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ORIGINAL RESEARCH

Biochemical, Histopathologic, and Genotoxic Effects of Ethanol Extract of *Salvia hypargeia* (Fisch. & Mey.) on Incisional and Excisional Wounded Diabetic Rats

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ABSTRACT

Purpose: Nonhealing wounds are a serious problem of diabetic patients. *Salvia* species are traditionally used for the treatment of wounds. The aim of the study was to investigate the effects of ointment prepared with ethanol extract obtained from the aerial parts of *Salvia hypargeia*, an endemic plant from Turkey, on diabetic rat incisional and excisional skin wounds. *Materials and Methods*: Male Wistar albino rats (*n*: 60) were divided into five groups. Diabetes was induced and two concentrations (0.5% and 1%) of the extract were used for ointments and applied on wounds for 7 and 14 days. Fito cream was chosen as a reference drug. *Results*: In excisional wounds, healing ratios of 0.5% (63.4% and 99.3%) and 1% (65.5% and 99.9%) *S. hypargeia* groups were higher compared to control (35.9% and 75.1%), and in incisional wounds, healing ratios of 0.5% (78.1% and 98.5%) and 1% (84.4% and 99.4%) *S. hypargeia* groups were higher compared to control (30.5% and 72.9%) ($p < .01$). Hydroxyproline (0.31 ± 0.3 and 0.34 ± 0.2) levels were lower and GSH (10.7 ± 3.1 and 7.6 ± 0.9) levels were higher in 0.5% and 1% *S. hypargeia* groups on the 14th day ($p < .01$). Histopathological results revealed re-epithelialization and formation of granulation tissue in all *S. hypargeia* groups. Genotoxicologic results indicated, GDI, DCP values, and MN frequency of 0.5% and 1% *S. hypargeia* groups did not reach to significant levels both on the 7 and 14 days. *Conclusions*: *S. hypargeia* may have a potential for therapeutic use in treatment and management of diabetic wounds with a successful topical application.

Keywords: wound healing; diabetes; Salvia hypargeia; GSH; histopathology; genotoxic effect

INTRODUCTION

Complications associated to diabetes have already become a serious health issue [1]. Hyperglycemia in diabetes is responsible for delayed or impaired wound healing [2]. Researchers performed human and animal models that have demonstrated several impairments associated with diabetes even at the molecular level in delayed wound healing [3]. Due to vascular disruption and high oxygen consumption by metabolically active cells, the microenvironment of the early wound is depleted of oxygen and becomes hypoxic. Diabetes prompts impaired vascular flow resulting with poor tissue oxygenation so a hypoxic wound occurs [4]. Wound healing is a complicated biological mechanism involving coagulation

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of blood, inflammation, cell migration, extracellular matrix deposition, and remodeling [5]. Finally, remodeling phase gives its place to scar tissue, matured tissue gradually regains its tensile strength with time [6]. These tightly regulated processes occur in a systematic manner [7].

Medicinal plants continue to be an important source of compounds being used for human health. Regarding their long use in traditional medicine, as well as their prophylactic properties, *Salvia* species are applied for their anti-inflammatory, antidiabetic, antioxidative, antiproliferative, antibacterial, antifungal, antiviral, and cytotoxic effects [8, 9] and also for the treatment of wound healing [10, 11]. *Salvia* species have active secondary metabolites such as flavonoids, phenolic acid, and terpenoids. Essential oil compositions of *Salvia* species are rich in oxygen-containing sesquiterpenes, monoterpene hydrocarbons, and oxygen-containing monoterpenes [12].

Salvia is one of the largest genera in the family of Lamiaceae [13]. There are 100 species recorded in the Flora of Turkey and the ratio of endemism in Turkey is 53% [14, 15]. *Salvia hypargeia* Fisch. & Mey. is one of the endemic species which belongs to the genus. According to literature, free-radical-scavenging [16] and antimicrobial [17] activities of aerial parts of the species were reported. But, we did not encountered any study on diabetic wound healing properties of endemic *S. hypargeia.* Therefore, the objective of the present study was to evaluate wound healing effects of ethanolic extract obtained from aerial parts of *S. hypargeia* on both excisional and incisional wound models in diabetic rats.

MATERIALS AND METHODS

Experimental Groups and Wound Creation

The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH Publication no. 85-23, revised 1996). All experimental protocols were approved by the Ethics Committee of Mersin University. Male Wistar albino rats 3–4 months aged and weighing 200–250 g were used in the study (*n*: 60), divided into 5 groups as shown in Table 1.

They were housed in separate cages and fed with standard chow diet and water *ad libitum* with light/dark cycle of 12 h/12 h at 25 ± 3 °C. A single dose of 45 mg/dL streptozotocin (STZ) (Sigma Chemical Co., USA) was given to rats (i.p.) to induce diabetes. Fasting blood glucose levels were measured using a rapid glucometer (Bayer, Germany) after 24 h of the injection by taking blood from the tail vein and animals with blood glucose levels above 250–300 mg/dL were used in the study [18]. None of the animals were excluded from the experiment and they remained hyperglycemic until the end of the experimental period [19].

Excisional and incisional wound models were created under intraperitoneal injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (30 mg/kg) anesthesia in all groups. In order to create *excisional wound model*; skin tissue of 1,5 cm diameter in a circular manner was removed by punch biopsy on the dorsal interscapular region of rats and left open.

Incision-type wound model; was created from 2 cm posterior to the excision-type wound with 4 cm in length and then three surgical sutures were placed 1 cm apart from each other [10].

Plant Material and Ointment Processing

The aerial parts of *S. hypargeia* were collected from Sivas province, between Akdagmadeni and Sarkısla, rockys areas, 39.58464°N, 36.20585°E, Turkey at an altitude of 1386 m (Voucher references; A. Kahraman 2112), Irano-Turanian element with 8.4% yield. The extraction yield was 8.4% and the extraction was performed manually as described. The powdered air-dried aerial parts of the plant were macerated with ethanol (96%), filtrated and evaporated using a vacuum evaporator (Heidolph-Rotar, Germany) and stored at 4 °C. Ethanol extract with two concentrations

TABLE 1. Description of experimental groups (*n*: 60)

Group name	Application type	Number of animals (*n*)a	Group code
Diabetic control group	Diabetic group treated with any material-negative control	12	Control
Diabetic vehicle group	Diabetic group treated with simple ointment base (glycol stearate:propylene glycol:liquid paraffin (3:6:1))	12	Vehicle
Fito® cream group	Diabetic group treated with Fito® cream-positive control	12	Fito
0.5% (w/w) *S. hypargeia* ointment group	Diabetic group treated with 0.5% (w/w) *S. hypargeia* ointment	12	SH-0.5
1% (w/w) *S. hypargeia* ointment group	Diabetic group treated with 1% (w/w) *S. hypargeia* ointment	12	SH-1

[a]For each group; *n*: 6 for 7-day applications and *n*: 6 for 14-day applications.

(0.5% and 1%) of *S. hypargeia* were added respectively in mixtures to prepare the simple ointment. Ointment base was prepared with glycol stearate: propylene glycol:liquid paraffin in the ratio of 3:6:1 [10]. Then, 0.5% and 1% (w/w) ointments of *S. hypargeia* were prepared (SH-0.5 and SH-1). 0.5 g ointment was topically applied on the wound area once a day for 7 and 14 days. Fito® cream (Tripharma Drug, İstanbul, Turkey) was used as a reference drug, containing 15% (w/w) *Triticum vulgare* L. aqueous extract.

