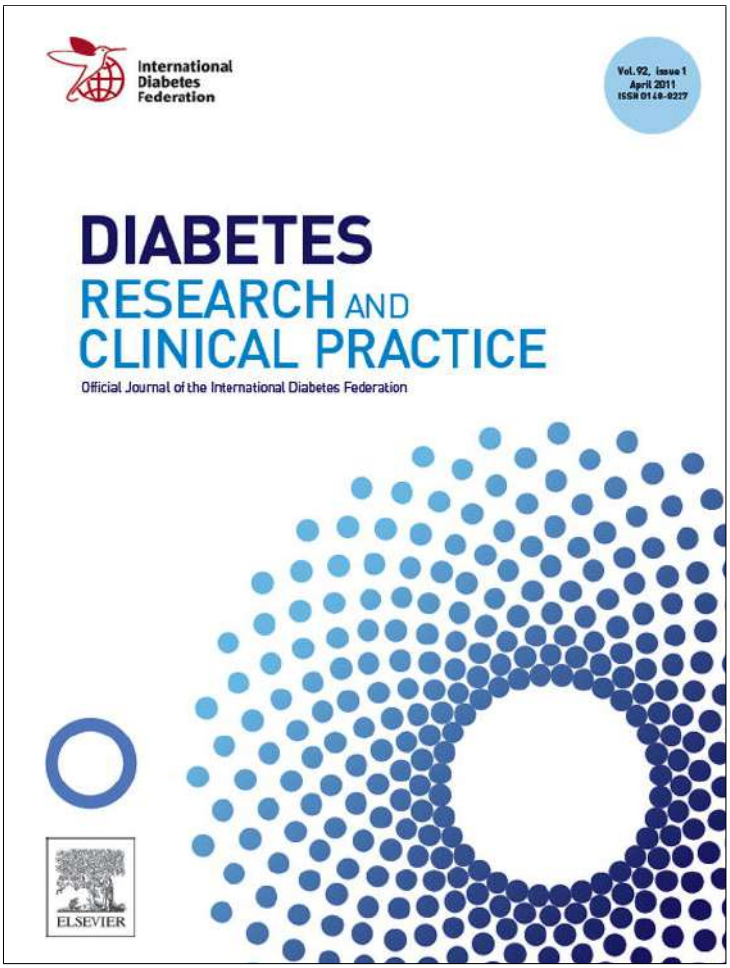

DIABETES RESEARCH AND CLINICAL PRACTICE 92 (2011) 92–99

Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Clinical evidence to demonstrate that simultaneous growth of epithelial and fibroblast cells is essential for deep wound healing

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ARTICLE INFO

Article history:
Received 30 March 2010
Received in revised form
3 December 2010
Accepted 13 December 2010
Published on line 17 January 2011

Keywords:
Deep wound
AS-21
Plant tannins
Protease activity

ABSTRACT

Objective: The aim of this study was to evaluate the chronic wound healing properties of tannin rich plant extracts.
Methods: The cell growth stimulating potential of 128 procyanidin rich plant extracts was evaluated in *in vitro* cell culture models. For clinical trial, a 3% solution of two plant extracts having synergistic effect on cell growth was prepared in glycerol and honey. Placebo test product contained only glycerol and honey. 93 adult patients with one or more lower extremity deep wounds were divided at randomly in two groups. 41 patients in the placebo (AS-22) and 52 in the active treatment (AS-21) groups having respectively 49 and 69 wounds of a mean surface area of 56.70 and 52.03 cm^2, and volume of 57.22 and 52.15 cm^3, were treated by applying the test products topically for a period of 6-weeks.
Results: A statistically significant difference was observed between the placebo and the AS-21 treated groups with respect to reduction in the wound surface area (33.37 vs 97.87%) and wound volume (29.45 vs 94.17%) after 6-weeks of treatment. Mean wound humidity and pain scores were also reduced.
Conclusion: Tannin rich plant extracts are highly interesting for the treatment of chronic wounds.

