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Exploring the toxicity and carcinogenic potential of 300 KDA "Mito Organelles"™ cellular extracts through MTT and BALB/C-3T3 cell transformation assays: a vital component of peptide and protein biomedical research and safety study

Abstract

The MTT assay, an essential component of our research, evaluates cellular metabolic activity to indicate cell viability, proliferation, and the cytotoxic effects of therapeutic products utilized as cellular therapy agents.

The **objectives** of this study were: to investigate and assess the potential cytotoxicity and carcinogenesis of the selected range of the medicinal biological products - 300 kDa cellular extracts "Mito Organelles" of the following types: heart, brain, kidney, cartilage, thymus, placenta, lungs, connective tissue, and a combo LPPSIMKE (liver, pancreas, placenta, kidney, intestines, retina); evaluate carcinogenic potential; compare toxicity; provide recommendations for biomedical research and application.

Materials and methods: The MCF-7 human breast cancer cell line was used for the MTT assay. Cells were cultured in standard MCF-7 medium (DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin) under controlled conditions at 37°C and 5% CO_2 in a humidified incubator. Different protein solutions (designated as MOs) were tested for their effects on MCF-7 cells. The BALB-CTA offers essential information to assess cell viability and carcinogenic potential following treatment with MOs 300 kDA cellular peptide/protein extracts. Material processing was carried out using MS Excel and Statistica EZR version 1.62-2023 statistical programs. Dunn's test, Kruskal-Wallis rank univariate analysis, Scheffe, Student's parametric t-test were used to assess differences between groups. The difference was considered statistically significant at $p < 0.05$.

Results: According to MTT assays, the 300 kDA peptide/protein cellular extracts "MitoOrganelles" (MOs) tested did not affect the viability of MCF-7 cells. In addition, cells that were stressed with low doses of H_2O_2 were able to improve their vitality through the addition of MOs 300 kDa. The BALB/c-3T3 two-stage in vitro transformation assay (CTAs), a model for studying carcinogenesis, is another important tool in our research. It showed chemical transformation with morphologically aberrant foci after treatment with MCA and TPA. In contrast, various 300 kDA peptide/protein cellular extracts were tested, and no carcinogenic activity was observed, reinforcing the safety profile of these cellular extracts. The confidence in our research methods, particularly the MTT and BALB/c-3T3 assays, is crucial in understanding the safety profile of these cellular extracts.

Conclusion: The comparative study conducted on the cytotoxicity and potential adverse effects of these extracts on cell viability and metabolic activity revealed that the selected range of medicinal biological products-specifically, the cellular extracts known as "Mito Organelles" from heart, brain, kidney, cartilage, thymus, placenta, lungs, connective tissue, and the combo LPPSIMKE (liver, pancreas, placenta, kidney, intestines, retina)-showed no cytotoxic effects on human cells. Additionally, no potential for malignant transformation or morphological changes were observed in the treated cell lines, and there were no negative impacts on cell viability or transformation rates. The results of these assays support recommendations for the safe use of Mito Organelles cellular extracts in biomedical research and therapeutic applications within regenerative medicine.

Keywords: peptides, cell therapy, stem cells, toxicity, carcinogenicity, mitochondria

Volume 9 Issue 1 - 2024

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Received: September 19, 2024 | **Published:** October 22, 2024

Introduction

Medicinal biological products, including stem cells, exosomes, peptides, and cellular extracts, have gained considerable interest in biomedical research and therapeutic development due to their remarkable abilities to self-renew, differentiate into various cell types, and promote tissue repair and regeneration, thereby enhancing healing. Their applications span from regenerative medicine to disease modeling, making it essential to understand their safety profiles. The unique characteristics of these products are not only fascinating but also hold significant promise for the future of biomedical advancements. However, there is increasing concern regarding the potential risks of cellular therapies, particularly related to their toxicity and carcinogenic potential.[1–10]

