Effects of a natural multi-component compound formulation on the growth, morphology and extracellular matrix production of human adult dermal fibroblasts

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Received January 30, 2019; Accepted July 16, 2019

DOI: 10.3892/etm.2019.7872

Abstract. The extracellular matrix (ECM) creates a tissue microenvironment able to regulate cellular signaling. The loss of ECM plasticity is associated with several pathologies, especially those involving chronic inflammation, therefore, the ECM represents a potential therapeutic target for certain conditions. The present study investigated the effects of a natural multi-component compound formulation, Galium-Heel®, on the growth, morphology and ECM production of human dermal fibroblasts (HDF). The effects of the formulation on HDF growth and morphology were assessed by sulforhodamine B assay, trypan blue exclusion staining, FACS and ultrastructural analyses. The effect of the compound on reactive oxygen species production by HDF was performed by dichlorofluorescin diacetate assay. The expression of ECM components, matrix metalloproteinases (MMPs) and signaling molecules was analyzed by western blot analysis. The present results demonstrated that Galium-Heel® did not significantly affect HDF growth, survival, cell cycle or morphology indicating the biocompatibility of the formulation. The formulation demonstrated antioxidant activity. Galium-Heel® was able to modulate ECM by regulating collagens (type I and III) and MMPs-3 and -7 expression. In addition, the formulation was able to regulate molecules involved in TGF-β signalling, including mitogen activated kinase-like protein, GLI family zinc finger 2 and pro-survival proteins such as AKT. The present results demonstrating the effects of a natural multi-component compound on ECM composition, highlighted the possibility of pharmacologically modulating ECM molecules. The recovery and the maintenance of ECM homeostasis might be considered as a potential therapeutic goal to ameliorate pathological conditions.

Introduction

Over the past decades, the connective tissue has been considered not only as a mechanical support structure for a tissue but also as a biologically active component of the tissue itself (1-3). The extracellular matrix (ECM) is a dynamic, living structure, which is continuously remodeled and involved in both physiological and pathological processes (1-3).

Accordingly, the analysis of ECM remodeling represents an attractive topic for investigation. The loss of ECM plasticity (i.e. the dysregulation of ECM turnover and barrier effect) is a key factor for the onset and the progression of a number of neoplastic, degenerative and inflammatory diseases (4,5). In these diseases, the structure and function of the ECM are affected by persistent low-grade chronic systemic inflammation (LGCSI). LGCSI contributes to the development of insulin resistance, dyslipidemia, atherogenesis, type II diabetes and hypertension thus promoting cardiometabolic disease (6). In addition, several cytokines are produced in tissues affected by LGCSI that can affect the homeostasis of ECM (6). These alterations trigger the pathological processes and promote both onset and progression of a disease (7). The loss of ECM homeostasis caused by LGCSI initially induces an increase of matrix solubility and then the deposition of disorganized collagens (types I, III and IV). The altered collagen turnover leads to tissue fibrosis (8). Accordingly, the ECM shifts from ‘plasticity’ to ‘rigidity’ (9,10). Within collagen types, collagen type III is directly responsible for the elastic properties of the connective tissue and it is involved in numerous fibrotic diseases (11,12). Various studies corroborated the pathophysiological role of the ECM, making it a potential therapeutic target (13-15). The analysis of the effects of some natural substances on ECM remodeling may lead to the development of a pharmacological approach for the modulation of ECM turnover.

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Key words: extracellular matrix, fibroblasts, Galium-Heel®, natural compound, collagen
The aim of the current study was to analyze the effect of a natural multi-component compound formulation named Galium-Heel® on the growth, morphology and ECM production of human skin fibroblasts. The present findings may provide basis for the development of a new pharmacological approach to counteract local abnormal ECM remodeling.

