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MOLECULAR MECHANISMS UNDERLYING WOUND HEALING AND ANTI-INFLAMMATORY PROPERTIES OF NATURALLY OCCURRING BIOTECHNOLOGICALLY PRODUCED PHENYLPROPANOID GLYCOSIDES

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Abstract- Two phenylpropanoid glycosides, verbascoside (VB) and teupolioside (TP), produced biotechnologically by *Syringa vulgaris* and *Ajuga reptans* plant cell cultures, were studied *in vitro* and *in vivo* for their anti-inflammatory and wound healing activities. It was shown that TP- and VB-containing extracts significantly accelerated wound healing and possessed remarkable anti-inflammatory action in the excision wound model. These effects correlated with the inhibition of reactive oxygen species release from the whole blood leukocytes and with the ferrous ion chelating capacity. On the other hand, they don't correlate either with free radical scavenging or with the inhibition of lipid peroxidation in the cell-free systems. Furthermore, both VB- and TP-containing extracts were extremely effective inhibitors of chemokine and growth factor expression by cultured human keratinocytes treated with pro-inflammatory cytokines, TNF-alpha and interferon-gamma.

Keywords: : phenylpropanoid glycosides, plant cell cultures, verbascoside, teupolioside, free radicals, metal chelators, experimental wounds, myeloperoxidase, glutathione peroxidase, glutathione-S-transferase, keratinocytes, inflammation, chemokines, cytokines.

INTRODUCTION

Phenylpropanoid glycosides (PPGs, also synonymous of phenylethanoid glycosides) are water soluble derivatives of phenylpropanoids (PPs), the largest group of natural polyphenols widely distributed in the plant kingdom. At present, more than two hundred different PPGs are known, extracted from aerial parts and roots of the plants belonging to the families of Labiateae, Asteraceae, Oleaceae, Liliaceae, and others. Both verbascoside (VB) and teupolioside (TP) belong to the PPGs group and are structurally characterized by caffeic acid (phenylpropanoid moiety) and 4,5 hydroxyphenylethanol (phenylethanoid moiety) bound to a β-(D)-glucopyranoside trough an ester and glycosidic links, respectively. In the VB molecule, rhamnose is linked in sequence to the glucose molecule (Figure 1). The monocarbohydrate chain of TP consists of three moieties, rhamnose, galactose, and glucose. There is a growing evidence that PPGs, like other plant polyphenols in general and PPs in particular, are powerful antioxidants either by direct scavenging of reactive oxygen and nitrogen species, or by acting as chain-breaking peroxyl radical scavengers (5). Polyphenols such as PPGs and bioflavonoids with two adjacent –OH groups, or other chelating structures, can also bind transition metals, first of all iron and copper, in forms poorly active in promoting free radical chain reactions (7). Recently, PPGs have been reported to possess multiple beneficial effects for human health. Indeed, they have been effective in the chemoprevention of tumors (15); some have anti-inflammatory activity (16), while others have anti-thrombotic (26), wound healing (11), and cardio-protective actions (14). These health effects of PPGs have been traditionally explained

in terms of the prevention of free radical-associated and transition metal-mediated cell and tissue damage. Natural accelerators of wound healing with anti-inflammatory action are of great interest for surgery, dermatology and modern cosmetology. For example, in the course of T cell-driven skin inflammatory diseases, activated Th1 lymphocytes infiltrating the dermis and the epidermis are the major source of potent pro-inflammatory cytokines TNF-α and IFN-χ (9). These cytokines initiate a program of increased keratinocyte expression of inflammatory mediators, including adhesion molecules, growth factors such as GCSF, cytokines and chemokines. In particular, prominent expression of CCL2 (Monocyte chemoattractant protein 1, MCP-1), CCL5 (RANTES), CXCL8 (interleukin 8, IL-8) and CXCL10 (IFN-χ-induced protein of 10 kDa, IP-10) in keratinocytes is a common feature of T cell-mediated skin inflammation, and mediates the recruitment of T cells, granulocytes, dendritic cells, and monocytes in the skin (23). The recruitment of activated granulocytes and monocytes leads to overproduction of reactive oxygen and nitrogen species (ROS and RNS, respectively). The prolonged overproduction of these reactive species can cause severe tissue damage, impair wound healing, and result in DNA mutations that can lead to tumorigenesis (4, 10). The endogenous protective mechanisms include induction of antioxidant and detoxifying phase II enzymes in the skin cells (keratinocytes and fibroblasts) and migrated inflammatory leukocytes (granulocytes and monocytes) (2, 10, 27). Two ROS-inducible enzymes, glutathione-S-transferase (GST) and heme oxygenase-1 (HO-1) are thought to be of extreme importance in the cytoprotection during cutaneous wound repair (2).

