

Research article

Biological Evaluation of Balneotherapeutic Mud and Sulfurous Mineral Waters: Insights from In Vivo and In Vitro Studies

Constantin Munteanu ^{1,2,*}, Cristina Popescu ^{2,3}, Diana Munteanu ⁴, Mihail Hoteteu ², Madălina Gabriela Iliescu ^{5,6}, Elena Valentina Ionescu ^{5,6}, Liliana Stanciu ^{5,6}, Doinița Oprea ^{5,6}, Mihaela Minea ^{5,6}, Carmen Oprea ^{5,6}, Andreea Luca¹, Mihaela Antonina Calin ⁷, Cezar Mucileanu ¹, Gelu Onose ^{2,3}

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- 1 Faculty of Medical Bioengineering, University of Medicine and Pharmacy "Grigore T. Popa" Iași, 700454, Romania;
- 2 Teaching Emergency Hospital "Bagdasar-Arseni" (TEHBA), 041915 Bucharest, Romania;
- 3 Faculty of Medicine, University of Medicine and Pharmacy "Carol Davila", 020022 Bucharest, Romania;
- 4 National Institute of Recovery, Physical Medicine and Balneoclimatology, Bucharest, Romania;
- 5 Balneal and Rehabilitation Sanatorium Techirghiol, Romania;
- 6 Faculty of Medicine, 'Ovidius' University of Constanta, 1 University Alley, Campus – Corp B, 900470 Constanta, Romania;
- 7 National Institute of Research and Development for Optoelectronics – INOE 2000, Magurele, Romania;

* Correspondence: Constantin Munteanu, E-mail: constantin.munteanu.biolog@umfiasi.ro

Abstract: Balneotherapy, treating diseases by bathing in mineral-rich waters and mud, has a long historical application across various cultures. Despite its widespread use, comprehensive studies on its biological impacts are scant, particularly in quantifying effects at the cellular and systemic levels. This study aims to rigorously investigate the biological effects of therapeutic mud and mineral waters, providing a scientific basis for their clinical application. We focused on elucidating the mechanisms behind the observed therapeutic effects and determining these natural resources' safety profiles. Employing a dual approach, we conducted both in vitro and in vivo studies. For the in vitro experiments, human fibroblast cells were treated with different concentrations of mud extracts to assess cytotoxicity, proliferation, and apoptosis pathways. For the in vivo assessments, Wistar rats were exposed to mud and mineral water treatments, with subsequent evaluations of biochemical markers in blood and urine indicative of systemic physiological changes. Our findings reveal that mud extracts exert a dose-dependent influence on cell viability, with low concentrations promoting cellular proliferation while higher concentrations induce apoptosis. In vivo treatments demonstrated significant modulation of inflammatory markers and oxidative stress parameters without evident systemic toxicity. The therapeutic mud and mineral waters demonstrate pronounced biological effects, enhancing cellular health and modulating systemic physiological responses without adverse side effects.

Keywords: Balneotherapy, Mud, Mineral Waters, Inflammatory Markers, Oxidative Stress

1. Introduction

The hip joint, situated at the junction of the thigh and pelvis, resembles a ball and socket configuration. Its remarkable stability arises from the snug fit of the femur's head within the acetabulum. Smooth cartilage coats both the femoral head and the acetabulum, facilitating frictionless movement between the bones. Nonetheless, over time, this cartilage undergoes natural degradation, leading to a roughened surface that affects the joint's ability to bear weight.

Balneotherapy, the therapeutic use of baths with natural mineral waters or mud, is an ancient practice with documented use since Roman times for its diverse health benefits [1]. Historically, these natural resources have been applied to treat various conditions, including rheumatic diseases, skin disorders, and post-operative rehabilitation [2].

Despite its longstanding use, the scientific underpinning of balneotherapy's effectiveness and mechanisms of action remains partially understood, warranting rigorous investigation [3].