**Materials and methods**

**Materials and reagents.** Galium-Heel® is manufactured by Biologische Heilmittel Heel GmbH. Sodium chloride 0.9% was obtained from Eurospital. Galium-Heel® (from here on termed Galium) contains: Acidium nitricum, Apis mellifica, argentum metallicum, aurum metallicum, Betula pendula, calcium fluoratum, Caltha palustris, Clematis recta, Echinaea, Galium mollugo, Galium aparine, Hedera helix, Juniperus communis, Ononis spinosa, phosphorus, Pyrogenium Nosode, Saponaria officinalis, Sedum acre, Sempervivum tectorum spp., Thuja occidentalis, Urtica urens (Table I). Components were dissolved in sodium chloride 0.9%.

Sulforhadamine B (SRB), trypan blue and Amicon centricon 100 and 30 micro concentrators were purchased from Sigma-Aldrich (Merck KGaA). SimplyBlue SafeStain was purchased from Invitrogen (Thermo Fisher Scientific, Inc.). Rabbit polyclonal antibody against collagen type I (cat. no. NB600-408; 1:1,000) was obtained from Novus Biologicals, LLC. Rabbit polyclonal antibody against collagen type III (cat. no. AB747; 1:300) was purchased from Chemicon International. Rabbit polyclonal antibodies against matrix metalloproteinase (MMP)-2, -3, -7 (cat. no. SA-384; 1:500) were obtained from Enzo Life Sciences. Rabbit monoclonal antibody against AKT (C67E7; cat. no. 4691; 1:1,000), rabbit monoclonal antibody against phosphorylated (p)-AKT (Ser473; D9E; cat. no. 4060; 1:1,000) were obtained from Cell Signaling Technology. Mouse monoclonal antibody against JNK/mitogen-activated protein kinase 9 (SAPK) 1 (cat. no. BD 610627; 1:250), mouse monoclonal antibody against JNK/SAPK (pT183/pY185; cat. no. BD 612540; 1:250), mouse monoclonal antibody against p38α/SAPK2A (cat. no. BD 612168; 1:1,000) and mouse monoclonal antibody against p38 mitogen activated kinase-like protein (MAPK; pT180/pY182; cat. no. BD 612280; 1:200) were obtained from BD Pharmingen (BD Biosciences). Rabbit polyclonal antibody against extracellular signal-regulated kinase (ERK)1/2 (C-14; cat. no. sc-154; 1:300), mouse monoclonal antibody against p-ERK (E-4; cat. no. sc-7383; 1:300) and rabbit polyclonal antibody against GLI family zinc finger 2 (GLI-2; H-300; cat. no. sc-26764; 1:200) were obtained from Santa Cruz Biotechnology, Inc. Rabbit polyclonal antibody against fibronectin (cat. no. F3648; 1:1,000), rabbit polyclonal antibody against actin (cat. no. A5060; 1:500) and peroxidase-conjugated goat anti-mouse polyclonal (cat. no. A4416; 1:5,000) or anti-rabbit polyclonal (cat. no. A6154; 1:10,000) immunoglobulin (IgG) were obtained from Sigma-Aldrich (Merck KGaA).

**Cell lines and treatments.** Human adult fibroblasts (HDF), purchased from Cell Applications, Inc. (cat. no. 106-05a), and previously characterized H-Meso-1 cells (16) were maintained in DMEM high glucose (Aurogene s.r.l) containing 10% fetal bovine serum (FBS) (Aurogene s.r.l), 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were cultured at 37°C in a humidified incubator with an atmosphere of 5% CO₂.

For the treatments, cells were incubated for the indicated times in the presence of Galium (dose range: 1:2 to 1:16) or vehicle control (sodium chloride 0.9%; CTR; 1:2 to 1:16) re-suspended in DMEM containing 0.2% bovine serum albumin (BSA) (Sigma-Aldrich; Merck KGaA), 100 U/ml penicillin and 100 µg/ml streptomycin.

