The Effect of Szigetvár Medicinal Water and Organic Matter Isolate on HaCaT Cells Exposed to Dithranol

Doctoral (PhD) Thesis

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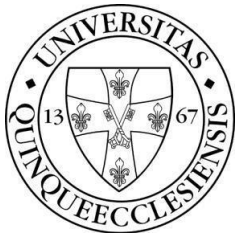

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1 Introduction

1.1 Ballneological aspects

Balneology is the therapeutic and recreational use of natural waters, peloids, and gases (referred to as agents). Beyond the everyday practice of operating bath facilities, balneology includes the scientific study of its tools. This involves analyzing the chemical composition of various agents and examining the effectiveness of therapies, as well as exploring possible mechanisms of action.

Balneology has a rich historical background, as humanity has utilized natural elements since ancient times, and their beneficial and healing effects have been recorded. The use of natural healing elements in everyday medical practice is called balneotherapy, which today is an integral part of modern medicine in Hungary. The documented effects on various diseases, their scientific evaluation, and the conduct of controlled human studies provide strong evidence to support the experiences accumulated over millennia.

In addition to practical applications, balneology is a multidisciplinary field of science that encompasses knowledge from geology, chemistry, physics, medicine, biology, and many other disciplines. Balneotherapy can complement classical treatment methods for a wide range of illnesses.

Waters are generally classified based on their inorganic composition for purely practical reasons and out of habit. The qualitative and quantitative analysis of inorganic components is a centuries-old science, and today the practical instrumental analysis of water is a routine task. Moreover, the characteristic inorganic anions and cations found in waters are present in quantities of a few dozen in number and on the scale of several thousand milligrams in concentration. What is often overlooked is that natural water, as it passes through the Earth's crust, does not only encounter inorganic substances but can also come into contact with organic materials, which are abundant along its path. These organic materials can dissolve into the water, resulting in waters with significant organic content becoming available.

This raises the question: what role do the organic compounds in medicinal waters play in affecting the human body when exposed to them, and how might they be responsible for any potential therapeutic effects?

Varga emphasizes that despite numerous research groups studying the organic composition of medicinal waters in recent decades, to this day, medicinal waters are

categorized based on their inorganic composition. The essence of Varga's organic hypothesis is that, when analyzing the therapeutic effects, one should not only consider the simpler inorganic composition (e.g., sulfurous, hydrogen carbonate-rich, or iron-containing medicinal water) but also examine the effects of the entirety of the medicinal water, including its organic components.

In our research, we studied the medicinal water and organic isolates of the Szigetvár Thermal Bath. Szigetvár area belongs to the porous and fissured thermal water layers of the South-West Transdanubian region, and additionally, thermal karst waters are also found in the vicinity. The medicinal water of the thermal bath is 62 °C, primarily sodium-chloride and alkaline hydrogen carbonate in composition.

1.2 The Onset, Treatment, and Balneotherapeutic Supportive Care of Psoriasis

Psoriasis is one of the diseases where adjunct balneotherapy significantly improves outcomes alongside conventional therapy. Psoriasis is a chronic, immune-mediated inflammatory skin disorder. Clinically, it manifests mainly as red, scaly plaques on the elbows, knees, scalp, and back. Besides these common areas, it can appear anywhere on the body. The disease greatly impacts the patient's quality of life. Psoriasis is now considered a systemic disease. In addition to localized symptoms, psychological, metabolic, musculoskeletal, and cardiovascular comorbidities may also occur.

The disease can develop at any age, including in children, but most cases appear before the age of 35. Among the etiological factors of psoriasis, genetic predisposition is significant. External factors include mechanical irritation of the skin, air pollution, side effects of certain medications, some vaccinations, infectious diseases, smoking, and alcohol consumption, all of which are associated with an increased prevalence of the disease. Internal factors include metabolic syndrome, obesity, diabetes, dyslipidemia, hypertension, and mental stress.

The cornerstone of psoriasis treatment is topical therapy with corticosteroid-containing preparations. Formulations combining betamethasone and calcipotriol (a vitamin D analog) are commonly used. UV phototherapy has become less prominent with the advent of biological treatments. In moderate to severe cases, the second-line treatment includes small-molecule systemic agents as part of the therapeutic protocol. These include active substances such as acitretin, methotrexate, and cyclosporine. Additionally, orally

administered drugs that inhibit intracellular signaling pathways, such as Janus kinase (JAK) inhibitors or phosphodiesterase-4 (PDE-4) inhibitors, have been developed.

The most advanced biological therapies have revolutionized psoriasis treatment. Biological therapeutic agents were first approved in Hungary 20 years ago. Currently used drugs target one of three potential pathways: they block TNF-α, interleukin-23 (IL-23), or IL-17 cytokines.

One of the classical compounds used in psoriasis therapy is dithranol (anthralin, DTH), a synthetic anthracene derivative known as hydroxyanthrone. This agent has largely fallen out of routine clinical practice but is still used in some cases. DTH is water-insoluble and is applied to the affected areas primarily in ointments, pastes, or creams. The main mechanisms of action of DTH are thought to include participation in free radical reactions, generation of oxygen-containing free radicals, and induction of keratinocyte apoptosis in plaques through oxidative stress.

However, its strong oxidative effect also causes its primary side effect: irritation and inflammation when applied to plaques or healthy skin. Additionally, the compound's yellow color permanently stains skin and objects it comes into contact with (e.g., clothing, bathroom items).

The clinical use and investigation of DTH have been accompanied by the paradigm that irritation and erythema arising during application are necessary components of its therapeutic effect. However, research by Benezeder et al. has provided strong evidence challenging this paradigm. Their findings indicate no correlation between DTH-induced perilesional and lesional erythema and its antipsoriatic effect. Irritation and erythema are merely side effects of DTH and not part of its therapeutic mechanism.

