



Antihyperglycemic, antihyperlipidemic and wound healing of *Boswellia serrata* on experimentally induced diabetic rats

Antihiper glucémico, antihiperlipidémico y cicatrización de heridas de *Boswellia serrata* en ratas diabéticas inducidas experimentalmente

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ABSTRACT

Diabetes mellitus is a metabolic disorder with complications such as metabolic syndrome and delayed wound healing. In this experimental study, 36 male Wistar rats were randomly divided into three groups: control, diabetic, and diabetic-extract-treated. Twenty four h after the wound was created in the diabetic group treated with topical *B. serrata* cream 2.5%, and the rats also received aqueous *B. serrata* extract (400 mg/kg) by oral gavage daily for 3 weeks. Diabetes was induced in the rats by subcutaneous injection of alloxan monohydrate (120 mg/kg). After anesthesia, the full-thickness of the dorsal skin (25 mm x 25 mm) was removed. On days 4, 7, 14, and 21, and wound specimens were collected to evaluate histopathological wound healing. After the treatments, blood samples were collected to measure biochemical factors. Consumption of *B. serrata* extracts in the diabetic group significantly decreased glucose, liver enzymes, kidney indicators and lipid profile compared to the diabetic control group ($P < 0.05$). Histopathologic studies showed that the rate of epithelial tissue and collagen fibers formation, as well as wound healing, was higher in the group treated with *B. serrata* than diabetic groups. Hence, it might be useful in diabetic patients, especially the ones with diabetic wounds.

Keywords: *Boswellia serrata*, alloxan, diabetic ulcer, glucose, healing, Rat.

RESUMEN

La diabetes mellitus es un trastorno metabólico con complicaciones como el síndrome metabólico y la cicatrización lenta de las heridas. En este estudio experimental, treinta y seis ratas Wistar machos se dividieron al azar en tres grupos: control, diabéticas y las tratadas con extracto diabético. Veinticuatro horas después de haberseles producido la herida a las ratas del grupo diabético tratadas con la crema tópica *B. serrata* al 2.5%, también se les administró extracto de serrata acuoso B. (400 mg/Kg) vía sonda oral por tres semanas. A las ratas se les indujo la diabetes por medio de una inyección subcutánea de monohidrato de aloxano (120mg/Kg). Luego de estar anestesiadas, se les removió el espesor total de la piel dorsal (25mm x 25mm). En los días 4, 7, 14 y 21 se recogieron muestras de las heridas para evaluar la curación histopatológica de la mismas. Al culminarse los tratamientos, se recogieron muestras de sangre para medir los factores bioquímicos. El consumo de B. extracto de serrata en el grupo diabético redujo significativamente la glucosa, las enzimas hepáticas, los indicadores renales y el perfil lipídico, en comparación con el grupo de control diabético ($P < 0.05$). Estudios histopatológicos mostraron que la tasa de formación de fibras de colágeno y tejido epitelial, así como la de cicatrización de heridas, fue más alta en el grupo tratado con *B. serrata* que en los grupos diabéticos. Por lo tanto, puede ser útil en pacientes diabéticos, especialmente aquellos con heridas.

Palabras clave: *Boswellia serrata*, aloxano, úlcera diabética, glucosa, cicatrización, rata.

INTRODUCTION

Diabetes mellitus is a metabolic disorder that affects approximately 5% of the world's population and is associated with hyperglycemia and abnormal changes in the metabolism of protein, fat, and carbohydrates (Wu and Parhofer, 2014). The destruction of pancreatic beta cells, oxidative stress, insulin resistance, and increased production of reactive oxygen species play important roles in the mechanism of diabetes induction (Chao *et al.*, 2009; Rodrigues *et al.*, 2012).

Chronic complications of diabetes are directly related to hyperglycemic conditions (Gomez-perez *et al.*, 2010). Wound healing is initially delayed by hyperglycemia, excessive expression of inflammatory cytokines, oxidative stress, delayed collagen synthesis, decreased angiogenesis, and microbial infections (Lerman *et al.*, 2003). Studies have shown that increased blood glucose levels in diabetic patients increase inflammation, prevent cell proliferation, and increase the levels of matrix metalloproteinases and inflammatory cytokines (Komesu *et al.*, 2004; Rahati *et al.*, 2016).

Today, commonly used treatments for diabetes include exercise, diet, insulin, and antidiabetic drugs. The use of biguanides and sulfonylurea leads to various side effects, including hypoglycemia, hepatotoxicity, and increased coagulation (Fisman and Tenenbaum, 2009). Over the past decade, medicinal plants in developed and developing countries have played an important role in the treatment of diseases due to their comparatively fewer side effects and various active ingredients (Kumar *et al.*, 2019; Roy *et al.*, 2019).

