The effects of *Feijoa sellowiana* fruit extract on wound healing in rats: a stereological and molecular study

Objective: The aim of this study was to evaluate the anti-inflammatory and wound-healing potential of *Feijoa sellowiana* fruit extract using stereological and molecular methods in experimental rat models.
Materials: Male Wistar rats were divided into four equal groups: non-treated, vehicle, *Feijoa sellowiana* fruit extract ointment (5% weight/weight) and the reference drug (madecassol). All animals were treated topically once per day. At the end of the study, wound samples were harvested for histological, stereological, immunohistochemical and molecular assessments to determine the in vivo healing potential and anti-inflammatory activity. A high-performance liquid chromatography (HPLC) analysis was performed for the characterisation of the phenolic acids in the extract.
Results: The study included 64 rats in total. Our results showed that the wound closure, volume of new epidermis and dermis, density of fibroblasts and blood vessels, and the deposition of collagen were significantly higher in both extract and madecassol groups compared to the non-treated and vehicle groups, with superior healing in the extract group. The transcript for the transforming growth factor (TGF)-β gene was significantly upregulated in both extract and madecassol groups compared to non-treated and vehicle groups and was highest for the extract group. The density of inflammatory cells and expression levels of the cyclooxygenase (COX)-2 protein and tumour necrosis factor (TNF)-α gene in the extract and madecassol groups, especially in the extract group, were significantly reduced compared to non-treated and vehicle groups.
Conclusion: Our results confirm that the *Feijoa sellowiana* fruit extract is a valuable source of antioxidant and anti-inflammatory activities and can allow for damaged tissue in wounds to recover markedly.
Declaration of interest: The authors declare no conflict of interest.

antioxidant • circular excision • *Feijoa sellowiana* fruit • linear incision • stereology • wound • wound care • wound healing

Wounds occur for various reasons, including surgery, physical, chemical and biological damage.[1] They are divided into acute and hard-to-heal according to the type and duration of repair.[2] In acute wounds, it is vital to maintain skin integrity and provide prompt treatment to prevent microbial contamination and dehydration, as well as to stop progressive inflammation.[3]

The wound healing process is complex and includes the inflammatory phase (presence of inflammatory cells at the site and secretion of inflammatory cytokines), proliferation (proliferation of local cells such as fibroblasts and keratinocytes to form granular tissue, and wound contraction) and the last stage is the remodelling phase.[4,5] However, one of the most important challenges in the treatment of acute and hard-to-heal wounds is the control of inflammation.[6] Prolonged inflammation by the abundant synthesis of factors such as cyclooxygenase (COX)-2[7] and tumour necrosis factor (TNF)-α[5] can disturb the balance between the synthesis and destruction of collagen, induce apoptosis in cells such as fibroblasts as well as prevent the wound from entering the proliferative phase.[8] On the other hand, some cytokines, such as transforming growth factor (TGF)-β, are important to the wound healing process as they play a key role in the proliferation of fibroblasts and keratinocytes and also promote the proliferative phase. Therefore, it should be possible to use a combination treatment that, in addition to being anti-inflammatory, can effectively cause the secretion of effective factors in wound healing from local cells.[9, 10]

For many years, herbal medicine has been considered for the treatment of numerous diseases.[11] The use of medicinal plants instead of chemical drugs, is reported to have fewer side effects, to be economical, safer and more accessible.[12] These benefits have accelerated the use of medicinal herbs in developed countries in recent years.[13] Iran is a country where the growth and consumption of medicinal plants have a long history

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due to favourable climatic and geographical conditions.[14] Feijoa (*Feijoa sellowiana,* Myrtaceae) is abundant in Northern Iran.[15]

Studies have shown that *Feijoa sellowiana* has various biological properties including antibacterial, antifungal,[16] antioxidant,[17] nephroprotective[18] and hepatoprotective.[19] It is rich in vitamin C, polyphenols, terpenes, tannins and flavonoids.[18–20,] Polyphenol compounds, such as flavonoid, proanthocyanidin and ellagitannin in leaves and fruits, have been shown to prevent blood clotting and cancer cell growth, reduce inflammation and regulate blood pressure.[21] Anti-inflammatory activity has been proven through the discontinuation of nitric oxide production by flavones.[18,19] Based on the properties and its availability, the present experimental study aimed to assess the anti-inflammatory and wound-healing potential of *Feijoa sellowiana* fruit extract.

Methods

Plant material and extraction

In order to prepare the ointment obtained from *Feijoa sellowiana*, the fruit was first collected in November 2017 from Mazandaran, Iran. Then, the collected fruits were dried for one month at room temperature and coarsely ground. The extract was made by macerating 100g of the prepared powder in 300ml of methanol as a solvent for 24 hours. The solution was separated using filter paper and rotated at 35–36°C under vacuum using a rotary evaporator until the methanol solvent was removed and the crude solid extract was obtained. Finally, a 5% ointment was prepared.