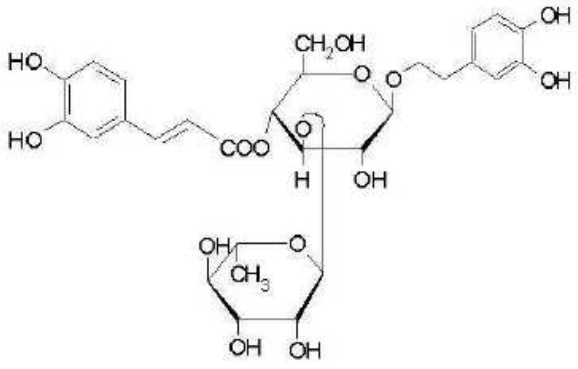

Figure 1. Chemical structure of verbascoside.

Naturally occurring PPGs seem to be excellent candidates to promote skin regeneration and ameliorate skin inflammation due to their ROS scavenging, antioxidant, iron chelating, and GST inducing properties (6, 13, 24). However, the industrial development and utilization of PPGs for medicinal use are limited because their chemical synthesis is extremely complex and expensive. Their extraction from mature plants has a very low yield, the final PPGs-containing products are poorly standardized due to unavoidable variations in the plant growth conditions, and they are often contaminated with environmental pollutants. There is now growing interest in the biotechnological approach to produce plant-derived active substances using non-genetically modified plant cell cultures (19). Plant cell cultures derived from medicinal plants are perfect sources of PPGs, biosynthesis of which could be specifically induced and directed to a certain compound depending on the nature of plant cells and a stimulus used (See the review by Korkina, present issue).

MATERIALS AND METHODS

Chemicals

All the reagents and solvents were of analytical grade. Most of chemicals for the enzyme, free radicals, chelating, and lipid peroxidation assays were purchased from Sigma-Aldrich. Medium for the keratinocyte cultivation, triggers for the keratinocyte activation and reagents for cytokine/chemokine analyses are specified below.

Animals

Healthy male Wistar rats, weighing 350-400 g were obtained from the animal house of Russian State Medical University. The rats were housed in polypropylene cages on normal food and water *ad libitum*. The rats were anaesthetized prior to infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (10 mg/kg). The study was approved by the Ethics Committee of Russian State Medical University.

Plant cell cultures and PPGs isolation and analyses

The extracts containing VB (97%, 56% w/w) and verbascoside-free (<1%) samples were obtained from *Syringa vulgaris* plant cell line. Both purified TP (95% w/w) and raw extract containing 70% w/w TP were obtained from *Ajuga reptans* plant cell line (19). Both *Syringa vulgaris* and *Ajuga reptans* have been recognized as European ethno-botanical medicinal herbs effective in accelerating of wound healing, as anti-inflammatory, anti-rheumatic, anti-pyretic and anti-fungal remedies since ages (12, 15). The stabilized and highly selected cell lines specified on the synthesis either VB or TP were obtained from sterilized dissected young *Ajuga reptans* leaves and *Syringa vulgaris* flowers, respectively. Cell cultures obtained in the industrial amounts were collected, homogenized, separated by centrifugation, and the solid residue discarded. The PPGs in the supernatant

were recovered by solid phase extraction on XAD4 resin, followed by elution with an 80/20 ethanol/water (v/v) mixture. The further purification was performed by repeated column chromatography on C18 silica gel and Sephadex LH20 and subsequent crystallization. The raw extracts of TP (70%) and VB (56%) contained other caffeic acid derivatives (approx. 10%) as was revealed by a chromatography-mass spectrometry analysis. The phenol content in PPG-containing extracts was determined by a modified Folin-Ciocalteu method (8).

