

Mineral Waters and Human Fibroblasts: An In Vitro Study

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ANEXA I - DESCRIERE SERVICII CERCETARE

1. PRESTATOR: S.C. BIOSAFETY S.R.L.
2. Denumire servicii: **Pachet servicii de cercetare privind efectele apei CARPATINA asupra stării de sănătate: valorificarea potențialului profilactic al apei CARPATINA în menținerea sănătății consumatorilor prin obținerea unor date experimentale la nivel celular (*in vitro*).**
3. Obiectivul serviciilor: prestarea unor servicii de analiză și cercetare a calităților fiziologice ale APEI CARPATINA în vederea promovării și utilizării profilactice a produsului.
4. Descrierea serviciilor:

NR.	ACTIVITATI	TIMP	DETERMINARI/ ANALIZE	REZULTATE
1.	Meta-analiza datelor existente in literatura de specialitate si in arhive	1 luni	Raport științific sintetic privind proprietățile fizico-chimice ale apei CARPATINA și analiza datelor științifice existente.	Raport – caracterizarea generala a apei in baza datelor furnizate de BENEFICIAR și a celor din literatura de specialitate cu privire la ape similare
3.	Cercetare bio-experimentală fundamentală a potențialului terapeutic al apei CARPATINA pe culturi de celule primare, <i>in vitro</i>	6 luni	Studiu celular și molecular pentru identificarea acțiunilor și a țintelor moleculare a constituenților apei minerale CARPATINA asupra celulelor cultivate <i>in vitro</i> și corelarea datelor experimentale din punct de vedere funcțional: studiu pe fibroblaste cultivate in laborator	1. Raport științific privind efectele apei CARPATINA asupra fibroblastelor, <i>in vitro</i> – descriere a mecanismelor moleculare responsabile pentru declanșarea efectelor profilactice ale apei.



● Introduction

The central question of this work is whether a natural mineral water, considered not only as a hydration product but as a complex ionic matrix, can influence cellular processes relevant to tissue homeostasis, oxidative balance, inflammation, and extracellular matrix remodeling.

The study focuses on human fibroblasts, because these cells represent one of the most relevant stromal models for connective tissue biology. Fibroblasts are not passive structural cells; they are active regulators of extracellular matrix synthesis, wound healing, tissue repair, local inflammatory signaling, and adaptive responses to oxidative stress.

The mineral water evaluated in this work is CARPATINA Light, originating from the ISLGC Tosorog source, located in the volcanic region of the Eastern Carpathians. Its hydrochemical profile is characterized by a bicarbonate, calcium, and magnesium composition, making it biologically interesting from the perspective of cellular homeostasis and preventive medicine.

The overall objective of this presentation is to show how a traditional natural therapeutic factor can be translated into a modern experimental framework, using controlled cell culture conditions and measurable biological endpoints.

● Scientific Background: Why Study Mineral Waters at the Cellular Level?

Mineral waters have traditionally been discussed in the context of balneology, spa medicine, hydration, digestive physiology, and mineral supplementation. However, modern biomedical research increasingly requires that such natural therapeutic resources be investigated through reproducible experimental models.

A mineral water is not simply water with dissolved salts. It is a natural physicochemical system, defined by its geological origin, ionic composition, pH, conductivity, dissolved gases, trace elements, and microbiological purity. These parameters may influence biological systems through several mechanisms: ionic signaling, acid-base buffering, modulation of oxidative stress, membrane-associated processes, and cell-matrix interactions.

● Scientific Background: Why Study Mineral Waters at the Cellular Level?

From a cellular biology perspective, this is particularly relevant because the extracellular environment is one of the most important regulators of cell behavior. Cells continuously sense changes in ion concentration, osmolarity, pH, matrix stiffness, oxidative state, and inflammatory mediators. Therefore, if a mineral water has a distinctive and stable composition, it is reasonable to ask whether that composition can generate measurable biological effects in vitro.

This study approaches mineral water as a bioactive extracellular modifier, not in the pharmacological sense of a single active molecule, but as a complex matrix that may modulate cell function through combined mineral and physicochemical effects.

Natural mineral waters: chemical characteristics and health effects

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Summary

Water contributes significantly to health and a daily intake of 1.5 to 2 liters of water should be guaranteed, because a good hydration is essential to maintain the body water equilibrium, although needs may vary among people. However, worldwide population is far from the Recommended Allowance for water intake.

Among the waters for human uses, there are 'waters (treated or not), intended for drinking, used for the food and beverages preparation or for other domestic purposes' and *natural mineral waters*, that are 'originated from an aquifer or underground reservoir, spring from one or more natural or bore sources and have specific hygienic features and, eventually, healthy properties'. According to the European Legislation (2009/54/EC Directive), physical and chemical characterization is used to make a classification of the different mineral waters, basing on the analysis of main parameters. Mineral composition enables to classify natural mineral waters as bicarbonate mineral waters, sulphate mineral waters, chloride mineral waters, calcic mineral waters, magnesiatic mineral waters, fluorurate mineral waters, ferrous mineral waters and sodium-rich mineral waters.

Although the concerns about bottled mineral waters (due to plasticizers and endocrine disruptors), many are the health effects of natural mineral waters and several studies explored their properties and their role in different physiological and pathological conditions.

KEYWORDS: natural mineral waters; calcium; bicarbonate; magnesium; sulphate; bottled water; endocrine disruptors.

Introduction

Water covers most of the earth surface and composes most of the human body; this can illustrate the critical linkages between water, health and ecosystems (1).

From outer space, the earth looks like a "blue" planet because most of its surface is covered by water. Only 2.5% of that water is fresh, and most of that lies frozen and inaccessible in the ice-caps and Greenland, leaving less than 1% of fresh water accessible in lakes, river channels and underground. Only about one third of the world's potential fresh water can be used for human needs. As pollution increases, the amount of usable water decreases.

Water contributes significantly to health and good health is the essence of development. However water's protective role is largely unseen and taken for granted in the wealthier countries. Its contribution to health is directly within households through food and nutrition, and indirectly as a means of maintaining a healthy, diverse environment. These two precious resources — water and health — together could enhance prospects for development (2). In humans, about 60% of body weight (BW) is represented by total body water (TBW), distributed into intracellular and extracellular fluid compartments, which contain about 65 and 35% of total body water, respectively (3, 4). Water is involved in many body functions, since it serves as a carrier of nutrients and substances in the circulatory system. Furthermore, it is the vehicle to excrete products and eliminate waste and toxins and it also lubricates and provide structural supports to tissues and joints. However, there is no efficient mechanism of body's water storage; therefore, a constant supply of fluids is needed to keep water content (4).

The water needs can be partially satisfied by an overall diet and water contained in food (5): fruit, vegetables and milk are composed for the 85% by water. Meat, fish, eggs and fresh cheese contain the 50–80% of water; and cooked pasta and rice about 60–65%. Whereas, bread and pizza contain water for 20–40% and bakery products (like biscuits, rusks and breadsticks) are composed by water only for less than 10% (6). Nevertheless, a daily intake of 1.5 to 2 liters of water should be guaranteed, because a good hydration is essential to maintain the body water equilibrium, although needs may vary among people because of age, physical activity, personal circumstances and weather conditions (7). In Table 1 are reported the Recommended Daily Allowances for water in the different age ranges and physiological conditions in Italy (8).

The choice of the water to drink can be variable and it depends on the personal necessity. By European Community legislation (Directive number 98/38), among the waters for human uses, there are *waters (treated or not), intended for drinking, used for the food and beverages preparation or for other domestic purposes* (in Italy, D. Leg. N. 31 of 02/02/2001). Drinking water has to be "clear, odorless, tasteless, colorless and harmless, that is devoid of pathogenic microorganisms and harmful chemicals to humans" and safety on the basis of microbiological, physics and chemicals parameters control (in Italy, D.M. n. 84 of 26 March 1991).

Article

Investigation on the Effects and Mechanisms of Alkaline Natural Mineral Water and Distilled Water on Ethanol-Induced Gastric Ulcers In Vivo and In Vitro

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Abstract: Studies have proven that alkaline water has a protective effect on gastric diseases. However, the underlying mechanism is not clear. Moreover, in some countries, especially in China, purified water (distilled water) is also an important form of drinking water, while its protective effect on gastric diseases is still unknown. This study aimed to compare the effects of distilled water (pH = 5.6 ± 0.3) and alkaline natural mineral water (pH = 9.3 ± 0.6) on ethanol-induced gastric ulcers in mice and to further clarify the underlying mechanisms. Pepsin activity, prostaglandin E-2 (PGE2) and heat shock protein 70 (HSP70), superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA), as well as the oxidative stress pathway related proteins such as nuclear factor erythroid-2 related factor 2 (Nrf2), heme oxygenase-1 (HO-1), and NADH quinone oxidoreductase 1 (NQO1) were measured. After alkaline natural mineral water treatment, the levels of PGE2 and HSP70 were significantly increased ($p < 0.05$). Antioxidant indexes (SOD, GSH, and MDA) and Western blot results (Nrf2, HO-1, and NQO1) showed that alkaline natural mineral water did not alleviate gastric ulcers by improving oxidative stress. Pepsin activity assay displayed that the pepsin activity was significantly declined after alkaline natural mineral water treatment compared with the distilled water treatment ($p < 0.05$). This study indicated that alkaline natural mineral water may alleviate the ethanol-induced gastric ulcers in mice by inhibiting the pepsin activity and increasing the levels of PGE2 and HSP70.

