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Abstract and Figures

Objective Panax notoginseng (P.notoginseng) has been used traditionally to treat traumatic injuries. Ginsenoside Rb1, a key active ingredient derived from P.notoginseng, has received a lot of interest due to its anti-inflammatory, bacteriostatic, and growth-promoting effects on cells. The therapeutic benefits of ginsenoside Rb1 on burn wounds in STZ-induced diabetic rats, as well as the probable underlying processes, were investigated in this work. Materials and Methods The skin wound healing effect of ginsenoside Rb1 (0.25 and 0.5% w/w) in a rat model of burn wounds in diabetic rats was observed at various time points after treatment. On days 5 and 19 following treatment, immunohistochemistry and Western blot analysis for Interleukin 1 beta (IL-1β), Tumour necrosis factor-α (TNF-α), Cluster of Differentiation 68 (CD68) and Cluster of Differentiation 163 (CD163) of biological tissues were done. The macroscopic observation was used to track the healing of skin wounds at various periods. The protein expression of CD68 and CD163, which serve as M1 and M2 macrophage markers, was examined in detail. More notably, the ability of ginsenoside Rb1 to alter inflammatory markers (IL-6) and anti-inflammatory markers (IL-10), influence on hydroxyproline and hexosamine was observed. Results As indicated by increased CD163 (M2) and reduced CD68 (M1) on day 5, ginsenoside Rb1 effectively flips the M1 to M2 phenotypic transition at the right time to improve burn wound healing in diabetic rats. Ginsenoside Rb1 (0.5 % w/w) treatment showed higher tensile strength, anti-inflammatory properties, antioxidant properties, increased tissue hexosamine and hydroxyproline levels. Skin tissue morphology was significantly improved following 19 days of ginsenoside Rb1 (0.5% w/w) therapy, according to hematoxylin-eosin and Masson's trichrome staining. Furthermore, Ginsenoside Rb1 (0.5% w/w) favoured the inflammatory phase of burn wound healing (IL-6), assisted the proliferation process (IL-10) and had considerably lower expression of IL-1β and TNF-α on the later stage of wound healing. Conclusion Overall, the data showed that ginsenoside Rb1 (0.5 % w/w) accelerates burn wound healing in diabetic rats through a mechanism that may be linked to the M1 to M2 phenotypic shift.

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ORIGINAL PAPER

Effects of Bioactive Compound, Ginsenoside Rb1 on Burn Wounds Healing in Diabetic Rats: Influencing M1 to M2 Phenotypic Trans

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Abstract

Objective *Panax notoginseng* (*P.notoginseng*) has been used traditionally to treat traumatic injuries. Ginsenoside Rb1, a ke active ingredient derived from *P.notoginseng*, has received a lot of interest due to its anti-inflammatory, bacteriostatic, an growth-promoting effects on cells. The therapeutic benefits of ginsenoside Rb1 on burn wounds in STZ-induced diabeti rats, as well as the probable underlying processes, were investigated in this work.

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Conclusion Overall, the data showed that ginsenoside Rb1 (0.5 % w/w) accelerates burn wound healing in diabetic rat through a mechanism that may be linked to the M1 to M2 phenotypic shift.

Keywords Ginsenoside Rb1 · Diabetic burn wound · Wound healing · Burn wound · M1 to M2 phenotypic transition

STZ	Streptozotocin
LPO	Lipid peroxidation
GSH	Glutathione
SOD	Superoxide dismutase
CAT	Catalase
VEGF	Vascular endothelial growth factor
PPAR	Proliferator-activated receptor

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The healing of a burn wound is a multi-step process tha begins with the inflammation and terminates with epithe lialization [1]. Diabetes may impact the length of a bur patient's hospital stay [2]. Moreover, hyperglycemia i linked to a higher risk of overall morbidity in burn patient [3].

Furthermore, as diabetes people can't distinguish betwee hot and warm due to a loss of sensation in their lowe extremities, they are more likely to get foot burns from elec tric heating, pads, water baths, and foot spas [5]. Burn dam age treatment is considered an unmet clinical need, with n

satisfactory solution available to date [5]. Because diabetes is a global epidemic, healthcare practitioners will face more challenges in treating diabetic burn victims [6]. The usage of Ginsenoside Rb1 in a diabetic thermal wound rat model was examined in this work.