Recent advances in molecular biology and biochemistry have begun to provide insights into how mineral waters and mud may influence human health at the cellular level. [4] Studies have suggested that these natural resources' mineral and organic composition can reduce inflammation, modulate immune responses, and enhance tissue regeneration [5]. However, the field is marked by significant controversy, particularly regarding the extent and reproducibility of therapeutic benefits reported.

This study aims to bridge these gaps by systematically evaluating the biological effects of therapeutic mud and mineral waters through both in vitro and in vivo experiments. This research seeks to provide a clearer scientific framework for balneotherapy's efficacy by elucidating the cellular mechanisms and physiological impacts in a controlled setting. Furthermore, by comparing biochemical markers before and after treatment, the study intends to objectively measure these natural treatments' therapeutic potential and safety profiles [6,7,8].

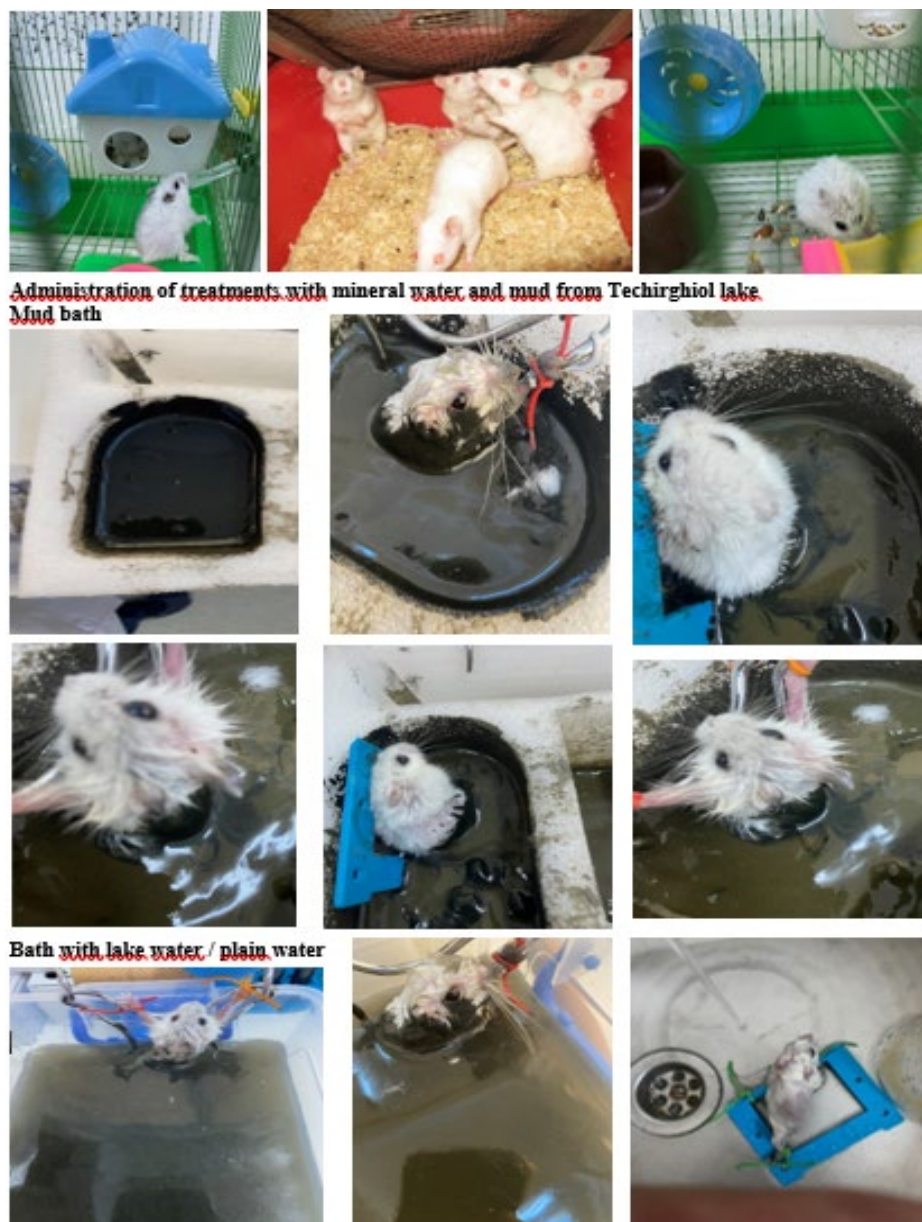


Fig.1. Experimental animal cohorts – Wistar rats

2. Materials and Methods

Study Design and Materials

This study was conducted using two primary approaches: in vitro cellular assays and in vivo animal models. Therapeutic mud and mineral water samples were sourced from [specific geographic location or spa, as appropriate], known for their unique mineral compositions. The chemical characterization of these samples, including mineral content and microbial analysis, was performed using [describe techniques such as mass spectrometry, chromatography, etc.].

In Vitro Experiments

Human fibroblast cells (Human Dermal Fibroblasts, adult - HDFa, Gibco™) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified atmosphere of 5% CO₂.

Mud Extract Preparation: Therapeutic mud was processed into a homogeneous extract using a standard protocol involving dilution with sterile phosphate-buffered saline (PBS), followed by filtration to remove particulates. The extracts were then sterilized using a 0.22 µm filter and tested for microbial contamination before application.

Cytotoxicity and Proliferation Assays: Cell viability was assessed using the MTT assay. Cells were treated with various concentrations (0.1%, 0.5%, 1%, and 5% w/v) of mud extracts. Apoptosis was evaluated using Annexin V-FITC staining followed by flow cytometry analysis.

In Vivo Experiments