Macroscopic Studies

Wounds were photographed by a camera (Spot Insight QE, Diagnostic Instruments, USA) on day 0, 7, and 14 days and evaluated with SPOT Advanced (Diagnostic Instruments) program to identify the size of wound healing. Incisional and excisional wound healing were evaluated by measuring the length and surface of wounds and equation below was used to calculate the wound healing ratio [19].

$$\text{Wound healing ratio } (\%)\text{: } 100 \times (1 - \text{specific day wound size/initial wound size})$$

Biomechanical Study

Tensile Testing System (Ilfa Electronic San.Tic.Ltd., Turkey) was used for biomechanical analyses of incisional wounds. Skin samples were placed between two clamps and pulled to rupture at a crosshead speed of 25 mm/min [20, 21]. The data was verified with the software (Ilfa Electronic San.Tic.Ltd., Turkey) and evaluated with Logger Pro Software Program (V 3.8.3, Vernier Software & Technology, Orlando, USA).

Biochemical Studies

On day 0, 7, and 14 day of excision, wound area from the rat skin were resected and biochemical analysis of hydroxyproline content, malondialdehyde (MDA), glutathione (GSH), and nitric oxide (NOx) were performed. To measure hydroxyproline levels, wound tissues were diluted, homogenized, and centrifuged. 25 µL from each of the sample was dissolved in the 1 mL of 50% (v/v) isopropyl alcohol. Chloramine-T was added to these samples 10 min later and incubated for 90 min at 50 °C after adding 1 mL Ehrlich's reagent. After the reaction, measurement of color changes were performed using spectrophotometer at 560 nm (Shimadzu UV 1601, Shimadzu, Tokyo, Japan). Sample concentrations were calculated with standard curve. Results were given as µg/mg dry weight of tissues [22]. The thiobarbituric acid; TBARS assay kit (Cayman Chemical, USA) was used to measure tissue MDA levels at 530 nm [23]. GSH assay kit (Cayman Chemical, USA) was used to measure tissue GSH levels at 410 nm [24]. Tissue NOx levels were measured using assay kit (Cayman Chemical, USA) at 540 nm [25].

Histopathological Studies

The entire wound area and peripheral intact tissue were removed from each animal for histopathological studies. Tissues were fixed in 10% neutral formaldehyde and embedded in paraffin wax after a routine tissue follow-up for histopathological examination. Tissue sections of 4–5 μm thick were cut then, stained with Hematoxylin eosin (H&E), Gomori Reticulin Stain Kit (Atom Scientific, UK), and Verhoeff-Van Gieson Stain Kit (Atom Scientific, UK) examined under a light microscope (Olympus, Japan). H&E staining and Van Gieson Stain kit were used for detecting re-epithelialization, angiogenesis, dermal inflammation. Gomori Reticulin stain kit was used for determining granulation tissue formation and observing type III collagen fibers.

A semi-quantitative histological evaluation scoring system was used to determine histopathological changes. Each specimen was scored using a scale ranging from 0 to 3. Re-epithelialization; 0 (no re-epithelialization), 1 (mild: less than 50%), 2 (moderate: >50%), and 3 (complete re-epithelialization) [26]. Angiogenesis; 0 (no angiogenesis), 1 (<5 vessels per one high power field), 2 (6–10 vessels per one high power field), and 3 (more than 10 vessels per one high power field). Depth of dermal inflammation; (0: no dermal inflammation, 1: in superficial dermis, 2: in reticular dermis and 3: involving subcutaneous fat tissue) granulation tissue thickness and Type 3 collagen fibers were scored as; 0 (none), 1 (scant), 2 (moderate), 3 (marked) [27]. Absence and presence of fibrosis were evaluated as 0 and 1, respectively.

Genotoxicologic Studies

The Comet Assay. Comet assay was performed on rat peripheral blood samples according to the method by Singh et al. [28] after 7 and 14 days applications. Blood samples were collected from rat heart by venipuncture and added 10% Fetal Bovine Serum (FBS, Capricorn Scientific GmbH, Germany) washed two times then, isolated cell samples were used. Microscopic slides were covered with 0.5% normal melting agarose (NMA, Sigma Chemicals

Co., USA) dissolved in PBS (Capricorn Scientific GmbH, Germany) at 50 °C. 100 µL of cell suspension was diluted with PBS (1 mL) and kept in water bath at 40 °C.30 µL of the mixture was stirred with 250 mL of Low Melting Agarose (LMA, Sigma Chemicals Co., USA) (0.5%). The prepared mixture was put on the NMA-coated slides. After preserving at +4 °C for 15 min., coverslips were carefully removed and slides were submerged in lysis solution and stored in dark at 4 °C for 2 h. Slides were washed with chilled distilled water and put on a horizontal gel electrophoresis filled with electrophoretic buffer (0.3 M NaOH + 1 mM EDTA) for 20 min for DNA unwinding Electrophoresis process was applied for 20 min (25 V, 300 mA). Slides were then washed and stained with Etidium Bromide and examined under a fluorescent microscope (BX51, Olympus, Tokyo, Japan). Mitomycin-C (MMC-2mg/kg) was used as a positive control. Two parameters were evaluated as *Genetic damage index* (GDI) and *Damaged Cell Percent* (DCP). The results were given as Arbitrary Units (AU) values which used to express the DNA damage. The equation given below were used for calculation of results. AU values indicating the comet assay scores were as: UD (undamaged, 0); Type 1 (low damaged, 1); Type 2 (moderate damaged, 2); Type 3 (high damaged, 3); Type 4 (ultrahigh damaged, 4).

N_i: Number of scored cells in i level.
i: Level of DNA damage (0, 1, 2, 3, and 4)
GDI: $[(0 \times \text{Type } 0) + (1 \times \text{Type } 1) + (2 \times \text{Type2}) + (3 \times \text{Type } 3) + (4 \times \text{Type } 4)]$
DCP: Type 2 + Type 3 + Type 4

The micronucleus assay. Micronucleus assay (MN) was applied according to Holden et al. on rat peripheral blood samples after 7 and 14 days applications. Blood samples were spread onto glass slides. They were air dried and fixed with methanol. Stained with acridine orange (125 mg/mL phosphate buffer, pH 6.8) and examined under fluorescence microscope [29]. 2000 polychromatic erythrocytes (PCE) per animal were used for micronucleated polychromatic erythrocytes (MNPCEs). The MMC was used as a positive control. MN assay results were evaluated according to color changes (PCEs in orange-red color, erythrocytes in green and micronuclei in yellowish color).

Statistical Analysis. Data analysis was performed by SPSS 20.0. Data were represented as the mean ± S.D. for 60 rats and were evaluated using ANOVA with Tukey post-hoc test. In genotoxicity studies, normality of the data was confirmed with Kolmogorov-Smirnov D test ($p \geq 0.05$) and student t-test. Values of $p < 0.05$ and $p < 0.01$ were considered to be significant.

RESULTS

Macroscopic Results

Wound healing ratios of 0.5% *S. hypargeia* group, SH-0.5, (63.4% & 99.3%) and 1% *S. hypargeia* group, SH-1 (65.5% & 99.9%) were higher ($p < .01$) compared to diabetic control group (35.9% and75.1%) in excisional skin wounds on the 7th and 14th days. Also, healing ratios in incisional skin wounds of SH-0.5, (78.1% & 98.5%) and SH-1 groups (84.4% & 99.4%) were higher ($p < .01$) compared to diabetic control group (30.5% and 72.9%) on the 7th and 14th days as shown in Table 2.

Photographs of excisional and incisional wounds on day 0, 7, and 14 are shown in Figures 1 and 2.

Biomechanical Results

When groups of SH-0.5 and vehicle groups were compared on the 7th day, results of SH-0.5 group were found to be higher in terms of stored energy, tensile strength, durability, and maximum stress parameters ($p < .05$).The average of maximum strain with the maximum deformation at the SH-1 group was higher than the vehicle group on the 7th day and the average of energy and endurance stored in the SH-0.5 on the 14th day group was higher than the vehicle group on the 14th day ($p < .05$). When groups of SH-0.5 and SH-1 on the 14th day, compared with fito group on the 7th day, the average of all the intrinsic (structural) and extrinsic (material) properties were observed to be similar to each other (Table 3).