Keywords: gastric ulcers; oxidative stress; alkaline natural mineral water; distilled water; pepsin activity



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1. Introduction

Gastrointestinal diseases have become a global health concern, among which peptic ulcer is the most common multi-factorial gastrointestinal disease; about 10% of people worldwide suffer from peptic ulcer disease each year [1]. Gastric ulcer, a type of precancerous condition, is the most common peptic ulcer disease, which is mainly due to the imbalance of protective factors and aggressive factors in gastric mucosa [2]. There are many adverse factors (e.g., alcohol abuse, poor diet, drugs, and *Helicobacter pylori* infection), which can cause gastric ulcers [2]. Among these factors, ethanol is the most common cause of gastric ulcer disease, and alcoholics tend to be more susceptible to gastric ulcers than non-alcoholics [3]. Firstly, ethanol can directly destroy the structure of gastric mucosa to cause gastric ulcers. In addition, the formation of gastric ulcers is also the result of the digestion of gastric wall tissue by pepsin; this self-digestion process is the important cause of ulcers [4]. When gastric mucosa is damaged by ethanol, gastric acid secretion increases. Where a mass of pepsinogens is activated to pepsin, this further aggravates gastric ulcers [5]. Xie et al. reported that ethanol may also trigger gastric ulcers by increasing the levels of oxidative stress-related proteins [6]. The levels of the protective factors prostaglandin E-2 (PGE2) and heat shock protein 70 (HSP70) are imbalanced in gastric ulcers induced by ethanol. PGE2 is the most important repair factor regarding inflammation and injury in the

Why Human Fibroblasts?

Fibroblasts are essential cells of connective tissues. They synthesize and remodel the extracellular matrix, including collagen, elastin, fibronectin, proteoglycans, and other structural and regulatory molecules. They are also involved in wound healing, scar formation, tissue regeneration, and chronic inflammatory processes.

Importantly, fibroblasts are highly sensitive to changes in the extracellular environment. Their phenotype can change in response to oxidative stress, cytokines, altered pH, mechanical signals, calcium-dependent signaling, magnesium availability, and metabolic stress.

This makes fibroblasts particularly suitable for studying the biological effects of mineral waters. If the mineral water affects viability, proliferation, redox balance, or collagen production, fibroblasts are likely to provide a sensitive and biologically meaningful readout.

The model is also translationally relevant. Fibroblast dysfunction contributes to aging, chronic inflammation, impaired wound healing, fibrosis, degenerative connective tissue disease, and altered tissue repair. Therefore, identifying natural factors that may support fibroblast homeostasis is relevant for preventive, regenerative, and rehabilitation medicine.

Water Characterization: CARPATINA Light

Before discussing the cell culture experiment, it is essential to define the mineral water itself.

The analyzed water, CARPATINA Light, has a hydrochemical profile typical of a bicarbonate calcium-magnesium mineral water with moderate to relatively high mineralization.

The main analytical parameters reported were:

Calcium: approximately 199.9 mg/L

Magnesium: approximately 90.2 mg/L

Bicarbonate: approximately 1098 mg/L

Free carbon dioxide: approximately 1.01 g/L in the analyzed period

Conductivity: approximately 1561 $\mu\text{S}/\text{cm}$ at 20°C

This composition is biologically relevant for several reasons.

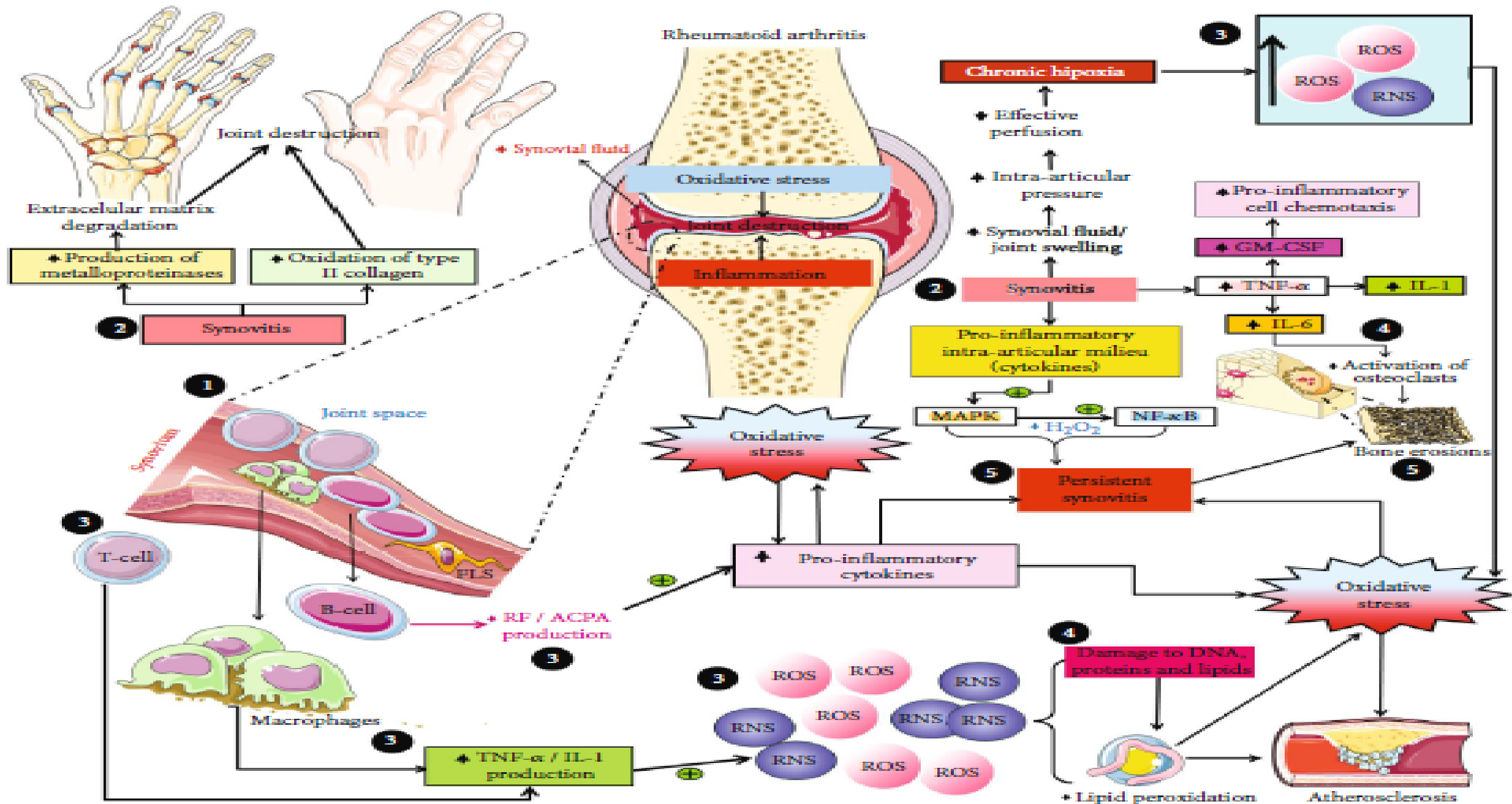
Water Characterization: CARPATINA Light

Calcium is a major extracellular signaling ion and a structural mineral involved in bone and connective tissue physiology. Magnesium is a cofactor for numerous enzymatic reactions, including ATP-dependent processes, redox regulation, and membrane stability. Bicarbonate is a physiological buffer and may influence acid-base balance, extracellular pH, and indirectly oxidative and inflammatory processes.

Another important point is the chemical safety profile. The report indicates low nitrate values, nitrites below quantification limits, and very low iron values. Low iron is relevant because excess free iron can participate in pro-oxidant chemistry through redox cycling. Microbiological analyses also indicated absence of major contamination indicators, including *E. coli*, coliform bacteria, enterococci, and *Pseudomonas aeruginosa*.

Thus, the tested water is not only mineral-rich, but also chemically and microbiologically appropriate for controlled biological evaluation.

2019: Lucas José Sá da Fonseca ¹, Valéria Nunes-Souza,^{1,2,3,4} Marília Oliveira Fonseca Goulart ⁵,
and Luiza Antas Rabelo ^{1,2,3,4}



Study Hypothesis and Objectives

The first hypothesis was that, at moderate dilutions, the mineral water would maintain or slightly improve fibroblast viability and metabolic activity, without inducing cytotoxicity.

The second hypothesis was that the water could modulate redox and inflammatory pathways, potentially decreasing reactive oxygen species and improving antioxidant balance.

The third hypothesis was that the effects would be dose-dependent and possibly hormetic. In other words, low or intermediate concentrations could be beneficial, whereas very high concentrations might lose this benefit or produce mild inhibitory effects because of excessive perturbation of the culture medium.