**SRB assay.** Cells were seeded at a density of 5x10⁴/well in 96-well plates and incubated at 37°C to allow cell attachment. After 24 h, the medium was changed and the cells were treated with Galium or sodium chloride and incubated for 24, 48 or 72 h at concentrations of 1:2, 1:4, 1:8 or 1:16 in DMEM 0.2% BSA. The cells were then fixed with cold trichloroacetic acid (final concentration 10%; Sigma-Aldrich; Merck KGaA) for 1 h at 4°C. After four washes with distilled water, the plates were air-dried and stained for 30 min at room temperature with 0.4% (w/v) SRB in 1% acetic acid (Sigma-Aldrich; Merck KGaA). After four washes with 1% acetic acid to remove the unbound dye, the plates were air-dried, and cell-bound SRB was dissolved with 100 µl/well of 10 mM unbuffered Tris base solution (Sigma-Aldrich; Merck KGaA). The optical density (O.D.) of the samples was determined at 540 nm with a spectrophotometric plate reader (Multiskan FC; Thermo Fisher Scientific, Inc.). The percentage survival of the cultures treated with Galium was calculated by normalizing their O.D. values to each control culture treated with sodium chloride at the same dilution (considered as 100%). The experiments were performed in triplicate and repeated three times. H-Meso-1 cells treated with 100 µM apigenin (Sigma-Aldrich; Merck KGaA) were used as the positive control for the inhibition of cell growth. The percentage of surviving cells treated with apigenin was calculated by normalizing the O.D. value to that of the control cultures treated with DMSO (≤0.1%), which is a solvent of apigenin (17).

**Trypan blue exclusion test.** Cells were seeded at a density of 5x10⁴/well in 24-well plates and incubated at 37°C to allow cell attachment. After 24-h, the medium was changed and the cells were treated with Galium or sodium chloride and incubated for 24, 48 or 72 h at concentrations of 1:2 and 1:4 in DMEM 0.2% BSA. After 24, 48 and 72 h of adherence, cells from each well were harvested and stained with trypan blue for 2 min at room temperature, then counted with an Olympus IX50 inverted light microscope (magnification, x100) (18). Percentage of cell death was determined by dividing dead cells by the total number of cells x100 (19). Experiments were performed in triplicate and repeated three times. H-Meso-1 cells treated with apigenin (100 µM) were used as the positive control for the inhibition of cells survival.

**Fluorescent measurement of reactive oxygen species (ROS).** To detect ROS production in HDF cells, 2,7'-dichlorofluorescin diacetate (DCF-DA; Sigma-Aldrich; Merck KGaA) was used. In brief, 2.5x10⁵ cells were seeded into six-well plates and incubated at 37°C to allow cell attachment before treatment. After two PBS washes, cells were incubated with 10 µM DCF-DA in PBS at 37°C and 5% CO₂ for 30 min (20). Following...
two washes, cells were treated with Galium or sodium chloride at the dilution of 1:2 in DMEM 0.2% BSA and incubated at 37°C and 5% CO₂ in the dark for 30 min. Then, cells were harvested, centrifuged at 300 x g for 10 min at 4°C and seeded in 96-well plates (2.5x10⁴ cells in 100 µl per well). Fluorescence intensity was measured after 30 min using a spectrophotometric plate reader at an excitation wavelength of 495 nm and an emission wavelength of 535 nm (Wallace 1420 Victor; Perkin Elmer Inc.).

