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Preparation of Anti-Inflammatory Ointments and Comparing Them with Existing Standards in the Market

Mansour Dolatabadi*, Rahmatollah Tavakoli

Department of Medical Engineering, Faculty of Engineering, Maziar Royan Nonprofit Institute, Royan Noor Iran.

***Corresponding Author**

Abstract: *Introduction: Previous studies had obviously indicated the adverse effects of steroid ointments on the skin whose unpleasant effects is inevitable. Recent studies have specifically paid attention to herbal medicine, since they have less complications and are cost-effective in comparison to chemical medicine. Methodology: The studied population included 50 mice, which were randomly divided into 5 groups including: 1-negative control group, 2-positive control group, 3- first experimental group, 4- second experimental group, 5- third experimental group.vChemical extracts and ointments were utilized in a specific range and measuring the level of inflammation as well as its improvement percentage was done using statistical analysis test.vResults: According to the obtained results of the current study, inflammation levels on the control groups showed significant differences towards each other, which had a similar effect to that of hydrocortisone ointment. Conclusion: The efficacy of five herbal medicine`s extract was similar or even better than hydrocortisone chemical ointment in reducing the inflammation of the treated group using our 5% extract of herbal medicine. Moreover, considering its effects on the inflammation, it had less side effects.*

Keywords: *Inflammation, Anti-Inflammation, Test, Pain, Wound, Skin, Eczema*

INTRODUCTION

Damage to the skin and the need for skin repair in order to preserve life have expanded the use and development of skin repair products.

The analysis of these studies demonstrates the continued complementarity of these studies. Of course, the process of skin regeneration involves the co-operation of several cell lines, such as blood cells (plackets, macrophages, neutrophils and lymphocytes), ectoderm (keratinocytes), and mesoderm cells (fibroblasts). All growth factors and secretory matrix markers involved in the natural stages of injury repair such as inflammation, growth and proliferation, migration, matrix synthesis, maturation and deformity respond to them to maintain the function and beauty of the skin (Singer and Simon, 2006).

Methods

After preparation of medicinal plants including (horsetail + quinine + ginger + sycamore + almond) and milling each of these herbs, 30 cc distilled water and 70 cc 96 % ethanol were added to 5 g of concentrated extract. The solutions were stirred for 3 hours at room temperature (24 ° C) on a magnetic stirrer (Shaker) and then centrifuged for 20 minutes at 4000 rpm with a rotary evaporator. Ethanol was extracted from these solutions and finally the extract was concentrated in two concentrations of 1% and 5%.
Each of the laboratory specimens (rats) was anesthetized with an anesthetic consisted of xylazine, ketamine, and eluting serum (sodium chloride) in an intraperitoneal injection of 0.3 ml by means of a syringe. The back of the animal was shaved using hair extinguishing cream, so the area was completely bare. Then the animal's skin was rinsed and disinfected with ethanol then using a forceps and sterile gas and cotton 50% NaOH was used as an inflammatory agent on the skin of the animal for 1 to 2 minutes in complete anesthesia. This caused severe inflammation on the back of our lab specimens. Preparation of 1 mM caustic soda with 50% concentration is prepared based on the following formula.

$$0.5 \text{ mol / lit} \times (40 \text{ gr}) / (1 \text{ mol (NaOH)}) \times 0.3 \text{ lit} = 6 \text{ gr}$$

At the moment of inflammation, which was also calculated as the first day of treatment, all samples were photographed on a smooth surface using a digital camera. A file is considered from start to end of the study for each different sample and group. All photographs of the specimens are kept in their own numbered animal file. Pictures of the inflammation were taken from the back of each animal on days 1, 4, 8, 12, ... and 34 and by calculating the level of inflammation and the percentage of recovery of these surfaces on the days of photography and measuring the length and the widths of these surfaces using the Digimaizer software, the inflammatory area of the back of the animal was calculated. Using the formula for calculating the area of inflammation as well as the formula for calculating the percentage of wound healing in below, the amount of inflammation and their recovery were measured on the photography days.

$$\text{The area of inflammation leading to wound at the day of X \%} = (\text{The area of wound on day } x * 100) / (\text{The area of wound on day zero})$$

The numbers obtained from the calculation of the percentage of wound area of each individual picture were put in the formula of recovery percentage as follows.

$$\text{Inflammation healing on day X} = 100 - \text{Percentage of wound area on day X}$$

Animal Classification

Experimental samples were divided into 5 groups, which are presented in the table below and treatment duration for these groups was 34 days. This study was also an experimental study.