HPLC analysis

High-performance liquid chromatography (HPLC) analysis was used to evaluate the phenolic compounds in the prepared extract, based on our recently published paper.[22] The HPLC system used includes a K-1001 solvent delivery system equipped with a Rheodyne injection valve (20µl sample loop inserted), a UV-vis spectrophotometric detector model set at 254nm (Knauer Assoc., Germany) and an ODS-C18 column (250mm×4.6mm I.D., 5µm particle size, Shim-pack VP-ODS). Solvents were filtered and degassed. Solvent A (H_2O with acetic acid (10%)) and solvent B (CH_3CN) (A: B, 95:5) were used as the mobile phase. The flow rate was 1ml/minute and measurements were performed at room temperature.

Antioxidant activity and total phenolic and flavonoid content

In order to analyse the free radical scavenging activity of the *Feijoa sellowiana* extract, 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used. For this purpose, different concentrations of the extract (6.25, 12.5, 25, 50, 100, 200 and 400µg/ml) in equal volumes were added to the methanolic solution of DPPH (100µM). Then, the compounds were placed in the dark at room temperature for 15 minutes. Finally, the absorbance was measured and recorded at 517nm.

As a positive control, butylated hydroxyanisole (BHA) was used. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.[23]

The Folin–Ciocalteu method was used to measure the total phenolic content.[23] For this purpose, 0.1ml of the extract was mixed with 0.5ml of Folin–Ciocalteu reagent (0.2N). After 5 minutes, 0.4ml of sodium carbonate prepared at a concentration of 75g/l was added to the solution. After 2 hours of incubation at room temperature, the absorbance was measured and recorded at 760nm. Results were reported as gallic acid equivalents (GAE).

In order to measure the total flavonoid content in the prepared extract, the $AlCl_3$ method was used .[23] For this purpose, 0.5ml of extract solution was mixed with 1.5ml of methanol, 0.1ml of 10% $AlCl_3$, 0.1ml of 1M potassium acetate, and 2.8ml of distilled water. After 30 minutes of incubation at room temperature, the absorbance was measured and recorded at 415nm. The total flavonoid content was calculated as quercetin equivalents (QE) from a calibration curve.

Study design and animals

A total of 64 male adult Wistar rats (200–250g, and eight weeks old) were obtained from the Pasteur Institute in Mazandaran, Iran. The housing and feeding conditions of the animals were based on the standard ethical principles of Mazandaran University of Medical Sciences (Ethic no. IR.MAZUMS.REC 1397-1354).

After the animals had been kept in the laboratory for two weeks in order to adapt them to the study conditions, they were randomly allocated into four equal groups (n=16), including: (I) non-treated group; (II) vehicle-treated group that received 1g of Vaseline topically (as ointment base); (III) extract-treated group that received *Feijoa sellowiana* fruit extract topically (1g of 5% concentration in Vaseline); and (IV) madecassol-treated group that received 1g of madecassol topically (Bayer, Korea) as a reference drug.

The present study was performed on two wound models, circular and surgical wounds. Therefore, 10 rats in each group were considered for circular excision and six rats for surgical incision. Also, all of the quantitative evaluations in the present study were performed for circular wounds, and the linear wound was solely for closure rate and similarity to the surgical wound.

Wound models

The rats were anaesthetised using intraperitoneal injections of ketamine and xylazine (50 and 5mg/kg, respectively). Then, the rats were placed on a surgical table in a prone position and their back thoracic hairs were shaved. Wound size was the same in all rats and was performed by an experienced person using a scalpel no. 15 blade. Also, the depth of wounds was full-thickness and included both dermis and hypodermis.

Circular excision wound model

A wound 20mm in diameter was made from an upper thoracic region on the back of the rats. Treatment was performed daily for 17 days. To measure the observational changes in wound site dimensions, we used a digital camera (FinePix S20, Japan). Photos were taken on days 1, 3, 7, 10, 14 and 17 after surgery in the same conditions for all rats.[7] The wound closure rate was calculated by the following Equation 1:

$$\text{Wound closure } (\%) = ((A_0 - A_n)/A_0) \times 100 \qquad (1)$$

where: A_n = wound area on day n, and A_0 = wound area on day 0.

