

The Wound Healing Effect of Various Extracts from *Onosma Microcarpum* Root in a Diabetic Animal Model

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ABSTRACT

Onosma microcarpum (Boraginaceae) locally known as “Tashnedary” is considered as one of important medicinal plants in west of Iran. Its roots have been used by the rural healers to treat the burns and wound healing.

In this study, different extracts of roots were used for the evaluation of its healing effect in diabetic wound model in rats. Diabetes was induced in Wistar rats, then wound incised on the back of rat, and subsequently they were divided into nine groups which eight of them contain eucerin with four concentrations of hexane extract (20%, 30%, 40%, 60%) and three other extract as acetone30%, ethanol30% and hydroEthanol30%, and one base eucerin without extract. Other formula was traditional formula and phenytoin. A photograph of the dorsum of the rat was taken from a standard height in days 0, 3, 6, 9, 15 and 20. Then, samples were processed in the pathologic surveys. Our study showed that the best result was demonstrated by Phenytoin cream treated group.

Our results indicated that like the general belief in west of Iran population, the ointment with n-Hex30% extracts of *O. microcarpum* could promote healing in described animal model, diabetic foot ulcer, compared to control.

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Introduction

The number of people triggered diabetes has soared from 108 million in 1980 to 422 million in 2014, with most residing in developing countries which consists of 8.5% of adult people in 2014 [1]. In 2012, an estimated 1.5 million deaths were directly caused by diabetes and another 2.2 million deaths apt to imputation because of excessive blood glucose. WHO estimates that diabetes will be the 7th chief reason of human expiry in 2030 [2]. Prevalence is growing universally, especially in low- and middle-earning nations. Obviously, it is a multifactorial issue mostly including obesity and lower corporal exercise [2]. Diabetes of all types can lead to problems in many sections of the body like sightlessness, heart and renal failure, stroke and lower limb cutting off and premature death [3]. According to the International Diabetes Federation, diabetics are 25 times more plausible to have a limb amputated than those without the ailment [4]. The foot ulcer is a principal reason of their hospital admittance which results in gangrene and amputation through peripheral neuropathy and arterial trigger and infection mostly leading to menace, suffering and mortality [4, 5].

The Boraginaceae family includes about 100 genera and 2000 species distributed in temperate and tropical zones [6]. *Onosma* is an important genus of this family with 150 species widespread in the east and the central Asia and in the Mediterranean area [7, 8]. The genus *Onosma* comprises about 85 species, occurring mainly in Iran, Syria, Turkey, and Europe [9]. Some *Onosma* spp have exerted antimicrobial [6, 10, 11] and wound healing effects [8, 11, 12]. Major constituents of *Onosma* spp are alkaloids, naphtoquinones, polyphenols, phytosterols, terpenoids and fatty acids [6, 8]. Wound healing property of this family attribute to antibacterial, antiviral, antioxidant and anti-inflammatory activities of phenolic compounds, such as alkannin and shikonin [8, 12]. *O. microcarpum*, with red root extract and locally known as "Tashnedari", is one of the most popular folk medicinal plants, which is used in west of Iran as anti-inflammatory and antiseptic in wounds

and burns. Therefore, the objective of this research was to investigate the effect of different roots extracts of *O. microcarpum* in diabetic rat foot ulcer.

Materials and Methods

Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich (USA). Diagnostic device for blood glucose determination was obtained from Gluco Dr brand (South Korea). Ketamine and xylazine was purchased from Alfasan (Netherlands). Diethylether, n-hexane, acetone, and ethanol was purchased from Merck (Germany).

Plant material

The roots of the plant were collected from Harsin, Iran, in June 2015 and dried in shadow. The plant was authenticated by Dr. Masumi, and by comparing with standard specimen in the Herbarium of the Faculty of Agriculture (909 (RuH) Razi University, Kermanshah, Iran).

