

**STUDY OF THE HEALING EFFECTS OF ALCOHOLIC FRACTIONS OF ROSA DAMASCENA ON THE EPITHELIALIZATION PROCESS OF THE NEW ZEALAND RABBIT'S EAR WOUNDS**

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**Summary**

The extracts and Fractions of Rosa Damascena are among the effective, herbal, and natural products which are currently used as treatment in different countries around the world. The present study aims to investigate the healing effects of Alcoholic Fractions of Rosa Damascena on the epithelialization process of the New Zealand rabbits' ear wounds.

This study was done on 6 New Zealand rabbits. wounds with 4 millimeter diameter were made in some areas of the left and right ears of the animals .In the test groups, N-Butanol(NB) and Ethyl Acetate(EA) which had a 5% density and in the control group, Normal Saline were used. Each of these compounds were poured on the wounds twice daily. After wounding, the percentage of the wound recovery in all the three groups and the speed of epithelialization were investigated on the day0, 5<sup>th</sup> day, 10<sup>th</sup> day ,15<sup>th</sup> day and 20<sup>th</sup> day .

The results of the study show that the effect of the Alcoholic Fractions of Rosa Damascena on the epithelialization process and on the speed of wound recovery in the treatment groups was not significantly different from the effect of the compounds used in the group (  $p \geq 0.05$ ). The statistical analysis (until the 20<sup>th</sup> day) shows that based on the measuring of the wound surface in the three groups, the percentage of recovery at the 5<sup>th</sup> day (for instance, in the Distal situation) in the control group was 45%, EA fraction was 42% and NB fraction was 50% but these percentages increased respectively to 87%, 80% and 84%.

The present study shows that the effect of the Alcoholic Fractions of Rosa Damascena on the epithelialization process and on the recovery of wounds was not as much as we expected. Thus, for explaining the effects of the fractions, it is suggested that the procedure and the dose of using these compounds be investigated.

**Key words:** Alcoholic Fraction, Wound Healing, Rabbit, Rosa Damascena

**Introduction**

A wound is an injury to the skin, mucous membrane or any surface of the body which may be caused as a result of physical, chemical or biological factors(1). Over the years, physicians from Egypt, India, Greece, and Europe have been trying to cure wounds immediately and with the least amount of complications(2).

Today in Iran, antiseptic liquids such as Betadine, Acetic acid, physiologic serum and etc are used in order to cure wounds. In chronic wounds and in some special conditions, biologic dressings such as skin autograft are used but recent studies have shown that such liquids are poisonous for fibroblast, lymphocytes and for cells which are necessary for the treatment of wound(3). So far, the effects of herbal drugs on wound recovery have been the subject of many studies. For example, A review of the effect of *Rhazya Stricta* (4); *Aloe vera*; (5, 6), *Centella Asiatic* (7, 8), The effect of honey (9), The effect of mummy (10). Recently, the therapeutic effects of *Rosa Damascena* have been widely studied (11, 12). The present study is an attempt to investigate the healing effects of alcoholic fractions- N-butanol (NB) and Ethyl acetate (EA) on the process of epithelialization of the skin wounds. To do this, an animal model is used in this study .

### **Methods and Materials**

**Animals:** this research was conducted on 6 New Zealand bucks which had a weight range between 2-2.5 kg. They were taken from Razi Vaccine and Serum Research Institute. The animals were kept individually in special cages in the Animal House of the Faculty of Medicine of Mashhad and natural light cycle and standard food and water were provided for them.

**Wounding:** in order to make wounds, punching method was used. First, the animal was put in the Restiner apparatus so that the animal couldn't move at all. Then, the target areas in the auricle of bucks' ear were completely sterilized by alcohol. Hairs of these areas was shaved . In order to anesthetize the ears, the common veterinary anesthetic (Lidocaine 10 % spray) was used. Having assured that the ears were anesthetized, we choose the positions of the holes in 5 areas. For that purpose, we considered the blood vessel system in the auricle of the ears within the central artery and circumferential veins and then punching was performed. All the holes were circular and had a 4mm diameter. Thus, there were 60 positions for sampling .

The animals were divided into three groups:

1- Control group( n=20): In this group, after wounding ,from the day 0 to the day 21 ,twice daily (both in the morning and afternoon of every day) Normal Saline was poured on the wound .

2- Treated group with Alcoholic Fraction NB (n=20): In this group after wounding, from the day 0 to the day 21 ,twice daily (8am and 4pm of every day), 3 drops of Fraction were poured on the wound with a dropper.