Surgical wound model

In order to create a surgical wound, two linear incisions 4cm long were made on the back of the rats (one on each side). Each wound was sutured in three areas (1cm apart) using VICRYL thread. Treatment was performed daily for nine days. A caliper was used for observational assessments of progressive changes on days 1, 3, 5, 7 and 9 after wound creation.[7]

Histological and stereological evaluations

Wound sampling was performed on day 18. After deep anaesthesia, the skin of the wound area along with adjacent skin was completely removed. The harvested tissues were immediately placed in 10% formalin fixative. After tissue processing, the samples were moulded in paraffin and then serial sections were prepared using microtomes. The thickness of the sections were of two sizes, 5µm (to assess the volumes of the newly formed epidermis and dermis and the density of collagen at the wound site) and 20µm (to evaluate the densities of cells and the length of blood vessels). From each sample 10 sections were selected, and they were selected at equal intervals.[24] Histological changes (including re-epithelialisation and tissue granulation) were evaluated using the standard scoring method (Table 1).[7]

For the calculation of collagen density in the newly formed dermis, trichrome Masson staining was used. In order to quantitatively evaluate collagen and make comparisons between the study groups, a total of five photos were taken from each sample using 20× magnification. Collagen density was measured for each photo using ImageJ software (National Institutes of Health, US) and the average density of five photos was reported per rat as a percentage.[25]

The volumes of the newly formed epidermis and dermis

To evaluate the volumes of the newly formed epidermis and dermis, the Cavalieri method was used. For this purpose, after selecting 10 photos (one photo from each section) of each rat, a grid was projected onto the photographs Next, all the points within the grid that were superimposed on the newly formed epidermis and dermis were counted. The total volumes of the new epidermis or new dermis were evaluated using the following Equation 2:

$$V_{total} = \Sigma P \times (a/p) \times t \qquad (2)$$

where: ΣP = the total number of points counted from 10 photos; a/p (mm^2) = the area related with each square formed between four points; and t (mm) = the real sectional thickness measured in every field.[25]

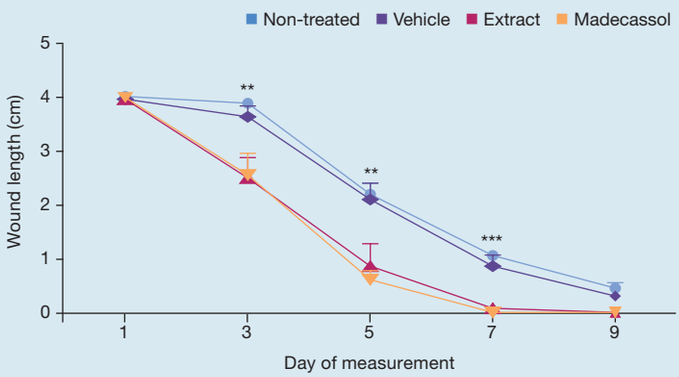
Cell density

For the determination of density (Nv) of the fibroblasts and inflammatory cells (mononuclear and polymorphonuclear leukocytes) in the wound site, the optical dissector method was used. For this purpose, after counting cells in tissue sections (10 per rat), the following Equation 3 was used:

$$N_v = ((\Sigma Q)/(\Sigma P \times h \times (a/f)) \times (t/BA)) \qquad (3)$$

where: ΣQ = number of nuclei; h (µm) = height of the dissector; ΣP = the total number of the counted frames; a/f (mm^2) = frame area; BA (µm) = block advance of the microtome (set at 20µm); and t (µm) = real sectional thickness.

Fig 1. The impact of treatment regimens on wound length. The wound length during the experiment period. Data are represented as mean±standard deviation



** p<0.01 extract and madecassol groups versus non-treated and vehicle groups.
*** p<0.001 extract and madecassol groups versus non-treated and vehicle groups.

Table 1. Scoring system for histological changes

Score	Epithelial regeneration	Granulation tissue thickness
1	Little epithelial organisation	Thin granular layer
2	Moderate epithelial organisation	Moderate granular layer
3	Complete epithelial organisation	Thick granular layer
4	Complete epithelial organisation	Very thick granular layer

The length density of the blood vessels

The following equation 4 was used to measure the length density of blood vessels in the newly formed dermis.

$$L_v = (2\Sigma Q)/(\Sigma P \times (a/f)) \quad (4)$$

where: ΣQ: total number of the blood vessel profiles counted per skin, ΣP: total number of the counted frames, and a/f (mm^2): counting frame area.

Immunohistochemistry

To determine the anti-inflammatory activity of *Feijoa sellowiana* fruit extract, immunohistochemistry against COX-2 protein was performed.[26, 27] Briefly, the sections were exposed to goat serum for 30 minutes. Then, anti-COX-2 rabbit polyclonal antibody was added to the samples (1:100 in phosphate-buffered saline (PBS), Abcam ab15291) and incubated overnight at 4°C. The next day, the sections were exposed to the secondary antibody (goat anti-rabbit IgG-HRP, Abcam) for one hour. Finally, diaminobenzidine tetrahydrochloride (DAB) was added to detect positive reactions. The sections were mounted and evaluated using a light microscope. For quantitative analysis, five photos of each sample were collected from all rats in each study group and evaluated by densitometry using MacBiophotonics ImageJ software. Data are represented as a percentage of total tissue area.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

