

# Effects of Shilajit Extract on Improving Burn in Rats

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**ABSTRACT:** The most important factor in healing wounds, especially, the wound of burn, is prevention of its infection. Shilajit is the natural compound derived from plants in the rocky mountainous. Researchs have shown it has inhibitory effect on Pseudomonas growth. According to role of this substance in traditional medicine and treatment of diseases, particularly bone fractures the aim of this study is to investigate the effects of Shilajit on burn wound healing in rats. Experiments were done on three groups of mice that were burned with 10 repetitions. The control group did not receive any restorative material. For other group, Shilajit solution with a concentration of 18 grams per deciliter was used for wound healing and for the third group was used Silver sulfadiazine ointment on the burns. Percent of improvement in different days of treatment, were calculated with the Feragson and Logan formula. Data were analyzed and compared by using SPSS software. The results showed that the use of Shilajit has statistically significant difference compared to the control and use of Silver sulfadiazine ointment. It seems this healing associated with Shilajit inhibitory effects on the growth of bacteria, elimination of inflammation, increasing growth factors and synthesis of collagen and other factors.

**Keywords:** Burn, Plant Extract, Rat, Shilajit, Silver Sulfadiazine, Traditional Medicine

## INTRODUCTION

In spite of the therapeutic effects of synthetic drugs, due to their unwanted side effects, the tendency of people to use these drugs has diminished on a daily basis, and their tendency to use drugs with a natural origin has increased (Shvetskii and Vorobeva, 1978; Farmahini Farahani et al., 2012).

Over the years, herbal remedies were, in some cases, the only remedy used and the materials used in the pharmaceutical industry were used. Today, the trend towards the use of medicinal plants is due to the low side effects, the variety of effective compounds in them, the development of medicinal plant cultivation, and in particular the proposal of the World Health Organization to use of pharmaceutical compounds has been created (Zargari, 1996).

Burn is one of the leading causes of death in the world (Chester and Papini, 2004). Burn is one of the most common injuries that annually affects a large number of people and is used in many ways to improve it (Farmahini Farahani et al., 2012). Since burn infections are the main cause of death in burns (Sankar, 2006), the use of silver sulfadiazine ointment (a group of sulfonamides) with a broad spectrum of antimicrobial properties is common in most burn centers (Branykardy, 2005). But due to the toxic effect of silver sulfadiazine ointment on the repair of keratinocytes due to adhesion to the surface of the wounds during dressing, the wound healing process is delayed (Dunn and Edwards-Jones, 2004). Other side effects of this drug include increased bacterial resistance, electrolyte imbalance, skin necrosis, skin discoloration, and leukopenia (Fraser et al, 2004). Given these conditions, it is important to find a drug with minimal side effects for the treatment of burn patients (Zargari, 1996; Masmoei et al,

2014 and Branykardy, 2005). from ancient times, preparing for the relief of pain and accelerating the onset of burn wounds has been considered by the medical practitioners, and based on this, various substances such as honey, fish oil, Aloe, potato, etc. are used to treat burn wounds (Visuthikosol et al, 1995).

Shilajit has been used in the folk medicine of different countries for almost 3,000 years. In its raw form, Shilajit is a semi hard, brownish black to dark, greasy resin that has a distinctive coniferous smell and bitter taste. is formed due to the long-term humification of Euphorbia and Trifolium plants (Jung et al, 2002). There is experimental evidence that it contains active chemical constituents that enhance processes of protein and nuclei acid metabolism, stimulate energy providing reactions in liver, promote the transfer of minerals, especially Ca, P, and Mg, into muscle tissue and bone, seem to possess anabolic properties, and increase muscle mass (Jung et al, 2002). Much attention was given to Shilajit action on bone regeneration after fractures.

Extensive research in the 1980s showed that the major organic mass of shilajit comprised humus (60–80%) along with other components such as benzoic acid, hippuric acid, fatty acid, ichthyol, ellagic acid, resin, triterpenes, sterol, aromatic carboxylic acid, 3,4-benzocoumarins, amino acids and phenolic lipids (Agarwal et al., 2007 and Ghosal et al., 1998).

so far, several possible effects have been reported for Shilajit, including Antiulcerogenic and anti inflammatory activity, Antioxidant activity, Learning augmentation, Antidiabetic activity, Antioxidant activity, Memory enhancement and anxiolytic activity, Anti stress activity, Antiallergic activity, Immunomodulatory activity and anti AIDS activity (Agarwal et al., 2007). Its curative potentials were found documented in ancient books and were used to treat many ailments since antiquity days (Aamir Mirza et al., 2010).