Tissue-specific cellular extracts, such as the "Mito Organelles" (MO) peptides, are biologically derived mixtures of cellular peptides with functions primarily associated with mitochondria.[11–15] Although there are common functions among cells from different organ systems, variations in cellular activities across organs result in distinct peptide expressions that can be leveraged for therapeutic purposes.[16] MO peptides are designed to target specific organs, aiming to enhance mitochondrial function and, consequently, support overall cellular and organismal regeneration. In our previous publications, we discussed the characterization of these peptides through MALDI-TOF analysis of various MO samples, as well as practical aspects of their clinical applications. We identified six major peptide groups in the extracts, categorized by molecular weights of 14,969 Da, 15,301 Da, 8,294 Da, 8,449 Da, 5,436 Da, and 6,214 Da, respectively.[17–21] This study is a significant step in addressing these concerns by investigating the toxicity and carcinogenic potential of MOs cellular extracts using two widely recognized assay systems: the MTT assay (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) and the BALB/c-3T3 cell transformation assay.[22] The MTT assay is a colorimetric method that evaluates cell viability and metabolic activity, offering insights into the cytotoxic effects of cellular extracts on various target cells. In parallel, the BALB/c-3T3 cell transformation assay assesses the carcinogenic potential of cellular extracts by detecting morphological and growth changes indicative of malignant transformation.[23,24]

Combining these two assays, our research aims to evaluate cellular therapy safety comprehensively. This dual approach not only helps in identifying potential adverse effects but also contributes to ensuring the safe application of stem cell technologies in clinical and research settings. Understanding these risks is essential for advancing stem cell science while safeguarding public health and promoting the responsible development of stem cell-based therapies.

Studying the toxicity of peptides and proteins in vitro is essential for understanding their potential therapeutic effects, side effects, and mechanisms of action. This research sheds light on the benefits and risks associated with biological therapeutics. The MTT assay plays a critical role in these investigations by measuring mitochondrial activity as an indicator of cell viability. A reduction in formazan production signals decreased cell viability, offering important insights into the effects of the substances being tested.

In vitro malignant cell transformation is a progressive process that occurs in several stages, mirroring the cellular and molecular events involved in in vivo carcinogenesis. This transformation includes phenotypic changes, such as spindle-shaped morphology and basophilic staining, as well as alterations in growth behavior, including immortality, multilayer growth, and anchorage-independent growth. Tumorigenicity is then assessed in susceptible animal models. These assays are designed to detect the induction of malignant traits in mammalian cells following chemical exposure, allowing for monitoring of the transition from normal to transformed states.

Recently, significant strides have been made to develop and validate alternative methods, such as the in vitro cell transformation assay (CTA), to minimize the reliance on animal models for carcinogenicity testing. While CTAs do not completely replicate the in vivo neoplastic process, they provide valuable insights into potential carcinogens and their mechanisms of action. Additionally, CTAs are faster and more cost-effective compared to traditional two-year rodent bioassays and are currently recognized as a reliable method for detecting both genotoxic and non-genotoxic carcinogens.

The MTT assay, a widely used colorimetric method, measures cell metabolic activity and is commonly applied to evaluate cell viability and cytotoxicity. It is frequently employed in studies involving cell proliferation, drug testing, and toxicity assessments.

One prominent CTA is the BALB/c-3T3 cell transformation assay (BALB-CTA), developed by Kakunaga, which detects early stages of carcinogenicity that lead to the formation of morphologically transformed colonies. In this study, we investigated the in vitro toxicity and carcinogenicity of peptide and protein extracts, finding no indications of toxicity or carcinogenic effects in the examined cells.

Objectives

a) **Assess cytotoxicity:** Evaluate the cytotoxic effects of a range of medicinal biological products, specifically the 300 kDa cellular extracts known as "Mito Organelles," sourced from heart, brain, kidney, cartilage, thymus, placenta, lungs, connective tissue, and the combination LPPSIMKE (liver, pancreas, placenta, kidney, intestines, and retina). Use the MTT assay to analyze the impact of these extracts on cultured cells, aiming to determine their effects on cell viability and metabolic activity, which will provide essential insights into any potential harmful effects.

b) **Evaluate carcinogenic potential:** Employ the BALB/c-3T3 cell transformation assay to assess the carcinogenic potential of the 300 kDa Mito Organelles extracts. This objective focuses on detecting any signs of malignant transformation or morphological changes in the BALB/c-3T3 cell line that could indicate a risk for cancer development.

c) **Compare toxicity across different biological sources:** Investigate how variations in the source tissue of the 300 kDa Mito Organelles extracts influence cell viability and transformation rates. This analysis will aid in establishing dose-response relationships and identifying threshold levels at which these extracts may present significant risks.

d) **Provide recommendations for biomedical research and application:** Based on the results from the MTT and BALB/c-3T3 assays, formulate recommendations for the safe utilization of 300 kDa Mito Organelles extracts in biomedical research and potential therapeutic applications. This objective aims to translate research findings into practical guidelines that minimize the risks associated with these extracts.plates without a stress medium (control). Each condition (with and without H_2O_2) was tested in duplicate, and for each protein solution, eight replicates were performed.