Free radical scavenging activity

Hydroxyl radical (OH^-) scavenging properties were analyzed by the Fenton reaction using luminol dependent chemiluminescence (LCL) as an analytical tool. Briefly, luminol (final concentration 2 x 10^{-4}M), $FeSO_4$ (final concentration, 9 x 10^{-4} M), and 50 µl of saline solution of phenylpropanoid (PPGs)-containing extracts or rutin, at various concentrations, or saline solution (0.155 M NaCl) as a vehicle, were mixed in 0,1 M potassium phosphate buffer (pH 7,4) placed into 1-mL plastic cuvette of a chemiluminometer (Wallach Oy 1251, Finland). The reaction was started by adding 10 µl of H_2O_2 (final concentration, 2,5 x 10-6 M). The intensity of LCL was monitored for two minutes under continuous mixing and temperature (20°C). The results were expressed as IC_{50}, concentration (µg/ml) of a substance, which induced 50% inhibition of LCL. *Superoxide* ($O_2^{\cdot-}$) scavenging properties were evaluated in the xanthine- xanthine oxidase system using lucigenin-dependent chemiluminescence (LgCL). Under our experimental conditions, 0,89 mL of 0,1M KH_2PO_4 buffer (pH 7,4) were mixed with 0,01 mL of lucigenin (final concentration, 5 x 10-5 M), 0,02 ml of catalase (final concentration, 5 µg/mL), 0.02 mL of xanthine oxidase (final concentration, 1000 IU), and 0.05 mL of PPGs-containing extract or rutin solutions in a physiological saline buffer. In the controls, 0.05 mL of pure saline solution were added. The reaction was started by addition of 0.01 mL of xanthine (final concentration, 10^{-4} M). The LgCL was monitored for 5 min. The total light yield, as an integrated area under the LgCL curve, was calculated. The results were expressed as a concentration of 50% inhibition of a 5-minute light yield in the control sample. *Peroxynitrite* (*OONO-)* scavenging properties were assessed by electron spin resonance (ESR) method using spin label PTIO and SIN-1 as a source of peroxynitrite (1). Spin label PTIO (10 µL; 1mM) and PP-containing extract or rutin (0-10 µg) and 0.1 M KH_2PO_4 buffer (pH 7.4) were placed in the 200 µL cell of a ESR spectrometer (LFR-30 Free radical Analyzer, JEOL, Tokyo, Japan). ESR spectra of spin adduct PTI was repeatedly registered for 1 hour after starting the reaction by the addition of SIN-1 (20 µL; 10mM). The results were expressed as IC_{50}, µg/mL.

Antioxidant activity

The inhibition of lipid peroxidation (antioxidant activity) was measured spectrophotometrically, using a Shimadzu 1770 UV spectrophotometer, by the thiobarbituric acid (TBA) assay in a system containing egg yolk lipoproteins as a substrate and $FeSO_4$ as a trigger of lipid peroxidation. The results were expressed as IC_{50}, the concentration of 50% inhibition of MDA formation in the presence of test compounds.

Oxygen radicals produced in the whole blood

Inhibition of oxygen free radical release from human white blood cells (WBC) was assessed by LCL. In brief, 10 µL freshly drawn human blood were mixed with 0.980 mL of Hanks' balanced salt solution (HBSS) containing 5 x 10^{-5} M luminol and a solution of PPGs-containing extracts or rutin at different concentrations. The oxygen free radical generation was induced by 10µL of PMA (final concentration, 10 nM). The LCL response to PMA was registered continuously for 30 min at 37°C and stirring. The results were expressed as IC_{50}, µg/mL.

Metal chelating activity

The chelating activities of TP and VB extracts in relation to Fe^{2+}, Fe^{3+}, and Cu^{2+} metal ions were determined by spectrophotometric method described previously (12). The results were expressed as an equilibrium constant Keq (M^{-1}).

Wound healing experiments

The rats were inflicted with full thickness excision wounds as described previously (20, 21). The rats were anaesthesized prior to wounding, with 1ml of intravenous ketamine hydrochloride (10mg/kg). The dorsal fur was shaved and a full thickness of the excision wound of 1.5 cm in width (square area = 2.25 cm^2) and 0.2 cm depth was done. Each wound was treated daily with 100 µL of either tested sample (0.2 mg/ml) or 0,9% NaCl and Tegaderm© dressing applied to maintain a moist wound environment and eliminate scab formation. The rats were briefly anesthetized with ether mask daily for 8 days, the dressings were removed, the wounds cleansed with sterile NaCl solution, the topical agents applied and the wounds re-dressed. Skin biopsies were taken in the close vicinity (less than 10mm) from the wound area on the days 4 and 8, homogenized, centrifuged, and enzyme activities were measured in the supernatant (18, 28). The animals were divided into five groups of 10 each. The group 1 animals were treated with NaCl (control). Animals of groups 2 and 4 were treated daily with aqueous solution of VB (0.2 mg/ml of the 56% or 97% extracts, respectively). Animals of groups 3 and 5 were treated daily with aqueous solution of TP (0.2 mg/ml of the 70% or 97%, respectively) for 8 days. The measurements of the wound areas were performed on the 4th, and 8th day using transparent paper and a permanent marker. The recorded wound areas were measured by planimetry using special computer program.

Keratinocyte cultures and analyses of pro-inflammatory mediators.