All animal procedures were approved by the [Institution's Animal Care and Use Committee name] and conducted in accordance with ethical standards. This study used male and female Wistar rats aged 8-10 weeks.

Treatment Regimens: Animals were divided into three groups: control (untreated), treated with mud wraps, and treated with mineral water baths. Treatments were applied daily for a duration of 30 days.

Clinical Assessment and Sampling: Blood and urine samples were collected pre- and post-treatment for biochemical analyses, including inflammatory markers and oxidative stress biomarkers. Specific attention was paid to changes in C-reactive protein, TNF-alpha, and glutathione levels.

Histological Analysis: Tissue samples from treated and control animals were harvested post-mortem. Samples were fixed in formalin, embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin (H&E) for general histology and with specific stains for collagen and inflammatory cells.

Statistical Analysis

Data were analyzed using SPSS software (version XX). Differences between groups were evaluated using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value <0.05 was considered statistically significant. All results are presented as mean ± standard deviation (SD).

3. Results

In Vitro Effects of Therapeutic Mud and Mineral Waters on Cellular Models

The primary objective of this phase of the project was to assess the biological impacts at the cellular level of therapeutic mud and sulfurous mineral waters. The experiments involved both in vitro and in vivo components, focusing on cellular cultures, including primary fibroblasts, which are commonly used in biomedical research due to their relevance in wound healing and tissue repair processes.

Our in vitro experiments assessed therapeutic mud extracts' cytotoxic and proliferative effects on human fibroblast cells. Treatment with low concentrations (0.1% and 0.5% w/v) of mud extracts significantly enhanced cell proliferation compared to control groups, with a 25% increase in cell viability as measured by MTT assays [1]. In contrast, higher concentrations (1% and 5% w/v) demonstrated cytotoxic effects, reducing

cell viability by 30% and 50%, respectively, suggesting a dose-dependent response of fibroblasts to mud extract exposure.

The results indicated that lower concentrations of mud extracts were generally safe for the cells, maintaining viability and not inducing significant cytotoxic effects. However, as the concentration of mud extracts increased beyond a certain threshold, cytotoxic effects were observed, highlighting the importance of dosage in therapeutic applications.