1. Introduction

Due to increasing life expectancy coupled with our modern way of life, more and more elderly people have conditions that confine them to wheelchairs and beds with decubiti as a major problem [1,2]. Usually, decubiti are caused by excessive and prolonged pressure or friction on a part of the body leading to poor blood circulation and localized tissue death. Patients with venous insufficiency and diabetes also develop deep ulceration/wounds on the lower extremities of the body [3–6]. Epidemiological studies indicate that the average hospitalization period for a patient with deep ulcer treatment is between 4 and 8 weeks and the average treatment cost is above 20,000 Euros per hospital admission [7]. Although poor nutrition and old age are mostly responsible for fatal outcome, the discomfort due to unhealing ulcers also plays a major role.

Deep wounds affect not only the superficial skin structures but also the underlying muscles and may reach up to the bone tissue [8]. Skin is predominantly composed of epithelial type of cells while muscles contain fibroblast type of cells. Any deep wound healing therefore requires optimal growth of fibroblast and epithelial cells simultaneously [9].

Except for some encouraging results with the growth factors, currently there is no treatment which specifically stimulate the growth of epithelial and fibroblast cells [10]. All the currently available treatments are directed at establishing

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0168-8227/$ – see front matter
doi:10.1016/j.diabres.2010.12.021

a clean, moist environment that will indirectly encourage cell regrowth. Due to their large surface area and deepness, deep wounds are often infected and require protection or the use of antiseptics and antibiotics. The use of antiseptics or other chemical agents over the wound has the disadvantage of creating an unfavorable environment for cell growth inside the wound cavity which delays further healing [6]. Due to wound surface contact with air, the drying of the wound is considered the main cause of pain and discomfort. This is the reason why almost all the currently available treatments, such as the hydrogels, are directed to keep the wound surface humid [11,12], but they have no direct effect on the cell growth. Several clinical trials are conducted since last 30-years using different types of growth factors but their clinical efficacy is limited and results are not encouraging [13].

As plants are less toxic to the cellular structures and many plant ingredients are traditionally used to accelerate healing [14–16], the aim of this study was to screen certain plant extracts rich in tannins (procyanidins) for their effect on the growth of fibroblast and epithelial cells *in vitro* and subsequently to evaluate their efficacy on the healing of deep wounds. As the growth of epithelial and fibroblast cells concerns all the types of deep wounds, the clinical trial was performed without taking into consideration the origin of wounds.

2. Materials and methods

2.1. Plant extract preparation

128 plants extracts were initially selected to study *in vitro* cell growth effects on the fibroblast and epithelial cell. Either whole plant or the parts of the plant which are traditionally used in phytomedicine were selected for extraction. For example, aerial parts of *Alchemilla vulgaris* (*A. vulgaris*), bark of *Mimosa tenuiflora* (*M. tenuiflora*), leaves and flowers of *Echinacea purpurea*, bark of *Azadirachta indica*, fruits of *Vaccinium* sp., leaves of *Camellia sinensis*, seeds of *Vitis vinifera* and roots of *Panax gingseng* were used for the extraction. The parts of the plant were collected, washed with distilled water, dried in air at 25 °C in the dark and stored at −20 °C until use.

Tannin rich plant extracts were prepared using standard extraction technique as described by Marja et al. [17]. Extracts were atomized for drying and diluted in the culture medium or in the test product base before use.

2.2. In vitro studies for cell growth

Epithelial cell cultures: MDBK (Madin–Durby Bovine Kidney) cells were purchased from ATCC (American Type of Culture Collection, USA) as a model of epithelial cells. Initial culture was prepared in 75 cm^2 tissue culture flasks (Corning, USA) in minimum essential medium (MEM) supplemented with 10% foetal calf serum (FCS). Complete and confluent cell monolayers were obtained within 6–8 days of incubation.

Primary smooth muscle fibroblast culture was prepared as described by Shrivastava et al. [18]. MDBK (Madin–Durby Bovine Kidney) cell line (ATCC, USA) was used as a model of epithelial cells. For experiments, cell monolayers were dispersed with trypsin and 100 μl cell suspension containing 3×10^5 cells/ml with 10% FCS was seeded in 96 well plates and incubated for 24 h at 37 °C to obtain a monolayer covering about 30% surface. After 24 h cultures, growth medium was replaced by a new maintenance medium (2% FCS) containing appropriate concentration of test product (0.3; 1.0; 3.0 and 5.0%). To study the effect of plant extract association, half of the most active concentration of the plant extract stimulating significantly the growth of either fibroblast, epithelial cells or both cell types, was associated to study combined effect on cell growth. Control cultures were treated identically with medium containing no test product.

