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Exploring the Influence of Tartrazine, Betanin, and Curcumin on Gene Expression of DNA Methyltransferase and Histone Deacetylase Enzymes in vivo and in vitro model

Ph.D. Thesis booklet

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1. Introduction

Color additives have had extensive usage since 1500 BC and remain a pivotal aspect for food producers concerning consumer preferences. Among these, synthetic food colorants are notably favored due to their stability, cost-effectiveness, and ability to impart vibrant colors. Available in diverse types and hues, these artificial additives often enter our diets surreptitiously, emphasizing the crucial need to explore their biological impact. Grasping their potential effects on our health is vital in making well-informed decisions about what we consume. Color additives serve several main functions in food:

1) Counteracting color loss triggered by factors such as light, air, temperature, and storage conditions 2) Enhancing natural tones to elevate the visual attractiveness and appetizing nature of the food. 3) Adding color to foods that lack it naturally. 4) Facilitating easy visual identification of products, especially in pharmaceuticals, for consumers.

Lately, there's been increasing attention toward the potential toxicity of food additives, notably azo dyes, renowned for their vivid colors. The primary apprehension limiting their use revolves around the potential for carcinogenic effects. This risk emerges when these dyes undergo azoreduction by intestinal microbiota, forming carcinogenic byproducts.

In this research we demonstrate the negative effect of Tartrazine on key enzymes mediating the epigenetic regulation of gene expression, namely DNA methyltransferase (DNMT) and histone deacetylase (HDAC) in both In vivo NMRI mouse and in vitro studies on various humans cell lines, such as hepatoblastoma cell line (HepG2), adenocarcinomic human alveolar basal epithelial cells (A549), and a spontaneously transformed aneuploid immortal keratinocyte cell line from adult human skin (HaCaT) as well as the measuring the dose dependent chemoprotective effects of Betanin and Curcumin on multiple human cell line mentioned above after exposure of the cells to ultraviolet radiation through down regulation of DNMTs and HDACs enzyme.

2. Objectives

1. We are postulating that we can ascertain the minimum concentration at which mono-azo dye food colorants elevate the levels of HDACs and DNMTs mRNA in both cancerous and normal human cell lines. In contrast, it is anticipated that natural dyes will either decrease these levels or have no discernible effect when compared to the control group.

2. Consequently, we are hypothesizing that the expression patterns of the genes under investigation will markedly differ from those in untreated controls. This is anticipated to be associated with the degree of methylation in the promoter regions of these genes.
3. One of our objectives is to be examining the connection between tartrazine and the expression level of HDAC2, HDAC3, HDAC8 along with DNMT1, DNMT3a and DNMT3b through qRT-PCR analysis on a range of human cell lines. These includes those originating from both normal and cencerous tissues to evaluate whether there are differences in the expression patterns among the cell lines such as human hepatocellular carcinoma (HepG2), lung adenocarcinoma (A549), and immortalized keratinocyte (HaCat) cell lines.
4. Assessing the relationship between tartrazine and the expression levels of HDAC2, HDAC3, HDAC8, as well as DNMT1, DNMT3a, and DNMT3b using qRT-PCR in the lungs, kidneys, liver, and spleen of young male and female mice following both prolonged and brief exposure periods. This led to the discernible augmentation of DNMT and HDAC gene activity due to tartrazine exposure.
5. Investigating the antioxidative and cytoprotective properties of curcumin and betanin on various cell lines, including those derived from both normal and cancerous tissues such as human hepatocellular carcinoma (HepG2), lung adenocarcinoma (A549), and immortalized keratinocyte (HaCaT) cell lines. In addition to oxidative stress performed by UV radiation exposure and estimating the impact of curcumin and betanin on diverse gene expression patterns, indicative of epigenetic modifications.

3. Materials and methods

3.1. In vivo treatment protocol

Male and Female NMRI mice, aged between 6 to 8 weeks consumed rodent feed containing 1xADI of equivalent of human dosage of tartrazine (Human ADI = 7.5 mg/kg/day, which corresponds to 1.845 mg/day of tartrazine, for a mouse with an average body weight of 20 g) both heated and unheated for the duration of 30 and 90 days. Following the designated treatment period, cervical dislocation was carried out. Subsequently, biopsies were obtained from different organs including the lungs, liver, kidneys, and spleen during necropsy for total RNA isolation.

