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Effects of plant lectin and extracts on adhesion molecules of endothelial progenitors

Research Article

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Abstract: Promise of cell therapy has advanced the use of adult stem cells towards the development of novel approaches to promote regeneration of injured endothelium. The aim of this study was to stimulate endothelial progenitor cells (EPCs) with lectin isolated from *Solanum tuberosum* (*potato*) *shoot* and *Calendula officinalis* (*marigold*) extracts, in order to increase EPCs proliferation and gene expression of molecules with roles in chemotaxis and adhesion for a better attachment to injured vascular tissue. EPCs were differentiated from umbilical cord blood-derived mononuclear cells and characterized by light microscopy, flow cytometry, and vascular tube-like structures formation on Matrigel. Cell proliferation was determined by MTS assay, and gene expression of molecules involved in EPCs adhesion (VCAM-1, VE-cadherin, ICAM-1, PECAM-1, P-selectin) and chemotaxis was determined (CXCR4, Tie-2) by RT-PCR. For the assessment of cell motility, wound-healing assay was employed. Both *potato shoot* lectin and *marigold* extracts stimulated EPCs proliferation in a concentration dependent manner and were able to increase expression of adhesion and chemotactic molecules. *Marigold flower* extract proved to be more efficient. This study demonstrates the usefulness of *potato* lectin and *marigold* extracts to increase EPCs proliferation and modulate gene expression of chemotactic and adhesion molecules, which may facilitate EPCs attachment to injured endothelium.

Keywords: *Endothelial progenitor cells • Lectin • Proliferation • Cell adhesion • Vascular repair*

1. Introduction

Lectins are carbohydrate-binding proteins that specifically recognize diverse sugar structures and mediate a variety of biological processes such as cell–to–cell and host–pathogen interactions, serum–glycoprotein turnover, and innate immune responses [1,2]. Cell surface carbohydrates are particularly involved in cell–to–cell signaling, cell adhesion, and various developmental processes [3]. For decades, legume lectins have been a paradigm in the area of protein–carbohydrate recognition. Despite the lack of knowledge on their *in vivo* activity, they have proven to be useful tools in immunology and glycobiology, because of their wide range of specificities for complex carbohydrates. Their carbohydrate specificity has also promoted their use in various applications, such as purification and characterization of complex carbohydrates and glyco-conjugates, as well as in bone marrow transplantation [4,5].

Solanum tuberosum (*potato*) lectin is a representative type for a large family of chitin-binding lectins, including *Lycopersicon esculentum* and *Datura stramonium* lectins. This family has a number of distinguishing features: (i) sugar-binding domain, which has a high affinity for *N*-acetyl-D-glucosamine oligomers (GlcNAc); (ii) high

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content of hydroxyproline (up to 30%); (iii) high extent of glycosylation, as compared with other plant lectins. It has been shown that administration of *potato tuber* lectin induces the immune system activation and also prevents the bacterial adhesion to intestinal mucosa by competing with them for sites involved in cell adhesion [6]. Lectin isolated from *potato shoot*, orally administered to chicken, induced visceral lymphoid tissue modifications [7]. *Calendula officinalis* (*marigold*) has been widely used in phytotherapy for the treatment of various disorders. It has been reported to possess numerous pharmacological activities, which include antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral. *Marigold* extracts induce healing effects on wounds, bruises, muscular contractions, as well as lesions associated to external bleeding. Several studies have shown that different *marigold* extracts can stimulate cellular proliferation and metabolism through an increased mitochondrial dehydrogenase activity [8,9].

Stem cells are unique cells that retain the ability to divide and proliferate throughout postnatal life to provide progenitor cells that can progressively commit to specialized cells. Endothelial progenitor cells (EPCs) identification has been an introductory emergence of stem cell biology in the field of vascular regeneration. Several groups have addressed the use of umbilical cord blood (UCB)-derived stem cells for *in vitro* generation of EPCs [10-13]. It has been extensively shown that cell therapy using EPCs may enhance the recovery of ischemic vasculature. EPCs recruitment and integration are essential steps for vascular regeneration. These processes require a coordinated sequence of multistep adhesive and signaling events, including chemoattraction (e.g., by SDF-1 / CXCR4), migration, and adhesion (e.g., by integrins), followed by their differentiation into mature endothelial cells [14-17]. EPCs attachment and adhesion to the injured endothelium are specifically important to promote vascular regeneration. Therefore, the aim of this study is to investigate the capacity of *marigold* extract and *potato shoot* lectin to stimulate EPCs proliferation and expression of surface molecules involved in their adhesion and chemotaxis.

2. Experimental Procedures

2.1 EPCs isolation and differentiation from umbilical cord blood

Human UCB samples were collected at term delivery by specialized staff at the "Polizu" Hospital, Bucharest, Romania with informed consent, according to European and Romanian legislation in force on the collection and handling of human biological samples and personal data protection. The use of UCB samples for research purposes were authorized by the Ethics Committee of the Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania. Immediately after collection, UCB samples were serologically tested for the absence of HIV1/2, HBV, HCV and HTLV, using dedicated kits (ChemWell ELISA, Awareness Technology, Palm City, USA), according to manufacturer guidelines, and processed within 4 h after collection.