All experiments were conducted in triplicate using 16 microwells per concentration per experiment ($n = 48$ per concentration).

After 72 h in culture, the number of live cells indicating cell growth was measured quantitatively through the MTT mitochondrial dehyrogenase indicator vital stain. The optical density (OD) of the solution was measured at 560 nm using an automatic micro-well reading apparatus (Dynatech MR 400).

2.3. Clinical study

This 6-week study was carried out by VITROBIO Pharmaceutical Research Center, ZAC de Lavaur, Issoire, France in collaboration with Taj hospital and Research Center at Panji in India according to the standard operating procedures and GCP standards of the institutes between December 2007 and September 2008.

2.3.1. Test products

The test product contained 1.5% dried extract of procyanidins from *M. tenuiflora* (13.5% polyphenols) and 1.5% dried extract of procyanidins from *A. vulgaris* (12% polyphenols) in an excipient containing 64% glycerol (vegetable origin, Pharma Grade, CAS no. 56-81-5, Undesa, Italy) and 33% honey (Acacia origin, Miels Villeneuve, France).

The placebo preparation contained 65% glycerol and 35% honey v/v. The product was filled in white 10-ml (approximately 9 g) plastic tubes with a 4-cm long application canula having an inbuilt security opening seal. A sticker containing product code, AS-21 for the plant extract containing tubes and AS-22 for the placebo glycerol–honey mixture containing tubes was sticked on each tube. For ethical reasons, except for the patients, the product identity was known to the treating staff.

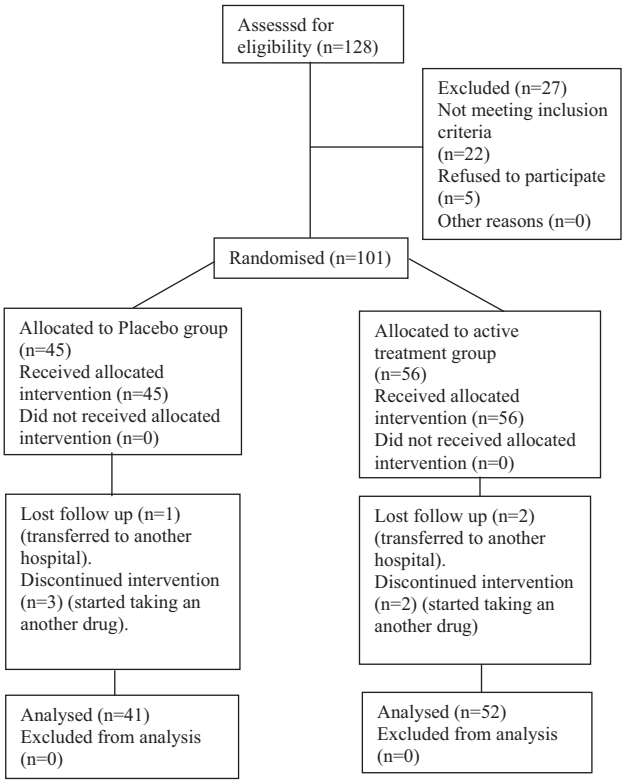
2.4. Patients

New patients with at least one lower-extremity chronic wound lasting longer than 2-months were recruited in the study on the basis of conformity with the inclusion and exclusion criteria and patients consent. The inclusion criteria were: subjects having at least one or more lower extremity wound for the last 2 months, not under any systemic antibiotic-therapy since last 2-weeks, not below 20 or above 80 years of age in which the primary etiology of the wound was either diabetic, pressure or venous ulcers. The main exclusion criteria were any systemic or topical therapy (corticosteroids, radiation or chemotherapy) or ayurvedic treatments which

may affect wound healing, pregnant and disabled persons or subjects not willing to participate in the study were not recruited. It was decided to stop the treatment in case of any critical event conform to the declaration of Helsinki/Tokyo/Venice and the law of 20th December 1988, concerning protection of volunteers for the biomedical research.

Relevant information about the history and severity of any medical conditions known to influence wound healing were also analyzed to select the patients.

Selected volunteers were randomly assigned in two groups on the basis of arrival in the clinic as shown below in the participant flow chart.