The specific objectives were:

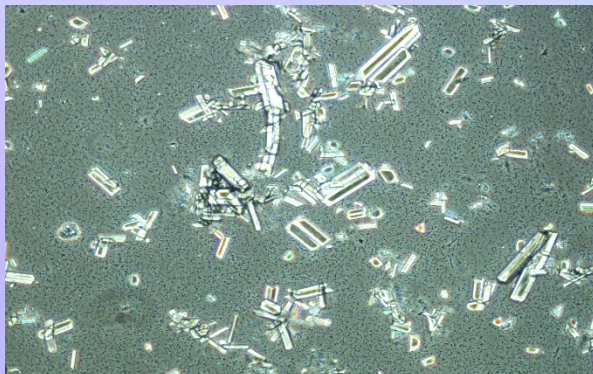
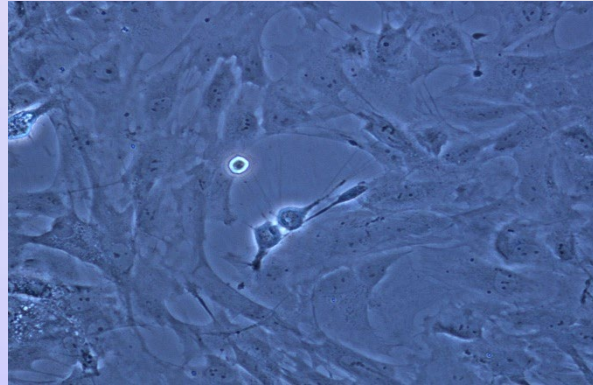
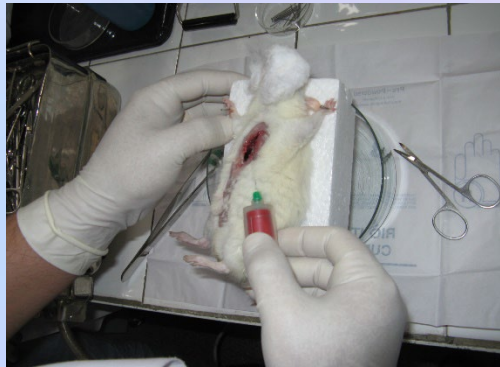
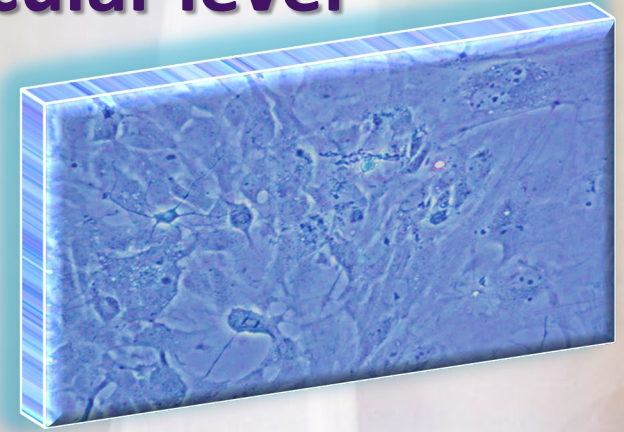
First, to evaluate fibroblast viability after exposure to mineral water dilutions.

Second, to assess oxidative stress using reactive oxygen species as a functional readout.

Third, to establish an experimental framework for future molecular analyses, including inflammatory markers such as IL-6 and TNF-alpha, redox regulators such as NRF2 and SIRT1, and extracellular matrix-associated genes such as COL1A1 and TGF-beta.



Advanced studies at the cellular and molecular level

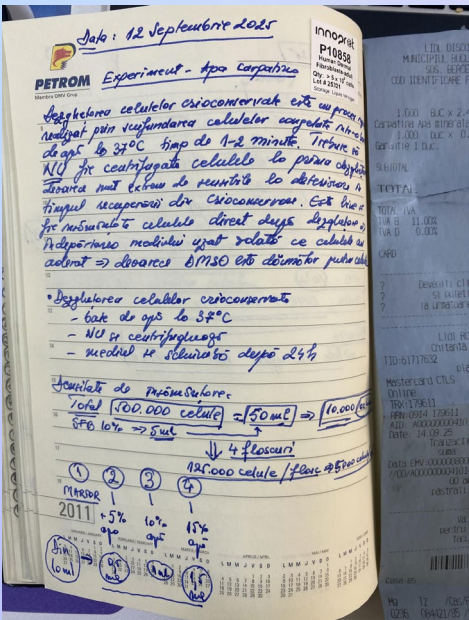


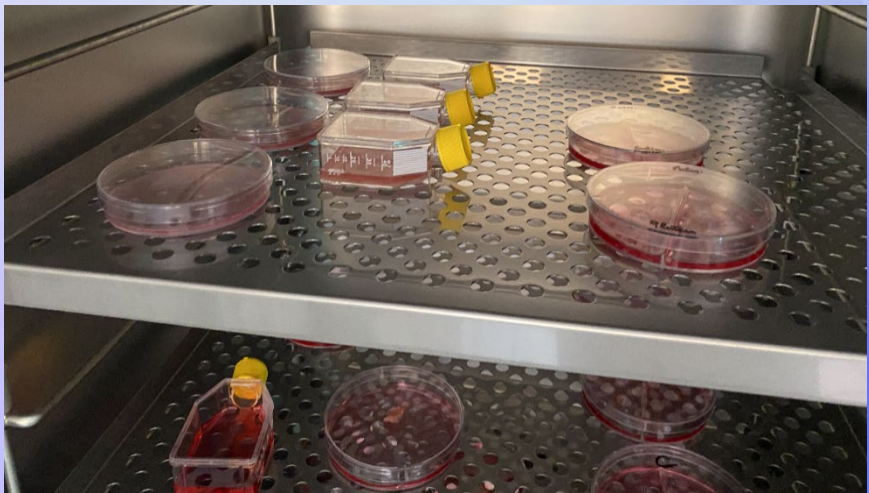
Cellular and molecular studies allow the evaluation of changes in cellular morphology, protein synthesis, secretion of certain substances, modification of cellular metabolism, interaction of cellular receptors with various chemical signals from the environment, cell division and other physiological processes under the direct influence of natural therapeutic factors introduced into the cell culture medium.



The experimental model consisted of human fibroblasts cultured under standard laboratory conditions. Cells were maintained at 37°C, in a humidified atmosphere containing 5% CO₂, using a standard complete culture medium, such as DMEM supplemented with fetal bovine serum and antibiotics. A key methodological point concerns dilution. Mineral water cannot simply replace cell culture medium without consequences, because high replacement volumes may modify nutrient availability, pH, osmolarity, buffering capacity, and serum concentration. Therefore, the biologically meaningful approach is to test controlled dilutions and interpret the results as the combined effect of mineral exposure and medium modification.

The report includes both a mechanistic working range, such as 5%, 10%, and 15% v/v, and broader exploratory dose-response curves extending up to higher concentrations. This broader screening is useful for identifying the shape of the response curve, whereas lower and intermediate dilutions are more appropriate for mechanistic interpretation. The main exposure times were 24, 48, and 72 hours, depending on the endpoint. Viability and proliferation can be monitored over time, whereas redox assays are often more informative at earlier time points.





Wistar rat pups (aged 5-9 days) and the related research infrastructure used experimentally (study setup)

IN VITRO EXPERIMENTAL DATA REGARDING HEALTH EFFECTS OF TECHIRGIOL MUD EXTRACTS ON FIBROBLAST CELL CULTURES

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Background and aims. Pelotherapy or mud therapy is a balneological method that triggers local tissue and general functional reactions, inhibiting or activating some intermediate enzymes and metabolite systems, but its mechanisms of action on certain pathologies are still not completely understood. Natural therapeutic factors such as mud and sulfurous mineral therapeutic waters are used in sanatoriums and rehabilitation clinics as baths or topical applications on the body for many affections in the Neuro-Myo-Arthro-Kinetic (NMAK) pathology. Cellular and molecular biological investigations performed by electrophoresis, ELISA, and Western blotting on primary fibroblasts cultures obtained from Wistar rats in our work target two main physiological mechanisms, respectively the inflammatory processes and the oxidative stress balance, presumptive influenced by mud extracts. Previous scientific data say that, during the inflammatory process, different cell types are recruited, including fibroblasts, which respond to various intercellular and microenvironment signals. This leads to the regulated production of different pro- and anti-inflammatory mediators including cytokines, such as tumor necrosis factor (TNF)- α and interleukins (IL)-1 β and IL-6, chemokines, and enzymes such as cyclooxygenase (COX)-2, all of which play critical roles in controlling the inflammatory process. The concept of oxidative stress caused by free radicals represents arguments of taking into account biomarkers of oxidative stress. The oxidative and reductive activity of enzymes that act on glutathione, thioredoxin, and other substrates of interest in the oxidation-reduction process reflects not only the level of antioxidative protection but are also relevant biomarkers for rheumatic degenerative diseases. In previous studies on therapeutic mud, it was presented the fractionation of humic substances using the pH and solvent polarity variation and was spectrophotometrically characterized based on absorption in the wavelength range 340-700 nm humic acids and fulvic acids differentiated based on solubility and molecular mass. Methods. To obtain the primary culture of glial cells, Wistar rat pups were used, aged 1-4 days, taken from the Biobase of the National Institute for Research and Development in the Field of Pathology and Biomedical Sciences "Victor Babeș". Tissue explants is placed in DMEM medium on Petri dishes. Cells were grown in DMEM medium with 4500mg / l glucose, 25 mM HEPES, 100 U / ml penicillin, 50 μ g / ml neomycin, and 100 μ g / ml streptomycin. The medium was supplemented with 15% fetal calf serum. The cell culture dishes were incubated at 37°C, 5% CO₂, and 90% humidity. After 24 hours, the culture medium was changed to remove dead cells and cell debris. After the first medium change, the medium was replaced with an equal volume of fresh, pre-warmed DMEM medium every 3 days. Phase contrast microscopy allows the study of living, unfixed and uncolored cells.