Protein solutions and treatments: Different protein solutions (designated as MOs) were tested for their effects on MCF-7 cells. On Thursday, MCF-7 cells were seeded in 96-well plates. On Friday, the medium was changed to fresh MCF-7 medium with or without adding H_2O_2 and the respective protein solutions. On Monday, a second medium change was performed, replacing the medium with 100 µL of fresh medium, with or without protein treatments, depending on the experimental group.

MTT assay protocol: After the second medium change on Monday, 10 µL of MTT reagent (5 mg/mL) was added to each well. The plates were incubated for 4 hours at 37°C with 5% CO_2 to allow for the formation of formazan crystals. After incubation, 100 µL of solubilization buffer was added to each well to dissolve the formazan crystals. The plates were incubated overnight under the same conditions.[22–26]

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Measurement and data analysis: The absorbance of each well was measured using an ELISA reader at wavelengths of 562 nm and 650 nm. Data were transferred to Excel for analysis, where the difference in absorbance (562 nm minus 650 nm) was calculated for each well. The mean absorbance values for each experimental condition were calculated from these differences. Diagrams were generated using GraphPad Prism, which was used to determine the standard deviation and perform T-value analysis for statistical comparisons between groups.[27]

Cell transformation assay (CTA)

The BALB-CTA is based on the immortalized embryonic mouse fibroblasts BALB/c-3T3, using the A31-1-1 subclone developed by Kakunaga and Crow. BALB/c-3T3 cells typically form a monolayer culture, with growth being contact-inhibited upon reaching confluence. After chemical treatment, some cells continue to increase, forming morphologically aberrant foci that overgrow the monolayer of normal cells. The standard protocol involves a 3-day chemical exposure, starting 24 hours after seeding. Cultures are maintained for 4 to 6 weeks, with biweekly medium changes, until fixation. An inversion microscope visualizes transformed foci.

The BALB-CTA offers essential information to assess cell viability and carcinogenic potential following treatment with MOs 300 kDA cellular peptide/protein extracts.

Cell culture and maintenance: The BALB/c 3T3 mouse fibroblast cell line was used for the CTA. Cells were cultured in DMEM/HAM's F-12 medium (Biochrom #T481-10), supplemented with 3 g/l D-glucose, 5% fetal bovine serum, and 1% penicillin/streptomycin to maintain consistent conditions throughout the experiment. Penicillin-streptomycin (1%) was optionally added to prevent contamination. Cells were kept in a humidified incubator at 37°C with 5% CO_2. BALB/c 3T3 cells were rapidly thawed in a 37°C water bath and transferred to a 15 mL centrifuge tube containing 10 mL of DMEM with 10% FBS for routine maintenance. Cells were centrifuged at 300g for 5 minutes to remove cryoprotectants. The cell pellet was re-suspended in DMEM (10% FBS), seeded into a 75 cm^2 flask, and incubated at 37°C in a humidified incubator. Cells were passaged at 70-80% confluence using trypsin-EDTA for detachment, and the split ratio was typically 1:5 or 1:10, depending on the growth rate.

Cell seeding for the assay: BALB/c 3T3 cells were seeded at a low density for the CTA to allow clonal expansion and focus formation. Cells were plated at 300 cells per well in 6-well plates or 1,000 cells per dish in 60 mm culture dishes. After seeding, cells were incubated in DMEM with 10% FBS for 24-48 hours to allow attachment before test compound treatment.

Post-confluence culture conditions: Once cells reached confluence (after 2-3 days), the serum concentration was reduced to 2-5% FBS in DMEM to promote cellular quiescence. Cells were kept in post-confluent conditions for the duration of the assay, typically for 2-4 weeks. Importantly, cells were not passaged after reaching confluence, as this would interfere with forming transformation foci, which are critical for the assay.