The importance of medicinal plants is well-known due to the presence of chemical structures with antioxidant activity, at high concentrations, for decreasing and preventing degenerative diseases such as tumors, neurological and cardiovascular diseases, and diabetes (Mentreddy, 2007). A group of plant species that have been used in traditional medicine and has been variously studied is the *Boswellia* genus from the *Burseraceae* family belonging to the Sapindales, which often occurs in tropical regions (Beheshti *et al.*, 2018).

Boswellia serrata, a species of the *Boswellia* genus, is also known, in some texts, as frankincense (Schmiech *et al.*, 2019). *B. serrata* is known as a useful drug for treating or accelerating the recovery of many patients. Based on the oldest sources, *B. serrata* has been used to treat asthma, digestive diseases, joint inflammation, and cancer (Assimopoulou *et al.*, 2005).

The resin of *B. serrata* has numerous pharmaceutical properties such as hypoglycemic (Mehrzadi *et al.*, 2018), antioxidant and peptic ulcer healing (Borrelli *et al.*, 2006). The resin has also shown colon ulcer healing, Crohn's disease treating (Raja *et al.*, 2011), antitumor (Bertocchi *et al.*, 2018), osteoarthritis symptoms reducing and memory and learning enhancing properties (Beheshti *et al.*, 2018). The resin contains certain chemical compounds such as boswellic acids, comprising a group of pentacyclic terpenoids that are found either free or in combination with other substances. Boswellic

acids are the active ingredients of *B. serrata*, the most important of which are beta-boswellic acid, keto-boswellic acid, and 3-acetyl-11-keto-beta-boswellic acid (Miao *et al.*, 2019).

B. serrata contains resin (a combination of terpenes) (60-85 %), gums (a combination of polysaccharides) (6-30 %), and also pentoses and hexoses along with some oxidizing agents and digestive enzymes. It also contains essential oil (5-9 %) that comprises monoterpenes, diterpenes, and sesquiterpenes (Hamidpour *et al.*, 2013).

The toxicological studies on the *B. serrata* resin in different animals showed no significant pathological, hematological, or genotoxic changes up to a dose of 1000 mg/kg (Sharma *et al.*, 2009). Since wound healing is not satisfactory in diabetic patients and studies on new drugs for the treatment of skin wounds in patients with diabetes mellitus to minimize inflammation and accelerate the healing process by increasing fibroblast and collagen synthesis and decreasing the complications of diabetes mellitus are very important; therefore, in the present study in alloxan-diabetic male rats, the hypoglycemic and hypolipidemic effects of oral administration of *B. serrata* extract (400 mg/kg) were investigated. The wound healing process after applying the cream of the extract was also evaluated.

MATERIAL AND METHODS

Experimental animals and protocol design

In this experimental study, 36 male Wistar rats weighing 150-180 g were purchased from the Pasteur Institute of Iran. The rats were randomly assigned to 3-member groups in a polycarbonate cage under standard conditions [20-22 °C Celsius, light/dark cycles (12/12 h), and 65% humidity] with *ad libitum* access to standard water and food at the Pathology Research Center of Islamic Azad University, Shahrekord Branch, Iran. The animals were transferred to the new environment at least one week before the experiments so that they could acclimatize to the environment. The Ethics Committee of the Islamic Azad University, Shahrekord Branch approved the entire protocol of the study. All ethical principles related to the studied animals were observed during the study (Namjou *et al.*, 2018).

Preparation of *Boswellia Serrata*: After obtaining of *B. serrata* resin from Goldaru Co. (Isfahan) and its identification by the botanist of the Medical Plants Research Center of Shahrekord University of Medical Sciences, the sections of the plant were pulverized by an electric mill. Two hundred g of the dried resin powder was macerated in 1000 ml of cooled boiled distilled water and stored in the refrigerator for 48 h. Next, the macerated *B. serrata* was heated in a bain-marie at 60°C until it was dissolved. The solution was then passed through a filter paper, flattened in a glass container, and then placed in an oven at 37°C to dry. From each 200 g of the dissolved powder, 40 g dried extract was obtained.

Afterwards, the dry extract on the dish was collected and, after being dissolved ([Jalili et al., 2014](#)), was used to prepare the *B. serrata* cream and fed the extract at a dose of 400 mg/kg body weight (BW) via gastric tube.

