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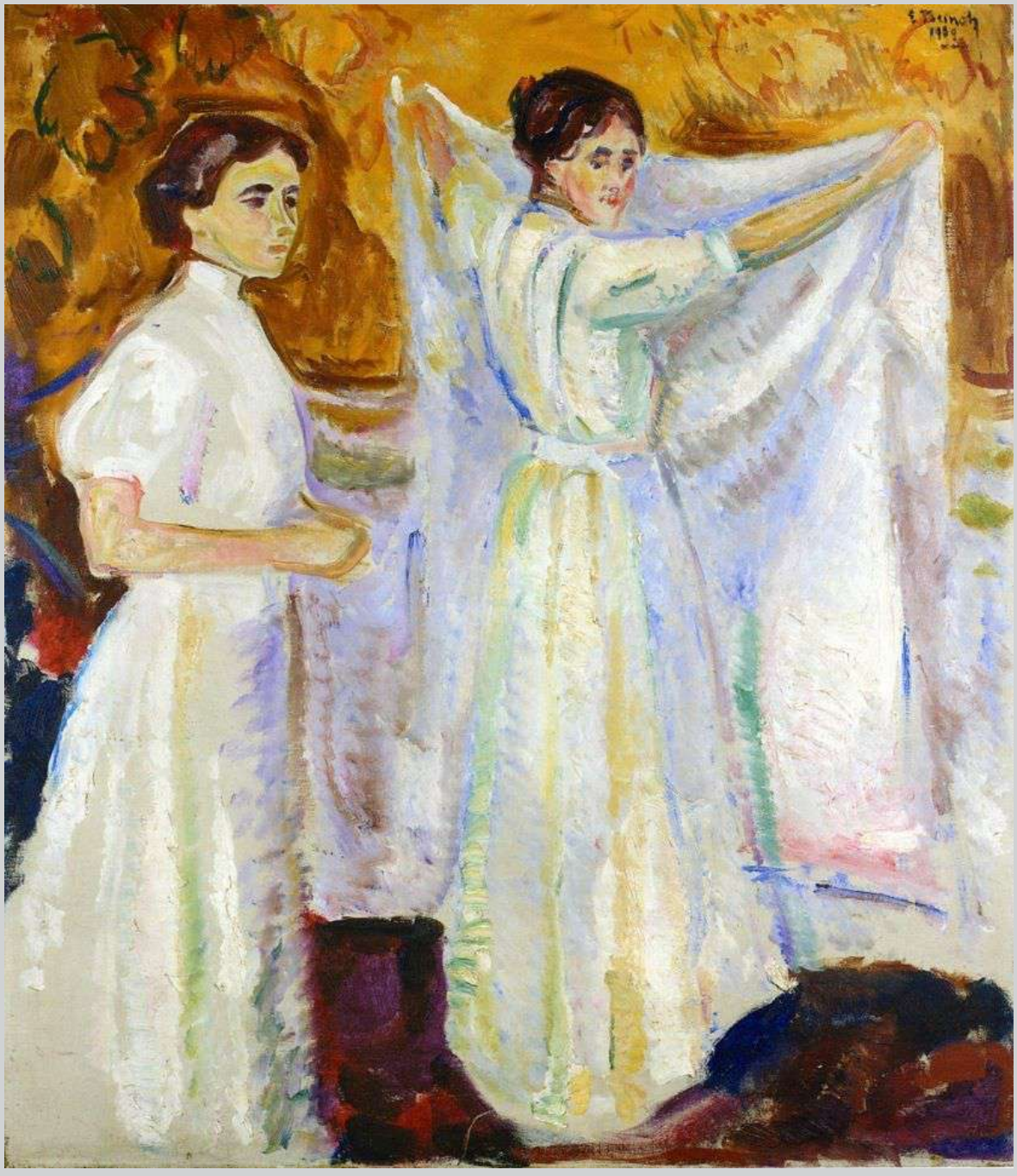

Edvard Munch
Two Nurses, 1909 / Oil on Canvas

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CONTENT

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COVER IMAGES

Edvard Munch,
Two Nurses, 1909 / Oil on Canvas

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EDITORIAL | Yeditepe Journal of Health Sciences, 13(3):110

A Year in Review: Reflections and Future Directions

Dear Colleagues,

We are pleased to present the third issue of the *Yeditepe Journal of Health Sciences*, which also constitutes the final issue of 2025. The past year has been both productive and inspiring, marked by dedicated efforts to establish the journal and ensure its continued development.

The cover artwork featured in this issue is *Two Nurses* by Edvard Munch, painted in 1909 during a period of hospitalization. Munch was a Norwegian painter and a prominent figure of the Expressionist movement, widely recognized for his iconic work *The Scream*. Throughout his life, he endured multiple illnesses, including severe and recurrent mental health conditions, which profoundly influenced his artistic expression. Regrettably, many of his works were criticized and undervalued during his lifetime; in some instances, patrons who commissioned his paintings were so dissatisfied that they returned them.

This issue includes a diverse selection of articles, ranging from drug-related research to studies involving medical students. As time passes swiftly—*verba volant, scripta manent*—our primary objective is to publish scholarly work grounded in knowledge and scientific rigor that will endure within the scientific record. Beyond publication, our foremost aspiration is for these articles to be widely read, critically evaluated, and, most importantly, cited by the scientific community. Increasing the visibility and citation of our publications is a key goal we look forward to pursuing in 2026.

As we bring the year to a close, I would like to extend my sincere gratitude to the authors who submitted their valuable work to our journal, to the reviewers for their meticulous and constructive evaluations, to the editorial team for their dedicated contributions, to **.doc,** the publisher of the journal, for its role in realizing this journal, and to our university administration for their continued support.

We extend our best wishes for a healthy, successful, and prosperous New Year, and hope that 2026 brings continued achievement and fulfillment to all.

Stay healthy,

Gülderen Yanıkkaya Demirel
Editor-in-Chief

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REVIEW ARTICLE | Yeditepe Journal of Health Sciences, 13(3):111-9

Testicular Seminoma and Hippo Signaling Pathway

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Abstract

The Hippo signaling pathway is a highly conserved regulator of tissue development and regeneration that controls organ size, primarily through the control of cell proliferation and apoptosis. Dysregulation of this pathway contributes to tumorigenesis in multiple human cancers; however, its role in testicular cancer—particularly seminoma—remains insufficiently characterized. Testicular germ cell tumors (TGCTs) are the most common malignancies in young adult men, with seminoma representing the predominant histological subtype. In this review, we summarize the molecular architecture of the Hippo signaling pathway and critically evaluate current evidence linking Hippo pathway components to testicular biology and seminoma pathogenesis, in accordance with the 2022 World Health Organization (WHO) classification of testicular tumors. Particular emphasis is placed on mixed germ cell tumors, the relative proportion of seminoma among TGCTs, and emerging therapeutic strategies targeting Yes-associated protein / Transcriptional coactivator with PDZ-binding motif (YAP/TAZ) signaling. We further integrate recent translational findings demonstrating the anti-cancer effects of verteporfin in human seminoma TCam-2 cells, highlighting the Hippo pathway as a promising and context-dependent therapeutic target in testicular seminoma.

Keywords: Testicular seminoma, Testicular germ cell tumors, Hippo signaling, pathway, YAP/TAZ, verteporfin.

TESTIS

The testis is the male gonad responsible for spermatogenesis and steroidogenesis (1,2). It consists of seminiferous tubules, where germ cell development occurs, and interstitial tissue containing Leydig cells that produce testosterone (3,4). Sertoli cells within the seminiferous tubules support germ cell differentiation and, through their tight interconnections, form the blood-testis barrier, which is essential for immune privilege and spermatogenic integrity (5-8). Leydig cells are the source of androgens or testosterone in males (9).

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Spermatogenesis and Spermiogenesis

Spermatogenesis is a highly regulated, multi-stage process involving mitotic proliferation, meiotic division, and post-meiotic differentiation (spermiogenesis), ultimately producing mature spermatozoa (4,10). Sertoli-germ cell interactions and tightly regulated signaling networks ensure proper progression of this process (11,12). The time required for the spermatogonia to develop into mature sperm is approximately 64 days (13). Spermiogenesis is the final phase of spermatogenesis which differentiation of haploid germ cells to motile, fertilization-competent spermatozoa occur.

TESTICULAR CANCER

Testicular cancer is the most common type of malignancy in men aged between 15 and 35 years (14,15) accounting for approximately 1% of all male cancers (16,17). The majority (>95%) are testicular germ cell tumors (TGCTs), whose pathogenesis involves a complex interplay between genetic susceptibility, disrupted germ cell differentiation, and environmental influences (16,17).

Testicular Cancer Development

Despite extensive research, the etiology of testicular germ cell tumors (TGCTs) remains incompletely understood. Both environmental and genetic factors have been implicated in TGCT development, with increased risk observed in individuals with cryptorchidism, familial TGCT history, Klinefelter syndrome, testicular dysgenesis, testicular atrophy, inguinal hernia, and hydrocele (18-21). Genome-wide association studies have further identified multiple single nucleotide polymorphisms associated with TGCT susceptibility, supporting a strong genetic contribution to disease risk (22,23). Although environmental influences such as androgen disruption and perinatal or lifestyle factors have been proposed, their direct relationship with TGCT remains unclear, suggesting that tumorigenesis likely arises from the combined effects of microenvironmental and epigenetic alterations (24-26).

Developmentally, TGCTs are thought to originate from germ cell neoplasia *in situ* (GCNIS), arising when primordial germ cells or gonocytes fail to differentiate into pre-spermatogonia during fetal or early postnatal development. This differentiation arrest may result from genetic abnormalities or exposure to endocrine-disrupting environmental factors, including anti-androgens and xenoestrogens (27-29). While it remains debated whether GCNIS originates from arrested spermatogonial cells or from reprogrammed adult germ cells, the high differentiation potential of adult spermatogonia lends support to the latter hypothesis (30).

Testicular Cancer Types

According to the 2022 World Health Organization (WHO) classification of tumors of the urinary system and male genital organs, testicular tumors are broadly categorized into germ cell tumors (GCTs), sex cord-stromal tumors, and a heterogeneous group of other rare tumors (31). Germ cell tumors account for more than 95% of all testicular malignancies and represent the most clinically significant category (16,32,33).

Testicular GCTs are further divided into two major biological groups based on their association with GCNIS:

- GCNIS-related tumors, which represent the vast majority of postpubertal TGCTs and include seminomas *and* non-seminomatous germ cell tumors *(*NSGCTs*)*, and
- Non-GCNIS-related tumors. which typically occur in prepubertal children or older adults and follow distinct pathogenetic mechanisms (34,35).

Seminoma is the most common histological subtype of TGCT, accounting for approximately 50–55% of cases (36). Seminomas are composed of relatively uniform cells resembling primordial germ cells or gonocytes and typically present in young to middle-aged adults. Histologically, seminomas are characterized by large polygonal cells with clear cytoplasm, centrally located nuclei, and prominent nucleoli, arranged in sheets or lobules separated by fibrous septa containing lymphocytic infiltrates (37). Serum tumor markers are usually normal, although mild elevation of β-human chorionic gonadotropin (β-hCG) may be observed due to the presence of syncytiotrophoblastic giant cells (36,37).

Non-seminomatous germ cell tumors comprise a heterogeneous group that includes embryonal carcinoma, yolk sac tumor, choriocarcinoma, and teratoma. These tumors may occur as pure forms but more commonly present as components of mixed germ cell tumors (38). Embryonal carcinoma is an aggressive malignant tumor composed of poorly differentiated epithelial cells with high mitotic activity (39). Yolk sac tumor is the most frequent TGCT in infancy and early childhood and is characterized by Schiller-Duval bodies and elevated alpha-fetoprotein (AFP) levels (40,41). Choriocarcinoma is a rare but highly aggressive tumor with early hematogenous dissemination and markedly elevated β-hCG levels (42-44). Teratomas consist of differentiated tissues derived from two or three embryonic germ layers and may be benign or malignant depending on patient age and associated tumor components (45).

Importantly, mixed germ cell tumors represent approximately 30–40% of all TGCTs and contain variable proportions of seminomatous and non-seminomatous elements

(33,38). The identification of mixed histology is clinically critical, as even a minor non-seminomatous component dictates treatment strategies and prognosis. Therefore, comprehensive histopathological sampling and accurate classification according to WHO criteria are essential for optimal patient management (42-44,46).

Sex cord-stromal tumors, including Leydig cell and Sertoli cell tumors, account for less than 5% of testicular neoplasms. These tumors are usually benign and hormonally active in some cases, leading to endocrine manifestations such as gynecomastia or precocious puberty (47). Malignant transformation is rare but has been reported, particularly in Leydig cell tumors (31,47).

Overall, the 2022 WHO classification emphasizes the biological heterogeneity of testicular tumors and highlights the importance of GCNIS status, mixed tumor composition, and precise histopathological diagnosis. This updated framework provides a critical foundation for understanding tumor behavior, guiding clinical decision-making, and interpreting molecular pathways—such as Hippo signaling—that may differentially contribute to seminoma and non-seminomatous TGCT pathogenesis (32,34).

HIPPO SIGNALING PATHWAY

The Hippo signaling pathway was originally identified in *Drosophila melanogaster* as a tumor-suppressive pathway and is highly conserved in mammals, including humans and mice (48). It functions as a central regulator of organ size and tissue homeostasis by integrating diverse upstream signals such as cell polarity, cell-cell contact, metabolic status, mechanical cues, and G-protein-coupled receptor signaling (48). Through these inputs, Hippo signaling limits cell proliferation, migration, and differentiation during development, while its dysregulation promotes abnormal tissue growth and tumorigenesis (48).

In mammals, Hippo signaling is mediated by a core kinase cascade in which mammalian STE20-like protein kinases 1/2 (MST1 and MST2 kinases), activated by autophosphorylation and upstream TAO kinases (TAOK1/2/3), phosphorylate and activate large tumor suppressor kinases 1/2 (LATS1/2) (48-52). The tumor suppressor neurofibromin 2 (NF2) (also known as Merlin) facilitates this process by recruiting LATS1/2 to the plasma membrane, enabling efficient mammalian STE20-like

Table 1. Hippo signaling pathway proteins and roles in cancer.

Protein	Class/Function	Role in active hippo pathway	Role in cancer	Reference
MST1/2 (STK4/3)	Serine/Threonine kinases	Phosphorylate and activate LATS1/2 and MOB1	Tumor suppressor (Inactivation promotes tumorigenesis)	(48)
SAV1	Adaptor protein/ Scaffold	Binds MST1/2 and LATS1/2 to facilitate LATS phosphorylation	Tumor suppressor	(49,50)
LATS1/2	Serine/Threonine kinases	Phosphorylate and inactivate YAP and TAZ	Tumor suppressor	(51)
MOB1A/B	Adaptor protein/ Cofactor	Associates with LATS1/2 to potentiate their kinase activity	Tumor suppressor	(52)
YAP	Transcriptional coactivator	Phosphorylated by LATS1/2, leading to cytoplasmic retention and degradation	Oncogene (Nuclear localization promotes cell proliferation/ survival)	(53-55)
TAZ (WWTR1)	Transcriptional coactivator	Phosphorylated by LATS1/2, leading to cytoplasmic retention and degradation	Oncogene (Nuclear localization promotes cell proliferation/ survival)	(56)
TEAD1-4	Transcription factors	Partner with YAP/TAZ in the nucleus to drive gene expression	Key oncogenic mediators of YAP/ TAZ activity	(57)

MST1/2: Mammalian STE20-like protein kinase 1/2, **STK4/3:** Serine/threonine kinase 4/3, **SAV1:** Salvador homolog 1, **LATS1/2:** Large tumor suppressor kinase 1/2, **MOB1A/B:** Mps one binder kinase activator-like 1A/1B, **YAP:** Yes-associated protein, **TAZ:** Transcriptional coactivator with PDZ-binding motif (WWTR1), **TEAD:** TEA domain transcription factor.

protein kinase 1/2 (MST1/2)-mediated phosphorylation (26). Activated LATS1/2 subsequently phosphorylate the transcriptional coactivators yes-associated protein (YAP) and (transcriptional coactivator with PDZ-binding motif) (TAZ), leading to their cytoplasmic retention or degradation and suppression of TEA domain transcription factor (TEAD)-dependent gene transcription (53,55-57). When Hippo signaling is inactive, unphosphorylated YAP and TAZ translocate to the nucleus, where they interact with TEAD transcription factors to induce genes involved in cell proliferation, migration, and survival (52-56) (Table 1).

The Hippo signaling pathway is organized by cell or tissue properties such as apicobasal polarity, mechanotransduction, cell-cell contact, and contact inhibition. Also, the Hippo signaling pathway and its components regulate very important processes such as cell viability, cell proliferation, cell competition, preservation of stem cell characteristics, regeneration, and metastasis (48). This pathway is conserved in mammals and has an important role in limiting tumor growth in cancer development. Regulation of the Hippo signaling pathway, therefore, presents a potential therapeutic case for treating cancer, but the targeted pathway needs to be explored in more detail (57-59).

Hippo Signaling Pathway in Male Reproductive System

Limited studies have examined Hippo signaling in the male reproductive system. In mice, genetic deletion of key Hippo components such as YAP, LATS1/2, or TAZ results in embryonic lethality, impaired postnatal development, or reduced fertility, highlighting their essential roles in testicular development and endocrine regulation (60-62). YAP and TAZ regulate genes involved in sex differentiation and early spermatogenesis, and Hippo pathway proteins have been identified in Sertoli cells across multiple species, where YAP controls cyclic AMP signaling, proliferation, and apoptosis (60,63). Although indirect evidence suggests a role for Hippo signaling in germ cell regulation, including miRNA-mediated inhibition of LATS2 and high YAP expression in spermatogonia (61). Its function in human testicular tissue remains unexplored, with no studies to date evaluating Hippo pathway protein expression in the normal human testis, aside from prostate cancer-related reports (64).

Hippo Signaling Pathway in Human Cancer

The Hippo signaling pathway functions as a central regulator of cellular homeostasis by integrating biochemical and mechanical cues to control proliferation, apoptosis, stemness, and tissue architecture. Canonically, activa-

Table 2. Hippo pathways inhibitors and key findings/status in cancer treatment.

Inhibitor/ Drug Class	Target(s)	Lead cancer indication(s) in trials	Key findings/Status	Clinical trial ID (NCT)	Reference
VT3989	YAP/TAZ-TEAD Interaction (Non-covalent TEAD Ligand)	Malignant mesothelioma (MPM), Non-small cell lung cancer (NSCLC), other advanced solid tumors	First-in-human Phase 1 trials showed early efficacy signals and durable responses in NF2-mutated and wild-type mesothelioma.	NCT04665206 (Clinical trial ID)	(77)
IAG933	YAP/TAZ-TEAD Interaction (Orally Bioavailable Inhibitor)	Various solid tumors with Hippo/YAP pathway activation.	Preclinical data shows potent, specific inhibition of the YAP/TAZ-TEAD axis and strong antitumor effects, positioning it for upcoming clinical studies.	NCT05590918 (Example trial ID for a related next-gen TEAD inhibitor)	(78)
VP	YAP/TAZ-TEAD Interaction (Disrupts complex, YAP degradation)	Glioblastoma, Pancreatic cancer, Ocular/Uveal melanoma (Mostly used in photodynamic therapy or PDT)	Preclinical studies show light-independent inhibition of YAP/TAZ function in many tumor types. Clinical trials mainly use it as a photosensitizer in PDT, but some Glioblastoma trials test its single-agent YAP-inhibitory activity.	NCT04590664 (Verteporfin for recurrent glioblastoma-light-independent)	(79)
MRK-A	YAP/TAZ-TEAD Interaction (Preclinical Compound)	Preclinical/Development (Focus on mesothelioma, glioblastoma, sarcoma)	Inhibits the YAP/TAZ-TEAD complex and suppresses tumor growth in NF2-deficient mesothelioma xenografts *in vivo*.		(80-82)

YAP: Yes-associated protein, **TAZ:** Transcriptional coactivator with PDZ-binding motif, **TEAD:** TEA domain transcription factor, **NF2:** Neurofibromin 2, **NSCLC:** Non-small cell lung cancer, **MPM:** Malignant pleural mesothelioma, **VP:** Verteporfin, **PDT:** Photodynamic therapy.

tion of the MST1/2-LATS1/2 kinase cascade restricts nuclear YAP/TAZ activity, thereby limiting TEAD-dependent transcription of genes that promote cell cycle progression and survival. Disruption of this regulatory axis through genetic mutations or functional suppression of upstream Hippo components leads to constitutive YAP/TAZ activation and uncontrolled cell proliferation, a phenomenon consistently observed in both *Drosophila* and mammalian tumor models (58,65-67).

Beyond proliferation, YAP and TAZ exert profound effects on tumor cell plasticity by promoting stem cell-like transcriptional programs. Elevated YAP/TAZ activity has been documented in embryonic, mesenchymal, and cancer stem cells, where it sustains self-renewal capacity and inhibits differentiation. Mechanistically, YAP/TAZ regulate pluripotency-associated gene networks and cooperate with TEAD transcription factors to maintain progenitor states, thereby increasing tumorigenic potential and resistance to therapy (65-68). These stemness-promoting effects are further reinforced by YAP/TAZ-mediated loss of contact inhibition and disruption of epithelial tissue architecture, both hallmark features of malignant transformation (69,70).

Hippo signaling is also a key mechanotransduction pathway that senses changes in extracellular matrix stiffness, cell-cell adhesion, and cytoskeletal tension. Mechanical inactivation of the Hippo kinase cascade results in nuclear accumulation of YAP/TAZ, which in turn drives transcriptional programs favoring invasion, migration, and metastatic progression. Increased YAP/TAZ activity has been correlated with aggressive and metastatic phenotypes in breast and prostate cancers, supporting a role for Hippo pathway dysregulation in tumor dissemination (70).

In regenerative contexts, transient suppression of Hippo signaling enables tissue repair by activating YAP/TAZ-dependent progenitor expansion. However, chronic or unrestrained activation of this regenerative program can promote oncogenesis, particularly in tissues with high regenerative capacity. Experimental models demonstrate that sustained YAP/TAZ activation during repeated injury or regeneration drives tumor formation, linking Hippo pathway dysregulation to regeneration-associated carcinogenesis (71-76).