**Table I. Concentration of Galium-Heel component.**

| Component                  | Concentration |
|----------------------------|---------------|
| Acidum nitricum            | 0.55 ng       |
| Apis mellifica             | 0.0055 pg     |
| Aurum metallicum           | 0.0055 pg     |
| Calthta palustris          | 1.1 µg        |
| Clematis recta             | 0.165 µg      |
| Echinacea                  | 0.0165 µg     |
| Galium aparine             | 0.88 µg       |
| Galium mollugo             | 0.88 µg       |
| Hedera helix               | 0.165 µg      |
| Juniperus communis         | 0.165 µg      |
| Ononis spinosa             | 0.165 µg      |
| Phosphorus                 | 5.5 pg        |
| Pyrogetium Nosode          | 5.5 ng        |
| Saponaria officinalis      | 0.55 µg       |
| Sedum acre                 | 1.1 µg        |
| Sempervivum tectorum ssp.  | 0.11 µg       |
| Thuja occidentalis         | 1.65 µg       |
| Urtica urens               | 4.4 µg        |
| Argentum metallicum        | 5.5 pg        |
| Calcium fluoratum          | 5.5 pg        |
| Betula pendula             | 0.011 mg      |

*One vial contains 1.1 ml (1.1 g)*

Fluorescence-activated cell sorting (FACS) analysis. Log-phase growing HDF cells (60% confluent; 2.5x10⁴/well in six-well plates) were treated with Galium or sodium chloride at the dilution of 1:2 in DMEM 0.2% BSA and incubated at 37°C and 5% CO₂ in the dark for 30 min. Then, cells were harvested, centrifuged at 300 x g for 10 min at 4°C and seeded in 96-well plates (2.5x10⁴ cells in 100 µl per well). Fluorescence intensity was measured after 30 min using a spectrophotometric plate reader at an excitation wavelength of 495 nm and an emission wavelength of 535 nm (Wallace 1420 Victor; Perkin Elmer Inc.). Malignant mesothelioma H-Meso-1 cells treated with apigenin (100 µM) were used as the positive control for the intracellular ROS production.

**Ultrastructural analysis.** Ultrastructural analysis was performed on HDF cells 24 h following treatment with Galium or sodium chloride at the dilution of 1:2 in DMEM 0.2% BSA. Cells were fixed with 2.5% glutaraldehyde in PBS at 4°C for 48-h, and processed for transmission electron microscopy (TEM), following routine procedures (23,24). Briefly, cells were post-fixed with 1.33% osmium tetroxide at 4°C for 1 h, dehydrated in graded alcohols (30%‑50%‑70%‑95%‑100% at 4°C), and then embedded in Epon 812 resin (Fisher Chemical Co.). Thin (1 µM) and ultrathin (900Å) sections were cut on a ultramicrotome, stained with uranyl acetate and lead citrate (3 min each at room temperature), and then contrasted with lead citrate (3 min each at room temperature), and observed under a Philips Morgagni 268D transmission electron microscope at magnifications between x7,000 and x28,000.

Statistical analysis. Statistical analyses were performed with GraphPad Prism 5.0 software (GraphPad Software, Inc.). Data were presented as the mean ± SD. Data distribution of cell survival and trypan blue exclusion test were
preliminarily verified by Kolmogorov-Smirnov test, and data sets were analyzed by one-way analysis of variance followed by Newman-Keuls post-hoc test. Data distribution of ROS production and FACS analysis, and differences in the intensity of immunoreactive bands were analyzed by two-tailed Student's t-test. P<0.05 was considered to indicate statistical significance.

Results

Galium does not significantly affect HDF cell growth or death. The survival of HDFs was evaluated by SRB assay after treatment with increasing doses of Galium or sodium chloride as control (1:2, 1:4, 1:8 and 1:16) for 24, 48 and 72 h. The percentage of cell survival following treatment with Galium was compared with cells treated with sodium chloride. No significant differences between Galium and CTR were observed (P>0.05; Fig. 1A). In addition, a Trypan blue exclusion test was performed to test cell viability after treatment with increasing doses of Galium or sodium chloride (1:2 and 1:4) for 24, 48 and 72 h. No significant differences between treatments were observed (P>0.05; Fig. 1B). H-Meso-1 cells treated with apigenin (100 µM) were used as control. Apigenin significantly inhibited H-Meso-1 cell survival: 65, 37, 22% for apigenin vs. 100% for control after 24, 48 and 72 h, respectively; P<0.01) and promoted cell death in a time-dependent manner (cell death: 49, 67, 84% for apigenin vs. 2, 2, 3% for control after 24, 48 and 72 h, respectively; P<0.01; data not shown).