Preparation of plant extracts

Dried roots powders (60 g) were sequentially extracted with n-Hexan (Hex) and Acetone (Act) by Soxhlet apparatus, and Ethanol (Eth) and hydroethanol (HdE) (50:50) by maceration methods. The extracts were concentrated to dryness *in vacuo*.

To make the traditional formula (TdF) (30%), the root was heated in the oil, then filtered and chilled.

Animals

Male Wistar rats (weighing 250-300 g) bred in the animal house of the School of Pharmacy, Kermanshah University of Medical Sciences were used. They were housed in polypropylene colony cages (5 rats per cage) at ambient temperature ($23 \pm 2^\circ\text{C}$) and 12/12 h light/dark cycles. They were fed *ad libitum* with normal laboratory chow. Before testing for blood glucose levels, rats were fasted overnight (at least 12 h) with free access to water. All experimental procedures involving

animals were approved by the Animal Research Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran. Animals were assigned randomly into groups of at least six rats.

Induction of Diabetes mellitus

Briefly, after a 12-h fast, rats received a single intraperitoneal (IP) injection of STZ (65 mg/kg; Sigma-Aldrich, USA) freshly prepared in 0.1 M sodium citrate buffer (pH 4.5). At 8 d after STZ injection, blood glucose measurement was performed on eye blood by using a glucometer (Gluco Dr, South Korea). Rats whose fasting blood glucose levels exceeded 250 mg/dL (13.9 mmol/dL) were considered diabetic. Water intake and weight were monitored throughout the study, and to confirm the diabetic state, fasting blood glucose measurement was repeated on the day of euthanasia.

Wound excision

On the day of *diabetes mellitus* confirmation, 60 diabetic rats were anesthetized by IP injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (25 mg/kg), and their dorsal surface hair was trimmed with an electric clipper. Rats then were divided into 10 groups depending on the type of the plant extracts formula.

Wounding

The anesthetized rats were separated into 6 groups depending on the type of plant extracts: Hex 30% in Eucerin, Act 30%, Eth 30%, HdE30%, TDF 30%, phenytoin cream 1% concentration.

Then, two symmetric full-thickness round wound were excised extending through the *Panniculus carnosus* in the interscapular region of the upper back of each rat, and the skin flap was cut by using iris scissors. The wounds were cured with extracts daily. A photograph of the dorsum of the rat was

taken from a standard height in days 0, 3, 6, 9, 15 and 20.

At 20 d after wounding, rats were euthanized by diethylether. Then, by using sterile surgical scissors, each wound was harvested in its entirety and placed in a sterile tube contain formalin. Samples were transported to the laboratory for immediate processing and pathologic surveys.

All the procedure was performed on the most active fractions in further concentrations as 20%, 30%, 40% and 60% concentrations.

Results

In this study, the therapeutic effects of different extracts of *O. microcarpum* were assessed on the reduction of wound area. The findings of wound area measurement demonstrated that the treated rats with ointment containing *O. microcarpum* n-Hex30% and HdE30% extract in spite of significant increase in the wound area from day 1 up to day 3, in such way, that the wound area in day 3 in groups n-Hex30% and HdE30% were 187.2 and 205.5 mm², respectively (**Table 1, Fig. 2**). It should be mentioned that the primary wound area in all of the samples was 156.3 mm² (**Fig. 1A**). In other groups, the wound area was gradually reduced during 20 day period, and it was shown that from all of these groups, the best results belong to n-Hex30% group (**Table1, Fig. 2**). In group n-Hex30%, all of six rats were completely recovered. In groups Act30%, the reduction of wound area was noticed, and the mean of wound area in day 20 was 0 mm², and the findings in this group indicated similar results with dose of groups n-Hex30%.

Table1. Mean of wound area in days 3, 6, 9, 15, 20 (Mean ± SD).