1.3 1.3 Possible (Molecular) Mechanisms of Action of Balneotherapy

The effects of balneotherapy on psoriasis have been the subject of numerous studies. While uncovering the exact mechanisms of action is a task for future research, several molecular processes, particularly those involving apoptotic and inflammatory pathways, have already been explored. Many research groups focus on the direct antioxidant effects of medicinal waters, their impact on antioxidant systems, and their influence on oxidative stress-related biomarkers.

The antioxidant effect is generally attributed to sulfur compounds, which is evident in sulfur-rich medicinal waters since many sulfur compounds, such as hydrogen sulfide, participate in redox reactions and exhibit strong antioxidant properties. However, this raises the question: can antioxidant effects be expected in medicinal waters with low sulfur content or in sulfur-free waters?

Water that comes into contact with the organic materials of bedrock dissolves various substances, and shallow groundwater often contains high concentrations of dissolved organic matter. The concentration of these substances can reach milligram levels, and their composition largely consists of humic and fulvic acids. Through thermocatalytic or microbial chemical degradation, these substances break down into smaller organic compounds and gases (e.g., CO2, hydrocarbons). While humic substances degrade in high-temperature thermal waters, cooler waters are rich in humic materials.

Patients with psoriasis often show significant deviations in inflammatory parameters from normal values. For example, serum malondialdehyde (MDA) levels, an indicator of oxidative stress, are significantly higher in psoriasis patients compared to healthy individuals. Szenczi et al. conducted a pilot study at the Harkány Thermal Bath Hospital, measuring serum MDA levels in 20 psoriasis patients. The patients underwent a three-week psoriasis treatment protocol, which included balneotherapy as an essential component alongside DTH therapy. The study found that plasma MDA levels in patients receiving balneotherapy with Harkány medicinal water were significantly lower than those in the placebo control group.

Varga et al. prepared organic matter isolates from five well-known Hungarian medicinal waters (Hévíz, Hajdúszoboszló, Gyopárosfürdő, Tiszakécske, Zalakaros). Using the Salmonella Ames test, they determined that the isolates had no mutagenic effects. However, the isolates provided significant protection against UV-induced lethality when tested in the Ames assay.

Gerencsér et al. investigated the UV protective effects of organic matter isolates from various medicinal waters on HaCaT human keratinocyte cell lines using the comet assay method. The HaCaT cells were exposed to the medicinal water isolates and UV radiation within the comet assay gel structure. Two medicinal water isolates, from Kakasszék and Gyopárosfürdő, showed significant UV protection under specific experimental conditions.

1.4 Objective of the Study

The effects of DTH on psoriasis have been extensively studied using cell culture models. Since abnormal keratinocyte behavior is a primary pathogenic factor in the disease, immortalized human keratinocyte cell lines (e.g., HaCaT) are widely used to investigate the effects of DTH exposure. The main mechanism of DTH is believed to involve participation in oxidative reactions within the mitochondria of cells, leading to the generation of reactive oxygen species (ROS), oxidative stress, and subsequent initiation of apoptotic processes.

In previous experiments, the dose range of DTH required to induce apoptosis without acute lethality was established. The temporal dynamics of early and late phases of apoptosis, changes in markers suitable for observing oxidative stress, and molecular responses of cells to oxidative stress were also determined. Evidence is available in the literature regarding the effects of medicinal water and its components, as well as the impact of balneotherapy on psoriasis. However, as far as current knowledge indicates, there is no evidence regarding the combined effect of DTH exposure and medicinal water exposure studied on HaCaT cell cultures. Furthermore, no evidence exists concerning the effect of Szigetvár medicinal water in the context of DTH exposure. The impact of Szigetvár medicinal water on specific inflammation- and oxidative stress-related markers has not been documented. Additionally, there is no evidence regarding the effect of the organic matter isolate of Szigetvár medicinal water (henceforth referred to as "isolate").

We prepared a cell culture medium for HaCaT cells that is tolerable, made from Szigetvár medicinal water and its organic matter isolate. We conducted acute toxicity tests on these culture media, determining the changes in HaCaT cell viability after a 3-hour exposure. Modeling a single DTH exposure followed by balneotherapy as performed in clinical practice, our short-term experimental setup consisted of 1 hour of DTH exposure, followed by 3 hours of exposure to Szigetvár medicinal water or the organic matter isolate. Our goal was to use this experimental protocol to examine how the gene expression of certain inflammatory cytokines (TNF-α, IL-6, IL-8) and the chemokine (GM-CSF), which respond to oxidative stress, changes in the cells. We aimed to investigate how the MDA concentration, considered a marker of oxidative stress, changes, as well as how the expression of miR-21, associated with the regulation of oxidative stress, is altered.

2 Materials and Methods

2.1 Szigetvár Medicinal Water and Isolate

The medicinal water samples were obtained from the Szigetvár Thermal Bath. The organic isolate was prepared using a method based on the 1992 patent by Varga et al., also utilized in Hanzel's Ph.D. work. The isolate does not contain the mineral components of the medicinal water but includes most of the organic matter, concentrated to 3000 times the original level.

The methodology described by Portalés et al. was used, involving the addition of powdered culture medium to native or diluted medicinal water. This method allows for higher concentrations of medicinal water in the exposure media compared to other dilution methods. The isolate was diluted with complete culture medium to achieve the following concentrations: 6x, 12x, 30x, 40x, 60x, 120x, 250x, 300x, 400x, 600x, 1500x, and 3000x (relative to the isolate). Experiments were conducted on HaCaT immortalized human keratinocyte cell lines.