Biochemical Results

Hydroxyproline levels on the 7th day were higher in SH-0.5 and SH-1 groups compared to control groups ($p < .01$) (Figure 3a). Hydroxyproline levels were also high in fito group ($p < 0.05$). At the end of the experimental process on the 14th day, vehicle group results showed higher levels of hydroxyproline ($p < .05$) (Figure 3b).

Antioxidant MDA results of SH-0.5 and SH-1 were lower than control on the 7th day ($p < .01$) and also, were lower in vehicle and fito groups ($p < .05$) (Figure 3a). On the 14th day; MDA levels were lower in fito, SH-0.5 and SH-1 compared to control and vehicle groups ($p < .01$) (Figure 3b.).

TABLE 2. Wound healing ratio of excisional and incisional wounds (% contraction)

Groups	Control	Vehicle	Fito	SH-0.5	SH-1
Excision					
7 Day (Min-Max)	35.9 ± 22.8a (9.8–60.2)	43.6 ± 13.8a,b (26.8–61.9)	68.7 ± 7.4c (55.8–79.8)	63.4 ± 7.7c (49.3–70.7)	65.5 ± 12.3c (43.2–70.7)
14 Day (Min-Max)	75.1 ± 18.8a (38.2–89.9)	80.4 ± 12.8a (58.1–90.7)	99.7 ± 0.2b (98–100)	99.3 ± 1.6b (95.9–9.9)	99.9 ± 0.01b (99.9–100)
İncision					
7 Day (Min-Max)	30.5 ± 17.1a (14.1–58.1)	44.7 ± 19.2a (14.1–64.6)	81.25 ± 10.1c (59.7–90.1)	78.1 ± 7.4c (71.8–89.5)	84.4 ± 15.9c (52.7–96.3)
14 Day (Min-Max)	72.9 ± 10.8a (55.8–87.2)	75.1 ± 10.7c (58.9–82.1)	99.5 ± 0.5c (99.3–100)	98.5 ± 2.2c (94.6–100)	99.4 ± 0.8c (98.2–100)

Control: Diabetic control group; Vehicle: Diabetic vehicle group; Fito: Fito cream group; SH-0.5: 0.5% (w/w) *S. hypargeia* ointment group; SH-1: 1% (w/w) *S. hypargeia* ointment group.
[a]Significantly different from DM-control group ($p < .05$).
[b]Significantly different from Fito group ($p < 0.05$).
[c]Significantly different from DM-vehicle group ($p < 0.05$).

On the 7th day, no difference was observed between the groups regarding GSH values (Figure 3a). On the 14th day of experiment, GSH values of SH-0.5 group were higher than other groups ($p < .05$) (Figure 3b).

No difference between the groups was observed regarding antioxidant NOx values on the 14th day ($p > .05$) (Figure 3b).

Histopathologic Results

Histopathological scores of the whole wound area in all groups were presented in Table 4.

Angiogenesis and re-epithelialization scores of ointment treated and fito groups were higher than the control group on the 7th and 14th days however, inflammation scores were lower in treated and fito groups compared to diabetic control group ($p < .001$). Type III collagen scores were lower on the 7th day in treated groups compared to diabetic control group ($p < .001$). Granulation tissue scores were lower in SH-0.5 and SH-1 groups on the 7 and 14 th days ($p < .05$). No difference between the groups were found regarding fibrosis scores ($p > .05$). Histological sections of groups were shown in Figure 4a and b. In the Figure 4a, healing of the dermis (d) with a loose distribution in diabetic control on the 7th day was seen. Epidermis and papillae were not formed and no hair follicles were observed. Collagen deposition (blue arrow head) and vascularization (yellow arrow head) were visible in fito group. Complete re-epithelialization (e), clear distinction between the papillary dermis (pd) and reticular dermis (rd) were observed in SH-0.5 group. No papillae and hair follicles were observed. Well-developed epidermis, dermis, and papillae were seen in SH-1 group. Hair follicles were highly developed *(H&E Stain).* No epidermal formation was visible in diabetic control on the 7th day. Collagen deposition in dermis was observed and papillae were seen in papillary dermis (pd) close to reticular dermis (rd) in diabetic control group. Re-epithelialization with a loose papillary dermis and no hair follicles (hf) and sparsely settled hair follicles were monitored in the deep parts of reticular dermis in fito group. Epidermis (e) with sparse and shallow papillae and primitive hair follicles were seen in SH-0.5 group. Healing in papillary dermis and dense collagen fibers in reticular dermis were observed. Arrow head, represents the inflammatory cells in papillary dermis. Papillae were rarely formed in SH-1 group. Epidermis (e) and dermis (d) were highly developed *(Verhoeff-Van Gieson Stain).* Reticular fibers around muscle fibers were seen in connective tissue of diabetic control tissue. Staining of dermis with reticulin stain was negative.

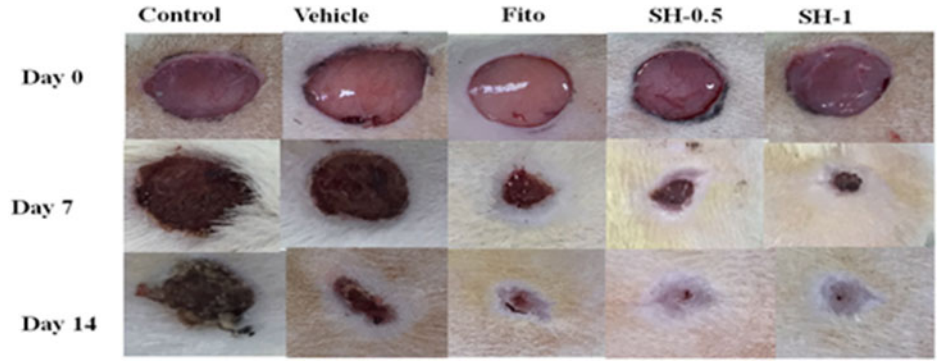

FIGURE 1. Wound area in different groups, excisional wounds treated with *S. hypargeia* in different days. Control: Diabetic control group; Vehicle: Diabetic vehicle group; Fito: Fito cream group; SH-0.5: 0.5% (w/w) *S. hypargeia* ointment group; SH-1: 1% (w/w) *S. hypargeia* ointment group.

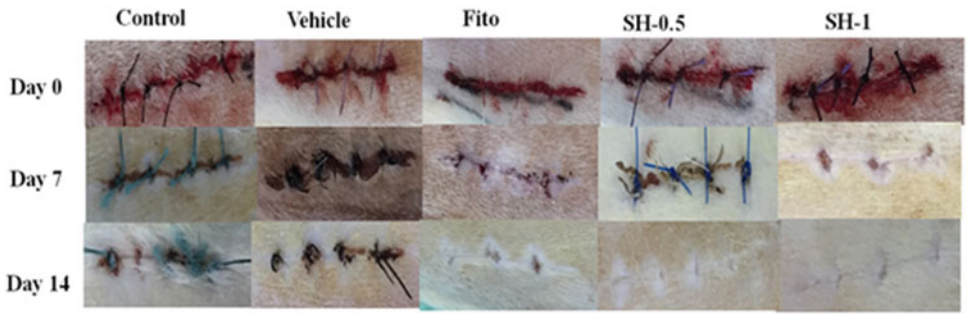

FIGURE 2. Wound area in different groups, incisional wounds treated with *S. hypargeia* in different days. Control: Diabetic control group; Vehicle: Diabetic vehicle group; Fito: Fito cream group; SH-0.5: 0.5% (w/w) *S. hypargeia* ointment group; SH-1: 1% (w/w) *S. hypargeia* ointment group.

