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ORIGINAL ARTICLE

The Effect of Hydroalcoholic Extract of *Berberis vulgaris* on Wound Healing of Diabetic Wistar Rats

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KEYWORDS

Diabetes;
Barberry;
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ABSTRACT: Diabetes mellitus is one of the most common diseases of the endocrine system. Barberry (*Berberis vulgaris*), as a common herb incorporated in treatment of various diseases, is used as anti-itch and anti-microbial medicine as well as lowering blood sugar and blood pressure. In this study, the effects of a hydroalcoholic extract of *B. vulgaris* on skin wound healing of diabetic rats were investigated. Forty eight male Wistar rats weighting 180 to 230 g were divided into four groups: control group (non-treated non-diabetic rats), sham group (oserin-treated diabetic rats), experimental 1 group (non-treated diabetic rats), experimental 2 group (*B. vulgaris* extract-treated diabetic rats) and then, 3-cm-length cuts were created on the skin of the dorsal lumbar region and then the wounds were treated on a daily basis. Lesion length measurements were conducted during the experiments using a caliper. For the purpose of histological studies, wound samples were taken from each group and finally, statistical data analysis was conducted using the one-way ANOVA test in addition to the Duncan test performed using the SPSS software. The results indicated that the second experimental group had the best influence on wound healing compared to others. Accordingly, the *B. vulgaris* extract might be able to improve the wound healing process along with reducing the lesion length due to its anti-diabetic and antimicrobial effects in rats after 21 days.

INTRODUCTION

Diabetes mellitus is known as one the most common endocrine diseases. This disease is caused by lack of cellular uptake of blood glucose due to decreased insuliln secretion or insulin resistance occurrence in body cells [1, 2]. Along with the development of diabetes, underlying clinical conditions like foot deformities, foot ulcers and infections become more common [3]. Barberry, with the scientific name *B. vulgaris*, belongs to the Berberidaceae family. For this plant, several effects including the treatment of various

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skin conditions, anti-itch, anti-microbial, reduction of blood sugar and blood pressure have been mentioned within the literature of traditional medicine [4-6]. Anthocyanin, as one of the alkaloids present in this plant, plays a significant role in preventing heart disease, neurological disorders, diabetes and cancer in addition to its color and antioxidant properties [7]. "Berberine is found in such plants as *Berberis*: e.g. *B. aquifolium* (Oregon grape), *B. vulgaris* (barberry), *B. aristata* (tree turmeric) and it is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids" [8]. Berberine is beneficial because of major anti-inflammatory activities [9].

In the present study, the hydroalcoholic extract of *B. vulgaris* effect on wound healing of male diabetic rats were investigated.

MATERIALS AND METHODS

Forty-eight male adult Wistar rats weighing 180-230 g were collected from the Animal House Department of the Pasteur Institute of Iran, Karaj, Alborz Province.

The procedures involving animals and their care were conducted in conformity with the Helsinki Declaration and guidelines for the care and use of laboratory animals approved by the Animal Care and Use Committee of Damghan Branch, Islamic Azad University.

All animals were kept under controlled conditions of light (12 h of light and 12 h of darkness) with the ambient temperature of 22±2 °C and relative humidity of 40-60% and free access to water and food in the Animals' Room of the Islamic Azad University, Damghan Branch, Iran, to adapt to the new environment. A caliper was used to measure the lesion length. In addition, a vacuum pump and rotary extractor were used for extracting purposes. Rats were divided into four groups (n = 12 each): control group (non-treated non-diabetic rats), sham group (oserin-treated diabetic rats), experimental 1 group (non-treated diabetic rats) and experimental 2 groups (*B. vulgaris* extract-treated diabetic rats). After keeping the rats for 10 days, 36 rats were subjected to 55 mg/kg streptozotocin (STZ) intraperitoneally injection and after 72 h, their blood sugar level was measured by glucometer. The bloods glucose levels in rats were 296 to 579, which was an indication of being diabetic.

A scalpel was used to create a 3-cm-length wound with a depth of epidermis, dermis and hypodermis according to relevant standards immediately after the anesthesia and shaving the animals' fur. The day of the surgery was considered as the first day and each rat was kept in a separate cage for sanitary purposes.

In order to prepare the barberry extract, 200 gr of dried, mixed barberry and l liter of 70% ethanol (with a proportion of 1 to 4) were mixed together and then, after the preparation of the extract, it was segregated with vacuum pump and rotary and eventually, a concentrated extract was prepared. Then, the extract was mixed with oserin with a proportion of 1 to 3.

In order to investigate the wound healing in control group, sham group, experimental 1 group and experimental 2 groups, a caliper was applied to measure the lesion length on days 3.5, 7, 14 and 21.

