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Histopathological Evaluation of Burdock (*Arctium lappa*) Root Hydroalcoholic Extract on Wound Healing

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Abstract

Background: Wound healing is a process that occurs following skin lesions. Shortening healing time is of critical importance as it reduces the risk of infection, complications, and costs.
Objectives: In this experimental study, 36 male Wistar rats each weighting approximately 200 - 220 g were studied in six groups, each with 6 animals for 21 days. Hydroalcoholic extract of Burdock root was prepared through the maceration method. The animals underwent a 2 × 2 cm diameter resection of cutaneous fragment on the dorsum. The first group was kept without treatment as the control group, the second group (negative control) was treated with Eucerin, and the third group (positive control) was treated with ointment of phenytoin (1%). Eucerin based-ointments from hydroalcoholic extracts of Burdock root with concentrations of 20%, 40% and 60% of weight/weight were administered to the animals in the other three groups twice a day. The lesion diameter and programmed euthanasia were analyzed through a surgical specimen resected for histopathology.
Results: The healing process was completed in 21 days in both no treatment and Eucerin groups. In the phenytoin 1% group, the healing time was 16 days and in hydroalcoholic extracts of Burdock root groups of 20%, 40% and 60%, complete wound closure was observed in 16, 16 and 14 days, respectively. All the treated groups and the control group showed significant differences when compared with the negative control and control group ($P < 0.05$). In addition, the histological study of the group treated with hydroalcoholic extracts of Burdock root showed that symptoms and improvement of skin tissues had a better status.
Conclusions: The crude hydroalcoholic extract of Burdock root was found to cause better outcomes in the healing process, acute inflammation, and fibrosis on the 7th, 14th, and 21st day postoperatively.

Keywords: Burdock Root, Wound Healing, Rat

1. Background

Wound healing is a treatment process that follows skin and other tissue lesions (1). Achieving faster healing with fewer side effects is still one of the most important medical goals. In addition, shortening the healing time is of critical importance because it can reduce the risk of infection, complications, and costs (2). Burdock with the scientific name of *Arctium lappa* belongs to the Asteraceae family. As a herbaceous, biennial plant with a thick and branched stem, it grows in some regions of Europe and Asia, as well as in North and North West of Iran. Harvesting is done in late March to May of the second year of its growth when roots are in their maximum growth (3, 4). Burdock roots contain chemical compounds such as inulin, volatile oil, tannin, resin, sugar, iron, calcium, quercetin, arctigenin, and vitamin C. In addition, burdock seeds contain bitter-yellow oils, known as linoleic and oleic acids (5, 6). The plant also contains flavonoids, and many researchers have demonstrated their role in treatment of diabetes (7). It is also beneficial for atherosclerosis due to its antioxidant properties and inhibitory effects on platelet adhesion (8). Burdock anti-inflammatory activity against diabetes and cerebral ischemia is attributed to its arctigenin ingredients (9, 10). Arctigenin also activates glucose uptake by mussels, inhabitation of golocenogenez, and lipogenez, and induction of AMP kinase activity (11). Some studies have mentioned the beneficial effect of Burdock on wound healing (12, 13). Pomari et al. (14) demonstrated the effect of burdock on cell adhesion and gene expression, which are of particular importance in the wound healing process.

2. Objectives

The main objective of the present study was to investigate the effects of hydroalcoholic extract of Burdock (*Arctium lappa*) root on skin wound healing in rats.

3. Methods

3.1. Preparation of the Plant Root

Burdock plants with their roots were collected in spring from surrounding areas of Karaj, and dried in the shade. The plant was identified by experts of agriculture faculty of Shiraz University and agriculture research center of agriculture organization of Fars province, Iran. A voucher specimen of *Arctium lappa* was deposited in the herbarium of the faculty of agriculture of Shahid Beheshti University (No. AR337E).

3.2. Preparation of Extract and Ointment

Active ingredients were extracted through the soaking method. To this end, 1000 mL of alcohol (70%) was added to 200 g of powdered roots and the mixture was kept for 3 days. The extract was then filtered using filter papers and funnels and concentrated as much as possible using a vacuum distillation device. After determination of density of the extract, it became concentrated, and then Eucerin based-ointments with concentration of 10%, 20%, 40%, and 60% weight/weight were prepared by the Pasteur Institute of Iran (15, 16).