In order to evaluate the effects of *Feijoa sellowiana* fruit extract on full-thickness wound healing, the expression of two genes, TNF-α (inflammatory cytokine and disruptive in wound healing) and TGF-β (effective in proliferation and regeneration) were evaluated. For this purpose, harvested tissue samples were immediately homogenised by a lyser device and total tissue RNA was extracted using TRIzol (Invitrogen, US). To evaluate the quality of the purified RNA, electrophoresis was performed on 1% agarose gel. Next, the cDNA was synthesised using 1µg of RNA and 20µl of the reaction mixture. The cDNA synthesis was based on a protocol derived from iScript cDNA synthesis kit (Bio-Rad, US). Finally, qRT-PCR was performed by a real-time PCR system (Applied Biosystems StepOne instrument) using SYBR Green Master Mix (three biological replicates for each sample). The sequence of primers used is reported in Table 2. The final analyses were performed using the comparative CT method (2-ΔΔct).[5]

Statistical Analysis

All quantitative data were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests by SPSS software (version 19, US). The data were expressed as the mean±standard deviation (SD) and $p<0.05$ was considered significant.

Fig 2. Wound closure, histological and immunohistochemical view of wound on day 17 **(a1–d1)**. The photographs represent healing of the treatment wounds at the end of study in comparison with the other two experimental groups. Representative micrographs of the healing wound tissues stained by haematoxylin & eosin (H&E) **(a2–d2)**; representative micrographs of the healing wound tissues stained by trichrome Masson methods to show collagen in a blue colour **(a3–d3)**; immunostaining photomicrographs against cyclooxygenase (COX)-2 protein (with dark brown nuclei) in newly formed dermis **(a4–d4)**. Magnification ×400; scale bars represent 75µm for each staining. Non-treated group **(a)**; vehicle group **(b)**; extract-treated group **(c)**; madecassol group **(d)**. Arrows point to wound-healing events. C—collagen; F—fibroblast; MNC—mononuclear cell; NV—neovascularisation; PMC—polymorphonuclear cell; P—positive COX-2 staining; Re—re-epithelialisation; S—scab; U—ulcer

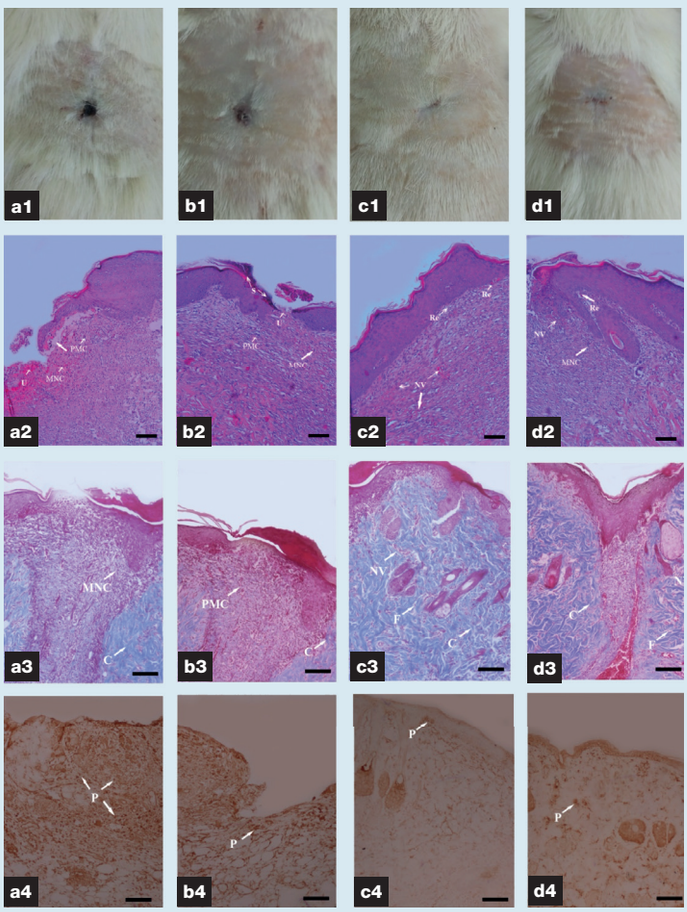
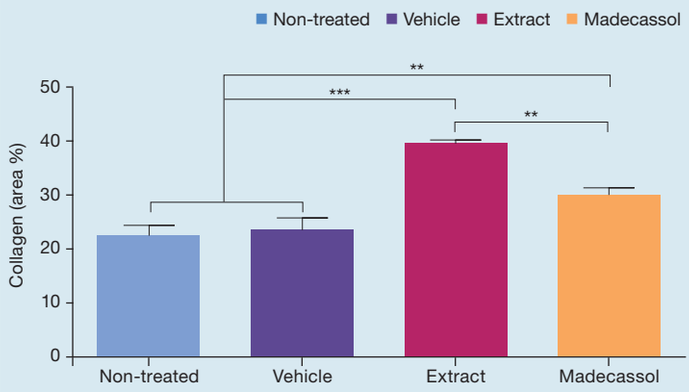


