ORİJİNAL MAKALE / ORIGINAL ARTICLE
Balıkesir Sağlık Bilimleri Dergisi / BAUN Sağ Bil Derg
Balıkesir Health Sciences Journal / BAUN Health Sci J
ISSN: 2146-9601- e ISSN: 2147-2238
Doi: https://doi.org/10.53424/balikesirsbd.1727811

Humic Acid Impairs Key Cellular Functions in Fibroblasts: Implications for Supplement Safety

Basak ISILDAR [1]

[1] Balikesir University, Faculty of Medicine, Histology and Embryology Department

Geliş Tarihi / Received: *26.06.2025,* ***Kabul Tarihi / Accepted:*** *24.07.2025*

ABSTRACT
Objective: Humic acid, the primary bioactive constituent of the traditional compound Shilajit, has been widely promoted for its therapeutic benefits. However, despite extensive interest in its anticancer effects, there remains a substantial lack of evidence regarding its cellular impact on non-cancerous cells. This study addresses this gap by evaluating the cytotoxic, apoptotic, migratory, and autophagic responses of 3T3 fibroblasts following humic acid exposure. **Materials and Methods:** 3T3 fibroblast cells were cultured under standard conditions and treated with various concentrations of humic acid for 24 hours to assess cytotoxicity using the MTT assay. The effects of humic acid on cell migration were evaluated using an in vitro scratch assay and apoptosis and autophagy were assessed by immunocytochemical detection of cleaved caspase-3 and Beclin-1, respectively. Data were analyzed statistically using appropriate tests. **Results:** Humic acid significantly reduced the viability of 3T3 fibroblast cells in a dose-dependent manner, with the lowest tested concentration (25 µg/mL) selected for further experiments. At this concentration, cell migration was markedly inhibited at 24 hours, although no significant difference was observed at 6 hours. Immunocytochemical analysis showed a significant increase in apoptosis, while autophagic activity tended to decrease, although the change was not statistically significant. **Conclusion:** These findings suggest that humic acid may have detrimental cellular effects and underline the importance of careful evaluation before its use in dietary supplements.
Keywords: Humic acid, 3T3 fibroblast cells, Cytotoxicity, Apoptosis, Autophagy.

Humik Asit, Fibroblastlarda Anahtar Hücresel Fonksiyonları Bozar: Diyet Takviyelerinin Güvenliği İçin Çıkarımlar

ÖZ
Amaç: Geleneksel bir bileşik olan Shilajit'in başlıca biyolojik etkili bileşeni humik asit, terapötik yararları nedeniyle yaygın şekilde kullanılmaktadır. Ancak antikanser etkilerine yönelik ilgi artmış olsa da, bağ dokusu gibi kanser dışı hücreler üzerindeki etkilerine dair literatürde ciddi bir bilgi eksikliği bulunmaktadır. Bu çalışma, humik asite maruz bırakılan 3T3 fibroblastlarında sitotoksik, apoptotik, migrasyon ve otofaji yanıtlarını değerlendirerek bu boşluğu doldurmayı amaçlamaktadır. **Gereç ve Yöntem:** 3T3 fibroblast hücreleri standart koşullarda kültüre edilerek 24 saat boyunca farklı konsantrasyonlardaki humik asit ile muamele edilmiştir. Sitotoksisite MTT testi ile, hücre göçü in vitro yara iyileşme testi ile; apoptoz ve otofaji ise sırasıyla kesilmiş kaspaz-3 ve Beclin-1'in immünositokimyasal tespiti ile değerlendirilmiştir. Veriler uygun istatistiksel testlerle analiz edilmiştir. **Bulgular:** Humik asit, 3T3 fibroblast hücrelerinin canlılığını doza bağlı olarak anlamlı şekilde azaltmıştır ve ileri deneylerde en düşük test edilen doz olan 25 µg/mL kullanılmıştır. Bu konsantrasyonda, 24. saatte hücre göçü belirgin şekilde baskılanmış, ancak 6. saatte anlamlı bir fark gözlenmemiştir. İmmünositokimyasal analizlerde apoptozda anlamlı bir artış saptanırken, otofajide gözlenen azalma istatistiksel olarak anlamlı bulunmamıştır. **Sonuç:** Elde edilen bulgular, humik asidin hücresel düzeyde zararlı etkiler oluşturabileceğini göstermekte ve bu maddenin diyet takviyesi olarak kullanılmadan önce dikkatli bir şekilde değerlendirilmesi gerektiğini ortaya koymaktadır.
Anahtar Kelimeler: Humik asit, 3T3 fibroblast hücreleri, Sitotoksisite, Apoptoz, Otofaji.