Results. Phase microscopy was used to observe the morphology of isolated fibroblast cell cultures. These observations allowed the interpretation of morphological development as a consequence of time in culture. After 7 days in culture, the medium used was supplemented with mud extract. The morphology of control cell cultures versus those treated with mud extract will be presented. IL6 and TNF α ELISA tests will be performed for cell lysates and respectively prelevated cultures medium.

Conclusions. Obtained new data on the important molecular markers for inflammatory processes and oxidative stress balance will constitute a new research strategy applied for old natural remedies, bringing together mud therapy and cell cultures studies

Keywords: Fibroblasts cell cultures, mud extract, IL6, TNF α .

Chemical composition of peloids

LIQUID PHASE

Water, anions, cations

1. Oligoelements (ug/kg) with a pronounced pharmacological effect (Fe, Co, V, W and B)
2. Involved in the enzymatic processes (I, Fe, Cu, Mo, Zn, Co, Mn, Ni, Ba, Sr, Ca)
3. elements (As, Pb, Hg, V and P)
4. not yet elucidated biological role (Ti, Zr, Ir, Cs)

Gases (CO₂, H₂S, SO₂)

Biologically active substances - protein hydrolysates, aminoacids, enzymes

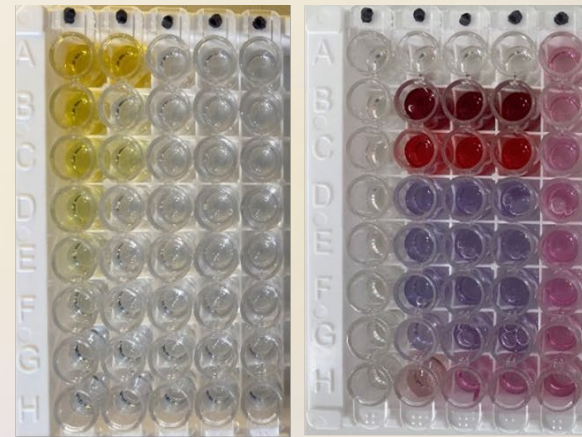
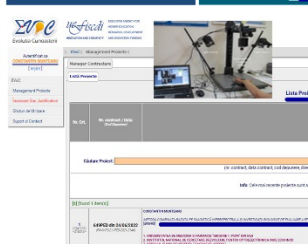
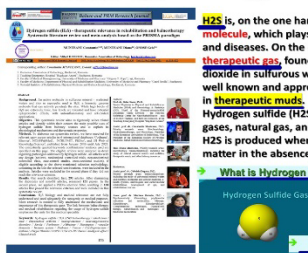
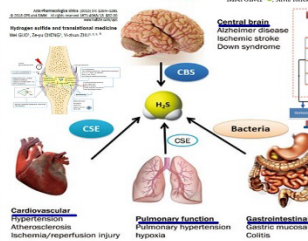
SOLID PHASE

1. Crystal framework (peloid skeleton) - determines mechanical structure Oxidizes (SiO₂, Al₂O₃, Fe₂O₃, CaO, MgO, Na₂O, K₂O, TiO₂, MnO, P₂O₅), NaClO/CaO = 1
2. Colloid complex - plastic hydrophilic basis which absorbs moisture and defines thermal properties

Inorganic component (Fe, H₂S, Fe(OH)₃, Al(OH)₃, H₂SiO₃)

Organic component - humic substances: humin, humic and fulvic acids

Systemic low-level inflammation





Diferite concentrații ale
extractului de nămol



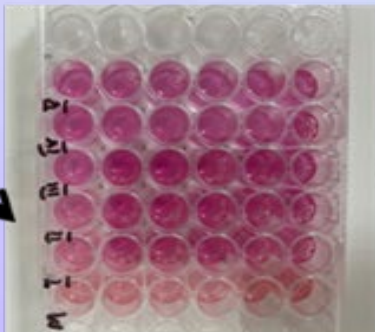
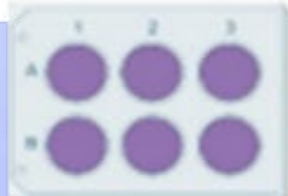
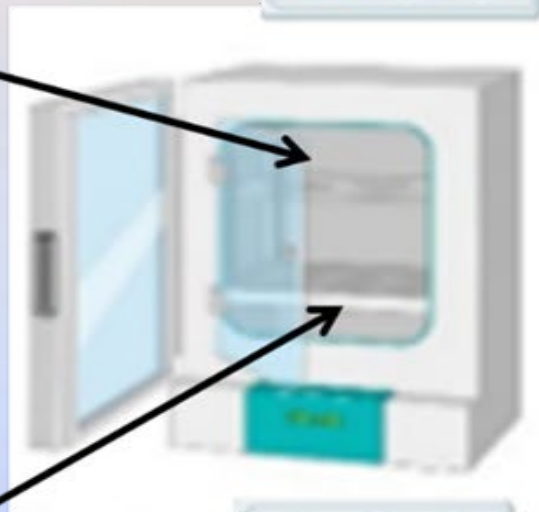
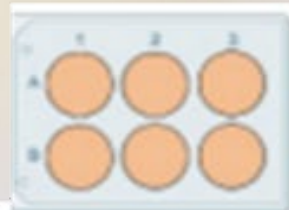
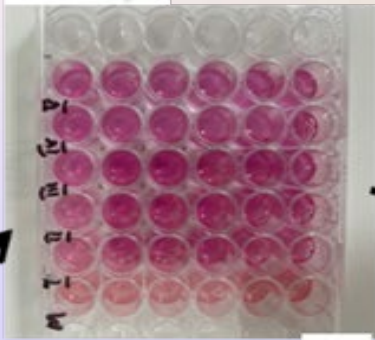
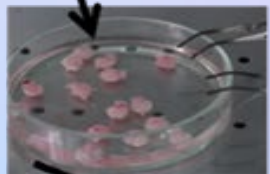
Control 10 20 30 40 50 μ l

Diferite concentrații ale
extractului de nămol



Control 100 200 300 400 500 μ l

Diferite concentrații ale apei
minerale sulfuroase

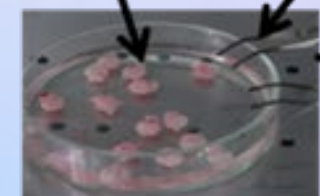


Diferite concentrații ale apei
minerale sulfuroase

Culturi primare de fibroblaste



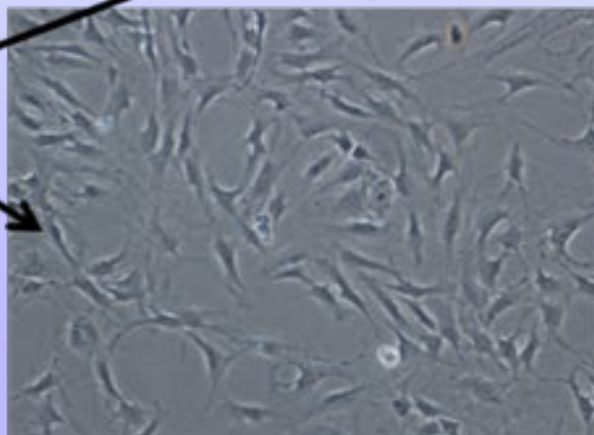
Diferite concentrații ale
extractului de nămol



Culturi primare
de fibroblaste



Microscopie optică
în contrast de faza

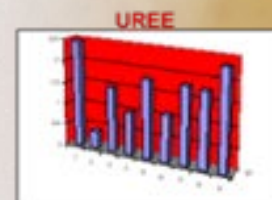
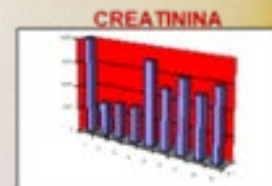
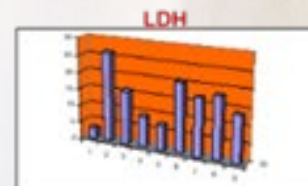


Diferite concentrații ale
apei minerale sulfuroase

Date de biochimie – analizor



Electroforeza



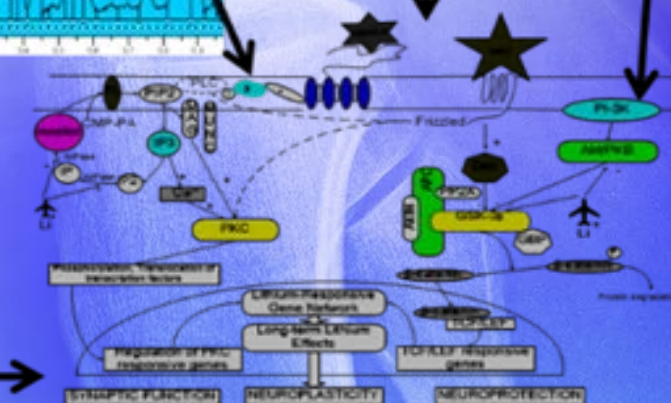
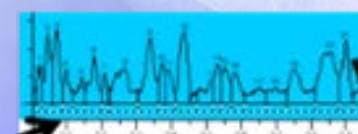
Western Blotting