3.2. In vitro treatment protocol and total RNA isolation.

A549, HepG2, HaCaT cell lines, refreshed with new media, underwent treatment using treatment solution containing one of the food colorants namely, tartrazine, betanin or curcumin solutions to reach the final concentrations of (20, 40, 80 μM). Following treatment, we have selected betanin and curcumin food colorants to measure their efficacy on the DNMTs and HDACs gene expression compared to housekeeping gene HPRT1 after direct ultraviolet radiation from the distance of 2cm at various times (15, 30, 60 seconds).

The cells were incubated at 37°C for 24 hours. Post-incubation, the cells' condition was observed using a light microscope before isolating the RNA. Apart from our research regarding the photoprotective effect of betanin on HaCat cells which utilized the Maxwell® RSC Instrument (Promega, Wisconsin, USA) using a Maxwell® RSC RNA FFPE Kit (AS1440, Promega, Wisconsin, USA).

All our other experiments RNA isolation followed The RNA extraction process employed the following reagents: ExtraZol Tri-reagent (Nucleotest Bio Kft), Chloroform (Merck Sigma), isopropyl alcohol (Merck Supelco), 75% alcohol (derived from absolute ethanol; BioTech Hungary Kft), and 0.1% DEPC water (Diethylpirocarboxylic acid; Merck Sigma). All reagents were utilized in strict accordance with the manufacturers' instructions to ensure optimal and high-quality RNA extraction.

3.3. Q-RT-PCR protocol and equipment

The Roche Lightcycler 480 system was used to assess the total RNA. Assays were conducted on the Roche 480 instrument employing the KAPA SYBR FAST One-Step qRT-PCR Master Mix kit from Sigma (Budapest, Hungary). Each amplification was carried out in a 20 μL reaction volume, involving 5 μL of RNA target (ranging from 50–100 ng) mixed with 15 μL of the master mix containing forward and reverse primers (10 μL KAPA SYBR FAST qPCR Master Mix, 0.4 μL KAPA RT Mix, 0.4 μL dUTP, 0.4 μL primers at 200 nM, and 3.8 μL sterile double-distilled water). The reactions followed a thermal profile: a 5-minute step at 42 °C for reverse transcription, a hot-start denaturing phase at 95 °C for 3 minutes, succeeded by 45 cycles at 95 °C for 10 seconds (denaturation) and 60 °C for 20 seconds (annealing/extension). Software was used to analyze the fluorogenic signal emitted during the annealing–extension step. Immediately post-amplification, a melting curve protocol was initiated, increasing the temperature by 0.5 °C per cycle for 80 cycles, starting from the set-point temperature of 55.0 °C, each cycle lasting 10 seconds. Integrated DNA Technologies (Bio-Sciences)

synthesized the primers, and their sequences were designed via primer express software.

Through the utilization of a qRT-PCR high-throughput method for detecting and quantifying specific DNA sequences, we assessed the relative gene expression levels of DNMT1, DNMT3A, DNMT3B, HDAC2.HDAC3, HDAC5, and HDAC6. The housekeeping gene HPRT1 served as our internal control in this experimental study. The PCR outcomes were presented as Cp values, representing the point of intersection between the amplification curve and the threshold value. These Cp values were instrumental in computing the fold changes of the target genes in comparison to the control sample, employing the 2-ΔΔCp method (commonly known as the Livak method).

3.4. Statistical analysis

To evaluate our results, we employed a range of statistical examinations, such as ANOVA, Levene's F test, and subsequent post hoc analyses utilizing the Scheffe and LSD tests. Additionally, we used the Kolmogorov–Smirnov test to determine distribution and standard deviation. When analyzing the comet assay data, we utilized the Mann–Whitney and Kruskal–Wallis tests for statistical evaluation. All statistical assessments were carried out using IBM SPSS Statistics Version 26.0 for Windows (Armonk, NY, USA), with significance established at $p < 0.05$.