Table 1: Animal Classification Table

Group 1	Negative control group	Including 10 rats. No treatment or ointment was used in this group.
Group 2	Positive control group	Including 10 rats. In this group, Eucerin was used to keep the site of inflammation and scars moist, and to treat this inflammation and scars 2 times a day at specified times during the day, Eucerin was used as a treatment.
Group 3	First experimental group	Including 10 rats. In this group 2 times daily and at specific hours hydrocortisone acetate ointment was used as treatment.
Group 4	Second experimental group	Including 10 rats. In this group 2 times daily and at specific hours 5% extract obtained from 5 medical herbs in our study was used as treatment.
Group 5	Third experimental group	Including 10 rats. In this group 2 times daily and at specific hours 10% extract obtained from 5 medical herbs in our study was used as treatment.

Results

In order to evaluate the wound healing process, the length and width of the inflammation leading to wound were determined on the determined days. The area of these inflammations and the percentage of wound healing in different groups were compared on the basis of statistical comparison using statistical software. The results of the tests and the wound removal were reported as mean mm^2. Finally, the relevant histograms were plotted using Minitab software.

The degree of controlling the healing area of inflammation leading to wound

The mean recovery time of 5% Eucerin ointment compared to the control group in terms of treatment days and comparing it at the 95% confidence level (Chart 1) indicated that there was a significant difference between the mean recovery rate in the treatment and control groups. Wound healing has improved over time as it can be seen in the chart. Given the extent of the recovery level on day 1, this level has been shown 70 mm^2. The recovery area was increasing over the photography days, and the wound was completely closed during the healing process and no effect of colloid and scar were not seen on the back of animals.

According to the table below, we might admit that the 5% ointment of our herbal extracts in this study performed at a level higher than the hydrocortisone ointment and the long-term use of five herbal extracts to heal inflammation leading to skin ulcers in experimental groups showed a significant increase in the recovery percentage (Chart 1).

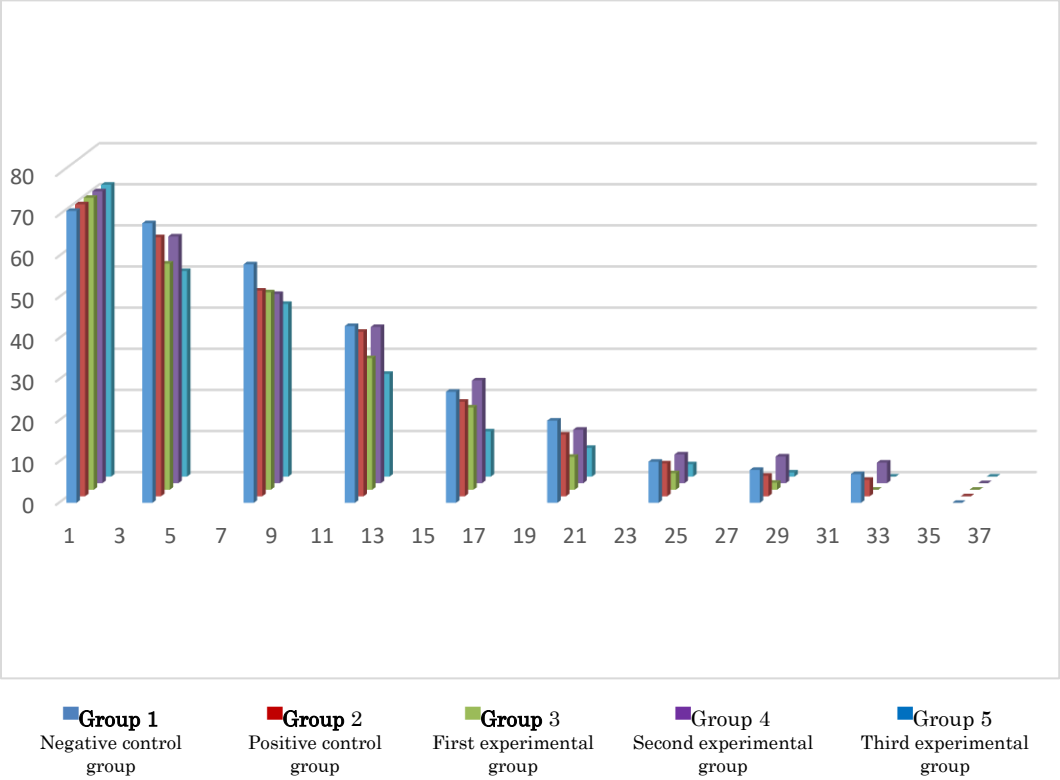

Chart 1: Improvement Level Control.