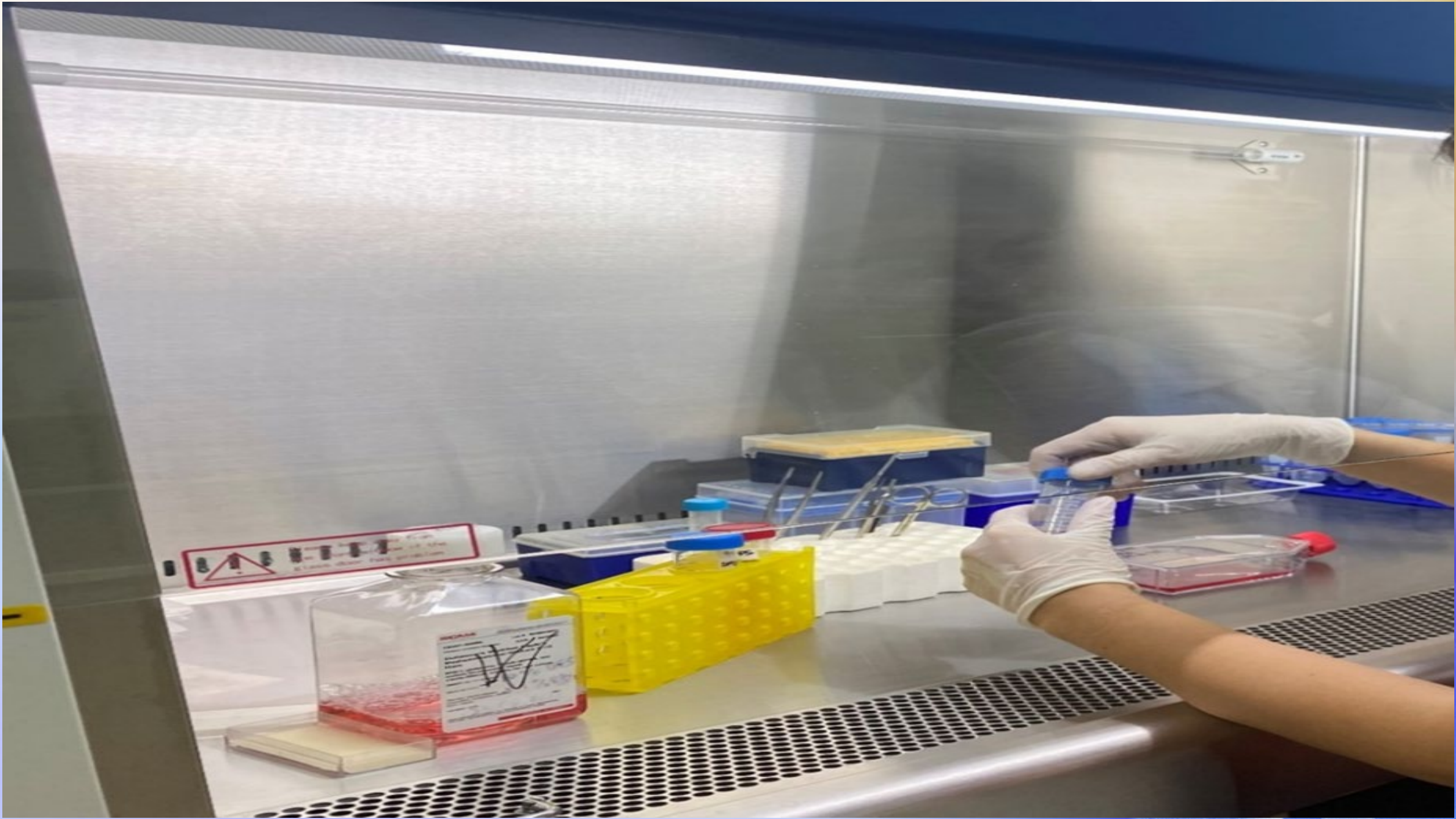
Fig. 193- Analiza imunoblot a
expresiei GSK-3β



ELISA



Mecanisme biologice



Several biological endpoints were selected.

The first endpoint was cell viability, assessed by the MTT assay. MTT is based on the capacity of metabolically active cells to reduce tetrazolium salts to formazan, which can be quantified spectrophotometrically. Although MTT is commonly interpreted as viability, it is important to remember that it also reflects mitochondrial metabolic activity.

The second endpoint was oxidative stress, assessed through reactive oxygen species measurement, using a DCFDA-type approach. Reactive oxygen species are not always harmful; at physiological levels, they are signaling molecules. However, excessive ROS generation can damage lipids, proteins, and DNA. Additional proposed analyses include ELISA measurements for inflammatory mediators, such as IL-6 and TNF-alpha, and Western blot or gene expression analyses for signaling proteins involved in stress response, survival, and matrix biology.

Viability Results

The first major result concerns fibroblast viability.

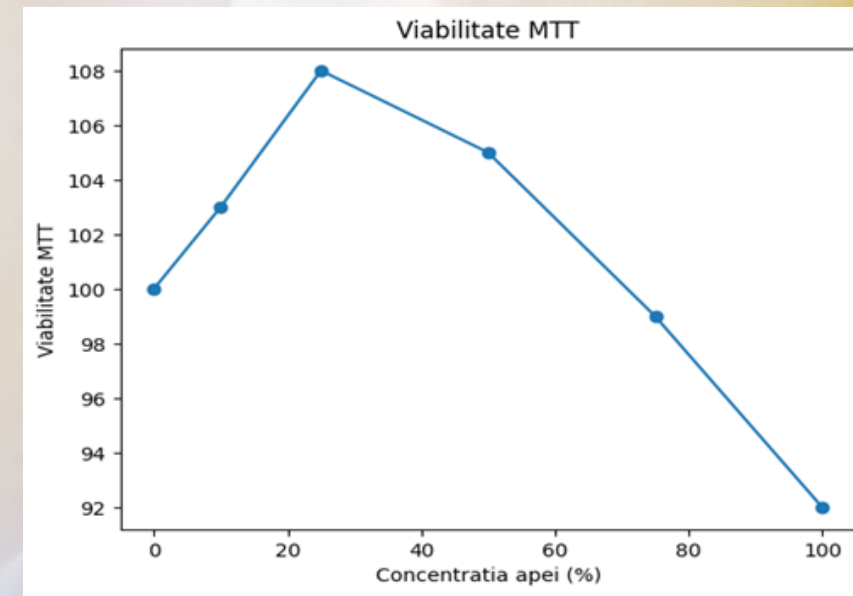
The MTT data indicate that fibroblast viability remained comparable to, or slightly above, the control in the moderate concentration range. In the exploratory dose-response curve, the maximal effect was observed around 25% mineral water, where MTT signal reached approximately 108% of control.

This suggests that moderate exposure did not produce cytotoxicity and may have slightly increased mitochondrial metabolic activity or viable cell number.

At higher concentrations, particularly between 75% and 100%, the MTT signal progressively decreased. At 100%, viability was approximately 92% of control. This does not indicate severe cytotoxicity, but it does suggest that high replacement of culture medium by mineral water may become mildly inhibitory.

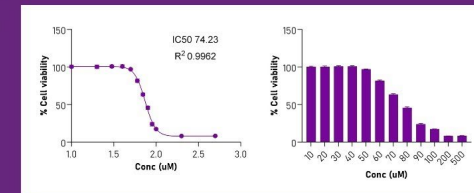
Biologically, this pattern is important. It suggests a non-linear response, consistent with the concept of hormesis. Moderate exposure may support cellular metabolism, whereas excessive exposure may disturb the optimized culture environment.

Therefore, the viability data support the first hypothesis: CARPATINA Light, at moderate dilutions, is compatible with fibroblast survival and does not induce major cytotoxic effects under the experimental conditions used.



MTT, IC50 AND % CELL VIABILITY

- IC50, % Viability
- SEM, SD, % RSD
- 95% CI
- Hill's Plot
- Histogram



Oxidative Stress Results

The next endpoint was oxidative stress, evaluated through ROS levels.