Cream formulation: An oil-in-water (O/W) emulsion-based cream (semisolid formulation) was prepared. The emulsifier (stearic acid) and other oil-soluble ingredients (acetyl alcohol) were dissolved in the oil phase (Part A) and heated to 75°C. The preservatives and other water-soluble components (methylparaben, propylparaben, triethanolamine, propylen glycol, *B. serrata* extract) were dissolved in the aqueous phase (Part B) and heated to 75°C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until the emulsifier was cooled (Table 1).

Table 1. Composition of *Boswellia serrata* seed cream

Material	% of material in formulation W/W
<i>Boswellia serrata</i>	2.5
Cetyl alcohol	5
Stearic acid	12
Glycerol	4
Methylparaben	0.02
Tri ethanolamine	Qs
Water	Qs

Determination of stability of formulation: Stability testing of drug products begins as a part of drug discovery and ends with the complete disappearance of the compound or commercial product. ICH guidelines were used to conduct drug and formulation stability studies. The cream was poured into a bottle and kept in the humidity chamber, maintained at 32 ± 2°C/70 ± 5% RH, and 42 ± 2°C/80 ± 5% RH for two months. After the experiments, samples were analyzed for their physical properties and viscosity and other physicochemical properties ([Nourbakhsh et al., 2016](#)) (Table 2).

Table 2. Physical properties of *Boswellia serrata* cream

pH of the Cream	Viscosity	Acid value	Saponification value	Homogeneity	After feel	Irritancy test	Appearance	Removal
7.35±6	30004±13	6.9±4	30.0±0.7	Good	Emollient	Not reaction	Yellow	Removed by washing with water

Induction of Diabetes in Rats: To induce experimental diabetes rats were injected subcutaneously in the back of the neck with alloxan (Sigma Aldrich, Germany) at 100-120 mg/kg BW in a cool physiologic serum solution. After 72 h, fasting blood sugar (FBS) of above 180 mg/dl was considered to indicate the development of diabetes ([Mostafavinia et al., 2016](#)).

The Method of Creating Wound: After induction of anesthesia in rats by intramuscular administration of xylazine 2% (10 mg/kg) and ketamine 10% (100 mg/kg), the rats were placed on the surgical table in a prone position. The central part of the spine of the dorsal region was scrapped, and 10% Povidone Iodine sterilized the area of interest. By using a Chlorhexidine and a scalpel, a wound was created in a way that full-thickness of the skin, including epidermis, dermis, and hypodermis, covering an area of 2.5 cm x 2.5 cm, was removed.

Grouping of The Rats: Rats were randomly divided into three groups of 12 each as follows:

Group I: Control group including healthy rats treated with distilled water via gastric tube and cream base for 21 days;

Group II: Diabetic control rats treated with aqueous *B. serrata* extract and cream base for 21 days; and

Group III: Rats treated with oral aqueous *B. serrata* extract at 400 mg/kg, divided into three doses, via a gastric tube, and cream 2.5% containing aqueous *B. serrata* extract for 21 days.

The duration of the experiment for all groups was 21 days, and the day of surgery was considered day 0. During the 21 days, each group underwent its respective treatment. Given that the day of surgery was considered day 0 and the size of the wound was fixed in all groups on day 0, on days 4, 7, 14 and 21, the length and width of the wound were measured using a caliper and also for more precision, a smart camera took the image of the wound (Figure 1). The images were converted to the AutoCAD software to measure the area of the wound, and the percentage of contraction or wound healing was calculated by using the formula below (Lee et al., 2012):

