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Investigation the effect of administration different levels of Licorice extract on cutaneous skin wound healing in New-Zealand rabbits

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Abstract: The aim of the current study wad to determine the effect of use different levels of licorice extract on New-Zealand rabbits cutaneous skin wounds healing. **Material and methods:** Twenty-one adult male rabbits by average age, 12 months and average weight of 3.5±0.750 kg were selected to evaluate cutaneous wound healing after treatment with 100, 200 and 300 mg/kg of licorice extract. Experimental wound excision model in rabbits was created by means of a skin punch of 2 cm diameter. The experimental rabbits were topically treated for seventeen days with a saline control or decoctions of mixed with common thistle and thyme derived extracts with carboxymethyl cellulose. The centripetal retraction, clinical, and histological aspects of the wounds were observed until complete wound healing. **Result:** The phototherapeutics agent presents in licorice extract improved cicatrization of cutaneous lesions in rabbit's skin during the first days of treatment. The treatments were beneficial to the reparation process of wound healing. The result showed that the administration the licorice extract has beneficial effects in the inflammatory phase and on the reparation process in experimental animal. Administration of 150 mg of licorice extract showed a good effectiveness on the macroscopic aspect of cutaneous lesions in rabbits but only during the first treatment days. **Conclusion:** In conclusion we may note that the use of different levels of licorice extract may have improved fibroplasia and pytotherapeutic activities and could be useful in topical treatment of cutaneous lesions in New-Zealand male adult rabbits without adverse effects.

Keywords: Wound healing, Licorice extract, Pytotherapeutic activities, New-Zeland Rabbits.

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Introduction

Licorice gained a great attention for its antioxidant and anti-inflammatory properties which expand its valuable effects as an herbal medicine (LANGMEAD AND RAMPTON,2001).

Liquorice root extract, derived from the plant "Glycyrrhiza glabra" has been used in traditional medicine in human and animals (DORAI, 2012, ZABIHI et al**.,** **20**03).

The clinical studies have shown that liquorice root extract has spasmolytic and beneficial influence on healing process of gastric ulcer (BAZZAZ AND HARIRIZADEH, 2003). Licorice extract, particularly its active component glycyrrhizin, has shown promise in promoting cutaneous wound healing (MUJAHID ET AL., 2025).

Studies indicate that licorice extract can accelerate wound closure, reduce inflammation, and enhance collagen production, potentially offering a natural approach to wound care (RIZZATO ET AL., 2017, OLOUMI et al., 2007).

The potential mechanisms of liquorice root extract for wound healing are antioxidant activity, promoting the angiogenesis and the formation of new blood vessels, stimulation the expression of growth factors like bFGF, VEGF, and TGF-β, which are involved in tissue repair and regeneration and synergistic effects to wound healing (TAN ET AL., 2018, HENRY AND GARNER, 2003).

The wound healing involves a series of well-coordinated biochemical and cellular events leading to the growth and regeneration of wounded tissue in a specific manner (SARABAHI ,2012).

The participation of various inflammatory cells such as macrophages and neutrophils is extremely crucial to the repair process (RODRIGUES et al., 2019).

These cells also promote the migration and proliferation of endothelial cells, leading to neovascularization of connective tissue cells, which synthesize the extracellular matrices including collagen;

and of keratinocytes leading to re epithelialization of the wounded tissue (HENRY AND GARNER,2003).

Hence all of these processes involved in tissue repair are altered during pathological conditions wound does not heal perfectly and it would be interesting to unravel the possible mechanisms involved in such cases as this would help to identify precisely newer agents, which may improve the delayed healing process. Natural compounds, especially those derived from various plant species (ADIKWU et al.,2008,

Pawar and Toppo, 2002), have been used successfully in studies on the treatment of cutaneous wounds and inflammatory changes in animal models. Recently the researches has been focused in herbal plants through their active compounds on wound healing and management without adverse effects on animals and although, licorice extract wound healing efficacy is already reported, little is known about the mechanism associated with its action, so the aim of current study was to investigation of administration different levels of licorice extract on New-Zealand rabbits cutaneous skin wounds healing.

Materials and methods

The current study was done at veterinary research clinic of Islamic Azad University Shahre-Kord Branch. The total of twenty-one New-Zeland Rabbits average weight of 3.5±0.750 kg were used in this study.

The rabbits were confined to individual and roofed cages and were fed by rabbit's diet and the food and water was available on free-choice form for them to fed. The animals were confined for two weeks to allow adaptation before initiation of the do the experiment. The licorice was cleared of dirt and dried under shade for about one month and using a mechanical grinder and powdered.

The obtained powder was sent to the laboratory to extraction with ethanol for extraction as many polar and non-polar compounds were extracted from the ethanol for five days, followed by hot percolation for 5 $^{hrs.}$ and filtered and distilled at 80$^{\circ C}$.