Despite extensive evidence implicating Hippo signaling in diverse human cancers, its role in testicular tumors has

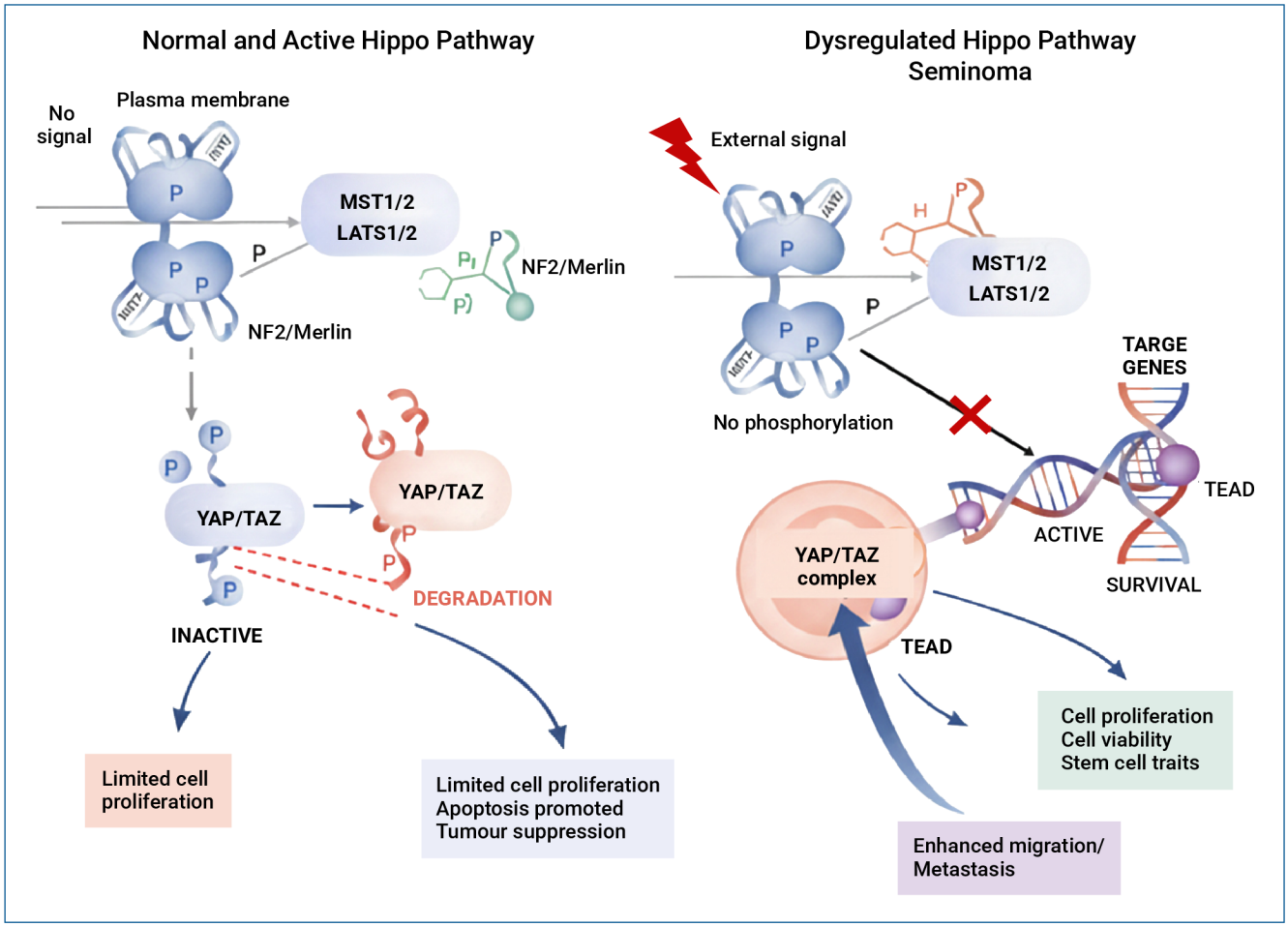

FIGURE 1. Normal and active Hippo signaling pathway with dysregulated Hippo pathway.

remained largely unexplored. In this context, our findings demonstrate that Hippo pathway components exhibit tissue-specific localization patterns in the human testis and that pharmacological inhibition of YAP-TEAD interaction by verteporfin suppresses proliferation and migration while inducing apoptosis in seminoma-derived TCam-2 cells. Notably, these effects occur primarily through post-transcriptional modulation and cytoplasmic sequestration of YAP/TAZ, highlighting a mechanistic vulnerability of seminoma cells to Hippo pathway targeting. Collectively, these data identify Hippo signaling as a context-dependent regulator of seminoma biology and support verteporfin as a promising therapeutic strategy for precision targeting of testicular cancer (77-82) (Table 2).

CONCLUSION

This review highlights the Hippo signaling pathway as a critical yet understudied regulator of testicular seminoma biology. Aberrant activation of YAP/TAZ has been implicated in tumor cell survival, proliferation, and therapy resistance across multiple cancer types, and emerging evidence suggests that similar mechanisms operate in seminoma. Importantly, experimental data using the seminoma-derived TCam-2 cell line demonstrate that pharmacological targeting of Hippo signaling with verteporfin exerts significant anti-cancer effects by suppressing YAP/TAZ activity and inducing apoptosis (77-82).

Collectively, these findings support the Hippo signaling pathway as a promising molecular target in testicular seminoma and provide a strong rationale for future translational and clinical studies aimed at precision therapy in TGCTs (Figure 1). Integration of WHO 2022 tumor classification with molecular pathway analysis will be essential for identifying patients most likely to benefit from Hippo pathway-directed therapeutic strategies.

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REFERENCES

1 Middendorff R, Müller D, Mewe M, Mukhopadhyay AK, Holstein AF, Davidoff MS. The tunica albuginea of the human testis is characterized by complex contraction and relaxation activities regulated by cyclic GMP. J Clin Endocrinol Metab. 2002;87(7):3486-99. [CrossRef]

2 Bertolotto M, Valentino M, Derchi LE. Imaging techniques and normal anatomy: scrotum. In: Hamm B, Ros PR, editors. Abdominal imaging. Berlin (Germany): Springer Berlin Heidelberg; 2013. p. 1851-60.

3 Heinrich A, DeFalco T. Essential roles of interstitial cells in testicular development and function. Andrology. 2020;8(4):903-14. [CrossRef]

4 Kaur G, Thompson LA, Dufour JM. Sertoli cells-immunological sentinels of spermatogenesis. Semin Cell Dev Biol. 2014;30:36-44. [CrossRef]

5 de Kretser DM, editor. Molecular biology of the male reproductive system. San Diego (CA): Academic Press; 1993.

6 França LR, Hess RA, Dufour JM, Hofmann MC, Griswold MD. The Sertoli cell: one hundred fifty years of beauty and plasticity. Andrology. 2016;4(2):189-212. [CrossRef]

7 Ni FD, Hao SL, Yang WX. Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis. Cell Death Dis. 2019;10(8):541. [CrossRef]

8 Cheng CY, Mruk DD. The blood-testis barrier and its implications for male contraception. Pharmacol Rev. 2012;64(1):16-64. [CrossRef]

9 Zirkin BR, Papadopoulos V. Leydig cells: formation, function, and regulation. Biol Reprod. 2018;99(1):101-11. [CrossRef]

10 Thakur RK, Yadav VK, Kumar A, Singh A, Pal K, Hoeppner L, et al. Non-metastatic 2 (NME2)-mediated suppression of lung cancer metastasis involves transcriptional regulation of key cell adhesion factor vinculin. Nucleic Acids Res. 2014;42(18):11589-600. [CrossRef]

11 Oatley JM, Brinster RL. Regulation of spermatogonial stem cell self-renewal in mammals. Annu Rev Cell Dev Biol. 2008;24:263-86. [CrossRef]

12 O'Donnell L. Mechanisms of spermiogenesis and spermiation and how they are disturbed. Spermatogenesis. 2015;4(2):e979623. [CrossRef]

13 Peschon JJ, Behringer RR, Brinster RL, Palmiter RD. Spermatid-specific expression of protamine 1 in transgenic mice. Proc Natl Acad Sci U S A. 1987;84(15):5316-9. [CrossRef]

14 Gilligan T. Testis cancer: rare, but curable with prompt referral. Cleve Clin J Med. 2007;74(11):817-25. [CrossRef]

15 Khan O, Protheroe A. Testis cancer. Postgrad Med J. 2007;83(984):624-32. [CrossRef]

16 Huyghe E, Plante P, Thonneau PF. Testicular cancer variations in time and space in Europe. Eur Urol. 2007;51(3):621-8. [CrossRef]

17 Regalado Porras GO, Chávez Nogueda J, Poitevin Chacón A. Chemotherapy and molecular therapy in cervical cancer. Rep Pract Oncol Radiother. 2018;23(6):533-9. [CrossRef]

18 Faja F, Finocchi F, Carlini T, Rizzo F, Pallotti F, Spaziani M, et al. PDE11A gene polymorphism in testicular cancer: sperm parameters and hormonal profile. J Endocrinol Invest. 2021;44(10):2273-84. [CrossRef]

19 Litchfield K, Levy M, Dudakia D, Proszek P, Shipley C, Basten S, et al. Rare disruptive mutations in ciliary function genes contribute to testicular cancer susceptibility. Nat Commun. 2016;7:13840. [CrossRef]

20 di Pietro A, Vries EG, Gietema JA, Spierings DC, de Jong S. Testicular germ cell tumours: the paradigm of chemo-sensitive solid tumours. Int J Biochem Cell Biol. 2005;37(12):2437-56. [CrossRef]

21 Lutke Holzik MF, Rapley EA, Hoekstra HJ, Sleijfer DT, Nolte IM, Sijmons RH. Genetic predisposition to testicular germ-cell tumours. Lancet Oncol. 2004;5(6):363-71. [CrossRef]

22 Chung CC, Kanetsky PA, Wang Z, Hildebrandt MA, Koster R, Skotheim RI, et al. Meta-analysis identifies four new loci associated with testicular germ cell tumor. Nat Genet. 2013;45(6):680-5. [CrossRef]

23 LeBron C, Pal P, Brait M, Dasgupta S, Guerrero-Preston R, Looijenga LH, et al. Genome-wide analysis of genetic alterations in testicular primary seminoma using high resolution single nucleotide polymorphism arrays. Genomics. 2011;97(6):341-9. [CrossRef]

24 Béranger R, Le Cornet C, Schüz J, Fervers B. Occupational and environmental exposures associated with testicular germ cell tumours: systematic review of prenatal and life-long exposures. PLoS One. 2013;8(10):e77130. [CrossRef]

25 Cook MB, Akre O, Forman D, Madigan MP, Richiardi L, McGlynn KA. A systematic review and meta-analysis of perinatal variables in relation to the risk of testicular cancer--experiences of the son. Int J Epidemiol. 2010;39(6):1605-18. [CrossRef]

26 Looijenga LH, Van Agthoven T, Biermann K. Development of malignant germ cells - the genvironmental hypothesis. Int J Dev Biol. 2013;57(2-4):241-53. [CrossRef]

27 Ponti G, Ponzoni M, Ferreri AJ, Foppoli M, Mazzucchelli L, Zucca E. The impact of histopathologic diagnosis on the proper management of testis neoplasms. Nat Clin Pract Oncol. 2008;5(10):619-22. [CrossRef]

28 Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16(5):972-8. [CrossRef]

29 Rajpert-De Meyts E. Developmental model for the pathogenesis of testicular carcinoma *in situ:* genetic and environmental aspects. Hum Reprod Update. 2006;12(3):303-23. [CrossRef]

30 Zhang XS, Zhang ZH, Jin X, Wei P, Hu XQ, Chen M, et al. Dedifferentiation of adult monkey Sertoli cells through activation of extracellularly regulated kinase 1/2 induced by heat treatment. Endocrinology. 2006;147(3):1237-45. [CrossRef]

31 Accardo G, Vallone G, Esposito D, Barbato F, Renzullo A, Conzo G, et al. Testicular parenchymal abnormalities in Klinefelter syndrome: a question of cancer? Examination of 40 consecutive patients. Asian J Androl. 2015;17(1):154-8. [CrossRef]

32 Sesterhenn IA, Davis CJ Jr. Pathology of germ cell tumors of the testis. Cancer Control. 2004;11(6):374-87. [CrossRef]

33 McIver SC, Roman SD, Nixon B, Loveland KL, McLaughlin EA. The rise of testicular germ cell tumours: the search for causes, risk factors and novel therapeutic targets. F1000Res. 2013;2:55. [CrossRef]

34 Looijenga LH, Gillis AJ, Stoop H, Biermann K, Oosterhuis JW. Dissecting the molecular pathways of (testicular) germ cell tumour pathogenesis; from initiation to treatment-resistance. Int J Androl. 2011;34(4 Pt 2):e234-51. [CrossRef]

35 Bahrami A, Ro JY, Ayala AG. An overview of testicular germ cell tumors. Arch Pathol Lab Med. 2007;131(8):1267-80. [CrossRef]

36 Reuter VE. Origins and molecular biology of testicular germ cell tumors. Mod Pathol. 2005;18 Suppl 2:S51-60. [CrossRef]

37 Marko J, Wolfman DJ, Aubin AL, Sesterhenn IA. Testicular seminoma and its mimics: from the radiologic pathology archives. Radiographics. 2017;37(4):1085-98. [CrossRef]

38 Ronchi A, Pagliuca F, Franco R. Testicular germ cell tumors: the changing role of the pathologist. Ann Transl Med. 2019;7(Suppl 6):S204. [CrossRef]

39 Pang S, Zhang L, Shi Y, Liu Y. Unclassified mixed germ cell-sex cord-stromal tumor with multiple malignant cellular elements in a young woman: a case report and review of the literature. Int J Clin Exp Pathol. 2014;7(8):5259-66.

40 Jamshidi P, Taxy JB. Educational case: yolk sac (endodermal sinus) tumor of the ovary. Acad Pathol. 2020;7:2374289520909497. [CrossRef]

41 Burns MJ, Zheng L, Dalla-Pozza L, Graf NS, Walton J, Tumuluri K. Yolk sac tumours of the orbit and sinonasal tract. Orbit. 2022;41(6):680-6. [CrossRef]

42 Jiang F, Xiang Y, Feng FZ, Ren T, Cui ZM, Wan XR. Clinical analysis of 13 males with primary choriocarcinoma and review of the literature. Onco Targets Ther. 2014;7:1135-41. [CrossRef]

43 Krege S, Beyer J, Souchon R, Albers P, Albrecht W, Algaba F, et al. European consensus conference on diagnosis and treatment of germ cell cancer: a report of the second meeting of the European Germ Cell Cancer Consensus group (EGCCCG): part I. Eur Urol. 2008;53(3):478-96. [CrossRef]

44 Krege S, Beyer J, Souchon R, Albers P, Albrecht W, Algaba F, et al. European consensus conference on diagnosis and treatment of germ cell cancer: a report of the second meeting of the European Germ Cell Cancer Consensus Group (EGCCCG): part II. Eur Urol. 2008;53(3):497-513. [CrossRef]

45 Gică N, Peltecu G, Chirculescu R, Gică C, Stoicea MC, Serbanica AN, et al. Ovarian germ cell tumors: pictorial essay. Diagnostics (Basel). 2022;12(9):2050. [CrossRef]

46 Chung P, Warde P. Testicular cancer: germ cell tumours. BMJ Clin Evid. 2016;2016:1807. [CrossRef]

47 Schultz KA, Harris AK, Schneider DT, Young RH, Brown J, Gershenson DM, et al. Ovarian Sex Cord-Stromal Tumors. J Oncol Pract. 2016;12(10):940-6. [CrossRef]

48 Ready D, Yagiz K, Amin P, Yildiz Y, Funari V, Bozdag S, et al. Mapping the STK4/Hippo signaling network in prostate cancer cell. PLoS One. 2017;12(9):e0184590. [CrossRef]

49 Zhao Z, Xiang S, Qi J, Wei Y, Zhang M, Yao J, et al. Correction of the tumor suppressor Salvador homolog-1 deficiency in tumors by lycorine as a new strategy in lung cancer therapy. Cell Death Dis. 2020;11(5):387. [CrossRef]

50 de Amorim ÍSS, de Sousa Rodrigues MM, Mencalha AL. The tumor suppressor role of salvador family WW domain-containing protein 1 (SAV1): one of the key pieces of the tumor puzzle. J Cancer Res Clin Oncol. 2021;147(5):1287-97. [CrossRef]

51 Moroishi T, Hayashi T, Pan WW, Fujita Y, Holt MV, Qin J, et al. The Hippo pathway kinases LATS1/2 suppress cancer immunity. Cell. 2016;167(6):1525-39.e17. [CrossRef]

52 Nishio M, Sugimachi K, Goto H, Wang J, Morikawa T, Miyachi Y, et al. Dysregulated YAP1/TAZ and TGF-β signaling mediate hepatocarcinogenesis in Mob1a/1b-deficient mice. Proc Natl Acad Sci U S A. 2016;113(1):E71-80. [CrossRef]

53 Abylkassov R, Xie Y. Role of Yes-associated protein in cancer: An update. Oncol Lett. 2016;12(4):2277-82. [CrossRef]

54 Yuan M, Tomlinson V, Lara R, Holliday D, Chelala C, Harada T, et al. Yes-associated protein (YAP) functions as a tumor suppressor in breast. Cell Death Differ. 2008;15(11):1752-9. [CrossRef]

55 Kim HS, Nam JS. The multifaceted role of YAP in the tumor microenvironment and its therapeutic implications in cancer. Exp Mol Med. 2025;57(10):2201-13. [CrossRef]

56 Chen F, Su J, Liu Y, Zhang Z, Li S, Yuan Y, et al. Targeting YAP/TAZ-TEAD and their protein-protein interaction for precision cancer therapy. Eur J Med Chem. 2026;302(Pt 2):118330. [CrossRef]

57 Pobbati AV, Kumar R, Rubin BP, Hong W. Therapeutic targeting of TEAD transcription factors in cancer. Trends Biochem Sci. 2023;48(5):450-62. [CrossRef]

58 Harvey KF, Zhang X, Thomas DM. The Hippo pathway and human cancer. Nat Rev Cancer. 2013;13(4):246-57. [CrossRef]

59 Liu AM, Wong KF, Jiang X, Qiao Y, Luk JM. Regulators of mammalian Hippo pathway in cancer. Biochim Biophys Acta. 2012;1826(2):357-64. [CrossRef]

60 Morin-Kensicki EM, Boone BN, Howell M, Stonebraker JR, Teed J, Alb JG, et al. Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65. Mol Cell Biol. 2006;26(1):77-87. [CrossRef]

61 Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell. 2006;124(6):1169-81. [CrossRef]

62 McPherson JP, Tamblyn L, Elia A, Migon E, Shehabeldin A, Matysiak-Zablocki E, et al. Lats2/Kpm is required for embryonic development, proliferation control and genomic integrity. EMBO J. 2004;23(18):3677-88. [CrossRef]

63 Kjærner-Semb E, Ayllon F, Furmanek T, Wennevik GE, Dahle M, Schulz RW, et al. The Hippo pathway co-factor VGLL3 regulates Sertoli cell function and testis maturation across vertebrates. Sci Rep. 2018;8(1):1912. [CrossRef]

64 Zhao B, Tumaneng K, Guan KL. The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. Nat Cell Biol. 2011;13(8):877-83. [CrossRef]

65 Pan D. The hippo signaling pathway in development and cancer. Dev Cell. 2010;19(4):491-505. [CrossRef]

66 Zhao B, Li L, Lei Q, Guan KL. The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version. Genes Dev. 2010;24(9):862-74. [CrossRef]

67 Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, et al. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell. 2011;147(4):759-72. [CrossRef]

68 Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the roots of cancer. Cancer Cell. 2016;29(6):783-803. [CrossRef]

69 Mo JS, Park HW, Guan KL. The Hippo signaling pathway in stem cell biology and cancer. EMBO Rep. 2014;15(6):642-56. [CrossRef]

70 Lamar JM, Stern P, Liu H, Schindler JW, Jiang ZG, Hynes RO. The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. Proc Natl Acad Sci U S A. 2012;109(37):E2441-50. [CrossRef]

71 Meng Z, Moroishi T, Guan KL. Mechanisms of Hippo pathway regulation. Genes Dev. 2016;30(1):1-17. [CrossRef]

72 Boggiano JC, Vanderzalm PJ, Fehon RG. Tao-1 phosphorylates Hippo/MST kinases to regulate the Hippo-Salvador-Warts tumor suppressor pathway. Dev Cell. 2011;21(5):888-95. [CrossRef]

73 Poon CL, Lin JI, Zhang X, Harvey KF. The sterile 20-like kinase Tao-1 controls tissue growth by regulating the Salvador-Warts-Hippo pathway. Dev Cell. 2011;21(5):896-906. [CrossRef]

74 Hergovich A, Schmitz D, Hemmings BA. The human tumour suppressor LATS1 is activated by human MOB1 at the membrane. Biochem Biophys Res Commun. 2006;345(1):50-8. [CrossRef]

75 Yin F, Yu J, Zheng Y, Chen Q, Zhang N, Pan D. Spatial organization of Hippo signaling at the plasma membrane mediated by the tumor suppressor Merlin/NF2. Cell. 2013;154(6):1342-55. [CrossRef]

76 Chan EH, Nousiainen M, Chalamalasetty RB, Schäfer A, Nigg EA, Silljé HH. The Ste20-like kinase Mst2 activates the human large tumor suppressor kinase Lats1. Oncogene. 2005;24(12):2076-86. [CrossRef]

77 Yap TA, Kwiatkowski DJ, Dagogo-Jack I, Offin M, Zauderer MG, Kratzke R, et al. YAP/TEAD inhibitor VT3989 in solid tumors: a phase 1/2 trial. Nat Med. 2025;31(12):4281-90. [CrossRef]

78 Chapeau EA, Sansregret L, Galli GG, Chène P, Wartmann M, Mourikis TP, et al. Direct and selective pharmacological disruption of the YAP-TEAD interface by IAG933 inhibits Hippo-dependent and RAS-MAPK-altered cancers. Nat Cancer. 2024;5(7):1102-1120. Erratum in: Nat Cancer. 2024;5(7):1130. [CrossRef]

79 Read RD. Repurposing the drug verteporfin as anti-neoplastic therapy for glioblastoma. Neuro Oncol. 2022;24(5):708-10. [CrossRef]

80 Papavassiliou KA, Sofianidi AA, Papavassiliou AG. YAP/TAZ-TEAD signalling axis: A new therapeutic target in malignant pleural mesothelioma. J Cell Mol Med. 2024;28(8):e18330. [CrossRef]

81 Cunningham R, Hansen CG. The Hippo pathway in cancer: YAP/TAZ and TEAD as therapeutic targets in cancer. Clin Sci (Lond). 2022;136(3):197-222. [CrossRef]

82 Cunningham R, Jia S, Purohit K, Fairley MN, Maniak MK, Lin Y, et al. Pipeline to evaluate YAP-TEAD inhibitors indicates TEAD inhibition represses *NF2*-mutant mesothelioma. Life Sci Alliance. 2025;8(10):e202503241. [CrossRef]

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Exploring the Antimutagenic and Antigenotoxic Potential of *Arbutus unedo* L. Fruits

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Abstract

Objective: This study aimed to comprehensively evaluate the phytochemical composition, antioxidant capacity, genoprotective potential, and genotoxic safety of *Arbutus unedo* L. (strawberry tree) fruit ethanolic (EtOH) and aqueous extracts.

Materials and Methods: Ethanolic and aqueous extracts of *A. unedo* fruits were analysed for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity using cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. High-performance thin-layer chromatography (HPTLC) was employed to check the presence of some specific phenolic compounds. Genotoxicity and genoprotection were assessed using the Ames test, alkaline comet assay, and cytokinesis-block micronucleus (CBMN) assay in Chinese hamster ovary (CHO-K1) cells.