In treating second-degree burns, silver sulfadiazine (SSD) is routinely used for the prevention of burn wound infections and helps to reduce symptoms [7]. However, considering the side effects of these medications (antibacterial activity-related side effects, cytotoxicity, and so on), [8] the prognosis of some patients remains bleak. As a result, new local topical medications for treating wounds and scalds are urgently needed, with proven therapeutic efficacy and fewer adverse effects than currently available treatments. *P.notoginseng* is a traditional herbal medication used to treat inflammatory diseases, cardiovascular illnesses, traumatic injuries, and external and internal bleeding caused by damage [9]. Because of its potential anti-inflammatory, antioxidant, and cell growth-promoting activities, ginsenoside Rb1, an essential active ingredient of *P.notoginseng*, has gotten a lot of attention [10–13]. But, the role of Ginsenoside Rb1has not been investigated in diabetic burn wounds. Thus, the effects of ginsenoside Rb1 (0.25 and 0.5% w/w) onhealing of burn wounds in diabetic rats and the processes behind these benefits were investigated in this work to contribute to a scientific foundation for the therapeutic use of ginsenoside Rb1 to treat burns in diabetic rats.

Materials and Methods

Ointment Processing

Ginsenoside Rb1 (purity > 99.0%) was procured from SelleckChem. A basic ointment base in the ratio of 1:6:3 will be prepared using liquid paraffin, propyleneglycol, and glycol stearate, respectively [14]. Adequate levels of test substance will be added to the base ointment for preparing two doses of test ointments-Ginsenoside Rb1 [High Dosage (0.5% w/w) and low dose (0.25% w/w)]. The ointment base will be applied topically to the vehicle group. For positive control,

commercial pellet diet for rats and adequate water for at leas one week before testing. The Institutional Animal Ethics Com mittee (IAEC) of Erode College of Pharmacy, Erode, Tamil nadu, India (565/02/CA/18/CPCSEA) approved all experi mental procedures. Experiments were carried out as per th guidelines for laboratory animal care and use.

Induction of Diabetes

Animals were kept starving overnight. Nicotinamid (HiMedia Labs Pvt. Ltd.) was given at a 110 mg/kg bod weight dose 15 min before streptozotocin (STZ) was giver A 65 mg/kg dose of STZ (Sigma, USA) solution dissolve in a citrate buffer with a pH of 4.5 was given intraperito neally (i.p). Further, to minimize hypoglycemia caused b increased pancreatic insulin secretion, a 10% glucose solu tions were given to rats for an additional 24 h followin STZ treatment. Blood was drawn from the rats' tail vein 72 h after they received STZ injections. Diabetic rats wer defined as those that have blood glucose level of > 200 mg dl at fasting and were included in this investigation [15].

Wound Healing Activity

Thermal Burn Wound Model

The rats have split into five groups of six rats for the ther mal wound model. The first group is non-diabetic (norma control), and the second group is diabetic (diabetic control receiving simple ointment. The third received silver sulfadi azine (1% w/w). The fourth and fifth groups receivedgin senoside Rb1 (0.25 and 0.5% w/w) respectively. The thir set was utilized to assess wound closure and to do furthe biochemical testing. The dorsal skin hairs were carefull removed one day before the burn. For 24 h, the animals wer monitored to check if shaving had caused any irritation. A metal rod of 2.5 cm diameter was heated to a temperature o 80–85 °C, and for 20 s, it was pressed on the dorsal skin o

or reference drug will be administered to the wound sites topically once a day.

Experimental Animals and Housing Conditions

Wistar albino rats of both sexes weighing 150–250 g were used to test burn wound healing capabilities. The animals were housed in poly-propylene cages with optimum humidity, light, and temperature [Temp: 25 ± 2 ºC, 75% relative humidity, and light/dark cycles (12/12 h)]. The animals were fed a

Biochemical Analyses

At the end of the test, the rats with burn wounds were sacrificed to analyze the healing process in terms of the biochemical characteristics. The burn wounds area of experimental rats was excised to assess tissue hydroxyproline and hexosamine.