The composition of shilajit is influenced by factors such as the plant-species involved, the geological nature of the rock, local temperature, humidity and altitude, etc (Agarwal et al., 2007).

Considering the increasing interest of people using natural drugs and aware of the therapeutic effects of Shilajit, and Considering the effect of environmental factors on the chemical composition of the Shilajit the purpose of this study was to investigate the effect of Shilajit obtained from Iran on the improvement of burn injuries in male rats.

## MATERIALS AND METHODS

**Preparing animals:** This experimental study was performed on 30 adult male Wistar rats weighing 200-250 g purchased from the Pasteur Institute of Iran. Animals received food and water in standard conditions. The temperature of the animal house and the laboratory was 23 Centigrade degree and humidity was 55 percentage and Animals were exposed to 12 hours of light and 12 hours of darkness (Jafari naveh et al., 2012).

**Create scars:** In order to cause burns, the rats were first anesthetized with ketamine and intraperitoneally. Then the hair of the animal was shaved. a circular burn with a 2 cm Diameter was created in the side bodies of the mice (Farmahini Farahani et al., 2012). Fig1.

**Groups under treatment:** 30 rats were randomly divided into 3 groups of 10. Group A (control), which during the research period was not treated by any method and only burns occurred, Group B (under treatment by Shilajit) in which the level of burns was determined by a Shilajit solution with a concentration of 18 g / dl and Group C where the burning surface was completely covered with silver sulfadiazine ointment (Masmoiei et al., 2014).

**Preparation of extract:** Shilajit was prepared from the Sarduyeh region of Jiroft in Kerman province of Iran. Then some of it was dissolved in boiling water at 100 ° C. Then 100 cc of the solution was placed in a 70 ° C incubator for 30 hours to dry completely. For the biological experiments, the dried Shilajit extract was suspended in a balanced salt solution and filtrated through a 0.2-mm filter (Jung et al, 2002).

**Recovery Measurement Method:** Burn recovery was measured by measuring the burn level, burn percentage, infection rate and duration of burn injuries. The level of burn was measured on days 1, 3, 6, 9, 12, 15, 18, and 21 after its creation using Ferguson and Logan method in Centimeter (Jafari naveh et al., 2012). Fig2

In short, in this method, on the days of above, the Transparent Paper was placed on the wound surface and then painted with a marker on the surface of the wound. Afterwards, the figure was transferred to the chessboard and measured by counting the existing squares of the wound surface.

The percentage of burn remission was calculated according to the formula below:

Percentage of wound healing = (Wound surface on the first day - Wound surface on x th day) / Wound surface on the first day (Jafari naveh et al., 2012).

Burning, measuring its surface, as well as measuring the weight of animals at a certain time and by a specific person.

**Statistical survey:** Data were analyzed by one-way ANOVA test to find out the differences between the groups and the Turkey test to determine the difference between the two groups.

## RESULTS

After wounding and starting treatment with the above compounds, the results were reviewed. Were Observed and reviewed the burn level, burn percentage, infection rate and duration of burn injuries. There was no significant difference in the level of burn in the day after the operation between the different groups, until 3 days. but on the third day there was a significant difference between the A group and B group, The A and C groups and B, C groups. It was observed that in the group treated with Shilajit (B group), a layer such as a blood clot included all the burns and there were no signs of infection, but in the group treated with sulfadiazine ointment and the control group, the surface of the burn was heterogeneously connected by a layer and there were signs of infection in it.

On days 3, 6, 9, 12, 15, 18, and 21, the percentage of healing was higher in the B and C groups than in the control group. Despite the fact that on the eighth day, the burns all samples treated with Shilajit and ointment of silver sulfadiazine were completely improved, the control group was the mean of 0.22 cm<sup>2</sup> surface of burns. the duration of complete burn treatment was 21, 18, and 15 days, respectively, in the control group, treated with silver sulfadiazine and treated with Shilajit. that is a significant difference between the control group and the others. (fig3 and table 1).