Results: The EtOH extract showed significantly higher TPC (46.46 ± 0.56 mg gallic acid equivalent [GAE]/g) and TFC (14.23 ± 1.18 mg catechin equivalent [CE]/g) than the aqueous extract (TPC: 33.66 ± 1.80 mg GAE/g; TFC: 8.90 ± 0.24 mg CE/g), which was consistent with stronger antioxidant activity in the CUPRAC (114.86 ± 5.33 mg Trolox equivalent [TE]/g), FRAP (47.48 ± 1.70 mg TE/g), and DPPH (89.86 ± 0.83 mg TE/g) assays. No mutagenic or genotoxic effects were detected in either extract in all genotoxicity tests. Moderate antimutagenic activity was observed only at the highest concentration (5000 µg/plate) of the EtOH extract in the TA100 strain with S9 metabolic activation in the Ames test. However, no significant DNA protection was observed against doxorubicin-induced damage in either the comet or micronucleus assays.

Conclusion: *Arbutus unedo* fruit extracts, particularly the EtOH extract, are rich in phenolic antioxidants and do not exhibit genotoxic effects under the tested conditions. These findings support the safe use of *A. unedo* fruits in the food industry and their potential as natural sources of antioxidants.

Keywords: *Arbutus unedo*, antioxidant activity, micronucleus assay, Comet Assay, Ames test

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INTRODUCTION

Arbutus unedo L., commonly referred to as the strawberry tree, belongs to the genus *Arbutus* in the *Ericaceae* family and is predominantly distributed throughout the Mediterranean region. Its distribution extends across Northeastern Africa, the Canary Islands and Western Asia, Western, Central, and Southern Europe as well as the Mediterranean region of Türkiye (1). The species typically thrives on arid, rocky slopes and hillsides or within pine forests, particularly in the Taurus mountains, from sea level up to elevations of 600 meters (2). *Arbutus unedo* is an evergreen perennial that can appear as either a shrub or a small tree. While its usual height ranges from 1.5 to 3 meters, it can reach up to 9 meters under favorable environmental conditions (3). Uniquely, the plant bears both fruit and flowers simultaneously during the winter months. Its inflorescences are composed of clustered, small, cream-colored, lantern-shaped flowers. The alternate leaves are simple and oblanceolate with a dark green hue, leathery texture, short petioles, and serrated margins. The fruits are globular, orange-red, rough in texture, and can reach up to 2 cm in diameter (1,4).

Wild edible plants like *A. unedo* play a significant role in traditional diets and community health practices. Its fruits have historically been incorporated into regional foods and beverages, including fermented drinks and fruit preserves. Extracts obtained from *A. unedo* fruits have demonstrated various pharmacological effects, such as antioxidant, anti-inflammatory, antimicrobial and antiproliferative activities (1,3). Triterpenoids such as lupeol and betulinic acid isolated from its fruit extracts have shown inhibitory effects on cyclooxygenase-2 (COX-2) activity (5). Additionally, the leaves are known for their astringent properties, antiplatelet effects, and potential urinary antiseptic, anti-inflammatory, antidiarrheal, antihypertensive, and antidiabetic activities. In traditional folk medicine, *A. unedo* has been used for its antiseptic, diuretic, and laxative properties, as well as for managing arterial hypertension (6). Despite its established health benefits and wide traditional use, the genotoxic safety profile of *A. unedo* fruit extracts remains insufficiently studied. Given that certain phytochemicals may exert genotoxic effects under specific conditions, a comprehensive safety evaluation is warranted. Therefore, the present study aims to assess the mutagenic/antimutagenic and genotoxic/antigenotoxic effects of aqueous and ethanolic extracts prepared from the ripe fruits of *A. unedo*, using pharmacognostic and toxicological approaches to provide a comprehensive safety profile.

MATERIALS AND METHODS

Plant Material

The ripe fruits of *A. unedo* L. were collected in October 2022 from the vicinity of the Yeditepe University campus, located in the Ataşehir district of Istanbul, Türkiye. The plant species was identified by one of us (HK) and a voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, Istanbul, Türkiye (Herbarium No: YEF 22043).

Preparation of Extracts

A 400 g of fresh fruits were cleaned and lyophilized in order to remove water. For the ethanol extract (EtOH), 20 g of ground sample was macerated in 200 mL of EtOH at room temperature for 3 days, followed by extraction at 40°C for 3 hours. The mixture was then filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator. The concentrated extract was dispersed in H2O, frozen in refrigerator and subsequently lyophilized (yield: 30.15%). For the aqueous extract (infusion), 20 g of ground material was mixed with 200 mL of boiling distilled water. After 15 minutes, the mixture was filtered, and lyophilized (yield: 44.14%).

Phytochemical Analysis

High-Performance Thin-Layer Chromatography (HPTLC)

Sample test solutions were prepared at a concentration of 20 mg/mL in methanol (MeOH) and applied as 5 µL bands onto 20 × 10 cm glass-backed HPTLC plates (Merck, Darmstadt, Germany) precoated with silica gel 60 F_{254}. Standard compound solutions of isoquercitrin, myricitrin, quercitrin and arbutin (50 µg/mL) was applied as 2 µL bands (8 mm in length) using a 100 µL Hamilton syringe and a semi-automated Linomat 5 sample applicator (CAMAG, Muttenz, Switzerland). Chromatographic separation was performed in a twin-trough chamber pre-saturated for 20 minutes with a mobile phase consisting of ethyl acetate, dichloromethane, acetic acid, formic acid, and water in a ratio of 100:25:10:10:11 (v/v/v/v/v). The plate was developed to a distance of 7 cm, then dried using a stream of cold air for 2 minutes. For derivatization, the developed plate was heated at 105°C for 3 minutes using a TLC plate heater III (CAMAG, Muttenz, Switzerland). Subsequently, it was sequentially derivatized by immersion in NP reagent (prepared by dissolving 1 g of 2-aminoethyl diphenylborinate in 200 mL of ethyl acetate) and PEG reagent (10 g of polyethylene glycol 400 in 200 mL of dichloromethane) using the chromatogram immersion

device (CAMAG, Muttenz, Switzerland). The derivatized plate was then photographed under 366 nm UV light using an HPTLC imaging system. All procedures were performed using WinCATS software (CAMAG, version 128 1.4.8.2031). Identification of the compounds in the samples was achieved based on their retardation factor (R_f) values and fluorescence band characteristics (7).

Total Phenolic Content (TPC)

In a 96-well plate, 25 µL of each sample (2.5 mg/mL), gallic acid (standard) or water (blank) was mixed with 125 µL of 10% Folin-Ciocalteu reagent and 100 µL of 7.5% NaHCO3. After 30 minutes incubation in the dark at room temperature, absorbance was measured at 760 nm. Results were expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g) (7,8).

Total Flavonoid Content (TFC)

Total flavonoid content was determined using sodium nitrite, aluminium chloride and sodium hydroxide. In a 24-well plate, 200 µL of aqueous extract or catechin (standard), and 50 µL of ethanolic extract (20 mg/mL) were tested. After sequential addition of 30 µL 5% $NaNO_2$, 30 µL 10% $AlCl_3$, 200 µL 1 M NaOH, and 340 µL water, mixtures were incubated at room temperature. Absorbance was measured at 510 nm. Results were expressed as mg catechin equivalents per gram dry extract (mg CE/g) (9).

Antioxidant Activity Assays

DPPH Radical Scavenging Capacity Assay

In a 96-well plate, 20 µL of extract, ethanol (blank), or Trolox (standard) was mixed with 280 µL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol. After incubation in the dark for 30 minutes, absorbance was measured at 520 nm. Results were expressed as mg Trolox equivalents per gram extract (mg TE/g) (10).

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

In a 96-well plate, 30 µL of sample, standard, or blank was mixed with 80 µL each of 1×10^{-2} M $CuCl_2$, 7.5×10^{-3} M neocuproine, and 1 M ammonium acetate buffer, followed by 30 µL methanol. After 30 min at room temperature, absorbance was measured at 450 nm. Results were expressed as mg TE/g extract (11,12).

Ferric Reducing Antioxidant Power (FRAP) Assay

In a 96-well plate, 20 µL of sample, Trolox standard, or blank were mixed with 280 µL of FRAP reagent ($FeCl_3$, TPTZ, sodium acetate buffer, 1:1:10). After incubation at room temperature for 6 minutes, absorbance was measured at 593 nm. Results were expressed as mg TE/g extract (13).

Genotoxicity Assessment

Mutagenicity and Antimutagenicity Assays

The bacterial reverse mutation assay (Ames test) was performed using *Salmonella typhimurium* strains TA98 (frameshift mutations) and TA100 (base-pair substitutions), with and without metabolic activation (±S9), following Maron and Ames (14). Aqueous and ethanolic *A. unedo* fruit extracts (10–5000 µg/plate) were tested. A maximum concentration of 5000 µg/plate was selected, in line with OECD Test Guideline 471 recommendations for non-cytotoxic and non-toxic substances. Serial dilutions were then applied to determine concentration-dependent effects. Dimethyl sulfoxide (DMSO) served as the negative control; 4-nitro-o-phenylenediamine (NPD) and sodium azide (SA) as positive controls for –S9 (TA98/TA100), and 2-aminofluorene (2-AF) for +S9.

Each test plate contained the test compound or control, bacterial culture, top agar, and either phosphate buffer (–S9) or S9 mix (+S9). After incubation at 37°C for 48 hours, revertant colonies were counted. All experiments were performed in triplicate; results were expressed as mean ± SD.

The mutagenic index (MI) was calculated as follows:
MI = A/B

where A = average number of revertant colonies in the presence of sample, B = average number of revertant colonies in the negative control. An MI value of ≥2 was considered indicative of a mutagenic effect.

For the antimutagenicity assay, the number of revertant colonies on plates containing only the mutagen was considered as 100% (0% inhibition). The percentage inhibition of mutagenicity was calculated using the following formula (Formula 1):

Formula 1= (A–B) / (A–C) × 100

where A = average number of revertants with mutagen only, B = average number of revertants with mutagen and test sample, C = average number of spontaneous revertants. Inhibition rate of 40% or more was defined as strong antimutagenicity, 25–40% inhibition as moderate antimutagenicity. Inhibitory effects of less than 25% were considered as weak and were not recognized as a positive result (15). Both mutagenicity and antimutagenicity experiments were performed in triplicate for all concentrations, as well as for the negative and positive controls.

Genotoxicity and Antigenotoxicity Assessment

Cell Line and Culture Conditions

Chinese hamster ovary (CHO-K1) cells (ATCC® CCL-61™) were cultured in Ham's F-12 medium (Gibco, NY,

USA) supplemented with 10 % fetal bovine serum (FBS; Gibco, NY, USA) and 1% penicillin-streptomycin (Gibco, NY, USA). Cells were maintained in a humidified incubator at 37°C with 5% CO_2 and sub-cultured every three days to ensure exponential growth.

The genotoxic and antigenotoxic potential of *A. unedo* extracts were evaluated in CHO-K1 cells using the alkaline comet assay and the cytokinesis-block micronucleus (CBMN) assay. The test concentrations were determined by first performing a range-finding cytotoxicity assay in CHO-K1 cells. The highest concentration tested (500 µg/mL) represented the maximum non-cytotoxic dose, and lower concentrations were selected in descending order to evaluate dose-response relationships. The comet assay was carried out to assess DNA strand breaks following the protocol previously described with slight modifications (16).

Alkaline Comet Assay

CHO-K1 cells (3 × 10^5 cells/well) were seeded in 6-well plates and treated with *A. unedo* extracts (10–500 µg/mL) for 4 hours. Dimethyl sulfoxide (DMSO; 0.5%) and 1 µM doxorubicin were used as negative and positive controls. After treatment, cells were centrifuged (1500 rpm, 5 minutes), resuspended in PBS and mixed with 1% low-melting agarose. This mixture was layered onto slides pre-coated with 1.5% high-melting agarose. After solidification, slides were lysed (1 h, cold lysis buffer), unwound in electrophoresis buffer (20 minutes, 4°C), and electrophoresed at 25 V and 300 mA for 20 minutes at 4°C. Slides were neutralized in 0.4 M Tris-HCl (pH 7.5), fixed with methanol, air-dried, and stained with ethidium bromide. DNA damage was scored under fluorescence microscope (BS 200 ProP, BAB Imaging System, Ankara, Türkiye) (17).

Cytokinesis-Block Micronucleus Assay

CHO-K1 cells (2 × 10^5 cells/well) were seeded in 6-well plates and treated with aqueous or ethanolic *A. unedo* extracts (10–500 µg/mL) for 24 hours. Dimethyl sulfoxide (0.5%) and 1 µM doxorubicin served as controls. After treatment, cells were rinsed and incubated with cytochalasin B (4.8 µg/mL) for 24 hours. Cells were then fixed in methanol:acetic acid (3:1), stained with 5% Giemsa, and examined microscopically (Nikon, Tokyo, Japan) at 40× magnification.

At least 1000 binucleated (BN) cells per condition were scored for micronucleus (MN) frequency and nuclear division index (NDI) using the following formula (Formula 2):

Formula 2= MN (%) = (Number of MN / Number of scored BN cells) × 100.

$$NDI = [M_1 + 2(M_2) + 3(M_3) + 4(M_4)] / N$$

where M_1- M_4 represent the number of cells with one to four nuclei, and N is the total number of cells scored (16).

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way, two-way, or three-way analysis of variance (ANOVA) followed by Dunnett's post hoc test in GraphPad Prism version 10.0.0 (GraphPad Software, San Diego, CA, USA). A p value <0.05 was considered statistically significant.

RESULTS

Phytochemical Analysis

The chemical fingerprint profile of *A. unedo* fruit extracts was evaluated using HPTLC and compared to standard reference compounds, as shown in Figure 1. Among the standards used, no spots were observed that match with myricitrin and arbutin in the HPTLC chromatograms. However, a band matching the migration position of isoquercitrin (Rf ≈ 0.26) was observed in both ethanolic and aqueous extracts, indicating its presence in these samples. Similarly, a fluorescent band corresponding to quercitrin (Rf ≈ 0.42) was detected in both extracts.

To confirm the presence of isoquercitrin and quercitrin, the UV spectra of the reference spots and the corresponding spots were recorded using a TLC Scanner 3

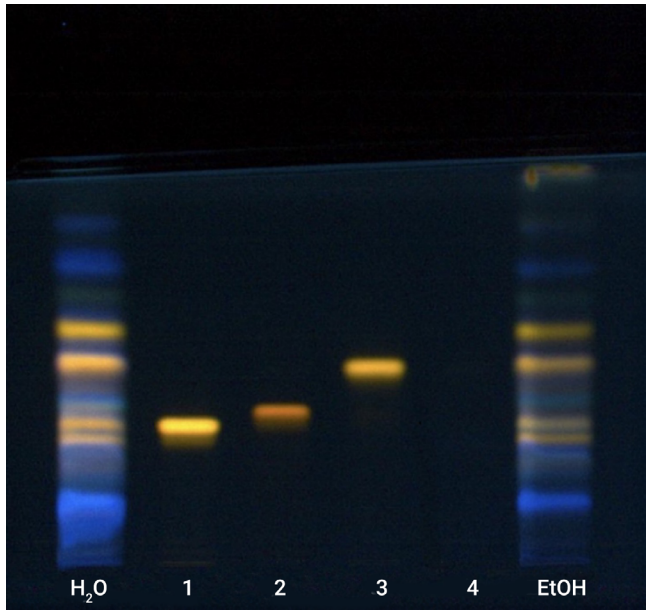

FIGURE 1. HPTLC chromatogram of aqueous and ethanolic extracts of *A. unedo* fruits and reference compounds. Mobile phase: ethyl acetate–dichloromethane–formic acid–acetic acid–water (100:25:10:10:11, v/v/v/v/v); detection at 366 nm. (1: isoquercitrin, 2: myricitrin, 3: quercitrin, 4: arbutin). Arbutin was detectable only at 254 nm.

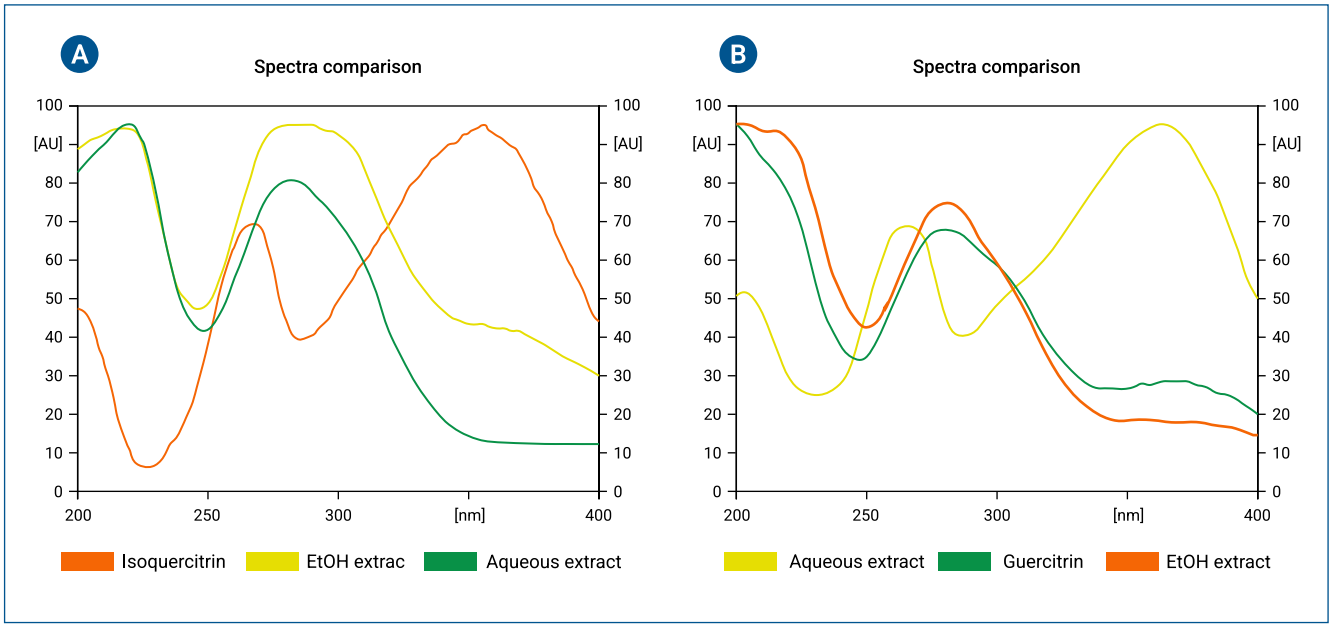

FIGURE 2. Overlay of UV spectra of isoquercitrin (A) and quercitrin (B) with the corresponding spots in the aqueous and ethanolic extracts of *A. unedo* fruits.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of *A. unedo* fruit extracts.

Sample	TPC (mg GAE/g)	TFC (mg CE/g)
Aqueous extracts (infusion)	33.7 ± 1.8	8.9 ± 0.2
Ethanolic extract	46.7 ± 0.6	14.2 ± 1.2

Values represent the mean ± standard deviation (SD) of three independent measurements (n = 3).

TPC: Total phenolic content, **TFC:** Total flavonoid content, **GAE:** Gallic acid equivalent, **CE:** Catechin equivalent.

(CAMAG, Muttenz, Switzerland). Interestingly, the UV spectra of the standard compounds did not overlay with those of the corresponding bands on the chromatogram of the extracts (Figure 2).

Accordingly, the combined evaluation of HPTLC chromatographic profiles and UV spectral overlay analyses indicated that none of the applied reference standards were detected in the *A. unedo* fruit samples collected from the university campus. High-performance liquid chromatography (HPLC) analysis may be more useful to confirm the occurrence of these compounds in the extract.

Total Phenolic and Flavonoid Content (TPC and TFC)

As shown in Table 1, the ethanolic extract of *A. unedo* fruits exhibited significantly higher TPC and TFC compared to the aqueous extract. Specifically, the TPC value of the ethanolic extract was 46.7 ± 0.6 mg GAE/g, whereas the aqueous extract (infusion) showed a lower value of 33.7 ± 1.8 mg GAE/g. Similarly, the TFC was 14.2 ± 1.2 mg CE/g in the ethanolic extract, which was markedly higher than the 8.9 ± 0.2 mg CE/g observed in the aqueous extract.

Antioxidant Activity of Extracts

The antioxidant capacity of A. unedo fruit extracts was assessed using DPPH, CUPRAC, and FRAP assays. In all tests, the ethanolic extract showed consistently higher activity than the aqueous extract (Table 2). The CUPRAC assay yielded the highest values: 114.9 ± 5.3 mg TE/g for the ethanolic and 94.4 ± 6.5 mg TE/g for the aqueous extract. In the DPPH assay, the ethanolic extract demonstrated stronger radical scavenging (89.9 ± 0.8 mg TE/g) than the aqueous extract (61.6 ± 7.3 mg TE/g). Ferric reducing antioxidant power results also favoured the ethanolic extract (47.5 ± 1.7 mg TE/g vs. 33.1 ± 2.3 mg TE/g).

Mutagenicity and Antimutagenicity Assays

As shown in Figure 3, the MI values for both the ethanolic and aqueous extracts remained below the threshold value of 2 in all tested conditions, indicating the absence of mutagenic effects. The reliability of the assay was confirmed by the significant responses of the positive controls— NPD, SA, and 2-AF.

The antimutagenic activity of *A. unedo* extracts was further evaluated using the same strains and conditions, in co-treatment with known mutagens. As illustrated in

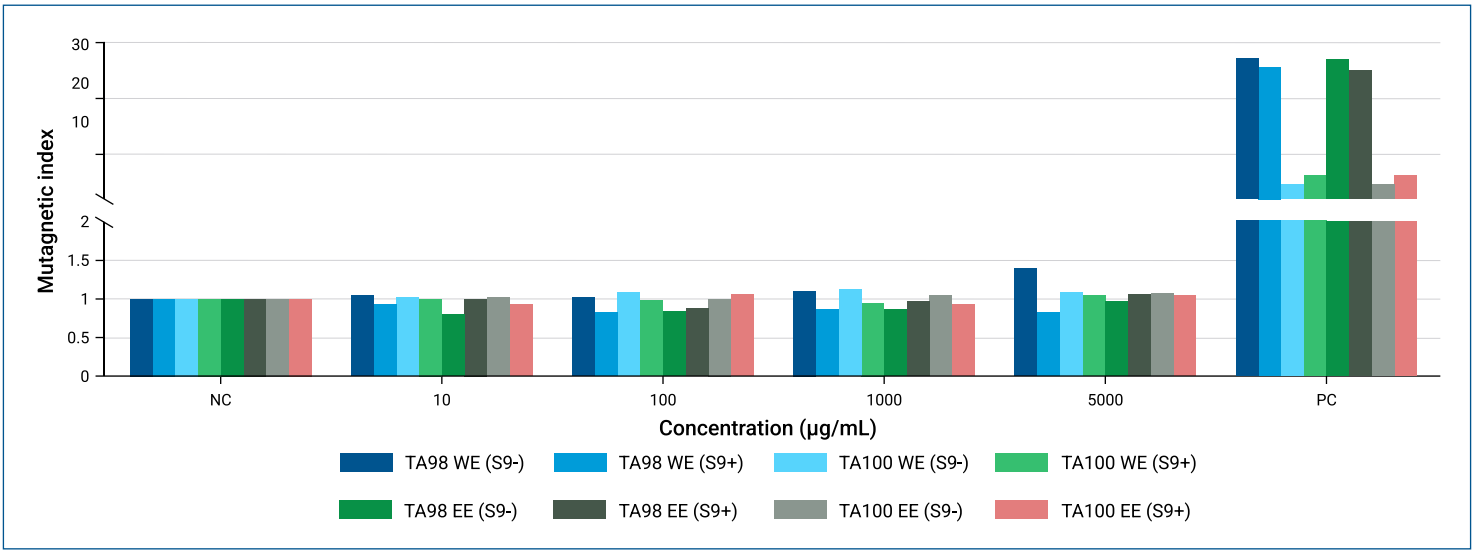

FIGURE 3. Mutagenic index (MI) values of ethanolic (EE) and aqueous (WE) extracts of *A. unedo* fruits at concentrations of 10–5000 µg/plate in *S. typhimurium* TA98 and TA100 strains, with and without S9 metabolic activation. Positive controls are included for comparison.