Control of the percentage of inflammation healing leading to wound

According to the photos taken from animals on specific days of treatment (1st, 4th, 8th, 12th, etc.) and the calculation of the percentage of recovery of all control groups using the corresponding formula stated in the preceding section, according to the (Chart 2), the percentage of healing in each of these groups is comparable.

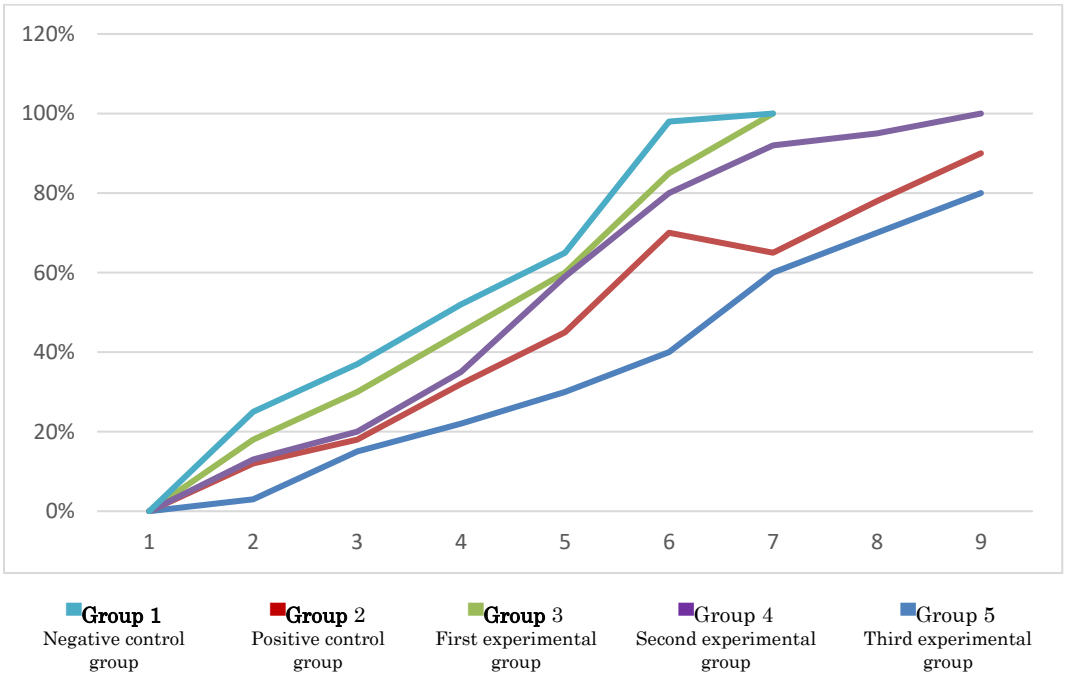

Chart 2. Percentage of recovery during the photography days

The effect of extracts

Given the duration of experiments on animals, the effect of the extracts made and their comparison with the hydrocortisone ointment compared to the day of inflammation-induced wound healing was obtained and is illustrated in Chart 3. The 5% recovery rate of the ointment 5 from the 5% extract of the medicinal plants in our study was the highest compared to other extracts, and it could be claimed that it was higher than ointment 3 which was hydrocortisone.