$$\text{Wound healing (\%)} = \frac{A_0 - A_t}{A_0} \cdot 100$$

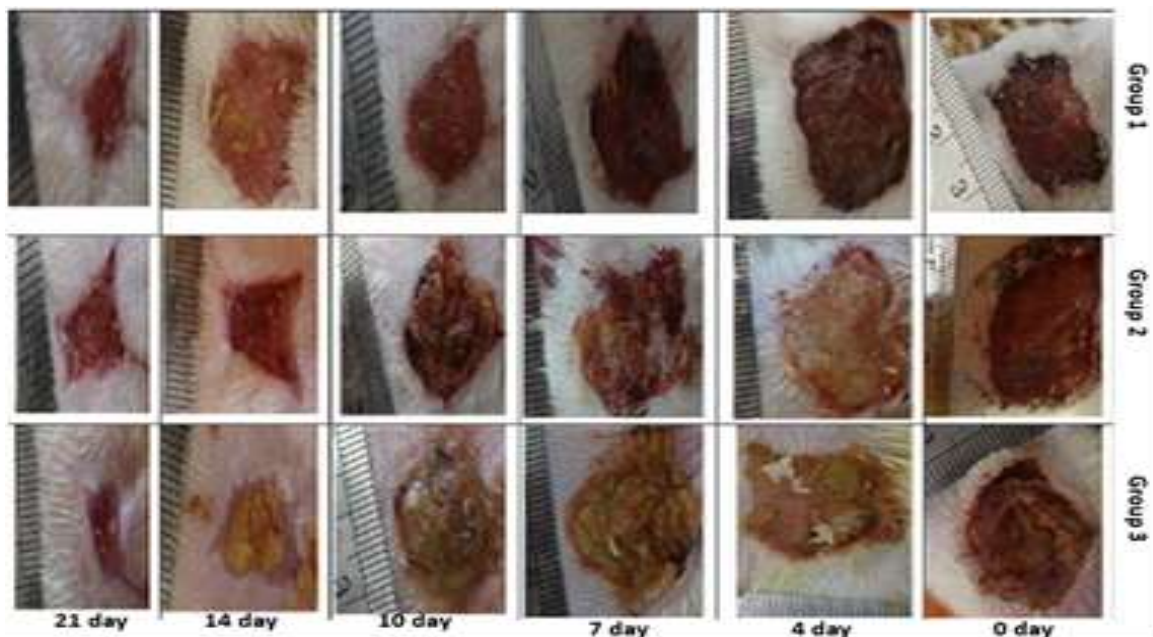


Figure 1. Macroscopic images of the wound healing process in non-diabetic (control), diabetic, and diabetic rats treated with *Boswellia serrata* extract cream 2.5% on days 0, 4, 7, 14 and 21.

Where: A_0 indicates the area of the wound on day 0; and A_t indicates the area of the wound on days 7, 14, and 21. To perform microscopic examinations, on days 4, 7, 14, and 21, in each group, three randomly selected rats were anesthetized, and dermal and epidermal tissue specimens of the wound were removed.

A full-thickness of approximately 4 mm in diameter from the area of the connective tissue, adjacent to the skin, was placed in 10% buffered formalin. After processing and preparation of paraffin blocks, 5 micron thick sections were obtained and stained with hematoxylin and eosin (Namjou *et al.*, 2018). Then infiltration of the inflammatory cells on the wound surface, the formation of granulation tissue, and regeneration of epithelial tissue were evaluated by a pathologist with using an optical microscope Table 3. At the end of the third week, the rats, after 14 h of fasting, were anesthetized with chloroform. After collection of the wound specimens, blood samples were also obtained from the hearts and transferred to 5 ml test tubes with a paraffin-covered bottom and kept at 45 °C for 45 minutes.

Serum was isolated by centrifuge at 3000 rpm for 10 min and kept at -20 °C until needed. Serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), creatine phosphokinase (CPK), urea, and creatinine were measured by standard biochemical kits (Pars Azmoon, Tehran, Iran), using an Auto-Analyst (3000 BT, Biotechnica Co., Italy).

Serum concentrations of triglyceride (TG), total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using the Pars Azmoon Kit (Iran) according to the manufacturer's instructions (Namjou *et al.*, 2018).

Besides, very LDL (VLDL) levels were determined by using the Friedewald formula, and the Atherogenic Index of Plasma (AIP) and Cardiac Risk Index were determined by the formulas below (Othman *et al.*, 2019):

Triglycerides/5 = Very low density lipoproteins

AIP = [Log (TG / HDL cholesterol)]

Cardiac Risk Index = Total cholesterol / HDL cholesterol

Table 3. Scoring system for the histological skin repair of rats.

Score	Epithelial regeneration	Inflammatory cell	Granulation tissue
0		High amount	None
1	Starting	Moderate	Low amount of immature
2	Partial-thickness	Low amount	Moderate degree of maturation
3	Full-thickness	None	Mature
4	Full-thickness organization		

Statistical analysis

Data analysis was performed by SPSS version 21. Biochemical data were analyzed by one-way ANOVA and expressed as mean ± standard deviation (SD). In case the

difference was statistically significant ($P < 0.05$), the data of each two groups were compared by Tukey's test. Histopathological data were analyzed by the nonparametric Kruskal-Wallis test ($p < 0.05$ was considered significant). When p was less than 0.05, then pairwise group comparisons were performed by the Mann-Whitney U test.