Table 2. Antioxidant capacity of *A. unedo* fruit extracts determined by CUPRAC, FRAP, and DPPH assays.

Sample	CUPRAC (mg TE/g)	FRAP (mg TE/g)	DPPH (mg TE/g)
Aqueous extracts	94.4 ± 6.5	33.1 ± 2.3	61.6 ± 7.3
Ethanolic extract	114.9 ± 5.3	47.5 ± 1.7	89.6 ± 0.8

Values represent the mean ± standard deviation (SD) of three independent measurements (n = 3).

CUPRAC: Cupric ion reducing antioxidant capacity, **FRAP:** Ferric reducing antioxidant power, **DPPH:** 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity, **TE:** Trolox equivalent.

Figure 4, the ethanolic extract exhibited notably higher inhibition percentages compared to the aqueous extract, indicating that ethanol-soluble phytochemicals may have stronger antimutagenic effects. Moderate antimutagenic activity was observed only at the highest concentration (5000 µg/plate) with metabolic activation, reaching 26.1% inhibition in TA98 and 39.1% in TA100. At all other tested concentrations, both extracts demonstrated weak antimutagenic activity in both strains, regardless of the presence or absence of metabolic activation.

Results of Alkaline Comet Assay

Figure 5 presents the genotoxicity and protective effects of *A. unedo* extracts in CHO-K1 cells using the alkaline comet assay. DNA damage was measured as the percentage of DNA in the comet tail.

Neither the aqueous nor the ethanolic extracts (up to 500 µg/mL) caused a significant increase in DNA strand breaks compared to the DMSO control, indicating no genotoxicity (Figure 5A).

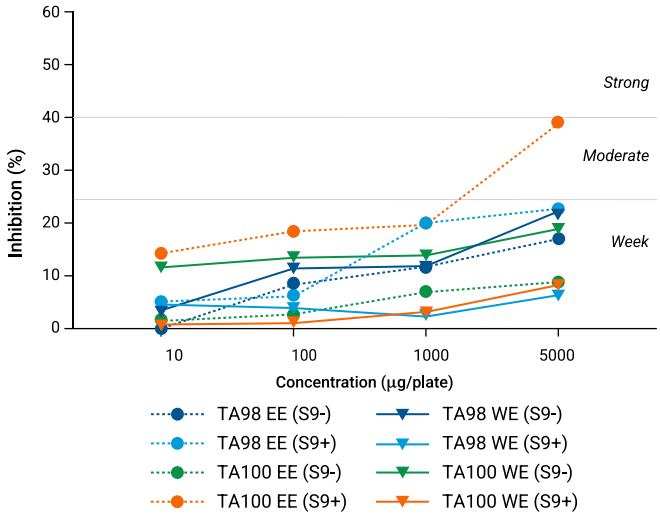

FIGURE 4. Percentage inhibition of mutagenicity by aqueous (WE) and ethanolic (EE) extracts of *A. unedo* fruits against known mutagens in *S. typhimurium* TA98 and TA100 strains, in the presence and absence of S9 metabolic activation.

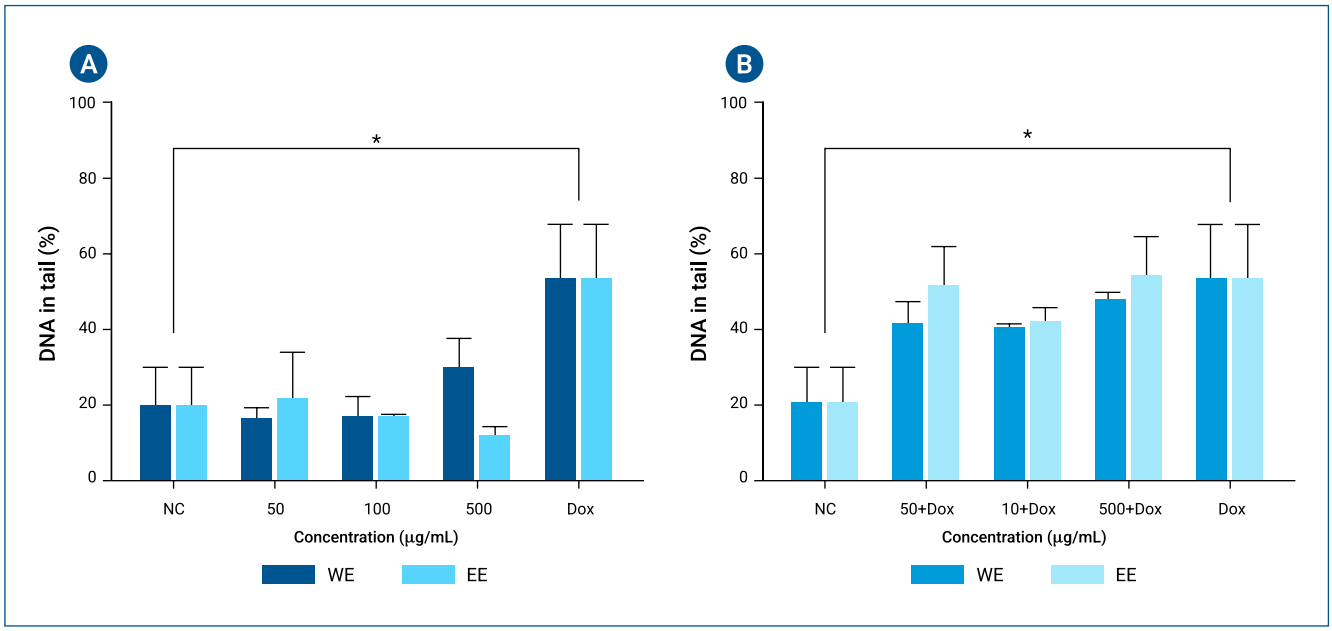

FIGURE 5. DNA strand breakage in CHO-K1 cells evaluated by the alkaline comet assay.

(A) DNA damage following treatment with *A. unedo* extracts alone.

(B) DNA damage after co-treatment with *A. unedo* extracts and doxorubicin.

Cells were treated with aqueous (WE) and ethanolic (EE) extracts of *A. unedo* at concentrations of 50, 100, and 500 µg/mL. Doxorubicin (Dox, 1 µM) was used as the positive control, and 0.5% (v/v) dimethyl sulfoxide (DMSO) served as the negative control. DNA damage is expressed as the percentage of DNA in the comet tail. Data are presented as mean ± standard error of the mean (SEM) from two independent experiments. $p<0.001$ versus the negative control group; for all other treatment groups, $p>0.05$.

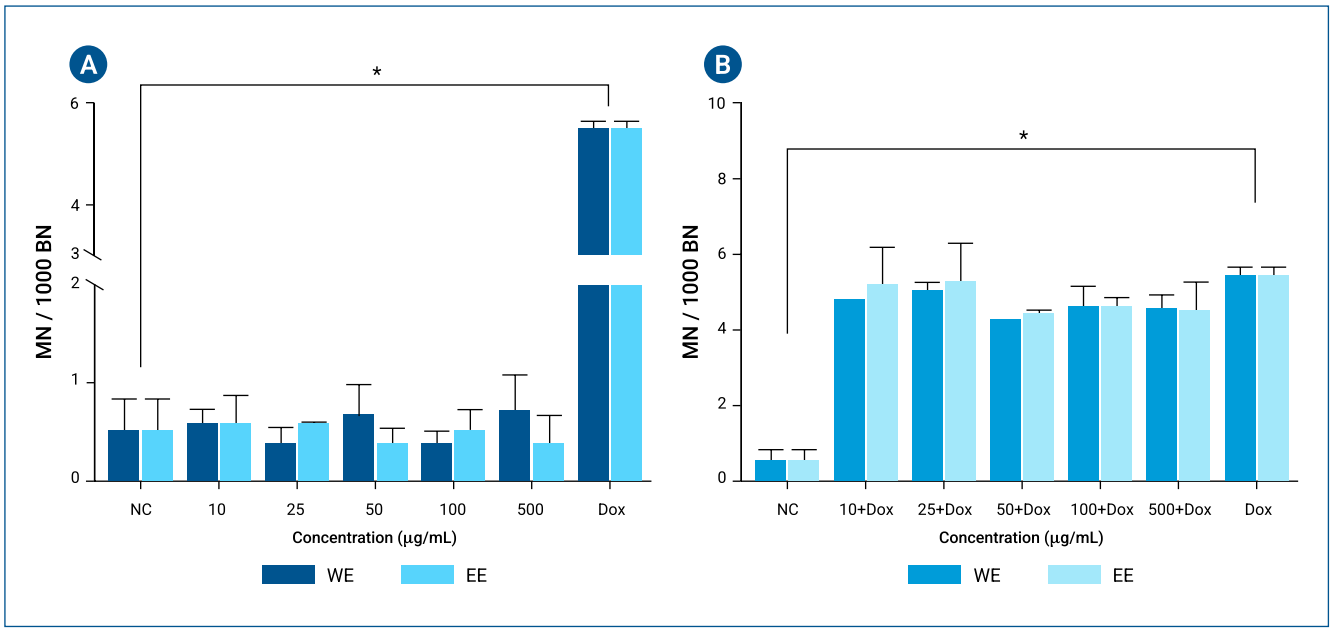

FIGURE 6. Frequency of micronucleus (MN) formation in CHO-K1 cells evaluated by the cytokinesis-block micronucleus (CBMN) assay.

(A) After treatment with *A. unedo* extracts alone.

(B) After co-treatment with *A. unedo* extracts and doxorubicin.

Cells were treated with aqueous (WE) and ethanolic (EE) extracts of *A. unedo* at concentrations of 10, 25, 50, 100, and 500 µg/mL. Doxorubicin (Dox, 1 µM) served as the positive control, and 0.5% (v/v) dimethyl sulfoxide (DMSO) as the negative control. Micronucleus frequency was determined by scoring 1,000 binucleated (BN) cells per sample. Results are expressed as mean ± standard error of the mean (SEM) from two independent experiments. $p<0.001$ versus the negative control group; for all other treatment groups, $p>0.05$

In the co-treatment with doxorubicin (Figure 5B), neither extract significantly reduced DNA damage relative to the positive control, suggesting no notable protective effect against doxorubicin-induced genotoxicity under the tested conditions.

Results of Cytokinesis-block Micronucleus Assay

Figure 6 shows the genotoxic and antigenotoxic evaluation of *A. unedo* fruit extracts in CHO-K1 cells using the MN assay.

As shown in Figure 6A, neither the aqueous nor the ethanolic extracts (10–500 µg/mL) significantly increased micronuclei frequency compared to the DMSO control, indicating no clastogenic or aneugenic effects.

In co-treatment with doxorubicin (Figure 6B), the extracts did not significantly reduce micronuclei formation. All reductions were below 25%, suggesting no meaningful antigenotoxic activity under the tested conditions.

DISCUSSION

In this study, the biological activities of the ethanolic and aqueous extracts prepared from the ripe fruits of *A. unedo* were evaluated through antioxidant, antimutagenic, and antigenotoxic assays. The safety profiles of the extracts were also assessed using standard mutagenicity and genotoxicity tests. Phytochemical characterization was performed *via* TPC and TFC analyses, while HPTLC was employed to detect specific phenolic compounds.

Previous studies on *A. unedo* leaves have identified phenolic compounds such as quercetin 3-*O*-rhamnoside (quercitrin), quercetin 3-O-glucoside (isoquercitrin), myricitrin, and arbutin as major bioactive constituents (18). However, these compounds were not detected in our fruit extracts, suggesting that the chemical profile of the fruits may differ from that of the leaves. It is also worth noting that more sensitive techniques such as HPLC or LC-MS/MS may be necessary to confirm whether these compounds are present at trace levels. The phenolic composition of *A. unedo* fruits has been previously reported to vary by region. For example, fruits collected in Samsun (Türkiye) were found to contain gallic acid as the dominant phenolic compound, followed by protocatechuic acid, gentisic acid, *p*-hydroxybenzoic acid, vanillic acid, and m-anisic acid (19). In another study, wild fruits from northeastern Portugal were rich in flavan-3-ols and galloyl derivatives, followed by anthocyanins and flavanols (20). Spanish samples were shown to contain delphinidin 3-galactoside, cyanidin galactoside, cyanidin glucoside, and cyanidin arabinoside, with cyanidin galactoside being the predominant anthocyanin. Similarly, cyanidin 3-O-glucoside was reported as the major anthocyanin in fruits from Portugal (21). Taken together, these previously reported phenolic compounds, particularly anthocyanins and phenolic acids, may contribute to the antioxidant and antimutagenic activities observed in the ethanolic extract.

Both aqueous and ethanolic extracts contained notable levels of phenolics and flavonoids. The TPC was 33.7 ± 1.8 mg GAE/g for the aqueous extract and 46.5 ± 0.6 mg GAE/g for the ethanolic extract. Total flavonoid content values were 8.9 ± 0.2 mg CE/g and 14.2 ± 1.2 mg CE/g, respectively. These results align with those of Asmaa *et al.* (8), especially for the aqueous extract. The higher flavonoid content in the ethanolic extract may reflect ethanol's greater efficiency in extracting flavonoids or variations in fruit composition.

Both extracts showed notable radical scavenging activity, with the ethanol extract displaying superior antioxidant capacity, consistent with its higher phenolic and flavonoid content. This supports previous findings on ethanol's effectiveness in extracting phenolic compounds. In contrast, water primarily dissolves polysaccharides and other polar molecules, resulting in lower yields of phenolic and lipophilic constituents. Overall, these results suggest ethanol is a more efficient solvent than water for extracting antioxidant compounds from *A. unedo* fruits.

In the antimutagenicity assay, activity was observed only at the highest concentration in the TA100 strain with metabolic activation (S9). This suggests that the ethanolic extract may protect against base-pair substitutions rather than frameshift mutations. The requirement for S9 activation indicates that biotransformation products of the extract may be responsible for its antimutagenic effect. This aligns with previous reports showing that certain polyphenols and flavonoids inhibit cytochrome P450 enzymes (e.g., CYP1A1, CYP1A2), reducing the formation of mutagenic metabolites from procarcinogens (22).

On the other hand, no protective effects were observed in the comet assay against DNA strand breaks or in the MN against doxorubicin-induced clastogenic or aneugenic damage. While the Ames test showed moderate antimutagenic activity of the ethanolic extract at the highest concentration with S9 activation, no protective effects were observed in the comet or MN assays. This discrepancy may stem from fundamental differences between the assays. The Ames test is a bacterial system detecting point mutations, whereas the comet and MN assays are mammalian cell-based and detect DNA strand breaks or chromosomal damage. Notably, this is the first study to evaluate the genoprotective potential of *A. unedo* fruit extracts using both comet and MN assays.

Although scientific interest in *A. unedo* is growing, toxicological data on its fruits remain limited. Most previous

genotoxicity assessments have focused on leaf extracts or isolated compounds rather than fruits. Jurica et al. (23, 24) investigated *A. unedo* leaf extracts and compounds such as arbutin and hydroquinone, reporting minimal cytotoxic and genotoxic effects in mammalian cell models. To our knowledge, this is the first study to comprehensively evaluate the safety of *A. unedo* fruit extracts using multiple genotoxicity bioassays.

In the Ames test, neither aqueous nor ethanol extracts exhibited mutagenicity in TA98 and TA100 strains, with or without metabolic activation (S9), indicating no direct or indirect mutagenic effects. These findings were supported by the MN assay, which showed no significant increase in micronuclei or change in the nuclear division index in CHO-K1 cells at any tested concentration, and by the comet assay, which revealed no significant DNA strand breaks compared to the positive control, doxorubicin. Collectively, these results indicate that *A. unedo* fruit extracts are non-genotoxic, in line with earlier reports on leaf extracts by Jurica et al. (23, 24).

This study presents the first comprehensive assessment of the phytochemical profile, antioxidant activity, and genotoxic/antigenotoxic potential of *A. unedo* fruits from Türkiye. The lack of mutagenic or genotoxic effects in both bacterial and mammalian systems supports their chemical safety. Moderate antimutagenic activity observed in the ethanolic extract at high concentrations, along with the absence of key reference compounds, points to a distinct fruit phytochemistry compared to the leaves. Overall, the findings confirm the non-genotoxic nature of *A. unedo* fruit extracts and suggest a limited, solvent-dependent chemopreventive potential.

However, the study is limited by the use of only two solvents for the extraction and a restricted set of chemical standards, which may overlook other active compounds. All tests were performed *in vitro*, which may not fully represent *in vivo* effects. Further research should include advanced techniques like HPLC, LC-MS/MS for broader phytochemical profiling and *in vivo* models to assess safety and efficacy. Seasonal, geographic, and dosage-related variations should also be explored to fully understand the biological potential of *A. unedo* fruits.

Ethical Approval: N.A.

Informed Consent: N.A.

Peer-review: Externally peer-reviewed

Author Contributions: Concept – D.H., H.K., M.H.; Design – D.H., A.Y., H.K., M.H.; Supervision – H.K., M.H.; Materials – M.A.O., H.K., M.H.; Data Collection and/or Processing – A.G.K., G.E., M.A.O.; Analysis and/or Interpretation – D.H., A.Y., A.G.K., G.E. M.A.O.; Literature Review – D.H., A.Y., A.G.K., G.E.; Writer – A.G.K., G.E. M.H.; Critical Reviews – H.K., M.H.

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REFERENCES

1 Miguel MG, Faleiro ML, Guerreiro AC, Antunes MD. *Arbutus unedo* L.: chemical and biological properties. Molecules. 2014;19(10):15799-823. [CrossRef]

2 Özcan MM, Hacıseferoğulları H. The strawberry (*Arbutus unedo* L.) fruits: Chemical composition, physical properties and mineral contents. J Food Eng. 2006;78(3):1022-8. [CrossRef]

3 Morgado S, Morgado M, Plácido AI, Roque F, Duarte AP. *Arbutus unedo* L.: From traditional medicine to potential uses in modern pharmacotherapy. J Ethnopharmacol. 2018;225:90-102. [CrossRef]

4 Bento I, Pereira JA. *Arbutus unedo* L. and its benefits on human health. J Food Nutr Res. 2011;50(2):73-85.

5 Carcache-Blanco EJ, Cuendet M, Park EJ, Su BN, Rivero-Cruz JF, Farnsworth NR, et al. Potential cancer chemopreventive agents from *Arbutus unedo*. Nat Prod Res. 2006;20(4):327-34. [CrossRef]

6 Mrabti HN, Bouyahya A, Ed-Dra A, Kachmar MR, Mrabti NN, Benali T, et al. Polyphenolic profile and biological properties of *Arbutus unedo* root extracts. Eur J Integr Med. 2021;42:101266. [CrossRef]

7 Güzelmeriç E, Ugurlu P, Çelik C, Şen NB, Helvacıoğlu S, Charehsaz M, et al. *Myrtus communis* L. (myrtle) plant parts: comparative assessment of their chemical compositions and antioxidant, anticancer, and antimutagenic activities. S Afr J Bot. 2022;150:711-8. [CrossRef]

8 Asmaa N, Abdelaziz G, Bakchiche B, Carbonell-Barrachina A, Cano-Lamadrid M, Noguera-Artiaga L. Chemical composition, antioxidant activity and mineral content of *Arbutus unedo* leaves and fruits. J Microbiol Biotechnol Food Sci. 2019;8:1335-9. [CrossRef]

9 Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: a critical evaluation. LWT. 2021;150:111932. [CrossRef]

10 Blois M. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199-200. [CrossRef]

11 Apak R, Güçlü K, Ozyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem. 2004;52(26):7970-81. [CrossRef]

12 Özyürek M, Güçlü K, Apak R. The main and modified CUPRAC methods of antioxidant measurement. TrAC Trends Anal Chem. 2011;30:652-64. [CrossRef]

13 Berker KI, Güçlü K, Demirata B, Apak R. A novel antioxidant assay of ferric reducing capacity measurement using ferrozine as the colour forming complexation reagent. Anal Methods. 2010;2:1770-6. [CrossRef]

14 Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983;113(3-4):173-215. [CrossRef]

15 Zengin G, Uysal A, Gunes E, Aktumsek A. Survey of phytochemical composition and biological effects of three extracts from a wild plant (*Cotoneaster nummularia* Fisch. et Mey.): a potential source for functional food ingredients and drug formulations. PLoS One. 2014;9(11):e113527. [CrossRef]

16 Helvacioglu S, Charehsaz M, Bankoglu EE, Stopper H, Aydin A. The ameliorative effect of rosmarinic acid and epigallocatechin gallate against doxorubicin-induced genotoxicity. Drug Chem Toxicol. 2024;47(6):1087-99. [CrossRef]

17 Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ Mol Mutagen. 2000;35(3):206-21. [CrossRef]

18 Oliveira I, Baptista P, Malheiro R, Casal S, Bento A, Pereira JA. Influence of strawberry tree (*Arbutus unedo* L.) fruit ripening stage on chemical composition and antioxidant activity. Food Res Int. 2011;44:1401-7. [CrossRef]

19 Ayaz FA, Kucukislamoglu M, Reunanen M. Sugar, non-volatile and phenolic acids composition of strawberry tree (*Arbutus unedo* L. var. ellipsoidea) Fruits. J. Food Compos. Anal. 2000;13:171-7. [CrossRef]

20 Guimarães R, Barros L, Dueñas M, Carvalho AM, Queiroz MJ, Santos-Buelga C, et al. Characterisation of phenolic compounds in wild fruits from Northeastern Portugal. Food Chem. 2013;141(4):3721-30. [CrossRef]

21 Pallauf K, Rivas-Gonzalo JC, del Castillo MD, Cano MP, de Pascual-Teresa S. Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo* L.) fruits. J. Food Compos. Anal. 2008;21:273-81. [CrossRef]

22 Charehsaz M, Sipahi H, Giri AK, Aydin A. Antimutagenic and anticlastogenic effects of Turkish Black Tea on TA98 and TA100 strains of *Salmonella typhimurium* (*in vitro*) and mice (*in vivo*). Pharm Biol. 2017;55(1):1202-6. [CrossRef]

23 Jurica K, Brčić Karačonji I, Kopjar N, Shek-Vugrovečki A, Cikač T, Benković V. The effects of strawberry tree water leaf extract, arbutin and hydroquinone on haematological parameters and levels of primary DNA damage in white blood cells of rats. J Ethnopharmacol. 2018;215:83-90. [CrossRef]

24 Jurica K, Brčić Karačonji I, Mikolić A, Milojković-Opsenica D, Benković V, Kopjar N. *In vitro* safety assessment of the strawberry tree (*Arbutus unedo* L.) water leaf extract and arbutin in human peripheral blood lymphocytes. Cytotechnology. 2018;70(4):1261-18. [CrossRef]

RESEARCH ARTICLE | Yeditepe Journal of Health Sciences, 13(3):130-8

Inhibition of mTORC1/2 by INK-128 Impairs *in vitro* Oocyte Maturation in Mice

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Abstract

Objective: Oocyte maturation is a complex process that encompasses both nuclear and cytoplasmic events, tightly regulated by multiple signaling pathways. The mammalian target of rapamycin (mTOR) has been implicated in follicular development and oocyte maturation, primarily through the activity of its two complexes, mTORC1 and mTORC2. While first-generation inhibitors such as rapamycin have been extensively studied, the simultaneous inhibition of both mTORC1 and mTORC2 by second-generation inhibitors remains poorly characterized in oocytes. In this study, we investigated the effect of INK-128, a dual mTORC1/2 inhibitor, on *in vitro* maturation (IVM) of mouse oocytes.