Table 2. Sequences of primers used to analyse molecular levels

Gene	Name	Sequence (5' > 3')
TGF-β	Transforming growth factor-beta	F: GGCTGAACCAAGGAGACGGA R: CCATGAGGAGCAGGAAGGGT
TNF-α	Tumor necrosis factor-alpha	F: AGCCTCTTCTCATTCCTGCTC R: GTTTGCTACGACGTGGGCTAC
β-Actin	Beta-actin	F: CCCATCTATGAGGGTTACGC R: TTTAATGTCACGCACGATTTC

Fig 3. The impact of treatment regimens on collagen deposition in the dermis of healing wound. Data are represented as mean±standard deviation; **p<0.01; ***p<0.001



Results

Antioxidant activity and total phenolic and flavonoid content

IC_{50} for DPPH radical scavenging activity was 18.3±0.88mg/ml. IC_{50} of BHA was 53.96±3.1µg/ml. Total phenolic contents (81.6±2.47 GAE) were reported as gallic acid equivalents by reference to standard curve (y=0.0058x, r^2=0.989). The total flavonoid contents (48.6±1.67 QE) were reported by reference to standard curve (y=0.0064x – 0.0076, r^2=0.998).

HPLC Analysis

The HPLC simultaneously separated phenolic acids within 30 minutes. The chromatograms of *Feijoa* fruit extract and our standard phenolic acids are reported elsewhere.[23] The extract contained at least five phenolic compounds including: catechin (188.5mg/g of extract), gallic acid (18.5mg/g of extract), rutin (15.8mg/g of extract), caffeic acid (3.2mg/g of extract) and p-coumaric acid (4.7mg/g of extract).

Wound closure rate

Wound closure rate results are shown in Table 3. Also, the photos of the wound site at the end of the study are shown in Fig 2 (a1–d1). By evaluating the wound closure rate on day 3, the results showed that the rate was significantly higher in the extract and madecassol groups compared to the non-treated and vehicle groups (p<0.01 and p<0.001, respectively). In addition, the wound area on days 7, 10 and 14 in both extract and madecassol groups was significantly reduced compared to non-treated and vehicle groups (p<0.01, p<0.05 and p<0.001, respectively). The wound repair was complete on day 17 in both extract and madecassol groups, while in non-treated and vehicle groups the wounds were not closed.

Evaluation of healing in surgical incision

Fig 1 represents the effect of topically applied *Feijoa sellowiana* fruit extract on the wound length. The results showed a significant reduction in wound length in extract and madecassol groups compared to non-treated and vehicle groups (both, p<0.01). Generally, the results showed that on day 7, the surgical wounds of five rats (83.33%) in the extract group and all of the rats (100%) in the madecassol group were completely healed, while non-treated and vehicle groups were still not completely healed (both, p<0.001).

Histological evaluation

Histological photos of the wound site are shown in Fig 2 (a2–d2). Evaluation of histological changes based on the standard scoring system (Table 1) showed that the re-epithelialisation score was significantly better in the extract and madecassol groups compared to non-treated and vehicle groups (both p<0.001). Furthermore, a comparison of granulation tissue formation showed that extract and madecassol groups had high scores in comparison with non-treated (both p<0.01) and vehicle groups (both p<0.01).

In the present study, trichrome Masson staining was used to evaluate the collagen density in the newly formed dermis at the wound site. The photos are shown in Fig 2 (a3–d3). Quantitative evaluation of the photos demonstrated that collagen density was significantly higher in the extract (both p<0.001) and madecassol (both p<0.01) groups compared to non-treated and vehicle groups. Moreover, the extract group had significantly higher collagen deposition compared to the madecassol group (p<0.01).

Table 3. Effect of *Feijoa sellowiana* fruit extract on wound closure. The percentage of wound closure during the study days

Group	Percentage wound contraction					
	Day 1	Day 3	Day 7	Day 10	Day 14	Day 17
Non-treated	2.67±1.22	19.25±3.49	34.49±10.49	62.5±10.08	81.5±2.25	88.5±3.3
Vehicle	1.94±0.88	13.72±3.12	34.46±4.89	74.48±2.99	84.3±3.52	89.96±3.62
Extract	6.11±2.29	40.5±1.93*†	75.35±1.7‡	93.41±1.05§	99.17±0.34§	100
Madecassol	4.55±1.71	45.00±2.67*†	79.9±4.52‡	97.15±0.81§	100§	100

Data are represented as mean±standard deviation; *p<0.01 versus non-treated group; †p<0.001 versus vehicle group; ‡p<0.01 versus non-treated and vehicle groups; §p<0.05 versus non-treated and vehicle groups

