLUENCE OF ETHANOLIC EXTRACT OF *JASMINUM GRANDFLORUM* LINN FLOWER ON WOUND HEALING ACTIVITY IN RATS

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Abstract : The influence of flower extract of *Jasminum grandiflorum* was studied for its wound healing activity at a dose of 250 mg/kg body weight, using excision and dead space wound models in rats. The animals were divided into three groups in excision wound model, the controls (n=10) were treated with 0.25% CM cellulose, reference standard (n=10) were treated with sulfathiazole ointment and the experimental (n=10) were treated with extract of *J. grandiflorum* flower till complete epithelialization. The animals in dead space wound models were divided into two groups, controls were given plain drinking water and the experimental animals were administered with extract orally for 10 days. The extract treated wounds were found to epithelize faster as compared to controls. Extract-treated rats exhibited 65% reduction in the wound area when compared to controls (54%). The wet and dry granulation tissue weight, and hydroxyproline content in a dead space wound model increased significantly ($P<0.001$) when compared to controls. Histological studies of the tissue obtained on day 10 from the extract-treated group showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells when compared to controls which showed inflammatory cells, scanty collagen fibres and fibroblasts. The demonstration of increased rate of wound contraction together with the biochemical and histological findings suggest the use of J. *grandiflorum* flower extract in the management of wound healing.

Key words : *jasminum grandiflorum* hydroxyproline excision and dead space wound model wound area

INTRODUCTION

Wound healing and tissue repair are complex processes that involve a dynamic series of events including clotting, inflammation, granulation tissue formation, epithelization, collagen synthesis and tissue remodeling (1, 2). These phases run either

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concurrently or intimately inter-linked through some chemical, biochemical and cellular pathways. A treatment could influence the healing of wounds by intervening in one or many phases of wound healing. No treatment, either systemic or local, could be considered inert on the healing process unless it is proved experimentally. Wounds are defined as breach in the continuity of living tissues. Thus, humans cannot escape from an event of injury in their lifetime. Depending upon the causation, site of injury, condition of the patient, extent of trauma etc., the wounds could be minor or major. Wound care and maintenance involve a number of measures including dressing and administration of painkillers, use of anti-inflammatory agents, topical and systemic antimicrobial agents and healing promoting drugs.

Jasminum grandiflorum Linn. (Oleaceae) is commonly knows as Jasmine. It is a well-known glabrous twining shrub widely grown in gardens throughout India. Its leaves are mostly ternate or pinnate; the flowers, usually white with a tubular, five- or eight-cleft calyx, a cylindrical corolla-tube, with a spreading limb and two stamens enclosed in the corolla-tube. The flower is acrid, bitter with a sharp taste. It is useful in treating diseases of the mouth and teeth, especially for toothache (3). The *J. grandiflorum* flowers and leaves are largely used in folk medicine to prevent and treat breast cancer. Flowers of *J. grandiflorum* are useful to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding (4). It is widely used in the Aurveda, as an antiulcerative, antileprotic, skin diseases and wound healing. The extensive studies on this species for its wound healing potential are yet to be ascertained.

A large number of materials have been reported to affect the healing differentially. However, the intensive research in wound healing has not yielded, until today, a safe, economic and efficacious pro-healing agent that could obviate the long hospitalization of patients following surgeries, wounds etc. Some rural communities still apply a paste made from the dried or wet leaves and flowers of several plants including *Jasminum*. There is however, a need to study and provide evidence for the efficacy of *Jasminum* extract in the treatment of wounds.

MATERIALS AND METHODS

Plant material and extract preparation

J. grandiflorum collected locally in August, 2004 and identified by the botanist, Department of Botany, Poorna Prajna Memorial College, Karnataka, India.

The ethanolic extract of *J. grandiflorum* flowers was prepared according to the method of Hossain *et al* (5). Two hundred grams of fresh flowers of *J. grandiflorum* were dried, powdered and then soaked in 400 ml of 95% ethanol overnight. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 h and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapour at 40–50°C under reduced pressure. A semisolid (8%) light yellow material obtained was stored at 0 – 4°C.