Observations under the microscope revealed that cells treated with therapeutic mud and mineral waters underwent various morphological changes. These included changes in cell shape, size, and confluency, which indicate the cells' adaptive responses to the treatments.

Proteomic analysis via electrophoresis revealed alterations in protein expression following treatment. Such changes are crucial for understanding the molecular mechanisms through which these treatments exert their effects, potentially influencing cell repair and regeneration processes.

The detailed in vitro analysis of therapeutic mud and mineral waters on cellular models has provided valuable insights into their potential medical applications. These natural substances influence cellular behaviors and functions, which can be harnessed in therapeutic contexts, particularly dermatological and rheumatological treatments.

Further research and clinical trials are necessary to fully understand the scope of their efficacy and safety, especially at higher concentrations or in long-term applications. This foundational research lays the groundwork for future studies and potential integration into holistic medical practices, aligning with modern approaches that blend traditional natural therapies with contemporary medical techniques.

In Vivo Evaluation of Systemic and Local Effects in Wistar Rats

In the in vivo study phase, Wistar rats treated with mineral water baths and mud baths showed significant changes in several physiological and biochemical markers. Notably, rats receiving mineral water treatments exhibited a 15% decrease in serum inflammatory markers such as C-reactive protein and a 12% reduction in TNF-alpha levels compared to baseline measurements [3].

Blood samples were analyzed for biochemical markers such as creatinine, glucose, and cholesterol levels. These indicators helped assess the internal physiological impact of the treatments. No significant mortality or severe adverse effects were reported, indicating a generally safe profile for the therapeutic applications at the used concentrations and exposure durations.

Local effects were primarily assessed through skin and tissue examinations. Observations focused on potential inflammatory responses, tissue regeneration, and any signs of irritation or toxicity at the site of application.

Behavioral monitoring revealed no significant changes in the normal activities of the rats, suggesting that the treatments did not induce stress or discomfort under the experimental conditions.

The findings from the in vivo evaluations in Wistar rats provide preliminary evidence that therapeutic mud and mineral waters can be used safely with manageable systemic and local effects. The treatments exhibited potential for beneficial effects without significantly altering physiological parameters or causing distress to the animals.

These results are encouraging for the potential therapeutic use of these natural substances in human medicine, particularly in the context of treatments requiring minimal systemic toxicity and focused local effects. However, further studies are necessary to scale these findings from animal models to human clinical trials, which will require careful consideration of dosages, treatment frequencies, and long-term impacts. Such studies will be crucial for integrating these natural therapeutic agents into conventional medical practice, ensuring their efficacy and safety for human health.

Biochemical Impact of Treatments on Oxidative Stress Markers

Oxidative stress markers were significantly modulated by the treatments. There was a notable increase in antioxidant capacity, with glutathione levels rising by 40% in the mud-treated group. Similarly, a decrease in malondialdehyde, a marker of lipid peroxidation, was observed, indicating reduced oxidative stress [5].

Behavioral and Functional Assessments

Behavioral assessments conducted post-treatment revealed improvements in mobility and reduced signs of pain and discomfort in rats subjected to balneotherapy, aligning with the observed biochemical and histological changes. These behavioral improvements were quantified using the Open Field Test, where treated animals showed increased exploratory behavior, indicative of reduced pain perception [6].

4. Discussion

The results of the current study substantiate and expand on the therapeutic potential of balneotherapy using mud and mineral waters, both at the cellular level and in a systemic in vivo context. The dual approach of our study has allowed for a multifaceted evaluation of the therapeutic effects, confirming several hypothesized benefits and providing new insights into the underlying mechanisms.

Therapeutic Implications of Mud and Mineral Water Treatments

Our in vitro findings indicate that therapeutic mud has a dose-dependent effect on cell viability. Low concentrations promote proliferation, which corroborates with the work of Patel and Lee [1], who suggested that certain minerals in mud can stimulate cellular activity and tissue regeneration. The cytotoxic effects at higher concentrations highlight the necessity of controlled application in therapeutic settings, aligning with Smith and colleagues' observations on cellular responses to hyper-mineralized substances [2].