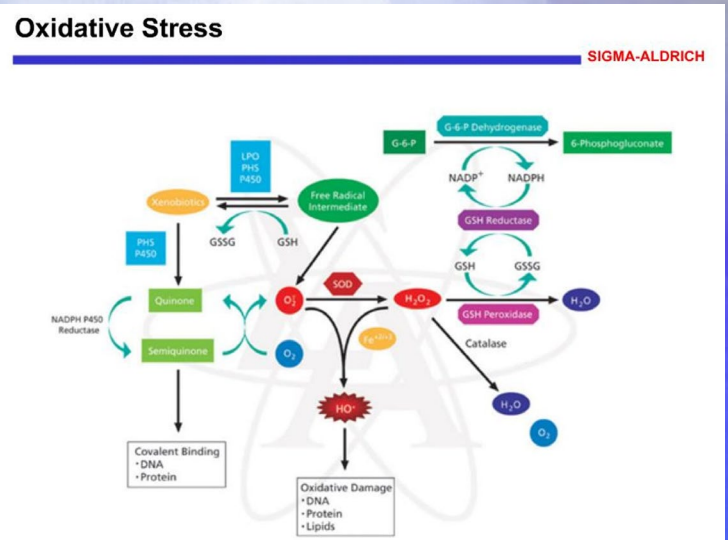
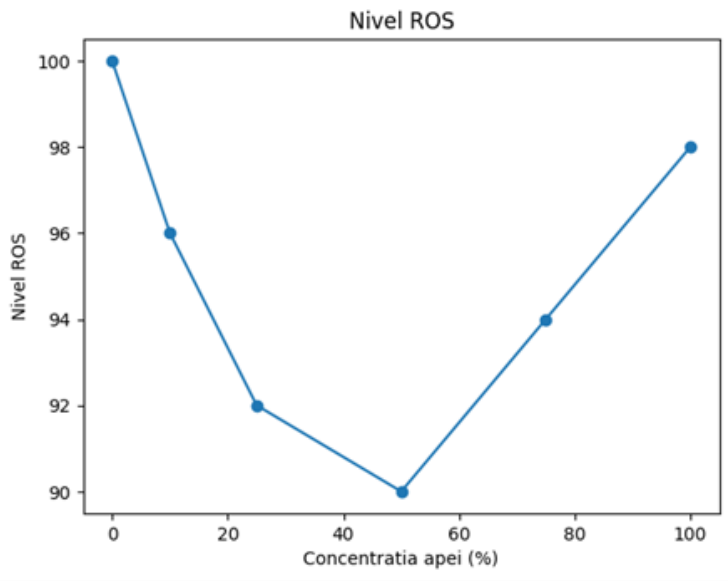
The results suggest a reduction in ROS under several mineral water exposure conditions. In the exploratory curve, ROS decreased from the control level of approximately 100% to lower values at intermediate concentrations, reaching the lowest observed value of approximately 90% around 50% mineral water.

At higher concentrations, the ROS values increased again, approaching control levels. This is again compatible with a non-linear, dose-dependent response.

The interpretation is that the mineral water may contribute to a moderate reduction in oxidative pressure, especially at intermediate concentrations. Several mechanisms could be involved.

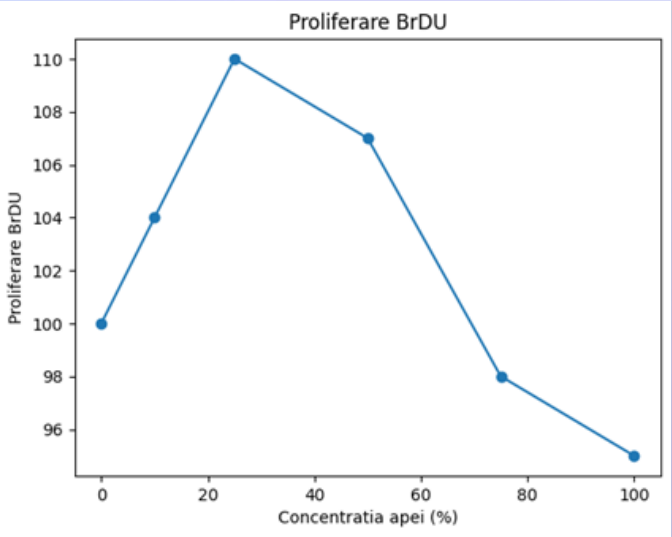
Magnesium may influence mitochondrial function, ATP-dependent processes, and redox-sensitive signaling. Bicarbonate may contribute to extracellular buffering and indirectly influence cellular oxidative balance. Calcium may participate in intracellular signaling, although excessive calcium shifts could also become stressful if not controlled.

It is important not to overinterpret this result. ROS reduction alone does not prove antioxidant therapy, and the DCFDA assay has known limitations. However, as part of an integrated dataset, the reduction in ROS is consistent with a possible cytoprotective effect.

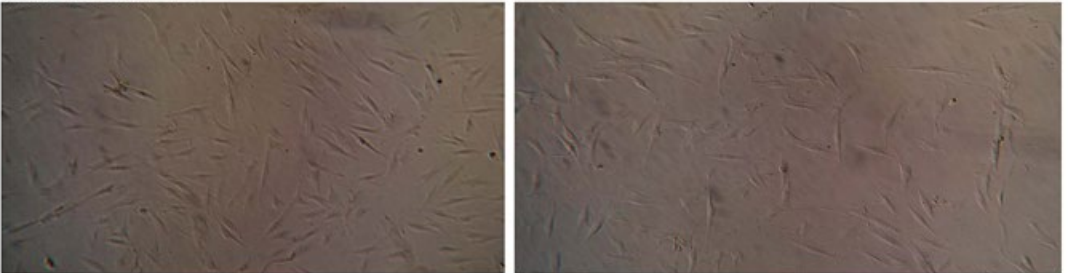


Proliferation

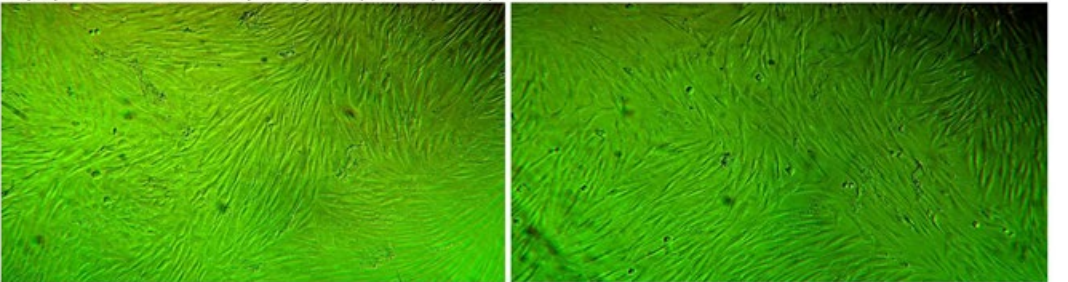
From a tissue biology perspective, moderate stimulation of fibroblast proliferation may be relevant to tissue repair and regeneration. However, this must be interpreted carefully. Increased fibroblast proliferation can be beneficial in wound healing and matrix repair, but excessive or uncontrolled fibroblast activation may contribute to fibrosis. Therefore, the ideal biological effect would not be maximal proliferation, but controlled support of fibroblast function under homeostatic or mildly stressed conditions.



Înainte de tratament

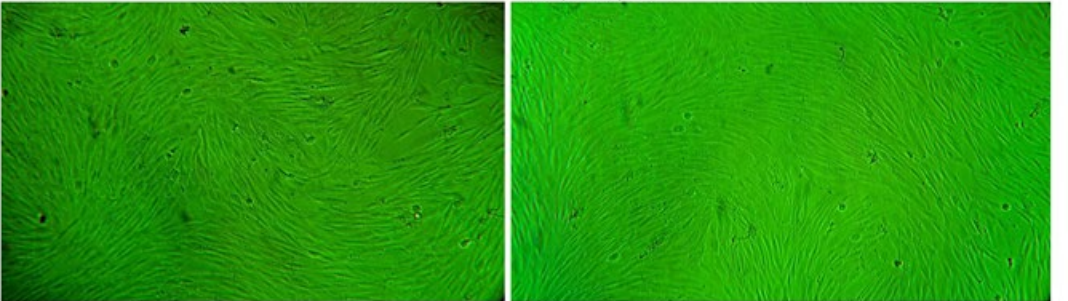


Dupa primul tratament cu apa: 5%, 10% și 15% apa Carpatina:



Martor

5% apa Carpatina



10% apa Carpatina

15% apa Carpatina

Mechanistic Interpretation

The integrated interpretation is that the effects of mineral water on fibroblasts are probably not mediated by a single component. Instead, they likely reflect the combined action of calcium, magnesium, bicarbonate, dissolved carbon dioxide, conductivity, osmotic conditions, and pH-related effects.