Mononuclear cells (MNCs) were obtained by Histopaque (Sigma-Aldrich, Saint Louis, MO, USA) density gradient centrifugation at 400*g*, for 30 min at room temperature. After centrifugation, the MNC layer was harvested and washed twice in Dulbeco's Modified Eagle's Medium (DMEM, Sigma-Aldrich St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS). The MNCs were plated on plastic dishes coated with fibronectin (1 μg/cm^3, BD Biosciences, San Jose, CA, USA) in endothelial differentiation medium (MV2, Promocell, Heidelberg, Germany), supplemented with 15% FBS, 40 ng/ml vascular endothelial growth factor (VEGF), 100 μg/ml endothelial cell growth supplement, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml neomycin (all purchased from Sigma-Aldrich). Cell cultures were maintained at 37°C with 5% CO_2 and 21% O_2 in a humidified atmosphere. One day after plating, the non-adherent cells were discarded and fresh medium was applied. To maintain optimal culture conditions, medium was changed twice a week. For the proposed studies, one characterized EPC line, at passage 6, was used.

2.2 EPCs characterization

2.2.1 Morphological characterization

Morphological characterization was done by light microscopy using an inverted fluorescence microscope (Eclipse TE300, Nikon, Tokyo, Japan) and a digital camera system for imaging (Digital Net Camera DN100, Nikon).

2.2.2 Expression of cell surface molecules

Expression of cell surface molecules on EPCs was assessed by flow cytometry (MoFlo FACS, Dako, Glostrup, Denmark) using 1 x 10^5 cells stained with fluorochrome-conjugated (Phycoerythrin, PE; Fluorescein isothiocyanate, FITC) antibodies against CD31, CD34, CD45, CD105, VEGF receptor (VEGFR)1, (Dako), and CD133 (MACS, Miltenyi Biotec, GmbH, Bergisch-Gladbach, Germany). Accutase-detached cells were washed in phosphate-buffered saline (1x PBS) and incubated for 30 min at 4°C with either PE- or FITC-conjugated antibodies. For negative controls, the cells were stained with the corresponding isotype-matched IgGs (IgG1, IgG2a/b,

Dako). Flow cytometry data were analyzed using the Summit 4.0 software (Dako).

2.2.3 Functional testing of EPCs for vascular tube formation

Functional testing of EPCs for vascular tube formation was performed using Matrigel extracellular membrane matrix (Sigma-Aldrich); 100 µl cellular suspension containing 2 x 10^4 EPCs was seeded onto 96-well tissue culture plates coated with 50 µl Matrigel. After 4-6 h of incubation at 37ºC, cells were assessed for capillary-like structures formation by light microscopy, using an inverted microscope (Eclipse TE300, Nikon).

2.3 Lectin isolation and purification

Lectin was extracted from *potato shoots*, at the same stage of vegetation, with 0.2 M acetate buffer, pH 5.0. Purification was performed by affinity chromatography on chitin as affinity support, as previously described [18-20]. Elution was done with 0.2 M acetic acid and affinity purified lectin was dialyzed against PBS. Lectin preparation presented a single band on SDS-PAGE electrophoresis [21]. Protein concentration assayed with Coomassie Plus Protein Assay Reagent (Thermo Scientific-Pierce Protein Research Products, Rockford, IL, USA), was 513 µg/ml. Lectin activity was evaluated by haemagglutiation test with rabbit erythrocytes. Briefly, this test was done by adding 2% suspension of rabbit red blood cells to the serially diluted lectin preparation, followed by incubation for 2 h at 37ºC; the degree of aggregation was evaluated at optical microscope. Hemagglutination titre (the reciprocal of the last dilution that produces agglutination) was 64. Agglutinating activity was inhibited by 0.2 M chitotriose (80%) and 0.2 M N-acetyl-D-glucosamine (60%, both chemicals were provided by Sigma Aldrich).

Both fresh *marigold* green plants and, separately, the flowers were washed, cut into small pieces, homogenized in PBS and left overnight at 4ºC. The crude unpurified extracts, obtained after centrifugation (3773 x g, 30 min), were assayed for lectin presence by agglutination of normal and trypsin-treated rabbit blood cells. Both extracts presented a weak lectin activity only on trypsinized erythrocytes. Hemagglutination titre was 8 for *green plant* extract and 4 for *flower* extract. The concentrations of *marigold green plant* and *flower* extracts (50 µg/ml and 20 µg/ml, respectively) were measured by Coomassie Plus Protein Assay Reagent (Thermo Scientific-Pierce Protein Research Products).

2.4 Cell Proliferation Assay

For cell proliferation assay (CellTiter96 Non-Radioactive Cell Proliferation Assay, Promega, Madison, USA), EPCs were seeded in 96-well plates at a density of 5 x 10^3 cells/well, in DMEM medium, supplemented with 10% FBS, and without growth factors. Cells were stimulated twice, at 24 and 48 h after seeding, with different concentrations of *potato shoot* lectin (50 ng/ml, 500 ng/ml, 5 µg/ml, 50 µg/ml, and 100 µg/ml), *marigold green plant* (50 ng/ml, 500 ng/ml, 10 µg/ml, and 25 µg/ml), and *marigold flower* extract (50 ng/ml, 500 ng/ml, 1 µg/ml, and 10 µg/ml). Controls were represented by EPCs grown in the same culture conditions, but without lectin stimulation. Cell proliferation assay was performed in triplicates, according to manufacturer's guidelines, at 24 h after the completion of lectin stimulation. Spectrophotometry measurement was performed at a wavelength of 570 nm (Mithras LB 940, Berthold Technology, Germany).