3.3. Gas Chromatography-Mass Spectrometry Analysis of Compounds

The prepared samples were analyzed through gas chromatography using Agilent HP6890 instrument coupled with a HP 5973 mass spectrometer. The gas chromatograph was equipped with a split-split less injector and a factor four TM VF-35ms 5% phenyl-methylpolysiloxane, 30 m, 0.25 mm, and 0.25 μm film thickness capillary column. Gas chromatography conditions were as follows, temperature range of 50 to 250°C at 40°C/minute, with a solvent delay of 5 minutes. The injector was maintained at a temperature of 250°C. The inert gas was helium at a flow of 1.0 mL/minute, and the injected volume in split less mode was 1 μL. The MS conditions were as follows, ionization energy of 70 eV, quadrupole temperature of 100°C, scanning velocity of 1.6 scan/s, and weight range of 40 to 500 amu. The percentage of the volatile compounds was calculated. Qualitative analysis was done based on the percentage area of each peak of the sample compounds. The mass spectrum of each compound was compared with the mass spectrum from the NIST 98 spectrum library (USA National Institute of Science and Technology Software).

3.4. Laboratory Animals

This experimental study was conducted in fall 2015 at the School of Veterinary Medicine, Kazerun University on 36 male Wistar rats each weighting approximately 200 to 220 g, that were prepared by research, proliferation, and maintenance center of laboratory animals in Kazerun University, Fars, Iran. The animals were kept in the animals center in separate protective aluminum cages under the following conditions, 12 hours of light and 12 hours of darkness, at 25 $\pm$ 2°C, and humidity of 45 to 55%. The experiments were conducted upon the approval of the state committee on animal ethics, Kazerun University, Kazerun, Iran (Ref: 1012-1013, 20-6-94). The recommendations of the European council directives (86/609/EC) of November 24th, 1986 concerning the guidelines for the protection of animals used for experimental purposes were followed in this study.

The animals were fed without restriction by compact food (made in Shushtar factory, Iran) and urban water. They were placed in 6 groups of six each, including control, Asrin group (negative control), phenytoin group 1% (positive control), and Burdock root 10% (experimental1), 20% (experimental 2), 40% (experimental 3), and 60% (experimental 4) groups.

3.5. Ulcers and Treatment Methods

The ulcer sites close to vertebral column were considered by a method proposed by Cross et al. (1995) (17). The rats were anesthetized with an intra peritoneal (IP) injection of 40 mg/kg sodium thiopental (UVB Pharma, Czech Republic). The skin of dorsum was shaved and thoroughly depilated and washed with povidone iodine. Then the animals were kept in a standard curved condition and after disinfection of the site, while the skin had been kept with a simple stretch between fingers, a square with dimensions of 2 $\times$ 2 was drawn using a metallic template. In the subsequent step, a full thickness of the drawing site was removed using a scalpel (Figure 1) (17, 18). Immediately after the injury, the considered site was washed with saline and the wounds were dressed with the specified material for each group. Thus, Asrin and phenytoin (1%) (Made in Daroo Pakhsh Company) were used for the negative control group and positive control, respectively. In addition, the Asrin based-ointments of hydroalcoholic extracts of Burdock root with various concentrations of 10%, 20%, 40%, and 60% of weight/weight were used in the other three groups. This process was repeated until complete wound closure and reformation of epidermis every 12 hours (18, 19). In addition, the wounded areas were measured every 24 hours until complete healing. The measurement error was minimized by measuring and obtaining averages of 3

trials (17, 18). The wounded area in the first day was considered 100% and the corresponding areas on subsequent days were compared with the area on the first day (19, 20). In addition, the wounded areas were imaged on the 1st, 7th, 14th, and 21st day. The healing percentage was calculated through the following formula:

The wound percentage on day x = the wound area on day x / the wound area on day 0 × 100.