Stereology parameters

The graphs for stereological assessments are shown in Fig 4a–c. The results indicated that the volumes of the newly formed epidermis and dermis in the extract and madecassol groups were significantly higher than the non-treated and vehicle groups (both p<0.01) (Fig 4a,b). Evaluation of inflammatory cell density showed that the levels of these cells in the extract group were significantly lower compared to madecassol, non-treated and vehicle groups (p<0.01, p<0.001 and p<0.001, respectively). In addition, comparison of fibroblast cell density showed that the extract group had considerably more cells than the madecassol, non-treated and vehicle groups (p<0.01, p<0.001 and p<0.001, respectively). Finally, the evaluation of length density of blood vessels showed that the number of vascular profiles in the extract group was significantly higher than the madecassol, non-treated and vehicle groups (p<0.05, p<0.001 and p<0.001, respectively) (Fig 4c).

Immunohistochemical assessment

Photographs of immunohistochemical staining against COX-2 protein at the wound site are shown in Fig 2 (a4–d4). Since this protein is an inflammatory factor, its detection and staining are signs of inflammation. Therefore, quantitative evaluation of colour density in the dermis of the studied groups showed that the density of COX-2 protein in the extract group was significantly lower than the madecassol, non-treated and vehicle groups (p<0.001, p<0.0001 and p<0.0001, respectively). Also, the colour density of COX-2 protein in the madecassol group was significantly lower compared to non-treated and vehicle groups (both p<0.01) (Fig 5).

Quantitative RT-PCR analysis

The graphs of expression of TNF-α and TGF-β genes between the studied groups are shown in Fig 6. The results showed a significant upregulation in the TGF-β gene in extract (both p<0.001) and Madecassol (both p<0.01) groups compared to non-treated and vehicle groups. Furthermore, expression for TGF-β gene (p<0.01) was significantly higher in the extract group in comparison to the Madecassol group.

Furthermore, evaluation of TNF-α gene transcript showed a significant downregulation in extract (both p<0.001) and madecassol (both p<0.05) groups compared with non-treated and vehicle groups. In addition, significantly lower expression of the TNF-α gene occurred in the extract group compared to the madecassol group (p<0.01).

Discussion

Rapid and effective treatment for acute wounds has been considered for many years.[28] However, one of the most important challenges in the treatment of these wounds is infection and prolonged inflammation, which delays their closure and becomes hard to heal.[29] However, in the present study, *Feijoa sellowiana* fruit

Fig 4. The impact of treatment regimens on stereological parameters. Volumes of new epidermis **(a)** and dermis **(b)** in the healing wounds, calculated by Cavalieri's method. Numerical density of inflammatory cells, fibroblasts, and length density of blood vessels in the wound bed **(c)**. Data are represented as mean±standard deviation, *p<0.05; **p<0.01; ***p<0.001