Sorumlu Yazar / Corresponding Author: Basak ISILDAR, Balıkesir University, Faculty of Medicine, Histology and Embryology Department, Balıkesir, Türkiye
E-mail: basakisildar@gmail.com

Bu makaleye atıf yapmak için / Cite this article: Isildar, B. (2025). Humic acid impairs viability, migration, autophagy, and apoptosis in murine fibroblasts: implications for the safety of dietary supplements. *BAUN Health Sci J, 14*(3), 574-580. https://doi.org/10.53424/balikesirsbd.1727811

INTRODUCTION

Humic acids are a group of high molecular weight polymers formed by the decomposition of plant material and are abundantly found in natural sources such as peat, soil, and groundwater (Lee et al., 2009). It is also the major active component of Shilajit (also known as Mumia), a traditional remedy used across cultures, now widely marketed as a natural dietary supplement. It consists of a complex mixture of structurally related aromatic polymers and typically appears as a pale brown to dark brown natural substance (Kamgar et al., 2023; Rahmani Barouji et al., 2020). Humic substances have been used for centuries in traditional medicine to treat a variety of health problems (Van Rensburg, 2015). When studies evaluating the pharmacological properties of humic acid are reviewed, it is observed that it has shown beneficial effects in the treatment of conditions such as inflammatory and cardiac disorders, viral infections, gastrointestinal diseases, dermatological conditions including dermatitis, and anemia (Güngen et al., 2012; Krzemiński et al., 2005; Wollina, 2009). In recent years, humic acids have gained renewed interest in the medical field owing to their broad range of biological activities, including anti-inflammatory, antiviral, antibacterial, anti-allergic, and anti-ulcerogenic properties (Schepetkin et al., 2002). Although humic acid is widely promoted for its therapeutic and detoxifying properties, emerging evidence suggests that it may also exert pro-carcinogenic effects under certain conditions (Lee et al., 2009). Several studies have shown that humic acid can enhance cancer cell proliferation, migration, and invasion, particularly at low or environmentally relevant concentrations. These effects appear to be mediated through oxidative stress, activation of multiple oncogenic signaling pathways (e.g., PI3K/Akt, ERK, JNK), and upregulation of metastasis-associated proteins such as MMP-2, MMP-9, and VEGF-A (Tsai et al., 2016). Although humic substances are often recognized for their ability to chelate heavy metals, questions remain regarding their own metal content and the limited evidence supporting their detoxifying capacity (Hussain & Saeed, 2024). These observations point to the need for further research into the complex nature of humic acid, particularly its cellular effects and the risks associated with its use in unregulated supplements.

Given the increasing use of humic acid in dietary supplements and the limited data on its cellular effects, this study aimed to evaluate the potential cytotoxic, apoptotic, migratory, and autophagic responses of murine fibroblast (3T3) cells to humic acid exposure. This investigation seeks to contribute to the growing body of evidence on the biological safety of natural supplements, particularly in the context of connective tissue cell models. The reason for evaluating the effects of humic acid in fibroblasts is that fibroblasts are among the human body's most widely distributed cell types, predominantly found in connective tissues throughout virtually all organs. They play a central role in maintaining tissue structure by producing and remodeling extracellular matrix components such as collagen, elastin, and glycosaminoglycans. In addition to their structural functions, fibroblasts actively participate in wound healing process, inflammation, and intercellular signaling, making them essential regulators of tissue homeostasis and repair (Plikus et al., 2021).