Materials and Methods: Germinal vesicle (GV) stage oocytes were isolated from 6-week-old Balb/c female mice following 5 IU pregnant mare serum gonadotropin (PMSG) stimulation. The oocytes were cultured *in vitro* for 18 hours in the presence of 10 nM, 50 nM, or 100 nM INK-128, while untreated oocytes served as controls. Maturation outcomes were assessed by morphological evaluation, total oocyte scoring (TOS), and immunofluorescence staining of α-tubulin and mTOR expression.

Results: Our results showed that INK-128 treatment reduced the overall maturation rate from GV to metaphase II (MII) stage. In particular, MII oocytes exhibited significantly decreased mTOR expression at 50 nM (p=0.0162) and 100 nM (p<0.0001) concentrations compared to controls. Furthermore, higher doses of INK-128 were associated with abnormal spindle organization and increased cytoplasmic granularity.

Conclusion: These findings suggest that dual inhibition of mTORC1/2 by INK-128 impairs oocyte maturation and highlights a potential role for mTOR in the MI-to-MII transition. Further investigations are required to elucidate the underlying mechanisms and to explore the translational relevance of mTOR modulation in assisted reproductive technologies.

Keywords: Oocyte maturation, mTORC1, mTORC2, INK-128, *in vitro* maturation

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INTRODUCTION

High-quality oocytes are essential for successful embryo development, implantation, and maintenance of pregnancy (1). Oocyte maturation is a highly orchestrated process involving both nuclear and cytoplasmic changes. Nuclear maturation refers to the resumption and completion of meiosis I, followed by arrest at metaphase II (MII), while cytoplasmic maturation includes the reorganization of organelles, accumulation of maternal transcripts, and storage of proteins required for fertilization and early embryogenesis (2). *In vitro* maturation (IVM) of germinal vesicle (GV) stage oocytes is an important clinical strategy for patients with limited follicular response or poor ovarian reserve, offering an alternative approach to conventional ovarian stimulation (3). However, the efficiency of IVM remains suboptimal, highlighting the need to better understand the molecular mechanisms underlying oocyte maturation (4).

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase of the phosphatidylinositol 3-kinase-related kinase (PIKK) family, playing critical roles in follicular development, oocyte maturation, and the maintenance of ovarian reserve (5,6). Dysregulation of this pathway has been implicated in impaired ovarian reserve and follicular development, highlighting its essential function in maintaining ovarian homeostasis (7-9). The regulatory role of mTOR signaling has also been demonstrated in polycystic ovary syndrome (10,11).

mTOR is expressed in granulosa cells, theca cells, ovarian stroma, surface epithelium, and oocytes at various stages of folliculogenesis (6). It forms the catalytic core of two structurally and functionally distinct multiprotein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (12,13). mTORC1 primarily regulates anabolic processes such as protein and lipid synthesis, ribosome biogenesis, and nucleotide synthesis, while inhibiting catabolic processes like autophagy and mRNA degradation (14,15). mTORC2, in contrast, controls actin cytoskeletal organization and activates Akt signaling through phosphorylation at Ser473 (12,16).

Rapamycin, a first-generation mTOR inhibitor, selectively suppresses mTORC1 activity but does not directly inhibit mTORC2 (17,18). Although rapamycin is highly specific, it does not fully block all mTORC1 actions and, upon long-term treatment, can indirectly suppress mTORC2 in certain cell types (19). Studies have shown that low-dose rapamycin enhances spindle integrity and chromosomal alignment in mouse oocytes, whereas high doses impair follicular growth, granulosa cell proliferation, and oocyte maturation (20-22). These findings highlight the role of mTOR signaling in oocyte quality regulation. Rapamycin treatment has been shown to increase the levels of key maturation factors in porcine oocytes (23). Moreover, recent evidence has shown that rapamycin can reduce DNA damage in *in vitro*-matured oocytes (24).

INK-128 (sapanisertib) is a second-generation ATP-competitive inhibitor that simultaneously suppresses both mTORC1 and mTORC2 by directly targeting the kinase domain (25). Unlike rapamycin, INK-128 provides a more complete inhibition of mTOR signaling and has been widely investigated in cancer research. However, its effects on ovarian physiology and oocyte maturation remain unexplored.

In this study, we aimed to investigate the impact of dual mTORC1/2 inhibition by INK-128 on mouse oocyte maturation *in vitro*. We hypothesized that simultaneous suppression of mTORC1 and mTORC2 would alter the efficiency and quality of oocyte maturation, as assessed by nuclear progression, morphological features, and mTOR expression.

MATERIALS AND METHODS

Animals and Ethical Approval

A total of six 6-week-old female BALB/c mice were obtained from the Yeditepe University Experimental Research Center. The animals were housed under controlled temperature and light/dark cycle conditions with ad libitum access to food and water. All procedures were approved by the Yeditepe University Local Ethics Committee for Animal Experiments (Protocol No. 2023-16, Decision No. 2023/03-08).

Superovulation and Collection of GV-Stage Oocytes

To stimulate follicular development, mice received an intraperitoneal injection of 5 IU pregnant mare serum gonadotropin (PMSG) (Cat. No. G4527; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). After 46–48 hours, the animals were euthanized by cervical dislocation under anesthesia, and ovaries were excised and transferred into HEPES-buffered medium (Cat. No. 51471C; Merck KGaA, Darmstadt, Germany). Germinal vesicle stage oocytes were isolated from Graafian follicles by puncturing the ovaries with a fine insulin needle under a stereomicroscope. Cumulus-oocyte complexes were collected using a mouth pipette, and cumulus cells were removed by gentle pipetting and brief exposure to hyaluronidase.

Experimental Design and *In Vitro* Culture

Isolated GV-stage oocytes were randomly allocated into four groups (n=20 per group) and cultured in SAGE 1-Step medium (Cat. No. 67010010; CooperSurgical, Inc., Trumbull, CT, USA) at 37°C in 5% CO_2 for 18 hours. The treatment groups were as follows:

Control group: GV oocytes cultured without treatment,
10 nM INK-128 group: GV oocytes cultured with 10 nM INK-128,
50 nM INK-128 group: GV oocytes cultured with 50 nM INK-128, and
100 nM INK-128 group: GV oocytes cultured with 100 nM INK-128.

Because INK-128 was supplied as a liquid and directly mixed into the SAGE 1-Step medium, an additional solvent control group was not required.

Oocyte Scoring

After 18 hours of incubation, oocytes were examined under an Axio Observer microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) and classified according to their developmental stage (GV, MI, or MII). Morphological quality was further assessed using the total oocyte score (TOS) system, which evaluates six parameters: oocyte shape, size, ooplasm characteristics, zona pellucida thickness, perivitelline space, and polar body morphology (26-31). Each parameter was scored as -1 (poor), 0 (average), or +1 (good), and the scores were summed to obtain the TOS. Maximum scores differed by maturation stage, with GV oocytes ranging from -4 to +4, MI oocytes from -5 to +5, and MII oocytes from -6 to +6.

Immunofluorescence Staining

Following culture, oocytes were fixed in 3% paraformaldehyde (Cat. No. 158127; Merck KGaA, Darmstadt, Germany) for 20 minutes at room temperature and washed three times with 1% bovine serum albumin (BSA) (Cat. No. A9418; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Permeabilization was performed with 0.01% Triton X-100 (Cat. No. X100; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) in 2% BSA for 1 hour, followed by blocking with 1% BSA. Oocytes were incubated overnight at 4°C with primary antibodies against α-tubulin (Santa Cruz Biotechnology, Dallas, TX, USA) (to assess spindle organization) and mTOR (to detect expression patterns).

Table 1. Distribution of oocytes at the GV, MI, MII in the control group and in groups treated with 10 nM, 50 nM, 100 nM INK-128.

Group	GV (%)	MI (%)	MII (%)
Control	3.77	33.96	62.26
10 nM	4.35	41.30	54.35
50 nM	14.29	53.57	32.14
100 nM	6.56	45.90	47.54

GV: Germinal vesicle, **MI:** Metaphase I, **MII:** Metaphase II.

After washing, samples were incubated with appropriate secondary antibodies conjugated to fluorophores for 1 hour at room temperature.

ProLong Gold Antifade Mountant with DAPI (Cat. No. P36931; Thermo Fisher Scientific, Waltham, MA, USA). Fluorescence images were acquired using a Zeiss LSM780 confocal microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany), and quantitative analysis was conducted with ImageJ software. Since we were unable to obtain consistent, high-quality, complete images of the meiotic spindle apparatus for all oocytes due to the limitations of the confocal microscopy setup, statistical quantification of spindle defects was not performed.

Statistical Analysis

Maturation rates, TOS values, and immunofluorescence intensities were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Homogeneity of variances was assessed with Levene's test. For comparisons among groups, one-way ANOVA followed by Tukey's multiple comparison tests were applied. A p-value of <0.05 was considered statistically significant.

RESULTS

Oocyte Maturation Rates

The overall maturation rates from GV to MII were 62% in the control group, 54% in the 10 nM group, 32% in the 50 nM group, and 47% in the 100 nM group. Compared to the control group, INK-128 treatment resulted in a decrease in the proportion of oocytes reaching the MII stage. The maximal inhibitory effect was observed at 50 nM (32% MII), with a partial recovery at 100 nM (47% MII), indicating a non-monotonic response (Table 1).

Morphological Evaluation of Oocytes

The effect of INK-128-mediated inhibition of mTORC1/2 on oocyte morphology was assessed under the ZEISS Axio Observer microscope. Increasing doses of INK-128 were associated with abnormal morphological features in MII oocytes, including enlarged polar bodies and increased cytoplasmic granularity (Figure 1).

Oocyte Scoring Results

Oocytes that progressed to MI and MII stages in each group were evaluated according to six morphological parameters (Table 2). Each parameter was scored as -1, 0, or +1, and the total oocyte score (TOS) was calculated. The distribution of TOS across experimental groups is summarized in (Table 3).

After the total oocyte scores of the experimental groups were calculated, the groups were classified based on oocyte quality. Oocytes were categorized as poor (-1), aver-

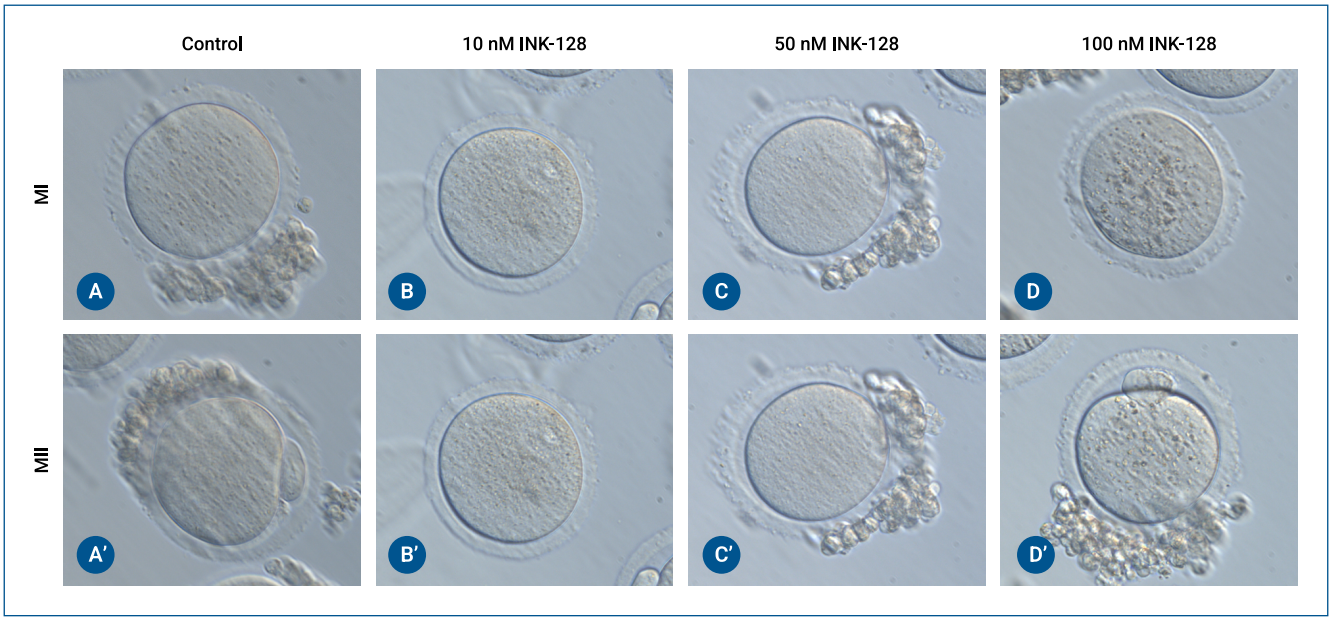

FIGURE 1. Representative images of mouse oocytes after 18 hours of *in vitro* maturation. (A, A') Control group MI and MII oocytes; (B, B') 10 nM group; (C, C') 50 nM group; (D, D') 100 nM group.

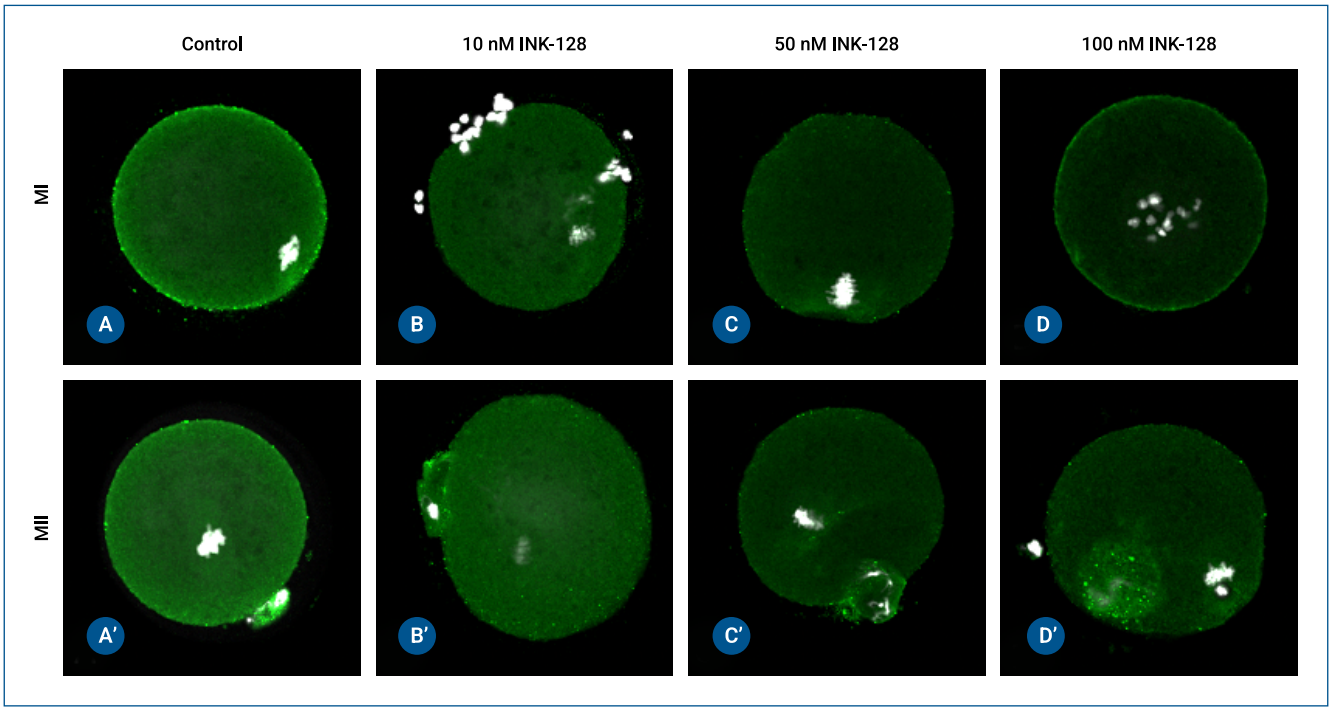

FIGURE 2. Immunofluorescence images of mTOR expression in MI and MII oocytes (control vs. INK-128 groups).

age (0), and good (1) (Table 4). Subsequently, the percentages of oocytes evaluated as poor (-1), average (0), and good (1) were calculated for each group (Table 5). In the control group, all oocytes that reached MI and MII stages received the highest positive morphological scores (+1). In the 10 nM group, all MI-stage oocytes scored +1, while 96.7% of MII-stage oocytes scored +1 and 3.3% scored -1. In the 50 nM group, MI oocytes were classified as 20% good (+1), 20% average (0), and 60% poor (-1), while MII oocytes were 56.5% good (+1) and 43.5% poor (-1). In the 100 nM group, MI oocytes were 64.3% good (+1), 14.3% average (0), and 21.4% poor (-1); MII oocytes were 78.6% good (+1) and 21.4% poor (-1).

These results demonstrate a decline in oocyte morphological quality with higher INK-128 treatment. The per-

Table 2. Oocyte scoring system (26-31).

Parameter	-1	0	+1
Oocyte shape	Dark general oocyte coloration and/or ovoid shape	Less dark general oocyte coloration and less ovoid shape	Normal
Oocyte size	Abnormally small or large	**For GV, MI and MII:** does not deviate from normal by more than 10 µm	**For GV:** > 75µm and < 85µm **For MI:** > 90µm and < 100µm **For MII:** > 100µm and < 110µm
Ooplasm characteristics	Very granular and/or very vacuolated and/or several inclusions	Slightly granular and/or only a few inclusions	Absence of granularity and inclusions
Zona pellucida characteristics	Very thin or thick	**For GV, MI and MII:** does not deviate from normal by more than 2 µm	**For GV, MI and MII:** > 7 µm and < 8 µm
Structure of the perivitelline space	Abnormally large PVS, an absent PVS, or a very granular PVS	Moderately enlarged PVS and/or small PVS and/or a less granular PVS	Normal size PVS with no granules
Polar body morphology	Flat and/or multiple PBs, granular and/or either abnormally small or large PBs	Fair but not excellent	Normal size and shape

GV: Germinal vesicle, **MI:** Metaphase I, **MII:** Metaphase II, **PVS:** Perivitelline space, **PB:** Polar body.

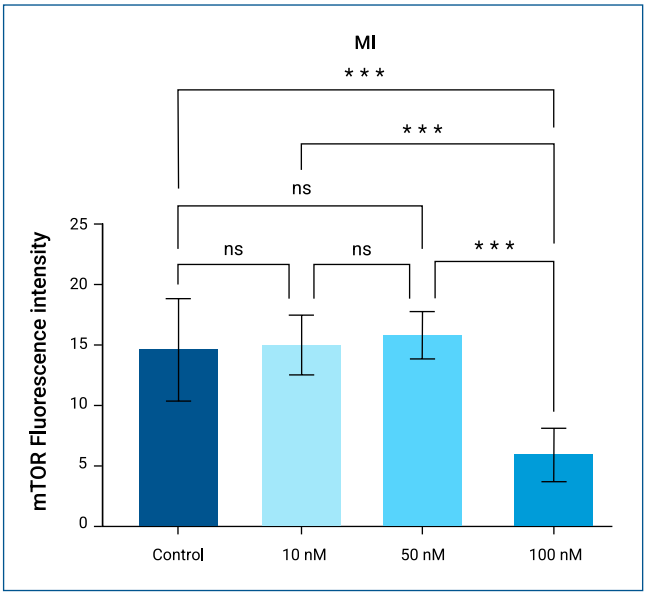

FIGURE 3. Quantitative comparison of mTOR expression in MI oocytes. (*p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001).

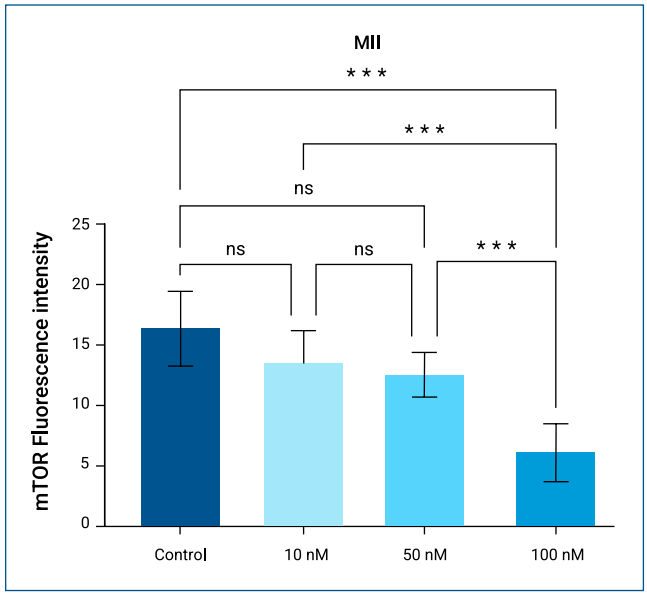

FIGURE 4. Quantitative comparison of mTOR expression in MII oocytes. (*p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001).

Table 3. Total oocyte scores (TOSs) across all groups.

TOS		Control	10 nM INK-128	50 nM INK-128	100 nM INK-128
MI	5	1	0	0	0
	4	1	1	0	0
	3	4	0	0	1
	2	1	2	0	2
	1	0	0	1	2
	0	0	0	1	2
	-1	0	0	0	1
	-2	0	0	0	1
	-3	0	0	1	0
	-4	0	0	0	0
	-5	0	0	0	0
MII	6	1	0	0	0
	5	0	3	0	0
	4	2	0	1	1
	3	3	3	0	0
	2	1	1	2	2
	1	0	3	5	3
	0	0	0	0	0
	-1	0	1	3	3
	-2	0	0	2	0
	-3	0	0	1	0
	-4	0	0	0	0
	-5	0	0	0	0
	-6	0	0	0	0

centage of morphologically poor (-1) oocytes peaked in the 50 nM group (43.5%), which was higher than that observed in the 100 nM group (21.4%), consistent with the non-monotonic effect on maturation rates.

Immunofluorescence Analysis of mTOR Expression

Immunofluorescence staining was performed to assess the effects of INK-128 on spindle morphology (α-tubulin) and mTOR expression (Figure 2).