The healing percentage on day x = 100 – the wound percentage on day x

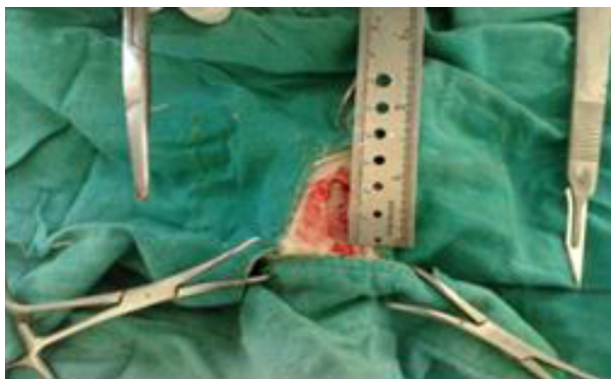

Figure 1. Skin Removing in 2 × 2 cm Dimension

3.6. Histological Studies

Hematoxylin and Eosin (H & E) staining method was used to conduct the histological studies. To this end, full thickness samples of skin on the 7th day of the treatment were taken from an additional animal kept for histological studies (16). The samples were washed first and then kept and fixed in 10% formalin solution. After the dehydration process in ascending alcohol concentration of 60% to absolute, the samples became transparent using Xylene and were molded with paraffin (20, 21). Samples with 5 microns of thickness were prepared and were stained with H & E sustaining method and were finally studied with an optical microscope.

3.7. Statistical Analysis

The data were expressed in SI units and analyzed by repeated measurements; analysis of variance (ANOVA), Duncan, Spearman, and t-test using SPSS software version 17 (15). All values were expressed as mean and standard error (SE) at the significance level of 0.05 ($P < 0.05$).

4. Results

4.1. Gas Chromatography-Mass Spectrometry Analysis

The global DBRE composition (percent of weight) was as follows: 11% of glucidic compounds, 86% of phenolic compounds, 1.2% proteins, and 1.8% others. However, the main ingredients of burdock (*Arctium lappa*) root were not identifiable using the GC-MS technique.

Wound healing of rats lasted 21 days in control and negative control groups (receiving Eucerin). Although the two groups showed statistical differences ($P < 0.50$) with all the others, there was no significant statistical difference between duration of wound healing in control and negative control groups. Wound healing of the skin in the treated rats with phenytoin ointment (1%) improved after 16 days. From the beginning of the treatment, this group showed significant statistical differences ($P < 0.05$) with the group, which was treated with Eucerin and the control group. Complete wound healing in the experimental groups receiving 20%, 40%, and 60% of Burdock root extract occurred after 16, 16, and 14 days, respectively. All these three groups had significant statistical differences ($P < 0.05$) with the group treated with Eucerin and control group. Table 1 shows the wound areas (mm^2) on days 0, 7, 14, and 21, respectively, in the treatment period:

The results of the histological study on the 7th day did not show any epidermis in skin samples of the groups untreated and treated with Eucerin. However, lesions with necrosis and hemorrhage and inflammation were observed in dermis (Figure 2). Epidermis with low thickness was formed in the samples treated with phenytoin (1%). The underlying connective tissue was formed but skin appendages did not appear (Figure 2). Hemorrhage, congestion, and inflammatory reactions were observed in the dermal site. Fibroblast cell proliferation and collagen deposition were observed as well (Figure 2). Images taken from the treated skin with Burdock root (20%) showed epidermal cell proliferation at the wound edge along with collagen fibers and fibroblasts deep in dermis with small degrees of inflammation and necrosis. However, in addition to formation of epidermis and dermis, fibroblast cells and enormous collagen fibers were observed in the samples treated with 40 and 60% of Burdock root extract. Inflammatory cells were observed in small numbers (Figures 3 - 5).