MATERIALS AND METHODS

Cell culture experiments

This study utilized the 3T3 Swiss albino cell line obtained from the American Type Culture Collection (ATCC). The cells were cultured at 37°C, under a humidified atmosphere with 5% CO_2 in a Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F12, Thermo Scientific, 11320074, USA) supplemented with 10% (v/v) Fetal Bovine Serum (FBS, Thermo Scientific, 10500064, USA) and 1% penicillin/streptomycin (Thermo Scientific, 15240062, USA). The humic acid solution used in the experiments was prepared by dissolving humic acid, sodium salt (Sigma, H16752-100G) in distilled water, followed by sterilization through a 0.22 µm filter prior to use in cell culture applications. Cells were observed under an inverted microscope (Euromex Oxion Inverso, Netherlands).

Dose analysis for humic acid

A dose-dependent cytotoxicity assay was conducted to assess whether humic acid exerts a cytotoxic effect on 3T3 fibroblast cells. The tested concentrations—0, 25, 50, 100, and 200 µg/mL—were selected based on previously published studies (Tsai et al., 2016), and cells were exposed to humic acid for 24 hours. The assay was performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (E-CK-A341, Elabscience) according to the manufacturer's protocol. The cells were seeded in 96-well plates at a density of 5×10^3 cells per well and were incubated under the specified culture conditions, and then treated with the determined dose of humic acid for 24 hours. At the end of the incubation period, 50 µL of working solution was added to each well and incubated for an additional three hours. Following the removal of the supernatant, 150 µL of dimethyl sulfoxide (DMSO) was added, and after brief shaking, absorbance was measured at 570 nm using a microplate reader (Thermo Labsystem, Franklin, ABD). Cell viability was calculated based on optical density (OD) values, and each condition was tested in three biological and three technical replicates to ensure reliability.

In vitro scratch assay

To evaluate the effects of humic acid on the migratory capacity of 3T3 fibroblast cells, an in vitro scratch assay was performed (Liang et al., 2007). Cells were seeded into 12-well plates under standard culture conditions, and scratch wounds were created using a

1000 µL pipette tip once the cells reached approximately 80% confluence. Subsequently, 25 µg/mL of humic acid was applied for 24 hours. Images of the wound area were captured using an inverted microscope at 0, 6, and 24 hours. The wound closure was quantified using Fiji ImageJ software, and migration distance was calculated as $\Delta X(\mu m) = X1 - X2$, where X1 is the initial wound width, and X2 is the wound width at the 6th/24th hour. The experiments were conducted in triplicate, and the data were analyzed accordingly.

Immunocytochemistry

The effects of humic acid on apoptosis and autophagy in 3T3 fibroblast cells were evaluated immunocytochemically. Cells were seeded in 24-well plates and cultured under standard conditions. At the end of the treatment period, the cells were fixed with 4% paraformaldehyde and processed for immunocytochemical analysis (Isildar et al., 2025). Following fixation, cells were washed with PBS, permeabilized using 0.1% Triton X-100 in PBS for 5 minutes, and washed again. A commercial blocking solution was applied to block nonspecific binding (Thermo Scientific TP-125-HL, USA). Primary antibodies specific to Beclin-1 (Thermo Scientific, PA116857, USA) and cleaved Caspase-3 (Thermo Scientific, 700182, USA) were diluted according to the manufacturer's instructions (1/100, 1/75, respectively) and incubated with the cells overnight at 4°C. After incubation, cells were rinsed with PBS, followed by the application of a biotinylated secondary antibody and streptavidin-peroxidase complex (Thermo Scientific TP-125-HL, USA). Visualization was achieved using a chromogenic substrate (Thermo Scientific TA-125-HA, USA), and the stained cells were examined under an Olympus BX53 microscope (Olympus, Tokyo, Japan) and photographed with a DP73 camera (Olympus, Tokyo, Japan) . Immunoreactivity was evaluated semi-quantitatively using the H-score method. For each staining, at least 100 cells were analyzed, and staining intensity was categorized as negative, weak, moderate, or strong. The H-score was calculated using the formula: (1 × % weakly stained) + (2 × % moderately stained) + (3 × % strongly stained) (Wen et al., 2024). Results were expressed as mean values and analyzed statistically.