2.2 XTT Cell Viability Test

The XTT tetrazolium salt (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxyanilide) was used, which is metabolically reduced in living cell mitochondria, producing a water-soluble, colored formazan product. This directly measures metabolic activity, correlating with the number of live cells. Cells were plated in 64-well cell culture plates. After 3 hours of incubation, color intensity was measured using a plate reader at 450 nm and 630 nm (background). Background intensity was determined using empty culture medium and empty wells. Untreated cells served as controls. Measurements were taken immediately after the 3-hour exposure period, and all experimental setups were repeated three times.

2.3 Lipid Peroxidation Malondialdehyde (MDA) Assay

Lipid peroxidation is a result of oxidative damage and serves as a quantitative indicator of oxidative stress. Polyunsaturated lipids are particularly prone to oxidative reactions, often driven by reactive oxygen species, resulting in characteristic end products like malondialdehyde (MDA). The Sigma-Aldrich Lipid Peroxidation (MDA) Assay Kit (Cat:

MAK085) was used to measure MDA levels in tissues, cells, and plasma. The reaction between MDA and thiobarbituric acid (TBA) produces a photometrically measurable product at 532 nm, with concentration proportional to the sample's MDA level.

2.4 Quantitative Real-Time PCR (qRT-PCR)

Exposures were conducted in 96-well cell culture plates. Preliminary studies confirmed that a single well contained sufficient RNA for subsequent measurements. Each experimental condition was repeated three times. Samples were extracted directly from the wells using the ExtraZol reagent (Qiagen, Blirt) following the manufacturer's protocol. Total RNA concentrations were measured using a Maestrogen MN-913 spectrophotometer. Primers were designed using NCBI Primer-BLAST software. PCR reactions were conducted using a LightCycler 480 PCR machine (Roche) with the qPCRBIO SyGreen 1-step Detect Lo-ROX kit (PCRBiosystems, Cat: PB25.11-3).

3 Results

We refer to the 120x, 600x, and 3000x dilutions of the organic matter isolate from Szigetvár medicinal water as 120x, 600x, and 3000x isolate dilutions or simply as dilutions. The culture medium containing 90% Szigetvár native medicinal water is referred to as SVNM.

3.1 Survival Studies

As an introduction to the experimental series, I examined the survival of HaCaT human keratinocyte cells in culture media prepared from Szigetvár medicinal water and supplemented with the organic matter isolate from Szigetvár. The experiments were conducted with a 3-hour exposure duration. For data representation, the untreated (negative control) cells' results were set as 100%. The results are shown in Figure 1.

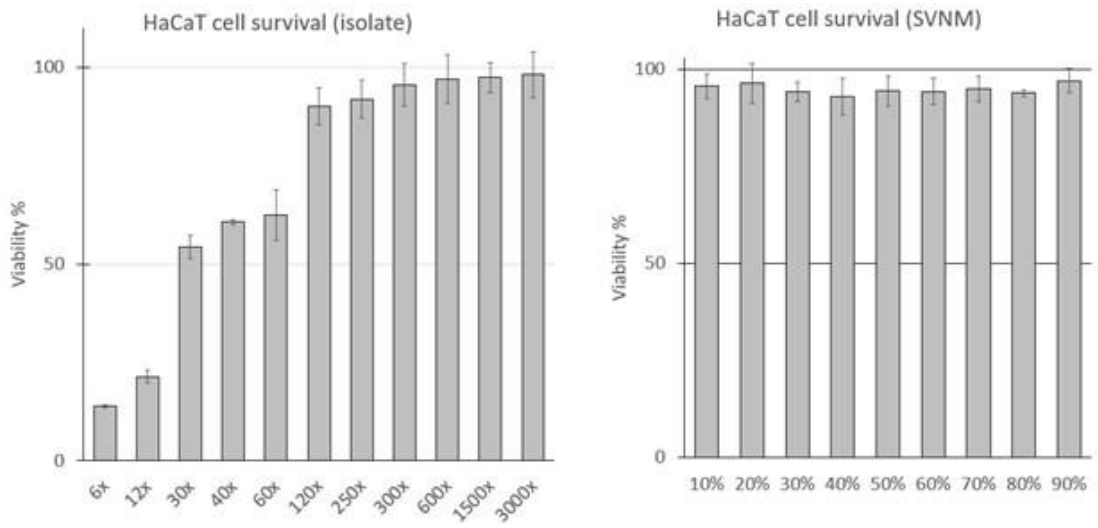

Figure 1. *(a) Survival of HaCaT cells in different dilutions of Szigetvár organic matter isolate in 3 hours. (b) Survival of HaCaT cell in different concentrations of Szigetvár medicinal water con-taining cell culture medium (SVNM). 6x-3000x – dilution of the isolate (Composition shown in Table 1.) 10-90% - Szigetvár medicinal water content of different studied SVNMs.*

Based on the survival curves, it is evident that starting from the 120x dilution of the isolate and moving toward higher dilutions, cell survival was not significantly reduced. Similarly, SVNM did not show any significant difference in cell survival compared to the control cells in any of the tested compositions.

3.2 Lipid Peroxidation Malondialdehyde (MDA) Assay

The measurement results for MDA concentrations are shown in Figure 2. The solvent controls, SVNM, and the isolate dilutions did not significantly increase MDA levels compared to the untreated cells. DTH, however, increased MDA levels in a concentration-dependent manner compared to untreated cells for both 1-hour and 4-hour exposures. SVNM showed significantly lower MDA concentrations in all three examined concentrations compared to the 4-hour DTH exposure. The isolate dilutions demonstrated significantly lower MDA concentrations in most experimental setups compared to the 4-hour DTH exposure.