RESULTS

Biochemical Evaluations. Intraperitoneal injection of alloxan in rats increased the level of FBS by 200% compared to the control group ($P < 0.001$). In diabetic rats treated with *B. serrata* extract, blood glucose levels decreased significantly when compared to the diabetic group ($P < 0.0001$). The levels of TG, cholesterol, LDL, VLDL, AIP, and the cholesterol/HDL ratio were significantly lower in diabetic rats treated with *B. serrata* extract than in diabetic rats ($P < 0.01$) (Table 4). The levels of CPK were significantly higher in diabetic rats and diabetic rats treated with *B. serrata* extract than in the control group ($P < 0.01$) (Table 5). Biochemical activities of blood urea nitrogen (BUN) and urea were higher in diabetic rats treated with *B. serrata* extract than in control and diabetic groups ($P < 0.01$) (Table 5). Biochemical activities of AST, ALT, and GGT were higher in the diabetic group treated with *B. serrata* extract than in the control group ($P < 0.05$). The results on other biochemical changes in the serum of rats showed no significant difference between the groups (Table 5).

Table 4. Changes in serum glucose and lipid profiles in rats on day 21 in rats of control, diabetic, and *Boswellia serrata* (400 mg/kg body weight)-treated diabetic groups

Groups parameters	Control (Group1)	Diabetic (Group 2)	Diabetic + Boswellia extract (400mg/kg) (Group 3)	p.value
Glucose(mg/dl)	61.200±4.76 ^b	190.25±11.47 ^a	64.40±18.02 ^b	0.001
Triglyceride(mg/dl)	62.4±19.28 ^b	117.5±16.01 ^a	32±13.43 ^c	0.001
Cholesterol(mg/dl)	64.6±11.48 ^a	78.25±4.99 ^a	38±8.12 ^b	0.001
HDL(mg/dl)	43.26±3.87 ^a	39.07±3.49 ^a	42.14±8.15 ^a	0.557
LDL(mg/dl)	30.74±6.98 ^b	40.90±3.3 ^a	27.76±4.47 ^b	0.01
vLDL(mg/dl)	12.48±3.85 ^b	23.500±3.2 ^a	6.40±2.68 ^c	0.001
Cholesterol/HDL	1.84±0.16 ^b	2.01±0.23 ^a	0.922±0.24 ^c	0.001
Atherogenic index	0.146±0.14 ^b	0.47±0.04 ^a	0.08±0.16 ^c	0.001

The number of samples in each group (n): 6, non-similar letters in each row indicate statistically significant differences ($P \leq 0.05$).

Table 5. Biochemical changes in the serum of rats on day 21.

Groups	Control (Group1)	Diabetic (Group 2)	Diabetic + Boswellia extract (400mg/kg) (Group 3)	p.value
Biochemical parameters				
ALT(IU/L)	75.60±11.01 ^a	76.00±6.27 ^a	179.40±102.27 ^a	0.038
AST(IU/L)	212.20±70.71 ^a	345.50±42.17 ^a	478.20±251.02 ^a	0.057
GGT(IU/L)	2.50±0.45 ^b	3.74±0.16 ^b	14.90±5.30 ^a	0.001
ALP(IU/L)	1146.400±243.53 ^a	1699.00±623.96 ^a	1517.40±153.71 ^a	0.698
CPK(IU/L)	826.60±486.47 ^b	1866.50±241.07 ^a	1965.40±495.52 ^a	0.003
Creatinine(mg/dl)	0.320±0.08 ^a	0.400±0.00 ^a	0.400±0.14 ^a	0.383
Urea(mg/dl)	138.80±6.45 ^b	144.50±11 ^b	272.00±44.66 ^a	0.001
BUN(mg/dl)	64.85±3.01 ^b	67.16±5.57 ^b	127.13±20.86 ^a	0.001
Albumin(g/dl)	4.68±0.58 ^a	4.57±0.05 ^a	4.32±0.34 ^a	0.398
protein(g/dl)	7.88±0.72 ^a	7.90±0.25 ^a	7.90±1.03 ^a	0.999

The number of samples in each group (n): 6, non-similar letters in each row indicate statistically significant differences ($P \leq 0.05$).

Evaluation of Wound Healing. Figure 2 shows that the percentage of wound healing or contraction on day 14 in the diabetic rats treated with the extract cream 2.5% was almost twice as high as that in the diabetic group. The percentage of healing on day 14 was also lower in diabetic rats than in the control group.