Statistical analysis

Statistical analyses were carried out using SPSS version 20.0. Data were expressed as mean ± standard error of the mean (SEM). The distribution of the data was assessed for normality. Depending on the number of groups and sample size, either an independent t-test or one-way ANOVA was applied. A p-value less than 0.05 was considered indicative of statistical significance.

Ethical approval

This study does not require ethics committee approval as it does not involve data collection from human or animal subjects. The data for this study were collected between January 2025 and May 2025.

RESULTS

Dose analysis results

Cytotoxicity analysis revealed that humic acid reduced the viability of 3T3 cells to 47.06% at 25 µg/mL, 50.56% at 50 µg/mL, 50.82% at 100 µg/mL, and 57.06% at 200 µg/mL. Statistical analysis showed a significant difference among the groups ($p<0.01$). Pairwise comparisons indicated that all treatment groups differed significantly from the control, while no significant differences were observed between the dose groups themselves. Therefore, the subsequent experiments were conducted using the lowest concentration (25 µg/mL), which is also closer to levels potentially achieved through dietary supplementation. Figure 1a displays the inverted microscope image of 3T3 cells, and Figure 1b presents the mean viability results from the dose-dependent cytotoxicity experiments.

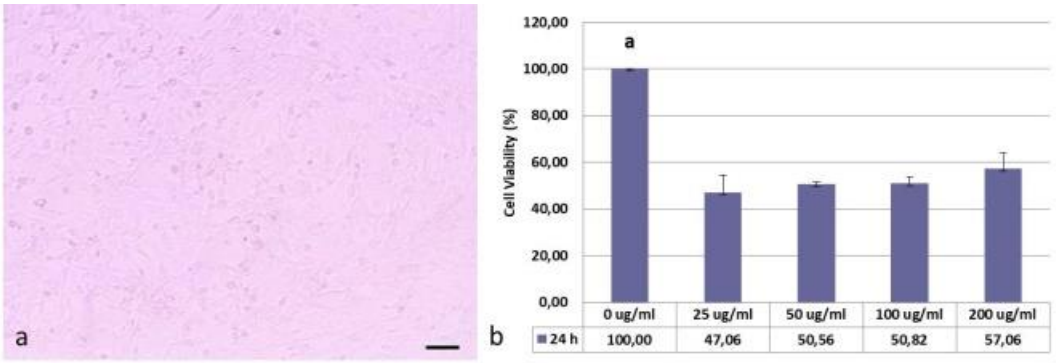

Figure 1. a) Inverted microscopic image of 3T3 fibroblast cells (10× magnification). b) Quantitative analysis of average cell viability (%) in 3T3 cells following humic acid treatment, [a]p<0.01. Scale bar = 40 µm.