TABLE 3. Skin biomechanical parameters of incision wound models in diabetic rats

	Skin biomechanical parameters							
Groups	Energy absorption capacity (U, mj)	Stiffness (S, N/mm)	Maximum load (F_U: N)	Maximum deformation (du: mm)	Young's (elasticity) modulus (E, MPa)	Toughness (u; MPa)	Maximum stress (σu; MPa)	Maximum strain (ε_U, mm/mm)
7-Day applications								
Control	84.51 ± 23.90	4.71 ± 2.14	18.96 ± 5.22	10.47 ± 2.45	0.06 ± 0.03	0.21 ± 0.06	0.09 ± 0.03	4.23 ± 1.23
Vehicle	83.16 ± 30.90	3.58 ± 1.61	19.84 ± 3.69	11.06 ± 1.51	0.04 ± 0.02	0.21 ± 0.08	0.10 ± 0.02	4.53 ± 0.76
Fito	121.82 ± 16.47a,c	3.20 ± 1.57	23.62 ± 3.30	15.05 ± 2.34a,c	0.03 ± 0.01	0.31 ± 0.04a,c	0.12 ± 0.02	6.52 ± 1.17a,c
SH-0.5	135.42 ± 30.84c	3.60 ± 0.44	25.86 ± 3.02c	13.94 ± 2.68	0.04 ± 0.01	0.34 ± 0.08c	0.13 ± 0.01c	5.97 ± 1.34
SH-1	103.48 ± 28.21	2.93 ± 1.89	20.07 ± 4.99	16.38 ± 3.67c	0.03 ± 0.02	0.26 ± 0.07	0.10 ± 0.02	7.19 ± 1.83c
14-Day applications								
Control	173.38 ± 48.67	3.46 ± 2.43	27.07 ± 6.72	16.15 ± 4.71	0.04 ± 0.03	0.43 ± 0.11b	0.13 ± 0.03	7.05 ± 2.29
Vehicle	98.04 ± 43.47	4.17 ± 2.76	25.86 ± 6.74	11.43 ± 3.94	0.04 ± 0.03	0.25 ± 0.12	0.13 ± 0.03	4.72 ± 1.97
Fito	172.81 ± 70.50	4.27 ± 2.23	30.34 ± 6.36	14.73 ± 3.93	0.04 ± 0.02	0.45 ± 0.18	0.15 ± 0.03	6.39 ± 1.97
SH-0.5	190.86 ± 25.32c	4.20 ± 0.85	33.11 ± 4.45	15.08 ± 1.55	0.04 ± 0.01	0.48 ± 0.07c	0.17 ± 0.02	6.54 ± 0.78
SH-1	153.16 ± 58.31	4.59 ± 2.63	27.93 ± 7.02	12.10 ± 0.86	0.05 ± 0.03	0.38 ± 0.12	0.14 ± 0.04	5.05 ± 0.43

[a]Significantly different from DM-control group ($p < .05$).
[b]Significantly different from Fito group ($p < .05$).
[c]Significantly different from DM-vehicle group ($p < .05$).
Measuring the average ± SD (Control: Diabetic control group; Vehicle: Diabetic vehicle group; Fito: Fito cream group; SH-0.5: 0.5% (w/w) *S. hypargeia* ointment group; SH-1: 1% (w/w) *S. hypargeia* ointment group).

Reticular fibers were strongly stained in the dermis and around the hair follicles were seen in fito group. Weaker staining of reticular fibers was seen in the dermis of SH-0.5 group. Reticular fibers were painted in black between collagen fibers were seen in the dermis of SH-1 group *(Reticulin Stain).*

On the 14th day, represented in Figure 4b, epidermis (e) was formed as a thin layer, papillae were shallow and hair follicles were seen in the deep parts of dermis in diabetic control group on the loosely distributed collagen fibers in dermis (d) were seen. Well-developed dermis (d) containing numerous hair follicles (hf) in fito group was visible. Epithelialization was completed and epidermis (e) was observed as a thin layer. Papillae are quite sparse with a shallow distribution and were completely formed in SH-0.5 group and well-developed in SH-1 group k; keratin (H&E Stain). Re-epithelization has started but not completed yet in diabetic control group. Dermis (d), papillae and reticular dermis cannot be distinguished. Intense collagen fiber deposition in the connective tissue under epidermis (e) was observed. Epidermis (e) was formed with rare papillae in fito group. Intensive collagen fiber accumulation in connective tissue of dermis was observed. Epidermis (e), papillary dermis (pd), and reticular dermis (rd) were well developed in SH-0.5 group. Hair follicles were seen. Epidermis (e) and dermis (pd) were completely developed, papillae (arrows) were deep and abundant in SH-1 group. Mild collagen fiber accumulation in the dermis layer was seen. Local loss of collagen fibers in dermis was observed (Verhoeff-Van Gieson Stain). Reticular fibers (for type III collagen) were observed in connective tissue, around fat cells and capillaries in diabetic control group. A weak reticular fiber staining in dark stained areas between the collagen fiber bundles was observed in fito group. The black areas painted around the hair follicles belong to the reticular fibers. Weak staining in the dermis of SH-0.5 group and SH-1 groups were observed (Reticulin Stain).

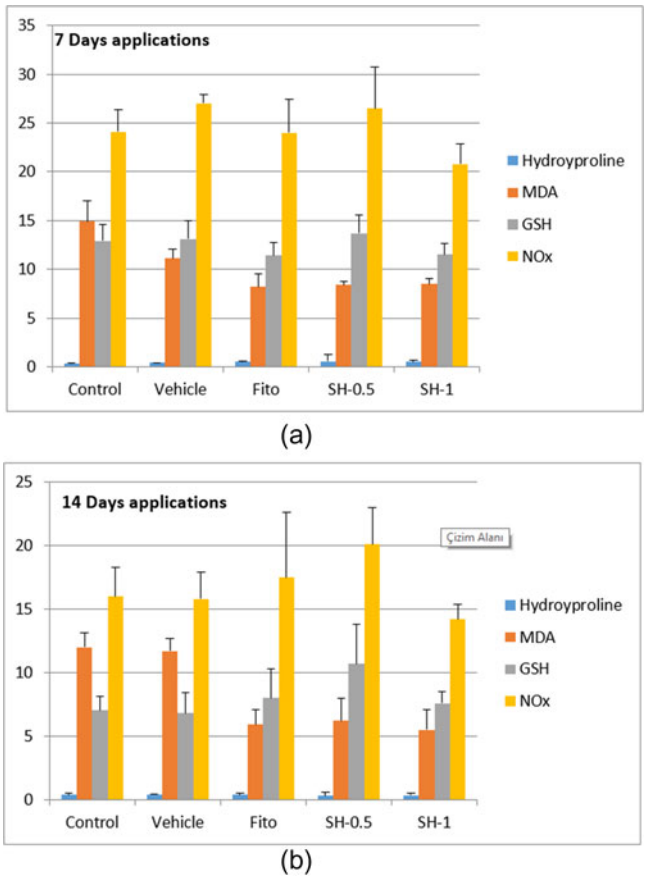

FIGURE 3. (a, b) Effects of extract from *S. hypargeia* on the levels of Hydroxyproline, MDA, GSH, NOx in streptozotocin (STZ)-diabetic rats.