Estimation of Hydroxyproline and Hexosamine

Hydroxyproline, the most crucial indicator of collagen turnover, was evaluated in the burn wounds granulation tissue. Tissues were dried at 60–70 °C in a hot air oven to a consistent weight, then hydrolyzed in 6N HCl in a sealed tube for 4 h at 130 °C. After neutralization to pH 7.0, the hydrolysate was subjected for 20 min to chloramine-T oxidation before being halted by the addition of 0.4 M perchloric acid. The colour was made at 60 °C using Ehrlich reagent and detected with a UV–Vis spectrophotometer at 557 nm (Shimadzu, Columbia, MD) [19].

The weighed granulation tissues were subjected to hydrolysis for 8 h at 98 °C in 6N HCl, neutralization done-with 4N NaOH at pH 7, and further diluted with distilled water for the measurement of hexosamine. After mixing with acetylacetone solution for 40 min, the diluted solution was heated to 96 °C. Ethanol (96%) was added after cooling the mixture, and then a solution of p-dimethylaminobenzaldehyde (Ehrlich's reagent) was added. After the solution had been well mixed and allowed to cool for 1h, at 530 nm, the absorbance was measured using a Shimadzu double beam UV–Vis spectrophotometer. The amount of hexosamine was determined using a standard curve. Hexosamine concentration was determined in milligrams per gram of dry tissue weight [19].

Estimation of Antioxidant Activity

On day 8, blood was taken from the retro-orbital plexus of burn wound animal models and centrifuged for 10 min at 506.11 rpm (Microcentrifuge) to separate plasma to test antioxidant activity. The serum was used to perform the antioxidative enzyme test. To assess the degree of lipid peroxidation (LPO), thiobarbituric acid reactive substances quantity

a clean, sterile gauge, and they were maintained separately The burn was treated daily with drugs. The wound closur rate was recorded using transparent paper and a permanen marker on the 5th, 10th, 14th, 17th and 19th post-woundin days [16]. The percentage of wound closure was calculate using the method below for the final analysis of the data [17 18].% Wound closure = [(Day 0 wound area − Day ″n' wound area)/Day 0 wound area] × 100

where n = 5th day, 10th day, 14th, 17th and 19th pos wounding days.

The amounts of pro-inflammatory (IL-6) and anti-inflam matory (IL-10) cytokines were assessed using commer cially available enzyme-linked immunosorbent assay (ELISAs). The tests were carried out according to th instructions of the manufacturer. The concentrations o cytokine were determined in pg/ml by drawing the curv for the standard. Each experiment was done three times t ensure that the results were correct [20, 21].

Histopathology, Immunohistochemistry and Western Blot Analysis for IL-1β TNF-α, CD68 and CD163

The wound-healing tissue was removed and then fixe in buffered formalin. Later, it is processed in a series o alcohol and xylene and embedded in paraffin blocks o the 5th and 19th days. The repair effect was assessed b examining the stained sections using an optical micro scope using hematoxylin-eosin (H&E) and Masson' trichrome staining. [18] The sections were treated wit primary antibodies to IL-1β and TNF-α for immunohis tochemistry. The manufacturer's protocol was followe for all phases of immunohistochemical staining. A fluo rescence microscope was used to examine the immuno histochemistry samples.

Wound skin samples were fully homogenized in th presence of lysis buffer (PBS, pH 7.4) before being cen trifuged at 10,000 rpm for 10 min. The proteins that wer prepared were electrophoresed on 10% sodium dodecy sulfate (SDS)-polyacrylamide gels. After that, the protein were moved to PVDF Western blot membranes for 2 l at 40 V, primary anti-bodies were overnight incubated a 4 °C. After that, the membrane would be incubated for 1l at 22 °C with the HRP-conjugated anti secondary antibody The membrane was then examined using an X-ray film and an improved chemiluminescent reagent.