3-Treated group with Alcoholic Fraction EA (n=20): In this group after wounding, from the day0 to the 21<sup>st</sup> day, twice daily (8am and 4pm of every day)3drops of Fraction were poured on the wound with a dropper.

**Procedure for making alcoholic and watery Fractions NB & EA:**

We put 200 g of *Rosa Damascena* in air in order to be dried. After pulverizing the petals of this flower, extracting was done by Soxhlet apparatus. Finally, after removing the solvent, we had 28 g alcoholic extract. 5 g of this extract was mixed with distilled water and then with 50cc Ethyl acetate. This mixture was then poured in the Decanter funnel and finally after making it transparent and removing solvent of it, 3.6 g alcoholic fraction Ethyl acetate was produced. This amount of alcoholic extract (5 g) was used for making Fraction N Butanol and finally after the similar stages, 2.3 g alcoholic fraction N Butanol was produced. Each fraction was diluted and was used with a 5% density.

**Analysis of the Wound Phenetic:**

In order to have an accurate assessment, the area of the samples both in the test and control groups were calculated by using a lab millimeter paper and this was done in time intervals- from the first punching to the 50<sup>th</sup> day .Macroscopic images were also taken at fixed time intervals. During the treatment ,the clinical situation of the wound was microscopically and macroscopically investigated in order to consider the possible inflammatory and infectious changes occurred in the treatment group in comparison with the control group.

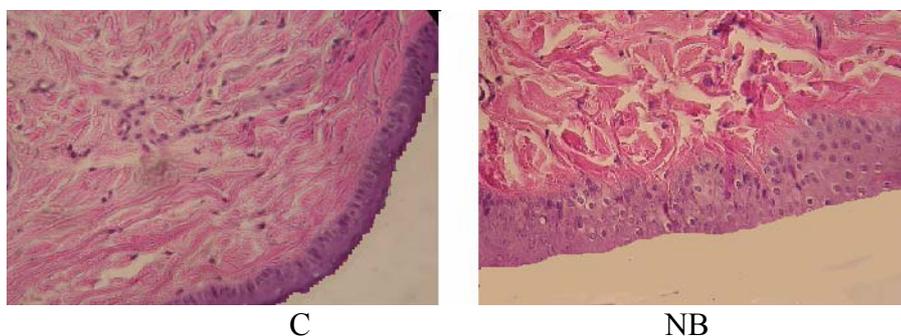
**Histological techniques:** In order to microscopically investigate the regeneration of the wounds in the bucks' ears ,in some stages (from the zero day to the 50<sup>th</sup> after punching) we did resampling from the areas which were regenerating and we provided some microscopic sections. After anesthetizing the area of wound, in order to take sampling, we removed the whole thickness of the wound by the use of a punch which had a 6 mm diameter. After washing that in the liquid of physiologic serum, we transferred that separately to the containers of Fixtor Formalin 10 % to be stabilized .After stabilizing all the samples, the tissue sections were found and were stained by Hematoxilin & Eosin. Two factors were in mind while doing the histologic study including: 1. the amount of epithelialization and wound regeneration and 2. .the amount of cells that were engaged in inflammatory processes such as Plasma tissue and Fibroblast cells.

**Statistical analysis**

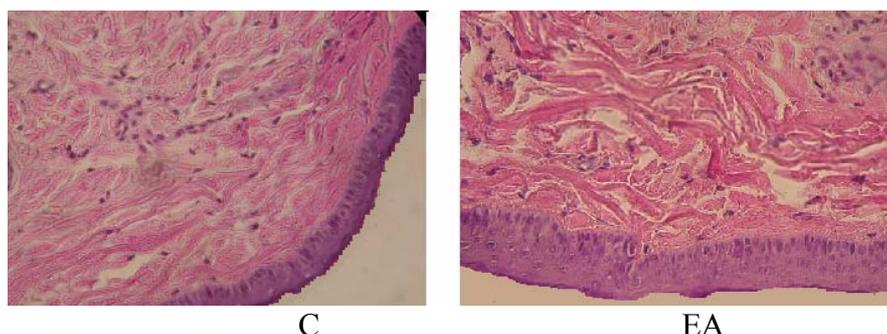
The data gathered were analyzed by JMP SOFTWARE and T-student test. JMP and Excel software were used to draw the required diagrams. The standard deviation was calculated with a possibility of( $p \geq 0.05$ )

**Results**

After doing the histological studies, we found out that epithelialization was quite simultaneous. Having investigated the cellular layers, we also found out that from the cellular distinction point of view the cells of the sample test (15<sup>th</sup> day) were closer to the stage of branching. On the other hand, the cellular layers of the samples in control group were still in the stages of growing and reproducing and they were far from the stage of branching) .Figure 1 and 2



Figures 1: The present cutting of injured tissue in the NB as well as control groups , indicating (from right to left) the rapidity of Epiderm repair from the edge of wound since it was received the injury in the fifteenth day .



