

The beneficial property of hydroalcoholic extract of Annab on burn healing

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Abstract: It has been suggested that Annab (*Zizipus vulgaris* L.) might be beneficial on burn healing, but there is not any evidence in literature for this effect of the plant. This study was carried out to evaluate the effect of hydroalcoholic extract of Annab on burn healing. In this study wounds were made by placing a hot plate with a surface area of 1.5 cm² on the back of animals, for 10s. Forty mice were designated in four groups and treated with vehicle or test substance two times per day for 21 days. The first group received nothing, the second group received Vaseline, the third and fourth groups received 1% and 10% Annab ointment two times/day, respectively. The percentage of burn healing and the total time required for complete healing was evaluated. Results showed that 1% dose of Annab extract had significant burn healing compared to control group (P<0.01). Annab has considerable effect on burn wounds and its usage might be beneficial in these patients.

Key words: Balb/c mice, Burn, *Zizipus vulgaris* L.

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Introduction:

Burns and their resulted damages are among the commonest causes of mortality and inability across the world. About 1200000 people are afflicted with burns in America every year that are in need of treatment (1). Healing dermal burns is a process that is conducted by the harmony of different tissues, cells, and factors (2). Among the most important reasons of delay in wound healing, it can refer to remaining inflammation or the insufficiency of veins secretions (3).

Although with the introduction of synthetic drugs, the usage of medicinal plants decreased (4, 5), however, the multiple side effects of these drugs caused people to return to medicinal plants (6-8). The present synthetic drugs for burn healing have low efficacy and high adverse effects. Medicinal plants are recently used for different diseases and have made good hope for various conditions (9). Recently, some herbal medicines have shown hopeful activities against burn wounds (10, 11). Traditional use of *Ziziphus vulgaris* as a therapeutic agent is common among the people in Chaharmahal & Bakhtiari province, and also the anti-inflammatory effects of this plant have been reported in recent years (12). *Ziziphus vulgaris* is a shrubby plant with stretched

egg like leaves, large fruits in 1.5 × 2.5 centimeter size, ellipse or almost sphere formed, sweet, and edible that grows in broad areas of Iran (13, 14). *Ziziphus vulgaris* fruit contains fat acids, beta-carotene, alpha tocopherol, and seven phenolic combinations including catechin, cafeic acid, epicatechin, ferolic acid, rutin, p-hydroxic benzoic acid and chlorogenic acid (15). There is about 69 milligrams of vitamin C in each 100 gram edible part of raw *Ziziphus vulgaris* (16). In addition, different studies have frequently proved the effects of fat acids and such antioxidants as vitamin C in accelerating wound healing (16, 17). However, no studies have been carried out about the effects of this fruit on the process of burn wound healing. Considering the point that wounds resulting from burns are among the late healable ones and finding natural substances accelerating wound healing with little side effects, can be regarded as a remarkable revolution in treating burn wounds. Consequently, this study was carried out to investigate the effects of hydro-alcoholic extract of *Ziziphus vulgaris* on the process of burn wound healing in Balb/c mice.

Materials and Methods:

In this experimental study, 40 Balb/c mice with approximate weights of 30 ± 3 grams were chosen. Having made burn wounds of 1.5 square centimeter area on the mice's backs by a hot round metal and got sure of type two burning, they were divided into four groups of control, sham and under treatment by two ointments containing extracts of one and ten percent *Ziziphus vulgaris* on Vaseline basis. The animals were kept in the conditions of 22-25 °C temperature, 50% moisture, 12 hour darkness lightness cycle, and usual feeding in separate cages (18).

In order to mark wound, first the animal was anesthetized by injection of Ketamine (50 mg/kg) and Zilazine (50 mg/kg), and then the hair on the back of the animal was removed and the skin thereof was completely cleaned by cotton and alcohol. Afterwards, surface burn wound of type two was made through putting a hot round metal of 1.5 square centimeter area on the fifth vertebra of thoracic for ten seconds. In the groups under treatment by vaseline or 1% and 10% *Ziziphus vulgaris* ointments, 1 gram of Vaseline or these ointments were respectively rubbed on the wounds twice a day, in such a way that the whole area of wound and some parts of its edges were covered. No substance was rubbed on the control wounds. All wounds were remained undressed and open. Microbiological experiments conducted on the utilized ointments showed that they were devoid of any microbial agents (19).

Having made the wound until the complete betterment, wounds were taken photos on 1st, 7th, 14th and 21st days after the animals' anesthesia. Photo taking conditions were the same during the whole experiment period. Using the taken pictures and also video image analysis software, wound area, and percentage of betterment on different days were calculated based on the following formula:

Wound percentage= wound area on the intended day/ wound area on the first day \times 100

And also, betterment percentage=100- wound percentage.

In order to compare the groups generally, first Kruskal wallis test and then Dan test were carried out by SPSS software. The level of $p < 0.05$ was considered statistically significant in the study.