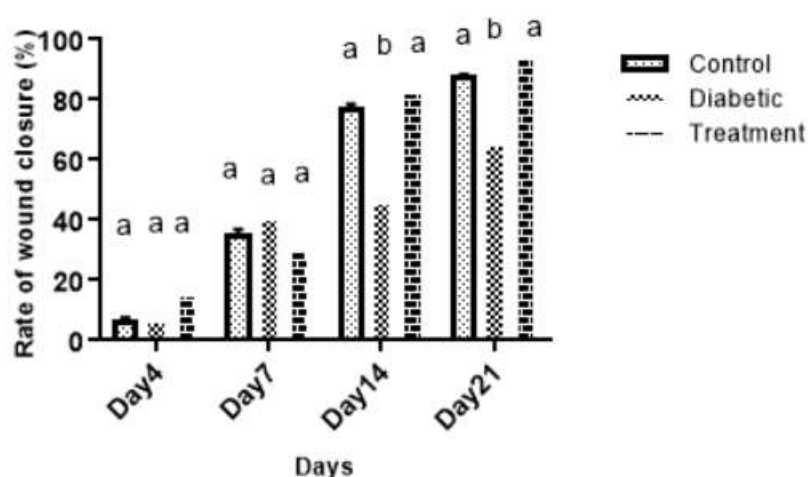


Figure 2. The percentage of wound healing (contraction) on different days in non-diabetic (control), diabetic, and diabetic rats treated with *Boswellia serrata* extract cream 2.5%.

The percentage of wound contraction on day 21 was higher in diabetic rats treated with the cream 2.5% than in the diabetic group (Fig. 3). The area of the wound in the second and third weeks was greater in diabetic rats than in the control group and the group treated with *B. serrata* extract cream 2.5% (Fig 3).

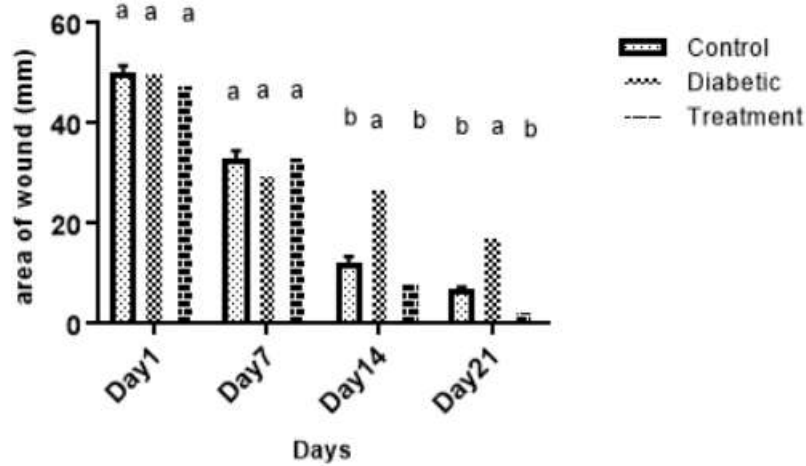


Figure 3. Area of the wound (mm) on days 1, 7, 14, and 21 in different groups; non-similar letters above each column indicate statistically significant differences ($P \leq 0.01$).

The area of the wound on day 21 was lower in diabetic rats treated with the cream 2.5% than in the diabetic group (Fig. 2). The area of the wound on day 21 was greater in diabetic rats than in the control group. Histopathologic studies of the wound tissues in the diabetic group on different days showed the presence of inflammatory cells, bleeding, and necrosis on the wound surface (Fig. 4b). The processes of epithelial tissue regeneration and connective tissue maturation, the density of collagen fibers, and contraction of the wound in the diabetic group took more time compared to those in the control group and the diabetic group receiving *B. serrata* cream (Fig. 4a, Fig. 4c, and Table 6.).

Table 6. Comparisons mean rank and median (Q1-Q3) histopathological evaluation of wound healing among the experimental groups of rats (n = 4/group) 4, 7, 14, 21 days after surgery.

Time	4 Day			7 Day			14 Day			21 Day		
Group	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
Mean Rank	6.5 ^{ab}	2.5 ^b	10.5 ^a	6.5 ^{ab}	2.5 ^b	10.5 ^a	6.5 ^{ab}	2.5 ^b	10.5 ^a	6.75 ^{ab}	2.5 ^b	10.25 ^a
Median(Q1-Q3)	2(0.25-4)			5(3-6)			5(3-7.75)			7.5(4-8.75)		
P value	0.005			0.005			0.006			0.008		

Significant difference compared to the diabetic group. $P \leq 0.01$. non-similar letters in each row indicate statistically significant differences ($P \leq 0.05$).

On day 4, 7, 14 and 21, the processes of formation of epithelial tissue, maturation of granulation tissue, and contraction of the wound progressed much faster in the diabetic group treated with *B. serrata* cream 2.5% than in the diabetic groups (Table 6) (Fig. 2 and 4 c).