The obtained extract was transferred into the previously weighed empty china dish and evaporated to get an ethanoic extract and kept in anhydrous calcium chloride containing desiccator and then the

percentage yield of the extract was calculated. The treatments were control, 150 and 300 mg/ kg of Wight of rabbits respectively. An isotonic solution of Na-Cl homogenized with six g of carboxymethyl cellulose was used as control therapy. Decoctions were prepared only once, and they were stored in amber glass bottles under refrigeration 4 until 8$^{\circ C}$.

Additionally, after 24^{h} food withdrawal and a 12^{h} water withdrawal wounds were surgically created. Thereafter, the heifers were sedated by 0.04 mg per kg of Xylazine HCL and their hair was clipped from an area of approximately 45×45 cm^2 in the lumbar region. After sedation, lidocaine was applied to the incision areas, and four full-thickness lesions were made by excising the skin to the level of loose subcutaneous tissue on each side in the lumbar region, using a punch of diameter 2 cm without antiseptics, thus preserving the resident microbiota. The circular wounds were located at ten cm from the spinal column, and were separated from each other by the same distance 5 cm. Clinical treatment was initiated twelve hours after the surgical wounds were made, and was administered on a daily basis until complete cicatrization of the lesions.

The treatments were as control without any herbal administration and administration of 100, 200 and 300 mg/kg of licorice extract. The pytotherapeutic plant decoction condensed with carboxymethyl cellulose were directly applied on the wounds daily with a syringe. The lesions on the right side in the lumbar region of each animal were clinically evaluated for local hemorrhage, presence of clots, crusts, granulation tissue, epithelization, and presence of exudate, and were classified as bad (1), regular (2), or good (3) by the same evaluator throughout the study. The macroscopic evaluation was performed on a daily basis until the 17th day after the surgery. For measure wound retraction, each wound area on days 3, 5, 7, 9, 11, 13, 15, and 17 days by placing a transparent plastic sheet on the lesion and marking the surrounding perimeter with a projector pen were measured. The lesions on the left side in the lumbar region of 20 randomly chosen animal were selected for biopsy.

Samples collected from the geometrical center of the lesions by using a

surgical punch of diameter 10 mm on days 7 and 17 after wound establishment and was fixed in formaldehyde for histopathological analysis. The fragments were stained with hematoxylin and Harris eosin, and analyzed by a pathologist who was blinded to the experimental methodology. For fragments obtained on day 7, inflammatory reaction was evaluated on the basis of cellularity and edema formation. The presence of young granulation tissue was also evaluated.

For cellularity, the following grades were attributed: present (1), moderately infiltrated (2), or severely infiltrated (3). Edema was classified as absent (1), slight (2), or severe (3). The young granulation tissue was classified as traces (1), moderate (2), or abundant (3), by using a semi-quantitative analysis. Treatment averages were obtained from the inflammatory reaction and granulation tissue deposition evaluation grades.

The material obtained on 17th day was evaluated for granulation tissue deposition by examining the presence and quantity of young or mature granulation tissue, and was graded as minimum (1), moderate (2), or abundant (3) deposition. Inflammatory reaction was classified as weak (1), strong (2), or severe (3). As on day 7, treatment averages were obtained from the evaluation grades for inflammatory response and granulation tissue deposition.

Statistical analysis

Data were gathered and analyzed by using the general linear model procedure of SAS (9.12) version. The means comparison differences between treatments were evaluated by t-test and ($p \leq 0.05$) was considered as a significant. Also, the qualitative parameters from the microscopic analysis were used for defining the healing quality response (STEEL ET AL., 1997).

Result 0

The grade of rabbits' skin wounds on days 3, 5, 7, 13, and 17 are presented in table 1. According to the data the wounds were treated with licorice extract showed the serous, smooth, and slender crusts, and borders with less edema and the significantly better macroscopic characteristics compared to the control ($p \leq 0.05$).

Table1- Effect of licorice extract administration on the wounds grade (Mean±SD)

Days / Grades	3	5	7	13	17
Control	1.88^{c*}±0.10	1.92^{c*}±0.11	1.98^{c*}±0.10	1.99^{c*}±0.09	2.01^{c*}±0.02
Licorice (100 mg)	1.91^{b*}±0.11	2.01^{b*}±0.08	2.10^{b*}±0.12	2.18^{b*}±0.15	2.14^{b*}±0.05
Licorice (200 mg)	2.21^{a*}±0.14	2.14^{a*}±0.12	2.25^{a*}±0.15	2.24^{a*}±0.18	2.29^{a*}±0.09
Licorice (300 mg)	2.54^{a*}±0.08	2.22^{a*}±0.16	2.41^{a*}±0.09	2.31^{a*}±0.10	2.37^{a*}±0.10
P- Value	**	**	**	**	**

*a,b,c = Means in the column significantly differed.

The wound area surface averages (cm^2) did not differed from average wound area of the control group at any time of experimental period.

Data showed that the lesion area was slightly smaller in the 300-gr licorice extract group (table 2).