Groups	3d	6d	9d	15d	20d
n-Hex30%	187.24 ± 23.8	49.11 ± 16.2	30.83 ± 12.8	1.88 ± 0.4*	0 ± 0*
Act30%	147.00 ± 39.6	52.05 ± 9.8	41.58 ± 14.8	2.78 ± 1.3	0 ± 0
Eth30%	72.31 ± 16.3	44.17 ± 12.4	22.18 ± 10.3	3.37 ± 0.8	0.61 ± 0.5
HdE30%	205.46 ± 12.7	81.63 ± 37.2	38.34 ± 12.1	5.52 ± 1.6	2.68 ± 1.4
TdF30%	80.48 ± 24.5	50.40 ± 16.0	27.77 ± 8.9	5.65 ± 2.6	1.44 ± 0.6
Eucerin	117.47 ± 63.7	51.66 ± 25.2	27.94 ± 11.0	4.92 ± 2.4	1.52 ± 1.3
Phenytoin cream	47.93 ± 17.5	35.33 ± 6.7	13.50 ± 3.7	2.95 ± 0.9	0.32 ± 0.4

d: Day, SD: standard deviation.

***: Significant difference between Hex30% group with Eucerin group(P value <0.05).**

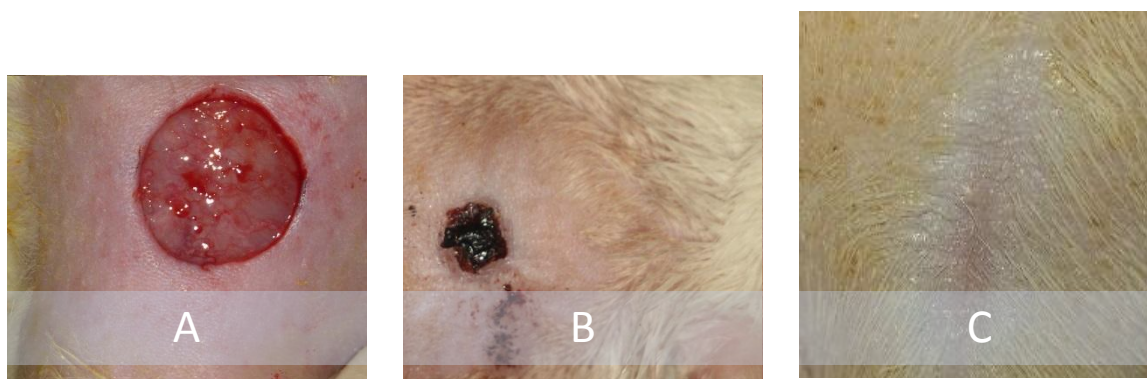


Fig 1. Rat dorsal wound area photograph after application of ointment containing n-Hex30% root extract, at day A: 0, B: 9 and C: 20.