4. Results

4.1. In vivo tartrazine

In this study on NMRI mice, tartrazine (TRZ) was observed to increase the expression of epigenetics-related enzymes, specifically DNMT1, DNMT3a, and DNMT3b, alongside HDACs, in various organs (spleen, liver, lungs, and kidneys). The duration and temperature of TRZ treatment influenced gene expression differently across these enzymes. DNMT1 showed increased expression in most organs with prolonged and high-temperature TRZ treatments. DNMT3a expression was notably elevated in multiple organs with both durations and temperatures of TRZ treatment. DNMT3b expression showed less consistent changes but was notably increased in certain organs with longer or high-temperature TRZ treatments. Overall, TRZ exposure seemed to affect the expression of these epigenetic enzymes, with variations depending on treatment duration and temperature.

In this study on the impact of tartrazine (TRZ) on gene expression in different organs of mice, heightened levels of HDAC2, HDAC3, and HDAC8 were observed in the spleen,

liver, lungs, and kidneys following both 30- and 90-day treatments with room temperature TRZ. High-temperature TRZ treatments showed significant activation of these genes, especially HDAC2, in various organs. HDAC3 expression, although generally lower than HDAC2, was notably increased by TRZ, particularly in the liver and lungs after 90 days. HDAC8 expression was increased in several organs with room temperature TRZ, except for the kidneys, and showed varied responses with increased treatment duration. Overall, the study indicates that TRZ influenced the activity of genes in the HDAC family across all tested organs, with varying strengths of response depending on treatment duration, organ, and gene studied.

4.2. In vitro results

Tartrazine

The research focused on assessing the expression levels of epigenetic-associated enzymes (DNMT1, DNMT3a, and DNMT3b) in HaCaT, HepG2, and A549 cell cultures. Relative RNA expression was measured using HPRT1 as a reference gene for normalization. Tartrazine exposure (20 to 80 μM) significantly increased DNMT1 gene expression across all cell lines. In HaCaT and HepG2 cells, the elevation was dose-dependent, while A549 cells exhibited a consistent 3-fold increase.

The analysis of DNMT3a indicated a dose-dependent and statistically significant increase in gene expression in the HaCaT cell line when exposed to varying concentrations of TRZ. A noteworthy 3-fold rise was observed at 80 μM compared to the control. Similarly, the HepG2 cell line showed a significant dose-dependent elevation in DNMT3a expression, ranging from 2-fold to 4-fold. In the A549 cell line, a consistent 3-fold increase in gene expression was noted across all tested TRZ concentrations.

Exposure to TRZ led to an increased level of DNMT3b in all tested cell lines. In HaCaT cells, the gene expression showed a dose-dependent increase, reaching approximately 6-fold at 80 μM. HepG2 cells exhibited a constant value, with a 4-fold increase compared to the control. In the A549 cell line, DNMT3b expression nearly reached an 8-fold increase at 80 μM, showing 2-fold and 4-fold higher expression compared to 40 μM and 20 μM concentrations, respectively.

In the assessment of TRZ's impact on HDAC5 gene expression in HaCaT and HepG2 cell lines, a nearly fourfold significant increase was observed at 20 μM, with the expression plateauing even at higher concentrations. In A549 cells, HDAC5 gene expression reached an almost sixfold increase after exposure to 80 μM of TRZ.

A similar pattern to HDAC5 expression was observed in the HDAC6 gene in HaCaT and HepG2 cell lines, with an almost fourfold overexpression. In the A549 cell line, overexpression was noted at fourfold following exposure to 20 μM and 40 μM of TRZ, and a significant fivefold increase was observed after exposure to 80 μM of TRZ.

Betanin

The results showed that betanin affected the activity of all the genes tested in the HDAC and DNMT families, demonstrating its photoprotective effect. In addition, DNMT3B and HDAC6 might be early response genes to betanin treatment even after short periods of radiation.