Table 2- Effect of licorice extract administration on the skin wound area surfaces (Mean±SD)

Days / Area surfaces (cm^2)	3	5	13	17
Control	2.20±0.02	1.71±0.12	1.26±0.07	0.95±0.11
Licorice (100 mg)	2.21±0.01	1.65±0.14	1.18±0.09	0.85±0.06
Licorice (200 mg)	2.18±0.02	1.55±0.19	1.15±0.04	0.98±0.08
Licorice (300 mg)	2.14±0.03	1.60±0.12	1.13±0.10	0.99±0.04
P- Value	n.s*	n.s	n.s	n.s

*n.s = Means in the column do not significantly differed.

According to the table 3 data the histological evaluation on 7^{th} day did not show significant differences for inflammatory response and granulation tissue deposition between treatments. The better macroscopic evaluation was not

associated with quantifiable alterations in microscopic evaluation in current study.

Additionally, the microscopic analysis on 17th day exhibited that licorice extract at 300 mg had more beneficial act to the wound healing process between treatments. Also, administration of licorice extract at 300 mg had led to greater mature conjunctive tissue than the others (table 3).

Table3- Result of histological evaluation numbering of rabbit's skin wounds (Mean±SD)

Response / Histological evaluation	Inflammation 7 days	Inflammation 17 days	Granulation 7 days	Granulation 17 days
Control	1.6±0.51	1.9±0.08	2.0±0.06	1.7±0.06
Licorice (100 mg)	1.7±0.11	2.0±0.11	1.9±0.10	2.1±0.02
Licorice (200 mg)	1.9±0.12	2.1±0.09	1.8±0.08	2.0±0.07
Licorice (300 mg)	1.9±0.14	2.3±0.13	1.9±0.05	1.9±0.05
P- Value	n.s*	n.s	n.s	n.s

*n.s = Means in the column do not significantly differed.

Discussion

Result of wound area measurements, indicated a healing potential for the licorice extract at 100,200 and 300 mg/kg dosage. The area measurements showed that there are significance differences between the different groups.

Data from (Afshar et al., 2015) study have been showed that on days 4 and 7, the numbers of inflammatory cells in the Malva administrated groups were significantly lower than the control group at the edges of the wound.

Pirbalouti et al (2010) study result showed that the significant reduction in the wound area in groups that they used herbal extract compared with other groups. Also, histopathological studies of the tissue obtained on days 6th, 9th and 16th from the extract-treated by Malva and increased well organized bands of collagen, more fibroblasts and few inflammatory cells.

Leite et al (2023) showed that dipotassium Glycyrrhizinate attenuates the inflammatory process by promoting skin wound healing through the modulation of distinct mechanisms and signaling pathways, including anti-inflammatory ones.

This involves modulation of the expression of anti-inflammatory cytokine expression; promotion of new granulation tissue; angiogenesis; and tissue re-epithelialization, all of which contribute to tissue remodeling. Some researchers have shown that the licorice extracts have the ability to down-regulate expression of pro-inflammatory cytokines as tumor necrotic factor alpha, interleukin 1 and interleukin (SHARMA et al., 2016)

Interestingly, in the (Doa et al., 2021) study they also demonstrated that licorice applications enhanced antioxidant status and notably declined the oxidative stress biomarker compared with the control untreated group.

In (Zabihi et al., 2023) study the rate of inflammation from the 3rd day to the 10th day, redness from the 6th day to the 15th day, pain on the 3rd day and burning from the 3rd day to the 15th day of the wound in the group that used the hydrogel-containing hydroalcoholic extract of licorice root was significantly lower than in the control group and the healing process was significantly faster than the control group.

Data from current study are in line with other study that they reported that use of may lead to better effectiveness on wound healing in experimental rats (Oloumi et al.,2007), result of many studies also have shown that application of herbal plant derived extract such as licorice led to better wound healing process in animal model experiments (LEITE ET AL., 2023, ZABIHI ET AL., 2003).

Conclusion

According to the result of current study we may demonstrated that administration different levels of licorice extract could improve cicatrization of cutaneous lesions in experimental New-Zeland rabbits' skin during the first days of treatment intervention period.

Use of licorice extract at 150 and 300 mg were beneficial act to the reparation and have beneficial effects to inflammatory phase and reparation process of wound healing processing experimental rabbits. The good effects on macroscopic aspect of cutaneous lesions in New-Zeland rabbits

during the treatment days by licorice extract have improved fibroplasia.

The biological activates of licorice active compounds specially at 300 mg dose was the most superior, and may be used in topical treatment of cutaneous lesions in New-Zeland male rabbits.

The possible reason for enhanced wound healing effect of licorice derived extracts may be due to the which may possess antioxidant, free radical scavenging properties and promote cell proliferating properties. More studies are needed for better explanation.

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Data availability

All data presented in this study will available free of charge for any researcher upon reasonable request from the corresponding author.

Declarations

All of the authors are involvement in the research process equally.

Competing interest's

The authors state that no known challenging financial benefits or personal elationships that would have seemed to affect the work described in this study.

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Conflict of interest

There are no conflicts of interest reported by all of the authors.

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