Research Article

The Wound Healing Activity of (Rue) *Ruta graveolens* L. Methanolic Extract in Rats

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ABSTRACT

This study was designed to evaluation the wound healing activity of *Rutagraveolens* L. methanolic extract in rats as a medicinal plant grown in Iraq. The dried powder of the leaves (360 gm) was extracted by adding 30gm in each of the twelve flasks with 200ml of methanol. Extract was concentrated using a rotary evaporator under vacuum and then dried. Eighteen Sprague Dawley (SD) rats were used in this study; the rats were divided into three groups each composed of six male rats of almost same weight. All rats were injured at their Para vertebral, straight incision of 2 cm length was made through the entire thickness of the skin, on other side of the vertebral column with the help of a sharp. First group treated with 5% methanol extract ones daily for three days, second group received nothing but the vehicle vaseline used as a paste for the extract in the first group and considered as negative control, third group treated with sulphasalaz in which considered as positive control. The results showed that methanol extract of *R. Graveo*lens significantly healed the wound in comparison to the positive and negative controls. The wound healing that methanol extract showed may be due to the plant active constituents. The alkaloids and terpines that R. *graveo*lens contain have important roles in wound healing.

Keywords: Methanol extract, Rutagraveolens, Wounds healing.

INTRODUCTION

The main function of the skin is to work as a defensive barrier against the environment. Loss of the integrity of large portions of the skin as a consequence of injury or disease may lead to major disability or even death.[1] The chief goals of the treatment of wounds are rapid wound closure and a functional and aesthetically satisfactory scar. Recent advances in cellular and molecular biology have greatly expanded our understanding of the biologic processes involved in wound healing and tissue regeneration[2], and have led to improvements in wound care. Wound healing is a dynamic, interactive procedure involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells; Wound healing has three phases:

Inflammation

A- Instant 2-5 days. B- Hemostasis: Vasoconstriction; Platelet aggregation; Thromboplastin makes clot. C- Inflammation: Vasodilatation; Phagocytosis.[3]

Tissue formation

A-Two days to three weeks. B- Granulation: Fibroblasts lay bed of collagen. Fills defect and produces new capillaries. C- Contraction: Wound edges pull together to reduce defect. D- Epithelialization: Crosses moist surface. Cell travel about 3 cm from point of origin in all directions tissue remodeling — that overlap in time.[3]

Remodeling phase

A- Three weeks to two years. B- New collagen forms which increases tensile strength to wounds. C- Scar tissue is only 80 percent as strong as original tissue.[3]

Rutagraveolens L.

isa native to southern Europe and northern Africa. Rue medicinal plant whose roots and aerial parts contain more than one hundred and twenty combinations of different classes of natural products such as acridone alkaloids, coumarines, essential oil, flavonoids and furoqinoline.[4,5] Many of these compounds are physiologically active and therefore of pharmacological importance.[6] The medicinal action of common rue is abortificient, antihelmeintic, antiseptic, antispasmodic, carminative, irritant and stomachic.[7] The main uses of rue are to relieve gouty and rheumatic pains and to treat nervous heart problems.[8]

Rue contains 1% of an essential oil, whose main constituents are 2-hendecanone 2-undecanone, methylnonylketone, up to 60% and 2-nonanone (methyl heptylketone) plus several more ketones and corresponding secondary alcohols. Methyl anthranilate and anethole glycol are also reported; terpenoids are represented mainly by limonene, α-pinene, cuminaldehyde and l,8-cineol.[9] Externally, Rue is an active irritant, being employed as a rubefacient. If bruised and applied, the leaves will ease the severe pain of backache. The expressed juice, in small quantities, was a noted remedy for nervous nightmare, and the fresh leaves applied to the temples are said to relieve headache.

Compresses saturated with a strong decoction of the plant, when applied to the chest, have been used beneficially for chronic bronchitis.[9] If a leaf be chewed, a refreshing aromatic flavour will infiltrate the mouth, giddiness, hysterical spasm, or palpitation will be quickly relieved. The objective of this study is to identify the wound activity of methanol extract of *Rutagraveolens* L. Extracts *in vivo* on Sprague Dawly (SD) rat.

MATERIALS AND METHODS

Extraction

The leaves were collected from the medicinal plants garden of Pharmacy College of Karbala University. Leaf specimen was labelled and annotated with date of collection and locality. The plant was oven dried at 40°C. The dried leaves were separated and then ground into powder. The dried powder leaves (360gm) were extracted by adding 30gm in each of the twelve flasks with 200ml of methanol. Extract was concentrated using a rotary-evaporator under vacuum and then dried. The lyophilized extract was then kept in desiccators at room temperature prior to the experiment.[10]

Animal Experiment

Male Sprague Dawley rats with 12-14 weeks of age were used in the experiments. All the animals were allowed to free access to food and tap water. The animals were obtained from the Animal House Facility, of Pharmacy College, university of Karbala, and kept at 28-30 °C. The experiments were approved by the Animal Ethical Committee University of Karbala.[10]

Incision wound

Para vertebral straight incision of 2cm length was made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. Animals were treated daily with extract; the process has been done under anaesthesia with diethyl ether. The animals were divided into three groups each composed of six SD rat of- 230 250g weight. First group received the methanol extract, group two received the vehicle (Vaseline) inert material; it consider as a negative control, while third group received sulphasalazin as a positive control the concentration used in the experiment was 5% of methanol extract.[10]

Wound Analysis

The traced wounds were measured by planimetry using an image analysis system (digital camera), and percentage wound closure was determined by using estimation method which state that for those of no healing the results should score zero and each 20% of wound closer were scored as 1,2,3,4 and 5 respectively. Wound closure is expressed as mean ± standard deviation, the healing expressed as percentile.[10]

Statistics

Mean ± standard deviation were used to express the wound healing percentage. The data compared to each other in comparison to their potency in wound closure by paired t-Test, using Sass software package version 16.