There are no significant differences in mTOR expression were detected between control, 10 nM, and 50 nM groups in MI oocytes. Notably, the 100 nM group exhibited a significant reduction in mTOR expression (p=0.0008) (Figure 3). While the 10 nM group showed no difference compared to controls, both 50 nM (p=0.0162) and 100 nM (p<0.0001) groups displayed significantly reduced mTOR expression in MII oocytes (Figure 4). These results indicate a suppression of mTOR in MII oocytes following INK-128 treatment. Additionally, α-tubulin staining revealed abnormal spindle organization in oocytes treated with higher doses of INK-128, suggesting impaired meiotic progression. However, due to the limited number of confocal images, statistical quantification of spindle defects could not be performed.

Table 4. Classification of oocytes based on poor (-1), average (0), and good (+1) scores.

Score		Control	10 nM INK-128	50 nM INK-128	100 nM INK-128
MI	1	23	8	1	9
	0	0	0	1	2
	-1	0	0	3	-3
MII	1	25	29	13	11
	0	0	0	0	0
	-1	0	-1	-10	-3

Table 5. Percentage distribution of oocytes by classification categories.

Score		Control %	10 nM INK-128 %	50 nM INK-128 %	100 nM INK-128 %
MI	1	100	100	20.00	64.29
	0	0.00	0.00	20.00	14.29
	-1	0.00	0.00	60.00	21.43
MII	1	100	96.67	56.52	78.57
	0	0.00	0.00	0.00	0.00
	-1	0.00	3.33	43.48	21.43

DISCUSSION

The present study investigated the impact of dual mTORC1/2 inhibition by INK-128 on mouse oocyte maturation *in vitro*. Our findings demonstrate that INK-128

treatment impairs oocyte maturation, reduces MII formation, alters morphological quality, and decreases mTOR expression. These results provide novel insights into the role of mTOR signaling in meiotic progression and oocyte competence.

Previous studies have shown that mTORC1 plays an essential role in folliculogenesis and oocyte maturation. mTOR is expressed in granulosa cells, ovarian stroma, theca cells, surface epithelium, and oocytes, and its phosphorylated form is enriched in dividing granulosa cells during M-phase (6). mTOR signaling has also been shown to regulate G1-S cell cycle transition in different cell lines, supporting its broader role in cell-cycle control (32). Consistent with these reports, our data indicate that suppression of mTOR activity impairs oocyte maturation, suggesting that both mTORC1 and mTORC2 may be involved in the regulation of meiotic progression.

First-generation inhibitors such as rapamycin have been widely studied in reproductive biology. Rapamycin treatment in bovine oocytes revealed that low concentrations (1 nM) improve maturation rates, while higher concentrations disrupt follicular development and granulosa cell proliferation (33). Similarly, in mouse oocytes, low-dose rapamycin improved spindle organization and reduced chromosomal misalignment, whereas high doses decreased maturation efficiency (20,21). Our results with INK-128, a second-generation ATP-competitive inhibitor, align with these observations: low concentrations (10 nM) had minimal impact compared to controls, while higher concentrations (50 nM and 100 nM) markedly reduced maturation rates and morphological quality. Importantly, the observed effect of INK-128 on both MII formation and morphological quality was non-monotonic (U-shaped), with the maximal inhibitory effect occurring at the 50 nM concentration and a partial recovery at 100 nM.

Alpha-tubulin staining, presented as a qualitative observation, suggested a higher incidence of abnormal spindle organization in oocytes treated with higher doses of INK-128, hinting at impaired meiotic progression. This observation is supported by evidence that proteins critical for cytokinesis, such as PRC1, require mTOR signaling for proper function during oocyte maturation (34). Thus, the decreased maturation efficiency observed here may be linked to impaired mTOR-dependent regulation of meiotic spindle assembly and chromosomal segregation.

Morphological analyses further confirmed the detrimental effects of INK-128. Oocytes treated with higher concentrations exhibited enlarged polar bodies, increased cytoplasmic granularity, and reduced total oocyte scores. The observation that the inhibitory effect peaked at 50 nM and partially reversed at 100 nM, creating a non-monotonic (or U-shaped) dose-response, is a notable finding. This non-monotonic pattern is a known phenomenon with mTOR inhibitors. It may be explained by concentration-dependent effects, such as a shift in target engagement (e.g., potential off-target binding at the highest 100 nM dose that partially compensates for mTORC1/2 inhibition) or differential regulation of downstream pathways at supra-maximal concentrations.

Collectively, our findings highlight that dual inhibition of mTORC1/2 impairs oocyte maturation, disrupts morphological integrity, and reduces mTOR expression. These results emphasize a potential role for mTOR not only in folliculogenesis but also in the MI-to-MII transition of mouse oocytes.

Future studies with larger sample sizes and functional assays (e.g., fertilization and early embryonic development) will be necessary to fully elucidate the mechanisms by which mTOR regulates oocyte competence. Furthermore, a key limitation of this study is the relatively low statistical power (n=20 per group), which may have contributed to the non-monotonic trend observed between the 50 nM and 100 nM groups; future investigations should utilize a larger sample size to confirm the non-monotonic nature of the response. Moreover, exploring the differential contributions of mTORC1 versus mTORC2 may help clarify how these complexes individually and collectively influence meiotic progression. Such insights could inform potential clinical applications in assisted reproductive technologies, where modulation of mTOR activity might be leveraged to improve oocyte quality and maturation outcomes.

Ethical Approval: The study was approved by the Yeditepe University Local Ethics Committee for Animal Experiments on March 27, 2023, with decision number 2023/03-08.

Informed Consent: N.A.

Peer-review: Externally peer-reviewed

Author Contributions: Concept – E.S.A., A.Y.; Design – E.S.A., A.Y.; Supervision – A.Y.; Fundings – E.S.A., A.Y.; Materials – E.S.A., A.Y.; Data Collection and/or Processing – E.S.A., A.Y.; Analysis and/or Interpretation – E.S.A., A.Y.; Literature Review – E.S.A., A.Y.; Writer – E.S.A., A.Y; Critical Reviews – A.Y.

Conflict of Interest: The author declares no conflict of interest.

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Scientific Presentation: Part of this work was presented as a poster at the 11th Congress on Reproductive Health and Infertility (TSRM 2023), held in Antalya, Türkiye, on 10–13 May 2023. The study received the Second-Best Poster Presentation Award and was published as a supplementary abstract in *Reproductive BioMedicine Online*.

REFERENCES

1 Wang Q, Sun QY. Evaluation of oocyte quality: morphological, cellular and molecular predictors. Reprod Fertil Dev. 2007;19(1):1-12. [CrossRef]

2 Pan B, Li J. The art of oocyte meiotic arrest regulation. Reprod Biol Endocrinol. 2019;17(1):8. [CrossRef]

3 Moor RM, Dai Y, Lee C, Fulka J Jr. Oocyte maturation and embryonic failure. Hum Reprod Update. 1998;4(3):223-36. [CrossRef]

4 Krisher RL. The effect of oocyte quality on development. J Anim Sci. 2004;82 E-Suppl:E14-23. [CrossRef]

5 Hua H, Kong Q, Zhang H, Wang J, Luo T, Jiang Y. Targeting mTOR for cancer therapy. J Hematol Oncol. 2019;12(1):71. [CrossRef]

6 Yaba A, Bianchi V, Borini A, Johnson J. A putative mitotic checkpoint dependent on mTOR function controls cell proliferation and survival in ovarian granulosa cells. Reprod Sci. 2008;15(2):128-38. [CrossRef]

7 Bora G, Önel T, Yıldırım E, Yaba A. Circadian regulation of mTORC1 signaling via Per2 dependent mechanism disrupts folliculogenesis and oocyte maturation in female mice. J Mol Histol. 2023;54(3):217-29. [CrossRef]

8 Önel T, Arıcıoğlu F, Yıldırım E, Zortul H, Yaba A. The effect of maternal separation stress-induced depression on ovarian reserve in Sprague Dawley Rats: The possible role of imipramine and agmatine through a mTOR signal pathway. Physiol Behav. 2023;269:114270. [CrossRef]

9 Onel T, Yıldırım E, Dogan S, Yaba A. Determination of mTOR signal pathway in MMTV-TGFα mice ovary at different ages. J Histotechnol. 2023;46(2):80-9. [CrossRef]

10 Yaba A, Demir N. The mechanism of mTOR (mammalian target of rapamycin) in a mouse model of polycystic ovary syndrome (PCOS). J Ovarian Res. 2012;5(1):38. [CrossRef]

11 Yildirim E, Onel T, Agus S, Gunalan E, Yilmaz B, Aydin MS, et al. The effect of rapamycin treatment on mouse ovarian follicle development in dehydroepiandrosterone-induced polycystic ovary syndrome mouse model. Zygote. 2024;32(5):386-95. [CrossRef]

12 Jacinto E, Loewith R, Schmidt A, Lin S, Rüegg MA, Hall A, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol. 2004;6(11):1122-8. [CrossRef]

13 Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol. 2004;14(14):1296-302. [CrossRef]

14 Dibble CC, Manning BD. Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. Nat Cell Biol. 2013;15(6):555-64. [CrossRef]

15 Laplante M, Sabatini DM. mTOR signaling at a glance. J Cell Sci. 2009;122(Pt 20):3589-94. [CrossRef]

16 Sabatini DM. mTOR and cancer: insights into a complex relationship. Nat Rev Cancer. 2006;6(9):729-34. [CrossRef]

17 Ballou LM, Lin RZ. Rapamycin and mTOR kinase inhibitors. J Chem Biol. 2008;1(1-4):27-36. [CrossRef]

18 Benjamin D, Colombi M, Moroni C, Hall MN. Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat Rev Drug Discov. 2011;10(11):868-80. [CrossRef]

19 Thoreen CC, Sabatini DM. Rapamycin inhibits mTORC1, but not completely. Autophagy. 2009;5(5):725-6. [CrossRef]

20 Lin FH, Zhang WL, Li H, Tian XD, Zhang J, Li X, et al. Role of autophagy in modulating post-maturation aging of mouse oocytes. Cell Death Dis. 2018;9(3):308. [CrossRef]

21 Yang Q, Xi Q, Wang M, Long R, Hu J, Li Z, et al. Rapamycin improves the quality and developmental competence of mice oocytes by promoting DNA damage repair during in vitro maturation. Reprod Biol Endocrinol. 2022;20(1):67. [CrossRef]

22 Yu J, Yaba A, Kasiman C, Thomson T, Johnson J. mTOR controls ovarian follicle growth by regulating granulosa cell proliferation. PLoS One. 2011;6(7):e21415. [CrossRef]

23 Lee SE, Lee HB, Yoon JW, Park HJ, Kim SH, Han DH, et al. Rapamycin treatment during prolonged *in vitro* maturation enhances the developmental competence of immature porcine oocytes. J Anim Sci Technol. 2024;66(5):905-19. [CrossRef]

24 Yang Q, Chen Y, Huang J, Tang J, Zhu L, Li J, et al. Rapamycin reduces DNA damage of in vitro matured oocytes by promoting Rad51-mediated homologous recombination. Reprod Biol Endocrinol. 2025;23(1):93. [CrossRef]

25 Yu K, Toral-Barza L, Shi C, Zhang WG, Lucas J, Shor B, et al. Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. Cancer Res. 2009;69(15):6232-40. [CrossRef]

26 Fan ZQ, Wang YP, Yan CL, Suo L, Zhu SE. Positive effect of partial zona pellucida digestion on in vitro fertilization of mouse oocytes with cryopreserved spermatozoa. Lab Anim. 2009;43(1):72-7. [CrossRef]

27 Griffin J, Emery BR, Huang I, Peterson CM, Carrell DT. Comparative analysis of follicle morphology and oocyte diameter in four mammalian species (mouse, hamster, pig, and human). J Exp Clin Assist Reprod. 2006;3:2. [CrossRef]

28 Jennings PC, Merriman JA, Beckett EL, Hansbro PM, Jones KT. Increased zona pellucida thickness and meiotic spindle disruption in oocytes from cigarette smoking mice. Hum Reprod. 2011;26(4):878-84. [CrossRef]

29 Lazzaroni-Tealdi E, Barad DH, Albertini DF, Yu Y, Kushnir VA, Russell H, et al. Oocyte scoring enhances embryo-scoring in predicting pregnancy chances with IVF where it counts most. PLoS One. 2015;10(12):e0143632. [CrossRef]

30 Liu X, Sun Y. Microfluidic devices for single-cell trapping and automated micro-robotic injection. In: Li X, Sun Y, editors. Microfluidic Devices for Biomedical Applications. Oxford: Woodhead Publishing (Elsevier); 2013. p. 351-65.

31 Wassarman PM, Josefowicz WJ. Oocyte development in the mouse: an ultrastructural comparison of oocytes isolated at various stages of growth and meiotic competence. J Morphol. 1978;156(2):209-35. [CrossRef]

32 Onel T, Erdogan CS, Aru B, Yildirim E, Demirel GY, Yaba A. Effect of rapamycin treatment in human seminoma TCam-2 cells through inhibition of G1-S transition. Naunyn Schmiedebergs Arch Pharmacol. 2023;396(5):1009-18. [CrossRef]

33 Kordowitzki P, Hamdi M, Derevyanko A, Rizos D, Blasco M. The effect of rapamycin on bovine oocyte maturation success and metaphase telomere length maintenance. Aging (Albany NY). 2020;12(8):7576-84. [CrossRef]

34 Guo J, Zhang T, Guo Y, Sun T, Li H, Zhang X, et al. Oocyte stage-specific effects of MTOR determine granulosa cell fate and oocyte quality in mice. Proc Natl Acad Sci U S A. 2018;115(23):E5326-33. [CrossRef]

RESEARCH ARTICLE | Yeditepe Journal of Health Sciences, 13(3):139-46

Perspectives of First-Year Medical Students on Physician Brain Drain and Associated Factors

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Abstract

Objective: Brain drain refers to the migration of qualified and educated professionals from developing or underdeveloped countries to developed ones, often without returning. It is a global issue, and Türkiye is among the most affected. This study aimed to examine first-year medical students' views on physician brain drain and identify factors associated with their intention to work abroad.

Materials and Methods: This cross-sectional study used a questionnaire including sociodemographic variables such as gender, age, socioeconomic status, and educational background. Participants were asked about their motivations to live and work abroad. Those who answered "Yes" or "No" to the question "Do you plan to go abroad after graduation?" were directed to different follow-up questions, including binary and 5-point Likert-type items. The minimum required sample size was calculated as 152 at a 95% confidence level. Data were collected online, and confidentiality was ensured. Analyses were conducted using SPSS 30.0. Categorical variables were presented as frequencies and percentages; Pearson's chi-square or Fisher's exact test was used for comparisons.

Results: A total of 156 first-year medical students participated (89 females, 57.1%; 67 males, 42.9%). Among them, 100 (64.1%) reported an intention to work abroad. A significant association was found between intention to go abroad and family economic status (p=0.029), with students reporting high economic status more likely to prefer continuing their careers in Türkiye. No significant associations were found with gender (p=0.089), foreign language proficiency (p=0.178), or previous experience abroad (p=0.417).

Conclusion: A considerable proportion of students expressed a desire to work abroad. Economic status appears to play a determining role, while gender, language proficiency, and international experience appeared to have lesser influence on the decision to work abroad.

Keywords: Brain drain, medical students, physicians

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INTRODUCTION

Medical brain drain refers to the loss of human capital resulting from the migration of highly educated individuals—particularly from developing to developed countries (1). Beyond individual preferences, this phenomenon reflects structural problems within healthcare systems and global inequalities, making it a multidimensional issue.

According to the Organisation for Economic Co-operation and Development (OECD) 2019 report, the proportion of physicians migrating to developed countries has been increasing each year. Countries such as India, Pakistan, and Nigeria are among the highest sources of physician migration, while the United States, the United Kingdom, Germany, and Canada are the main destination countries (2).

In countries such as Türkiye, where healthcare resources are relatively limited, unfavorable working conditions, heavy workload, and low income—known as "push" factors—have been shown to increase students' tendency to seek career opportunities abroad (3). About 18% of physicians in OECD countries were trained abroad, demonstrating the extent of workforce mobility and the resulting strain on source countries' health systems. (4). Moreover, systematic reviews indicate that while push factors such as low remuneration, limited career progression, and infrastructure deficits drive out-migration, "pull" factors like advanced technology access and favorable working conditions in destination countries exert significant influence as well (5).

A similar trend is observed in Türkiye. According to data from the Turkish Medical Association (*Türk Tabipleri Birliği*, TTB), applications for the "Certificate of Good Standing" increased from 1405 in 2021 to 3025 in 2023. This indicates physicians' dissatisfaction with current working conditions and their growing inclination to work abroad (6).

Studies conducted among medical students have shown a high desire to work abroad after graduation. For example, 52.9% of students at Pamukkale University and 77.5% of students at Çanakkale Onsekiz Mart University reported that they intend to pursue a career abroad after graduation (7,8). In a study carried out with students from Cumhuriyet University by Filiz et al. (10), students' attitudes toward brain drain were evaluated as one of the notable investigations on this topic, and the relationships between these attitudes and various sociodemographic variables were examined. Such studies are important for understanding the expectations and concerns of the young population regarding the future.

Thus, physician brain drain is not only an individual decision but also a societal and systemic indicator. Properly analyzing the causes of this phenomenon and developing solution-oriented policies are crucial for both Türkiye and global healthcare systems.

This study aimed to determine the perspectives of first-year medical students at Marmara University regarding migration as future physicians and the factors influencing these perspectives.

MATERIALS AND METHODS

This cross-sectional study was conducted among first-year students at the Marmara University Faculty of Medicine during the 2024–2025 academic year. The study population consisted of 255 students. Based on a prevalence rate of 50% and a 95% confidence level, the minimum sample size was calculated as 152; ultimately, 156 students participated. Eligible participants were all actively enrolled medical students during the data collection period. Students who declined participation, provided incomplete surveys, or were temporarily not enrolled (e.g., taking a leave of absence) were excluded from the analysis. Among the participants, 42.9% were male (n=67) and 57.1% were female (n=89), with no participant selecting the "other" option.

Data collection was carried out online. The questionnaire, developed by the research team, was distributed *via* the Google Forms. Items were generated based on a review of the existing literature on migration intentions among medical students and early-career physicians. Additional items were informed by expert input from faculty members in medical education and public health. The questionnaire was piloted with a small group of students (N=5) to ensure clarity and comprehensibility, and minor wording adjustments were made accordingly. The average completion time was approximately 10 minutes, and participation was entirely voluntary. No personal data were collected, and confidentiality principles were strictly observed. Verbal informed consent was obtained prior to participation.

The study was conducted in accordance with established ethical principles, and ethics approval was granted by the Marmara University Faculty of Medicine Non-Drug and Non-Medical Device Research Ethics Committee on March 21, 2025, with decision number 09.2025.25-0224.

The questionnaire consisted of two main sections. The first section included sociodemographic questions such as age, gender, parents' educational levels, family economic status, type of high school, and reasons for choosing the medical faculty. The second section focused on

evaluating students' willingness to live and work abroad after graduation. Participants were asked, "Would you like to pursue your professional career abroad after graduating from medical school?" and were divided into two groups ("Yes" and "No") according to their answers. For both groups, statements were presented in "Yes–No" format as well as on a five-point Likert scale. The Likert scale was rated as "no effect," "slightly effective," "moderately effective," "very effective," and "extremely effective."

Data analysis was performed using SPSS version 30.0 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequencies and percentages, and relationships between variables were examined using the Pearson chi-square test. Fisher's exact test was applied when expected frequencies were insufficient. A p-value <0.05 was considered statistically significant.

Several variables were recoded prior to analysis. Categories with very small cell counts were merged to ensure adequate subgroup sizes, improve statistical stability, and allow for more meaningful comparisons. Recoding decisions were based solely on distributional considerations, and no conceptual categories were altered. During analysis, some variables were recategorized for clarity. Parental education levels were grouped as "primary," "secondary," and "higher education." Economic status was categorized as "low," "medium," "high," and "unknown/prefer not to answer." The reason for choosing medicine was classified as "voluntary" or "other," while the post-graduation career goal was divided into "specialization" and "other." Foreign language proficiency was coded as "low," "medium," and "high." Satisfaction with the healthcare system and quality of life was simplified into three categories: "satisfied," "neutral," and "dissatisfied." Responses from Likert-type items were merged into three categories: "not effective," "moderately effective," and "effective."

Microsoft Excel was used during data organization and tabulation, and all analyses were conducted by the research team. The study was designed based on scientific principles, including sample size determination, standardized data collection, and appropriate statistical analyses. It aims to objectively reveal the attitudes of Marmara University Faculty of Medicine students toward physician brain drain in relation to sociodemographic variables.

During the preparation of this work, the author(s) utilized ChatGPT version GPT-5.1 (OpenAI, San Francisco, CA, USA) to generate summaries of research articles related to the topic.

RESULTS

Most first-year medical students (64.1%) stated that they intend to pursue their professional careers abroad after graduation. In contrast, 35.9% reported that they wish to continue their careers in Türkiye.

A statistically significant relationship was found between family economic status and the preference for or against physician brain drain after graduation, based on the chi-square analysis (p=0.029). Specifically, students from low and middle-income families were more likely to prefer pursuing their professional careers abroad after graduation compared to those from high-income families. In the research survey, 6 participants chose to not disclose their family's economic status; these responses were excluded from the related analysis, and Table 1 reflects this adjustment.

Among the students who stated that they wish to pursue their professional careers abroad after graduation (N=100), the factors influencing their intention to emigrate were evaluated on a 5-point Likert scale. The highest mean scores were observed for the following factors: "the regulatory conditions in Türkiye" (mean=4.24), "the economic conditions in Türkiye" (mean=4.23), and "working conditions abroad" (mean=4.23) (Table 2).

Table 1. The most influential factors in brain drain intention.

Economic status	Students wishing brain drain n (%)	Students wishing to stay in Türkiye n (%)	Total n (%)	p-value*
Low	13 (76.5)	4 (23.5)	17 (100.0)	0.02
Moderate	54 (70.1)	23 (29.9)	77 (100.0)	
High	28 (50.0)	28 (50.0)	56 (100.0)	
Total	95 (63.3)	55 (36.7)	150 (100.0)	

*Pearson chi-square test; p<0.05 considered statistically significant.

Table 2. The most influential factors in brain drain intention.

Factor	Mean	Standard Deviation
The regulatory conditions in Türkiye	4.24	0.96
The economic conditions in Türkiye	4.23	0.85
Working conditions abroad	4.23	0.88
Economic expectations	4.22	0.82
Expectations regarding incidents of violence against healthcare workers and respect for healthcare professionals' rights	4.18	0.99
Social life and quality of life opportunities abroad	4.14	0.93
Opportunities for professional development and access to innovation abroad	3.91	1.03
Desire to live in a country that I consider more developed overall	3.90	1.11
Research and academic career opportunities abroad	3.87	1.11
The state of psychological comfort in working environments abroad	3.85	0.95
Workload abroad and the time available for individual patient follow-up	3.82	1.05
My general feelings about living in Türkiye	3.80	1.14
Comparison of burnout risks in medical professions in Türkiye and abroad	3.71	1.14
Current state of the healthcare system in Türkiye	3.62	1.09
Desire to experience different cultures	3.53	1.21
Opportunities abroad for my children / future family	3.51	1.36
Availability of medical technology abroad	3.40	1.07
Perceived professional prestige abroad	3.34	1.24
The desire to improve myself and return to my country	3.15	1.39

The table presents the mean and standard deviation scores of factors influencing the intention to pursue a medical career abroad among first-year medical students who stated that they wish to work abroad after graduation (N=100). These values were calculated using a 5-point Likert scale, where 1 indicates "Not influential at all" and 5 indicates "Extremely influential."