Animals

Healthy inbred male albino rats of Wistar strain weighing 180–200 g were used for the study. They were individually housed and

maintained on normal food and water *ad libitum*. Animals were periodically weighed before and after experiments. The rats were anaesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine hydrochloride as anesthesia (120 mg/kg) body weight. Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study. An acute toxicity study was conducted for the extract by the stair-case method (6). The ethical committee clearance was obtained to carry out this work.

Phytochemical screening tests

Test for saponins: Extract (300 mg) was boiled with 5 ml water for two minutes; the mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicates the presence of saponins.

Test for tannins: To an aliquot of the extract sodium chloride was added to reach 2% strength, filtered and mixed with 1% gelatin solution. Precipitation indicates the presence of tannins.

Test for Triterpenes: Extract (300 mg) mixed with 5 ml chloroform and warmed for 30 minutes, was then treated with a small volume of concentrated sulphuric acid and mixed well. The appearance of red color indicates the presence of triterpenes.

Test for alkaloids: Extract (300 mg) was digested with 2 M HC1, and the acidic filtrate was mixed with amyl alcohol at room temperature, and the alcoholic layer was examined for a pink colour which indicates the presence of alkaloids.

Test for flavonoids: The presence of flavonoids was determined by using 1% aluminum chloride solution in methanol, concentrated HC1, magnesium turnins, and potassium hydroxide solution.

Wound healing activity

Excision and dead space wound model was used to evaluate the wound-healing activity of *J. grandiflorum*.

Excision wound model

The rats were inflicted with the excision wounds (7). The rats were anaesthetized prior to creation of the wounds, with 1 ml of intravenous ketamine hydrochloride (120 mg/kg body weight). The dorsal fur of the animal was shaved with an electric clipper, and the area of the wound to be created was outlined on the back of the animal with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area of about 300 mm^2 and 2 mm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The animals were distributed into three groups of ten each: group 1-control-treated with 0.25% CM cellulose, group 2–reference standard-treated with sulfathiazole ointment, group 3-experimental-treated with extract of *J. grandiflorum* flower (250 mg kg^{-1} day^{-1}) till complete epithelialization. The parameters studied were wound closure and epithelialization time. The measurements of the wound areas of the wound were taken on day 1, 5 and 11 post-wounding using transparent paper and a permanent marker. The recorded wound areas were measured with AutoCAD RL 14 computer analysis since it was more accurate, reliable and less time consuming.

Dead space wound model

Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg each), one on either side of the groin and axilla on the ventral surface of each rat by the technique of D'Arcy et al as described by Turner (8). The animals were distributed into two groups of 10 each. The test group rats were given flower extract orally in their drinking water at a dose of 250 mg kg^{-1} daily for 10 days. An average, rat consumes 110 ml of water/kg/day, we dissolved 250 mg of flower extract in 100 ml of drinking water. The control group animals were administered with plain drinking water. On the 10th post-wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anaesthesia. The wet weight of the granulation tissue was noted. These granulation tissues were dried at 60°C for 12 hours, weighed and recorded the dry weight. To the dried tissue 5 ml of 6N HC1 was added and kept at 110°C for 24 hours. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline (9). Additional piece of wet granulation tissue was preserved in 10% formalin for histological studies.

Estimation of Hydroxyproline

Dry granulation tissue from both control and treated group was used for the estimation of hydroxyproline. Hydroxyproline present in the neutralized acid hydrolysate were oxidized by sodium peroxide in presence of copper sulfate and subsequently they were complexed with para-dimethylaminobezaldehyde to develop a pink color which was measured at 540 nm by spectrophotometery.

Histological study

The granulation tissues were obtained on day 10 from the test and control group animals for the histological study. For the better appreciation of collagen deposition Van Geison stain was used which stain the fibres pink.