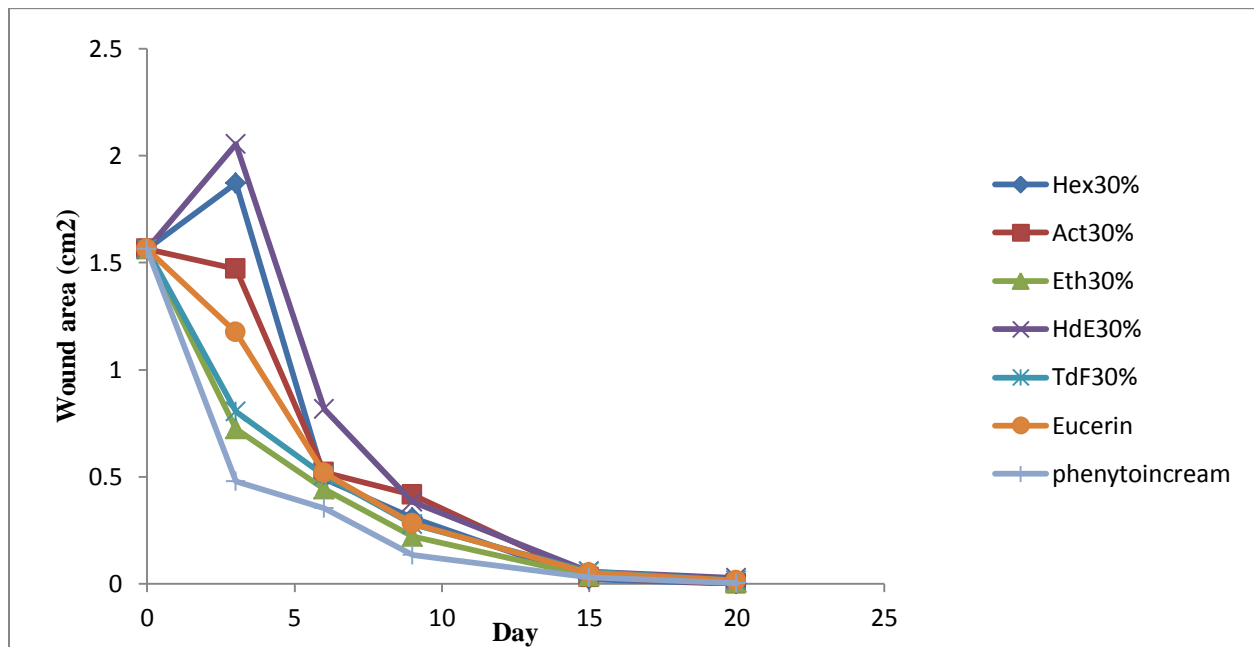


Fig. 2. Comparison of wound area at different days.

In group n-Hex 30%, in spite of significant increase in the wound area from day 1 up to day 3, have shown a suitable effect on diabetic wounds. Mean of the wound area in day 20 was 0 mm² (Table 2, Fig. 3). The ointment containing n-

Hex40% reduction of wound area was 0.884 mm² (Table 2, Fig. 3) that indicated similar results with groups n-Hex30%.

Table2. Mean of wound area in days 3, 6, 9, 15, 20 (Mean ± SD).

Groups	3d	6d	9d	15d	20d
n-Hex20%	73.64 ± 15.6	46.07 ± 21.7	22.24 ± 11.1	3.92 ± 3.0	1.19 ± 1.3
n-Hex30%	187.24 ± 23.8	49.11 ± 16.2	30.83 ± 12.5	1.88 ± 0.4*	0 ± 0*
n-Hex40%	24.19 ± 9.8	27.43 ± 29.9	13.99 ± 14.7	3.59 ± 2.5	0.88 ± 1.4
n-Hex60%	19.32 ± 8.6	29.55 ± 30.1	15.84 ± 15.7	4.20 ± 3.1	1.36 ± 1.4
Eucerin	117.47 ± 63.7	51.66 ± 25.2	13.50 ± 3.7	4.92±2.4	1.52 ± 1.3
Phenytoin cream	47.93 ± 17.5	35.33 ± 6.7	27.94 ± 11.0	2.95±0.9	0.32 ± 0.4

d: Day, SD: standard deviation.

*: Significant difference between n-Hex30% group with Eucerin group (P value <0.05).