Treatment with test compounds: To maintain consistent conditions for analysis during the initiation (days 1–4), promotion (days 8–21), and post-promotion (days 21–42) phases, only DMEM/HAM's F-12 medium was used, as it had proven effective in promoting foci formation. The assay duration was set to 42 days to allow sufficient colony formation. 5000 cells per well were seeded into 4 replicate wells of 6-well plates and cultured under standard conditions (37 °C, 5% CO2, 95% humidity) for 42 days. Media changes occurred every 3 to 4 days (according to the treatment schedule), with 1) of 0.5 µg/ml MCA (3-Methylcholanthrene) and 0.3 µg/ml TPA (12-O-Tetradecanoyl-phorbol-13-acetate) or 2) MOs administered on days 1 to 21.

Control groups included untreated cells as the negative control and cells exposed to a known transforming agent as the positive control. Test compounds were prepared by dissolving them in an appropriate solvent and added to cells 24-48 hours after attachment. For continuous exposure, cells were treated with the test compound for 3-5 days, with the media (containing the compound) replaced every 48-72 hours. In the case of pulse exposure, cells were treated for 24 hours, followed by removal of the compound and replacement with fresh medium without the compound.

Description of MOs

Biological micro and ultrafiltration in the preparation of cellular therapy products: Biofiltration is a method employed to separate very small particles—such as peptides, microvesicles, exosomes, and growth factors—from cultures of progenitor stem cells. This separation primarily relies on the size of the molecules; however, the filter's efficiency can also be affected by the chemical, molecular, or electrostatic properties of the sample. For successful separation via ultrafiltration, a molecule typically needs to differ in size by at least an order of magnitude, with a molecular weight ranging from 1 kDa to 1000 kDa.

The therapeutic potential of progenitor or precursor stem cells has been well-documented in numerous studies. In contrast, cell-free biotherapeutics address compatibility and survival challenges often associated with cellular therapies. The secretome of progenitor cells is abundant in bioactive factors derived from amino acids, lipids, and nucleic acids that promote regenerative processes in target cells. Various types of cellular extracts, including extracellular vesicles and exosomes, contain many of these factors within a lipid bilayer, which shields them from enzymatic degradation and facilitates their delivery to both adjacent and distant cells. Cellular extracts from precursor stem cells are typically categorized by size and tissue origin, though their final composition is influenced by the purification method used. Exosomes, measuring between 50 and 100 nm, form through endosomal pathways and are released when multivesicular bodies fuse with the plasma membrane. In contrast, microvesicles, which range from 100 nm to 1 µm, are produced through the budding of the cytoplasmic membrane. Despite the growing interest in enhancing therapeutic cellular extracts, the precise level of purity required for effective therapy remains uncertain. Given that the regenerative signals from progenitor stem cells comprise a complex array of components, thorough characterization, as well as toxicity and tumorigenicity assessments of the cellular secretome, along with in vitro and in vivo analyses, may contribute to the safety and efficacy profiling of these extracts.

In this experiment, we evaluated a variety of Mito Organelles cellular tissue extracts derived from different cultures of progenitor stem cells, including heart, brain, kidney, cartilage, fetal connective tissue, thymus, placenta, and lungs, to assess their potential toxicity and tumorigenicity.

The cellular extracts were obtained through a series of filtration processes. Initially, the progenitor cell culture medium was concentrated using 3 kDa centrifuge filter units for 45 minutes at 2800 x g. This step captured both extracellular vesicles and soluble proteins larger than 3 kDa. The culture medium was then further

concentrated using 100 kDa centrifuge filter units for 20 minutes at 2800 x g, yielding a fraction labeled as "EV+," which was enriched with extracellular vesicles and proteins or complexes larger than 100 kDa. Finally, the medium that passed through the 300 kDa filter was concentrated again with 3 kDa centrifuge filter units for 40 minutes at 2800 x g to isolate proteins smaller than 300 kDa but larger than 3 kDa that are independent of extracellular vesicles.

Monitoring and media changes: Throughout the assay, cells were monitored microscopically for morphological changes indicative of transformation, such as multilayered growth, loss of contact inhibition, and altered cell shape. The media was changed every 2-3 days to prevent nutrient depletion, ensuring the cells remained covered with fresh media at all times, particularly during the post-confluent phase.

Foci development and scoring: After 4-6 weeks of culture, the plates were examined for the development of transformed foci. Transformed foci were identified as dense, multilayered regions of cells that were irregularly shaped with evidence of cell piling. Foci were manually counted using an inverted microscope. Optionally, foci were stained with crystal violet to aid in visualization and scoring.