Among 45 patients recruited in the group 1 (placebo) and 56 in the group 2 (active treatment), the data of 4 patients from each group were discarded from the study as they either took some indigenous treatment (5 patients) or were transferred to another hospital (3 patients). All patients were recruited over a period of 4 weeks.

The primary cause of wounds was diagnosed as diabetic ulcers, pressure ulcers and venous insufficiency respectively in 67%, 18% and 18% cases in the placebo group-1 and 64%, 16% and 20% in the active treatment group-2. Among these 93 patients (41 in group 1 and 52 in group 2), 49 wounds were treated in the group-1 with 5 having a surface area below 20 cm^2, 19 between 20 and 40 cm^2, 13 between 40.1 and 80 cm^2 and 12 above 80 cm^2. Among 69 wounds treated in the group-2, 10 had a surface area below 20 cm^2, 24 between 20 and 40 cm_2, 21 between 40.1 and 80 cm^2, and 14 above 80 cm^2.

The placebo test product contained honey as honey is traditionally used to accelerate wound healing and is also used as excepient in the active treatment product. The product identity was known to the hospital staff but not to the patients.

2.5. *Posology and treatment method*

After patient recruitment, on the first day of the study, the wound surface was cleaned thoroughly with saline and any excess growth was removed with a scalpel. Measurements of wound surface and depth were taken and first treatment was applied 2 h after cleaning.

The test product tube was opened and the product was applied drop by drop on the boundary of the wound in such a way that product forms a thin film over the entire wound surface. The wound was then covered with sterile cotton gauze allowing some air circulation. Wound was examined twice a day, cleaned if necessary and fresh product was applied in an identical fashion. No antiseptics or the compression therapy was used during the study.

2.5.1. *Parameters measured*

Local tolerance, side effects and any undesirable reaction was checked and noted at the time of each product application. Each patient was asked to evaluate pain intensity on a 0 (no pain) to 4 (very painful) rating scale at the time of first product application (day 0) and thereafter at weekly intervals (days 7, 14, 21, 28, 35, 42) during the 6-week treatment period only in the AS-21 group 2. Similarly, wound surface humidity was evaluated on a 0 (completely dry surface) to 4 (very humid surface) scale and recorded by nurses at weekly intervals in the AS-21 group only.

Wound surface was determined for all the wounds at weekly intervals in both groups by placing a transparent cm^2 graduated film over the wound and by tracing the wound border with a permanent marker pen. The cm^2 area inside the transparent film represented the wound surface area.

Wound volume was measured in all the patients of both groups for all the wounds on days 0, 21 and 42 ($\pm$1) by covering the wound surface with a transparent adhesive film and by injecting a saline solution into the wound cavity with the help of a graduated syringe (20-ml). The amount of saline required to fill the wound cavity completely was recorded (ml solution) and considered as wound volume.

2.6. *Data analysis*

For *in vitro* studies, mean percent changes in cell number compared to controls are presented as mean $\pm$ s.d. For the clinical study, mean scores were calculated in each treatment group, at each time point by for each parameter. The results of AS-21 treated group were compared with the placebo group data. In each case, statistical analysis was performed with one way ANOVA with the paired Student's *t* test *post hoc*.

3. Results

3.1. *Effects of plant extracts on cell growth parameters*

Results are expressed as % change in cell number compared to corresponding untreated control cultures. Among 128 plant

Table 1 – Effect of *A. vulgaris* and *M. tenuiflora* extracts alone and in association (50–50%) on the growth of epithelial and fibroblast *cells in vitro*. Values are means of 48 wells ± SEM.

Plant	Concentration							
	% change epithelial cells ($n = 48$)				% change fibroblast cells ($n = 48$)			
	0.3%	1.0%	3.0%	5.0%	0.3%	1.0%	3.0%	5.0%
M. tenuiflora alone	2.0 ± 0.94	10.04 ± .66	17.95 ± .75	3.08 ± .73	5.16 ± 1.06	11.19 ± 1.19	19.72 ± 0.72	4.40 ± 1.07
A. vulgaris alone	8.98 ± 0.45	21.01 ± 0.47	26.67 ± 0.73	8.54 ± 0.43	1.15 ± 0.82	5.55 ± 0.75	10.35 ± 0.76	0.64 ± 0.76
A. vulgaris 50% + *M. tenuiflora* 50%	13.61 ± 0.63	34.69 ± 0.57	57.7 ± 0.54	9.08 ± 0.69	5.08 ± 1.37	15.42 ± 1.11	43.09 ± 1.22	11.11 ± 1.39