2.5 Reverse Transcription – Polymerase Chain Reaction (RT-PCR)

To assess the expression of adhesion markers and molecules involved in EPCs chemotaxis and angiogenesis, cells grown in DMEM medium supplemented with 10% FBS were stimulated with lectin concentrations that induced the highest proliferation (5µg/ml for *potato shoot* lectin; 10 µg/ml and 1 µg/ml for *marigold green plant* and *marigold flower* extract, respectively). Total RNA extraction was performed using GeneElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich) and reverse-transcription reaction was performed using M-MLV polymerase (Invitrogen, Carlsbad, CA, USA); RT-PCR was performed using a PCR kit (Promega, Madison, USA), following manufacturer's protocol. The sequences of GAPDH, CXCR4, Tie-2, VE-cadherin, platelet endothelial cell adhesion molecule 1 (PECAM-1), intercellular adhesion molecule-1 (ICAM-1), P-selectin, and vascular cell adhesion molecule (VCAM-1) primers (Metabion GmbH, Martinsried, Germany) are listed in Table 1. PCR reactions were carried out in a Corbett Thermal Cycler (Qiagen, Hilden, Germany) with the following schedule: denaturation step at 95ºC for 5 min, 35 cycles of amplification (denaturation at 94ºC for 45 s, annealing at 60ºC for 45 s, extension at 72ºC for 45 s), and incubation step at 72ºC for 10 min.

Synthesized DNA fragments were detected by 1.5% agarose gel electrophoresis with ethidium bromide staining.

Quantification was performed by densitometry using an Image Master Total Lab Software (Pharmacia Biotech, Buckinghamshire, UK). The experiments addressing the assessment of adhesion, chemotactic and angiogenic molecules expression were done in triplicates, with controls represented by samples not stimulated with lectins.

Gene	GeneBank accession number	Sequences of oligonucleotide primers (S, sense; A, antisense)	Predicted size (bp)
GAPDH	NM_002046	S: 5'-ACCACAGTCCATGCCATCAC-3' A: 5'-TCCACCACCCTGTTGCTGTA-3'	450
ICAM-1	NM_000201	5'-GCAAGAACCTTACCCTACGC-3' 5'-CTGAGACCTCTGGCTTCGTG-3'	590
VCAM-1	NM_080682	5'-GTCGTGATCCTTGGAGCCTC-3' 5'-TTCGTCACCTTCCCATTCAG-3'	205
PECAM-1	NM_000442	S: 5'-AGCACCACCTCTCACGTCA-3' A: 5'-CTTGGATGGCCTCTTTCTTG-3'	250
VE-cadherin	NM_001795	S: 5'-CCTTGGGATAGCAAACTCCA-3' A: 5'-CTTTGCCTCCAGGCAGATAG-3'	283
P-selectin	NM_003005	5'-GGATTGTTCTGACACTCGTGG-3' 5'-GAGGTTGGAGCAGTTCATCG-3'	415
CXCR4	NM_003467	S: 5'-GATGACAGATATATCTGTGACCGC-3' A: 5'-TTAGCTGGAGTGAAAACTTGAAGA-3'	519
Tie-2	NM_000459	S: 5'-CATACTGGGGAAAGCAATGAAAC-3' A: 5'-ACCACTGTTTTTCACCTTCCAAA-3'	281

Table 1. Sequences of the oligonucleotide primers used for RT-PCR.

2.6 *In vitro* wound-healing assay

An equal number of cells (1 x 10^5) were plated in triplicates on 6-well plates. Cells were grown in DMEM medium with 10% FBS, until the cells reached the confluence. The monolayer of confluent cultures was lightly scratched with a 1000 µl pipette tip and photographed by phase-contrast microscopy at timed intervals for up to 24 h. The assay was performed in triplicates with controls represented by EPCs grown in the same culture conditions, but without lectin stimulation. Quantification was done using the AxioVision software (4.8.1 version, Carl Zeiss MicroImaging GmbH, Jena, Germany) by measuring the number of pixels in the wound area and calculating the decrease in the scratched areas. This was achieved by subtracting the number of pixels at the 6 and 12 h time points from the number of pixels in the corresponding wound area at the 0 h time point.

2.7 Statistical analysis

Statistical analysis was performed by a one-way analysis of variance (ANOVA) using the OriginPro 7.5 software. Differences were considered statistically significant when $P<0.05$. The results are presented as means ± standard error (SE), where *n* represents the number of experiments.

3. Results

3.1 EPCs isolation and characterization

Colonies of adherent cells with a cobblestone, epithelial-like phenotype (Figure 1) started developing after approximately 10 to 14 days after umbilical cord blood-derived MNCs culture under endothelial growth factors stimulation.

Flow cytometry analysis showed that the cells were markedly positive for CD31 and CD105, less positive for CD133 and VEGFR1, and negative for CD34 and CD45 (Figure 2). Capillary tube formation assay showed that EPCs organized robustly into tube-like structures, as early as 4 h after plating on Matrigel (Figure 3).

3.2 Lectins effect on EPCs proliferation

Both *potato shoot* lectin and *marigold* extract stimulated cell proliferation, in a concentration-dependent manner. EPCs stimulation with *potato shoot* lectin significantly increased cell proliferation at lectin concentrations ranging from 50 ng to 5 µg,; the highest cell proliferation was observed at a lectin concentration of 5 µg/ml, whereas a 20-fold higher concentration lead to decreased cellular proliferation (Figure 4).

