Effects of *Achillea millefolium* L. And *Pimpinella anisum* L. Extracts on Wound Healing in rats

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Abstract: This experiment was conducted in order to compare the effects of Achillea millefolium and Pimpinella anisum Extracts on healing of wounds in rats. Twenty-four adult, male Wistar-albino rats were divided into four groups of six rats each. Group A received a Achillea millefolium extract and Group B received a Pimpinella anisum extract, Group C, as the control group, didn't receive any treatment and finally Group D received a silver sulfadiazine ointment. The results show that Achillea millefolium extract served to accelerate the wound healing process and specifically increased epithelialization in treatment groups compared to the other groups. Thus, this study demonstrates that Achillea millefolium may be effective in stimulating the enclosure of wounds. Results from our investigation suggest a positive effect of Achillea millefolium on aseptic surgical skin wound healing.
[Maryam Rezaei, Mohamad Dalvand, Forozan Hadipor, Mahbobe Pirhemat, Mohamad Alipor, Omolbanin. **Effects of *Achillea millefolium* L. And *Pimpinella anisum* L. Extracts on Wound Healing in rats.** *Rep Opinion* 2013;5(6):1-4. (ISSN: 1553-9873). http://www.sciencepub.net/report. 1

Keywords: wound healing, rat; silver sulfadiazine; Achillea millefolium; Pimpinella anisum.

1. Introduction

The healing process occurs in 3 overlapping but distinct stages: inflammation, new tissue

formation, and remodeling [Gurtner et al., 2008]. During wound healing, fibroblasts produce extracellular matrix, predominantly in the form of collagen [Werner et al., 2007].

Infection following traumatic injuries is a common problem that can occur further hinder wound healing. Infection may delay the healing process, as well as cause other complications [Edwards et al., 2004]. Therefore, current treatment options for infected wounds often require therapeutic approaches that both enhance regeneration and limit infection [Jiang et al., 2012].

Achillea millefolium (Asteraceae) is native to Europe and Western Asia and widely naturalized in temperate regions. It grows vigorously and is often considered a troublesome weed (Armitage, 1987).

Pimpinella anisum is a flowering plant in the family Apiaceae, native to the India and southwest

Asia. It is a herbaceous annual plant growing to 1m tall (Kosalec et al., 2005).

Silver sulfadiazine (SSD) is the topical agent of choice in Wound Healing and is used almost universally today in preference to compounds such as silver nitrate and mafenide acetate. SSD cream, while being effective, causes some systemic complications including neutropenia, erythema multiforme, crystalluria and methemoglobinemia (Gracia, 2001; Gregory et al., 2002; Hosnuter et al., 2004).

The use of Achillea millefolium And Pimpinella anisum Extracts has a long tradition in the Lorestan folk medicine. However, the effect of these herbs to improve skin wound healing has never been experimentally verified.

Several studies showed a antiseptic, antimicrobial, antioxidant, sedative, hepatoprotective, and anti-inflammatory effects of the Achillea millefolium And Pimpinella anisum(Armitage,1987; Kosalec et al., 2005). Alternatively, less is known about the role of these in dermal wounds. Hence, this study was undertaken to evaluate the wound healing activity of the ethanol extracts of Achillea millefolium And Pimpinella anisum in experimentally-induced wound in rats.

2. Materials and methods

2.1. Preparation of the plant extract

100 gm of powdered seeds (dry) was soaked in 250 ml of aqueous alcoholic solution (95%) for 24 hrs followed by cold maceration for further 48 hrs with occasional shaking. The mixture was filtered using muslin cloth followed by removal of excess of solvent by means of Rotatory evaporator.

2.2. Animals

In this study, a total of 24 male Sprague-Dawley rats (weight 200-220 g) were used and they were randomly divided into four experimental groups of 6 rats each.

The Rats were obtained from the Pastor Institute, Tehran, Iran and kept in animal house in

standard conditions. The animals were acclimatized to the laboratory conditions for one week prior to the onset of experiment. They were provided food and water ad libitum during the whole period of the experiment.

2.3. Excision wound model

The dorsal skin of the rats was shaved. An area of about 200 mm2 was defined with marker on the depilated back of the rat, in the dorsal interscapular region, 1cm away from the ears of the animals. The circular marked area was excised with full thickness using a surgical sterile blade and scissors under Ketamine anesthesia [Goodson et al., 1977; Church et al., 2000].

Wounds were left undressed to the open environment and the animals were kept individually in separate cages. After the making of surgical wounds, all rats were randomly coloured with a non-toxic colour and divided into four groups. Group A received a Achillea millefolium extract and Group B received a Pimpinella anisum extract, Group C, as the control group, didn't receive any treatment and finally Group D received a silver sulfadiazine ointment. All rats were monitored daily for 21 days and any wound fluid or evidence of infection or other abnormalities were noted. Percentage of wound healing was computed at the beginning of experiments and the next 2, 4, 6, 8, 10, 12 ,14 and 16 days.

2.4. Measurement of wound area

The progressive changes in wound area were measured in mm at every 2 days interval. Progressive decrease in the wound size was monitored periodically. The evaluated surface area was then employed to calculate the percentage of wound contraction by using the following equation:

$$= \frac{\text{Initial wound size - specific day wound size}}{\text{Initial wound size}} \times 100$$

All the wounds were digitally photographed in the presence of a standard reference ruler.