extract tested for cell growth effects on the epithelial and fibroblast cells *in vitro*, none of the plant extract stimulated significantly the growth of fibroblast as well as of epithelial cells simultaneously. Slight (<10% compared to corresponding controls) fibroblast growth enhancing effects were observed with the plant extracts of *E. purpurea* and *Commiphora mukul* while *Asculus hippocastanum*, *Vaccinium* sp., *C. sinensis* and *V. vinifera* extracts stimulated slightly the growth of epithelial cells (results not shown). Only the plant extracts of *A. vulgaris* and *M. tenuiflora* stimulated the growth of epithelial and fibroblast cells (Table 1).

These results show that both plant extracts stimulate the growth of epithelial and fibroblast cells in a concentration dependent fashion between 0.3 and 3% concentrations in the culture medium but a higher concentration of 5% alters the composition of culture medium and has no significant effect on the cell growth. *A. vulgaris* alone specifically stimulated the growth of epithelial cells attaining 26.67% ± 0.73% at 3% concentration while *M. tenuiflora* alone specifically stimulated the growth of fibroblast cells with 19.72 ± 0.72% extra cell growth at 3% compared to the corresponding controls. Surprisingly, the association of half the most active concentration (1.5% each) of each of these two plant extracts produced a highly synergistic statistically significant ($p < 0.05$ for all 1 and 3% concentrations compared to controls) effect on cell growth with 57.70 ± 0.54% and 43.09 ± 1.22% increase in epithelial and fibroblast cell growth, respectively. The association of *C. sinensis* either with *Vaccinium* sp. or with *V. vinifera* also stimulated epithelial and fibroblast cell growths to some extent but the best results were obtained with *M. tenuiflora* and *A. vulgaris* tannins. This is the reason why a test product containing 1.5% concentration of each of these two plant tannins was used to apply on deep wounds which require the growth of superficial epithelial and deeper fibroblast cell for wound healing.

3.2. *Clinical results*

Among total of 101 patients initially enrolled (45 in placebo and 56 in active treatment group), 8 patients were excluded from the study (4 in the placebo group and 4 in the active treatment group) as 5 patients started taking an ayurvedic medicine, 2 were transferred to another hospital and 1 left the city during the study period. The exclusion of these patients was not considered to be related with the test product application.

The baseline profile of the 41 subjects who completed treatment in the placebo group and 52 in the AS-21 treated group was similar as shown in Table 2.

The primary cause of wounds was diagnosed as diabetic origin (65%), pressure ulcers (17%) or venous insufficiency ulcers (18%).

3.3. *Effect on wound surface area*

In both the groups a progressive reduction in the wound surface area was observed during the 6-weeks treatment period (Fig. 1).

Table 2 – Baseline profile of the patients who completed the study.

Profile	Placebo group	Active treatment group
No. of patients	41	52
Mean age	69.03 ± 7.68	67.44 ± 9.69
Males	34	43
Females	7	9
No. of lesions treated	49	69
Patients with 2 wounds	4	8
Patients with 3 wounds	2	4
Wound surface area at the start		
Below 20 cm^2	5	11
Between 20 and 40 cm^2	19	23
Between 40 and 80 cm^2	13	21
Above 80 cm^2	12	14
Wound volume at the start		
Between 0 and 40 cm^3	20	33
Between 40 and 80 cm^3	20	26
Above 80 cm^3	9	10

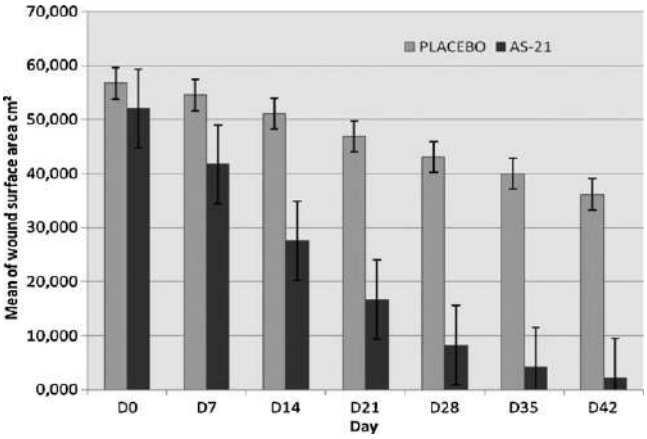


Fig. 1 – Mean wound surface area ($cm^2 \pm SEM$) in the placebo ($n = 49$ wounds) and in the AS-21 treated ($n = 69$ wounds) during the 6 week treatment period.