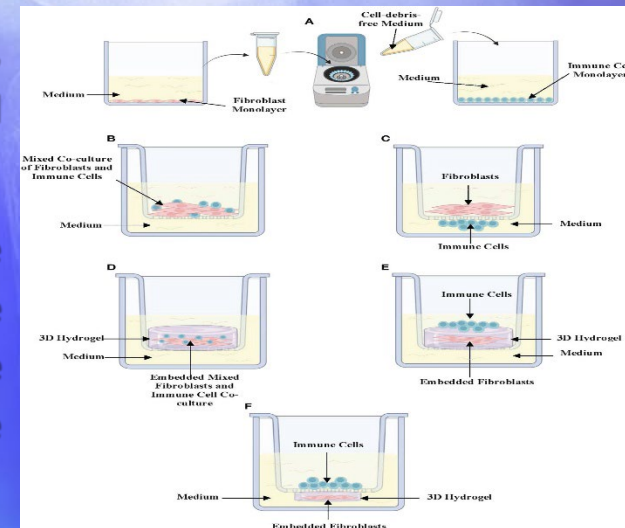
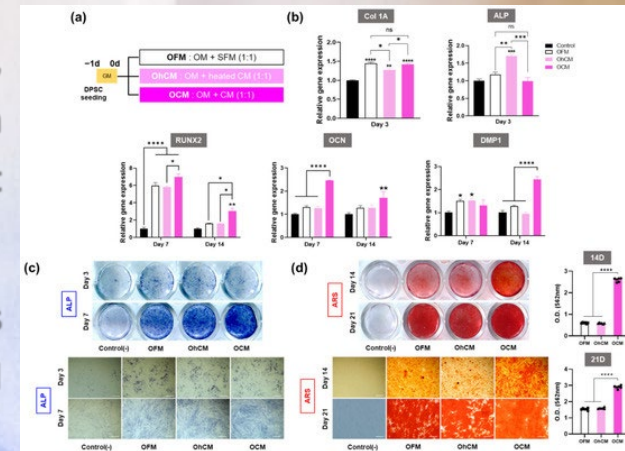
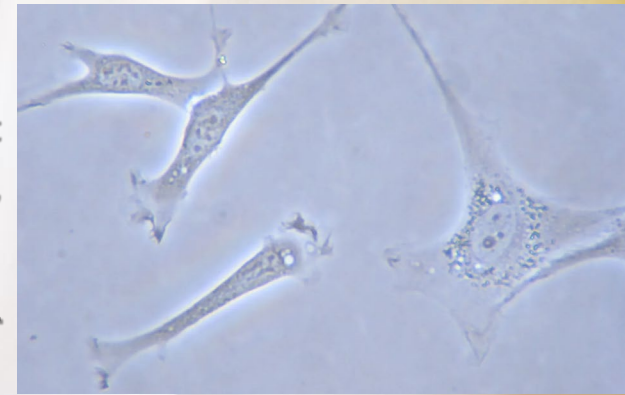
Calcium can influence intracellular signaling, adhesion, cytoskeletal organization, and extracellular matrix-related processes.

Magnesium is particularly important for ATP-dependent enzymatic reactions, mitochondrial stability, kinase signaling, membrane excitability, and inflammatory regulation. Magnesium deficiency is often associated with increased inflammatory tone and oxidative stress, so its presence in biologically relevant concentrations may contribute to cytoprotective effects.

Bicarbonate can influence acid-base balance and extracellular buffering. In cell culture, pH regulation is especially important, because fibroblast function is sensitive to both intracellular and extracellular pH shifts.

The proposed molecular framework includes modulation of redox-sensitive pathways such as NRF2, stress adaptation pathways such as SIRT1, inflammatory mediators such as IL-6 and TNF-alpha, and matrix remodeling pathways involving COL1A1 and TGF-beta.

The working model is therefore the following: moderate mineral water exposure modifies the extracellular ionic environment in a way that supports fibroblast metabolic activity, reduces oxidative pressure, improves glutathione redox balance, and modestly supports proliferative behavior. Excessive replacement of culture medium, however, may reduce these benefits because it disturbs optimal culture conditions.



Translational Significance

What is the translational significance of these findings?

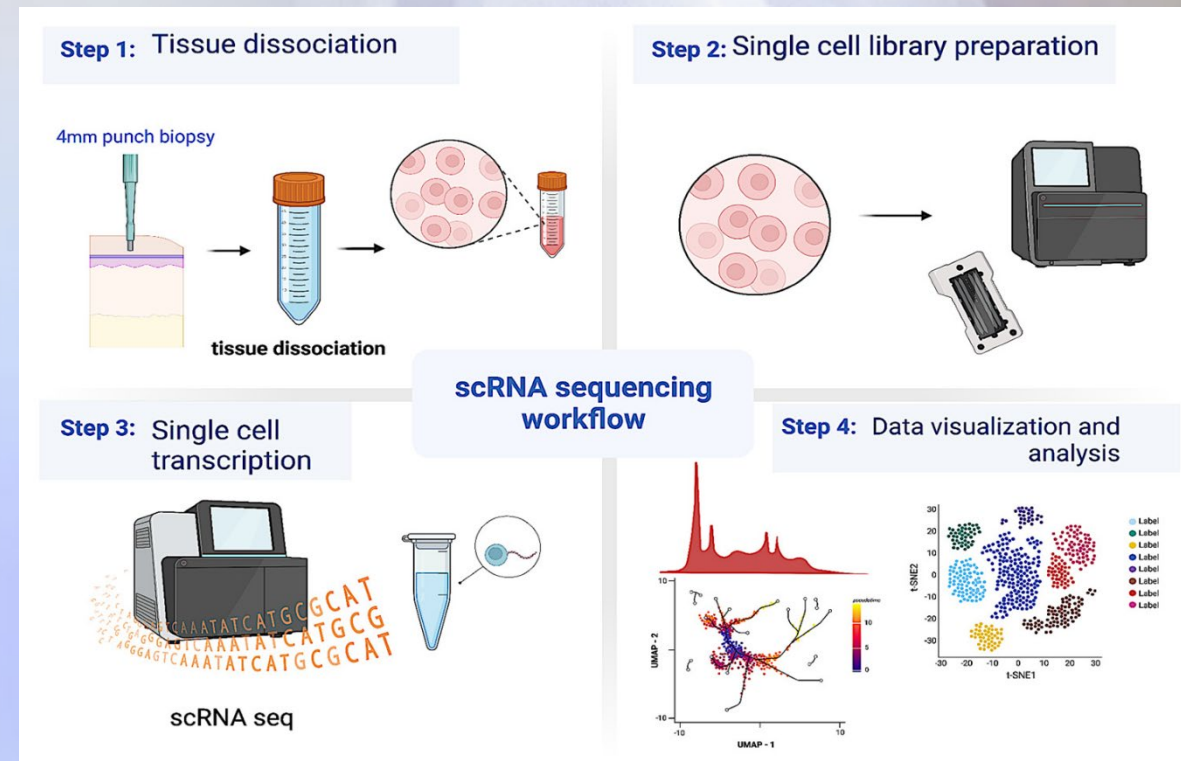
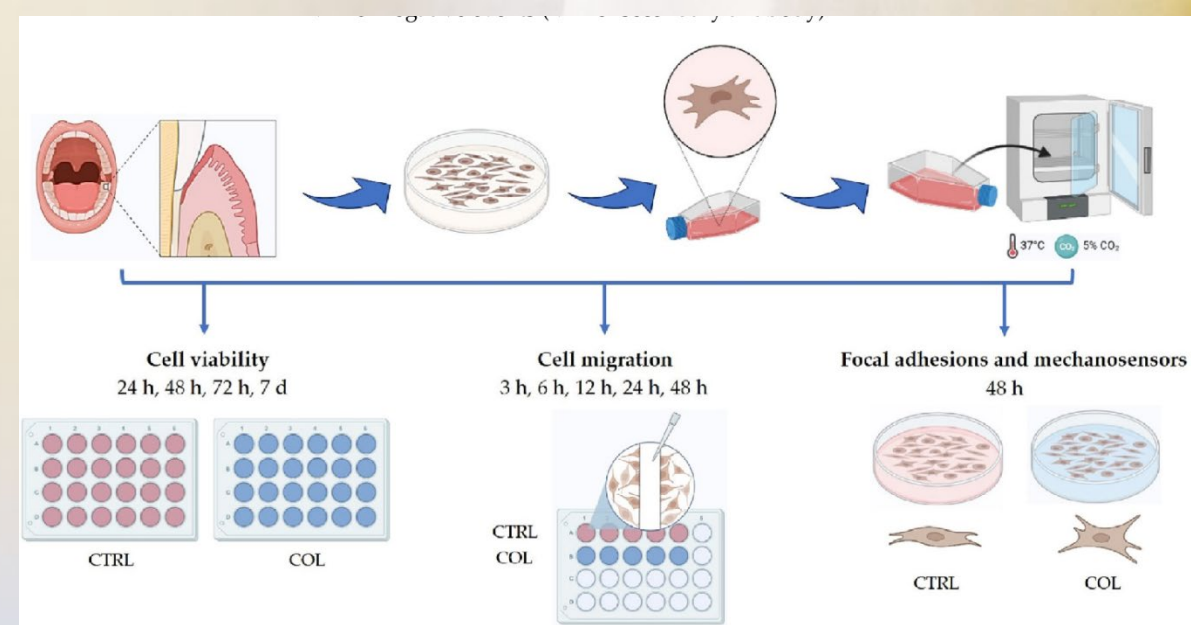
First, the study provides a modern experimental rationale for evaluating mineral waters beyond traditional descriptive balneology. Instead of relying only on historical therapeutic indications, we can test cellular mechanisms under controlled laboratory conditions.

Second, the results are compatible with the idea that certain mineral waters may support biological processes relevant to recovery medicine, tissue maintenance, and preventive health.

Third, the study links the hydrochemical profile of the water to measurable cellular outcomes. This is important because mineral waters are heterogeneous. Two waters may both be called “mineral water,” but their biological effects may differ substantially depending on calcium, magnesium, bicarbonate, sodium, iron, trace elements, pH, dissolved gases, and total mineralization.

Fourth, the findings suggest that CARPATINA Light may be positioned as a bicarbonate calcium-magnesium water with potential relevance for mineral intake, redox homeostasis, and low-grade inflammation models.

However, this must be stated cautiously. In vitro data do not establish clinical efficacy. They provide mechanistic plausibility and generate hypotheses for subsequent translational studies.



Limitations

Several limitations must be acknowledged.