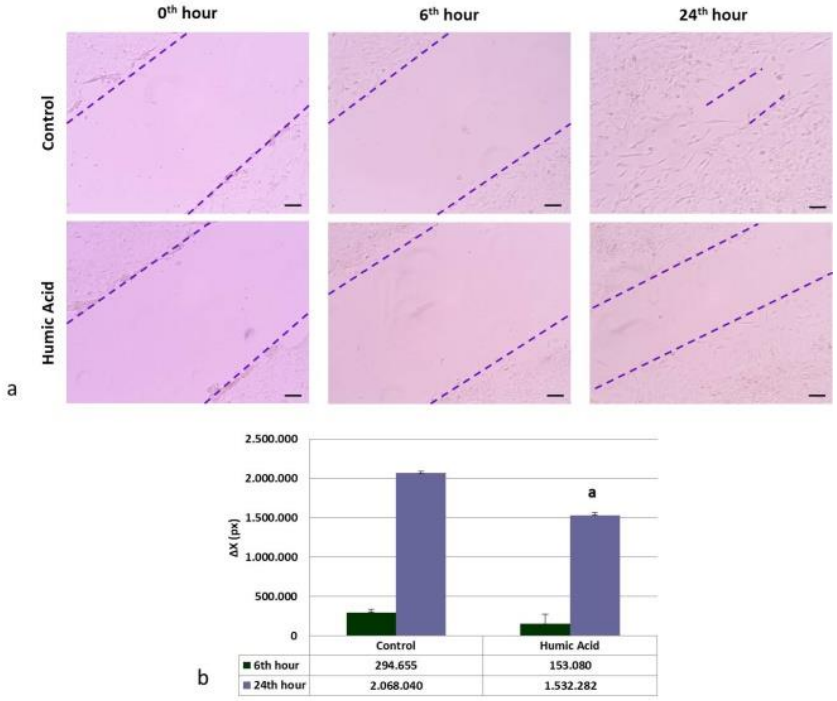

Figure 2. Representative micrographs (a) (10× magnification) and corresponding mean wound closure (Δx, in pixels) (b) from the scratch assay, [a]p<0.001. Scale bar = 40 µm.

Effects of humic acid on migration

The effect of humic acid on 3T3 cell migration was evaluated using an in vitro scratch assay. No significant difference was observed between the control and treatment groups at the 6th hour; however, a statistically significant difference was detected in wound closure at the 24th hour ($p<0.001$). Cell migration was markedly reduced in the humic acid-treated group. Representative images of the scratch assay and the corresponding graph illustrating the average migration distances (ΔX values in pixels) are presented in Figure 2.

Effects of humic acid on apoptosis and autophagy

The effects of humic acid on 3T3 cells were assessed via immunocytochemistry, focusing on apoptosis and autophagy.

Evaluation of the cleaved caspase-3 immunoreactivity using the H-score revealed a significant increase in apoptosis in humic acid-treated 3T3 cells ($p<0.05$) (Figure 3a–c). In contrast, Beclin-1-based analysis of autophagy showed a reduction in autophagic activity following humic acid exposure; however, this decrease did not reach statistical significance (Figure 3d–f).

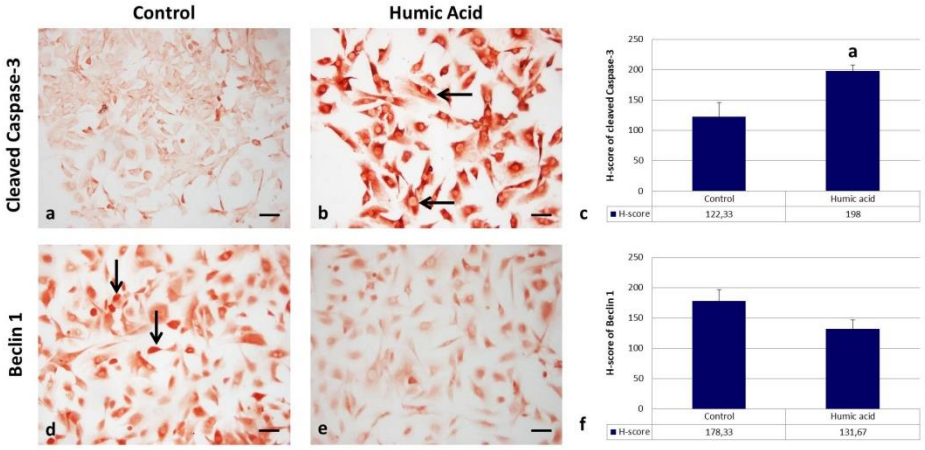

Figure 3. a-c) Apoptosis evaluation via cleaved caspase-3 immunocytochemical staining. a) Control group, b) Humic acid-treated group, c) H-score analysis of caspase-3 immunoreactivity, [a]p<0.05. d-f) Autophagy evaluation via beclin 1 immunocytochemical staining. Arrows indicate cells with strong expression. 20× magnification, scale bar = 50 µm.