Epidermal sheets for keratinocyte cultures were obtained from healthy individuals undergoing plastic surgery (n = 4, two females and two males; age 25-45 y). Primary cultures were established as described (22). Keratinocytes were sub-cultured in the serum-free medium Keratinocyte Growth Medium™ (KGM; Cambrex, San Diego, CA, USA), prepared from the essential solution supplemented with 10 ng/ml epidermal growth factor, 0.4 µg/ml hydrocortisone, 5 µg/ml insulin, 2 ml bovine pituitary extract and antibiotics. Second- or third-passage keratinocytes were used in all experiments. In the 24 h preceding the experiments, 80% confluent keratinocyte cultures were switched to hydrocortisone-depleted medium. Then, cells were treated with 100 ng/ml TNF-α and/or 100 U/ml IFN-γ for 24 h. Recombinant human TNF-α and IFN-γ were from R&D Systems (Abingdon, United Kingdom). Pro-inflammatory mediators were measured in the cell supernatants by ELISA using correspondent kits from BD Biosciences (San Diego, CA, USA). All experiments were

repeated with keratinocytes derived from at least three different donors.

RESULTS AND DISCUSSION

Wounding of skin induces a complex reaction, including early inflammation, new tissue formation, and extracellular matrix remodeling, which finally should result in the reconstruction of the wounded tissue (17). In the earliest stage of inflammation, the release of a variety of chemotactic factors that attract neutrophils and macrophages occurs. The recruitment of these cells to a wound is beneficial because they play an important role in the defense against bacteria and in the destruction of damaged tissue with the help of proteinases and reactive oxygen and nitrogen species produced by these inflammatory cells (10). However, ROS and RNS released from the skin cells in excess can cause severe oxidative damage to the skin itself, including both cellular and extracellular elements. Therefore wounded skin should be properly protected against the toxic effects of ROS and RNS. For this purpose, three major endogenous strategies have been evolved such as: (i) a sacrifice of pre-existent small antioxidant molecules in the skin (ascorbate, tocopherol, glutathione, bilirubin, uric acid, etc.); (ii) the induction of ferritin as the cellular trap of redox active free iron (26); and (iii) the induction of ROS- metabolizing enzymes, including superoxide dismutases, catalase, glutathione peroxidase, glutathione-S-transferase, and hemoxygenase-1 (2, 25). In order to accelerate wound healing and diminish the risk of inflammation-associated tissue damage, exogenous substances, which could interfere with wound-associated ROS (RNS)-mediated processes should be developed and applied. In the present study, the biotechnologically produced PPGs-containing extracts with different concentrations of verbascoside (VB) and teupolioside (TP) were tested for their superoxide, hydroxyl radical, and peroxynitrite scavenging activity in the cell-free systems generating ROS and RNS as well as for their ability to inhibiting Fe^{+2}-induced lipid peroxidation and ROS release from the whole blood leukocytes (Table 1). The results were compared to "classic" free radical scavenger – rutin, a glycosilated bioflavonoid. Both, TP- and VB-containing extracts with 97% of PPGs were more effective scavengers of superoxide and peroxynitrite as compare to rutin. From more than 20 flavonoids listed by Denisov and Afanas'ev (5), we found only three other molecules as effective as VB and TP in scavenging of superoxide radicals (epigallocatechin, kaempferol, and myricetin). The scavenging activity of extracts decreased with the decrease of PPGs concentrations and total phenol content in them. Thus, deprived from VB and phenols in general plant cell extract (> 1% VB and > 0.10 mg/g phenols) did not exhibit any free radical scavenging action. At the same time, both PPGs extracts were less effective than rutin in the hydroxyl radical scavenging. VB-containing extracts inhibited lipid peroxidation in a concentration dependent manner and were more effective than TP extracts and rutin. As compared to some well known water soluble polyphenols-antioxidants, the IC50 values of quercetin, hesperetin, naringenin, and rutin for iron-induced linoleate peroxidation were equal to 28.6, 17.2, 565, and 6.8 µM, respectively (5). In our hands, the IC50 values for 97% VB and TP extracts, and rutin were equal to 9.3, 11.6, and 19.7 µM, respectively. The inhibition of ROS release from the whole blood leukocytes decreased in the following range: 56% VB > 70% TP > 97% VB > 97% TP > rutin. It is evident that the inhibition of ROS release from leukocytes does not correlate with the findings in both cell-free ROS-generating and lipid peroxidation systems. However, the data obtained in a whole blood correlated with Fe^{+2} chelating capacity of PPGs extracts (Table 2). The greatest affinity towards ferrous ions was found for 56% VB and 70% TP extracts. It could be explained by the presence of other than VB and TP strong and selective iron chelators of polyphenolic origin, for example, caffeic acid derivatives, VB and TP analogues in the raw extracts. In the excision wound model, VB and TP containing extracts, being applied topically, showed different wound healing activity. The greatest one was found for 56% VB and then, for 70% TP-containing extracts (Table 3). On the other hand, highly concentrated 97% PPGs extracts were practically ineffective in the acceleration of wound closure. Our data confirmed (Table 4) that injury to the skin resulted in a sharp increase in the recruitment of neutrophils (assessed by the MPO activity) as well as in the activity of two glutathione metabolizing ROS-induced enzymes, GST and GPx. The maximal activity of these enzymes was observed at the 4^{th} day after wounding that corresponded to the maximum of inflammatory reaction in the excision wound model (10). The