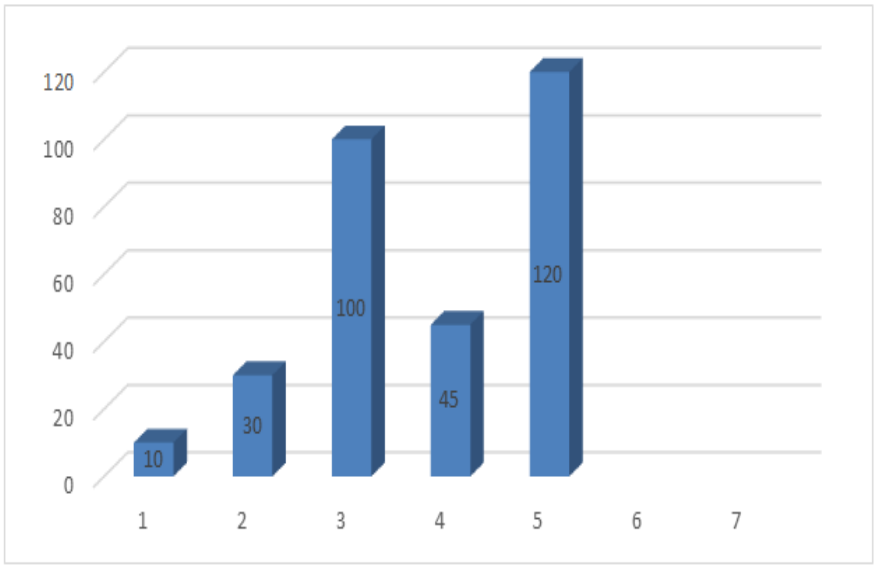

Chart 3: Comparison of the effect of ointments in terms of the wound healing day

1. Negative control group

2. Positive control group
3- Hydrocortisone ointment group
4- 1% ointment group
5- 5% ointment group

According to Chart (3), the ointment made from 5% extract in the treated experimental group performed similarly to hydrocortisone, while in the 1% group all the aforementioned events were delayed compared to the other two groups. However, no significant changes were observed in terms of wound healing in the Eucerin received group (positive control group) as they were treated only with the Eucerin ointment to keep the wound moist. It should be noted that keeping the wound moist and greasy is not enough for wound recovery.

Discussion

Horsetail is an effective drug for the treatment of inflammatory wounds due to its free silica. Contemporary American herbal medicine uses horsetail for healing the external wounds (Khorasani, 1982). In fact, the horsetail can rapidly heal cutaneous wounds by reducing the dermatitis and inflammation caused by surgical wounds and a variety of chemical mediators affecting inflammation, strengthening the cellular immune system, inhibiting cellular and bacterial enzymes and mediators (Cowan, 1999). Also quinine bark used in this research has many therapeutic properties. The active substances in quinine bark contain alkaloid quinoline (15% -5%) such as quinine, cinchonine , Saponins Triterpenoid . Quinine has been registered as an effective medicinal plant in all prestigious pharmacies worldwide.

Quinidine has inhibitory effect on sodium channels and is used as an antiarrhythmic agent . Ginger is also one of the herbs used in this study, especially in Iran, which is introduced as an anti-inflammatory herbal medicine in ancient Iran (Phan et al., 2005). The root of this plant, called rhizome, is edible and used as a spice. It is one of the most experienced medicinal herbs in medical science, especially in the treatment of inflammation in arthritis (Surh et al., 1999). Despite numerous reports of the antitumor effects of this plant, it has been reported that most of these effects are through inflammatory-reducing mechanisms. (Grzanna et al., 2004) Also, many researches have shown that at the cellular level, the herbal extract is capable of modulating inflammation exacerbating immune responses (Tripathi et al., 2008; Ojewole, 2006).

Regarding the anti-inflammatory effects of this plant, numerous reports have suggested that the active ingredients of this plant, such as gingerol, shogaol and curcumin, are well capable of inhibiting the production of prostaglandines, nitric oxide and eveni involved in inflammation (Shimoda et al., 2010).

In addition to the mentioned items, enzymes producing these inflammatory mediators are specifically inhibited by the active ingredients of ginger (Gupta and Sharma, 2001). The antioxidant effects of ginger have been proven, also it is used in medicine, spices and delicacies worldwide (Bhattarai and Duke, 2001). Bitter almonds also have antioxidant activity, and sweet almonds are used as softening, moisturizing and cleansing products for dry skin (Herbal medicine brochure). In external use, crushed almonds with a little water added could be helpful in relieving nerve pain, and rheumatic pains. Its emulsion has a decisive effect on the elimination of cutaneous itching. The other plant used in this study was Russian olive. Russian olive trees and shrubs that are often thorny and consisted of three genera and more than 20 species. Russian olive tree is scientifically named Elaeagnus Angustifolia (Speroni et al., 2002). It is used as a medicinal plant worldwide. The anti-inflammatory (Gertsch et al., 2004) and cytotoxic effects of Russian olive on the malignant cells (Goncharova and Glushenkova, 1990) are of the therapeutic effects of this plant. This plant has positive anti-inflammatory effects (Hengge et al., 2006). Russian olive has anti-hemorrhagic effects due to vitamin K. Russian olive contains compounds such as phytochemicals which are good antioxidants. Russian olive cooked in olive oil relieves joint pain and itching of the skin and is helpful for hair growth. Also extract of Russian olive can treat arthritis and inflammation of the joints.

Conclusion

The extracts used in this study to improve the quality and speed of the healing of the wound caused by inflammatory (caustic soda) chemicals on animal specimens not only accelerated the healing of wounds so that at the end of the work not only the wound on the back of the specimen healed well but no evidence of colloid and scar was observed in any of the specimens. Therefore, these herbal extracts can be used at different concentrations to improve the surface of the skin with inflammation and ulcers. Finally, it is worth noting that the results of this study are derived from laboratory work and further research is needed.

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