The wound area was measured immediately by placing a transparent tracing paper over the wound and tracing it. The tracing paper was placed on a 1 mm^2 graph sheet and traced accordingly. The squares were counted and the area was recorded. The wound area was assessed by the same blinded observer.

Using this excisional wounding method, the epidermal, dermal and hypodermal layers were removed completely. The study was approved by the ethics committee for animal experiments.

2.5. Statistical analysis

The relative wound area results were compared using one- way analysis of variance (ANOVA). P values less than 0.05 were considered statistically significant.

3. Results and discussion

There are significant differences between groups. Average the wounds treated with silver sulfadiazine and Achillea extract significance level of 0.001, the difference was not statistically, But in others it groups at a significant level, the difference was significant.

There was a significant reduction in the wound surface area of the treated lesions on days 15 and 17 compared to those of the untreated ones.

The four graphs draw for six different times, the average general is the minimum amount, For the group treated with silver sulfadiazine, extract, Pimpinella and control (Figure 1).

The most reduction of the three groups belong to the control group (Figure 2).

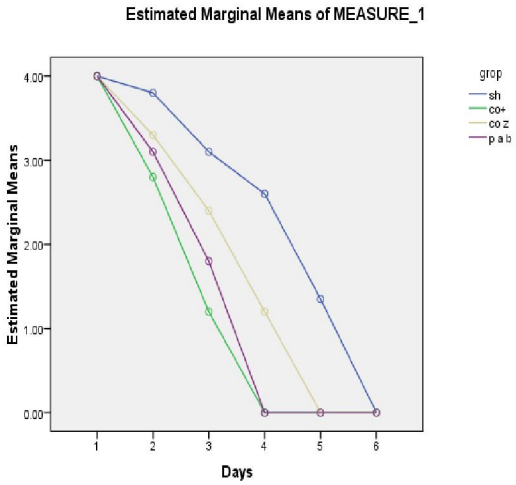

Fig. 1. The mean lesion length in the control group, positive control or silver sulfadiazine, Pimpinella and Achillea extracts in various days after wounding.

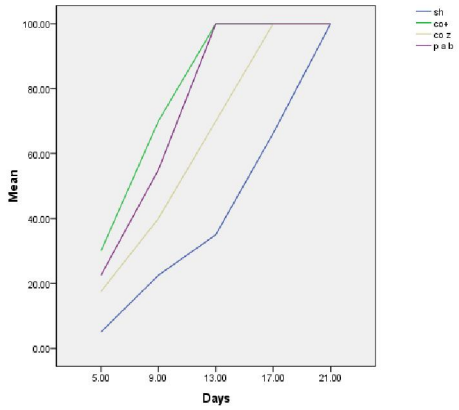

Fig. 2. Percentage of wound healing in the control group, positive control or silver sulfadiazine, Pimpinella and Achillea extracts in various days after wounding.

Wound healing, or wound repair, is the body's natural process of regenerating dermal and epidermal tissue. The sequence of events that repairs the damage is categorized into separate inflammatory, proliferative and maturation phases. There are numerous reports of positive effects of different medicinal herbs published in literature (Biswass et al., 2003). in his study is looking toward the Ayurveda, the Indian traditional system of medicine. It reports about 130 medicinal herbs that have positive influence on wound healing.

Achillea millefolium L. is a widely distributed medicinal plant that has been used for over 3000 years (Mitich L, 1990). Popular indications of this species include treatment of wounds, hemorrhages headaches, inflammation, pain, spasmodic diseases, flatulence and dyspepsia

(Correia P, 1974; Chandler R, 1982; Blumenthal M, 2000). Some of these reputed traditional effects have been determined showing the potential medicinal use of the plant. The medicinal properties of Achillea millefolium are worldwide recognized and the plant is included in the national Pharmacopoeias of countries such as Germany, Czech Republic, France and Switzerland (Bradley P, 1992; Alonso J, 1998).

Recently, the spasmolytic activity of the flavonoids and topical anti-inflammatory activity of the sesquiterpenes have been already shown being caused by inhibition of the arachidonic acid metabolism (Kastner U, 1993). B Benedek (2007) showed anti-inflammatory action of crude plant extract, flavonoid and dicaffeoylquinic acids fraction of Achillea millefolium (Benedek B, 2007). The late phase seems to be an inflammatory pain due to the inflammatory response that can be inhibited by topical anti-inflammatory activity of the flavonoids.

As a medicinal plant, Pimpinella anisum has been used as a stimulating effect of digestion and antiparasitic, antifungal (Soliman and Badea, 2002) and antipyretic (Afifi et.al.,1994). Additionally, the plant and especially its fruit essential oil have been used for treatment of some disease including seizures and epilepsy .

Furthermore, it has been shown to have anticonvulsant effects and has been used for the treatment of constipation and possesses muscle relaxant effect. Recently its oil has been reported to be used as antibiotic substitute in broiler ration (Chicouri and Chicouri, 2000).

Acknowledgements:

The cooperation and support of the Herbal Medicine Research Center, Lorestan University of Medical Sciences and all the participants who helped we in conducting this research are highly appreciated.

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5/12/2013