Table 1 Phenol content, free radical scavenging, and antioxidant capacity of verbascoside- and teupolioside-containing extracts (mean ± SEM)

Extract	Parameter, units					
	Phenols, mg/g	IC_{50}, μg/mL				
		Hydroxyl	Superoxide	Peroxynitrite	Lipid peroxidation	Whole blood LDCL
Verbascoside, 56%	0.52	14.0±1.7	2.5±0.1	2.0 ±0.1	7.4±0.6	0.2±0.1
Verbascoside, 97%	0.65	10.0±0.9	2.2 ±0.1	1.6 ±0.2	5.8± 0.4	1.2±0.5
Verbascoside, < 1%	< 0.10	>250	> 19.0	> 16.0	no activity	no activity
Teupolioside, 70%	0.45	18.0±1.2	3.1± 0.3	2.1 ±0.2	12.0 ±0.7	1.00±0.2
Teupolioside, 97%	0.52	10.0±1.2	1.0± 0.1	1.8 ±0.1	9.4± 0.6	1.4±0.4
Rutin, 100%	0.54	8.8±0.7	4.5 ±0.5	2.3± 0.1	10.3±1.0	2.1±0.4

Table 2 Chelation equilibrium constants (Keq, M^{-1}) for verbascoside- and teupolioside-containing extracts.

Extract	**Metal ions**		
	Fe^{+2}	**Fe^{+3}**	**Cu^{+2}**
Verbascoside, 56%	$(19.0\pm6.8)\times10^5$	$(1.3\pm0.4)\times10^5$	$(1.1\pm0.3)\times10^5$
Verbascoside, 97%	$(6.3\pm1.7)\times10^5$	$(4.2\pm1.3)\times10^5$	$(1.4\pm0.5)\times10^5$
Verbascoside, <1%	no activity	no activity	no activity
Teupolioside, 70%	$(18.6\pm5.7)\times10^5$	$(1.2\pm0.4)\times10^5$	$(1.4\pm0.4)\times10^5$
Teupolioside, 97%	$(3.7\pm0.1)\times10^5$	$(4.6\pm1.0)\times1.0^5$	$(0.9\pm0.2)\times10^5$
Rutin	$(1.4\pm0.3)\times10^5$	$(1.6\pm0.4)\times10^5$	$(1.1\pm0.1)\times10^5$

Table 3 The effect of VB and TP-containing extracts on the full thickness excision wound healing (wound square, mm^2)

Group	**0 days**	**4 days after operation**	**8 days after operation**
Control	459.16±15.68	252.96±51.31*	150.16±65.46*;**
56% VB	459.16±15.68	127.31±28.21*;[a]	46.29±12.21*;**;[a]
70% TP	459.16±15.68	219.76±35.40*;[b]	78.39±21.75*;**;[b]
97% VB	459.16±15.68	261.25±22.99*;[b]	124.29±31.23*;**;[b]
97% TP	459.16±15.68	287.02±44.33*;[b]	98.45±24.26*;**;[b]

*- $p<0.05$ *vs* 0 day
**-$p<0.05$ *vs* 4th day after operation
a- $p<0.05$ *vs* control
b- $p<0.05$ *vs* 56% VB

Table 4 Enzyme activities in the skin (wound healing experiments)