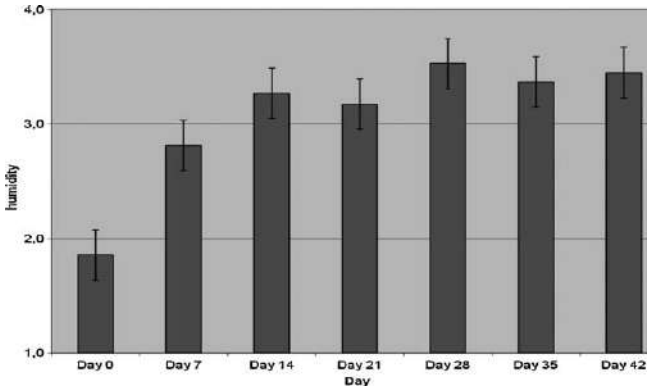


Fig. 3 – Score of wound humidity recorded in the AS-21 treated group at weekly intervals on a scale between 0 (completely dry) and 4 (humid and soft wound surface). Values are mean ± SEM.

In the placebo group, the mean surface area was decreased from $56.70 \pm 38.29\ cm^2$ on the day 0 to $46.84 \pm 38.07\ cm^2$ (−17.39%) on the day 21 and $36.08 \pm 30.97\ cm^2$ (−36.37%) on the day 42. Complete healing was observed in 2/49 wounds (4.08%) after 4 weeks and in 7/49 wounds after 6 weeks of treatment.

The reduction of the mean wound surface area was much faster in the AS-21 treated group, reducing from $52.03 \pm 36.66\ cm^2$ on the day 0 to $16.7 \pm 20.5\ cm^2$ (−67.90%) on the day 21 and $2.13 \pm 3.86\ cm^2$ (−97.87%) on the day 42. Complete healing was observed in 19/69 wounds (27.53%) after 4 weeks and in 41/69 wounds (59.4%) after 6 weeks. All results are statistically significant compared to the corresponding placebo values from the day 14 onwards ($p < 0.005$). The granulation tissue formation was extremely rapid in the AS-21 treated group and a large proportion of wound volume was already filled with mesenchymal tissue within the first 3-weeks of treatment. Although the development of granulation tissue formation in the placebo group was slow, the healing was slow and extremely progressive. The consistency and the physical appearance of the mesenchymal tissue were not significantly different in the two groups.

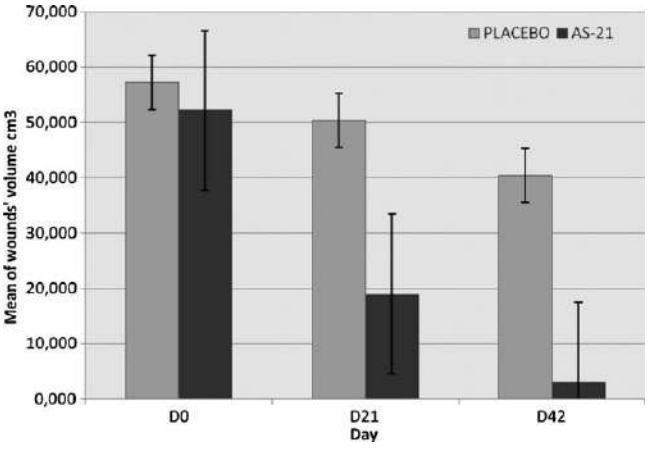


Fig. 2 – Mean wound volume ($cm^3 \pm SEM$) in the placebo ($n = 49$) and in the AS-21 treated ($n = 69$) groups during the 6 week treatment period.

3.4. *Effect on wound volume (Fig. 2)*

The placebo group had a slow but progressive reduction in the wound volume which was reduced from $57.22 \pm 42.14\ cm^3$ on the day 0 to $50.31 \pm 40.01\ cm^3$ on the day 21 (−12.08% reduction compared to the start; $p < 0.5$) and $40.37 \pm 33.04\ cm^3$ on the day 42 (−29.45%; $p < 0.2$) showing that the placebo product containing honey in saline is a good healing agent and helps to reduce the volume of the wound by stimulating cell growth.