Data analysis: The number of foci in treated wells or dishes was compared to the control groups. The frequency of focus formation in treated cells reflected the carcinogenic potential of the test compounds. Material processing was carried out using MS Excel and Statistica EZR version 1.62-2023 statistical programs. The Shapiro-Wilk W test was used to test the distribution for normality. For indices with a normal distribution, the results are presented in the form of M ± σ, where M is the average value, and σ is the mean square deviation, as well as in the form of Me [P25; P75] for variables with a distribution that was different from the normal one, Me – the median, and the interquartile range [IQR] of QI÷QIII indices were used. Dunn's test, Kruskal-Wallis rank univariate analysis, Scheffe, Student's parametric t-test were used to assess differences between groups. The difference was considered statistically significant at $p < 0.05$.

Controls and replicates: Each treatment condition was tested in triplicate wells or dishes. Each assay run included negative (untreated) and positive (known transforming agent) controls. The experiment was repeated in independent assays to ensure reproducibility of the results.

Results and discussion

Summary of findings on cellular extracts and their impact on cell viability and carcinogenic potential

The data demonstrate that organ-specific extracts can improve cell survival during oxidative stress induced by H_2O_2. In the absence of H_2O_2, some extracts also enhance cell viability, though this effect is less significant than under oxidative conditions (see Figure 1). The results from the MTT assay, conducted both with and without H_2O_2, are illustrated in three panels (top left, top right, and bottom), measuring the metabolic activity of cells treated with various extracts, which correlates with cell viability through absorbance readings at 562 nm - 650 nm.

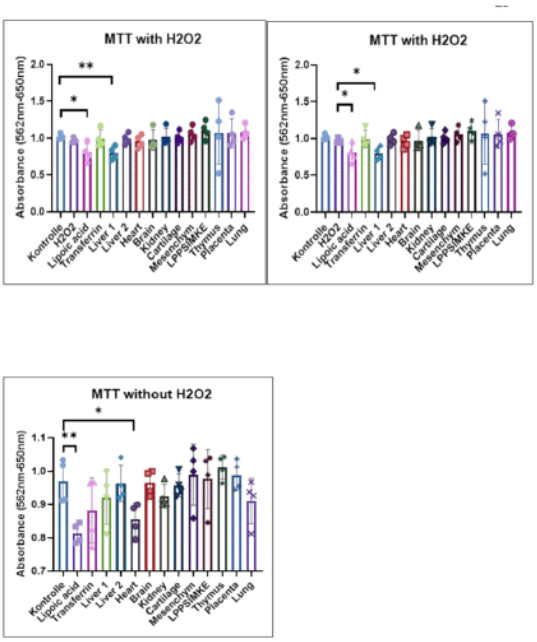

Figure 1 MTT Assay Analysis of Different Extracts in the Presence and Absence of cellular stress with H_2O_2.

The figure displays three bar graphs representing the MTT assay results with and without H_2O_2 treatment, comparing the metabolic activity of cells treated with various extracts. The assay measures cell viability through absorbance values at 562 nm - 650 nm, reflecting mitochondrial activity. Top Left Panel: MTT Assay with H_2O_2 (First Experiment) – Cells were treated with H_2O_2 alongside different extracts, including "control," "H_2O_2," "Lipofect," and several compound extracts such as "Liver" and others. Statistical significance is highlighted by the asterisks (* and **), indicating significant differences between treatment groups. Extracts "Lipoic acid shows a significant reduction in absorbance compared to the control group, implying reduced cell survival in the presence of oxidative stress induced by H_2O_2. Top Right Panel: MTT Assay with H_2O_2 (Second Experiment) – A repeat experiment with a similar setup, showing how different extracts protect cells from H_2O_2-induced oxidative stress. Notable extracts and others, are compared, with specific extracts showing significant differences (denoted by * and **). These results are consistent with those in the first experiment, reaffirming the protective effect of all extracts, particularly. Bottom Panel: MTT Assay without H_2O_2 – This panel represents the MTT assay in the absence of oxidative stress (without H_2O_2), focusing on the basal effect of the extracts on cell viability. "Lipoic acid," show significant differences in absorbance compared to the control. Asterisks denote statistically significant variations, suggesting that some component naturally enhance or reduce cell viability. Statistical Significance: Asterisks denote statistical significance between groups: *P < 0.05 (single asterisk, *) indicates significant differences; *P < 0.01 (double asterisks, **) indicates highly significant differences.