In our comet assay experiments, we observed that the number of DNA lesions (tail moments) in the HaCaT cell line significantly increased after exposure to UV radiation. 2-fold and nearly 4-fold after 15 seconds and 60 seconds, respectively. Treatment with betanin dose-dependently ameliorated tail-moment growth under UV irradiation for 15 seconds, UV irradiation for 30 seconds, and UV irradiation for 60 seconds. The most significant reduction was detected in the 80 μM betanin treatment group compared to the 30 second positive control group. However, the 20, 40, and 80 μM betanin treatments significantly decreased the tail moments in a dose-dependent manner after 60 seconds of UV irradiation, which was the greatest increase.

Curcumin

HaCat cell line

This passage explores the effects of different durations of UV treatment on gene expressions (DNMT1, DNMT3a, DNMT3b, HDAC5, and HDAC6) and the subsequent impact of curcumin at various concentrations. Overall, UV exposure increased the expression of these genes, notably DNMT1, DNMT3a, DNMT3b, HDAC5, and HDAC6. However, curcumin, particularly at concentrations of 20, 40, and 80 μM/ml, demonstrated a dose-dependent ability to decrease the heightened gene expressions induced by UV treatment across these genes, except for certain cases in DNMT3a and HDAC6 expressions where resistance to curcumin's effects was observed in longer UV exposures.

HepG2 cell line

In this paragraph, the effects of different durations of UV treatment and curcumin on gene expression are discussed. DNMT1 expression increased with UV exposure, which was reduced by curcumin, particularly in longer exposures. DNMT3a expression

increased with 30- and 60-second UV treatments, not affected by curcumin. DNMT3b expression rose with UV exposure and was reduced by curcumin, especially in shorter exposures. HDAC5 expression increased with UV exposure, curcumin reduced it in varied degrees across exposure times. HDAC6 expression increased with UV exposure, and curcumin generally decreased it, particularly in shorter exposures, except for some cases with 60-second exposure where specific curcumin concentrations were effective.

A549 cell line

Interestingly, the control group, which was treated with only DMSO, had significantly higher expression of the *DNMT1* gene. Then, 20, 40, and 80 μM/ml curcumin solution significantly reduced the *DNMT1* expression. The DNMT1 expression inhibition was similar in UV light-treated groups.

measuring gene expression changes in response to treatments with curcumin and UV light exposure. DNMT3a expression was reduced by curcumin in UV-treated groups, while DNMT3b expression increased with extended UV exposure but was reversed by curcumin treatment. HDAC5 expression increased with longer UV exposure and was decreased by curcumin in those groups. HDAC6 expression increased with UV exposure duration and was reduced by curcumin treatment in a dose-dependent manner across all UV-treated groups.

5. Discussion

5.1. Tartrazine

The study delved into the effects of tartrazine, an azo dye commonly used in foods and cosmetics, on DNA epigenetic modifiers in various organs. Tartrazine, at acceptable daily intake (ADI) levels, showed potential risks related to DNA damage, oxidative stress, and alterations in biochemical profiles in organs like the liver and kidneys. It was found to increase the expression of DNMTs and HDACs, potentially leading to DNA hypermethylation and various health implications.

Tartrazine's breakdown products have been linked to oxidative stress, inflammation, and altered biochemical markers in rats' hepatic and renal structures, raising concerns about its impact on organ health. Additionally, there are suggestions that tartrazine might have negative effects on lung health, potentially exacerbating respiratory conditions like asthma by inducing oxidative stress, inflammation, and upregulating pro-inflammatory factors. Although limited, some studies hint at the possibility of tartrazine affecting neurological function due to its ability to cross the blood-brain barrier and impact DNMTs and HDACs. This connection prompts the need for further

investigation into tartrazine's potential long-term impact on brain function, especially at everyday doses within current regulatory limits.

The findings draw attention to the potential risks associated with tartrazine consumption, even at ADI levels, urging the need for comprehensive research to understand its effects on various organ systems, including the brain, and its potential role in health conditions like neurological disorders and behavioral deficits.