EPCs stimulation with *marigold green plant* extract induced the same effects on cell proliferation compared to those obtained upon stimulation with extract from *marigold flowers*, but at higher concentrations. Different concentrations of *marigold green plant* extract, ranging from 50 ng/ml to 10 µg/ml, were able to significantly stimulate cell proliferation, (Figure 5; P-values are: for 50 ng/ml: 0.00105; 500 ng/ml: 0.0130; 10 µg/ml: 0.0108). When EPCs were treated with different concentrations of *marigold flowers* extract, cell proliferation was significantly stimulated at concentrations ranging from 50 ng/ml to 1 µg/ml, whereas an inhibitory effect,

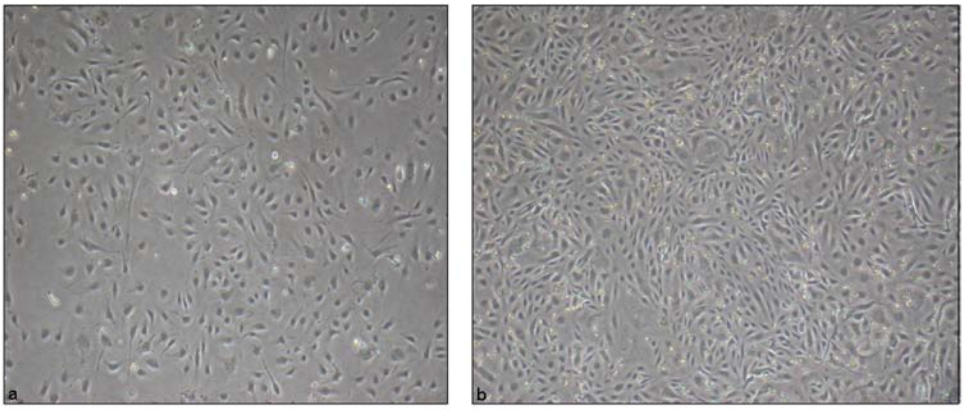

Figure 1. Epithelial-like morphology of adherent cells derived from umbilical cord blood-derived MNCs under endothelial growth factors stimulation. a- primary culture, b- EPCs at passage 6. Cobblestone-like clusters start to appear around 10 to 14 days after planting the MNCs. Nikon, (a, b, 4x).

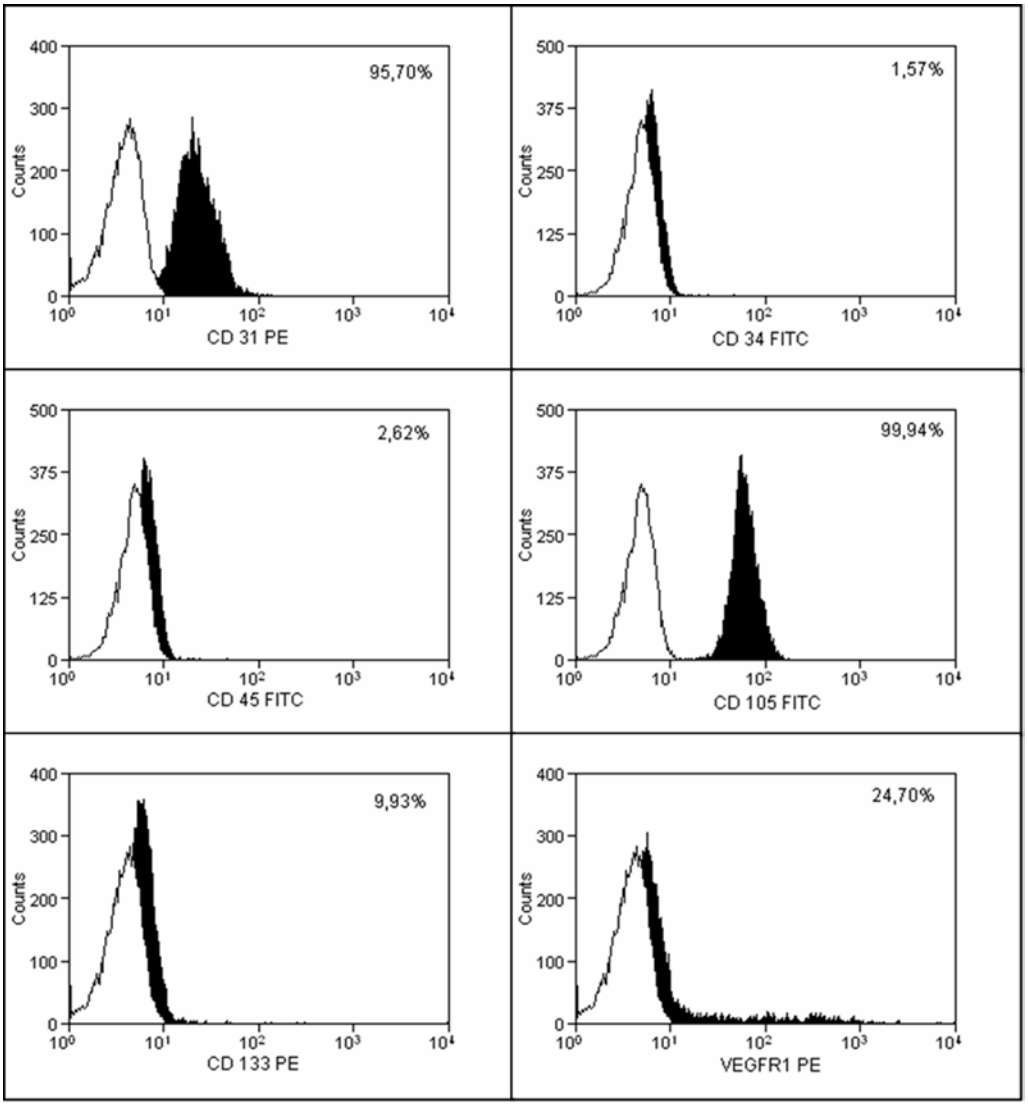

Figure 2. Flow cytometry analyses of EPCs revealed an endothelial-like phenotype: $CD31^{+}/CD105^{+}/CD133^{low}/VEGFR_{1}^{low}/CD34^{-}/CD45^{-}$.