Among the students who stated that they wish to continue their professional careers in Türkiye after graduation (N=56), the highest mean scores for factors influencing their preference to remain in the country (based on a 5-point scale) were as follows: "my desire to stay close to my family and friends in Türkiye" (mean=4.73), "my liking for living in Türkiye" (mean=4.18), and "my cultural ties in Türkiye" (mean=4.14) (Table 3).

Gender-based differences in the factors influencing the intention to work abroad were also examined among students who wished to pursue their careers abroad (N=100).

Impact of violence in healthcare: 94.2% of female students rated this factor as "highly influential," compared with 56.3% of male students. This difference was statistically significant (p<0.001) (Table 4).

Access to professional development and innovation: 82.7% of female students and 54.2% of male students rated this factor as "highly influential," demonstrating a statistically significant difference (p=0.008) (Table 5).

Working conditions abroad: 90.4% of female students and 70.8% of male students rated this factor as "highly

Table 3. The most influential factors in the intention to stay in Türkiye.

Factor	Mean	Standard Deviation
My desire to stay close to my family and friends in Türkiye	4.73	0.82
My liking for living in Türkiye	4.18	1.20
My cultural ties in Türkiye	4.14	1.21
My belief that living abroad could cause the feeling of loneliness	4.14	1.32
My desire to serve my country	3.98	1.40
My sense of responsibility toward my country	3.98	1.40
The difficulties of living abroad	3.61	1.37
Family-related or personal reasons	3.48	1.57
The challenges of adapting to different healthcare procedures abroad	3.43	1.34
My desire to bring change to the healthcare system in Türkiye	3.32	1.55
The length and difficulty of the migration process	3.30	1.45
My wish to take a stance against brain drain	3.29	1.62
Immigration policies applied abroad	3.29	1.62
My state of satisfaction with the social life and quality of life conditions in Türkiye	3.18	1.37
Job opportunities in Türkiye after graduation	3.07	1.38
Financial reasons (moving abroad, costs of the process, etc.)	3.05	1.51
My state of satisfaction with working conditions for doctors in Türkiye	3.04	1.47
Language barrier	2.91	1.38
My level of trust in the healthcare system in Türkiye	2.84	1.39
Religious reasons	2.70	1.62
The difficulties of passing professional competency exams abroad	2.66	1.36

The table presents the mean scores of factors influencing the desire to remain in Türkiye among 56 first-year medical students who stated that they wish to pursue their professional careers in the country after graduation. The values were calculated using a 5-point Likert scale, where "1=Not influential at all" and "5=Extremely influential," and are reported along with their standard deviations.

influential," and the association was statistically significant (p=0.022).

The satisfaction levels of 156 first-year medical students with the healthcare system in Türkiye were measured using a 5-point Likert scale. The overall mean satisfaction score was calculated as 2.61 out of 5. When group differences were examined, the mean satisfaction score for students who wish to pursue their professional careers abroad after graduation was 2.52, whereas it was 2.77 for those who prefer to continue their careers in Türkiye.

The overall satisfaction levels of 156 first-year medical students regarding living in Türkiye were measured using a 5-point Likert scale. Based on the collected data, the overall mean satisfaction score was calculated as 2.58 out of 5. When comparing groups, the mean satisfaction score for students who wish to pursue their professional careers abroad after graduation was 2.29, whereas it was 3.09 for those who prefer to continue their careers in Türkiye.

No statistically significant relationship was found between the participants' gender and their preference to

Table 4. Impact of violence in healthcare on the decision of brain drain.

Level of the Impact	Female n (%)	Male n (%)	Total n (%)	*p*-value*
Low impact	0 (0.0)	5 (10.4)	5 (5.0)	0.001
Moderate impact	3 (5.8)	16 (33.3)	19 (19.0)	
High impact	49 (94.2)	27 (56.3)	76 (76.0)	
Total	52 (52.0)	48 (48.0)	100 (100.0)	

*Pearson chi-square test; $p<0.05$ considered statistically significant.

Table 5. Impact of the opportunities for professional development and access to innovation abroad on the decision of brain drain.

Level of the Impact	Female n (%)	Male n (%)	Total n (%)	*p*-value*
Low impact	3 (5.8)	9 (18.8)	12 (12.0)	0.008
Moderate impact	6 (11.5)	13 (27.1)	19 (19.0)	
High impact	43 (82.7)	26 (54.2)	69 (69.0)	
Total	52 (52.0)	48 (48.0)	100 (100.0)	

*Pearson chi-square test; $p<0.05$ considered statistically significant.

pursue their professional careers abroad after graduation, according to the chi-square analysis ($p=0.089$). Similarly, no statistically significant association was identified between participants' prior experience of living abroad and their intention to pursue physician brain drain after graduation ($p=0.417$). In addition, the chi-square analysis showed no statistically significant relationship between participants' foreign language proficiency and their preference to emigrate after graduation ($p=0.178$).

DISCUSSION

This study aimed to evaluate the attitudes of first-year medical students at Marmara University Faculty of Medicine toward brain drain and to identify the factors influencing these preferences. The findings shed light on the sociodemographic and structural determinants shaping students' decisions to work abroad.

Family economic status emerged as a key factor influencing the tendency toward brain drain. Students from middle- and low-income families showed a stronger desire to pursue a career abroad. This result is consistent with the findings of Altun Güzelderen et al. (9), who reported a relationship between income level and migration intention. Differences reported in other studies may be attributed to variations in sampling or measurement methods (10,11).

In our study, foreign language proficiency, prior experience abroad, and gender were found to have no statistically significant effect on migration preference. This finding contrasts with some studies that identified language proficiency as a decisive factor (10,11). The discrepancy may be explained by the fact that Marmara University provides medical education in English, which may reduce language-related barriers among its students. Regarding gender, the results indicated no significant effect on the decision to migrate, which aligns with previous studies reporting limited gender-based differences in brain drain decisions (1).

Among the factors influencing students' inclination to migrate, regulatory and economic factors in Türkiye, as well as working conditions abroad, were prominent. The increasing number of "Good Standing Certificate" applications reported by the TTB reflects this general trend

(9,12). In countries like Türkiye, where healthcare resources are relatively limited, unfavorable working conditions, heavy workload, and low income — known as "push" factors — have been shown to increase students' tendency to seek career opportunities abroad (13). Conversely, among those who preferred to stay in Türkiye, a sense of belonging to family, friends, and cultural ties was found to be a major determinant. This finding is consistent with studies emphasizing the mitigating role of social support in reducing brain drain (9,14).

Moreover, recent research in Türkiye indicates that not only structural factors like salary and workload, but also psychological and ethical dimensions contribute significantly to physicians' migration intentions. For instance, a qualitative study found that professional-ethical concerns such as maintaining autonomy, avoiding harm, and upholding beneficence in patient care play an important role in physicians' decisions to emigrate (15). This suggests that efforts to retain future physicians may benefit from addressing not only economic and systemic issues, but also professional values and workplace culture.

Another relevant observation from the literature is that mental health and stress levels among medical students and physicians are increasingly linked to migration intention. A cross-sectional study of Turkish medical students found that higher levels of depression and stress were significantly associated with the intention to migrate abroad (16). These findings underscore the need for retention strategies that incorporate psychosocial support, reduce burnout, and promote resilience among health-care students and professionals.

This study has several limitations. First, it was conducted at a single medical school, which may limit the generalizability of the findings to medical students in other regions or institutional contexts in Türkiye. Second, the sample consisted only of first-year students; migration intentions may evolve throughout medical training, and therefore the results may not reflect the perspectives of more advanced cohorts. Third, participation was voluntary, introducing the possibility of selection bias, as students with stronger opinions about studying or working abroad may have been more likely to participate. In addition, the data were based on self-reported measures, which are subject to recall bias and social desirability effects. The cross-sectional design also prevents causal inference. Finally, some unmeasured factors—family migration history—may have acted as potential confounders. Despite these limitations, the study provides important preliminary insights into early-stage brain drain intentions among medical students in Türkiye.

This study demonstrates that family economic status plays a decisive role in the intention to migrate, whereas individual characteristics such as language proficiency and gender have a more limited influence on migration decisions. Economic and working conditions in Türkiye and living standards abroad strengthen students' inclination to seek opportunities outside the country. Conversely, family ties, cultural belonging, and the desire to live in Türkiye emerge as protective factors supporting students' decisions to remain in their home country.

The findings indicate that the phenomenon of brain drain arises not solely from individual preferences but from the intersection of social, economic, and structural factors. Therefore, policies aimed at reducing brain drain should not only address financial conditions but also focus on broader dimensions such as overall life satisfaction, opportunities for academic advancement, and professional prestige.

Ensuring economic stability within the healthcare system, clarifying career pathways for young physicians, reducing workload and burnout, preventing workplace violence, and implementing fair wage policies may contribute to mitigating brain drain. Likewise, educational institutions can strengthen students' sense of belonging by offering programs that foster national commitment, mentorship systems, and career planning support, thereby enhancing young physicians' motivation to remain in Türkiye.

Future research should be designed to include students from different faculties and academic years and incorporate variables such as international experience, career goals, and professional satisfaction. Longitudinal studies could track changes in brain drain tendencies from medical education through post-graduation, providing a more comprehensive understanding of the process.

CONCLUSION

This study shows that the tendency toward brain drain among medical students is strongly shaped by socioeconomic background and perceptions of the national regulatory and economic climate. Students from middle- and low-income families expressed a greater desire to pursue careers abroad, highlighting the role of financial insecurity in shaping career aspirations. Additionally, dissatisfaction with local working conditions and perceived professional opportunities abroad further reinforced migration intentions.

These findings have several implications for policy and medical education. At the policy level, strategies aimed at improving economic stability, strengthening social support for students from lower-income families, and enhancing working conditions in the healthcare system

may help reduce the push factors driving young physicians to consider leaving the country. At the educational level, medical schools could integrate structured career counseling, mentorship programs, and well-being initiatives to help students navigate uncertainties, develop realistic career expectations, and foster a stronger sense of professional belonging within the national context. Addressing these factors in a coordinated and sustained manner may contribute to reducing the early formation of brain drain intentions among medical students.

Ethical Approval: The study was approved by the Marmara University Faculty of Medicine Non-Drug and Non-Medical Device Research Ethics Committee on March 21, 2025, with decision number 09.2025.25-0224.

Informed Consent: Verbal informed consent was obtained from all participants before data collection.

Peer-review: Externally peer-reviewed

Author Contributions: Concept – M.Ö.; Design – M.A., Ö.T.; Supervision – M.A., Ö.T., S.H.; Materials – S.H., Ö.T.; Data Collection and/or Processing – M.A., M.Ö., İ.Ş., E.F.A., Ş.A., H.E.M. Analysis and/or Interpretation – M.A., M.Ö., İ.Ş., E.F.A., Ş.A., H.E.M.; Literature Review – M.A., M.Ö., İ.Ş., E.F.A., Ş.A., H.E.M.; Writer – M.A., M.Ö., İ.Ş., E.F.A., Ş.A., H.E.M.; Critical Reviews – M.A., M.Ö., İ.Ş., E.F.A., Ş.A., H.E.M., S.H., Ö.T.

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REFERENCES

1 Shakil S. Stemming the medical brain drain: A personal perspective on a global problem. Einstein J Biol Med. 2016;31:11-6. [CrossRef]

2 Organisation for Economic Co-operation and Development (OECD). Recent trends in international migration of doctors, nurses and medical students [Internet]. Paris: OECD Publishing; 2019. [cited November 12, 2025]. Available from: https://www.oecd.org/en/publications/recent-trends-in-international-migration-of-doctors-nurses-and-medical-students_5571ef48-en.html

3 Toyin-Thomas P, Ikhurionan P, Omoyibo EE, Iwegim C, Ukueku AO, Okpere J, et al. Drivers of health workers' migration, intention to migrate and non-migration from low/middle-income countries, 1970-2022: a systematic review. BMJ Glob Health. 2023;8(5):e012338. [CrossRef]

4 Saluja S, Rudolfson N, Massenburg BB, Meara JG, Shrime MG. The impact of physician migration on mortality in low and middle-income countries: an economic modelling study. BMJ Glob Health. 2020;5(1):e001535. [CrossRef]

5 Kollar E, Buyx A. Ethics and policy of medical brain drain: a review. Swiss Med Wkly. 2013;143:w13845. [CrossRef]

6 Taşkın S. Hemşire Göç Eğilimi Çalışması Raporu [Internet]. Ankara: Turkish Nurses Association (THD). March 26, 2023. [cited November 12, 2025]. Available from: https://www.thder.org.tr/uploads/subeler/THD%202023/hemsire_goc_egilimi_calismasi_ra.2023-.pdf

7 Uzun SU. Attitudes and related factors toward brain drain among Pamukkale University medical students. Presented at: 5th International and 23rd National Public Health Congress; December 2021; Türkiye.

8 Kaya S, Toraman Ç, Tekin M. [Examining the views of medical faculty students about working abroad in the future: The case of Çanakkale]. TED. 2023;22(66):47-60. Turkish. [CrossRef]

9 Altun Güzelderen YB, Yurdakul Ş, Altundal EG., Ünal E, Mollahaliloğlu S. (2024). [Medical faculty students' perspectives on brain drain - a descriptive study]. ESTÜDAM Halk Sagligi Derg. 2024:9(3):245-53. Turkish. [CrossRef]

10 Filiz M, Karagöz MB, Karagöz N. [Evaluation of attitudes of medical faculty students towards brain drain]. Karadeniz Sos Bilim Derg. 2022:14(27):679-92. Turkish. [CrossRef]

11 Güner ME, Şengelen M, Ünal BB, Karabıçak C, Cakıcı D, Karadoğan E, et al. [Evaluation of the tendency of medical faculty term 1 and term 5 students to migrate abroad]. TED. 2024;23(69):59-60. Turkish. [CrossRef]

12 Köse Tosunöz V. [Brain drain among health professionals: A review study]. Dent Med J – R. 2023:6(2):97-108. Turkish.

13 Bener A, Ventriglio A, Almas F, Bhugra D. Determinants of *brain drain* among physicians in Turkey: Findings from a national exploratory study. Int J Soc Psychiatry. 2025;71(1):179-87. [CrossRef]

14 Mollahaliloğlu S, Çulha Ülger A, Koşdak M, Öncül HG. The migration preferences of newly graduated physicians in Turkey. Medical Journal of Islamic World Academy of Sciences. 2014;22(2):69-75. [CrossRef]

15 Önal FG, Akay FE. Are Turkish doctors in deep water? The role of professional ethics and factors affecting the medical brain drain: A qualitative study from Turkey. Dev World Bioeth. 2024;24(4):284-95. [CrossRef]

16 Sancak B, Selek SN, Sarı E. Depression, anxiety, stress levels and five-factor personality traits as predictors of clinical medical students' migration intention: A cross-sectional study of brain drain. Int J Health Plann Manage. 2023;38(4):1015-31. [CrossRef]

RESEARCH ARTICLE | Yeditepe Journal of Health Sciences, 13(3):147-153

The Safety Evaluation of Verbascoside from the Viewpoint of Genotoxicity

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Abstract

Objective: This study aimed to evaluate the genotoxic potential of verbascoside using a standard battery of assays across bacterial and mammalian cell systems.

Materials and Methods: The genotoxic potential of verbascoside was evaluated using a standard battery of assays, including the bacterial reverse mutation (Ames) test, cytokinesis-block micronucleus (CBMN) assay, and alkaline Comet assay. The Ames test was performed on *Salmonella typhimurium* TA98 and TA100 strains, with and without S9 metabolic activation, at concentrations ranging from 1 to 1000 µg/plate. Chinese hamster ovary (CHO-K1) cells were used for the CBMN and Comet assays at concentrations between 25 and 200 µg/mL.

Results: The mutagenic index remained below 2.0 across all tested concentrations, showing no significant variation with increasing dose in the Ames test. No significant differences were observed in micronucleus frequency between the negative control and any concentration of verbascoside. The Comet assay results revealed no significant difference in DNA tail percentage between the negative control and verbascoside-treated groups.

Conclusion: Under the tested conditions, verbascoside showed no mutagenic or genotoxic effect in bacterial or mammalian cell models, supporting a favorable genotoxicity safety profile and warranting further pharmacological development.

Keywords: Micronucleus assay, Comet assay, Ames test, verbascoside, genotoxicity

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INTRODUCTION

Verbascoside (acteoside) is a phenylethanoid glycoside composed of a hydroxyphenethyl (hydroxytyrosol) moiety linked to a disaccharide (α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl) *via* a glycosidic bond, which is also esterified with a caffeic acid (Figure 1). First isolated in 1968 from *Verbascum* species, it has since been reported in more than 200 plant species across 23 plant families, including Scrophulariaceae, Lamiaceae, Plantaginaceae, Verbenaceae, Oleaceae, and Buddlejaceae (1-3).

Pharmacologically, verbascoside exhibits a broad range of biological activities, including anti-inflammatory (4-6), anti-ulcerogenic (7), antioxidant (8-10), antimicrobial (5), and analgesic effects (11). Mechanistic studies indicate attenuation of TAK1/JNK/AP-1 signaling, accompanied by increased SHP-1 phosphorylation, down-regulation of cyclooxygenase and nitric oxide synthase expression, and calcium-dependent inhibition of arachidonic acid and histamine release through phospholipase modulation (12-15). In line with its antioxidant potential, verbascoside strongly suppresses reactive oxygen species (ROS)-driven oxidation and has been shown to outperform vitamin C in comparative assays (16). Preclinical data further demonstrated its antidepressant and neuroprotective actions *via* enhanced dopamine biosynthesis and modulation of neuronal stress-response pathways (17), as well as vascular protection through NO pathway dependent effects and modest angiotensin-converting enzyme (ACE) inhibition (18,19).

Beyond its central and cardiovascular effects, verbascoside contributes to glycemic regulation by inhibiting α-amylase and sodium–glucose cotransporter 1 (SGLT1)-mediated glucose absorption (20,21), alleviates β-cell endoplasmic reticulum stress through PERK/eIF2α suppression (22), and reduces inflammatory signaling *via* MAPK/NF-κB pathway modulation in chondrocytes and hepatic cells (23). Verbascoside has also shown cytotoxic and antiproliferative effects in various cancer cell lines, including human myeloma, leukaemia, gastric carcinoma, colorectal carcinoma, oral squamous cell carcinoma, and glioblastoma (24).

Medicinal plants have long been important sources of pharmacologically active compounds, and many modern drugs originate from natural products. Nevertheless, the safety and efficacy of numerous herbal preparations remain insufficiently validated (25). As global use of herbal medicines increases, objective toxicological evaluation is essential. The assumption that "natural" equates to "safe" is misleading, since some plants produce toxic secondary metabolites capable of serious adverse effects. Accordingly, systematic safety testing, including *in vitro* cytotoxicity, toxicokinetic, and genotoxicity assessments, is critical to define safety margins for plant-derived compounds. Although verbascoside has been widely investigated for pharmacological potential, data on its genotoxic and mutagenic safety are limited and inconsistent, with some studies reporting no genotoxicity (26,27) and others indicating genotoxic effects (28). These discrepancies underscore the need for comprehensive evaluation. In this study, we therefore assessed the genotoxic potential of verbascoside using a standard assay battery comprising the Ames test, the micronucleus assay, and the Comet assay.

MATERIALS AND METHODS

Chemicals

Verbascoside was purified from *Globularia sintenisii* and its chemical structure was elucidated by NMR and MS analysis (29).

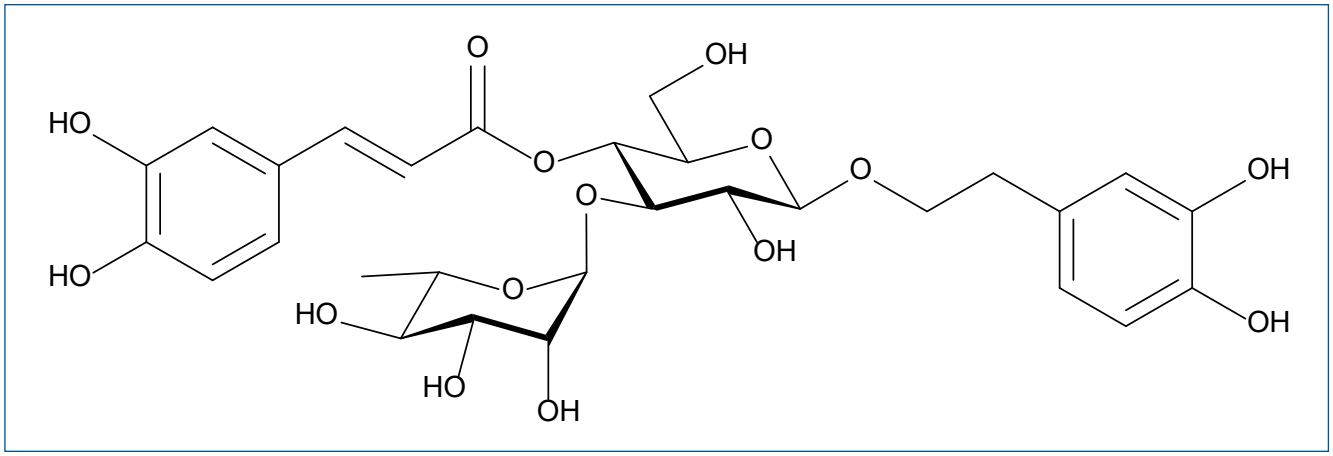

FIGURE 1. Chemical structure of verbascoside.

Genotoxicity Assessment

Mutagenicity Assays

The bacterial reverse mutation (Ames) plate-incorporation assay was carried out in a Turkish Accreditation Agency (*Türk Akreditasyon Kurumu*, TÜRKAK)-accredited facility (TS EN ISO/IEC 17025; AB-1764-T) in accordance with the method described by Maron and Ames (1983) (30). Histidine-auxotrophic *Salmonella typhimurium* strains TA98 and TA100 were maintained and used as recommended by the supplier. Testing was performed in triplicate at four concentrations (1–1000 µg/plate) across two independent experiments, both with and without metabolic activation (S9 from Aroclor™ 1254-induced rat liver). Dimethyl sulfoxide (DMSO; 50 µL/plate) was used as the vehicle control.

Positive controls were included as follows: −S9: 4-nitro-o-phenylenediamine (20 µg/plate) for TA98; sodium azide (1 µg/plate) for TA100. +S9: 2-aminofluorene (5 µg/plate) for TA98 and TA100. A result was judged positive when the revertant count showed at least a two-fold increase over the concurrent negative control. The mutagenic index (MI) was calculated as follows:

$$MI = A/B$$

where *A* represents the mean number of revertant colonies in the presence of the test compound and *B* represents the mean number of revertant colonies in the negative control. An MI value of ≥2 was considered indicative of a mutagenic effect.

Genotoxicity and Antigenotoxicity Assessment

Cell Line and Culture Conditions

Chinese hamster ovary (CHO-K1) cells (ATCC® CCL-61™) were grown in Ham's F-12 medium (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Gibco, NY, USA) and 1% penicillin–streptomycin (Gibco, NY, USA). Cultures were kept at 37°C in a humidified 5% CO_2 atmosphere and passaged approximately every 3 days to maintain exponential growth.