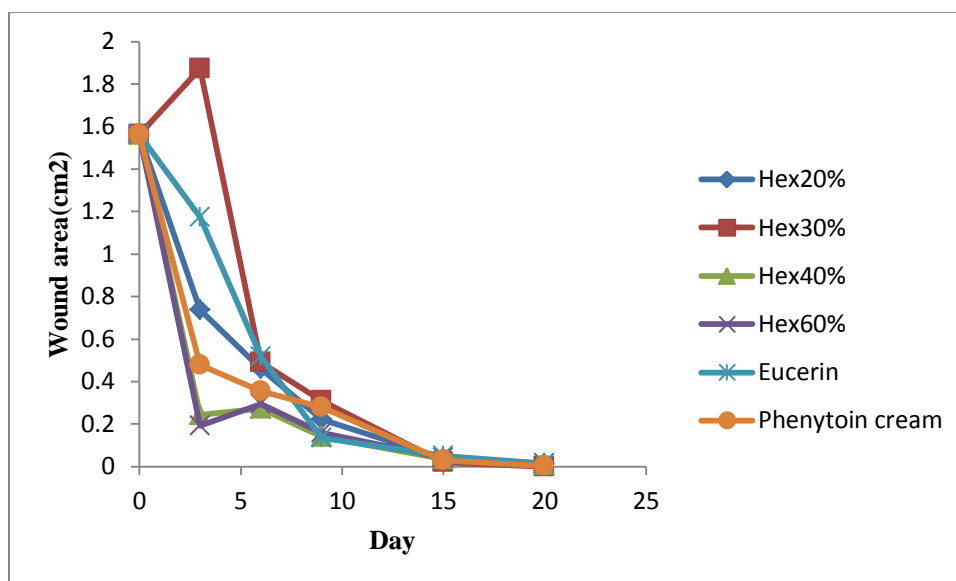


Fig. 3. Comparison of the wound area at different days between various concentrations of n-Hexane extracts.

Histological results

The groups n-Hex30% had most fibroblasts and neutrophils that were 1500 and 1525 respectively (Table 3). Blood vessel in n-Hex 30% and n-

Hex40% was 88 and 200, respectively. Phenytoin cream has shown a similar effect.

Table3. Mean of blood vessels, neutrophils, fibroblasts in 1mm² in day 20.

Groups	Fibroblast	Blood vessel	Neutrophil
n-Hex30%	1500 ± 707.1	88 ± 17.6	1525 ± 2085.9
Act30%	500 ± 0.0	125 ± 106.0	30 ± 28.2
Eth30%	875 ± 176.7	125 ± 106.0	275 ± 318.1
HdE30%	750 ± 353.5	88 ± 17.6	130 ± 169.7
TdF30%	875 ± 176.7	175 ± 35.3	175 ± 106.0
n-Hex20%	400 ± 141.4	75 ± 35.3	55 ± 63.6
n-Hex40%	1000 ± 0.0	200 ± 0.0	38 ± 17.6
n-Hex60%	300 ± 0.0	100 ± 0.0	10 ± 0.0
Eucerin	487 ± 194.8	57 ± 50.0	257 ± 496.2
Phenytoin cream	1250 ± 353.5	150 ± 70.7	275 ± 318.1

d: Day, SD: standard deviation.