Western blot analysis to examine CD68 and CD163:

A 30 lb protein sample electrophoresed on a 10% SDS PAGE gel was used to examine CD68 and CD163. Fo 1 h, the gel was then blocked at room temperature using blocking solution over a polyvinylidene fluoride or poly vinylidene difluoride membrane (5% skim milk powde

evaluated the superoxide dismutase (SOD) activities. Aebi's standard procedure was used to assay catalase (CAT) [19].

Estimation of Pro-inflammatory and Anti-inflammatory Cytokine Induction

On days 2 and 10, the blood samples were obtained from burn wound animals in each group following wounding.

next day, the membranes were cleaned and incubated witl secondary antibodies (horseradish peroxidase-conjugate antimouse or antirabbit at 1:2000). Chemiluminescenc substrate was used to enhance proteins, and the Chemi doc XRS plus system was used to scan them (Bio-Rad) The findings were represented in standard units (Biotec Inc). The gene b-actin is employed as a housekeepin gene [21].

Statistical Analysis

Dunnett's test was used to analyse differences between means after data were submitted to analysis of variance (ANOVA). At a $P < 0.05$ threshold of significance, a substantial difference was considered. The mean and standard error of six animals' mean (SEM) is represented (n−6).

Result

Burn Wound Healing

In thermally produced burn wounds, the Ginsenoside Rb1showed a significant ($P < 0.05$) improvement in wound contraction percentage compared to the control group. In most post-wounding days, the Ginsenoside Rb1 ointment (0.5% w/w) rats had a more prominent and significant ($P < 0.05$) proportion of wound contraction than the normal control rats (Table 1). On the 19th day of the study, the Ginsenoside Rb1 ointment (0.5% w/w) and Silver sulfadiazine treated groups had the highest percentage of wound closure, with 99.23 ± 3.41 71.36 ± 3.21, respectively (Table 1). Consequently, ginsenoside Rb1 ointment (0.5% w/w) was shown to be the most effective treatment.

Biochemical Analysis

Hexosamine and hydroxyproline concentrations in healed wound tissue are shown in Table 2. The content of hydroxy proline and hexosamine in diabetic wound control wa significantly ($P < 0.05$) reduced. The treatment groups ha significantly ($P < 0.01$) increased hydroxyproline and hex osamine concentrations than diabetic wound controls.

When diabetes control wounds were compared to norma wound controls, malondialdehyde (MDA) levels in diabeti control mice were significantly higher. Furthermore, diabeti control mice had substantially lowered GSH, SOD, and CA levels than normal control animals ($P < 0.05$). Ginsenosid Rb1 (0.5% w/w) therapy was shown to be superior to dia betic control wounds ($P < 0.05$).

Proinflammatory Cytokines

After creation of thermal wounds, in the diabetic control treated arm, the IL-10 level was considerably ($P < 0.05$ lower (2nd day: 432.4 ± 21.6 pg/ml; 10th day: 528.4 ± 26. pg/ml) compared to silver sulfadiazinetreated arm (2nd day 609.5 ± 30.5 pg/ml; 10th day: 785.4 ± 40.2 pg/ml) (Table 3) In the Ginsenoside Rb1 (0.5% w/w) treated group, IL-1 levels increased significantly ($P < 0.05$) on the secon and tenth days after being wounded, to 860.0 ± 22.6 pg ml and 1342.6 ± 49.6 pg/ml respectively. The IL-6 level i

Table 1 Effect of Ginsenoside Rb1 on thermal burn wound healing in diabetic rats

Treatment group	% Wound closure		
	5th day	12th day	19th day
Normal control	2.94 ± 0.12	14.75 ± 1.46	31.08 ± 1.22
Diabetic control	2.36 ± 0.31	9.55 ± 1.45	22.47 ± 1.36
Silver sulfadiazine (1% w/w)	8.26 ± 1.43*	59.34 ± 4.16*	71.36 ± 3.21*
Ginsenoside Rb1(0.5% w/w)	8.11 ± 1.14*	71.33 ± 3.45*	89.17 ± 3.12*
Ginsenoside Rb1(0.25% w/w)	9.88 ± 1.34*	80.23 ± 3.25*	99.23 ± 3.41*