5. Discussion

Burdock root contains chemical compounds such as inulin, volatile oil, tannin, resin, sugar, iron, calcium, quercetin, arctigenin, and vitamin C (5, 6, 22, 23). However, the mentioned compounds were not recognized using the

Table 1. The wound Areas (mm^2) on Days 0, 7, 14, and 21 During the Treatment Period[a]

	Groups					
Day	**Control**	**Positive Control**	**Negative Control**	**Experimental 20%**	**Experimental 40%**	**Experimental 60%**
0	0.43 ± 42.44	0.43 ± 42.44	0.43 ± 42.44	0.43 ± 42.44	0.43 ± 42.44	0.43 ± 42.44
7	0.61 ± 40.13	0.65 ± 39.42	0.54 ± 39.16	0.11 ± 14.82[b]	0.02 ± 10.87[b]	0.04 ± 9.92[b]
14	0.63 ± 11.22	0.25 ± 5.27[b]	0.13 ± 8.21	0.05 ± 4.11[b]	0.03 ± 2.81[b]	0.03 ± 1.95[b]
21	0.48 ± 3.53	0.50 ± 1.75[b]	0.76 ± 2.95	0	0	0

[a]Values are expressed as standard error of mean (mean ± SD).
[b]A significant difference ($P \leq 0.05$) between groups.

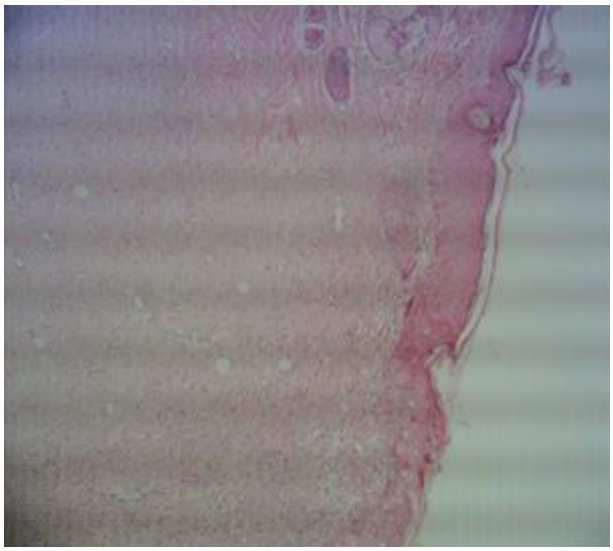

Figure 2. Control Group (Magnification 40 ×)

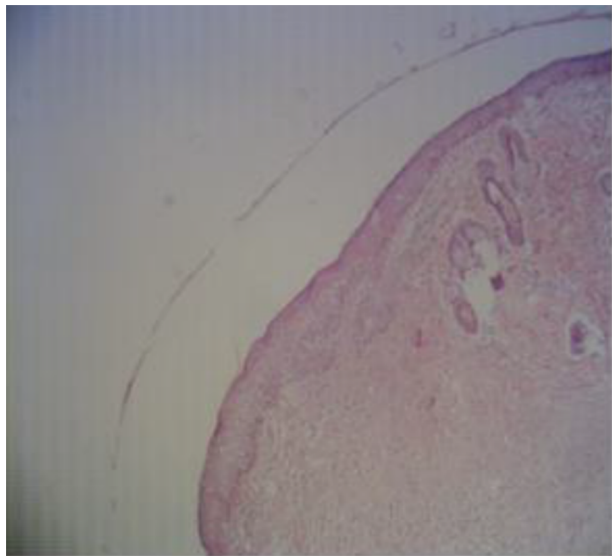

Figure 3. The Group Treated With 20% of the Extract (Magnification 40 ×)

GC tool in the present study, because all these compounds remained in the extract and they did not penetrate in the solvent. This can be attributed to the temperature differences in the extracting process and the removal of solvent through rotary. Therefore, the temperature differences can be regarded as the main research for the non-presence of the compounds in the solvent (23).

Given that no study with a histology approach has been conducted both in Iran and abroad, it is not possible to compare the results of the present study with the literature.