Galium significantly decreases intracellular ROS production in HDF cells. A DCF-DA assay was performed to determine the effect of Galium on the intracellular ROS production in HDF. Galium induced a significant decrease of intracellular ROS production when compared with the control (P<0.01; Table II). H-Meso-1 cells treated with apigenin (100 µM) were used as a positive control for monitoring the intracellular ROS production. Apigenin significantly increased the production of ROS in H-Meso-1 cells (3.353±3.530 for control; P<0.001; data not shown).

Galium does not significantly affect cell cycle distribution. In order to evaluate the effect of Galium on cell cycle distribution, FACS analysis of DNA content was performed on HDF cells treated with Galium or sodium chloride at the dilution of 1:2. No significant changes were observed in any of the different phases of the cell cycle following Galium treatment compared with control cells treated with sodium chloride (P>0.05; Table III).

Effect of Galium on the expression of extracellular matrix molecules and MMPs. The effect of Galium on the expression of extracellular matrix molecules (collagen type I and III, fibronectin) and metalloproteinases (MMP-2, -3, -7) in HDF was evaluated by western blot analysis 48 h following Galium or sodium chloride treatments. The analysis was performed both in HDF lysates (Fig. 2A and C) and in the conditioned medium (Fig. 2B and D). Galium treatment significantly decreased the expression of collagen type III both in the cell lysate (P<0.05) and in the conditioned medium (P<0.01). Conversely, collagen type I was increased following Galium treatment both at the intracellular level (P<0.01) and in the extracellular space (P<0.001). Collagen type III/I ratio was 0.81 for CTR vs. 0.43 for Galium in the cell lysate and 1.0 for CTR vs. 0.59 for Galium in the extracellular space. The expression of fibronectin was not affected by Galium treatment. MMP-3 (P<0.01) and MMP-7 (P<0.01) significantly decreased in the cell lysates following Galium treatment. Conversely, MMP-3 (P<0.05) increased in the extracellular space compared with CTR (Fig. 2). Galium treatment did not affect the expression of MMP-2 (Fig. 2).

Galium upregulated expression and activation of pro-survival signaling pathway molecules. To evaluate whether Galium affected the expression and phosphorylation of MAPKs, western blot analysis was performed after treating HDF with Galium or sodium chloride for 48 h.

The levels of p-ERK1, p-ERK2, p-p38-MAPKα, p-JNK p46 and p-JNK p54 proteins were compared with their respective total proteins. Results demonstrated that Galium treatment significantly decreased the levels of phosphorylation of ERK1/2 (p-ERK1, P<0.05; p-ERK2, P<0.01) compared with CTR (Fig. 3). In addition, Galium-treated cells demonstrated significantly reduced phosphorylation of JNK p54 (P<0.01) and JNK p46 (P<0.05) compared with the CTR-treated cells (Fig. 3). Conversely, p38-MAPKα phosphorylation was increased following Galium treatment (P<0.001) compared with sodium chloride treatment (Fig. 3). Furthermore, the effect of Galium treatment on the expression of pro-survival kinase AKT was evaluated. Results demonstrated that Galium significantly increased the p-AKT protein levels compared with CTR (P<0.05; Fig. 3). Finally, GLI-2 expression was