With the application of AS-21, the reduction in the volume of the wound was extremely rapid with 63% reduction within 21 days and as much as 94% within 42 days. This difference is statistically significant ($p < 0.005$) compared to the placebo values for days 21 and 42.

There was no difference in the rate of healing of deeper compared to shallower wounds as the wound volume reduction was proportional with time in all the treated wounds in both the groups.

No side effect, local irritation or infection was seen in any of the wounds showing that the products were locally well tolerated. The results of wound humidity scores are given in the Fig. 3.

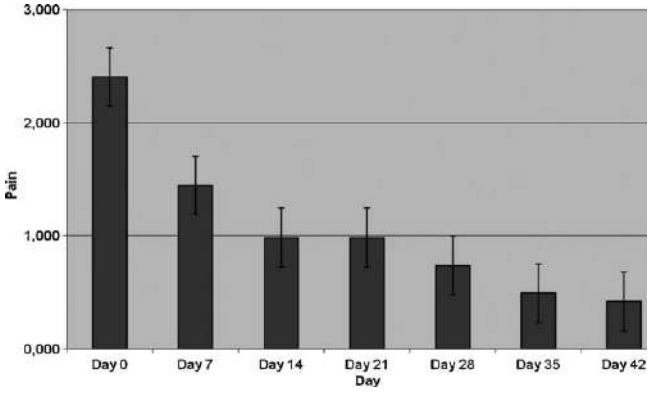


Fig. 4 – The score of pain intensity in the AS-21 group recorded on a scale between 0 (no pain at all) and 4 (highly painful wound) on days 0, 7, 14, 21, 28, 35, and 42. Values are mean ± SEM.

Wound humidity score: Which was rated 1.7 on a scale of 4 on the day 0 went up to 2.7 within one week and remained in a range 3.2–3.3 up to the end of the study (all values $p < 0.05$ compared to day 0) indicating that the application of AS-21 increases wound humidity without any application of additional wound humidifying products.

Pain score: The intensity of wound pain was measured only in AS-21 active treatment group which showed a progressive reduction throughout the 6-week treatment period ($p < 0.5$) with a marked drop during the first week of treatment (2.4/4 on the day 0 and 1.5/4 after one week) as shown in Fig. 4.

4. Discussion

Deep wound healing is a very complex process and may take months or even years to heal completely. Deep wound healing involves expression of numerous growth factors, particularly PDGF (platelet derived growth factor), FGF (fibroblast growth factor) and EGF (epithelial growth factor), which are directed to stimulate the growth of fibroblast cells or epithelial cells. Simultaneous growth of both these cell types is essential to reduce the wound volume and to close the wound cavity. Unfortunately, up till now, there is no specific drug which can stimulate simultaneously the growth of these two cell types.

The currently used most common treatment is hydrogel bandages which protects the wound and keeps the wound cavity humid. They avoid wound surface drying and pain but have no direct effect on the growth of fibroblast and epithelial cells. The second most commonly employed treatment is antiseptics and antibiotics to reduce microbial load [19]. Unfortunately, topical use of antiseptics, which remain in direct contact with the fibroblast and epithelial cells, creates an unfavorable environment for the cell growth which consequently delays healing process.

A number of other agents are developed or are under development for the treatment of deep chronic wounds. The use of growth factors such as PDGF [20,21], EGF [22–24], FGF [25] was considered scientifically appropriate and promising but the recent analysis shows that their efficacy is limited [26]. Despite the use of different types and different origins of growth factors, still there is no scientific evidence explaining the reasons behind their poor activity in stimulating chronic wound healing. It is known that all the cells require a matrix, composed of collagen, elastin, hyaluronic acid and other proteins for initial attachment and growth. Growing cells detached from the matrix during the G_2M phase of the cell cycle when two daughter cells are born and again attach immediately to the matrix surface to mature and multiply. Even in *in vitro* tissue cultures the culture surface requires to be treated with a matrix to facilitate cell attachment. This proves that the presence of optimal amount of matrix is a preliminary requirement for cell growth and wound healing and probably this condition is not fulfilled when only growth factors are used to treat chronic wounds.