Wound healing has different stages including inflammation, proliferation, and reconstruction, each consisting of several other steps, some of which overlaps with each other and thus are not easily separable (24, 25). Therefore, the qualitative and quantitative improvement of each of these stages can accelerate wound healing and reduce its complications. Furthermore, anti-inflammatory effect is one of several effects of flavonoids in Burdock root (23, 26, 27). A variety of methods have been proposed to examine the presence of inflammation in damaged tissues. The experiments conducted in the present study showed that wound healing duration was longer in control and negative control groups, while this period was shorter in positive control group and the groups treated with 20, 40, and 60% of Burdock root extract. Besides, all groups showed significant statistical differences compared to control and negative control groups. These differences were also clearly distinct in microscopic histological figures. In addition, by increasing fibroblasts growth, one will prepare the area for excretion of collagen and as a result for faster wound healing (28). The results of the experiments carried out on the above four groups on 7th day of treatment and at the end of treatment showed that the epider-

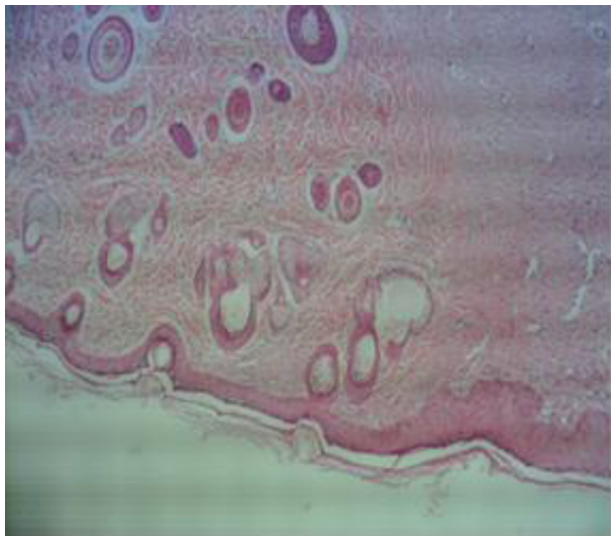

Figure 4. The Group Treated With 40% of the Extract (magnification 40 ×)

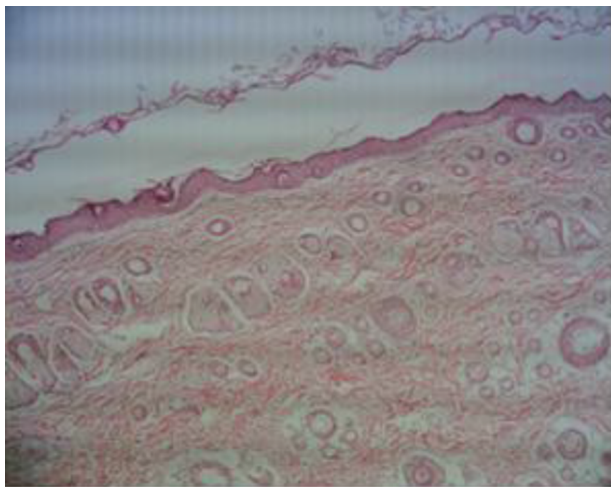

Figure 5. The Group Treated With 60% of the Extract (magnification 40 ×)

mis has been formed and granulation of tissue was completed. The observation of more collagen fibers deposition and higher density of fibroblasts, hair follicles and blood vessels in the groups treated by 40% and 60% of Burdock root ointment as well as lower duration of wound healing in these groups suggests that 40% and 60% of Burdock root ointment causes faster and better healing when compared with other treated groups. All of the treated groups showed significant statistical differences ($P < 0.05$) compared to control and negative control groups. However, the statistical and histological results showed that 40% and 60% of Burdock root ointment had the best effect on wound healing of mice skin compared with the other groups.

5.1. Conclusion

The findings of macroscopic and microscopic studies suggest that effective components of Burdock root stimulate collagen synthesis and faster wound contraction, angiogenesis, vascular dilation, reduction of inflammation, bleeding, and edema of the wound. However, research on this plant is not yet completed and due to its widespread use in the region, future researchers are recommended to investigate its effects on other types of wounds, burns, and its effect on fibroblast growth. Later studies may also attemp to isolate and identify active ingredients within the plant.

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Footnotes

Authors' Contribution: All of the authors approved the content of the manuscript, contributed significantly to the research, and were involved in the writing of the manuscript. Fereshteh Ghorat, Mohammad Azizkhani, Shahriar Naji, Ali Ghorbani Ranjbary, and Farzad Doostishoar participated in various stages of this study including design, data collection and analysis, preparing the draft of manuscript, and making critical revisions to the paper for important intellectual content and English editing.

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