Figure 1. Effects of Galium on HDF cell survival and death. (A) Survival and viability of HDF were assessed by the sulforhodamine B and (B) trypan blue exclusion assays 24, 48 and 72 h post-treatment with CTR or Galium in DMEM 0.2% BSA. The percentage of surviving cells treated with Galium was calculated by normalizing the optical density value to each control culture treated with sodium chloride at the same dilution (considered 100%). The percentage of cell death was compared with the total number of cells. Results are expressed as the mean ± SD of three independent experiments performed in triplicate. Galium, Galium-Heel®; HDF, human dermal fibroblasts; CTR, sodium chloride.
The ECM produces a tissue microenvironment able to affect and regulate cellular signalling (1-3). It is fundamental for optimal cell crosstalk to maintain ECM homeostasis (1-3). The loss of ECM plasticity, which is dependent on the anabolic and catabolic phases, is associated with the onset of several pathologies, especially those involving chronic inflammation. Persistent LGCSI causes the ECM to lose its plasticity, making it become rigid with consequent fibrotic degeneration (4-6). Accordingly, it is important to counteract the vicious cycle of inflammation and ECM turnover alterations by primary therapeutic intervention. However, the therapy needs to affect several different aspects involving both the inflammatory processes leading to reactive oxygen species production and ECM turnover mechanisms.

The present study aimed to investigate the activity of a natural multi-component compound formulation named Galium-Heel® on HDF growth, morphology and ECM production. The results demonstrated that Galium-Heel® had no significant effect on the cell cycle, cell growth, cell survival and cell morphology, which highlighted the biocompatibility of Galium-Heel®. The observed antioxidant activity of Galium-Heel® on HDF might be due to its components Galium aparine, Sedum acre, Sempervivum tectorum or Urtica urens, which are able to regulate redox processes (25-27). The modifications of collagen type I and type III and MMP-3, observed after the treatment of HDF with Galium-Heel® suggested that the multi-component compound formulation had a role in the modulation of ECM anabolic and catabolic phases. Galium-Heel® treatment significantly decreased the expression of collagen type III and MMP-3, whilst collagen type I expression increased at the intracellular level. In addition, Galium-Heel® significantly decreased the expression of collagen type III and increased collagen I and MMP-3 expression in the extracellular space. Increased MMP-3 expression might support subsequent ECM remodelling (28). Tumour necrosis factor-α (TNF-α) is able to increase the expression of MMP-3 and subsequent ECM remodelling with the process dependent on p38 phosphorylation (29). This effect can be reversed by TGF-β1 via activation of MAPKs namely ERK1/2 (30). The present results demonstrated that Galium-Heel® may have a similar effect to TNF-α acting as a TGF-β inhibitor, inducing activation of p38 and increasing the expression of MMP-3 whilst inhibiting ERK1/2 activity. Increased expression of MMP-3 could be responsible for the decrease in collagen type III/I ratio observed in the cell lysate and extracellular space following Galium-Heel® treatment. TGF-β1 is able to induce fibrosis by upregulating the expression of collagen type III and increasing the collagen type III/I ratio (31). Collagen type III is considered a marker of lung fibrosis (32). In addition, it has been demonstrated that collagen type III expression is increased in liver cirrhosis and this increased expression is related to the development of portal hypertension (33).

GLI proteins are the effectors of the Hedgehog signalling pathway (34). GLI-2 is an early gene target of the TGF-β1/SMAD cascade independent of Hedgehog signalling (34). Hedgehog and TGF-β1 signalling pathways overlap in GLI-2, which integrates those signals to promote tissue fibrosis (35). The present study observed that Galium-Heel® decreased the expression of GLI-2 therefore, it could potentially prevent the early stages of fibrosis by maintaining homeostasis of the ECM. The effects of Galium-Heel® on ECM modulation might be due to certain components contained in the formulation including: i) Acidum nitricum, which participates as a nitric oxide (NO) donor through the nitrate/nitrite/NO pathway, and is involved in the collagen synthesis mechanism (36-39); ii) argentum metallicum and aurum metallicum are donors of the trace elements silver and gold respectively, and exert an antioxidant effect (40); iii) aurum metallicum regulates MMP expression (41); iv) phosphorus contributes to the tissue inhibitor of metalloproteinases synthetic pathways (42); v) calcium fluoratum is involved in the synthesis of MMP-2 and -9 (43); vi) Calitha palustris promotes collagen synthesis (44); vii) Juniperus communis controls the synthesis of collagen type I and III (45); viii) Betula pendula reduces the expression of MMP-3 (46,47); and ix) Pyrogenium and Thuja occidentalis exert an immunomodulating effect through stimulation of T helper type I immune response and the synthesis of MMPs (48-50). In addition, it should be considered that the various components contained in the formulation could have an additive or a synergistic effect. Previous studies by our group demonstrated that the simultaneous administration of polyphenols exerts additive or synergistic anticancer effects (51-53). Finally, an increase in the polymerization of intermediate filaments was observed in Galium-treated cells by ultrastructural analysis.