The complex process of wound healing involves an optimal balance between the activities of several type of cells (thrombocytes, neutrophils, macrophages, keratinocytes, epithelial and fibroblast cells); growth factors (PDGF, EGF, FGF) and proteases such as matrix metalloproteinases (MMPs), plasmin, elastase, collagenase, and the cell matrix forming substances such as collagen, elastin and hyaluronic acid. A close harmony between the amounts and activities of these substances is essential to create a proper environment for cellular growth and healing. But now it is fairly well established that a marked cellular disfunctioning exist in chronic wounds with a great imbalance in levels of key proteases, cytokines and growth factors [27,28]. In contrast to normal wound healing, the inflammatory reaction in chronic wounds is prolonged which generates a corresponding intensified protease response, in particular MMPs, collagenase and elastase [29].

Bacterial contamination, endotoxins, fragments of cells and extracellular matrix maintain the inflammatory process in the wound which attracts neutrophils, granulocytes which in turn secrets different cytokines capable of increasing the synthesis of MMPs [30]. The high levels of proteases lead to destruction of matrix forming ingredients such as collagen and elastin. In the absence of a proper matrix surface for cell attachment, the cells cannot attach and the wound healing process is delayed.

We observed that only 2 plant extracts among 128 screened stimulated the growth of either fibroblast or epithelial cells with a remarkable synergistic effect on the growth of both cell types. The mode of action of these plant extracts to specifically stimulate the cell growth is not known but probably they act by neutralizing the excess of proteolytic enzymes involved in the destruction of intercellular matrix forming constituents.

A. vulgaris is traditionally used for the treatment of varicose vein and *M. tenuiflora* for wound healing [15,31]. This is the first time that the synergistic effect of these two plant extract to enhance the growth of fibroblast as well as epithelial cells was discovered (patent PCT/EP2008/064214) [32].

Both these plant extracts are rich in proanthocyanidins (condensed tannins) which are polymers made up of multiple anthocyanidin-like molecules. These plant extracts contain hundred of different proanthocyanidins [33], having very high affinity for proteins [34,35]. It has already been shown that the procyanidins have a strong affinity for proteolytic enzymes such as elastase [36], xanthine oxydase, β-glucuronidase, collagenase, and hyaluronidase [31,33,37,38], which are involved in the destruction of matrix components. It was shown that procyanidins interact with cell wall membrane through weak energy bonds of type hydrogen bonds and hydrophobic interactions [39,40]. It is therefore postulated that the procyanidins of *A. vulgaris* binds specifically to certain proteases which degrade matrix components essential for epithelial cell growth while the procyanidins from *M. tenuiflora* neutralize certain other enzymes involved in the degradation of intercellular matrix necessary for the growth of fibroblast cells. The synergistic inhibition of different MMPs by these two plant procyanidins in chronic wounds probably helps to stop the destruction of extracellular matrix leading to enhanced cell growth [29,41].

The results of clinical trial conform these *in vitro* findings as the healing rate was much faster in AS-21 treated compared to the placebo group. We postulate that the procyanidins of *A. vulgaris* and *M. tenuiflora* neutralize excess MMPs in the deep wound thereby stopping the degradation of intercellular matrix and creating a favorable environment for the growth

of fibroblast cells located deep into the wound and the epithelial cells situated at wound boundary. A progressive reduction in the depth of the wound due to the proliferation of fibroblast cells and in wound surface due to the growth of epithelial cells, accelerate the healing process. Current research efforts to search wound healing plants [42,43], especially with protease inhibiting and antimicrobial properties [44] prove that the future of diabetic ulcer treatment lies in natural substances.

5. Conclusion

The results of this study clearly show that a rapid deep wound healing requires simultaneous growth of epithelial and fibroblast cells. In chronic wounds, due to excessive production of protease type of enzymes, the cellular matrix constituents are destroyed leading to poor cell attachment and retarded cell growth. Therefore, plant extracts rich in tannins may play a vital role in deep wound healing. Further studies are recommended to elucidate the synergistic mode of action of plant tannins for the treatment of chronic wounds.

Conflicts of interest

There are no conflicts of interest.

Acknowledgements

This study was sponsored by VITROBIO Research Institute. This product and patents are the properties of VITROBIO Research Institute.

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