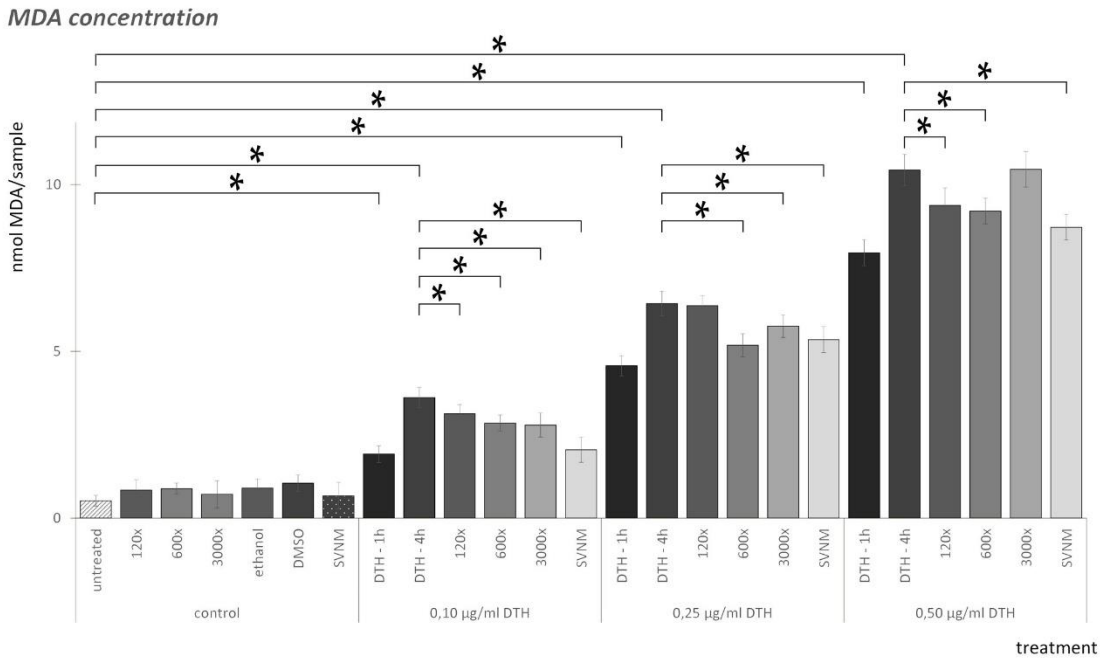

Figure 2. *Amount of MDA (mmol/sample) in HaCaT cells in different experimental settings. (* = p < 0.05)*

3.3 Examination of Inflammatory Cytokine mRNA Expression Using RT-qPCR

The relative gene expression values of the TNF-α, IL-6, and IL-8 cytokines, as well as the GM-CSF chemokine, are shown in Figures 3–6 in the experimental setup used in this study.

In summary, we observed no significant increases in any of the examined cytokines or chemokines for the solvent controls, SVNM, or the isolate dilutions. In line with literature data, relative gene expression values slightly increased after 1-hour DTH exposure and markedly increased after 4-hour DTH exposure.

The effects of SVNM and the isolates on DTH exposure were always compared to the 4-hour DTH exposure values. Generally, in several DTH concentrations, SVNM exposure following DTH exposure resulted in lower mRNA expression levels compared to the groups exposed to DTH alone. In many cases, groups exposed to the isolate showed significantly lower mRNA expression than the 4-hour DTH exposure groups.

Overall, expression levels were generally lower in the groups exposed to SVNM than in those exposed to the isolates. Among the three isolate dilutions, no clear concentration-dependent trends could be identified.

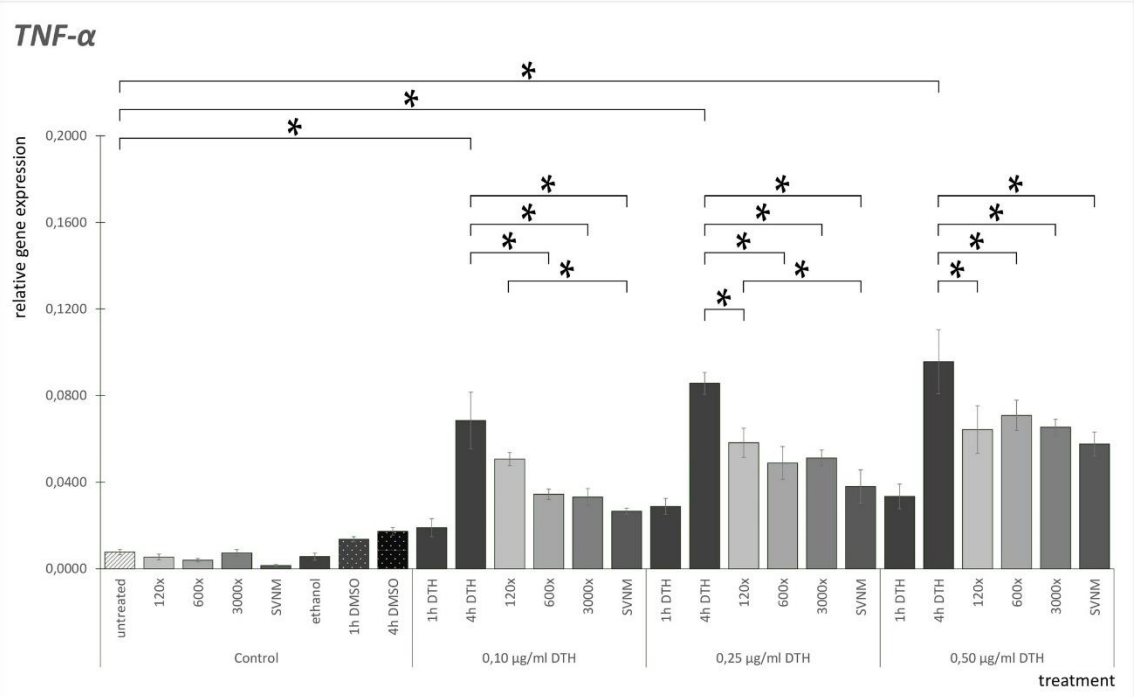

Figure 3. *Relative gene expression level of TNF-α in HaCaT cells treated according to the ex-perimental protocol. (* = p < 0.05). DTH – dithranol; 120x, 600x, 1200x - dilution of the tested or-ganic matter isolate; SVNM – Cell culture medium with 90% Szigetvár medicinal water content*