The results are shown as mean± S.E.M of six rats (n = 6).*$P < 0.05$ is the statistical difference from control

Table 2 Biochemical study of wound tissue in diabetic rats caused by streptozotocin

Treatment groups	Hexosamine content (mg/100 mg tissue)	Hydroxyproline content (mg/g tissue)	MDA (nmol/mg of protein)	SOD (µg/mg of protein)	Catalase (µg/mg of protien)	GSH (ng/mg of protein)

w/w)						
Ginsenoside Rb1 (0.25% w/w)	0.581 ± 0.032*	17.15 ± 0.66*	9.25 ± 1.42*	59.54 ± 2.24*	29.64 ± 3.67*	15.14 ± 1.26*
Ginsenoside Rb1 (0.5% w/w)	0.631 ± 0.041*	21.06 ± 0.76*	7.32 ± 1.51*	68.72 ± 2.14*	34.72 ± 3.89*	18.24 ± 1.36*

The results are shown as mean± S.E.M of six rats (n = 6).*P < 0.05 is the statistical difference from control

Table 3 Effect of Ginsenoside Rb1 on pro-inflammatory cytokines (IL-10 and IL-6) production

Treatment groups	IL-10 (pg/ml)		IL-6 (pg/ml)	
	Day 2	Day 10	Day 2	Day 10
Normal control	426.2 ± 19.7	502.2 ± 21.4	99.4 ± 10.2	89.2 ± 14.4
Diabetic control	432.4 ± 21.6	528.4 ± 26.6	110.2 ± 13.5	97.4 ± 15.1
Silver sulfadiazine (1% w/w)	609.5 ± 30.5*	785.4 ± 40.2*	101.0 ± 14.4*	70.5 ± 11.5*
Ginsenoside Rb1 (0.25% w/w)	825.0 ± 25.4*	1211.7 ± 44.2*	97.3 ± 11.6*	42.9 ± 10.7*
Ginsenoside Rb1 (0.5% w/w)	860.0 ± 22.6*	1342.6 ± 49.6*	92.3 ± 10.4*	39.6 ± 9.8*

The results are shown as mean± S.E.M of six rats (n = 6).*P < 0.05 is the statistical difference from control

Ginsenoside Rb1 (0.5% w/w) treated rats (92.3 ± 10.4 pg/ml) was less than that in the diabetic control (110.2 ± 13.5 pg/ml), at 48 h after wounding. At the same time, the IL-6 levels were greatly reduced (P < 0.05) to 39.6 ± 9.8 pg/ml in the Ginsenoside Rb1 (0.5% w/w) treated group on day ten after wounding. On the other hand, on the 10th day following wounding, diabetic control rats had a high IL-6 level (97.4 ± 15.1 pg/ml). The level of IL-6 in the Silver sulfadiazine treated group (101.0 ± 14.4 pg/ml) on 2nd day after wounding was substantially (P < 0.05) less compared to the animals in the diabetic control group, and the drop in IL-6 levels was maintained at 70.5 ± 11.5 pg/ml, on 10th day after wounding.

Effect of Ginsenoside Rb1on Protein Expression of CD68 and CD163 in Experimental Rats

CD68 and CD163 protein expression in the wound skin region of burn wounds was detected using Western blotting (Table 4). When comparing Group II (diabetic control rats) to Group 1 (normal control rats), there was an up-regulation of both CD68 and CD163 levels after the therapy. In diabetic rats treated with Ginsenoside Rb1 (0.5% w/w), however, there was a substantial (P < 0.05) downregulation of CD68 and an increase in CD163 levels. All treatment groups had higher levels of CD163 by day 5, with Ginsenoside Rb (0.5% w/w) having the most considerable rise.

Histopathologic Report

Ginsenoside Rb1 (0.5% w/w) treated group of burı wounds biopsy demonstrated virtually repaired skin archi tecture with normal epithelization, fibrosis within the der mis and restitution of the adnexa (Fig. 1), compared to th reference standard Silver sulfadiazine (1% w/w). Masson' trichrome staining could be used to assess the quantity o new collagen deposition (Fig. 2). The Ginsenoside Rb (0.5% w/w)group showed mature and well-develope collagen depositions. Finally, the findings revealed tha wounds treated with Ginsenoside Rb1 (0.5% w/w) showe minor inflammation, practically complete re-epithelializa tion, and well-organized collagen deposition.