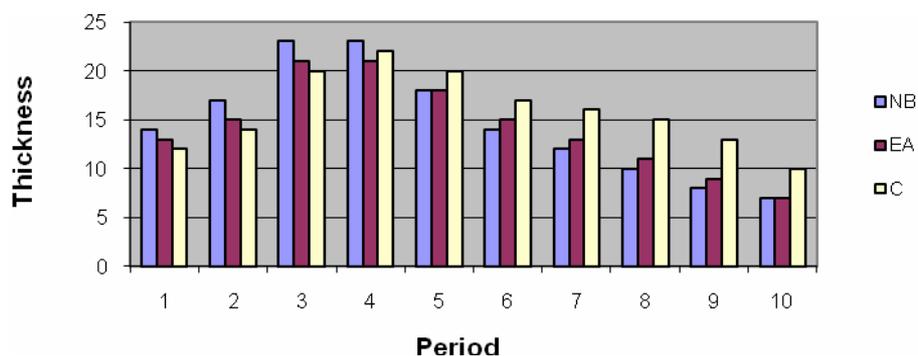
Figures 2: The present cutting of injured tissue in the EA as well as control groups , indicating (from right to left) the rapidity of Epiderm repair from the edge of wound since it was received the injury in the fifteenth day.

The diagram shows the comparison of the changes of the epiderm drop in the holes in every 3 groups of this experiment. In this study, reviewing the average of the wound surface in different days of the study shows that recovery of the wounds in the group which received NB and EA alcoholic fractions was faster than the recovery of the wounds in the group which received Normal Salin treatment. However ,there was still no significant difference. ( $p \geq 0.05$ ) Table and graph 1

Table 1: The comparison of Epiderm Thickness( $\mu\text{m}$ ) among experimental groups over the specific period of therapy

DAY50	DAY45	DAY40	DAY35	DAY30	DAY25	DAY20	DAY15	DAY10	DAY5	
7	8	10	12	14	18	23	23	17	14	NB
7	9	11	13	15	18	21	21	15	13	EA
10	13	15	16	17	20	22	20	14	12	C

Graph 1 :Statistical analysis of the comparisons between Epiderm thickness( $\mu\text{m}$ ) among three groups of NB, EA, and C



The statistical analysis (until the 20<sup>th</sup> day) shows that based on the measuring of the wound surface in the three groups ,the percentage of recovery at the 5<sup>th</sup> day (for instance, in the distal situation) in the control group was 45%, EA fraction was 42% and NB fraction was 50% but these percentages increased respectively to 87%, 80% and 84% . (table and diagram 2)

Table 2: The comparison of the repair of wound in percentage among the three experimental groups up to 20 days

C	EA	NB	DATE									
D	D	D	M2	M2	M2	M1	M1	M1	P	P	P	
0	0	0	0	0	0	0	0	0	0	0	0	DAY0
45	42	50	41	47	61	44	46	51	46	48	59	DAY5
70	67	65	73	72	67	73	70	73	68	68	70	DAY10
87	80	84	83	87	82	84	85	81				DAY15
												DAY20

### Discussion

The successful healing of the wound was indebted to the regeneration of epiderm and derm (1). (So far, different herbal products have been proved to influence the healing of the wound (8). Extracts and fractions of the Rosa Damascena are among the effective, natural and herbal products (15, 12 and 14). In this study, it seems that in microscopical study of the wound regeneration, there have been positive effects on the regeneration and closing processes of the wound. However, in the statistical analysis there was no significant difference between the area of the wounds in both control and treatment groups. The speed of the wound closing in which the size of the open wound decreases through moving to the center of the skin around it can be as a result of the systolic features of the active fibroblasts which are available in the wound tissue. (16, 17, 18).

We observed the thickness of the epiderm and we found that there was no difference among the epithelial thicknesses of the three groups. Therefore, here it seems that in comparison with Normal Saline, these fractions have no effect on the reproduction of the epithelial cells and this may be dependent on the application of the fractions. On the other hand, it is possible that treatment with doses more than 5% (which was used in this study) have more reproduction effects on the epithelial cells. Thus, it is suggested that future studies will be done on the effects of these fractions

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