Group	MPO, μmol/ g protein **Day 0** – 123±35		GPx, units/mg protein **Day 0** – 0.16±0.03		GST, μmol CDNB/g protein **Day 0** – 2.8±1.0	
	4th day	**8th day**	**4th day**	**8th day**	**4th day**	**8th day**
Control	237±56*	218±74*	0.56±0.32*	0.22±0.06*	5.7±1.0*	3.6±1.3
Verb., 56%	153±40**	170±29*	0.25±0.06**	0.22±0.03*	3.6±0.5**	3.0±1.3
Teup., 70%	229±25*	209±36*	0.31±0.04*	0.24±0.07*	3.2±0.9**	3.4±1.3
Verb., 97%	202±57*	185±68*	0.56±0.07*	0.18±0.05	5.3±0.7*	5.1±1.3*
Teup., 97%	186±47*	228±45*	0.52±0.20*	0.23±0.04*	8.4±1.5**	5.9±1.2**

*-$p<0.05$ *vs* Day 0 (before operation)
**-$p<0.05$ *vs* Control

56% TP-containing extract with the best wound healing action (Table 3) statistically significant prevented skin against excessive influx of pro-inflammatory neutrophils and against oxidative damage by recruited neutrophils that was revealed by normal activities of MPO, GST and GPx even at the maximum of inflammation. Interesting, that 97% VB and TP extracts did not affect highly increased activities of MPO, GPx and GST in the wounded skin. Moreover, the 97% TP extract application led to further increase of GST activity (Table 4). The experiments with primary cultures of human keratinocytes activated by pro-inflammatory cytokines, TNF-alpha and interferon-gamma taken alone or in combination, showed that the expression of chemokines for monocytes (MCP-1), for granulocytes and monocytes (IL-8), for dendritic cells (IP-10), and growth factor GCSF was effectively inhibited by both 97% VB and TP extracts within micromolar range of concentrations (1-50μM) (2 and 3). Both extracts were more effective inhibitors than hydrocortisone and triamcinolone, two "classical" anti-inflammatory agents (data not shown). Taken together, our data allowed us to suggest that biotechnologically produced VB and TP extracts exhibited remarkable anti-inflammatory and wound healing activity, which correlated with their ability to inhibit the ROS release from the whole blood granulocytes and monocytes, and with their chelating capacity towards Fe^{+2} ions. The diminished production of ROS by pro-inflammatory cells recruited to the damaged skin led to anti-inflammatory effect of PPGs and accelerated wound healing. The selective chelation of intracellular redox active "free" iron would further facilitate endogenous cytoprotection by increased levels of ferritin in the stressed skin cells (10, 27). Another important target for these natural anti-inflammatory agents is keratinocytes, which, being triggered by pro-inflammatory cytokines, produce growth factors and chemoattractants for pro-inflammatory cells such as granulocytes, monocytes, and T lymphocytes. Inhibition of pro-inflammatory cell migration to damaged skin would result in the moderate inflammatory response. The concentrated PPGs, being strong free radical scavengers and antioxidants, induce GST, the major phase 2 detoxifying enzyme, which could be of greater importance for tumor chemoprevention than for wound healing action (2, 6).