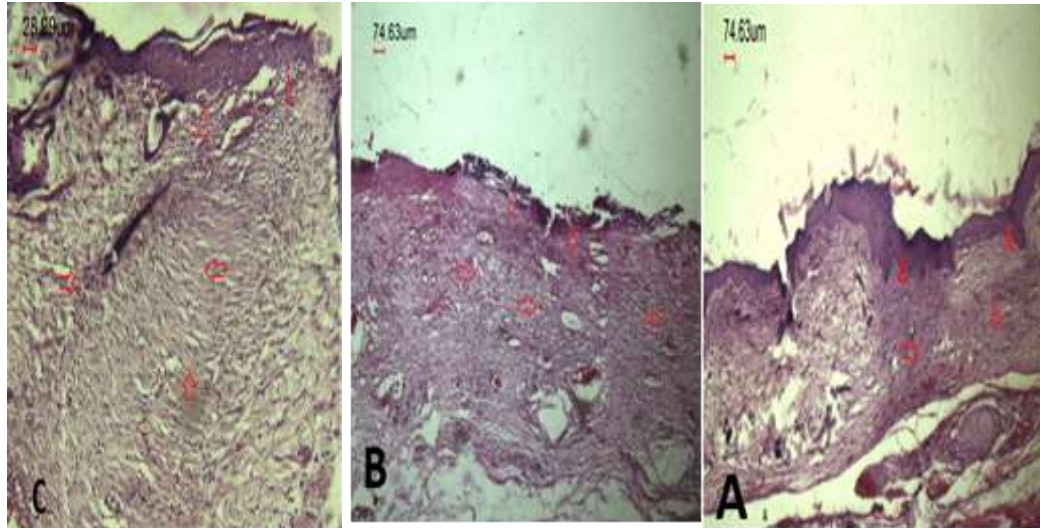


Figure 4. Microscopic images of the process of skin wound healing on day 21. In the control group. (A); epithelial tissue formation on the wound surface (arrowhead), and increased connective tissue along with the atrophy of blood vessels in the dermis (arrow) (H&E × 40); diabetic group (B): The presence of purulent exudate and blood coagulation on the wound surface (arrowhead) and immature connective tissue formation (high-degrees vascularity and full of cells) in the dermal region (arrow) (H&E × 40). the diabetic group treated with *Boswellia serrata* extract cream 2.5% (C): Full epithelial tissue formation (arrowhead) and mature granulation tissue formation with dense collagen fibers (H&E×100). (arrowhead) and mature granulation tissue formation with dense collagen fibers (H&E×100).

DISCUSSION

In this study, alloxan monohydrate was used to induce diabetes in rats. The action mechanism of this substance in the development of diabetes has been frequently studied. It has been determined that alloxan (2, 4, 5, 6, tetraoxypyrimidine) (Elsner *et al.*, 2006), via glucose transporter 2 in the plasma membrane, enters the beta cell (Dra *et al.*, 2018), and by specifically destroying the pancreatic beta cells and producing free radicals in these cells (Bhawali *et al.*, 2019), leading to the rapid release of insulin from these cells. This leads to a rapid drop in blood glucose and then increase in blood glucose and diabetes mellitus in mature rats (Elsner *et al.*, 2006). In the present study, *B. serrata* extract treatment significantly decreased glucose in diabetic rats. Various researchers have pointed to the hypoglycemic effects of *B. serrata* (Mehrzadi *et al.*, 2018).

In an experimental study, the use of a *B. serrata* -containing herbal formula reduced blood glucose levels in alloxan-induced diabetic rats, which was similar to Phenformin hydrochloride induced hypoglycemic effects (Roy *et al.*, 2019). The hypoglycemic

effects of *B. serrata* in diabetic patients have been attributed to the antioxidant properties of the plant (Yang *et al.*, 2020).

The study of Mehrzadi *et al.*, (2018), on the use of *B. serrata* -containing supplements in type 2 diabetic patients showed a decrease in fasting blood glucose and an increase in insulin levels. Evaluation of the use of *B. serrata* extract on lipid profile in diabetic rats showed a significant reduction in TG, cholesterol, LDL, and cholesterol-to-HDL ratio compared to control and diabetic rats.

Also, LDL decreased in diabetic rats treated with *B. serrata* compared with the diabetic control group. In diabetic rats, insulin deficiency causes fat degradation and increase in lipid profiles and free fatty acids (Ahangarpour *et al.*, 2014). In the present study, reduced concentration of TG, LDL, and AIP in diabetic rats treated with *B. serrata* extract compared to the diabetic and control groups, showed a positive role of the extract in preventing an increase in lipid profiles in diabetic rats.

The extract of *B. serrata* appears to improve with insulin secretion by beta cell regeneration (Mehrzadi *et al.*, 2018). In the present study, the reduction in lipid profiles was consistent with a study on the hypolipidemic properties of *B. serrata* resin (Gomaa *et al.*, 2019).