DISCUSSION

Humic acid is a naturally occurring, high-molecular-weight organic compound derived from the decomposition of plant materials and commonly found in soil, peat, and groundwater. Traditionally used in folk medicine, it has gained attention for its broad pharmacological activities, including anti-inflammatory, antiviral, and antibacterial effects (Vašková et al., 2023). However, recent studies suggest that humic acid may also promote cancer cell proliferation and migration through oxidative stress mechanisms and activation of oncogenic signaling pathways (Lee et al., 2009). These findings underscore the need for further research into its cellular effects and potential health risks. Here, we evaluated the effects of humic acid on 3T3 murine fibroblast cells to assess its safety as a dietary supplement. Together, these findings highlight the need for caution regarding the unregulated use of humic acid-containing supplements, especially in the absence of standardized safety assessments and dose guidelines. The humic acid concentrations used in this study were selected based on data from the literature. In the study by Tsai et al., the applied doses (10–100 µg/mL) are considered environmentally relevant, as humic acid concentrations up to 200 µg/mL were detected in artesian well water, with daily exposure reaching 400 mg (Tsai et al., 2016). In another study, Swidsinski et al. reported oral supplementation with humic acid at doses up to 2400 mg/day in humans (Swidsinski et al., 2017). While direct extrapolation from oral intake to in vitro concentrations is not possible, these findings suggest that the selected doses broadly represent levels encountered through environmental exposure or dietary supplementation. All doses of 25, 50, 100, and 200 ug/mL used in this study showed cytotoxic effects on 3T3 cells and reduced viability by 50% on average. Notably, while some studies report the cytotoxic and apoptosis-inducing effects of humic acid, others suggest that it may promote cancer progression. This duality highlights that uncontrolled and medically inappropriate use of humic acid may not only contribute to carcinogenesis but also accelerate the progression of existing malignancies. In line with these concerns, Lu et al., in one of the few studies conducted on non-cancerous cells, showed that humic acid at 100 µg/mL induced neoplastic transformation in mouse epidermal JB6 Cl41 cells via reactive oxygen species (ROS) generation (Lu et al., 2006). Evaluation of the migration results revealed that humic acid treatment slowed the migration of 3T3 cells. Although in vivo studies have reported that humic acid accelerates wound healing, these effects are primarily attributed to its antibacterial and anti-inflammatory properties, as well as its ability to generate and bind oxygen radicals, rather than a direct influence on cell migration (Çalişir et al., 2018; Gheibi et al., 2024). Wound healing is a complex and multifactorial process. Therefore, while humic acid may contribute to tissue repair in vivo, it appears not to promote, and may even reduce, cell migration under culture conditions that do not fully reflect the biological complexity of wound healing.

Previous studies have shown that humic acid induces apoptosis in several cancer cell lines, including A375 human melanoma cells (Salehi et al., 2022), HepG2 hepatocellular carcinoma cells, and HL-60 promyelocytic leukemia cells (Yang et al., 2004). In HepG2 cells, this apoptotic response was also associated with reduced Beclin-1 expression and decreased autophagosome formation, indicating suppression of autophagy (Pant et al., 2016). In a study on macrophages, prolonged exposure to humic acid (25–200 µg/mL for 72 hours) led to increased apoptosis and cell cycle arrest, along with elevated p53 levels and signs of DNA damage (Yang et al., 2014). These results suggest that humic acid may trigger cell death pathways in immune cells and play a role in disease-related processes. Similarly, this study demonstrated that humic acid induces apoptosis and suppresses autophagy in the non-cancerous 3T3 fibroblast cell line. In another study, humic acid was shown to induce dose- and time-dependent apoptosis in human umbilical vein endothelial cells (HUVECs), as evidenced by DNA fragmentation, chromatin condensation, and caspase activation. This effect was mediated by oxidative stress, calcium signaling, p53 upregulation, and Bcl-2/Bax imbalance, and was reversed by antioxidants and calcium chelators (Hseu et al., 2002). Overall, these findings support the notion that humic acid can promote apoptosis and modulate autophagy-related pathways through oxidative and stress-related mechanisms across different cell types.

CONCLUSION

This study demonstrated that humic acid exerts cytotoxic effects even at low concentrations, slows cell migration, promotes apoptosis, and suppresses autophagy in 3T3 fibroblast cells. These findings highlight the potential risks associated with the uncontrolled use of humic acid as a dietary supplement—often consumed without medical supervision, precise dosing, or regulatory oversight. On the other hand, due to its potent biological activities, humic acid may hold promise as a candidate compound in the development of therapies aimed at inducing cell death in tumor cells. Future studies may further explore the dose-dependent cellular effects of humic acid in both normal and cancer cells, and assess its potential for therapeutic use when applied in a controlled and targeted way.

