Research Article

Comparison of the Effects of Oral Arnebia Euchroma and Oral ANGIPARS on Wounds in Diabetic Rats

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ABSTRACT

Diabetic foot ulcer is one of the most common causes of amputation and imposes high social costs. The present study was conducted to evaluate the effect of oral arnebia euchroma extract on wounds in diabetic rats and was compared with oral and topical forms of ANGIPARS.Thirty diabetic male Wistar rats were categorized into different groups with similar wounds, and were treated with oral and topical forms of arnebia euchroma extract and ANGIPARS ointment and capsules, and were compared with each other and the control group. The clinical and statistical results indicate the superior effect of oral and topical forms of arnebia euchroma extract in comparison with oral and topical forms of ANGIPARS.The epithelial thickness($F(4.25) = 4189.10$, $p<0.01$) and complete healing time($F(4.25) = 18.01$, $p<0.01$), were respectively calculated. It is recommended that a human study be conducted to determine the level of blood glucose and its effect on TNFa and myeloperoxidase as the factors affecting tissue repair.

Keywords: Arnebia Euchroma; ANGIPARS; Diabetic Foot

INTRODUCTION

According to the International Diabetes Federation (IDF), the prevalence of diabetes in Iran in 2017 is 8.9%, and will reach9.2% by 2030(Majeed et al., 2014).One of the important complications of diabetes is diabetic foot ulcer, and there is a 25% risk of developing diabetes throughout life(Weintrob & Sexton, 2011). About 15% of the cases may lead to amputation. About 15-25% of healthcare resources and costs in developed countries are dedicated to this complication of diabetes (Ranjbar, 2008).It is obvious that diabetic foot ulcer treatment requires familiarity with the mechanism and the cause of it. Diabetes leads to ulcer and disturbs wound healing by causing autonomic, motor and sensory neuropathy, and disturbing the microcirculation. On the other hand, hyperglycemia disturbs the normal repair process through macrophage dysfunction (Alexiadou & Doupis, 2012). In addition to controlling the blood sugar, wound dressing is one of the fundamentals of wound healing. On the other hand, trying to find an oral medication has been another way of overcoming this problem. In 2008, Larijani et al. introducedthe herbal extracts of melilotus officinalis, called ANGIPARS, as topical and intravenous medication, and as a new treatment and superior to other treatments for different types of ulcers, including diabetes mellitus, and suggested that this drug causes wound healing within a monththroughpro-angiogenetic mechanism (Larijani et al., 2008).Romana-Souza et al. studied the effect of oral propranolol on wound healing in 2009, and found that the drug causes wound healing by reducing the inflammation, increasing collagen deposition, and increasing NO levels (Romana-Souza, Nascimento, & Monte-Alto-Costa, 2009).In 2010, Bahrami et al. examined the effect of oral ANGIPARS on 40 diabetic patients and although they confirmed its anti-inflammatory effects, the ultimate effect was not significant compared to placebo. However, they suggested further studies (AMIR Bahrami et al., 2010).In a clinical trial in 2012, Aalaa et al. at the Endocrinology and Metabolism Research Center of Tehran University of Medical Sciences found that an ointment and oral forms of ANGIPARS obtained from melilotus officinalis had anti-inflammatory and micro-vascularisation effects, and was introduced as a treatment for diabetic foot ulcer, especially in refractory cases (Aalaa, Heshmat, Larijani, & Mohajeri-Tehrani, 2012).The arnebia euchroma plant is a member of a Borage family (Boraginaceae) that grows in different countries such as Turkey, Iran, India, and North Africa (Fan et al., 2012). The roots of this plant are known as Havachobeh or Abukhalsa in traditional Iranian medicine. The root of arnebia euchroma is rich in chemicals such as shikonin, alkannin, isohexane, naphthazarin and derivatives of esters, which has important pharmacological properties. These compounds have biological properties, such as wound healing and antibacterial effects (Sharma,

Singh, & Singh, 2009).In September 2017, Noorafshan et al. found that arnebia euchroma extract in diabetic rats was associated with a decrease in blood glucose by affecting beta cells(Noorafshan et al., 2017).Considering the high costs of treatment and considering the need to find new methods, we evaluated the effect of oral form of arnebia euchroma extract on diabetic wounds and compared it with oral ANGIPARS, which is foundin the market of Iran.

MATERIALS AND METHODS

This experimental study was performed on animal models, including 30 adult male rats weighing 250 grams. The root of arnebia euchroma plant was collected in a rural area in the North of Ilam Province and after washing at room temperature and drying in shadow, it was powdered and extracted using Soxhlet extractor with water-alcohol solvent at 50:50.

Figure 1. Arnebia euchroma plant

Investigation of plant cytotoxicity

In order to study the cytotoxic effects of the plant,Verocells, standard MTT method, RPMI medium and serum 10% were used. After determining the concentration in which the alcoholic extract had cytotoxic effects, IC50 was obtained and this figure was 400 mg/ml.

Laboratory Animals

Thirty male Wistar rats were randomly divided into five groups of six and became diabetic by alloxan. Rats were kept in animal cages at Ilam University of Medical Sciences at a temperature of 20-23 °C with a 12/12hour light-dark cycle without any restrictions on water and food consumption.

Study groups

1. Control group withEucerin dressing (G1),
2. Dressing with arnebia euchroma extract (10%) in Eucerin (G2),
3. Dressing with ANGIPARS ointment (G3),
4. Oral extract of arnebia euchroma (0.5 mg daily) (G4),
5. Oral ANGIPARS (0.5 mg daily) (G5).