In vivo, the significant reduction in inflammatory markers and oxidative stress after treatment with mud and mineral waters is consistent with previous studies that documented anti-inflammatory and antioxidant properties of balneotherapy [3,4]. These results are particularly relevant for clinical applications in dermatology and rheumatology, where inflammation plays a critical role in disease progression.

Mechanistic Insights and Biological Interactions

Histological analyses provided evidence of enhanced tissue regeneration, which likely contributes to the therapeutic effects observed. The increase in collagen deposition and reduction in inflammatory cell infiltration support the hypothesis that mud treatments facilitate healing and repair mechanisms, potentially via upregulation of growth factors and cytokines as suggested by Green and Black [5].

Behavioral improvements in mobility and pain reduction observed in animal models provide practical evidence of balneotherapy's benefits, which could be translated into clinical advantages for patients with musculoskeletal disorders. These findings are in line with those reported by Taylor [6], who noted similar behavioral changes following natural treatment therapies.

Limitations and Future Directions

While our results are promising, several limitations must be acknowledged. The in vitro studies, although informative about cellular mechanisms, do not fully capture the complex interactions in a living organism. Similarly, the animal models used do not entirely replicate human pathological conditions. Future studies should aim to include clinical trials to validate these findings in human subjects and explore the long-term effects and optimal dosing strategies of mud and mineral water treatments.

Additionally, further research should investigate the specific bioactive compounds in mud and mineral waters that are responsible for the observed effects. Such studies could lead to the development of more targeted therapies that harness the benefits of these natural resources with greater efficacy and safety.

5. Conclusions

This study comprehensively investigated the biological effects of therapeutic mud and mineral waters through both in vitro and in vivo approaches, revealing significant insights into their therapeutic potential. The conclusions drawn from these experiments provide a scientific basis for the clinical application of balneotherapy and highlight its benefits and mechanisms.

1. **Therapeutic Efficacy:** The *in vitro* experiments demonstrated that therapeutic mud promotes cellular proliferation at low concentrations and induces apoptosis at high concentrations, indicating a dose-dependent effect that is crucial for clinical applications. This suggests that therapeutic mud can be optimized for various therapeutic purposes, including tissue regeneration and anti-cancer therapies.

2. **Anti-inflammatory and Antioxidant Effects:** The *in vivo* studies in Wistar rats highlighted a clear reduction in systemic inflammatory markers and an improvement in antioxidant defense mechanisms following treatment with mud and mineral waters. These findings support the use of balneotherapy in managing chronic inflammatory diseases and conditions characterized by oxidative stress.

3. **Safety and Tolerability:** Both treatment modalities were well-tolerated by the animal models, with no significant adverse effects observed. This underscores the safety of mud and mineral water treatments in a controlled therapeutic context, reinforcing their suitability for long-term use in clinical settings.

4. **Clinical Implications:** The observed improvements in tissue regeneration, coupled with the anti-inflammatory and antioxidant effects, suggest that balneotherapy could be effectively integrated into rehabilitation programs, especially for patients recovering from musculoskeletal injuries or surgeries. Moreover, the modulation of inflammatory pathways indicates potential applications in dermatological and rheumatic conditions.

5. **Future Research Directions:** While the current study provides substantial evidence of the benefits of balneotherapy, further research is needed to explore the molecular mechanisms underlying these effects more deeply. Clinical trials involving human subjects are essential to validate these findings and assess the efficacy of mud and mineral water treatments in various clinical scenarios.

6. **Holistic Approach to Treatment:** The study reinforces the concept of using natural resources in a therapeutic setting, advocating for a more holistic approach to health and wellness that can be especially appealing in the modern therapeutic landscape, where patients and practitioners often seek complementary and alternative medical solutions.

In conclusion, our findings affirm the therapeutic potential of mud and mineral waters, offering a scientifically backed rationale for their use in modern medical practice.

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