Cytokinesis-Block Micronucleus Assay

CHO-K1 cells were seeded in 6-well plates (2×10^5 cells/well, 24 h), then exposed to test samples at 25–200 µg/mL. After 24 hours, cytochalasin B (3 µg/mL) (Sigma-Aldrich, St. Louis, MO, USA) was added for 24 hours to block cytokinesis. Cells underwent hypotonic treatment (0.075 M KCl), fixation (methanol:acetic acid 3:1, twice), slide preparation, and Giemsa staining (5% in Sorensen buffer, 5 min) (Sigma-Aldrich, St. Louis, MO, USA).

For each culture/treatment, 1000 binucleated cells were scored for micronuclei and the nuclear division index (NDI) were calculated using formulas described previously (31).

Alkaline Comet Assay

CHO cells were seeded in 6-well plates (3×10^5 cells/well) 24 hours before treatment. Cells were then exposed for 4 hours to the samples. Following treatment, cells were collected, centrifuged (5 minutes, 1000 rpm), and resuspended.

20 µL of the cell suspension was mixed with 180 µL prewarmed low-melting-point agarose (Sigma-Aldrich, St. Louis, MO, USA); 45 µL was layered onto high-melting-point agarose-precoated slides (Genaxxon Bioscience, Ulm, Germany), covered, set 3 minutes, and coverslips removed (two replicates/treatment).

Slides were lysed at 4°C in the dark, equilibrated 20 minutes in cold electrophoresis buffer, then electrophoresed at 25 V with current adjusted to 0.3 A for 20 minutes. After electrophoresis, slides were rinsed (distilled water → Tris, 5 min), fixed in ice-cold methanol (-20°C, 5 min), air-dried, stained with ethidium bromide (Bio Basic Inc., Markham, ON, Canada), and cover slipped.

DNA damage was scored under fluorescence microscope (BS 200 ProP; BAB Imaging System, Ankara, Türkiye) (31).

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical comparisons were performed using analysis of variance (ANOVA), followed by Dunnett's post hoc test for multiple comparisons against the control group. Analyses were conducted using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). A p value <0.05 was considered statistically significant.

RESULTS

Mutagenicity and Antimutagenicity Assays

As shown in Table 1, the positive controls yielded MI values >2 in both S9+ and S9− conditions for TA98 and TA100, confirming assay performance. Across all four verbascoside doses, MI values for TA98 and TA100 remained <2 regardless of metabolic activation, with no dose-related increase (Table 1). In summary, verbascoside showed no mutagenicity in either strain under S9+ or S9− conditions within the tested concentration range.

Results of Cytokinesis-Block Micronucleus Assay

As summarized in Table 2, verbascoside did not increase micronucleus (MN) frequency relative to the negative control at any concentration tested ($p>0.05$) and NDI values remained within the acceptable range of 1.3-2.2, indicating no relevant cytotoxicity under assay conditions.

Table 1. Ames mutagenicity results in *Salmonella typhimurium* TA98 and TA100, with and without metabolic activation (S9).

	TA98 (revertants/plate)	TA100 (revertants/plate)	MI (TA98)	MI (TA100)
Without S9				
Negative control	28.5 ± 4.5	192.5 ± 3.5	-	-
Positive control	998.0 ± 76.4 *	976.5 ± 17.7*	35.5	5.1
1000	23.0 ± 8.5	192.5 ± 19.1	0.8	1.0
100	27.5 ± 4.9	178.0 ± 19.8	1.0	0.9
10	34.0 ± 2.8	188.0 ± 14.1	1.2	1.0
1	27.5 ± 3.5	198.0 ± 5.7	1.0	1.0
With S9				
Negative control	39.5 ± 0.7	140.0 ± 22.6	-	-
Positive control	1451.5 ± 67.2 *	888.0 ± 5.7*	50.9	5.8
1000	32.5 ± 3.5	144.0 ± 12.7	1.1	1.0
100	29.0 ± 2.8	141.0 ± 5.7	1.0	1.0
10	38.0 ± 5.7	140.5 ± 21.9	1.3	1.0
1	39.0 ± 4.2	133.0 ± 7.1	1.4	1.0

Positive controls: in the experiment without metabolic activation, TA98; 4-nitro-o-phenylenediamine (20 µg / plate), TA100; sodium azide (1 µg /plate), in the experiment with metabolic activation, 2-aminofluorene (5 µg / plate) for both strains. Dunnett's multiple comparison test was carried out for statistical analysis. *p<0.01 versus negative control group. For all other treatment groups, p-values were found to be above 0.05.

Table 2. Percentage of micronuclei observed in CHO cell cultures treated with various concentrations of verbascoside and with doxorubicin as a positive control.

Sample	MN%	NDI
Negative control	1.04 ± 0.05	2.00 ± 0.01
Positive control (doxorubicin, 1 µM)	33.3 ± 2.05*	1.33 ± 0.04
Verbascoside 25 µg/mL	0.98 ± 0.01	2.01 ± 0.01
Verbascoside 50 µg/mL	0.86 ± 0.25	1.97 ± 0.09
Verbascoside 100 µg/mL	1.17 ± 0.31	2.00 ± 0.02
Verbascoside 200 µg/mL	1.28 ± 0.19	2.07 ± 0.10

MN%: Percentage of cells with micronuclei, **NDI:** Nuclear division index.

*p<0.01 versus the negative control (Dunnett's test). All verbascoside-treated groups did not differ significantly from the negative control (p>0.05).

In contrast, doxorubicin produced the expected significant elevation in MN frequency (p<0.01), confirming assay sensitivity. Overall, verbascoside showed no MN induction and no cytotoxicity up to 200 µg/mL in CHO cells.

Results of Alkaline Comet Assay

DNA strand-breaks were evaluated in CHO cells by the alkaline Comet assay and expressed as %DNA in tail (100 cells/condition; analyzed with the BAB microscope software). Typical Comet images observed with ethidium bromide staining, are shown in Figure 2.

As shown in Figure 3, verbascoside produced no significant change in %DNA in tail versus the negative control across the tested concentrations ($p>0.05$). In contrast, doxorubicin (positive control) caused a significant increase in %DNA in tail ($p<0.01$). Although modest rises were noted at 100 and 200 µM verbascoside, these did not reach statistical significance ($p>0.05$).

DISCUSSION

Verbascoside, one of the most common phenylethanoid glycosides, possesses numerous biological activities, including analgesic, anti-inflammatory, anticancer, neuroprotective, antiulcer and antispasmodic. Given its increasing interest as a potential therapeutic and nutraceutical compound, the evaluation of its genotoxic safety is essential, as untested herbal products may pose toxicological risks despite their natural origin.

Previous studies evaluating the genotoxic potential of verbascoside have yielded inconsistent results. Santoro et al. (28) showed that verbascoside isolated from *Kigelia africana* induced structural chromosome aberrations and sister chromatid exchanges in human lymphocytes, accompanied by a reduction in mitotic index, suggesting a potential clastogenic effect. However, contribution of co-isolated constituents or extraction related artefacts cannot be completely excluded in this study. In contrast, Henn et al. (26) reported that verbascoside (1–50 µg/mL) was non-genotoxic in human fibroblasts and V79 cells using the alkaline Comet assay and that extracts containing verbascoside from *Aloysia* species were non-mutagenic in the Ames test. These findings were further supported by *in vivo* and alternative model studies. For instance, a long-term dietary rabbit study revealed no induction of chromosome aberrations or sister chromatid exchanges in peripheral lymphocytes and even showed a tendency toward reduced cytogenetic damage over time in treated groups (32).

Similarly, negative results were obtained in the *Drosophila melanogaster* SMART assay (27). Acute and subacute toxicity studies in mice further indicated a high safety margin, with an intraperitoneal LD_{50} exceeding 5 g/kg and no treatment related systemic toxicity following 21-day administration (33).

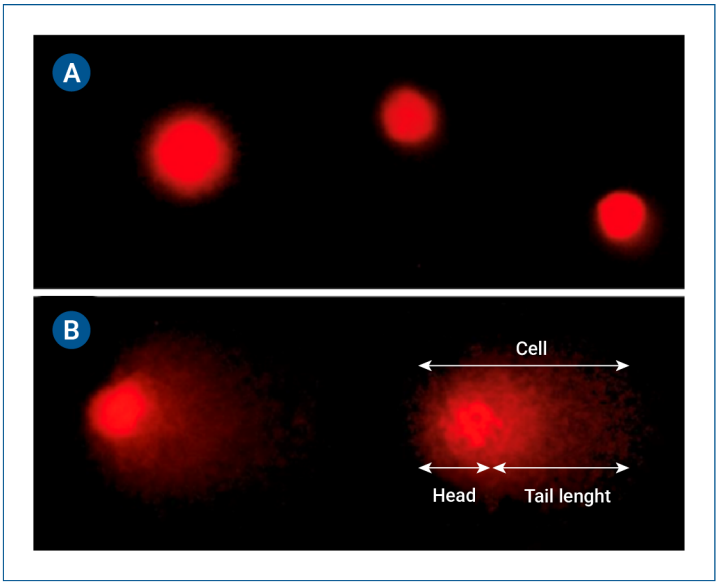

FIGURE 2. Example images of the Comet assay on CHO-K1 cells. A) Control group, B) Cells exposed to 1.5 µM doxorubicin, displaying increased DNA damage, as indicated by the presence of Comet tails.

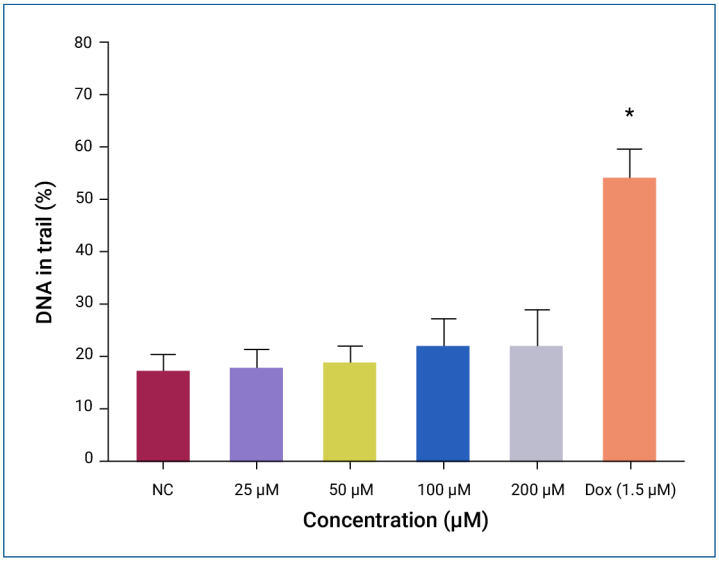

FIGURE 3. Percentage of DNA in tail observed in the Comet assay for CHO cell cultures treated with different concentrations of verbascoside and with doxorubicin (Dox) as a positive control.

*$p<0.01$ versus negative control group. For all other treatment groups, p-values were found to be above 0.05.

In the present study, verbascoside was evaluated using a battery of complementary genotoxicity assays, including the Ames test, the cytokinesis-block micronucleus assay, and the Comet assay. In the Ames test, the mutagenic index remained below the threshold value of 2.0 at all tested concentrations, indicating a lack of mutagenic effect in bacterial reverse mutation systems. In the micronucleus assay, verbascoside did not induce a statistically significant increase in micronucleus frequency compared with the negative control, suggesting the absence of clastogenic or aneugenic effect in mammalian cells. Furthermore, Comet assay analysis did not reveal a significant increase in DNA strand breaks, as reflected by unchanged DNA tail percentages across treatment groups. Although slight numerical increases were observed at higher concentrations (100 and 200 µM), these

changes were not statistically significant and did not exhibit a clear dose response relationship, limiting their biological relevance.

A major strength of the present study is the integrated use of three distinct genotoxicity endpoints, enabling the detection of gene mutations, structural/numerical chromosomal aberration and DNA strand breaks within a single experimental framework. This approach strengthens the reliability of the negative findings and reduces the likelihood of false negative interpretation that may arise from reliance on a single assay. Nevertheless, several limitations should be acknowledged. First, the present study was restricted to *in vitro* test systems and therefore does not account for complex *in vivo* factors such as absorption, metabolism, tissue distribution and long-term exposure. Second, mechanistic endpoints such as oxidative DNA base damage or DNA repair modulation were not specifically investigated and may warrant further targeted exploration.

Several mechanistic considerations may explain why verbascoside consistently appears non-genotoxic. Verbascoside is a strong antioxidant and radical scavenger capable of reducing intracellular ROS, chelating transition metals and stabilizing free radicals through its phenolic structure. Since oxidative stress is a major driver of DNA strand breaks and chromosomal damage, its ROS modulating activity may inherently limit DNA lesion formation (34). Additionally, verbascoside has been shown to enhance endogenous antioxidant defenses (e.g., SOD, CAT, GSH systems) and suppress inflammatory signaling, further reducing oxidative stress related genotoxicity (35). Thus, the biochemical properties of verbascoside are consistent with the absence of mutagenic or clastogenic findings observed in the present study's assays.

Taken together, the current findings in conjunction with published *in vitro* and *in vivo* studies, indicate that verbascoside does not exhibit mutagenic or genotoxic effect under the tested conditions. The single report suggesting clastogenicity appears to be an exception rather than the prevailing trend and may reflect experimental or matrix specific factors. Importantly, to the best of our knowledge, this is the first study to evaluate the genotoxic safety of verbascoside using the combined application of the Ames test, micronucleus assay and Comet assay within a single experimental design. Therefore, the present work provides a substantial and methodologically rigorous contribution to the toxicological characterization of verbascoside and supports its continued investigation as a bioactive phytochemical with a favorable genotoxic safety profile.

Ethical Approval: Ethical approval was not required for this study, as all experiments were conducted exclusively *in vitro* using established bacterial strains and commercially available cell lines, without the involvement of human participants or experimental animals.

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REFERENCES

1 Jiménez C, Riguera R. Phenylethanoid glycosides in plants: structure and biological activity. Nat Prod Rep. 1994;11(6):591-606. [CrossRef]

2 Scarpati ML, Delle Monache F. Isolation from *Verbascum sinuatum* of two new glucosides, verbascoside and isoverbascoside. Ann Chim. 1963;53(4):356-67.

3 Isacchi B, Iacopi R, Bergonzi MC, Ghelardini C, Galeotti N, Norcini M, et al. Antihyperalgesic activity of verbascoside in two models of neuropathic pain. J Pharm Pharmacol. 2011;63(4):594-601. [CrossRef]

4 Wang YN, Wu X, Shan QY, Yang Q, Yu XY, Yang JH, et al. Acteoside-containing caffeic acid is bioactive functional group of anti-fibrotic effect by suppressing inflammation via inhibiting AHR nuclear translocation in chronic kidney disease. Acta Pharmacol Sin. 2025;46(11):2975-88. [CrossRef]

5 Zhang FK, Jia KX, Wang H, Liu RP, Xue XY, Huo ZX, et al. Acteoside as a rising star for clinical treatment: Current fundamental research and future outlooks. J Integr Med. 2025:S2095-4964(25)00134-7. [CrossRef]

6 Guo D, Mao Q, Fang X, Huang L, Tian H, Yang W, et al. Synergistic modulation of microglial polarization by acteoside and ferulic acid via dual targeting of Nrf2 and RORγt to alleviate depression-associated neuroinflammation. Adv Sci (Weinh). 2025;12(43):e03889. [CrossRef]

7 Guo W, Wang X, Liu F, Chen S, Wang S, Zhang Q, et al. Acteoside alleviates dextran sulphate sodium induced ulcerative colitis via regulation of the HO 1/HMGB1 signaling pathway. Mol Med Rep. 2022;26(6):360. [CrossRef]

8 Díaz AM, Abad MJ, Fernández L, Silván AM, De Santos J, Bermejo P. Phenylpropanoid glycosides from *Scrophularia scorodonia*: *in vitro* anti-inflammatory activity. Life Sci. 2004;74(20):2515-26. [CrossRef]

9 Hausmann M, Obermeier F, Paper DH, Balan K, Dunger N, Menzel K, et al. *In vivo* treatment with the herbal phenylethanoid acteoside ameliorates intestinal inflammation in dextran sulphate sodium-induced colitis. Clin Exp Immunol. 2007;148(2):373-81. [CrossRef]

10 Penido C, Costa KA, Futuro DO, Paiva SR, Kaplan MA, Figueiredo MR, et al. Anti-inflammatory and anti-ulcerogenic properties of *Stachytarpheta cayennensis* (L.C. Rich) Vahl. J Ethnopharmacol. 2006;104(1-2):225-33. [CrossRef]

11 Hara K, Haranishi Y, Terada T. Verbascoside administered intrathecally attenuates hyperalgesia via activating mu-opioid receptors in a rat chronic constriction injury model. Eur J Pain. 2022;26(6):1322-32. [CrossRef]

12 Pesce M, Franceschelli S, Ferrone A, De Lutiis MA, Patruno A, Grilli A, et al. Verbascoside down-regulates some pro-inflammatory signal transduction pathways by increasing the activity of tyrosine phosphatase SHP-1 in the U937 cell line. J Cell Mol Med. 2015;19(7):1548-56. [CrossRef]

13 Lee JH, Lee JY, Kang HS, Jeong CH, Moon H, Whang WK, et al. The effect of acteoside on histamine release and arachidonic acid release in RBL-2H3 mast cells. Arch Pharm Res. 2006;29(6):508-13. [CrossRef]

14 Song HS, Choi MY, Ko MS, Jeong JM, Kim YH, Jang BH, et al. Competitive inhibition of cytosolic Ca2+-dependent phospholipase A2 by acteoside in RBL-2H3 cells. Arch Pharm Res. 2012;35(5):905-10. [CrossRef]

15 Motojima H, Villareal MO, Iijima R, Han J, Isoda H. Acteoside inhibits type I allergy through the down-regulation of Ca/NFAT and JNK MAPK signaling pathways in basophilic cells. J Nat Med. 2013;67(4):790-8. [CrossRef]

16 Liu M, Tan H, Xie H. Phenylethanoid glycosides from *Michelia champaca* leaves. Phytochemistry. 2024;226:114118. [CrossRef]

17 Zhao Y, Wang S, Pan J, Ma K. Verbascoside: A neuroprotective phenylethanoid glycosides with anti-depressive properties. Phytomedicine. 2023;120:155027. [CrossRef]

18 Lau CW, Chen ZY, Wong CM, Yao X, He Z, Xu H, et al. Attenuated endothelium-mediated relaxation by acteoside in rat aorta: Role of endothelial [Ca2+]i and nitric oxide/cyclic GMP pathway. Life Sci. 2004;75(10):1149-57. [CrossRef]

19 Bento I, Pereira JA. *Arbutus unedo* L. and its benefits on human health. J Food Nutr Res. 2011;50(2):73-85.

20 Lu Y, Zhou W, Feng Y, Li Y, Liu K, Liu L, et al. Acteoside and acyl-migrated acteoside, compounds in Chinese Kudingcha Tea, inhibit α-amylase *in vitro*. J Med Food. 2017;20(6):577-85. [CrossRef]

21 Shimada H, Urabe Y, Okamoto Y, Li Z, Kawase A, Morikawa T, et al. Major constituents of *Cistanche tubulosa*, echinacoside and acteoside, inhibit sodium-dependent glucose cotransporter 1-mediated glucose uptake by intestinal epithelial cells. J Funct Foods. 2017;39:91-5. [CrossRef]

22 Galli A, Marciani P, Marku A, Ghislanzoni S, Bertuzzi F, Rossi R, et al. Verbascoside protects pancreatic β-cells against ER-stress. Biomedicines. 2020;8(12):582. [CrossRef]

23 Lim H, Kim DK, Kim TH, Kang KR, Seo JY, Cho SS, et al. Acteoside counteracts interleukin-1β-induced catabolic processes through the modulation of mitogen-activated protein kinases and the NFκB cellular signaling pathway. Oxid Med Cell Longev. 2021;2021:8684725. [CrossRef]

24 Şenol H, Tulay P, Ergören MÇ, Hanoğlu A, Çalış İ, Mocan G. Cytotoxic effects of verbascoside on MCF-7 and MDA-MB-231. Turk J Pharm Sci. 2021;18(5):637-44. [CrossRef]

25 Bent S. Herbal medicine in the United States: review of efficacy, safety, and regulation: grand rounds at University of California, San Francisco Medical Center. J Gen Intern Med. 2008;23(6):854-9. [CrossRef]

26 Henn JG, Steffens L, de Moura Sperotto ND, de Souza Ponce B, Veríssimo RM, Boaretto FBM, et al. Toxicological evaluation of a standardized hydroethanolic extract from leaves of *Plantago australis* and its major compound, verbascoside. J Ethnopharmacol. 2019;229:145-56. [CrossRef]

27 Anter J, Tasset I, Demyda-Peyrás S, Ranchal I, Moreno-Millán M, Romero-Jimenez M, et al. Evaluation of potential antigenotoxic, cytotoxic and proapoptotic effects of the olive oil by-product "alperujo", hydroxytyrosol, tyrosol and verbascoside. Mutat Res Genet Toxicol Environ Mutagen. 2014;772:25-33. [CrossRef]

28 Santoro A, Bianco G, Picerno P, Aquino RP, Autore G, Marzocco S, et al. Verminoside- and verbascoside-induced genotoxicity on human lymphocytes: involvement of PARP-1 and p53 proteins. Toxicol Lett. 2008;178(2):71-6. [CrossRef]

29 Kırmızıbekmez H, Çalış İ, Piacente S, Pizza C. Iridoid and phenylethyl glycosides from *Globularia sintenisii*. Helvetica chimica acta. 2004;87(5):1172-9. [CrossRef]

30 Maron DM, Ames BN. Revised methods for the *Salmonella* mutagenicity test. Mutat Res. 1983;113(3-4):173-215. [CrossRef]

31 Helvacioglu S, Charehsaz M, Bankoglu EE, Stopper H, Aydin A. The ameliorative effect of rosmarinic acid and epigallocatechin gallate against doxorubicin-induced genotoxicity. Drug Chem Toxicol. 2024;47(6):1087-99. [CrossRef]

32 Perucatti A, Genualdo V, Pauciullo A, Iorio C, Incarnato D, Rossetti C, et al. Cytogenetic tests reveal no toxicity in lymphocytes of rabbit (*Oryctolagus cuniculus*, 2n=44) feed in presence of verbascoside and/or lycopene. Food Chem Toxicol. 2018;114:311-5. [CrossRef]

33 Etemad L, Zafari R, Vahdati-Mashhadian N, Moallem SA, Shirvan ZO, Hosseinzadeh H. Acute, sub-acute and cell toxicity of verbascoside. Res J Med Plant. 2015;9(7):354-60.

34 Goncalves S, Grevenstuk T, Martins N, Romano A. Antioxidant activity and verbascoside content in extracts from two uninvestigated endemic *Plantago* spp. Ind Crops Prod. 2015;65:198-202. [CrossRef]

35 Khorashadizadeh N, Neamati A, Moshiri M, Etemad L. Verbascoside inhibits paraquate-induced pulmonary toxicity via modulating oxidative stress, inflammation, apoptosis and DNA damage in A549 cell. Drug Chem Toxicol. 2022;45(5):2212-20. [CrossRef]

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