Table II. Effects of Galium on the intracellular ROS production in HDF.

| Treatment                  | Intracellular ROS production |
|----------------------------|------------------------------|
| Sodium chloride 1:2 (CTR)  | 320,564±2,251                |
| Galium 1:2                 | 283,354±11,411\(^a\)         |

\(^a\)P<0.01 vs. control. The results are reported as the mean of the fluorescence intensity ± SD values from three experiments performed in triplicate. **Galium, Galium-Heel®**, HDF, human dermal fibroblasts; ROS, reactive oxygen species.
Table III. Effect of Galium on cell cycle distribution.

| Treatment          | Sub-G1  | P-value | G0/G1     | P-value | S       | P-value | G2/M    | P-value |
|--------------------|---------|---------|-----------|---------|---------|---------|---------|---------|
| Sodium chloride 1:2 (CTR) | 1.70±0.71 | >0.05   | 89.70±1.44 | >0.05   | 3.05±0.11 | >0.05   | 5.65±0.86 | >0.05   |
| Galium 1:2         | 1.90±1.05 | >0.05   | 90.89±1.93 | >0.05   | 2.67±0.03 | >0.05   | 4.66±0.92 | >0.05   |

Results are expressed as the mean ± SD of two independent experiments performed in duplicate. Galium, Galium-Heel®.
It has been established that fibroblasts within the connective tissue are able to remodel the ECM (54). Upon ECM remodelling, fibroblasts adapt their cytoskeleton to a different extracellular microenvironment (54), which can affect the viscoelastic and stretching properties of the connective tissue (55). Therefore, the present results suggested that polymerization of the cytoskeleton in Galium-treated HDF might be a consequence of ECM remodelling or that
ECM remodelling might be a consequence of the fibroblast cytoskeleton polymerization.

Based on the fundamental role of the tissue microenvironment in the onset and maintenance of many pathological conditions, the importance of ECM turnover is crucial, and the recovery of ECM homeostasis can be considered as a potential therapeutic goal. The present results demonstrating the beneficial effects of Galium-Heel® on ECM plasticity highlight that it may be possible to pharmacologically control some mechanisms of ECM metabolism. The present findings may provide basis for the development of novel pharmacological approaches to counteract local abnormal ECM remodeling.

Acknowledgements

The authors would like to thank Mrs. Barbara Bulgarini (Department of Clinical Sciences and Translational Medicine, University of Rome ‘Tor Vergata’) for editorial assistance in the preparation of the manuscript.

Funding

This study was supported by a grant from GUNA S.p.a. (UPB: BeiRCtGUNA) which provided the Galium-Heel®. However, this did not influence the authors’ scientific neutrality.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

MB performed cell proliferation, western blot analysis, FACS, statistical analysis and analyzed the results. RM performed cell death experiments, reaction oxygen species production, western blot analysis and analyzed the results. MTM performed statistical analysis and analyzed the results. MGG, IT, LA, VM and AM analyzed the results and critically revised the manuscript. LM performed ultrastructural analysis and analyzed the results. RB supervised the project, analyzed the results and wrote the manuscript. All the authors have critically revised the manuscript and gave the final approval of the version to be published.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The present study was partially funded by GUNA S.p.a which provided Galium-Heel®. However, this did not influence the authors’ scientific neutrality. No products, patents or other commercial interests relate to this study.

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