5.2. Betanin

The study focused on DNA methylation's significance in chromatin stability, gene expression, and genetic imprinting, particularly regarding DNA methyltransferases (DNMTs) like DNMT1, DNMT3a, and DNMT3b. Research indicates DNMT deficiencies contribute to tumor development, linking epigenetic changes to carcinogenesis. While UV radiation's impact on DNA mutations is known, its connection to epigenetic changes (epimutations) remains unclear, though UV exposure can alter DNA methylation in skin cells.

Their research specifically highlights the benefits of betanin treatment in HaCaT cell cultures, showing its ability to downregulate DNMT1, DNMT3a, and DNMT3b expressions. Betanin's high-concentration treatment exhibited radioprotective effects by inhibiting these target genes, potentially countering photo carcinogenesis induced by UV radiation and its associated epigenetic modifications.

Additionally, the study emphasizes DNMT3a and DNMT3b's roles in embryogenesis and cancer development, connecting increased DNMT expression with cell mitosis and carcinogenesis. Betanin is presented as a promising photoprotective compound against UV exposure, downregulating DNMT genes effectively.

The research also delves into histone deacetylases (HDACs), particularly HDAC5 and HDAC6, discussing their roles in cellular functions and diseases. Betanin was found to be an effective inhibitor of HDAC5 and HDAC6, indicating its potential as a therapeutic agent, offering photoprotective immune responses against UV irradiation.

In summary, the study underscores the influence of DNA methylation and histone deacetylation in gene regulation and disease development, presenting betanin as a promising agent for modulating epigenetic processes and countering the detrimental effects of UV radiation.

5.3. Curcumin

In this research, the focus was on analyzing the effects of curcumin, derived from turmeric, on gene expression patterns in different cell lines under UV radiation. The study found that curcumin treatment significantly countered the increase in gene expression induced by UV radiation in various cell lines. Specifically:

- In HaCat cells, curcumin suppressed the UV radiation-induced increase in gene expression.

- HepG2 cells showed decreased mRNA levels of specific genes related to UV radiation after curcumin treatment.

- A549 lung adenocarcinoma cells exhibited increased gene expression under UV radiation, which curcumin was able to suppress in a dose-dependent manner.

Other studies by Huiyang Deng and colleagues highlighted curcumin's ability to protect against UV radiation-induced photoaging in HaCat cells through antioxidant defense and NRF2 signaling pathways. Meanwhile, research by Masumeh Sanaei and team explored HDAC inhibitors' impact on inducing apoptosis in hepatocellular carcinoma cells, revealing that valproic acid treatment triggers apoptosis by activating specific pathways.

Overall, these findings suggest that curcumin may have a protective effect against UV radiation-induced damage and that HDAC inhibitors like valproic acid could potentially induce apoptosis in certain cancerous cells.

In a recent research, Youwei Zhang and colleagues explored the impact of epigallocatechin-3-gallate (EGCG) on small cell lung cancer cells resistant to cisplatin. Pre-treatment with EGCG followed by cisplatin led to significant tumor inhibition in vivo, suggesting demethylation induced by EGCG could offer a novel therapeutic approach for cisplatin-resistant cells.

Additionally, the study highlighted that elevated NF-κB levels in pancreatic cell lines increased DNMT1 expression. Targeting NF-κB, which is prevalent in pancreatic cancers, might impede DNMT1 and methylation processes. Curcumin, known for inhibiting NF-κB, shows promise in this regard.

The findings collectively support the idea that curcumin, a common food-derived substance, might counteract the effects of carcinogens across different cell lines and concentrations. It holds potential as a chemopreventive agent, possibly reducing cancer risk. However, translating in vitro results to in vivo conditions poses challenges due to

curcumin's limited solubility in water and lower concentrations when used as a food coloring. Ongoing experiments aim to improve dosing methods, like nano-curcumin formulations, to potentially enhance its chemopreventive effects even at lower doses.

6. Summary of new findings

6.1. Tartrazine can cause epigenetically alteration in animal model

After subjecting experimental animals to the human equivalent of the accepted daily intake of Tartrazine for 30 and 90 days, we noted alterations in the activity of all tested genes within the HDAC and DNMT families. Furthermore, the organs of the animals, including the liver, spleen, lungs, and kidneys, exhibited noticeable effects. However, the intensity of the response varied based on the specific time point, organ, and gene under consideration.