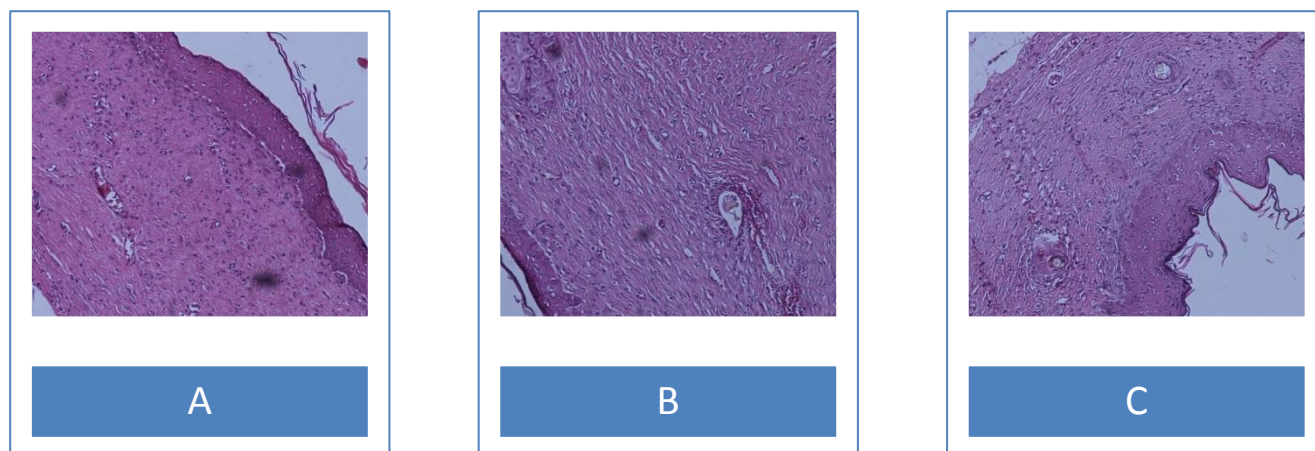


Fig. 4. Histology of granulation tissue of *O. microcarpum*. (A) n-Hex30%; fibroblasts 1500 ± 707.1 , neutrophils 1525 ± 2085.9 and blood vessels 88 ± 17.6 (most effective), (B) n-Hex40%; fibroblasts 1000 ± 0.0 , neutrophils 200 ± 0.0 and blood vessels 38 ± 17.6 , (C) Phenytoin cream; fibroblasts 1250 ± 353.5 , neutrophils 150 ± 70.7 and blood vessels 275 ± 318.1 .

Discussion

Traditional therapy of *Diabetes mellitus* aims not only on attaining a euglycemic condition but also to treat the allied morbidities. Traditional herbal medicines are accessible and better tolerable, much less expensive with less side effects as compared to synthetic prescription drugs [13]. To the best of our knowledge, no published study is available for the evaluation of wound healing effects of *O. microcarpum*, thus present investigation is aimed to study wound healing effects of *O. microcarpum* in animal model.

Previously, healing effects of Boraginaceae family have been studied. For example, hydroethanolic extract of *Arnebia euchroma* which is rich in naphthoquinones, alkannins and shikonins showed wound healing, antibacterial, antifungal and anti-inflammatory effects [14-16]. Besides, Pirbalouti et al. reported the anti-inflammatory effect of *A. euchroma* and *Malva officinalis* in goat lipid as well as its significant impact on fibroblast proliferation and collagen synthesis in burn wounds based on pathological analyses [17].

Similar effects were reported from 10 and 20 percent hydroethanolic extract of *A. euchroma* [18] and aqueous and MeOH extracts of *Heliotropium indicum* [19]. Root and leaf extract of the *Arnebia euchroma* showed wound healing activity by neovascularization, collagenation, anti-inflammatory effect and induction of fibroblast proliferation. The root extract has also been proved to increase re-epithelialization, collagen synthesis, fibroblasts and extracellular matrix [13]. Anyway, roots of *O. dichroanthum* showed negative effect on healing process of burn wound in rats [13]. Ozgen et al. findings support the traditional use of a lipophilic extract consist of n-hexane-dichloromethane (1:1) and 5,8-o-dimethyl acetyl shikonin of *O. argentatum* roots for wounds and burns by stimulation of the growth of human fibroblasts on cell culture [20]. Wound healing effects were reported from a naphthoquinone dimer from *O. echioides* and MeOH extract of *O. hispidum* as well [21, 22].

On controversy, Zarghami-Moghaddam et al. indicated that neither the traditional preparation nor the ointment with acetone extract of *O.*

dichroanthum Boiss. exerted effects on burn wound [23].

Our study is in consistent with the latter research, and shows that like the general belief in west of Iran folk medicine, the traditional preparation of *O. microcarpum* as well as *n*-hexane extract prepared from the plant can promote healing in animal model diabetic foot ulcer. The clinical studies or other animal model of diabetic foot ulcer are proposed in further investigations.

Conclusion

Our results indicated that like the general belief in west of Iran population, the ointment with *n*-Hex30% extracts of *O. microcarpum* could promote healing in described animal model, diabetic foot ulcer, compared to control.

Conflict of Interests

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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