Genotoxicologic Results

Genotoxicologic studies revealed that parameters of GDI, DCP, and MN/2000 were decreased in the *S. hypargeia* ointment treated groups of SH-0.5 and

TABLE 4. Histological scores of groups in excision wound model

Application periods	Groups	Parameter (mean ± standard deviation)					
		Re-epithelialization	Granulation tissue	Angiogenesis	Inflammation	Fibrosis	Type III collagen
7 Days	Control	0.16 ± 0.4	1.0 ± 0.8	0.3 ± 0.5	2.2 ± 0.4	0.2 ± 0.4	2.3 ± 0.5
	Vehicle	0.16 ± 0.4	1.0 ± 0.8	0.3 ± 0.5	2.0 ± 0.6	0.3 ± 0.5	2.2 ± 0.4
	Fito	1.5 ± 0.5b	0.7 ± 0.5	1.7 ± 0.5c	0.3 ± 0.5c	0.2 ± 0.4	1.5 ± 0.5
	SH-0.5	1.8 ± 0.4c	0.5 ± 0.5a	2.2 ± 0.7c	0.8 ± 0.4c	0.5 ± 0.5	0.5 ± 0.6c
	SH-1	2.2 ± 0.8c	0.1 ± 0.4a	1.8 ± 0.7c	0.0 ± 0.0c	0.0 ± 0.0	0.2 ± 0.5c
14 Days	Control	0.5 ± 0.54	0.7 ± 0.5	0.5 ± 0.6	1.8 ± 0.9	0.3 ± 0.5	1.8 ± 0.8
	Vehicle	0.5 ± 0.54	0.8 ± 0.9	0.5 ± 0.6	2.0 ± 0.6	0.5 ± 0.5	1.7 ± 0.5
	Fito	2.2 ± 0.4c	0.0 ± 0.0a	1.5 ± 0.5b	0.2 ± 0.4c	0.2 ± 0.4	0.5 ± 0.5c
	SH-0.5	2.0 ± 0.6c	0.1 ± 0.4a	2.2 ± 0.7c	0.3 ± 0.5c	0.2 ± 0.4	0.2 ± 0.4c
	SH-1	2.6 ± 0.5c	0.0 ± 0.0a	2.5 ± 0.6c	0.0 ± 0.0c	0.0 ± 0.0	0.0 ± 0.0c

Measuring the average ± SD and Min-Max.
[a]ANOVA analysis was performed $p < .05$.
[b]ANOVA analysis was performed $p < .01$
[c]ANOVA analysis was performed $p < .001$.
Control: Diabetic control group; Vehicle: Diabetic vehicle group; Fito: Fito cream group; SH-0.5: 0.5% (w/w) *S. hypargeia* ointment group; SH-1: 1% (w/w) *S. hypargeia* ointment group.

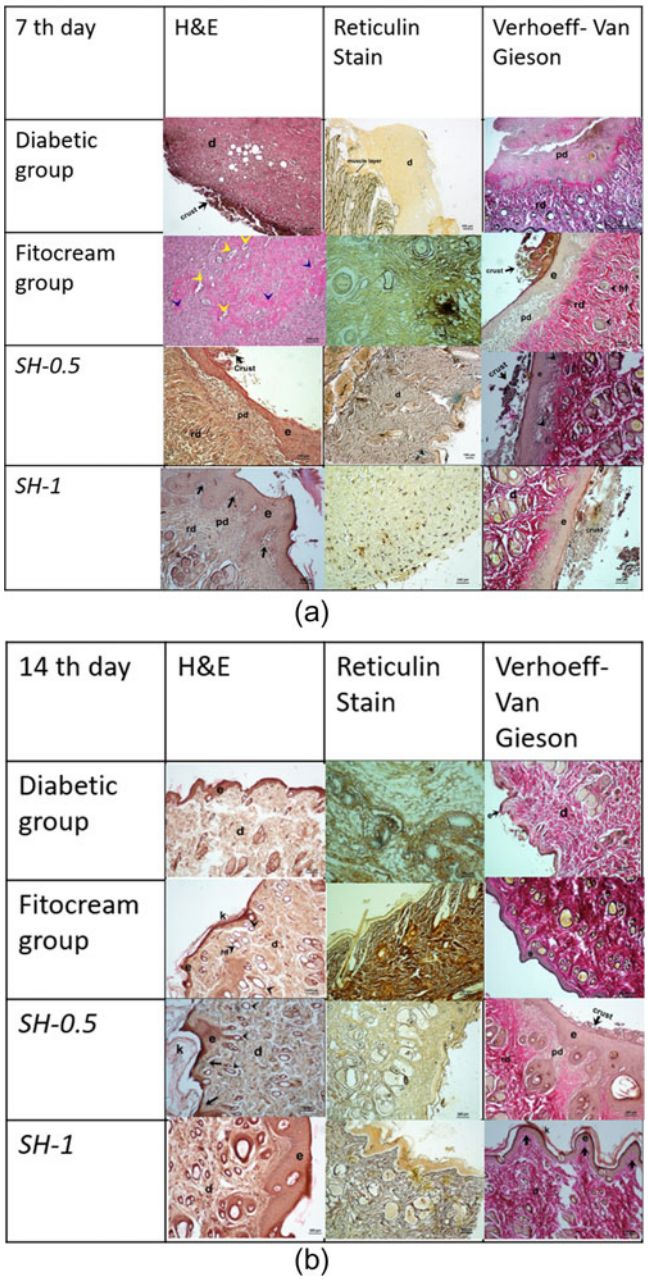

FIGURE 4. (a). Histologic sections of groups on the 7th day. (b). Histologic sections of groups on the 14th day (hf, hair follicle; rd, reticular dermis; pd, papillary dermis; e, epidermis; d, dermis; arrows, k: keratinization; papillae; blue arrowheads: collagen deposition, yellow arrowheads: vascularization).

SH-1 at the end of 7 and 14 days. The results of MN and Comet assays in studied groups were shown in Table 5 and MN photograph of the control group was seen in Figure 5.

Comet assay photographs were shown in Figure 6. Genotoxicologic results indicated that *S. hypargeia* has no adverse effects on genetic material. GDI values of SH-0.5 group (99.3 ± 9.3 & 28.7 ± 5.64) and SH-1group (22.67 ± 5.3 & 52.2 ± 2.2) and DCP values of SH-0.5 group (18.0 ± 4.2 & 6.8 ± 6.2) and SH-1 group (2.5 ± 0.61 & 4.3 ± 0.55) did not reach to significant levels on the 7th and 14th days. MN frequency of SH-0.5 (1.8 ± 0.16 & 2.16 ± 0.16) and SH-1 group (1.67 ± 0.2 & 1.5 ± 0.22) did not reach to significant levels on the 7th and 14th days.

DISCUSSION

Wound healing and anti-inflammatory effects of *S. hypargeia* extracts on diabetic wounds were investigated in this study using biochemical, biomechanical, histopatological, and genotoxicological parameters. In diabetic wounds, delay in wound healing and biomechanical parameters of skin in comparison with healthy rats were reported [30]. Researchers still consider natural biologically active herbal compounds as an alternative medicine [31, 32]. Consequently, a great deal of interest has been focused on medicinal plants regarding their long use in folk medicine, as well as their prophylactic properties, mainly in developing countries [33]. *Salvia* species are still being used in traditional medicine all around the world as anti-inflammatory, antidiabetic, anti-tuberculosis, antiseptics, diuretics, stimulants, sedatives, antitumor, and wound healing agents [34–36]. The local use of *Salvia* species includes the treatment of wounds because of their anti-inflammatory properties [36]. A study of Estakhr and Javdan (2011) on wound healing activity of *Salvia hypoleuca* Benth. in rats using chemically induced wound model and revealed that *S. hypoleuca* had strong wound healing activity [8]. The components isolated from the *Salvia* species and their bioactivities have been published in several articles [10, 11]. Salvia species are reported to be rich in bioactive compounds mainly diterpenoids, triterpenoids, flavonoids, phenolic acids, and essential oils [35, 37–40]. Previous studies demonstrated the presence of several diterpenoids, two triterpenes and β-sitosterol, δ-oleanol 3-acetate and also palmitoleic acid (6.4%) and palmitic acid (51.6%) in the roots of *S. hypargeia* [41, 42] showing strong free radical scavenging capacity [43]. Karagoz et al. also found that the aerial parts of *S. hypargeia* exhibited potent free radical scavenging activity with 86.64%–95.69% which is even higher than that of the antioxidant vitamin E used as a reference [16]. In a study on wound healing properties, antimicrobial and antioxidant activities of *Salvia kronenburgii* and *Salvia euphratica* on excision and incision wound models in diabetic rats indicated that these plants showed wound healing effects, herein Fito cream was used for comparison [44]. Guzel et al. indicated that ethanol extract of aerial parts of *S. hypargeia* had antimicrobial activity with different MIC values against some microorganisms including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Aeromonas hydrophila*,