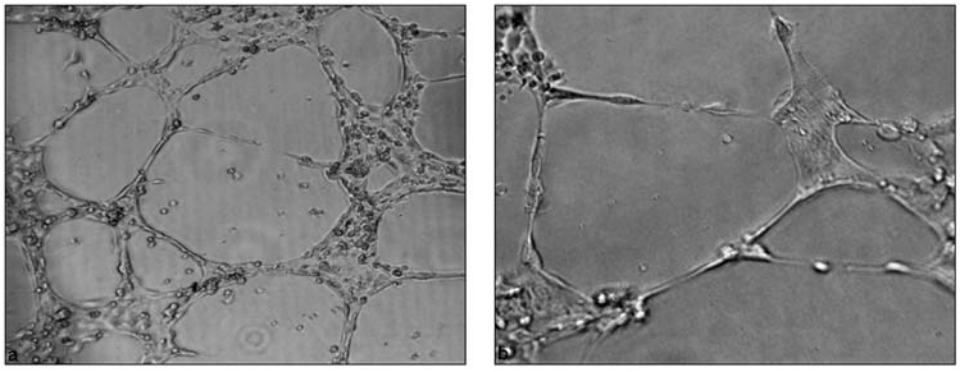

Figure 3. Capacity of EPCs to form capillary-like structures in a Matrigel artificial basement membrane matrix after 4 h of incubation. Nikon, (a) 4x, (b) 10x.

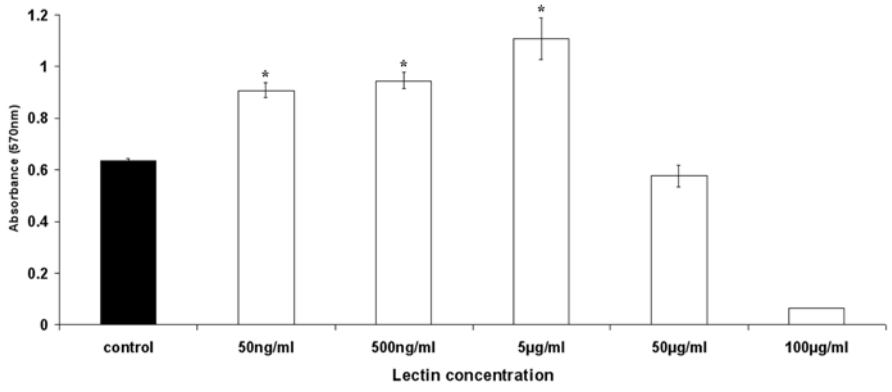

Figure 4. EPCs proliferation in the presence of different concentrations of *potato shoot* lectins. *Potato shoot* lectin significantly stimulated EPCs proliferation at concentrations ranging from 50 ng/ml to 5 µg/ml. Results are represented as mean ± standard error, n=3, * P<0.05, ** P<0.005.

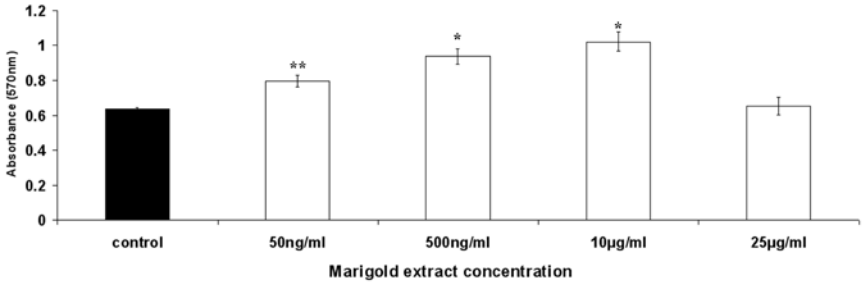

Figure 5. EPCs proliferation in the presence of different concentrations of *marigold green plant* extract. *Marigold green plant* extract significantly stimulated EPCs proliferation at concentrations ranging from 50 ng/ml to 10 µg/ml. Results are represented as mean ± standard error, n=3, * P<0.05, ** P<0.005.

although not statistically significant, was seen at a concentration of 10 µg/ml (Figure 6; P –values are: for 50 ng/ml: 0.000127; 500 ng/ml: 0.0189; 1 µg/ml: 0.0101).

3.3 Expression of adhesion molecules

Both *potato shoot* lectin and *marigold* extracts were able to significantly increase gene expression for VCAM-1 and VE-cadherin; a significantly increased expression

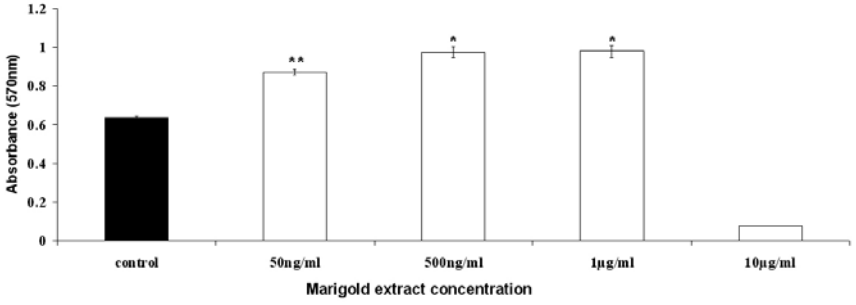

Figure 6. EPCs proliferation in the presence of different concentrations of *marigold flower* extract. *Marigold flower* extract significantly stimulated EPCs proliferation at concentrations ranging from 50 ng/ml to 1 μg/ml Results are represented as mean ± standard error, n=3, * P<0.05, ** P<0.005.

of ICAM-1 was observed only after stimulation with *marigold* extracts, as compared to controls (Figure 7; P –values are: for VCAM-1: 0.0100, 0.000120, 0.00693; VE-cadherin: 0.00109, 0.00203, 0.00270; ICAM-1: 0.0839, 0.00302, 0.00155).