Results:

The percentage of wound betterment on 7th, 14th, and 21st days in groups of control, were 8.06%, 36.47%, and 92.97%, Vaseline 12.57%, 13.90%, and 97.69%, 1% *Ziziphus vulgaris* ointment 30.52%, 96.32%, and 99.96%, and in 10 % *Ziziphus vulgaris* ointment group 12.86%, 75.06%, and 99.38%, respectively.

According to the test of Kruskal wallis, the wound betterment percent was significant among the groups ($p < 0.001$). Using the test of Dan, the percentage of wound betterment in the group of 1% *Ziziphus vulgaris* was higher than the control ($p < 0.01$) and Vaseline groups ($p < 0.05$) (Table 1).

Table 1: Comparison of the betterment percentage of the under study groups on the basis of the test of Dan

Groups	Rank Difference	P Value
Control with Vaseline	-33.3	$p > 0.05$
Control with 1% <i>Ziziphus vulgaris</i>	-36.67	$p < 0.001$
Control with 10% <i>Ziziphus vulgaris</i>	-20	$p > 0.05$
Vaseline with 1% <i>Ziziphus vulgaris</i>	-33.33	$p < 0.001$
Vaseline with 10% <i>Ziziphus vulgaris</i>	-16.67	$p > 0.05$

Discussion:

Healing results in this study were indicator of the superiority of the under treatment groups with 1% *Ziziphus vulgaris* over the control and 10% *Ziziphus vulgaris* groups.

Factors that bring forth inflammation reduction and disinfection have positive effects on burn betterment and healing (20-21). Disinfectant and anti-inflammatory effects of *Ziziphus vulgaris* have been observed in the other studies, so it may be said that the disinfectant and anti-inflammatory effects of *Ziziphus vulgaris* have positive effects on burn betterment and healing. (22-23)

Studies conducted on *Ziziphus vulgaris* fruit have shown 9 fat acids, 2 Saponins, lots of vitamin C,

alpha tocopherol, and 7 phenolic compounds including catechine, cafeic acid, epicatechine, ferolic acid, Rutin, p-hydroxicbenzoic acid, and chlorogenic acid (9,19). On the other hand, it was known based on the conducted studies that fat acids cause the increase in collagen synthesis and also acceleration in wound healing through increasing the level of interleukin 6 (24). Accordingly, the existence of such fat acids in *Ziziphus vulgaris* is considered a positive agent, and also as the cause of the anti-inflammatory property of olive oil is known due to the existence of fat acids inside that can be replaced by arachidonic acid in cells membranes and decrease the substrate needed for the inflammatory enzymes, some parts of its anti-inflammatory effects can be justified for *Ziziphus vulgaris* plant, too. Miscellaneous studies have

frequently proved the strong antioxidant effects of vitamins A and C in accelerating the wound healing (16,25), and as it was mentioned there are such other antioxidants besides vitamin C in *Ziziphus vulgaris* as fat acids, alpha tocopherol, b-caroten, and phenolic compounds like ferolic acid. On the other hand, the study carried out by John et al. shows that the existence of antioxidant vitamins C and E as well as ferolic acid together have better protective effects against the dermal damages resulting from the ultra-violet ray (26). Perhaps, it can also be said in our study that the adjacency of vitamin C and ferolic acid in *Ziziphus vulgaris* fruit has been effective in wound healing acceleration. In addition, the existence of alpha tocopherol, vitamin C, and polyphenols together have synergistic effects on their antioxidant properties (27), and it is perhaps the existence of these three substances together in *Ziziphus vulgaris* has been effective on wound healing acceleration. Ivoone et al. stated in their study that such antioxidants as vitamins C and E, carotenoids, and felenoids take peroxidant property under special conditions and high dosages (28). Since *Ziziphus vulgaris* consists also of these vitamins, carotenoids, and the other antioxidants, the better effect of 1% *Ziziphus vulgaris* than the 10% one may be justified. Meanwhile, some studies have revealed vitamin C has antioxidant and peroxidant properties in the concentrations of 60 to 100 micromollars as well as lower or higher than this range, respectively (29). Furthermore, it has been expressed in studies that the effects of α -tocopherol antioxidants, scorbic acid, and beta-caroten decrease and their peroxidant properties increase under the circumstances they contain a lot of oxygen (30-31).

Conclusion:

Considering the fact that *Ziziphus vulgaris* fruit contains high volumes of unsaturated fatty acids, vitamins A and C, ferolic acid, α -tocopherol, and betacaroten, as well as the remarkable treating effects of such combinations in improving inflammation, the theory of synergistic effects of vitamins C and E antioxidants and polyphenols together, and also the wide availability of *Ziziphus vulgaris* in Iran with cheap prices, it was attempted in the current study to investigate and examine the ointment taken out of *Ziziphus vulgaris* to be used as an ointment effective on burn healing. However, this important point should be taken into consideration that the healing effect of *Ziziphus vulgaris* decreases in the case the dosage of its extract increases. Accordingly, it seems the other concentrations of this extract should be studied to reach better effects and results.

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