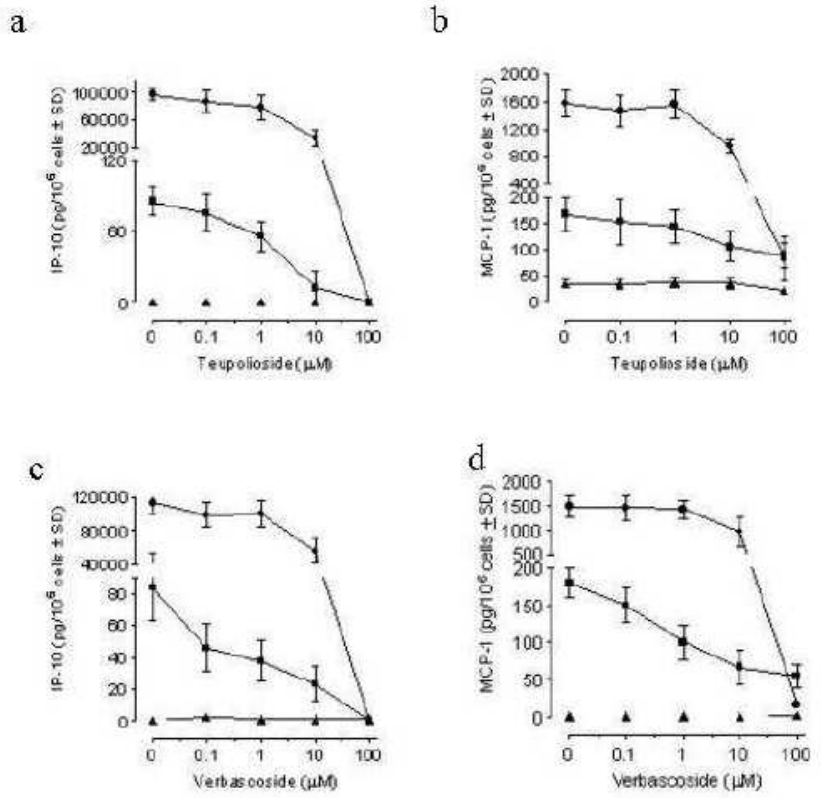

Figure 2. Effect of 97% verbascoside (VB) and teupolioside (TP) containing extracts on the IP-10 and MCP-1 expression in cultured human keratinocytes.
A and B – TP extracts; C and D – VB extracts.
Spontaneous expression of chemokines (▲), TNF-alpha induced (■), and TNF-alpha + interferon gamma induced (♦)

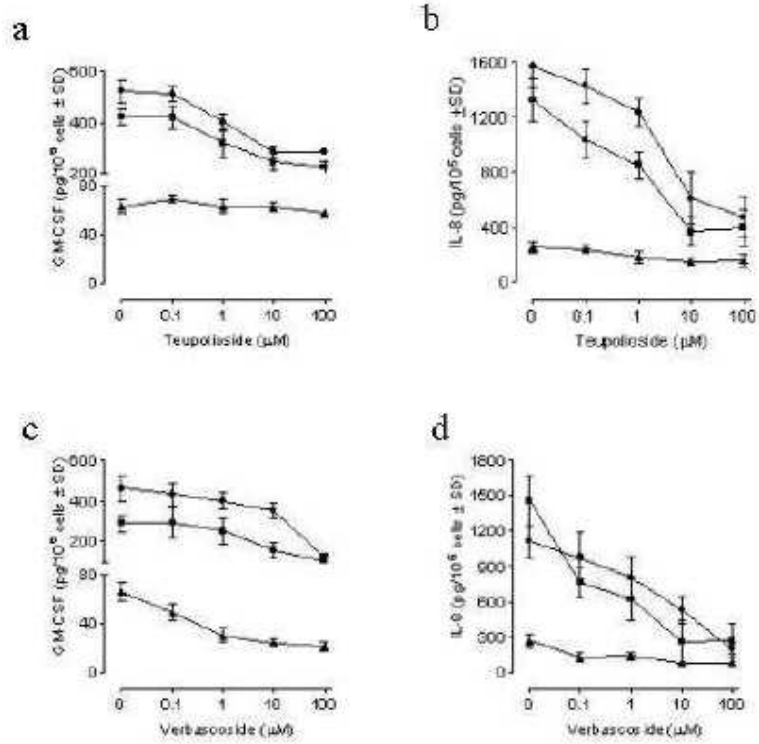

Figure 3. Effect of 97% verbascoside (VB) and teupolioside (TP) containing extracts on the GM-CSF and IL-8 expression in cultured human keratinocytes.
A and B – TP extracts; C and D – VB extracts.
Spontaneous expression of chemokines (▲), TNF-alpha induced (■), and TNF-alpha + interferon gamma induced (♦)