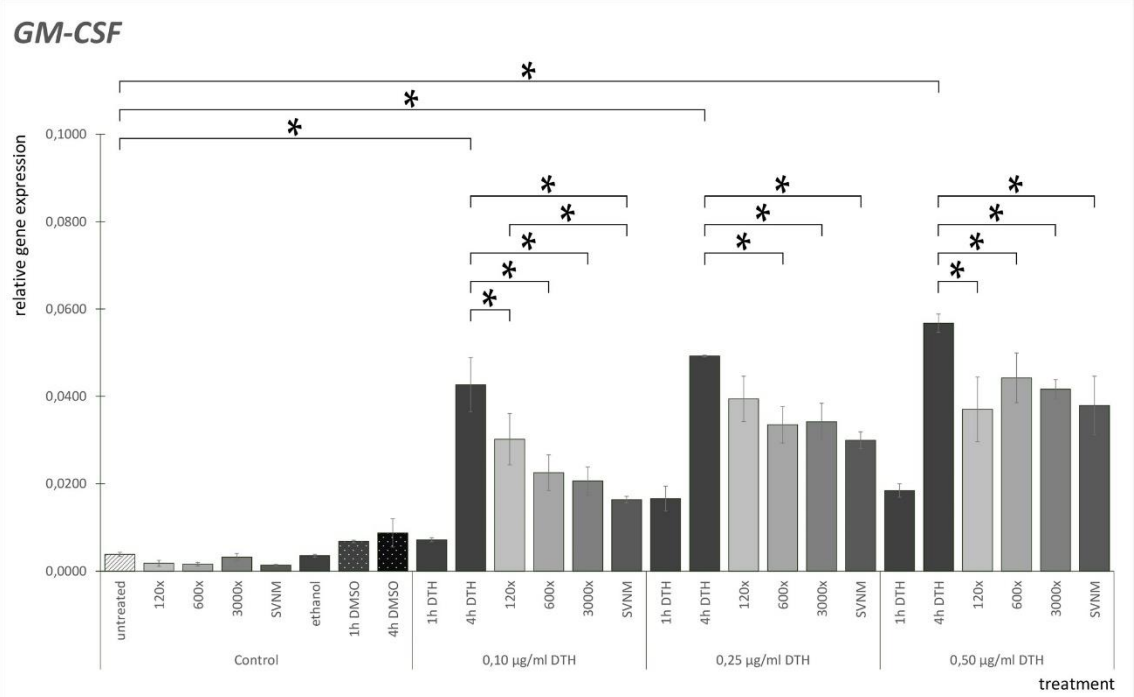

Figure 4. *Relative gene expression level of GM-CSF in HaCaT cells treated according to the ex-perimental protocol. (* = p < 0.05). DTH – dithranol; 120x, 600x, 1200x - dilution of the tested or-ganic matter isolate; SVNM – Cell culture medium with 90% Szigetvár medicinal water content*

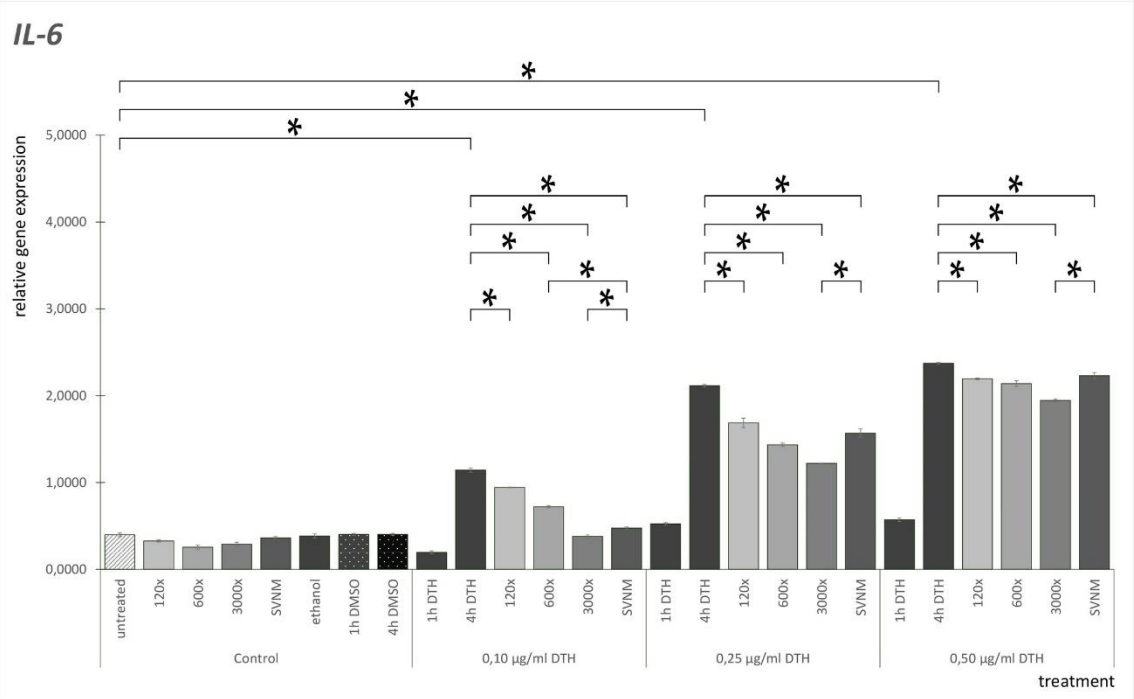

Figure 5. *Relative gene expression level of IL-6 in HaCaT cells treated according to the experi-mental protocol. (* = p < 0.05). DTH – dithranol; 120x, 600x, 1200x - dilution of the tested organic matter isolate; SVNM – Cell culture medium with 90% Szigetvár medicinal water content*