PECAM-1, P-selectin, and Tie-2 gene expression were significantly increased after stimulation with *marigold flower* extract, while *potato shoot* lectin and *marigold green plant* extract significantly down-regulated expression of these molecules. However, both *marigold* extracts were able to increase gene expression of chemokine receptor CXCR4, whereas *potato shoot* lectin significantly decreased its expression comparative with control (Figure 8; P –values are: PECAM-1: 0.00295, 0.0415, 0.00238; P-selectin: 0.00246, 0.0489, 0.00250, Tie-2: 0.0412, 0.0302, 0.00119, CXCR4: 0.0123, 0.608, 0.00441).

3.4 Wound healing assay

Cell motility, evaluated by classical wound-healing assay, at 2 time intervals (6 and 12 h) revealed that both *potato shoot* lectin and *marigold flower* extract enhanced the migration ability of the cells. At 6 h after plate scratching, EPCs migration ability compared to control was increased with a rate of 24% and 38% after stimulation *with potato shoot* lectin and *marigold flower* extract, respectively; at 12 h after plate scratching, the highest increase in EPCs migration activity (~45%) was observed after stimulation with *marigold* extract (Figure 9).

4. Discussion

Since their initial description, approximately a decade ago, EPCs have raised great enthusiasm given their therapeutic promises for maintenance of endothelial integrity, function, and postnatal neovascularization [12,17,22]. A major problem for *in vivo* studies is the homing of EPCs after systemic/local delivery in animal models of cardiovascular ischemia [23-26]. A significant amount of these cells are lost while crossing the systemic circulation, and only a small fraction can reach the injured endothelium [27,28]. Exogenous cells and/or exogenous factors delivery, to produce activated EPCs that can be more robust than non-activated cells, seems to be a promising therapeutic strategy [29]. It has previously been shown that *ex vivo* priming of EPCs with chemokines, such as SDF-1, before transplantation, could increase their proangiogenic potential [30]. For that reason, priming of EPCs with plant lectin or extracts prior to their systemic/local delivery may stimulate their adhesion to endothelial cells and thus augment the efficiency of cell therapy for ischemic vascular diseases. Lectins are a unique group of proteins that share specific carbohydrate binding sites, which play a very broad range of biological roles. Here we demonstrate the capacity of lectin from *potato shoot* and *marigold* extracts to increase EPCs proliferation and modulate expression of molecules involved in their chemotaxis and adhesion. Both *potato shoot* lectin and *marigold* extracts stimulated EPCs proliferation, in a concentration-dependent manner.

Marigold extract from flowers stimulated EPCs proliferation at concentrations lower than the *marigold green plant* extract, which can be explained by the presence of more abundant active compounds contained in *marigold flowers*, such as pentacyclic triterpene, trihydroxyalcohols, flavonoids, saponins, carotenoids, and phenolic acids that have been suggested to present anti-inflammatory and wound-healing properties [9,31,32].

EPCs recruitment and incorporation requires a coordinated sequence of multistep signaling events including chemotaxis, adhesion, integration, and maturation [15]. VE-cadherin is the major determinant of endothelial cell contact integrity; regulation of its activity is an essential step that controls the permeability of the blood vessel wall for different cells and substances

[33,34]. VCAM-1 can mediate both rolling-type and firm adhesion, depending on the avidity status of integrins [35]. Furthermore, intercellular adhesion molecule ICAM-1 may act in endothelial cells as a signaling receptor to induce expression of molecules with key roles in angiogenesis [36]. Both, *potato shoot* lectin and *marigold* extracts were able to increase gene expression of VE-cadherin, VCAM-1, and ICAM-1 adhesion molecules. It has been reported that various plant lectins stimulate VEGF production [37], a pro-angiogenic factor that up-regulates ICAM-1 expression in microvascular endothelial cells [38]. The extracellular domain of VEGFR consists of seven immunoglobulin homology domains, that upon VEGF binding through sugar moieties, promote receptor dimerization [39]. A high proportion of immunoglobulin glycosidic structures contain GlcNAc and/or core fucose residues. Some cytokines have lectin-like properties *via* their carbohydrate-binding capacity [40]. Therefore, *potato shoot* lectin with specificity for GlcNAc oligomers might have mimicked VEGF in its ability to bind to GlcNAc-containing glycoreceptors, such as VEGFR, this way up-regulating adhesion molecules expression on EPCs.

We have shown that EPCs stimulation with *marigold flower* extract also led to increased expression of PECAM-1, P-selectin and chemokine receptors, such as Tie-2 and CXCR4.

PECAM-1 has been implicated in angiogenesis through its involvement in endothelial cell-to-cell and cell-to-matrix interactions and signals transduction [41]. It has been indicated that endothelial injury/activation leads to the production of certain factors that release P-selectin of endothelial intracellular storage, which mediates cell adhesion [42,43]. Furthermore, the angiopoietin receptor Tie-2 is expressed exclusively in endothelial cells and promotes anigiogenesis [44,45]. It has also been shown that CXCR4 overexpression contributes to *in vivo* reendothelialization capacity of EPCs [46]. Therefore, up-regulation of chemokine receptors in human EPCs by stimulation with plant extracts, such as *marigold flower* extract, may become a novel therapeutic target for endothelial repair.