Serum activities of AST, ALT, and GGT is an important enzymes indicator for liver damage (Rikhi *et al.*, 2020). Damage to liver cells causes the ALT and AST to enter from liver cytosol to bloodstream. In this study, AST and ALT significantly increased in diabetic rats treated with *B. serrata* extract compared to the control and diabetic groups, which may be related to the dosage of the extract.

Research on the protective effects of hexane *B. serrata* extract on carbon tetrachloride-induced damage showed that the oral use of the extract at 87.5 mg/kg for nine days was better than the extract at 175 mg/kg (Jyothi *et al.*, 2006).

Urea and creatinine are reliable indicators of renal function (Glastras *et al.*, 2016), so that a significant increase in urea in diabetic rats treated with *B. serrata* extract compared to other groups may be due to kidney dysfunction due to high doses of *B. serrata*. Increased levels of CPK in alloxan-induced diabetic rats treated with *B. serrata* extract may be due to damage to the myocardium (Baird *et al.*, 2012). The acute lethal dose or LD50 of this resin has been reported above 2000 mg/kg (Kumar *et al.*, 2019). The process of wound healing as a natural biological process in the human body consists of four programmed stages: hemostasis (bleeding stop), inflammation, proliferation, enlargement, and regeneration (Lodhi *et al.*, 2013).

Increased blood glucose causes some of the body proteins, such as collagen, to be sweetened, which reduces the flexibility and stability of collagen and delays the wound healing process in diabetic patients (Rahati *et al.*, 2016).

The results of the recovery process and the wound healing percentage on days 14 and 21 in the non-diabetic control group and the diabetic group treated with cream and extract of *B. serrata* were higher than those in the diabetic control group.

The area of the ulcer surface on days 14 and 21 in diabetic rats was greater than those in non-diabetic and diabetic rats treated with cream 2.5% and extract of *B. serrata*. The results showed that the use of cream 2.5% and extract of *B. serrata* had beneficial

effects on the wound healing process in diabetic rats. Which is probably due to the presence of the derivatives of boswellic acid (molecular formula: C₃₅H₅₂O₄) containing pentacyclic triterpene acid with anti-inflammatory properties, and with a reduction in the production of NO, the wound healing process improves ([Ahangarpour et al., 2014](#)).

[Shehata et al. \(2011\)](#) showed that intraperitoneal injection of 150 mg/kg *B. serrata* extracts for ten days resulted in a significant decrease in pro-inflammatory cytokines, such as IL-1A, IL-1B, IL-2, IL-6, interferon-gamma, and tumor necrosis factor-alpha in streptozotocin-induced diabetic rats.

It is not surprising to assume that the resin *B. serrata* extract prevents the transcription and production of cytokine by reducing the function of granulocyte-macrophage colony-stimulating factor, which is the source of production of nuclear factor-kappa B ([Shehata et al., 2011](#))

The mechanism of anti-inflammatory activity of the *B. serrata* extract is due to boswellic acid with the molecular formula C₃₅H₅₂O₄. This compound contains pentacyclic triterpene acid ([Al-Harrasi, 2008](#)), which is very similar to steroid ([Zhang et al., 2013](#)). The function of pentacyclic triterpene acids is different from those of non-steroidal anti-inflammatory analgesics and is related to immune system constituents and 5-lipoxygenase ([Ammon, 2006](#)). Pentacyclic triterpene acids reduce inflammation by blocking the synthesis of leukotrienes ([Koeberle et al., 2018](#)).

One of the main reasons for the delay in the wound healing process in diabetic rats was increased glucose and prolonged inflammation and infection period. This was observed in the macroscopic and microscopic findings of the wound surface of the rats on day 21 of the inflammatory reaction, which delayed the process of wound surface epithelization.

In the diabetic group, the maturation of the granulation tissue and the epithelization of the wound surface of rats are completed in a far more delayed time than those in the other groups.

Extract of *B. serrata* contains various compounds such as tannin, alkaloids, and various flavonoids, each of which alone or in combination is effective to reduce glucose and lipid profiles as well as to heal the wound. Therefore, additional studies are needed on each of the active ingredients to determine the recovery mechanism.

CONCLUSION

The findings on wound morphology and biochemical changes in diabetic rats treated with cream 2.5% and extract of *B. serrata* showed that oral use of the extracts is beneficially effective in reducing blood glucose and lipid profiles. The results also show that the synergistic effect of its topical and oral use increases contraction shortens the time of repair and epithelization of the exposed wound, which is one of the main components of wound healing. *B. serrata* is a safe herbal remedy with antioxidant properties, and efficient in the treatment of wounds in patients with diabetes. However, using the extracts of this plant at high doses may cause liver and kidney damage.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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