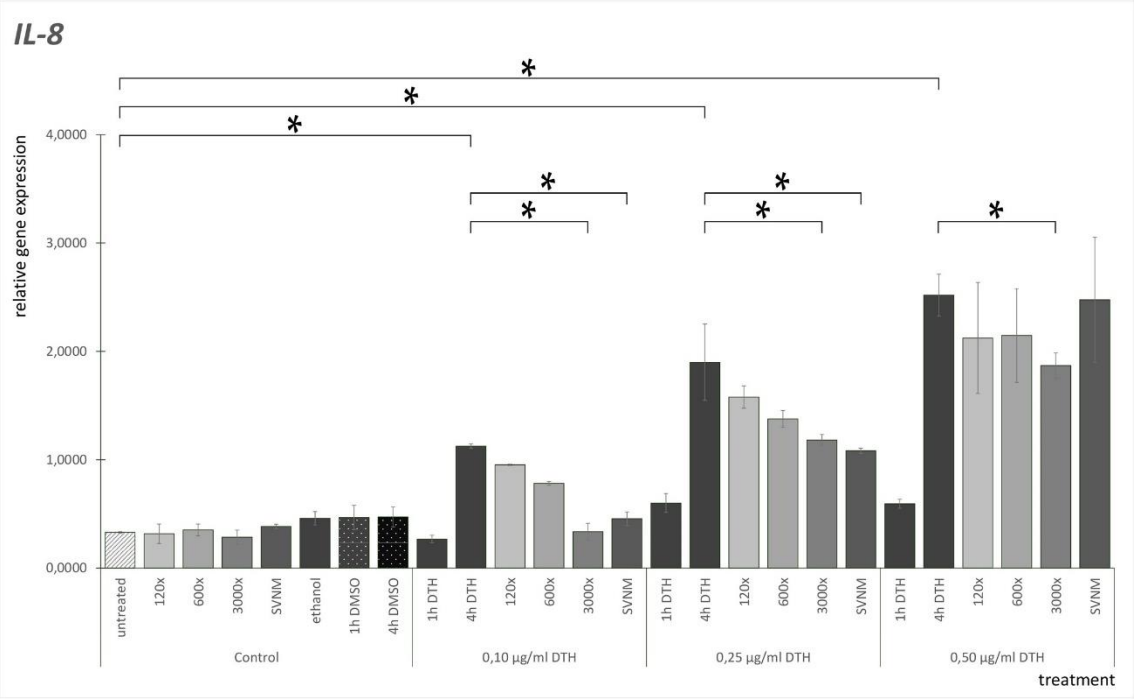

Figure 6. *Relative gene expression level of IL-8 in HaCaT cells treated according to the experi-mental protocol. (* = p < 0.05). DTH – dithranol; 120x, 600x, 1200x - dilution of the tested organic matter isolate; SVNM – Cell culture medium with 90% Szigetvár medicinal water content*

When analyzing the relative expression of miR-21 (Figure 7), neither the solvent controls, SVNM, nor the isolate showed an increase compared to the untreated control cells. No significant differences were observed among the three concentrations of DMSO controls, nor among the ethanol controls.

A 1-hour DTH exposure significantly increased miR-21 levels at all concentrations (this is not marked in Figure 12). Examining the 4-hour DTH exposures, all values were significantly higher than the corresponding 1-hour DTH exposures.

Comparing SVNM and isolate exposures to the 4-hour DTH exposures, at 0.5 µg/ml DTH, the 600x dilution reduced miR-21 expression, but the decrease was not significant. At all DTH concentrations, SVNM exposure showed an increase in miR-21 relative expression compared to the 4-hour DTH exposures. However, this increase was only significant at 0.1 µg/ml DTH.