Marigold extracts were previously reported to produce both inhibitory and stimulatory *in vitro* effects on different cell lines [47]. Our study also revealed that *potato shoot* lectin and *marigold green plant* extract significantly decreased PECAM-1, P-selectin, Tie-2 and, CXCR4 gene expression. This observation is in line with other groups findings, showing that lectins and plant extracts also induced inhibitory effects on adhesion molecules expression [48]. In endothelial cells, it has been claimed that these inhibitory effects of plant extracts may be exerted by blocking of nuclear factor kappa B activation

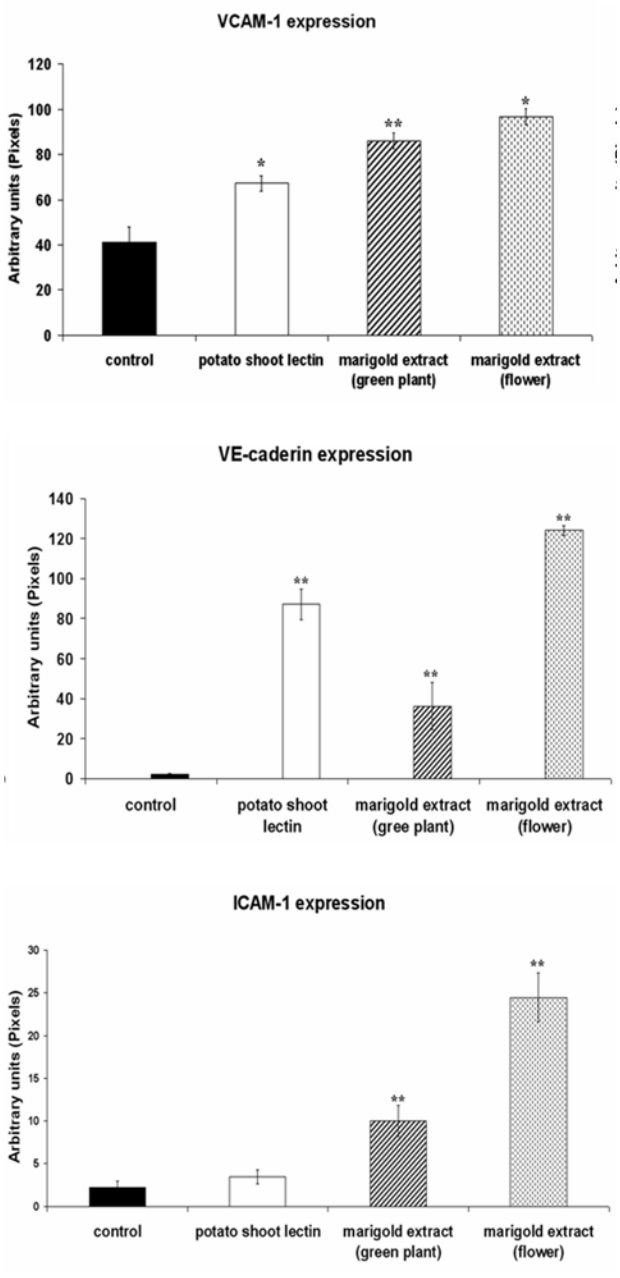

Figure 7. VCAM-1, VE-cadherin, and ICAM-1 mRNA expression in EPCs after 48 h of stimulation with either *potato shoot* lectin (5 μg/ml), *marigold green plant* extract (10 μg/ml) or *marigold flower* extract (1 μg/ml). Both *potato shoot* lectin and *marigold* extracts were able to significantly increase expression of VCAM-1 and VE-cadherin; ICAM-1 expression was significantly increased only after stimulation with *marigold* extracts. Results are represented as mean ± standard error, n=3, * $P<0.05$, ** $P<0.005$.

[49]. These findings suggest that different plant extracts have different modulatory properties on expression of different adhesion molecules.

Taken together, our observations suggest that both *potato shoot* lectin and *marigold* extracts may have