Finally, statistical data analysis was conducted using the one-way ANOVA test along with the Duncan test that was performed using the SPSS software (Chicago, IL, USA). To evaluate wound healing in the groups of control, sham, experimental 1 and experimental 2 over the days 3.5, 7, 14 and 21, the length of wound was measured by caliper and the results were analyzed using SPSS and ANOVA and Duncan test

RESULTS

The variations in the wound healing percentage of studied groups on different days are shown in Table 1. There was a significant reduction ($P \leq 0.008$) in the wound healing percentage of sham and experimental 1 groups compared to those of control group and there is a significant increase ($P \leq 0.01$) in the wound healing

percentage of experimental 2 group compared to those of control group on day 3.5 (Figure 1). There was a significant reduction ($P \leq 0.03$) in the wound healing percentage of sham and experimental 1 groups compared to those of control group on day 7 (Figure 1).

There was a significant reduction ($P \leq 0.05$) in the wound healing percentage of sham and experimental 1 groups and increase ($P \leq 0.001$) in the wound healing percentage of experimental 2 group compared to those of control group on days 14 and 21 (Figure 1).

Microscopic studies showed that the percentage of wound healing on day 14 in experimental 2 groups (*B. vulgaris* extract-treated) was higher than those of other groups (Figure 2). On day 21, full recovery was observed in experimental 2 groups which was an indication of the fact that the wound healing process in experimental 2 groups had a more rapid progress compared to others.

Wound healing in the experimental 2 group treated with *B. vulgaris* ointment is higher than the other groups (Figure 1). Full recovery was observed on day 21 in the experimental 2 group that this indicates that the process of wound healing in experimental 2 groups had a more rapid progress. Figures 2 and 3 show the histological sections of wound in control and experimental 2 groups on day 21.

Table 1. Mean ± SD changes in wound healing percentage in studied groups on different days.

Group \ Days	Day 3.5	Day 7	Day 14	Day 21
Control	6.218 ± 1.953 d	21.863 ± 1.1 c	65.382 ± 3.284 b	94.873 ± 1.178 a
Sham	3.625 ± 1.241 a	18.475 ± 0.794 b	48.358 ± 0.562 c	69.158 ± 0.726d
Experimental 1	3.315 ± 0.407 a	17.635 ± 0.814 b	48.04 ± 0.692 c	67.798 ± 0.417d
Experimental 2	8.897 ± 1.178 b	21.803 ± 0.77 c	69.125 ± 3.365 d	100 ± 0 c

Different letters show the significant difference between groups ($P \leq 0.05$).

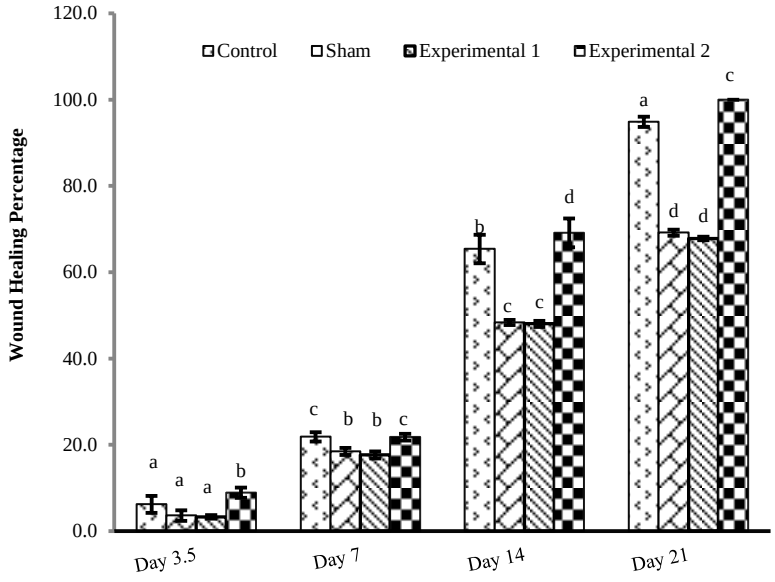

Figure 1. Mean ± SD changes in wound healing percentage in studied groups on different days.

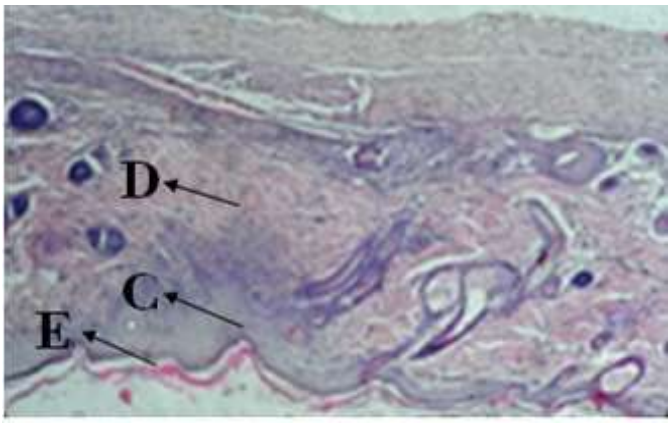

Figure 2. Microscopic study of wound healing in control group on day 21 (×100)
E: the epidermis, D: dermis, C: collagen fibers. (Original)