TABLE 5. Comparison of the results from MN and comet assays of studied groups

Groups						
	Control	Vehicle	Fito	SH-0.5	SH-1	Positive control (MMC—2 mg/kg)
7-Day applications						
GDI	329.1 ± 6.04[a]	157.46 ± 3.4[b]	279.3 ± 18.34[a]	99.3 ± 9.3	22.67 ± 5.3	237.00 ± 15.64[a]
DCP	98.7 ± 2.42[b]	49.5 ± 2.63[b]	78.8 ± 6.1[b]	18.0 ± 4.2	2.5 ± 0.61	84.83 ± 3.18[b]
MN/2000	4.01 ± 0.26[a]	4.4 ± 0.61[a]	3.46 ± 0.3[a]	1.8 ± 0.16	1.67 ± 0.21	5.33 ± 0.33[a]
14-Day applications						
GDI	323.7 ± 11.5[a]	201.7 ± 5.61[a]	82.1 ± 3.5[b]	28.7 ± 5.64	52.2 ± 2.2	237.00 ± 15.64[a]
DCP	96.2 ± 4.2[a]	57.1 ± 1.4[b]	10.1 ± 0.6	6.8 ± 6.2	4.3 ± 0.55	84.83 ± 3.18[a]
MN/2000	5.01 ± 0.5[a]	4.9 ± 0.1[a]	1.7 ± 0.1	2.16 ± 0.16	1.5 ± 0.22	5.33 ± 0.33[a]

Measuring the average ± SD.
[a]$p < .001$.
[b]$p < 0.01$.
GDI: Genetic Damage Index; DCP: Damaged Cell Percent; MMC: Mitomycin-C (Control: Control group; Vehicle: Vehicle group; Fito: Fito® cream group; SH-0.5: 0.5% (w/w) *S. hypargeia* ointment group; SH-1: 1% (w/w) *S. hypargeia* ointment group).

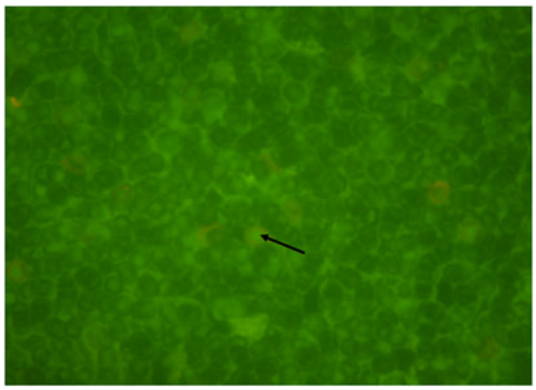

FIGURE 5. MN photograph of the control group. Arrow indicates the micronucleated cell.

Mycobacterium tuberculosis, *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis*, and also showed strong antimicrobial activity to *Acinetobacter baumannii* which causes serious infections especially in surgical wounds with 62.5 μg/mL MIC value when compared to reference drug Ampicillin (125 μg/mL MIC value) [17]. This finding demonstrates that *S. hypargeia* extract may protect against bacterial invasion and facilitate wound healing.

The present study was also demonstrated that ointment prepared with the aerial parts of *S. hypargeia* facilitates wound healing in a rat model of diabetic excisional and incisional wounds, supported not only with biomechanical methods but also with biochemical, histopathological and toxicological methods. As seen in Figures 1 and 2, the wound area treated with *S. hypargeia* ointment recovered completely, which was almost difficult to distinguish from the uninjured one.

Results of biochemical analysis supported the advanced healing effect of *S. hypargeia*. There is much experimental evidence that cells produce reactive oxygen species under hyperglycemic conditions [45]. Glutathione is a non-enzymatic antioxidant that serves to detoxify free radicals both by scavenging them or acting as a co-substrate in glutathione peroxidase catalyzed reduction of hydrogen peroxide and lipid peroxides [46]. Oxidative stress due to diabetes leads to a decrease in the serum levels of antioxidants such as GSH [45] confirming that, in the ointment treated groups it was found to be increased especially on the 14th day contrary to diabetic group. Malondialdehyde, a free radical, is increased in diabetic tissue [47], however, in our study it was decreased in all treated groups similar to fito cream group. A healing tissue synthesizes collagen, which is a constituent of regenerating cell. Concentration of hydroxyproline indicates the concentration of collagen as hydroxyproline participates in collagen structure. Higher the concentration of hydroxyproline indicates facilitated wound healing thus, increased in wound healing process [46]. Increased in ointment treated groups on the 7th day indicates wound healing activity. Nitric oxide is a free radical, regulating collagen formation, increases at the early phase of wound healing [48]. We found decreased levels of nitric oxide on the 7th day in ointment treated groups, suggesting the facilitation of healing period.

When the durability parameters obtained from skin biomechanical tests that measure resistance to disintegration of tissue and also stored energy giving information about the tendency to disruption were taken into consideration, it can be said that *S. hypargeia* extract contributed to the improvement of wound healing. *S. hypargeia* extract increases the deformation capacity of the diabetic rat skin so the elasticity of skin returns to normal values. In addition, the average of parameters showing intrinsic (structural) and extrinsic (material) properties was seen to be similar to the fito cream means that

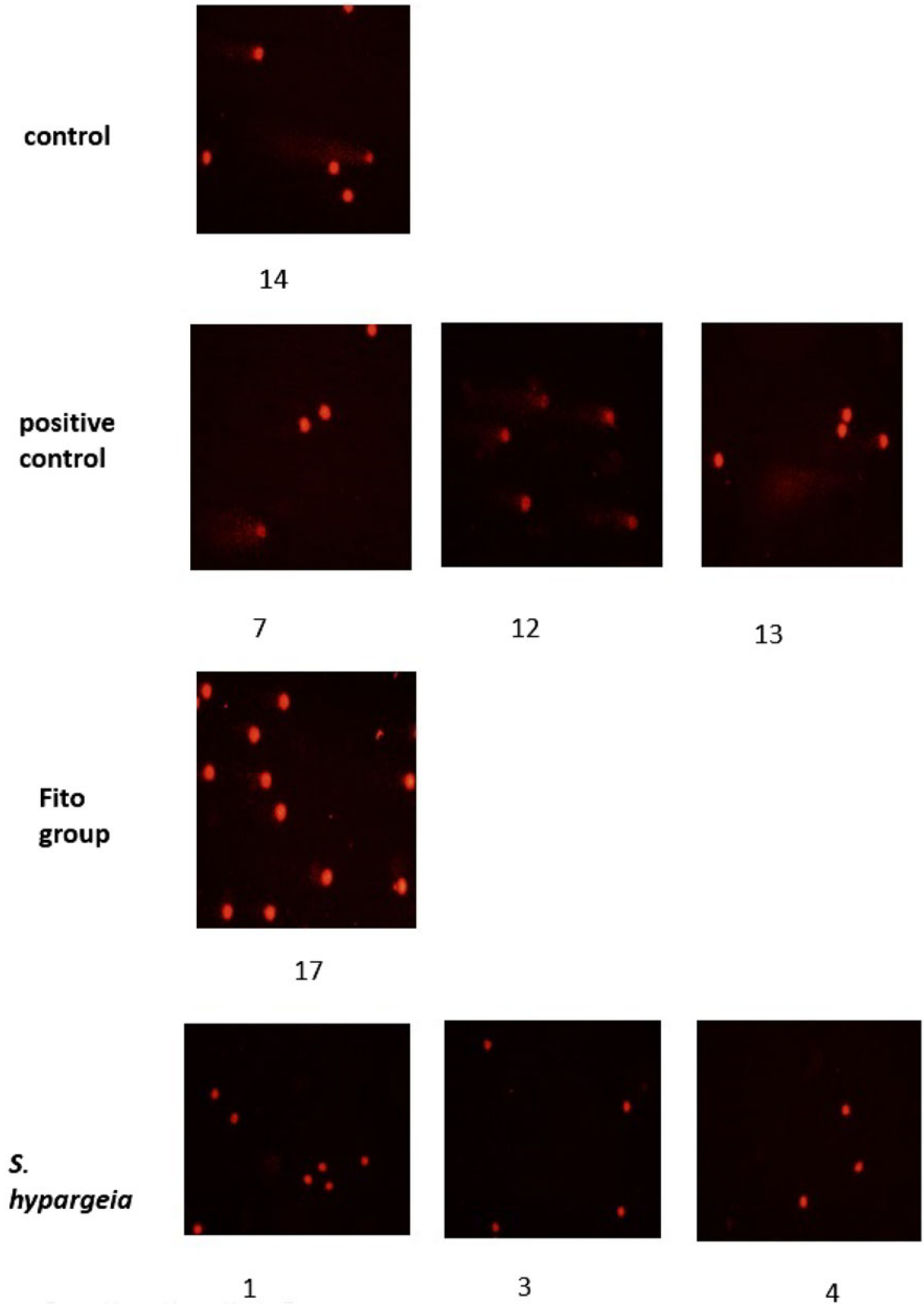

FIGURE 6. Comet photographs of all studied groups.

application of the extract contributed to the wound healing.