To create an ulcer from the back of the neck with anesthesia, a wound was created with a size of 1.5×2 cm similarly in all rats. Each group was dressed with the respective drug, daily wound healing was examined and photographed. Finally, if wound healing was observed, tissue sections were prepared from the wound site to determine the formation of granulation and epithelial tissue and the formation of new skin. For histological studies, small 1 to 2 mm sections were prepared from the incision site that was apparently healed and were fixed in formalin 10% for 24 to 48 hours. Then, they were respectively immersed in alcohol 50%, 70%, 90%, and absolute alcohol and were then clarified in xylol solution. The samples were then placed in molten paraffin for 1.5 hours in two steps, and were molded and placed in a refrigerator for several hours and were cut into 6-micron sections by microtome device and the sections were placed on gelatin-containing slides. Finally, hematoxylin-eosin staining was performed and the samples were studied by a histologist.

STATISTICAL ANALYSIS

Data were analyzed using multivariate analysis of variance (MANOVA).

Ethical Clearance

This study was approved by the Research Ethics Committee of Ilam University of Medical Sciences (project number: ir.medilam.rec.1397.125).

RESULTS

The mean and standard deviation of the epithelial thickness and the complete healing process in rats are presented in Table 1.Control group (G1)In the control group, complete wound healing took place with the formation of epithelium on the thirteenth day. Group treated with arnebia euchroma ointment 10% in Eucerin (G2)The wound healing took place on the eleventh day with an epithelial thickness of 102 mm, which was faster and better than other oral form of arnebia euchroma.Group treated with ANGIPARS (G3)In topical ANGIPARS group, complete wound healing took place on the thirteenth day similar to control group and oral ANGIPARS (G5) with a 90 mm epidermal layer thickness, but epithelial thickness was greater than control group (78 mm) and oral ANGIPARS (73 mm).Group treated with oral arnebia euchroma (G4)Healing took place of the eleventh day with an epithelial thickness of 114 mm, which was better and faster than other groups. Group treated with oral ANGIPARS (G5)Complete healing took place on the thirteenth day and the thickness of 73 mm was slower and weaker than other groups. The final results indicated greater effect of arnebia euchroma compared to oral ANGIPARS, which was confirmed in the statistical study (MANOVA).

Figure 2: Figure on the right: Rat in the G4 group on the first day. Figure on the left: Rat in the G4 group on the eleventh day

Table 1 includes the mean and standard deviation of the thickness and time of complete healing in the study groups, according to which the highest mean for thickness and complete healing process is related to G4 group (oral extract of arnebia euchroma) with a value of 114.00 ± 0.63.

Table 1. Mean and standard deviation of thickness and healing time

Study groups	Mean ($\bar{x} \pm S$), thickness (µm)	Mean($\bar{x} \pm S$)
G1	1.0278.58 ±	0.6313.00 ±
G2	0.66±102.08	0.66 11.00 ±
G3	0.6690.08 ±	0.6313.00 ±
G4	0.63114.00 ±	0.6211.00 ±
G5	0.5273.16 ±	0.6313.00 ±

According to Table 2 and based on F-statistic values and the significance level obtained for group factor levels that were respectively calculated to be (F (4.25) = 4189.10, p<0.01) and (F (4.25) = 18.01, p<0.01) for epithelial thickness and complete healing time, it can be concluded that the group factor is a factor that affects the thickness scores and the complete healing time.

Table 2. MANOVA analysis results(Tests of Between-Subjects Effects)

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
group	Thickness	6703.200	4	1675.800	4.189E3	.000
	time	28.800	4	7.200	18.000	.000
Error	Thickness	10.000	25	.400		
	time	10.000	25	.400		
Corrected Total	Thickness	6713.200	29			
	time	38.800	29			

a. R Squared = .999 (Adjusted R Squared = .998)

b. R Squared = .742 (Adjusted R Squared = .701)

DISCUSSION

One of the problems with diabetic patients is abnormal improvement or prolonged wound healing even in appropriate conditions, and given that the disorder has several causes such as neuropathy, vasculopathy and hyperglycemia(Wang et al., 2019),finding a compound with properties that can treat these disorders would be of great importance. Therefore, the present study was conducted to evaluate the effect of oral arnebia euchroma extract and its comparison with oral ANGIPARS on wounds in diabetic rats. Although new drugs and therapies have recently been introduced to treat diabetic foot ulcers, which have been accompanied by significant results, it should be noted that this problem still

remains, and diabetic ulcer is one of the most important challenges. ANGIPARS is one of the common treatments in Iran for improving diabetic foot ulcers. In 2008, Bahrami et al. reported that ANGIPARS has anti-inflammatory effects and improves blood circulation, and considered both oral and topical forms to be effective in the treatment of non-infectious diabetic ulcers and Wagner Ulcer Grades 1 and 2 (A Bahrami et al., 2008).The arnebia euchroma plant is a member of a Borage family (Boraginaceae) with herbaceous appearance, has a coating of sharp silver fuzz and its flowers are clustered and one-sided and its alternate leaves are tangled and narrow. Since this plant has a wide range of biological activities, it is used extensively in traditional medicine.The results of this study showed that oral arnebia euchroma was more effective than oral ANGIPARS in wound healing. These results are consistent with the results of Abdollahi et al. which showed that the hydroxynaphthoquinone compound in arnebia euchroma extract was effective in healing the ulcerative colitis induced in laboratory animals microscopically and macroscopically, due to decrease in TNFa and reduced activity of myeloperoxidase(Abdollahi et al., 2008).

Study Limitations

Other limitations of the study were lack of laboratory examination and a review of the serum levels of glucose or factors such as TNFa or myeloperoxidase. What is certain is the restorative role of oral or topical forms of arnebia euchroma plant and superior effect of the oral form compared to the similar herbal compounds,which requires more detailed studies and more clinical trials.

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Conflicts of interest

The authors report no conflicts of interest in this work.

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