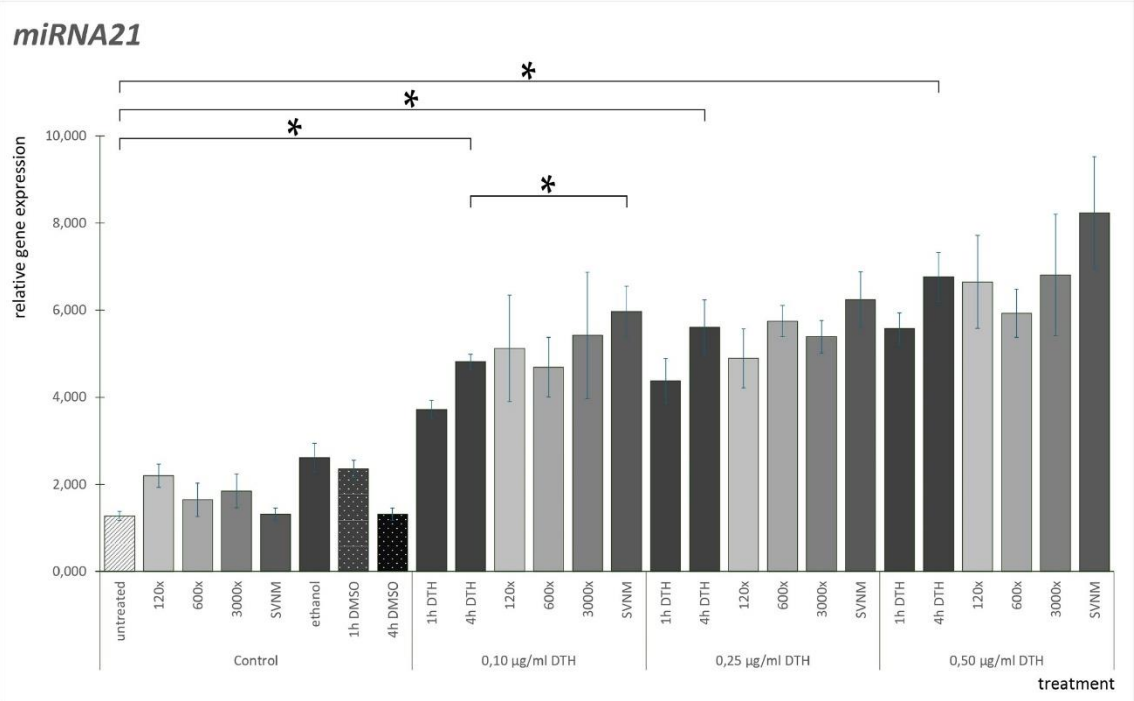

Figure 7. *Relative gene expression level of miR-21 in HaCaT cells treated according to the ex-perimental protocol. (* = p < 0.05). DTH – dithranol; 120x, 600x, 1200x - dilution of the tested or-ganic matter isolate; SVNM – Cell culture medium with 90% Szigetvár medicinal water content*

4 Discussion

Balneotherapy associated with clinical treatment for psoriasis has shown positive results regarding healing. The main aim of the present study was to investigate DTH, a substance whose effectiveness in psoriasis treatment remains widely used today despite being questioned due to its side effects, using HaCaT keratinocyte cell culture. DTH induces inflammation in psoriatic plaques, and this inflammatory response is still considered by many as a necessary therapeutic mechanism for DTH. Even when applied to healthy skin, DTH causes irritation, a burning sensation, redness, and discoloration, triggering inflammatory responses. Numerous studies have discussed the potential and proven antioxidant effects of mineral waters and balneotherapy.

Our results are in line with the trends described in the literature. DTH exposure significantly increased MDA concentrations. A significant increase was observed after both 1-hour and 4-hour DTH exposures, with a dose-dependent increase. Several studies have discussed the possible direct antioxidant effects of mineral waters or the modification of the cell's response to oxidative stress. Antioxidant activity is often attributed to the presence of reducing sulfhydryl groups (thiol, SH), due to the natural sulfur content of the waters. In our experiment, we selected a thermal water with low sulfur content and organic material. The MDA test results correlate with the literature data. DTH exposure increased the MDA levels in untreated cells. A more significant increase was observed in the first hour than in the subsequent three hours, suggesting that oxidative damage processes occur early during treatment. It is important to note that neither the SVNM nor the isolate dilutions significantly increased MDA levels, which is consistent with previous findings, where thermal waters exhibited a protective effect against oxidative damage without inducing oxidative stress.

According to our findings, SVNM shows a significant protective effect against the MDA increase induced by DTH exposure at all three concentrations of DTH. Interestingly, the organic isolates also showed significantly lower MDA levels, suggesting that the organic content of thermal water carries the same effect as the full water, but the complete protective effect is likely due to the combination of organic and inorganic materials. Antioxidants affect DTH damage in a dose-dependent manner. In our case, the effect of the organic material concentrate was not clear. The 120x dilution did not show significant protective effects, while the 600x dilution demonstrated greater protection than the 3000x dilution.

Recent research has shown that normal keratinocytes respond to different types of oxidative stress. These responses often induce adhesion molecules that increase inflammation and the migration of immune cells to the inflammatory site. The noxa leads to the induction, production, and release of inflammatory mediators (including cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-8, and GM-CSF) through various signaling pathways, such as the mitogen-activated protein kinase (MAP) pathway or activation via protein kinase C. According to the literature, balneotherapy has a beneficial effect on cells involved in inflammatory processes, but the water composition must also be considered.

Our results are consistent with literature findings, suggesting that balneotherapy reduces the levels of molecular markers associated with inflammation. All of this supports the observed effects of mineral waters and balneotherapy in practice. In our experiments, we proved that Szigetvár thermal water has similar properties. For the first time, we demonstrated that when the organic materials are separated from the inorganic components and examined individually, the organic content alone positively influences the excessive expression of the inflammatory cytokines we studied. The Szigetvár thermal water-based medium consistently showed better results compared to the organic isolates, suggesting that the combined presence of organic and inorganic components is responsible for the effect. The 3000x dilution, which has the same organic material concentration as the original thermal water, showed similar trends but more moderate results in reducing elevated cytokine levels compared to SVNM. The 600x dilution significantly reduced cytokine levels in most cases. On the other hand, the 120x dilution often did not provide protection against the cytokine level increase induced by DTH. According to our findings, Szigetvár Thermal Water and the isolate alone did not increase the gene expression of the studied markers, suggesting that the exposure itself does not cause oxidative stress.

MicroRNAs (miRNAs) are short, non-coding RNA sequences that influence numerous physiological and pathological processes. They act as post-transcriptional regulators of gene expression, binding to mature mRNAs in a sequence-specific manner and interacting with them. miR-21 is a critical regulator of ROS homeostasis and responses to non-physiological oxidative stress. Our findings regarding miR-21 expression are consistent with results found in the literature. miR-21 expression increased following DTH exposure

compared to untreated cells. Neither SVNM nor the isolates increased miR-21 expression compared to untreated cells. SVNM seemingly increased miR-21 levels compared to the 4-hour DTH exposures, but this was only significant at 0.1 μg/ml DTH exposure. SVNM and isolate exposures reduced TNF-α mRNA expression, but this was not directly reflected in changes in miR-21 expression. Based on our results, the increase in miR-21 expression is not significantly influenced by either Szigetvár thermal water or isolate exposure. However, the DTH-induced miR-21 expression increase was not significantly affected by SVNM and isolate exposure, except for a significant miR-21 expression increase observed when SVNM was combined with 0.1 μg/ml DTH exposure. Our results do not sufficiently support our seventh hypothesis.