Acknowledgments

The author gratefully acknowledges the contributions of those involved in the establishment of the Meryem Şeremet Cell Culture Laboratory, Faculty of Medicine, Balıkesir University, where this study was conducted.

Conflict of Interest
The authors declare that they have no competing interests.

Author Contributions
Plan, design: BI; **Material, methods and data collection:** BI; **Data analysis and comments:** BI; **Writing and corrections:** BI.

Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Approval
This study does not require ethics committee approval as it does not involve data collection from human or animal subjects.

REFERENCES

Çalişir, M., Akpinar, A., Talmaç, A. C., Lektemur Alpan, A., & Göze, Ö. F. (2018). Humic Acid Enhances Wound Healing in the Rat Palate. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2018/1783513

Gheibi, N., Samiee-Rad, F., Sofiabadi, M., Mosayebi, E., & Shalbaf, Z. (2024). The Effect of Combining Humic and Fulvic Acids Poultice on Wound Healing in Male Rats. *Journal of Cutaneous and Aesthetic Surgery*, *17*(2), 105–111. https://doi.org/10.4103/JCAS.JCAS_92_23

Güngen, G., Ardic, F., Findkoglu, G., & Rota, S. (2012). The effect of mud pack therapy on serum YKL-40 and hsCRP levels in patients with knee osteoarthritis. *Rheumatology International*. https://doi.org/10.1007/s00296-010-1727-4

Hseu, Y. C., Huang, H. W., Wang, S. Y., Chen, H. Y., Lu, F. J., Gau, R. J., & Yang, H. L. (2002). Humic acid induces apoptosis in human endothelial cells. *Toxicology and Applied Pharmacology*. https://doi.org/10.1006/taap.2002.9429

Hussain, A., & Saeed, A. (2024). Hazardous or Advantageous: Uncovering the Roles of Heavy Metals and Humic Substances in Shilajit (Phyto-mineral) with Emphasis on Heavy Metals Toxicity and Their Detoxification Mechanisms. In *Biological Trace Element Research*. https://doi.org/10.1007/s12011-024-04109-4

Isildar, B., Ozkan, S., Neccar, D., & Koyuturk, M. (2025). Preconditioning and post-preconditioning states of mesenchymal stem cells with deferoxamine: A comprehensive analysis. *European Journal of Pharmacology*, *996*(April), 177574. https://doi.org/10.1016/j.ejphar.2025.177574

Kamgar, E., Kaykhaii, M., & Zembrzuska, J. (2023). A Comprehensive Review on Shilajit: What We Know about Its Chemical Composition. In *Critical Reviews in Analytical Chemistry*. https://doi.org/10.1080/10408347.2023.2293963

Krzemiński, T. F., Nozyński, J. K., Grzyb, J., Porc, M., Zegleń, S., Filas, V., Skopińska-Rózewska, E., Sommer, E., & Filewska, M. (2005). Angiogenesis and cardioprotection after TNFα-inducer-Tolpa Peat Preparation treatment in rat's hearts after experimental myocardial infarction in vivo. *Vascular Pharmacology*. https://doi.org/10.1016/j.vph.2005.06.003

Lee, W. J., Lu, F. J., Wang, S. F., Chen, Y. R., & Tseng, T. H. (2009). In vitro enhancement effect of humic acid on the progression of lung cancer cells. *Chemico-Biological Interactions*. https://doi.org/10.1016/j.cbi.2009.08.009

Liang, C. C., Park, A. Y., & Guan, J. L. (2007). In vitro scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. *Nature Protocols*. https://doi.org/10.1038/nprot.2007.30

Lu, F. J., Tseng, T. H., Lee, W. J., Yen, C. C., Yin, Y. F., Liao, C. W., & Liu, K. M. (2006). Promoting neoplastic transformation of humic acid in mouse epidermal JB6 Cl41 cells. *Chemico-Biological Interactions*. https://doi.org/10.1016/j.cbi.2006.07.007