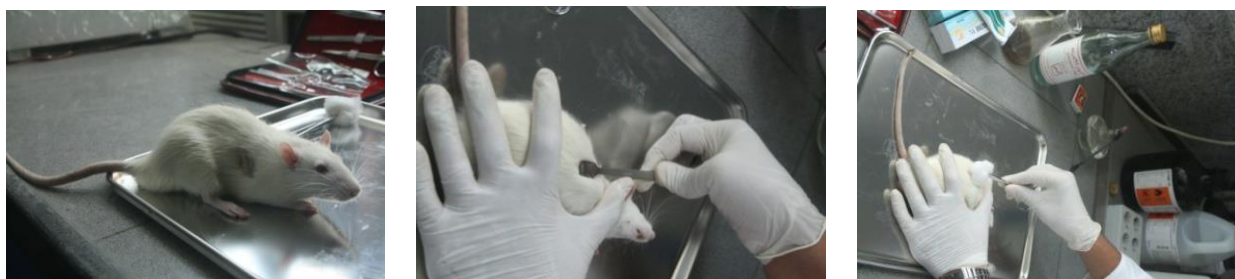


Fig1: step of causing burns: Right to left



Fig2: 3 days after the recovery begins in treatment by Shilajit (right) and Silver Sulfadiazine (center) and control(left)

The mean weight of animals in control groups, the treatment with silver sulfadiazine and shilajit pre-operative was respectively  $206 \pm 8.2$ ,  $213 \pm 7.4$ ,  $198 \pm 8.5$ . Which did not show significant differences in the weight of animals after the operation.  $207 \pm 3.2$ ,  $213 \pm 4.2$ ,  $217 \pm 6.2$

Table1: Burn surface and Percentage of burn remission in different groups on different days after surgery

Percentage of burn remission			Burn surface $cm^2$			
Shilajit	Silver Sulfadiazine	Control	Shilajit	Silver Sulfadiazine	Control	day
–	–	–	$0.2 \pm 2$	$0.2 \pm 2$	$0.3 \pm 2$	1th day
$0.54 \pm 15\%$	$0.22 \pm 15\%$	$1.2 \pm 0.5\%$	$0.2 \pm 1.7$	$0.3 \pm 1.8$	$0.18 \pm 1.99$	3th day
$0.42 \pm 30\%$	$0.34 \pm 15\%$	$0.23 \pm 5\%$	$0.1 \pm 1.4$	$0.1 \pm 1.7$	$0.1 \pm 1.9$	6th day
$0.36 \pm 62.5\%$	$0.48 \pm 35\%$	$0.17 \pm 15\%$	$0.2 \pm 0.75$	$0.3 \pm 1.3$	$0.3 \pm 1.7$	9th day
$0.29 \pm 89\%$	$0.24 \pm 65.5\%$	$0.43 \pm 35\%$	$0.1 \pm 0.22$	$0.2 \pm 0.69$	$0.2 \pm 1.3$	12th day
$0.43 \pm 95\%$	$0.27 \pm 87.5\%$	$0.5 \pm 74.5\%$	$0.1 \pm 0.1$	$0.2 \pm 0.25$	$0.1 \pm 0.59$	15th day
100%	$0.44 \pm 90\%$	$0.39 \pm 89\%$	0	$0.1 \pm 0.2$	$0.1 \pm 0.22$	18th day
100%	100%	$0.28 \pm 95\%$	0	0	$0.1 \pm 0.1$	21th day

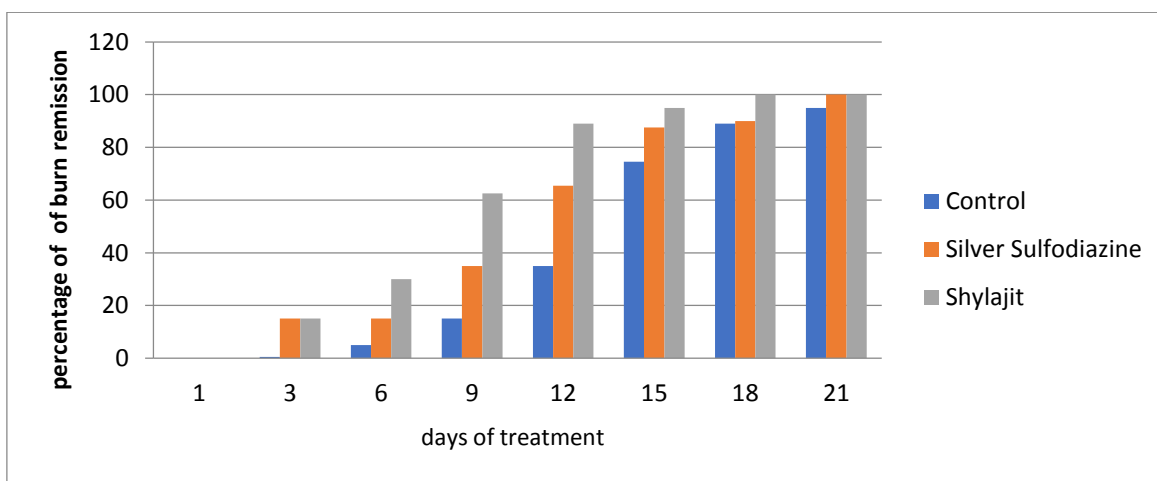


Fig3- diagram of Percentage of burn remission in days of treatment

**DISSCUSSION**

Despite the advances made in the treatment of burns and the production of various drugs in its treatment, there are still many studies on medicinal plants and chemical drugs to find the compounds that affect the process of burning up (Masmoei et al., 2014). The results of this study showed that the level of burns in the group treated with Shilajit and silver sulfadiazine on days 3, 6 and 9 was significantly lower than the control group.