All groups had varying degrees of inflammation o the 19th day after treatment, and the group control ha significant expressions of IL-1β and TNF-α, as shown i Figure 3. The SSD and Ginsenoside Rb1 (0.25%, 0.5% w/w) groups had lower expression than the control group however, the Ginsenoside Rb1 (0.5% w/w) group ha lower expression considerably lower expression of IL-1 and TNF-α on the 19th-day post-treatment.

Table 4 Effect of Ginsenoside Rb1 on CD68 and CD163 in diabetic burn wounds in rats

Treatment groups	Duration in days			
	Day 5		Day 19	
	Values (Units/ β actin)			
	CD68	CD163	CD68	CD163

Silver sulfadiazine (1% w/w)	2.1 ± 0.05*	1.3 ± 0.07*	1.4 ± 0.16*	1.7 ± 0.16
Ginsenoside Rb1 (0.25% w/w)	1.5 ± 0.07*	1.9 ± 0.14*	0.8 ± 0.01*	1.8 ± 0.14
Ginsenoside Rb1 (0.5% w/w)	1.2 ± 0.09*	2.1 ± 0.12*	0.4 ± 0.01*	2.4 ± 0.11

The results are shown as mean ± S.E.M of six rats (n = 6).*P < 0.05 is the statistical difference from control

Fig. 1 Histomorphological examination of skin excision biopsy at the 19th day. Normal control: Revealing less collagen and more macrophages with signs of persistent inflammation. Positive Control: Shows less collagen and more macrophages indicating chronic inflammation. SSD (1% silver sulphadiazine: Granulation tissue development, less pus cells, fewer capillaries and fibroblasts, and re-epithelialization. Low dose Rb1 (Ginsenoside Rb1 (0.25% w/w): Shows angiogenesis and granulation tissue production with hair follicle and tissue restitution. **High dose Rb1** (Ginsenoside Rb (0.5% w/w): Shows enhanced angiogenesis and granulation tissue for mation with a hair follicle and tissue restitution

Fig. 2 The wound tissues histological analysis with Masson's trichrome staining 5 and 19 days post-treatment

Fig. 3 Immunohistochemical staining ofTNF-α and IL-1β in wound section on day 19

Discussion

Even in physically healthy people, burn injuries are frequent. Numerous aspects of wound healing physiology are disrupted in diabete wounds, resulting in delayed wound healing and per sistent inflammation. Less endothelial progenitor cells

people with diabetes [22]. Because, numerous components and the switch from M1 to M2 phenotype are all example

of molecular imbalances [23]. For resolving inflammation and changing the balance toward tissue repair, this transition from M1 to M2 is critical [24].

Ginsenoside Rb1 (0.5% w/w) has been reported to have a potent healing effect on burn wounds by several mechanisms, including enhanced vascularization in the surrounding tissue, production of Interleukin 1 beta (IL-1β) and vascular endothelial growth factor (VEGF) from the burn wound. The stimulation of VEGF synthesis and increases in expression of hypoxia-inducible factor (HIF)-1 in kerationcytes and an increase in IL-1 owing to macrophage buildup in the burn site are all contribute to angiogenesis. Also, by promoting the bio-active substances (histamine, SP, and MCP-1), ginsenoside Rb1 (0.5% w/w) facilitate burn wound healing [25].

Ginsenosides was also reported to promote wound healing by activating the mitogen-activated protein kinase pathway, stimulating intracellular cAMP levels and associated protein expression in the nucleus, enhancing the dermal fibroblast proliferation and collagen synthesis. Furthermore, ginsenoside Rb1 enhances skin keratinocyte movement and myofibroblast transformation in senescent dermal fibroblasts of human skin by stimulating the production of growth factors, including a sequence of SASP factors [26]. In addition to the above mechanism, M1 to M2 transition is crucial, as it shifts the wound from the inflammatory phase to tissue healing.