6.2. In various human cell lines Tartrazine can cause over expression of DNMTs and HDACs

In our study, we investigated the impact of different concentrations of tartrazine on human cell lines, specifically HaCat, HepG2, and A549. We assessed the expression levels of DNMTs and HDACs genes in comparison to the housekeeping gene HPRT1. Our findings indicate a noteworthy increase in gene expression levels for the mentioned genes upon exposure to tartrazine. At higher concentrations, this increase could reach up to eightfold in the tested cell lines.

6.3. Betanin Mitigates Epigenetic Processes and UV-Induced DNA Breakage in HaCaT Cells: Significance for Chemoprevention of Skin Cancer.

Treatment with betanin dose-dependently reduced DNA damage in cells exposed to 30 and 60 seconds of UV radiation. Within the tested concentration range, betanin exhibited no toxicity (20-80 μM). This finding suggested that betanin has potential as a protective agent against UV-induced DNA damage by reactivating silenced tumor suppressor genes in keratinocytes through epigenetic mechanisms.

6.4. Curcumin modulates gene expression alterations induced by UV radiation and exhibits antioxidant and chemopreventive capabilities in human cell lines.

The study found that curcumin alone did not affect gene expression, but exposure to UV radiation for 15, 30, and 60 seconds increased the expression of DNMT1, DNMT3a, DNMT3b, HDAC5, and HDAC6 genes. Adding curcumin at 20, 40, and 80 μM/ml doses dose-dependently reduced these elevated gene expressions. The results varied based on the UV exposure duration in HaCat, HepG2, A549 cell lines.

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8. Publikációs lista
List of Publication

8.1. A disszertáció témájához kapcsodó publikációk
List of publications related to dissertation

1. Zand, Afshin, Sodbuyan Enkhbilguun, John M. Macharia, Ferenc Budán, Zoltán Gyöngyi, and Timea Varjas. 2023. "Tartrazine Modifies the Activity of *DNMT* and *HDAC* Genes—Is This a Link between Cancer and Neurological Disorders?" *Nutrients* 15, no. 13: 2946.

2. Zand, Afshin, Sodbuyan Enkhbilguun, John M. Macharia, Krisztina Varajti, Istvan Szabó, Gellért Gerencsér, Boglárka Bernadett Tisza, Bence L. Raposa, Zoltán Gyöngyi, and Timea Varjas. 2024. "Betanin Attenuates Epigenetic Mechanisms and UV-Induced DNA Fragmentation in HaCaT Cells: Implications for Skin Cancer Chemoprevention" Nutrients 16, no. 6: 860.

8.2. A disszertáció téméjában konferencián elhangzott előadások és poszterek listája
Conference oral and poster presentation related to dissertation

1. Varjas, Timea; Zand, Afshin; Szabó, István; Ónozó, Réka; Raposa, Bence; Nowrasteh, Ghodratollah. Természetes és mesterséges színezékek hatásának összehasonlítása DNS-metil-transzferázok és hiszton-deacetilázok génexpressziós mintázatára mRNS szinten in vitro (2022) Magyar Higiénikusok Társasága - XLVII. vándorgyűlés 2022.

2. Afshin, Zand; Bence, Raposa; Timea, Varjas. The role of artificial and natural origin food dye in altering gene expression patterns of DNMTs and HDACs in vitro In: Biró, Lajos; Gelencsér, Éva; Lugasi, Andrea; Rurik, Imre (szerk.) Magyar Táplálkozástudományi Társaság XLV. Vándorgyűlése: Program füzet és összefoglalók Budapest, Magyarország: Magyar Táplálkozástudományi Társaság (2022) 77 p.p. 12

3. Zand, Afshin; Sodbuyan, Enkhbilguun; Varjas, Timea. CHEMOPROTECTIVE EFFECT OF BETANIN ON HUMAN KERATINOCYTES In: Biró, Lajos; Gelencsér, Éva; Lugasi, Andrea; Rurik, Imre; Simonné, Sarkadi Lívia (szerk.) Táplálkozástudományi kutatások XI. PhD konferencia Budapest, Magyarország: Magyar Táplálkozástudományi Társaság (2023) 37 p.pp. 13-13., 1 p.