REFERENCES

1. Akaike, T. and Yoshida, M., Antagonistic action of imidazolineoxyl N-oxides against EDRF/NO through a radical reaction. *Biochem.* 1993, **32**: 827-832.
2. Applegate, L.A., Luscher, P. and Tyrrell R.M., Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. *Cancer Res*. 1991, **51**: 974-978.
3. Braun, S., Hanselmann, C., Gassmann, M.G., auf dem Keller, U., Born-Berclaz, C., Chan, K., Kan, Y.W. and Werner S., Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. *Mol. Cell. Biol*. 2002, **22**: 5492-5505.
4. Cerutti P.A. and Trump B.F. Inflammation and oxidative stress in carcinogenesis. *Cancer Cells* 1991, **3**: 1-7.
5. Denisov, E. and Afanas'ev, I., Oxidation and Antioxidants in Organic Chemistry and Biology 2005; CBC Taylor & Francis Group, Boca Raton-London- New York-Singapore.
6. Feng, R., Lu, Y., Bowman, L.L., Qian, Y., Castranova, V. and Ding, M., Inhibition of AP-1, NF-kB and MAPKs and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. *J. Biol. Chem*. 2005, **280**: 2888-2895.
7. Galey, J-B., Potential Use of Iron Chelators against Oxidative Damage. *In: Advances in Pharmacology, Academic Press*, Inc. 1997, **38:** 167- 203.
8. George, S., Brat, P., Alter, P. and Amiot, M.J., Rapid determination of polyphenols and vitamin C in plant-derived products. *J.Agric.Food Chem*. 2005, **53**: 1370-1373.
9. Girolomoni, G., Sebastiani, S., Albanesi, C. and Cavani, A. T-cell subpopulations in the development of atopic and contact allergy. *Curr. Opin. Immunol.* 2001, **13**: 733-737.
10. Hanselmann, C., Mauch, C. and Werner, S., Haem oxygenase-1: a novel player in cutaneous wound repair and psoriasis? *Biochem. J*. 2001, **353**: 459-466.
11. Hsu, S., Green tea and the skin. *J. Am. Acad. Dermatol*. 2005, **52:** 1049-1059.
12. Korkina, L.G. and Afanas'ev, I.B. Antioxidant and chelating properties of flavonoids. *Adv. Pharmacol*. 1997, **38**: 151-63.
13. Korkina, L., Mikhal'chik, E., Suprun, M., Minghetti, A., Crespi Perellino, N., Boninsegna, S., Pressi, G. and Dal Toso, R., Phenylpropanoids as a new class of natural chelators and antioxidants Feasibility for dermatology and cosmetology, 4th world Congress of "Cellular and molecular biology", 7-12 October, Poitiers, France, 2005: p. 72.
14. Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binoski, A.E., Hilpert, K.F., Griel, A.E. and Etherton T.D., Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med*. 2002, **113:** 715-885.
15. Kurkin, V.A., Phenylpropanoids from medicinal plants: distribution, classification, structural analysis, and biological activity. *Chem Nat Compd*. 2003, **39**: 123-153.
16. Lee, J.Y., Woo, E.R. and Kang, K.W., Inhibition of lipopolysaccharide-inducible nitric oxide synthase expression by acteoside through blocking of AP-1 activation. *J. Ethnopharmacol*. 2005, **97:** 561-566.
17. Martin, P. Wound healing-aiming for perfect skin regeneration. *Sci.* 1997, **276:** 75-81.
18. Mikhal'chikm, E.V., Ivanovam, A.V., Anurovm, M.V., Titkova, S.M., Pen'kov, L.Y., Kharaeva, Z.F. and Korkina L.G., Wound-healing effect of papaya-based preparation in experimental thermal trauma. *Bull. Exp. Biol. Med*. 2004, **137**: 560-562.
19. Minghetti, A., Una tecnologia innovativa per la produzione di principi attivi da piante. Colture cellulari di origine vegetale. *Natural 1*, 2003, **20**: 54-57.
20. Morton, J.J.P. and Malone, M.H., Evaluation of vulnerary activity by an open wound procedure in rats. *Arch. Int. Pharmacodyn.* 1972, **196**: 117-126.
21. Nayak, B.S., Vinutha, B., Geetha, B. and Sudha, B. Experimental evaluation of *Pentas lanceolata* for wound healing activity in rats. *Fitotherapia* 2005, **76**: 671-675.
22. Pastore, S., Fanales-Belasio, E., Albanesi, C., Chinni, L.M., Giannetti, A. and Girolomoni, G. Granulocyte macrophage-colony stimulating factor is overproduced by keratinocytes in atopic dermatitis. Implications for sustained dendritic cell activation in the skin. *J. Clin. Invest.* 1997, **99**: 3009-3017.
23. Pastore, S., Mascia, F., Mariani, V. and Girolomoni, G., Keratinocytes in skin inflammation. *Expert Rev. Dermatol.* 2006, and: 279-291.
24. Prestera, T., Talalay, P., Alam, J., Ahn, Y.I., Lee, P.J. and Choi, A.M. Parallel induction of heme oxygenase-1 and chemoprotective phase 2 enzymes by electrophiles and antioxidants: regulation by upstream antioxidant-responsive elements (ARE). *Mol. Med*. 1995, **1**: 827-837.
25. Steiling, H., Munz, B., Werner, S. and Brauchle, M., Different types of ROS-scavenging enzymes are expressed during cutaneous wound repair. *Exp. Cell Res.* 1999, **247**: 484-494.
26. Tognolini, M., Barocelli, E., Ballabeni, V., Bruni, R., Bianchi, A., Chiavarini, M. and Impicciatore, M., Comparative screening of plant essential oils: phenilpropanoid moiety a basic core for antiplatelet activity. *Life Sci*. 2006, **78:** 1419-1432.
27. Vile, G.F., Basu-Modak, S., Waltner, C. and Tyrrell, R.M., Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. *Proc. Natl. Acad. Sci. USA* 1994; **91**: 2607-2610.
28. Xia, Y. and Zweier, J.L., Measurement of myeloperoxidase in leukocyte-containing tissues. *Anal. Biochem.* 1997, **245**: 93-96.