5 New Scientific Results

Using a method based on the addition of powdered cell culture medium, we created a culture medium that contains 90% Szigetvár thermal water and is non-toxic to HaCaT cells. The culture medium supplemented with the organic matter isolate from Szigetvár thermal water, at dilutions of 120x, 600x, and 3000x, was also non-toxic to HaCaT cells.

The cell culture medium with 90% Szigetvár thermal water did not significantly increase malondialdehyde (MDA) concentration in HaCaT cells during a 3-hour exposure period. This indicates that the thermal water alone does not induce oxidative stress. None of the tested organic matter isolate dilutions (120x, 600x, and 3000x) caused a significant increase in MDA concentration during the 3-hour exposure period compared to untreated cells. This demonstrates that the isolate alone does not induce oxidative stress in HaCaT cells.

After a 1-hour dithranol exposure, we performed a 3-hour exposure with either Szigetvár thermal water or the organic matter isolate from Szigetvár thermal water. In the groups where dithranol exposure was followed by Szigetvár thermal water or isolate exposure, significantly lower MDA concentrations were measured in several experimental setups compared to control groups. This suggests a protective effect against oxidative stress.

Neither the 90% Szigetvár thermal water medium nor its organic matter isolate dilutions significantly increased the relative mRNA expression of the inflammatory cytokines (TNF-α, IL-6, IL-8) and the chemokine (GM-CSF) in any experimental condition. This supports the hypothesis that the exposure media alone do not induce oxidative stress.

Exposure to dithranol at three concentrations (0.1, 0.25, and 0.5 µg/ml) increased the mRNA expression of the oxidative stress-related cytokines (TNF-α, IL-6, IL-8) and chemokine (GM-CSF). Following 1-hour dithranol exposure, a 3-hour exposure to Szigetvár thermal water or its isolate dilutions resulted in significantly lower mRNA expression levels of the examined cytokines and chemokine in several experimental setups compared to cells exposed only to dithranol. This suggests a protective effect against oxidative stress.

6 Future Objectives

In the current series of experiments, I examined the short-term effects of DTH exposure in relation to Szigetvár medicinal water and its isolate. Our long-term plans include investigating the effects of DTH exposure over a 24-hour period. After a 6-hour exposure, apoptotic processes begin in the cells, which can already be observed through morphological changes. In this context, cell survival studies may provide new insights; improved survival compared to DTH exposure alone could indicate the protective effect of the medicinal water.

In the literature, the expression of several apoptosis-related regulators (e.g., BCL-2, BCL-XL) has been studied in relation to DTH exposure. It would be worthwhile to examine the changes in these markers under the influence of medicinal water exposure, as inhibition of apoptotic processes could indicate a protective effect. Additionally, the process of apoptosis can be monitored by examining DNA fragmentation, for which the comet assay method is suitable. Any significantly lower levels of DNA fragmentation could also indicate a protective effect against DTH-induced damage.

It would also be valuable to study DTH exposure and medicinal water exposure initiated simultaneously or with pre-treatment using medicinal water. The organic compounds in the medicinal water may accumulate in the cells and exert a direct protective effect against subsequent DTH exposure. To substantiate the hypothesized antioxidant effect, it is worth conducting MDA tests and other assays that directly demonstrate antioxidant effects under the aforementioned conditions.

Furthermore, to better support the organic matter hypothesis and investigate the separation of effects between inorganic and organic components, we consider it important to prepare and test a preparation that is stripped of its organic content but retains the salts in their original form. This preparation would be tested alongside the original medicinal water and the organic matter isolate.

7 List of publications

Publications related to this PhD Thesis:

Szabó, I., Varga, Cs. (2020). Finding possible pharmacological effects of identified organic compounds in medicinal waters (BTEX and phenolic compounds). *International Journal of Biometeorology*, 64(6), 989-995. Q2, IF: 2,68 (2019)

Szabó, I., Szenczi, Á., Zand, A., Varjas, T., Varga, Cs. (2024) The effect of Szigetvár medicinal water on HaCaT cells exposed to dithranol. *Life (Basel)* 17,14(10), 1318. Q1, IF: 3,2 (2024)

Publications not related to this PhD thesis:

Gerencsér, G., **Szabó, I.,** Szendi, K., Hanzel, A., Raposa, B., Gyöngyi, Z., & Varga, C. (2019). Effects of medicinal waters on the UV-sensitivity of human keratinocytes - a comparative pilot study. *International Journal of Biometeorology*, 63(10), 1417-1423.

Varga, Cs., Szendi, K., **Szabó, I.,** Gerencsér, G., & Németh, B. (2024). "Gyógyhatású víz" – Egy (két) elhibázott rendeletről. *Orvosi Hetilap*, 165(33), 1303-1304.

Lectures:

Szabó, I., Varga Cs. (2013). Hazai gyógyvizeink szervesanyagainak hatástani áttekintése tömegspektrometriával nyert adatok alapján. Magyar Balneológia Egyesület 2013. Évi Nagygyűlése Mezőkövesd (2013. november 15-17.).

Szabó, I., Varga, Cs. (2019) Gyógyvizek szervesanyag-tartalmának lehetséges farmakológiai hatásai: BTEX és fenolos vegyületek. Magyar Balneológia Egyesület 2019. Évi Nagygyűlése Egerszalók (2019. november 15-17.).

Szabó, I., Varga, Cs. (2021) Gyógyvizek szervesanyag-tartalmának lehetséges farmakológiai hatásai 2. Magyar Balneológia Egyesület 2021. Évi Nagygyűlése Harkány (2021. november 12-14.)