Angiogenesis and re-epithelialization scores were found to be higher in the ointment treated groups as this is a remarkable finding in favor of wound healing since insufficient angiogenesis is known as not only lead to decreased blood flow and oxygen but also limit the migration of inflammatory

cells to the wound, resulting in delayed wound healing [49]. It is suggested that improved angiogenesis by the *S. hypargeia* ointment would respond sufficiently to the metabolic needs of the healing tissue, improving wound healing. The expression of Type III collagen increases in the early healing processes i.e. on the 3–4th days in many tissues, including the skin and then, Type I collagen becomes dominant [50]. Therefore, increased amounts of collagen III relative to collagen I means an immature scar [6]. According to Merkel et al., Type III collagen is expressed in early granulation tissue, and the fact that we found decreased Type III collagen amount in the treated group, is consistent with their finding, suggesting that our treatment with *S. hypargeia* caused the maturation of granulation tissue, also demonstrating another evidence for its wound healing effect as well [50]. Comet and MN analysis were used in previous studies to demonstrate genotoxic effects [51–53]. One of the prominent features of this study is that DNA damage induced by experimental diabetes [54, 55], seem to be reduced in *S. hypargeia* ointment treated groups in comparison to control group. This was noted in our genotoxicological results, supporting that *S. hypargeia* has no toxic effect when topically applied to the wound. It can even be said that extracts of *S. hypargeia* reduced genotoxic effect both in 7 and 14 days applications due to its antioxidant properties that prevented oxidative damage on DNA.

All the results obtained from the present study such as increased tensile strength, hydroxyproline content and antioxidant activity explain the reputed wound healing effect of *S. hypargeia* in a diabetic wound model described herein.

Overall, authors recommend that *S. hypargeia* may have a potential for therapeutic use in the treatment and management of diabetic wounds with a successful topical application, and further studies are required for the bioavailable use as a drug.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

[1] Chamberlain JJ, Rhinehart AS, Shaefer CF, Neuman A. Diagnosis and management of diabetes: synopsis of the 2016 American Diabetes Association standards of medical care in diabetes. *Ann Intern Med.* 2016;164(8): 542–552. doi:10.7326/M15-3016.

[2] Guo S, DiPietro LA. Factors affecting wound healing. *J Dent Res.* 2010;89(3):219–229. doi:10.1177/0022034509359125.

[3] Nuutila K, Katayama S, Vuola J, Kankuri E. Human wound-healing research: issues and perspectives for studies using wide-scale analytic platforms. *Adv Wound Care.* 2014;3(3):264–271. doi:10.1089/wound.2013.0502.

[4] Tandara AA, Mustoe TA. Oxygen in wound healing-more than a nutrient. *World J Surg.* 2004;28(3):294–300. doi:10.1007/s00268-003-7400-2.

[5] Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest.* 2007;117(5): 1219–1222. doi:10.1172/JCI32169.

[6] Xue M, Jackson CJ. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. *Adv Wound Care.* 2015;4(3):119–136. doi:10.1089/wound.2013.0485.

[7] Ponrasu T, Suguna L. Efficacy of *Annona Squamosa* on wound healing in streptozotocin-induced diabetic rats. *Int Wound J.* 2012;9(6):613–623. doi:10.1111/j.1742-481X.2011.00924.x.

[8] Alimpić A, Knežević A, Milutinović M, et al. Biological activities and chemical composition of *Salvia Amplexicaulis* Lam. extracts. *Ind Crops Prod.* 2017;105:1–9. doi:10.1016/j.indcrop.2017.04.051.

[9] Estakhr J. Estakhr and Javdan 622 evaluation of wound healing activity of S*alvia hypoleuca e*xtract on rats. *Pharmacologyonline.* 2011;3:622–625.

[10] Suntar I, Akkol EK, Senol FS, Keles H, Orhan IE. Investigating wound healing, tyrosinase inhibitory and antioxidant activities of the ethanol extracts of *Salvia cryptantha* and *Salvia cyanescens* using in vivo and in vitro experimental models. *J Ethnopharmacol.* 2011;135(1): 71–77. doi:10.1016/j.jep.2011.02.022.

[11] Narayan S, Sasmal D, Mazumder PM. Evaluation of the wound healing effect of herbal ointment formulated with *Salvia splendens* (scarlet sage). *Int J Pharm Pharm Sci.* 2011;3:195–199.

[12] Kamatou GPP, Makunga NP, Ramogola WP, Viljoen AM. South African *Salvia* species: a review of biological activities and phytochemistry. *J Ethnopharmacol.* 2008; 119(3):664–672. doi:10.1016/j.jep.2008.06.030.

[13] Hedge IC. Labiatae of South-West Asia: diversity, distribution and endemism. *Proc Sect B Biol Sci.* 1986;89:23–35. doi:10.1017/S0269727000008873.

[14] Kocak A, Bagci E. Chemical composition of essential oil of local endemic *Salvia kronenburgii* Rech. Fil. to Turkey. *J Essent Oil Bear Plants.* 2011;14(3):360–365. doi:10.1080/0972060X.2011.10643946.

[15] Er M, Tugay O, Özcan MM, Ulukuş D, Al-Juhaimi F. Biochemical properties of some *Salvia* L. Species. *Environ Monit Assess.* 2013;185(6):5193–5198. doi:10.1007/s10661-012-2935-z.

[16] Karagoz A, Artun FT, Ozcan G, Melikoglu S, Anıl S. In vitro evaluation of antioxidant activity of some plant methanol extracts. *Biotechnol Biotechnol Equip.* 2015;29(6): 1184–1189. doi:10.1080/13102818.2015.1080600.

[17] Guzel S, Ozay Y, Ulger M, Kahraman A, Gonul A. evaluation of antimicrobial activity of three endemic *Salvia* species growing in Turkey. *Hacettepe Univ J Fac Pharm.* 2018;38:4–10.