4. Varjas, Tímea; Szabó, István; Nowrasteh, Ghodratollah; Raposa, Bence; Zand, Afshin. Betanin és azorubin hatásának összehasonlítása DNS-metil-transzferázok és hiszton-decetilázok génexpressziós mintázatára mRNS szinten in vitro (2023) Magyar Higiénikusok Társasága XLVIII. Vándorgyűlés 2023-09-05 Szentendre.

8.3. A disszertáció témájához közvetlenül nem köthető publikációk és konferencia részvételek jegyzéke
List of publication and posters not related to dissertation

1. Miklós, Poór; Afshin, Zand; Lajos, Szente; Beáta, Lemli; Sándor, Kunsági-Máté Interaction of α- and β-zearalenols with β-cyclodextrins MOLECULES 22: 11Paper: 1910 , 14 p. (2017) DOI WoS Scopus PubMed.

2. Poór, Miklós; Faisal, Zelma; Zand, Afshin; Bencsik, Tímea; Lemli, Beáta; Kunsági-Máté, Sándor; Szente, Lajos Removal of Zearalenone and Zearalenols from Aqueous Solutions Using Insoluble Beta- Cyclodextrin Bead Polymer TOXINS 10 : 6Paper: 216 , 12 p. (2018)

3. Hammoud, Sahar; Khatatbeh, Haitham; Zand, Afshin; Kocsis, Béla A survey of nurses' awareness of infection control measures in Baranya County, Hungary NURSING OPEN 8 : 6pp. 3477-3483. , 7 p. (2021)

4. Nyakundi, Patrick Nyamemba; Némethné Kontár, Zsuzsanna*; Kovács, Attila; Járomi, Luca; Zand, Afshin; Lohner, Szimonetta Fortification of Staple Foods for Household Use with Vitamin D: An Overview of Systematic Reviews NUTRIENTS 15 : 17Paper: 3742 , 29 p. (2023)

5. Nowrasteh, Ghodratollah; Zand, Afshin; Raposa, László Bence; Szabó , László; Tomesz, András; Molnár, Richárd; Kiss, István; Orsós, Zsuzsa; Gerencsér, Gellért; Gyöngyi, Zoltán et al. Fruit Extract, Rich in Polyphenols and Flavonoids, Modifies the Expression of DNMT and HDAC Genes Involved in Epigenetic Processes NUTRIENTS 15 : 8Paper: 1867 , 11 p. (2023)

6. Macharia, John M.; Kaposztas, Zsolt; Varjas, Tímea; Budán, Ferenc; Zand, Afshin; Bodnar, Imre; Bence, Raposa L. Targeted lactate dehydrogenase genes silencing in probiotic lactic acid bacteria: A possible paradigm shift in colorectal cancer treatment? BIOMEDICINE & PHARMACOTHERAPY 160 Paper: 114371 , 10 p. (2023)

7. Macharia, John M.; Mwangi, Ruth W.; Szabó , István; Zand, Afshin; Kaposztas, Zsolt; Varjas, Tímea; Rozmann, Nóra; Raposa, Bence L. Regulatory activities of Warbugia ugandensis ethanolic extracts on colorectal cancer-specific genome expression dose-dependently BIOMEDICINE & PHARMACOTHERAPY 166 Paper: 115325, 10 p. (2023)

8. Raposa, László Bence; Varjas, Tímea; Afshin, Zand; John, Machariaa. Warburgia ugandensis etanolos kivonatainak szabályozó hatása a vastag és végbélrák- specifikus genom dózisfüggő génexpressziójára in vitro In: Biró, Lajos; Gelencsér, Éva; Lugasi, Andrea; Rurik, Imre (szerk.) MAGYAR TÁPLÁLKOZÁSTUDOMÁNYI TÁRSASÁG XLVI. VÁNDORGYŰLÉSE PROGRAMFÜZET ÉS ÖSSZEFOGLALÓK (2023) Paper: 1170