Statistical analysis

The means of wound area measurements between groups at different time intervals was compared using a one-way ANOVA, followed by Tukey's post-hoc tests. One-way ANOVA was used to examine the mean differences in wound healing between the groups in incision and dead space wound models. Data were analyzed using the SPSS (Version 12.0, Chicago, USA) and P value was set at 0.05 for all analyses.

RESULTS AND DISCUSSION

Wound healing is a process by which the damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound. It is mainly dependent upon the type and extent of damage, the general state of health and the ability of the tissue to repair. The granulation tissue of the wound is primarily composed of fibroblasts, collagen, edema, and new small blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblasts, which start migrating into the wound gap along with the fibrin strands. The collagen, composed of the amino acid, hydroxyproline, is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of the hydroxyproline could be used as an index for collagen turnover.

The LD_{50} of *J. grandiflorum* flower extract was found to be 2500 mg/kg, b.w. In the excision wound model (Table I), extract-

treated rats showed 65% reduction in the wound area when compared to controls which was 54%. The extract treated wounds were found to epithelize faster as compared to controls. This was comparable to the study done by Popova et al in which they reported the physiological regeneration and epithelialization using fractions isolated from *Calendula officinalis* (10). The wet and dry granulation tissue weight, and hydroxyproline content in a dead space wound model increased significantly when compared to controls (P<0.001). Upadhya and others noticed the similar wound healing effects with the leaf extract of *J. grandjflorum* (11). Our earlier work also reported the similar effects with *Pentas lanceolata* flower extract (12). The estimated increase in hydroxyproline content of the granulation tissue indicated rapid collagen turnover thus, leading to rapid healing of wounds (13). The above mentioned pro-healing actions of the extract may be due to the constituents present in it. Many researchers reported the similar type of pro-healing actions of constituents present in *Aloe Vera, Peperomia galioides, Anredera diffusa* and *Jatropha curcas* (14, 15). Histological studies of the tissue obtained on day 10 from the extract-treated animals showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells when compared to controls which showed inflammatory cells, scanty collagen fibres and fibroblasts.

TABLE I: Wound healing activity of *J. grandiflorum* flowers in the excision wound model.

Parameter	*Control*	*Standard*	*Experimental*
Wound area (mm^2)			
Day 1	260.40±0.160	260.60±0.22	260.60±0.22
Day 5	176.44±0.17 (32%)	171.40±0.15 (35%)	163.44±0.17 (37%)
Day 11	80.91±9.50 (54%)	59.36±11.16 (65%)	57.00±0.51** (65%)
Epitheli-alization time (days)	16.60±0.30	14.2±0.13	12.30±0.13**

n=10 *P.<0.05, **P<0.001 vs. control.
Values are mean±SE.

TABLE II: Wound healing activity of *J. grandiflorum* flowers in the dead space wound model.

Parameter	*Control*	*Experimental*
We granulation tissue weight (mg/100 g rat)	128.2±4.20	395.9±3.4**
Dry granulation tissue weight (mg/100 g rat)	30.3±0.68	60.0±1.2**
Hydroxyproline (mg/g tissue)	48.3±2.29	95.1±1.4**

n=10 *P.<0.05, **P<0.001 vs. control.
Values are mean±SE.

The *J. grandiflorum* is known to contain methyl anthranilate, indol, benzyl alcohol, benzyl acetate, and the terpenes linalol and linalyl acetate. Our preliminary phytochemical study confirmed the presence of triterpenoids. The flavonoids, (16) and triterpenoids (17) are known to promote the wound-healing process mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and increased rate of epithelialisation. The anti-inflammatory action of triterpene alcohols present in the compositae flowers have been well documented (18). Our results lend credibility to these observations. We cannot rule out the presence of other phytochemical which promote wound healing. Further phytochemical studies are suggested to isolate, characterize and identify the specific active compounds in this plant responsible for wound healing activity.

The present study has demonstrated that an ethanol extract of *J. grandiflorum* flower has properties of promoting wound healing activity compared with controls. Wound contraction and increased hydroxyproline content support the *J. grandiflorum* in the topical treatment and management of wounds.

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