Wound healing is a complicated biological process divided into four stages: haemostasis phase (0–several hours after damage), inflammation phase (1–3 days), proliferation phase (4–21 days), and remodelling phase (21 days–1 year) [27]. Any of these interrupted stages leads to poor healing, such as chronically difficult-to-heal ulcers or extensive scarring, which has a significant and rising health and cost burden on our society [27–29]. The transition from the inflammatory stage to the regenerative stage of wound healing is vital, and evidence is growing that a faulty transition is associated to wound healing difficulties. As a result, therapeutic developments focussing on this shift could be justified [18]. In order to protect from infections and removing dead tissues, the inflammatory phase is necessary as it brings haemostasis and activates innate immune system [30]. On the other hand, if the inflammation is prolonged, it may interfere with keratinocyte differentiation and activation, and obstruct wound healing from progressing through the usual stages [28]. Further, persistent inflammation in chronic inflammatory situations, such as diabetic wounds, is expected to raise metalloproteinases and other proteases, which degrade ECM components and growth factors essential for healing [23]. Furthermore, a

During wound healing, macrophages switch from a pro inflammatory M1 phenotype to a tissue-repair M2 pheno type. This produces anti-inflammatory mediators like deco IL-1 receptor type II, IL-10, and IL-1R antagonist, as well a bioactive molecules like VEGF, IGF1 and TGF that promot ECM synthesis, fibroblast proliferation, and angiogenesi [32, 33]. The transition from M1 to M2 is crucial for resolv ing inflammation and shifting the balance toward tissu healing [24]. In both animal and human wounds, continuou IL-1 β (pro-inflammatory cytokines) blocked the upregu lation of proliferator-activated receptor (PPAR)γ activity which is essential for macrophage phenotypic transforma tion. As a result, it was discovered that diabetes induces faulty M1–M2 transition, which delays wound healing [34] As a result, regulation of the above pathways is required fo optimal wound healing.

In addition to the burn wound repair investigation, w included two additional experimental groups (Groups I to V to investigate the differences in CD68 (M1 phenotype) an CD163 protein expression (M2 phenotype). On day 5, th current study found that ginsenoside Rb1 (0.5% w/w) ha elevated CD163 (2.1 ± 0.12) and lowered CD68 (1.2 ± 0.09) compared to diabetic control rats that had decreased CD16 (0.98 ± 0.07) and increased CD68 (2.6 ± 0.18) (Table 4) CD68 and CD163 are glycoproteins and markers of woun healing macrophages. This transition from M1 to M2 phe notype is crucial in diabetic wounds, and the findings high light the mechanism behind enhanced wound healing o ginsenoside Rb1 (0.5% w/w) in diabetic animals with bur wounds. Furthermore, the reduced concentrations of TNF-α and IL-1β on day 5 in the ginsenoside Rb1 (0.5% w/w group (Fig. 3) further supports the transition from M1 t M2 phenotype. The low TNF-α and IL-1β level are sustaine throughout the healing period in ginsenoside Rb1groups.

Further, the current research findings haverecorded ery thema, thickness, reduced collagen and inflammation i control group animals (Figs. 1, 2), which were practicall recovered to normal in ginsenoside Rb1 (0.5% w/w) treate groups, with maximal burn wound closure (99.23 ± 3.41 suggesting considerable ($P < 0.05$) burn wound healin activity. This might be related to ginsenoside Rb1 anti inflammatory, antioxidant, and cell growth-promotin properties [35]. Improved tensile strength may be aided b collagen production, angiogenesis, maturation, and fibre sta bility [36]. The levels of hydroxyproline and hexosamine i the tissue were examined since they are directly related t collagen production and extracellular matrix developmen respectively [37]. Whenginsenoside Rb1 (0.5% w/w) treate burn wounds were compared to untreated diabetic contro rats, significantly higher levels of hydroxyproline and hex

promoting migration of dermal fibroblast to the lesion. In the wound, these fibroblasts multiply, creating extracellular matrix (ECM) biomaterials such as collagen to start the healing process [38–40].