[18] Jacob J, Aleykutty NA, Harindran J. Evaluation of wound healing activity in streptozotocin induced diabetic rats by ethanolic extract of *Blepharis maderaspatensis* (L.). *B Heyne Ex Roth Int J Herb Med.* 2017;5(6):45–47.
[19] Ozay Y, Guzel S, Erdogdu IH, et al. Evaluation of the wound healing properties of luteolin ointments on excision and incision wound models in diabetic and non-diabetic rats. *Recnatprod.* 2018;12(4):350–366. doi:10.25135/rnp.38.17.08.135.
[20] Paul RG, Tarlton JF, Purslow PP, et al. Biomechanical and biochemical study of a standardized wound healing model. *Int J Biochem Cell Biol.* 1997;29(1):211–220.
[21] Reddy GK, Stehno-Bittel L, Enwemeka CS. Laser photostimulation accelerates wound healing in diabetic rats. *Wound Repair Regen.* 2001;9(3):248–255.
[22] Jamall IS, Finelli VN, Que Hee SS. A simple method to determine nanogram levels of 4-hydroxyproline in biological tissues. *Anal Biochem.* 1981;112(1):70–75.
[23] Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am. J Obstet Gynecol.* 1979;135(3):372–376. doi:10.1016/0002-9378(79)90708-7.
[24] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959;82(1):70–77.
[25] Bories PN, Bories C. Nitrate determination in biological fluids by an enzymatic one-step assay with nitrate reductase. *Clin Chem.* 1995;41(6 Pt 1):904–907.
[26] Khoshneviszadeh M, Ashkani-Esfahani S, Namazi MR, Noorafshan A, Geramizadeh B, Miri R. Topical Simvastatin enhances tissue regeneration in full-thickness skin wounds in rat models. *Iran J Pharm Res.* 2014;13(1):263–269.
[27] Abramov Y, Golden B, Sullivan M, et al. Histologic characterization of vaginal vs. abdominal surgical wound healing in a rabbit model. *Wound Repair Regen.* 2007;15(1):80–86. doi:10.1111/j.1524-475X.2006.00188.x.
[28] Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res.* 1988;175(1):184–191.
[29] Holden HE, Majeska JB, Studwell D. A direct comparison of mouse and rat bone marrow and blood as target tissues in the micronucleus assay. *Mutat Res.* 1997;391(1–2):87–89.
[30] Brem H, Kodra A, Golinko MS, et al. Mechanism of sustained release of vascular endothelial growth factor in accelerating experimental diabetic healing. *J Invest Dermatol.* 2009;129(9):2275–2287. doi:10.1038/jid.2009.26.
[31] Bindseil KU, Jakupovic J, Wolf D, Lavayre J, Leboul J, Pyl D. Pure compound libraries; a new perspective for natural product based drug discovery. *Drug Discov Today.* 2001;6(16):840–847.
[32] Wang BQ. *Salvia miltiorrhiza*: chemical and pharmacological review of a medicinal plant. *J Med Plants Res.* 2010;4(25):2813–2820.
[33] Maroyi A. Traditional use of medicinal plants in South-Central Zimbabwe: review and perspectives. *J Ethnobiol Ethnomed.* 2013;9(1):31. doi:10.1186/1746-4269-9-31.
[34] Culhaoğlu B, Hatipoglu SD, Donmez AA, Topcu G. Antioxidant and anticholinesterase activities of Lupane triterpenoids and other constituents of *Salvia trichoclada*. *Med Chem Res.* 2015;24(11):3831–3837.
[35] Hatipoglu SD, Zorlu N, Dirmenci T, Goren AC, Ozturk T, Topcu G. Determination of volatile organic compounds in fourty five *Salvia* species by thermal desorption GC-MS technique. *Rec Nat Prod.* 2016;10:659–700.
[36] Wu YB, Ni ZY, Shi QW, et al. Constituents from *Salvia* species and their biological activities. *Chem Rev.* 2012;112(11):5967–6026. doi:10.1021/cr200058f.
[37] Bahadori MB, Dinparast L, Valizadeh H, Farimani M, Ebrahimi SN. Bioactive constituents from roots of *Salvia syriaca* L. acetylcholinesterase inhibitory activity and molecular docking studies. *South African J Bot.* 2016;106(106):1–4. doi:10.1016/j.sajb.2015.12.003.
[38] Bahadori MB, Dinparast L, Zengin G, et al. Functional components, antidiabetic, anti-Alzheimer's disease, and antioxidant activities of *Salvia syriaca* L. *Int J Food Prop.* 2017;20(8):1761–1772. doi:10.1080/10942912.2016.1218893.
[39] Hasan MR, Al-Jaber HI, Al-Qudah MA, Abu Zarga MH. New sesterterpenoids and other constituents from *Salvia dominica* growing wild in Jordan. *Phytochem Lett.* 2016;16:12–17. doi:10.1016/j.phytol.2016.02.009.
[40] Li B, Zhang C, Peng L, et al. Comparison of essential oil composition and phenolic acid content of selected *Salvia* species measured by GC–MS and HPLC methods. *Ind Crops Prod.* 2015;69:329–334. doi:10.1016/j.indcrop.2015.02.047.
[41] Topcu G, Turkmen Z, Schilling JK, Kingston DGI, Pezzuto JM, Ulubelen A. Cytotoxic activity of some Anatolian Salvia extracts and isolated abietane diterpenoids. *Pharm Biol.* 2008;46(3):180–184. doi:10.1080/13880200701735411.
[42] Ulubelen A, Topcu G, Chai HB, Pezzuto JM. Cytotoxic activity of diterpenoids isolated from *Salvia hypargeia*. *Pharm Biol.* 1999;37(2):148–151. doi:10.1076/phbi.37.2.148.6082.
[43] Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem.* 2006;95(2):200–204. doi:10.1016/j.foodchem.2004.12.031.
[44] Guzel S, Ozay Y, Kumaş M, et al. Wound healing properties, antimicrobial and antioxidant activities of *Salvia kronenburgii Rech. f. and Salvia euphratica Montbret, Aucher & Rech. f. var.euphratica* on excision and incision wound models in diabetic rats. *Biomed. Pharmacother.* 2019;111:1260–1276. doi:10.1016/j.biopha.2019.01.038.
[45] Matough FA, Budin SB, Hamid ZA, et al. The role of oxidative stress and antioxidants in diabetic complications. *Squmj.* 2012;12(1):5–18. doi:10.12816/0003082.
[46] Dwivedi D, Dwivedi M, Malviya S, Singh V. Evaluation of wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in Wistar rats. *J Tradit Complement Med.* 2017;7(1):79–85. doi:10.1016/j.jtcme.2015.12.002.
[47] Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. *J Biomarkers.* 2013;2013:1–8. doi:10.1155/2013/378790.
[48] Witte MB, Barbul A. Role of nitric oxide in wound repair. *Am J Surg.* 2002;183(4):406–412.
[49] Ud-Din S, Sebastian A, Giddings P, et al. Angiogenesis is induced and wound size is reduced by electrical stimulation in an acute wound healing model in human skin. *PLoS One.* 2015;10(4):e0124502. doi:10.1371/journal.pone.0124502.
[50] Merkel JR, DiPaolo BR, Hallock GG, Rice DC. Type I and Type III collagen content of healing wounds in fetal and adult rats. *Proc Soc Exp Biol Med.* 1988;187(4):493–497.
[51] Celik A, Ogenler O, Comelekoğlu U. The evaluation of micronucleus frequency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate. *Mutagenesis* 2005;20(6):411–415.
[52] Celik A, Eke D, Ekinci SY, Yıldırım S. The protective role of curcumin on perfluorooctane sulfonate-induced

genotoxicity: single cell gel electrophoresis and micronucleus test. *Food Chem Toxicol.* 2013;53:249–255. doi:10.1016/j.fct.2012.11.054.

[53] Eke D, Celik A, Yilmaz MB, Aras N, Kocatürk Sel S, Alptekin D. Apoptotic gene expression profiles and DNA damage levels in rat liver treated with perfluorooctane sulfonate and protective role of curcumin. *Int J Biol Macromol.* 2017;104:515–520. doi:10.1016/j.ijbiomac.2017.06.075.

[54] Togay VA, Sevimli TS, Sevimli M, Celik DA, Ozcelik N. DNA damage in rats with streptozotocin-induced diabetes; protective effect of Silibinin. *Mutat Res Toxicol Environ Mutagen.* 2018;825:15–18. doi:10.1016/j.mrgentox.2017.11.002.

[55] Vikram A, Tripathi DN, Ramarao P, Jena GB. Evaluation of streptozotocin genotoxicity in rats from different ages using the micronucleus assay. *Regul Toxicol Pharmacol.* 2007;49(3):238–244. doi:10.1016/j.yrtph.2007.09.006.