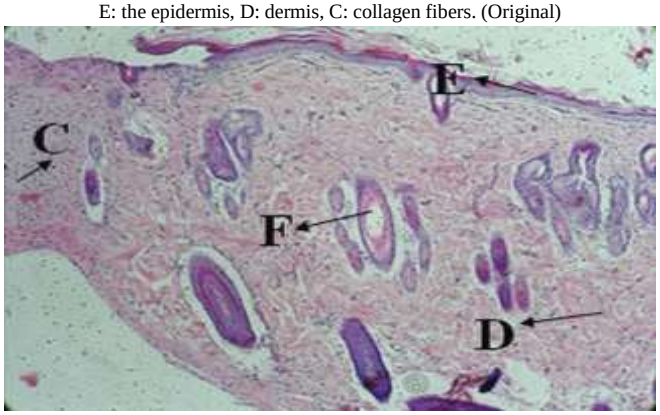

Figure 3. Microscopic study of wound healing in experimental 2 group on day 21 (×100)
E: the epidermis, D: dermis, F: the hair follicle, C: collagen fibers (Original)

DIASCUSSION

For the treatment and healing of diabetic ulcers, plants are the potential source of anti-diabetic medications [10].

The original chemical composition of plants that cause the reduction of blood sugar levels are glycoside, alkaloids, polysaccharides, glycans, mucilages, terpenes, vitamins, saponins, oils, glycoproteins, peptides, amino acids and proteins [11]. Due to lack of having an effective drug for treatment of wounds, it is essential to investigate the effects of herbs for healing of wounds [12-14].

In this study, *B. vulgaris* extract was used for diabetic wound healing treatment. Nowadays, many scientific studies have been conducted regarding the specific properties and effects of this plant on physiological and biochemical systems of the human body.

Barberry fruit contains sugar, malic acid, citric acid, tartaric acid, pectin, gum and vitamin C [15]. The acidity of the fruit has the holding effects and it will prevent microorganisms from growing in large amounts [16]. The therapeutic effects of this herb include reduction of blood pressure, reduction of blood sugar, strengthening heart and liver, anti- itching and other skin disorders [4-6]. One of alkaloids in this plant is anthocyanins which in addition to the characteristic of coloring and being an antioxidant, plays a significant role in preventing heart disease, neurological disorders, diabetes, and cancer [7].

Flavonoids are known to be the common pigment color of plants. In addition, production of some flavonoids begins only as the plant reacts towards a wound. In this

case, flavonoids are referred to as "protective materials" [17].

In this study, the healing effect of the *B. vulgaris* extract on wounds of diabetic rats was investigated and showed that in STZ-induced diabetic rats, the wound healing percentage was lower than control group after 21 days and although the wound length decreased over time, it was never healed. Yet, in experimental 2 groups, full recovery was observed after 21 d and the lesion length almost got zero compared to those of control and sham groups.

Alkaloids have healing effects due to their antioxidant activities. In addition, flavonoids and terpenes increase wound concentration and epithelialization [18]. Such compounds exist in barberry which led to decreased lesion length in experimental 2 group compared to those of control and sham groups and this is probably achieved through development and proliferation of the epidermis layer of the skin, leading to faster wound healing in diabetic rats and since the side effects of diabetes include reduced angiogenesis and cell proliferation, the epithelialization rate mechanism could be counted as a key factor [19].

Plants with high levels of flavonoids have anti-inflammatory properties [20]. Hence, it could be inferred that barberry has anti-inflammatory properties due to having flavonoids where such compounds along with anthocyanins have antioxidant activities and therefore, neutralizes free radicals and improve diabetes and consequently, diabetic wounds.

The berberine present in the barberry extract was successfully used and tested in experimental diabetes mellitus [21, 22].

Similar to metaformin, berberine has demonstrated the ability to lower effectively elevated blood glucose [23]. The mechanisms of action is composed of inhibition of aldose reductase, [24], preventing insulin resistance (25), inducing glycolysis [26] via increasing insulin receptor expression [27] and functioning like incretins [28]. According to the abovementioned functions of berberine, it is probable that berberine could also be effective in wound healing process due to its anti-inflammatory effects.

CONCLUSIONS

Barberry has the ability to heal wounds and increase the wound healing percentage along with reduced lesion length based on the abovementioned reasons and since prevalence of diabetes is increasing in our communities, identification of effective methods of wound healing and reduced amputations would lead to improved quality of life and reduced health care costs.

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