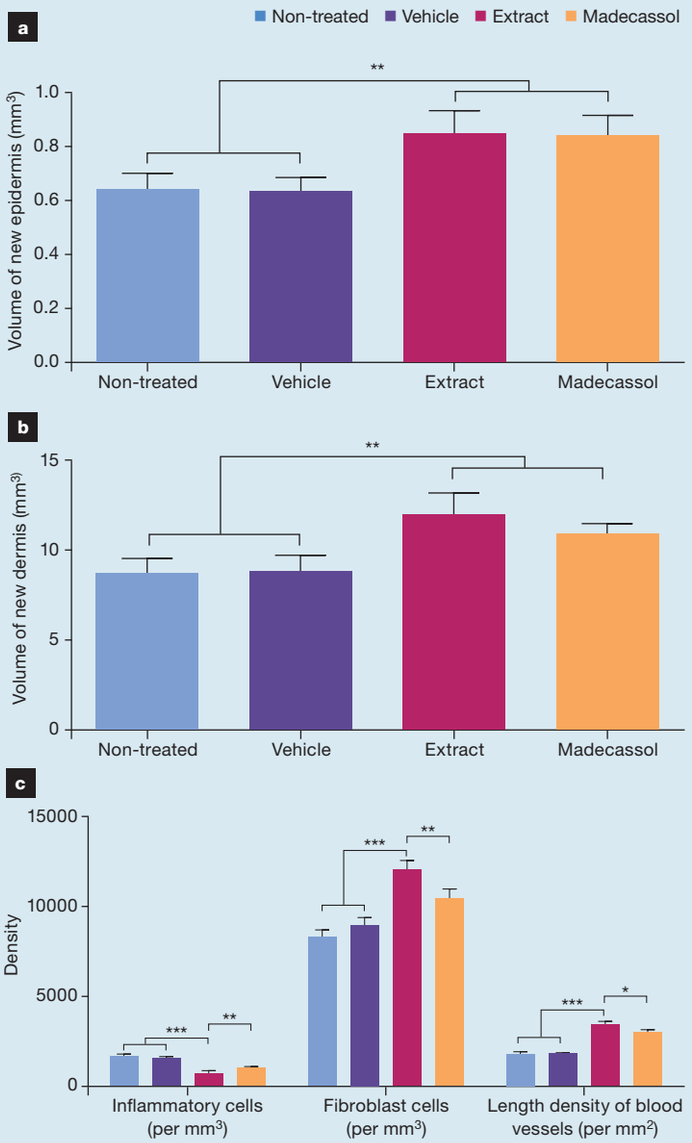


Table 4. Effect of *Feijoa sellowiana* fruit extract on histological changes

Wound healing scaling group	Epithelial regeneration	Granulation tissue thickness
Non-treated	1.5±0.44	1.2±0.56
Vehicle	1.5±0.73	1.75±0.3
Extract	4.0±0.0*	3.5±0.8†‡
Madecassol	4.0±0.0*	3.5±0.22†‡

Data are represented as mean±standard deviation; *p<0.001 versus non-treated and vehicle groups; †p<0.001 versus non-treated group; ‡p<0.01 versus vehicle group

Fig 5. The impact of treatment regimens on cyclooxygenase (COX)-2 protein expression in the dermis of a healing wound. The quantitative examination of COX-2 -positive cells in the dermis of healing wounds. Data are represented as mean±standard deviation; **p<0.01; ***p<0.001; ****p<0.0001

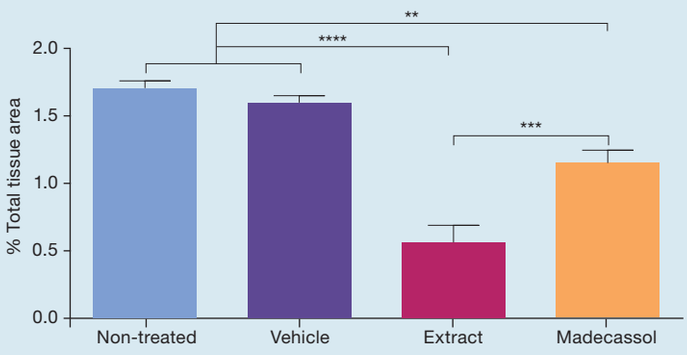
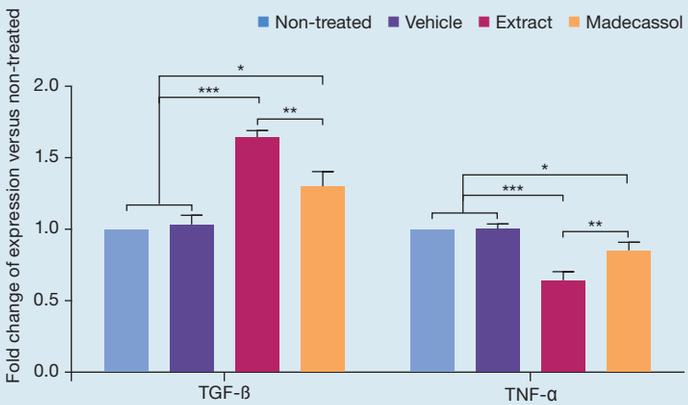


Fig 6. The impact of treatment regimens on gene expression of a healing wound. The number of transcripts for a gene contributing to regeneration (TGF-β) and a gene involved in inflammation (TNF-α) were analysed using qRT-PCR. Data are represented as mean±standard deviation; *p<0.05; **p<0.01; ***p<0.001



extract was used for the first time and its anti-inflammatory and antioxidant properties were confirmed experimentally. We observed that it accelerates wound healing and improves the quality of the wound closure.

In the present study, we used two models of circular and linear wounds, because the effect of therapeutic compounds in circular and surgical wounds can vary. Therefore, to confirm the effectiveness of a compound in wound healing, its effects should be investigated in both wound models.[30] Also, to evaluate the wound healing effects of *Feijoa sellowiana* fruit extract, histology, immunohistochemistry and molecular studies were applied to assay its effects in treated and untreated animals. We recently reported several biological activities of *Feijoa sellowiana* and its compounds, such as nephroprotective, hepatoprotective, antioxidant and anti-fungal properties.[18–20,31] The majority of these properties are directly related to iron-chelating activity, and its phenol and flavonoid contents.[23]

Our results regarding linear incision showed that the group receiving the *Feijoa sellowiana* fruit extract had a faster wound closure rate than the other groups. Also, findings from the evaluation of circular wounds showed similar results. In the groups receiving the extract and madecassol, we observed complete wound closure on day 15, compared to the other groups at the end of the study that still did not show complete wound healing. Previously, we examined the wound-healing effects of *Cantharellus cibarius* extract, similar results were obtained and these were concluded to be due to the antioxidant and anti-inflammatory effects of the compound used.[7] It seems also to be the reason in our present study.