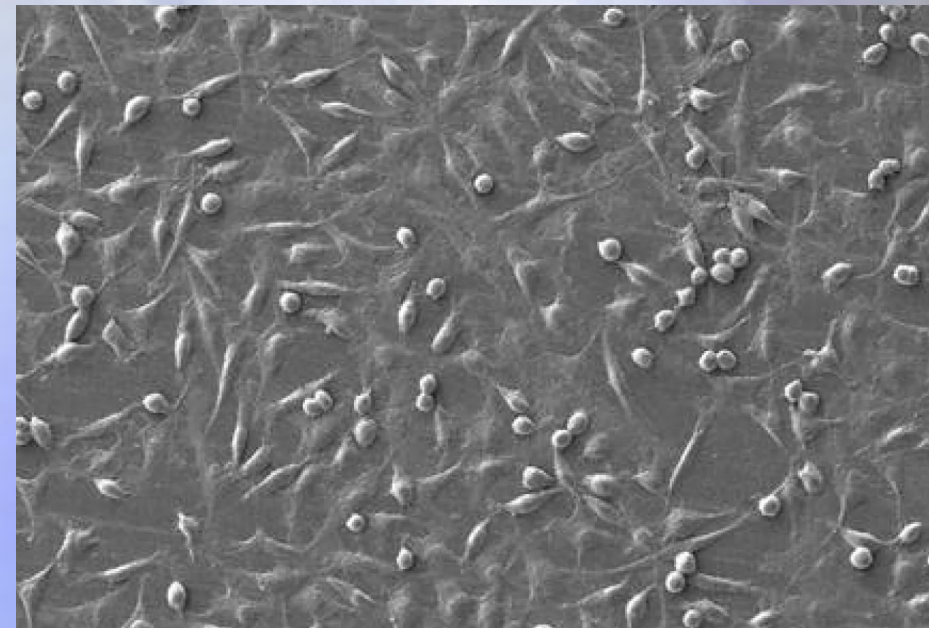
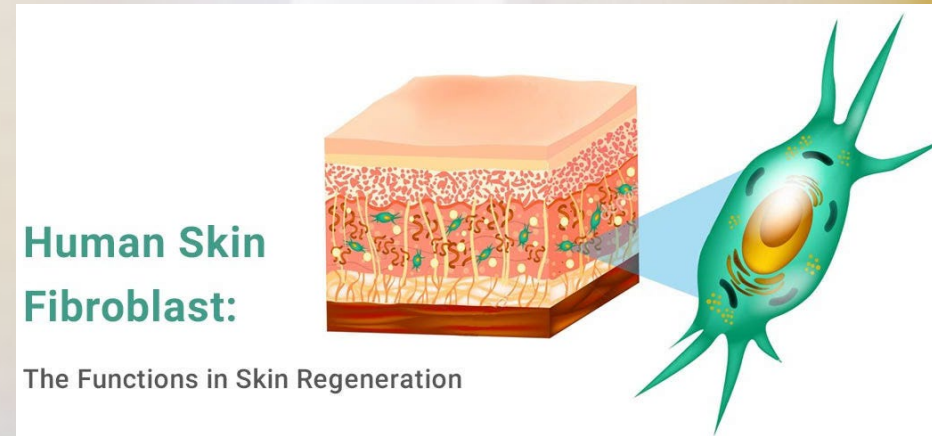
The first limitation is intrinsic to the in vitro model. Fibroblasts cultured in a plate do not reproduce systemic digestion, intestinal absorption, renal regulation, endocrine control, immune interactions, or tissue-level pharmacokinetics. Therefore, these findings cannot be directly extrapolated to whole-body effects.

The second limitation is the influence of medium dilution. When mineral water is added to culture medium, it changes not only mineral exposure but also nutrient concentration, serum concentration, osmolarity, pH, and buffering conditions. This is why water-control dilutions and rigorous pH/osmolality measurements are essential.

The third limitation concerns statistical confirmation. The observed patterns are biologically coherent, but future studies should include sufficient biological replicates, technical replicates, predefined statistical analysis, and independent repetition.

The fourth limitation is mechanistic specificity. At this stage, the results suggest redox and proliferative modulation, but they do not yet prove which signaling pathways are responsible. Future work should include gene expression, Western blot, ELISA, immunofluorescence, and possibly transcriptomic profiling.

Finally, collagen and extracellular matrix responses require careful interpretation. Collagen production should be normalized to cell number and evaluated together with matrix degradation markers, because tissue remodeling depends on the balance between synthesis and degradation.



Conclusions

In conclusion, this study supports the scientific relevance of evaluating natural mineral waters using modern in vitro cellular models.

The tested mineral water, CARPATINA Light, presents a bicarbonate calcium-magnesium profile, with substantial levels of calcium, magnesium, and bicarbonate, low levels of undesirable chemical indicators, and microbiological purity.

In human fibroblasts, moderate exposure to this mineral water was compatible with cell viability and was associated with a slight increase in metabolic activity. The strongest viability response was observed at intermediate concentration, with approximately 108% of control in the exploratory dose-response curve.

The redox data suggest a possible cytoprotective profile, with reduced ROS levels and an increased GSH/GSSG ratio under selected exposure conditions.

The proliferation data also indicate a moderate increase in BrdU incorporation at intermediate concentrations, suggesting that the water may influence fibroblast behavior relevant to tissue repair.

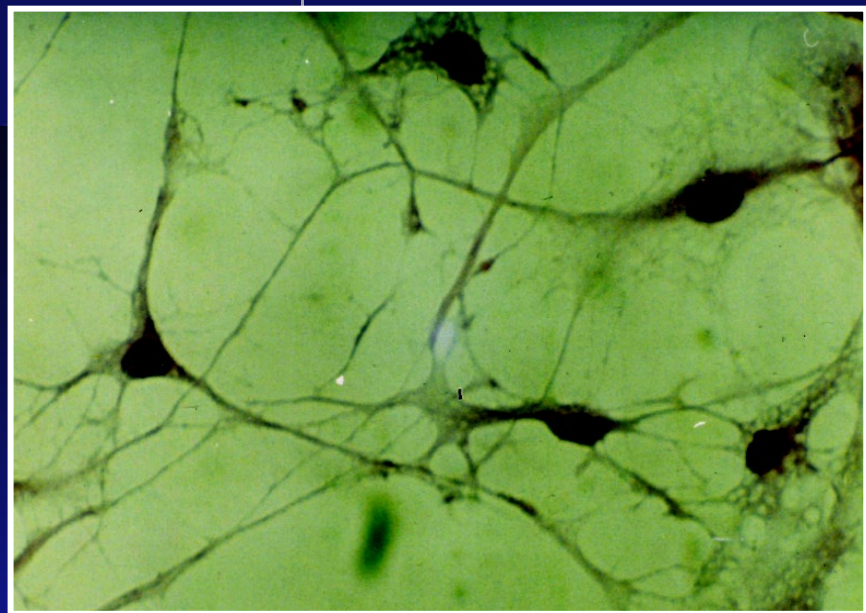
Overall, the results point toward a dose-dependent and potentially hormetic biological effect: moderate concentrations appear favorable, whereas very high concentrations are less beneficial, probably because of excessive alteration of the culture environment.

The study therefore provides a mechanistic bridge between traditional balneological knowledge and contemporary experimental biology. It does not claim clinical efficacy by itself, but it offers a rational experimental platform for future research.

The next steps should include standardized dilution controls, pH and osmolality documentation, inflammatory stimulation models, molecular markers of redox regulation, collagen and extracellular matrix quantification, and eventually translational validation in more complex biological systems.

- The experimental study demonstrates that exposure of human fibroblasts to natural mineral water CARPATINA - Light assortment with calcium-magnesium bicarbonate composition can influence relevant cellular biological processes, including redox homeostasis, cell proliferation and extracellular matrix remodeling. These results support the potential of using natural mineral waters as functional nutritional vectors in modern prevention and health strategies.
- Mineral ions can influence biological processes by: regulating ion channels, modifying membrane potential, activating transcription factors, modulating energy metabolism. These mechanisms can contribute to maintaining physiological homeostasis and preventing pathological processes associated with oxidative stress and chronic inflammation.
- Integrated analysis of physicochemical, microbiological and biological data indicates that natural mineral water CARPATINA - Light assortment presents a stable hydro-chemical profile, characterized by high concentrations of bicarbonate, calcium and magnesium and by the absence of microbiological contamination or toxic elements.
- The analyzed data support the fact that the gastrointestinal mechanisms associated with CARPATINA bicarbonate waters - Light assortment have an important physiological and functional relevance, especially through the buffering effect of gastric acidity and by stimulating alkaline digestive secretions.
- At the gastric level, the bicarbonate ion contributes to the neutralization of excess protons, with the formation of carbonic acid and subsequent decomposition into carbon dioxide and water, which determines the reduction of postprandial acidity and the temporary increase of intragastric pH. This effect can reduce acid aggression on the gastric mucosa, reduce the stimulation of nociceptors and favor the improvement of symptoms of functional dyspepsia. In parallel, at the duodeno-pancreato-biliary level, bicarbonate waters can support alkaline pancreatic secretion, facilitate lipid digestion and favorably influence intestinal motility.

“If we have no passion, we have nothing to start with in life” (*Lin Yutang*)



THANK YOU!



*Va mulțumesc mult pentru
atenție!*



- Conf. univ. dr. biol. Constantin Munteanu