According to the above results, it was found that the 18.3 g/dl of Shilajit solution treatment improved burn treatment especially on days 3, 6 and 9 after starting the treatment. These effects have also been shown to reduce burns and increase the recovery rate, as well as reduce the amount of time needed for complete burn treatment. The study is consistent with the report that eating and also rubbing the Shilajit on the Healing of bone fracture, dislocation and coronation is useful (Tavakoli et al., 2003).

Due to the mechanisms that contribute to the improvement of surface burn, the Shilajit may be due to reduced swelling or inflammation, has led to accelerate recovery in burns. Because modulating the stages of inflammation, accelerates its recovery. Regarding the main role and application of shilajit in the treatment of fractures and the elimination of inflammation caused by it, anti-inflammatory effect on wound healing can be considered for Shilajit (Jung et al, 2002). Another possible mechanism for accelerating the recovery of burns by using a Shilajit may be the increase in oxygen supply to the wound site, because it's reported that there is oxygen in the composition of Shilajit (Jung et al, 2002). also, given that antibiotics control the infection at the site of the wound, they can accelerate the recovery of the burn, and by preventing infections and sterilizing the burning surface, it speeds up recovery. and also given that some results have already been reported on anti-fungal and anti-Bacterial effects of Shilajit (Jaffary et al., 2017). therefore, Shilajit restorative effects can be attributed to its antimicrobial effects. In a comparison between the groups treated with silver sulfadiazine and the groups treated with Shilajit, it was found that the percentage of burn injuries of different days in the group treated with Shilajit was more than burn treatment in the group treated with silver sulfadiazine. Therefore, mechanisms for silver sulfadiazine are used to accelerate burn repair including the Increasing growth factors (Dill and Lacopino, 1997), increasing the number of macrophages (Song and Cheng, 1997), increasing the pulling strength, increasing the formation of new vessels, increasing the synthesis of collagen, and increasing the filtration of fibroblasts (Dacosta et al., 1998), can be considered as possible mechanisms for the Shilajit action.

Similar research has been done on the improvement of burn wounds, which is consistent with the results of this research. Fish oil rich in omega-3 through the formation of collagen from fibroblast produces interleukin (Mousavi et al., 2006; Jafari naveh et al., 2012). Its effect on wound healing is consistent with the results of this research. Also, the anti-inflammatory effect of olive oil on the healing of wound healing is consistent with the results of this study (Farmahini Farahani et al., 2012). Considering the role of anti-inflammatory of fatty substances and the fact that these compounds have been reported in Shilajit (Carrasco-Gallardo et al., 2012), can be considered for its anti-inflammatory role, Various studies have demonstrated the role of antioxidants and elements such as zinc, copper and selenium in accelerating wound healing and Studies have shown that the amount of selenium, zinc and copper in serum decreases in tissue damage and burn wounds (Farmahini Farahani et al., 2012). Due to the presence of many mineral elements in shilajit (Jung et al, 2002), the role of burn restoration can be considered for it.

In general, the results of this study showed that Shilajit accelerates the repair of superficial burns in rats. It was also found that the Shilajit had a more effective effect than sulfadiazine ointment in improving superficial burns. Therefore, it is recommended that a clinical trial be performed before human consumption and the exact recognition of all the possible mechanisms mentioned above such as the effect on the synthesis of collagen, reducing inflammation, increasing oxygenation, increasing the formation of new vessels, and filtration of fibroblasts at the burning position, is required.

## CONCLUSIONS

Shilajit is a humus rich blackish-brown substance, which is very useful in many diseases and serves as a potent tonic. The results of this study showed that the effect of this substance on the healing of wound caused by burn is very strong and compared with the sulfadiazine ointment used to heal the wound, the results are better. Both in terms of acceleration in time of recovery and in terms of the extent of improvement. More physiological tests are needed to suggest that it is used in humans. Therefore, it is recommended that a clinical trial be performed before human consumption.

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