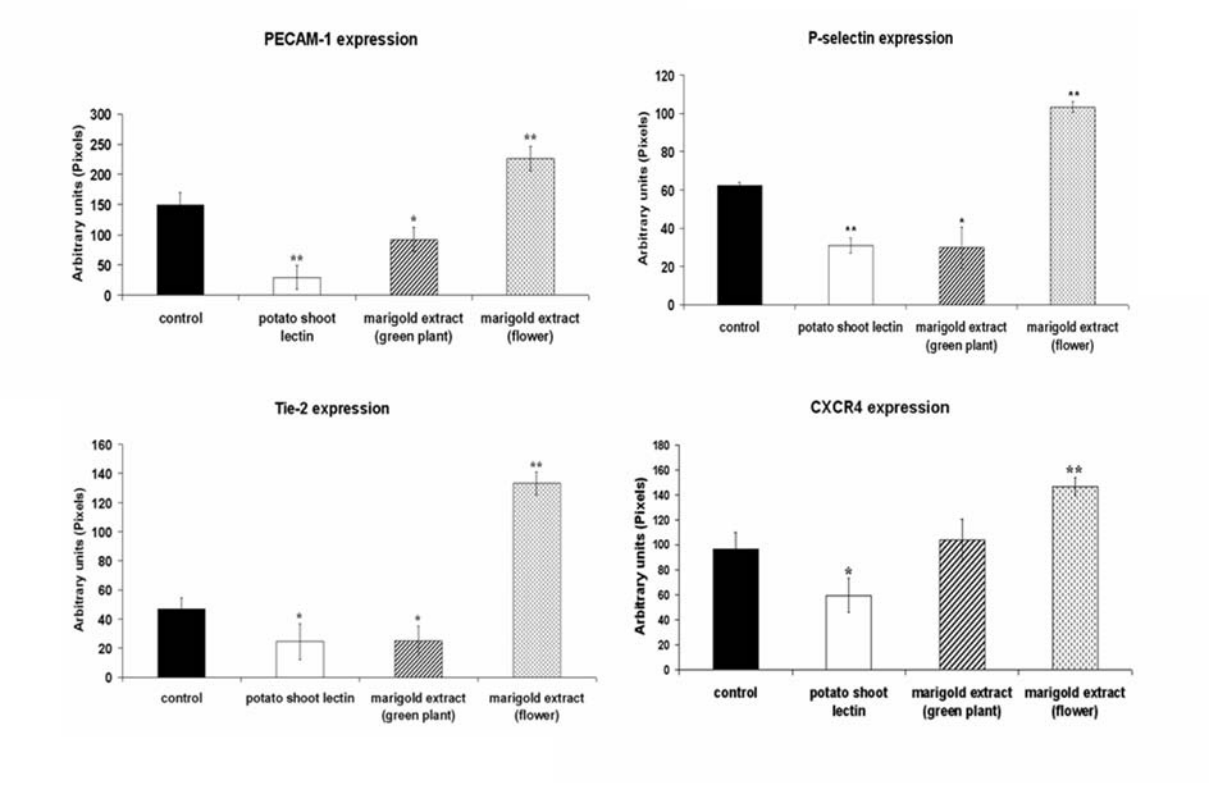

Figure 8. PECAM-1, P-selectin, Tie-2, and CXCR4 mRNA expression in EPCs after 48 h of stimulation with either *potato shoot* lectin (5 μg/ml), *marigold green plant* extract (10 μg/ml) or *marigold* flower extract (1 μg/ml). PECAM-1, P-selectin, and Tie-2 expression was significantly increased after stimulation with *marigold flower* extract, and decreased after stimulation with *potato shoot* lectin and *marigold green plant* extract. Both *marigold* extracts increased the expression of CXCR4, whereas *potato shoot* lectin significantly decreased its expression. Results are represented as mean ± standard error, n=3, * P<0.05, ** P<0.005.

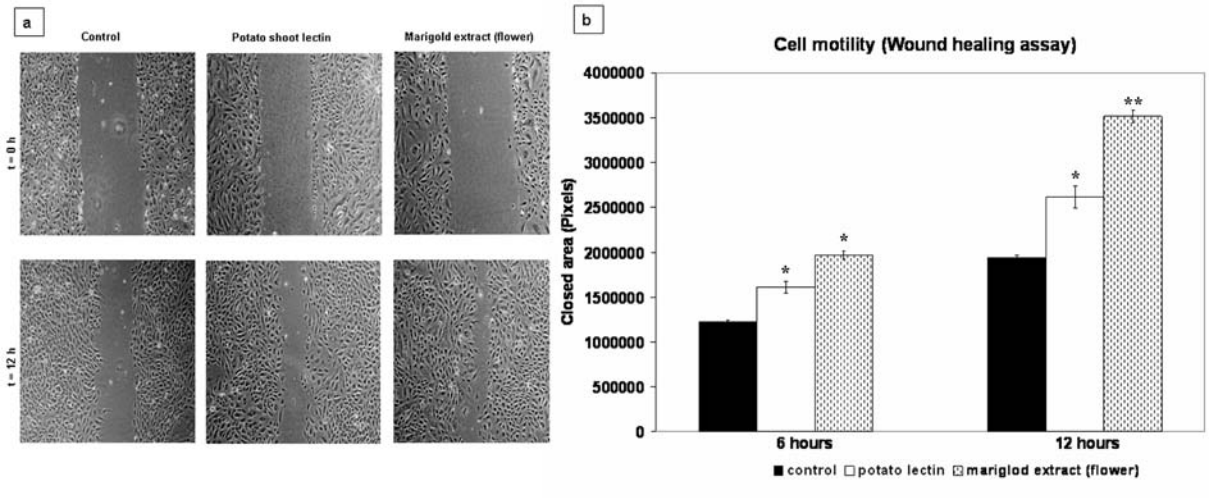

Figure 9. Study of EPCs migration by wound healing assay, upon stimulation with either *potato shoot* lectin (5 μg/ml), or *marigold flower* extract (1 μg/ml). Cell migration was quantified at 6 h and 12 h after stimulation, by measuring the wounded area (in pixels) that was covered by the cells during the indicated time points (a, Nikon, 4x). Both *potato shoot* lectin and *marigold flower* extract enhanced EPCs migration ability. Results are represented as mean ± standard error, n=3, * P<0.05, ** P<0.005 (b).

modulatory effects on expression of adhesion and chemotactic molecules, opening a new avenue in the investigation of lectin therapeutic properties. Therefore, these properties deserve to be further investigated in animal models of vascular ischemia. The *in vivo* experiments would bring new insights into lectins' ability to promote EPCs-induced vasculogenesis at the level of injured endothelium for vascular repair, and will prove their real value for clinical cell therapy.

Although further work is needed, the approach of activating the progenitor cells may circumvent the problems of insufficient cell number and low efficiency of incorporation that have been encountered in recent clinical pilot studies [29]. Thus, our work opens a new possibility for investigating how lectins could regulate progenitor cell behavior by uncovering novel mechanisms involved in their activation, proliferation, differentiation and survival. These insights would result in new tools to manipulate EPCs both *in vitro* and *in vivo* in order to improve cell-based therapy.

5. Conclusions

Lectin from *potato shoot* and *marigold* extracts showed the capacity to stimulate EPCs proliferation and modulate expression of molecules involved in their chemotaxis and adhesion. Interestingly, EPCs exposure to *marigold flower* extract resulted in a more pronounced increase in expression of adhesion and chemotactic molecules than *marigold green plant* and *potato shoot* lectin. These observations may be further investigated in the setting of cardiovascular regeneration, as a novel approach to promote proliferation and adhesion of cells at sites of vascular injury.

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