Pant, K., Yadav, A. K., Gupta, P., Rathore, A. S., Nayak, B., & Venugopal, S. K. (2016). Humic acid inhibits HBV-induced autophagosome formation and induces apoptosis in HBV-transfected Hep G2 cells. *Scientific Reports*. https://doi.org/10.1038/srep34496

Plikus, M. V., Wang, X., Sinha, S., Forte, E., Thompson, S. M., Herzog, E. L., Driskell, R. R., Rosenthal, N., Biernaskie, J., & Horsley, V. (2021). Fibroblasts: Origins, definitions, and functions in health and disease. In *Cell*. https://doi.org/10.1016/j.cell.2021.06.024

Rahmani Barouji, S., Saber, A., Torbati, M., Fazljou, S. M. B., & Yari Khosroushahi, A. (2020). Health Beneficial Effects of Moomiaii in Traditional Medicine. *Galen Medical Journal*. https://doi.org/10.31661/gmj.v9i0.1743

Salehi, M., Piri, H., Farasat, A., Pakbin, B., & Gheibi, N. (2022). Activation of apoptosis and G0/G1 cell cycle arrest along with inhibition of melanogenesis by humic acid and fulvic acid: BAX/BCL-2 and Tyr genes expression and evaluation of nanomechanical properties in A375 human melanoma cell line. *Iranian Journal of Basic Medical Sciences*, *25*(4), 489–496. https://doi.org/10.22038/IJBMS.2022.60651.13444

Schepetkin, I., Khlebnikov, A., & Kwon, B. S. (2002). Medical drugs from humus matter: Focus on mumie. In *Drug Development Research*. https://doi.org/10.1002/ddr.10058

Swidsinski, A., Dörffel, Y., Loening-Baucke, V., Gille, C., Reißhauer, A., Göktas, O., Krüger, M., Neuhaus, J., & Schrödl, W. (2017). Impact of humic acids on the colonic microbiome in healthy volunteers. *World Journal of Gastroenterology*. https://doi.org/10.3748/wjg.v23.i5.885

Tsai, M. L., Yen, C. C., Lu, F. J., Ting, H. C., & Chang, H. R. (2016). Environmentally relevant concentration of arsenic trioxide and humic acid promoted tumor progression of human cervical cancer cells: In vivo and in vitro studies. *Environmental Toxicology*. https://doi.org/10.1002/tox.22121

Van Rensburg, C. E. J. (2015). The antiinflammatory properties of humic substances: A mini review. In *Phytotherapy Research*. https://doi.org/10.1002/ptr.5319

Vašková, J., Stupák, M., Vidová Ugurbaş, M., Žatko, D., & Vaško, L. (2023). Therapeutic Efficiency of Humic Acids in Intoxications. In *Life*. https://doi.org/10.3390/life13040971

Wen, Z., Luo, D., Wang, S., Rong, R., Evers, B. M., Jia, L., Fang, Y., Daoud, E. V., Yang, S., Gu, Z., Arner, E. N., Lewis, C. M., Solis Soto, L. M., Fujimoto, J., Behrens, C., Wistuba, I. I., Yang, D. M., Brekken, R. A., O'Donnell, K. A., … Xiao, G. (2024). Deep Learning–Based H-Score Quantification of Immunohistochemistry-Stained Images. *Modern Pathology*. https://doi.org/10.1016/j.modpat.2023.100398

Wollina, U. (2009). Peat: A natural source for dermatocosmetics and dermatotherapeutics. *Journal of Cutaneous and Aesthetic Surgery*. https://doi.org/10.4103/0974-2077.53094

Yang, H. L., Hseu, Y. C., Hseu, Y. T., Lu, F. J., Lin, E., & Lai, J. S. (2004). Humic acid induces apoptosis in human premyelocytic leukemia HL-60 cells. *Life Sciences*. https://doi.org/10.1016/j.lfs.2004.02.033

Yang, H. L., Huang, P. J., Chen, S. C., Cho, H. J., Kumar, K. J. S., Lu, F. J., Chen, C. S., Chang, C. T., & Hseu, Y. C. (2014). Induction of macrophage cell-cycle arrest and apoptosis by humic acid. *Environmental and Molecular Mutagenesis*. https://doi.org/10.1002/em.21897