RESULTS AND DISCUSSION

Table (1) showed the wound healing scores for the SD for the three groups group 1 (GP1) represent the rats which received the methanolic extract, group 2 (GP2) represent rats received the vehicle, group 3 (GP3) represent rats received the positive control. So the wound healing percentage for the three groups was 92%, 6% and 50% for GP1, GP2 and GP3 respectively.

Figure 1 showed the wound healing potency for the three groups of SD rats each group composed of six rats first group received methanol extract while second and third received vehicle and Sulphasalazine respectively.

Group 1 showed significant difference in comparison to group 2 and 3 $P< 0.05$ Group 2 significantly higher efficacy than group 2, but less than group 1.

Table 1: Healing scores for the rats groups, group1 (GP1) represent the rats which received the methanolic extract, group2 (GP2) represent rats received the vehicle, group3 (GP3) represent rats received the positive control.

GP1	GP2	GP3
4	0	2
4	0	2
5	0	2
5	1	3
5	1	3
5	0	3
MEAN ± SDEV	MEAN ± SDEV	MEAN ± SDEV
4.6 ± 0.5	0.3 ± 0.05	2.5 ± 0.5

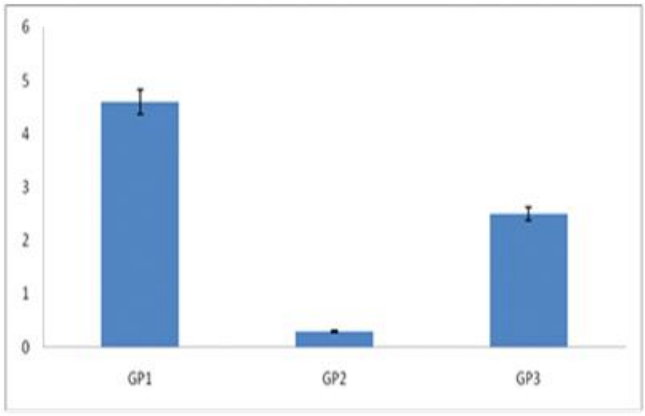

Figure 1: The wound healing potency for the rats groups

DISCUSSION

Wound healing process consists of different steps such as granulation, collagenization, collagen maturation and scar maturation which are harmonize but independent to each

other.[11] In this experiment methanol extract of R. Graveolens was used to evaluate its activity in wounds healing. This plant containing a diversity of biologically active compounds such as alkaloids, coumarins, flavonoids and essential oil.[12] Quinoline alkaloids are the main biologically active compounds in the aerial parts of plant. The methanol extract of R.graveolens showed increasing in the rate of wound healing in comparison to the negative and even positive controls used in the experiment and the reason may be due to the existence of the active constituents in the extract such as triterpene and alkaloids; these active constituents have been identified and isolated long ago by scientists.[12] The rate of epithelialization and weight of the granulation tissue (Granulation tissue formed in the final part of the proliferative phase) is primarily composed of fibroblasts, collagen, oedema and new small blood vessels. The proper reason behind the higher pharmacological activity of the extract in comparison to the positive control may be related to the synergistic activity between the active ingredients in the methanol extract, the synergism showed significantly higher efficacy.[13] Previous studies showed that triterpenoids and flavonoids are known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound healing and increased rate of epithelialisation.[13] Moreover, the anti-inflammatory activity of rue plant play important role in wound healing. This activity mediated by rutin and quercetin, both constituents of rue have been shown to decrease nitric oxide along with the reduction in nitric oxide synthase (iNOS) in vitro and quercetin has also been shown to have the same effect *in vivo.*[14] The whole plant extract has been observed to inhibit the nitrite level in lipopolysaccharide challenged murine macrophage cells and the inhibition was much more significant than with pure rutin.[14] In an endotoxin-induced inflammatory model, the methanol extract of the whole plant as well as a coumarin of rue have been shown to inhibit the expression of inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-induced macrophage cells. Reduction in cycloxygenase-2 (COX-2) gene expression was also observed.[15] Expression of IL-1β gene was significantly inhibited by the whole plant extract as well as its diethyl ether fraction and the purified compound.[15] IL-1β has been shown to induce chondrocytes to produce several types of reactive oxygen species, including H2O2 and hydroxyl and superoxide radicals.[15] The most probable mechanism of action is its potent anti-oxidant activity, this finding has agreed with other study had been conducted on antioxidant compounds showed good wound healing activity.[15] Furanocoumarins are one of the main active constituents of this plant are also reported to be potent antioxidants.[16,17] Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. Flavonoids are one of the most diverse and widespread group of natural compounds and are one of the most important natural phenolics present in R graveolens. These compounds possess a broad spectrum of activities including radical scavenging properties.[18] Antioxidants properties of flavonoids may protect tissues against oxygen free radicals and lipid peroxidation.[19,20]

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