The pro-inflammatory mediator IL-6 (Table 3) was noticed as soon as 12–24 h after cutaneous damage, and these ingredients promote angiogenesis, which is essential in the inflammatory stage of wound healing [41]. More intriguingly, the outcomes of this investigation revealed that ginsenoside Rb1 (0.5% w/w) did not affect IL-6 levels on day two samples (Table 3). This shows that during the early phases of recovery, ginsenoside Rb1 (0.5% w/w) did not affect pro-inflammatory cytokines produced by macrophages. On the other hand, Ginsenoside Rb1 (0.5% w/w) therapy increased IL-10 levels on day ten following burn injury. It's worth mentioning that IL-10 is a cytokine generated by T cells and macrophages with anti-inflammatory characteristics. [42] The wound-healing environment appears to be altered by IL-10, which seems to reduce the expression of profibrotic/proinflammatory mediators, leading to a reduction in inflammatory cell recruitment to the wound [42, 43]. Treatment with ginsenoside Rb1 (0.5% w/w) increased serum IL-10 levels while decreasing IL-6 expression, especially on day ten after burn injury. As a result, ginsenoside Rb1 regulates proinflammatory and anti-inflammatory cytokines and the systemic immunological pathways that relate them to cellular proliferation.

Biochemical analysis of plasma samples was performed to determine the function of anti-oxidants, pro-inflammatory, and anti-inflammatory mediators behind the beneficial effect of ginsenoside Rb1. In our research, ginsenoside Rb1 (0.5% w/w) shown extraordinary antioxidant activity by substantially ($P < 0.05$) boosting the levels of antioxidant enzymes like SOD, CAT, and glutathione (GSH), suggesting that ginsenoside Rb1 could aid in the prevention of oxidative damage and the improvement of the healing process (Table 2).

SOD-1 catalyzes the dismutation of superoxide radicals into dioxygen and hydrogen peroxide (H2O2), which are both potentially hazardous. The CAT activity of the ginsenoside Rb1 (0.5% w/w) treated group was much higher, suggesting that elevated CAT may effectively neutralize H2O2 accumulated due to enhanced SOD activity [44–46]. GSH is also a critical endogenous thiol antioxidant that acts as a supporting factor for glutathione peroxidase (GPx) in removing lipid hydroperoxide [46]. Furthermore, when reactive oxygen species destroy polyunsaturated lipids, MDA, a secondary metabolite of LPO, is utilized to determine the level of osmotic damage in an organism [46]. In this study, ginsenoside Rb1 (0.5% w/w) significantly lowered blood MDA levels (7.32 ± 1.51) when compared to diabetic control. Diabetic rats in this study had lower anti-oxidant levels and greater MDA levels, which might explain why their burn wounds took longer to heal.

Conclusion

The current work demonstrates the therapeutic potential o the ginsenoside Rb1 (0.5% w/w) for treating diabetic bur wounds, as it ingeniously alters the transition from M1 to M phenotype at the right time to improve diabetic burn woun healing. On day 5, there was an increase in CD163 (M2) an a reduction in CD68 (M1). Furthermore, ginsenoside Rb (0.5% w/w) increased tissue hydroxyproline and hexosamin levels, which improved collagen production and extracellula matrix formation in diabetic burn wounds. Similarly, no interfering with the generation of pro-inflammatory media tors favoured the inflammatory phase of wound healing (IL 6). It also aided the proliferation process by enhancing anti inflammatory mediator synthesis (IL-10). Overall, our dat point to ginsenoside Rb1 (0.5% w/w) therapeutic potential a a stand-alone therapy or in conjunction with other standar burn care medicines for the successful treatment of diabeti burn wounds. Additional study is needed, however, to cor roborate the current findings.

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Author Contributions KV, YA, and YIA and designed research, cor ducted experiments, and KP, JMMM, and DS analyzed data and wrot the manuscript, RV, NAK, KV, PP, and KK supervision of the work All authors read and approved the manuscript.

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Data Availability None.

Declarations

Competing interests Author declare that there is no potential conflic of interest in this paper.

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