Evaluation of histological changes showed a significant improvement in the extract group compared to non-treated and vehicle groups in terms of re-epithelialisation and tissue granulation variables (Tables 1 and 4).

On the other hand, the angiogenesis in the current study was determined by the length and density of blood vessels. Studies have shown that the formation of new blood vessels plays a key role in oxygenation, as well as the presence of stem cells at the wound site to accelerate wound healing ([5,32–34]). Our results showed a significant difference between the extract-treated group and non-treated and vehicle groups. Angiogenesis can significantly contribute to the wound-healing activity of *Feijoa sellowiana* fruit. The determination of fibroblast cell density in the extract group was significantly higher in comparison to the non-treated and vehicle groups. Also, regarding the volumes of the new epidermis and dermis, significantly greater volumes were observed in the extract group compared to the non-treated and vehicle groups. As previously reported, phenolic compounds stimulate the connective tissue growth factor gene (e.g., TGF-β, epidermal growth factor (EGF), fibroblast growth factor (FGF)).[35–37] These growth factors stimulated the proliferation of fibroblasts and keratinocytes, led to the new formation of vessels in the granulation tissue and modulated collagen deposition and reconstitution of the injured area.[32] Therefore, it seems that the findings of the present study were consistent with previous reports.

Collagen is one of the most important components of the extracellular matrix and its effective presence in the wound bed plays a vital role in healing.[25] Studies have shown that in the absence of adequate and effective collagen fibres in the repaired dermis, even if the wound is completely closed, the wound will reoccur after a short period of time.[5,38] On the other hand, Nasiry et al. and Sharifi et al. showed that the use of flavonoid-rich plant compounds can have a synergistic effect on collagen synthesis in the wound bed.[7,13] In the present study, it was clearly shown that the use of *Feijoa sellowiana* fruit extract, as a rich source of phenolic and

flavonoid compounds, significantly increased collagen synthesis in the wound bed compared to other experimental groups.

Regarding the gene expression results of TGF-β, considerable expression was determined in the extract group in comparison with other groups. This property is probably due to the antioxidant properties of *Feijoa sellowiana* extract and the stimulation of TGF-β growth factor secretion, followed by fibroblast cell proliferation and collagen synthesis by those cells, and ultimately contraction and wound closure.

As mentioned, one of the main challenges in treating acute or hard-to-heal wounds is prolonged inflammation. Studies have shown that even if the most ideal compounds are used to heal wounds, if the inflammation is not controlled, quality and effective wound healing cannot be achieved. In this regard, Nasiry et al. documented that even if dermal tissue engraftment is used at the wound site, controlling inflammation is vital.[5] Siqueira et al. showed that if TNF-α gene expression was inhibited, cell apoptosis at the wound site was significantly reduced and fibroblast cell proliferation was increased.[39] Malekshah et al. reported that if anti-inflammatory compounds were used, the excessive presence of inflammatory cells such as neutrophils and macrophages at the wound site would be reduced, resulting in reduced production of some enzymes, such as metalloproteinases and the wound would be effectively healed.[40] In this regard, in the present study, three methods were used to evaluate the anti-inflammatory properties of *Feijoa sellowiana* fruit extract: stereological (inflammatory cell density), immunohistochemical (COX-2 protein) and molecular (TNF-α gene expression level). In all three parameters, the group receiving the extract had a better and more significant status compared to other groups.

Limitations of the study

In rodent wounds, due to the presence of the panniculus carnosus muscle in the hypodermic area, the wound closed spontaneously after creation. However, this muscle is absent in human skin, except in the face and neck. In other words, if it were possible to prevent the spontaneous contraction of wounds in rats, the results might be more reliable and generalisable to human wounds. However, due to the identical conditions for all the animals of the present study, it can be hypothesised that the effects of the *Feijoa sellowiana* extract are superior in wound healing.

Reflective questions

- What differentiates acute and hard-to-heal wound healing?
- What factors distinguish the ability of medicinal plants in wound healing?
- Why is it important to evaluate different wound healing mechanisms (inflammation, proliferation and angiogenesis, remodelling)?

Conclusion

The present study investigated the wound healing potential of *Feijoa sellowiana* fruit extract in experimental models. The HPLC analyses revealed the abundance of the phenolic compounds catechin, caffeic acid, p-coumaric acid, gallic acid and rutin, which have been related to the extract's antioxidant properties. In general, the results showed that *Feijoa sellowiana* extract effectively increased the volume of the newly formed epidermis and dermis, increased collagen synthesis, increased blood vessels at the wound site, reduced inflammation (COX-2 protein and TNF-α gene) and increased secretion of an effective factor in wound healing (TGF-β gene). The safety and effectiveness of the *Feijoa sellowiana* fruit extract in human wounds needs further investigation. JWC

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