Abbreviations: Kontrolle - Control group without treatment. H_2O_2 - Hydrogen peroxide-induced oxidative stress model. Lipofect - A commercial reagent used as a control. Various "Liver" and other terms refer to plant extracts or compounds being tested for their protective effects. Each data point represents the mean absorbance with error bars indicating standard deviation across multiple replicates.

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MTT assay with H_2O_2 (Top left panel): Cells subjected to oxidative stress from H_2O_2 and treated with different extracts—including "control," "H_2O_2," "Lipofect," and various compound extracts-exhibited varying viability levels. Notably, "Lipoic acid" resulted in a significant decrease in absorbance (*P <0.05), indicating lower cell survival compared to the control group. The detrimental impact of oxidative stress on cell viability is clear, although some extracts provided minimal protective effects.

MTT assay with H_2O_2 (Top right panel): A second independent experiment was conducted under similar conditions to validate the initial findings. Consistent with the first assay, certain extracts demonstrated significant protective effects against H_2O_2-induced oxidative stress (*P <0.05), with the most substantial protection noted in extracts labeled as "Liver." These results suggest that these extracts reliably enhance cell viability under oxidative stress.

MTT assay without H_2O_2 (Bottom panel): In the absence of H_2O_2, the baseline metabolic activity of cells treated with the same extracts was evaluated. "Lipoic acid" showed a significant decrease in absorbance relative to the control (*P <0.05), indicating a reduction in basal cell viability. The peptide/protein extracts exhibited milder effects, suggesting that their impact on viability is more pronounced under stress conditions.

Overall, the MTT assay results indicate variability among the tested extracts in their ability to protect cells from oxidative stress caused by H_2O_2. The repeatability of these findings in the second experiment supports the protective properties of these extracts, especially under oxidative conditions. This consistency highlights the potential for certain compounds to mitigate oxidative damage, which is valuable for therapeutic applications focused on oxidative stress-related cellular harm.

Interestingly, "Lipoic acid" showed a marked reduction in cell viability without H_2O_2, implying it may inhibit mitochondrial activity or cell proliferation in normal conditions. However, under oxidative stress, this extract did not provide protection, indicating a complex interaction that warrants further investigation. Future research should aim to elucidate the mechanisms by which these extracts confer protection and explore their therapeutic potential in diseases characterized by oxidative stress.

The BALB/c-3T3 Cell Transformation Assay (BALB-CTA) mimics specific stages of in vivo carcinogenesis and assesses the formation of morphological aberrant foci induced by various chemicals. In this two-stage model (Figure 2A), cells are treated with a known tumor initiator for 72 hours, one day after seeding. Following a four-day recovery in standard medium, a tumor promoter is applied from day 8 to day 20, leading to the transformation of cells that overgrow the contact-inhibited monolayer. Cells are then maintained in standard medium until day 42, after which they are fixed with methanol for Giemsa staining, revealing morphological aberrant foci classified into three distinct types. The reproducibility and efficacy of the BALB-CTA have been established using known carcinogens. The combination of MCA and TPA significantly increased cell transformation, resulting in multilayer colony growth (see Table 2B).

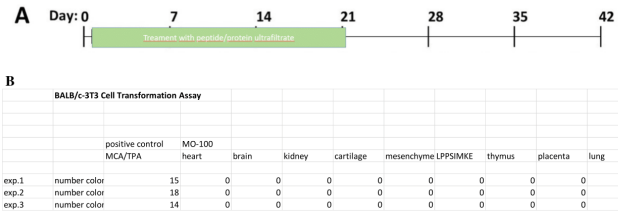

Figure 2 A) Schedule of the BALB/c-3T3 Cell Transformation Assay and B) Results for Various Tissue Extracts.

The **Table 2B** presents the results of the BALB/c-3T3 cell transformation assay, which measures the ability of various tissue extracts and treatments to induce transformation in BALB/c-3T3 cells, as indicated by the formation of colonies. The assay is performed across three independent experiments (exp. 1, exp. 2, and exp. 3), and the number of colonies observed in each condition is recorded. Positive Control (MCA/TPA): The positive control group treated with MCA/TPA (a known tumor promoter and inducer of cell transformation) shows consistent colony formation across all three experiments, with 15, 18, and 14 colonies in experiments 1, 2, and 3, respectively. This confirms the assay's sensitivity to transformation stimuli.

MO-100 and Tissue Extracts (Heart, Brain, Kidney, Cartilage, Mesenchyme, LPPSIMKE, Thymus, Placenta, Lungs): No colonies were observed for MO-300 and the various tissue extracts (heart, brain, kidney, cartilage, mesenchyme, LPPSIMKE, thymus, placenta, and lung) in any of the experiments. This suggests these extracts do not induce transformation under the experimental conditions.

The positive control (MCA/TPA) successfully induces colony formation in BALB/c-3T3 cells, validating the assay. However, none of the tested tissue extracts MO-300 treatment induced transformation in this system, as evidenced by the absence of colonies across all three experiments.

Abbreviations: MCA/TPA: 3-Methylcholanthrene/12-O-tetradecanoylphorbol-13-acetate, known carcinogens and tumor promoters; MO-300: A tested compound or treatment.

The consistency of results across three independent experiments strengthens the conclusion that the tested tissues and compounds do not exhibit transformative properties under these conditions.

The BALB/c-3T3 Cell Transformation Assay serves as a biological method for assessing the oncogenic potential of chemical compounds or physical agents, examining their ability to induce malignant transformation in vitro using BALB/c 3T3 cells, a fibroblast cell line derived from BALB/c mice. The BALB-CTA provides a valuable in vitro model for simulating key stages of in vivo carcinogenesis, particularly for evaluating the oncogenic potential of chemical compounds and biological extracts like peptides and proteins. By assessing both tumor initiation and promotion phases, the BALB-CTA is a comprehensive tool for evaluating the transforming ability of various agents.

The assay's reproducibility and efficacy have been validated, particularly with known carcinogens like the positive control-MCA combined with TPA-which consistently enhances the transformation process, as evidenced by multilayer colony growth. This growth indicates transformed cells that have lost contact inhibition, a hallmark of malignancy. The ability of MCA/TPA to consistently induce cell transformation further validates the sensitivity and robustness of the assay for detecting carcinogenic agents.

Given its capacity to identify both initiating and promoting effects, the BALB-CTA is an essential tool in carcinogenicity testing. Its use of the BALB/c 3T3 mouse fibroblast cell line enhances its relevance, as this line is particularly susceptible to transformation and offers a reliable system for evaluating potential oncogenes. The increased colony formation with MCA/TPA treatment emphasizes the assay's utility in identifying chemicals capable of inducing malignant transformation.

Conclusion

The comparative study on the cytotoxicity and potential negative impacts of the tested extracts on cell viability and metabolic activity indicates that the selected range of medicinal biological products specifically, the 300 kDa cellular extracts known as "Mito Organelles" from heart, brain, kidney, cartilage, thymus, placenta, lungs, connective tissue, and the combination LPPSIMKE (liver, pancreas, placenta, kidney, intestines, retina) showed no cytotoxic effects on human cells.

The assessment of the carcinogenic potential of these MOs cellular extracts via the BALB/c-3T3 cell transformation assay did not reveal any signs of malignant transformation or morphological changes in treated cell lines, suggesting no risk for cancer development.

Furthermore, the toxicity comparisons across different MOs cellular extracts did not indicate any negative effects on cell viability or transformation rates. The results obtained from both the MTT and BALB/c-3T3 assays support the safe use of MOs cellular extracts in biomedical research and therapeutic applications within regenerative medicine.

Funding

This research conducted in Institute of Anatomy and Cell Biology of the University of Heidelberg (Germany) was funded by European Wellness Biomedical Group.

Institutional review board statement

Not applicable.

Data availability statement

The data presented in this study are available in the study outlined.

Acknowledgments

None.

Conflicts of interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Citation: Chan MKS, Wong MBF, Klokol D, et al. Exploring the toxicity and carcinogenic potential of 300 KDA "Mito Organelles"™ cellular extracts through MTT and BALB/C-3T3 cell transformation assays: a vital component of peptide and protein biomedical research and safety study. *J Stem Cell Res Ther.* 2024;9(1):9–15. DOI: 10.15406/jsrt.2024.09.00166