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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Anticancer Potential of Lyophilised Medicinal Leech (*Hirudo verbana*) of Saliva Extract Against Pancreatic Cancer (MIA PaCa-2) Cell Lines [*]

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Abstract: Cancer is the second leading cause of death worldwide, after cardiovascular disease. It can affect any part of the body and spread to other organs. Pancreatic cancer is a tough disease to diagnose and treat. It is the fourteenth most common and the seventh deadliest cancer worldwide. New technology and innovative methods, combined with a range of therapeutic agents, have led to promising new anticancer treatments. The presence of various bioactive components in the secretions of medicinal leeches has prompted a re-evaluation of these organisms as a popular method in traditional medicine. In this study, the effect of the anticancer potential of lyophilised medicinal leech secretion on pancreatic cancer cell line (MIA PaCa-2) was investigated using XTT assay. A cell viability test was conducted to ascertain the degree of cytotoxicity following the administration of varying concentrations of medicinal leech secretion to cell lines over a period of 24, 48, and 72 hours. The percentage viability of cancer cells was determined at each concentration. The doses were adjusted using dilution procedures with ratios of 75 µg/ml, 150 µg/ml, 300 µg/ml, 600 µg/ml, and 1200 µg/ml. IC_{50} value was determined at 24th hour: 484.48 µg/ml; at 48th hour: 330.92 µg/ml; at 72nd hour: 542,75 µg/ml. It was observed that the effect of *Hirudo verbana* leech secretion on pancreatic cancer (MIA PaCa-2) cell viability was not linear. It was determined that 600 µg/ml and 1200 µg/ml extracts had cytotoxic and anti-proliferative effects. These results indicate that the leech saliva extract has anti proliferative and cytotoxic effects and may have a promising role in developing new anticancer drugs.

Keywords: Anti-cancer, *Hirudo verbana*, Pancreatic Cancer, MIA PaCa-2, XTT.

Liyofilize Tıbbi Sülük (*Hirudo verbana*) Tükürük Ekstraktının Pankreas Kanseri (MIA PaCa-2) Hücre Hatlarına Karşı Antikanser Potansiyeli

***Sorumlu yazar:**
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Öz: Kanser, kardiyovasküler hastalıklardan sonra dünya çapında ikinci önde gelen ölüm nedenidir. Vücudun herhangi bir bölümünü etkileyebilir ve diğer organlara yayılabilir. Pankreas kanseri teşhis ve tedavisi zor bir hastalıktır. Dünya çapında en yaygın on dördüncü ve en ölümcül yedinci kanserdir. Yeni teknoloji ve yenilikçi yöntemler, bir dizi terapötik ajanla birleşerek umut verici yeni antikanser tedavilerine yol açmıştır. Tıbbi sülüklerin salgılarında çeşitli biyoaktif bileşenlerin bulunması, bu organizmaların geleneksel tıpta popüler bir yöntem olarak yeniden değerlendirilmesine neden olmuştur. Bu çalışma, liyofilize tıbbi sülük salgısının pankreas kanseri hücre hattı (MIA PaCa-2) üzerindeki antikanser potansiyelinin etkisi XTT testi kullanılarak incelenmiştir. Hücre hatlarına 24, 48 ve 72 saatlik bir süre boyunca değişen konsantrasyonlarda tıbbi sülük salgısının uygulanmasının ardından sitotoksisite derecesini tespit etmek için bir hücre canlılık testi yapılmıştır. Kanser hücrelerinin canlılık yüzdesi her bir konsantrasyonda belirlenmiştir. Dozlar 75 µg/ml, 150 µg/ml, 300 µg/ml, 600 µg/ml ve 1200 µg/ml oranlarında seyreltme prosedürleri kullanılarak ayarlanmıştır. IC_{50} değeri 24. saatte: 484,48 µg/ml; 48. saatte: 330,92 µg/ml; 72. saatte 542,75 µg/ml olarak belirlenmiştir. *Hirudo verbana* sülük salgısının pankreas kanseri (MIA PaCa-2) hücre canlılığı üzerindeki etkisinin doğrusal olmadığı gözlenmiştir. 600 µg/ml ve 1200 µg/ml ekstraktların sitotoksik ve anti-proliferatif etkileri olduğu belirlenmiştir. Bu sonuçlar, sülük tükürüğü ekstraktının anti proliferatif ve sitotoksik etkilere sahip olduğunu ve yeni antikanser ilaçların geliştirilmesinde umut verici bir role sahip olabileceğini göstermektedir.

Anahtar Kelimeler: Anti-kanser, *Hirudo verbana*, MIA PaCa-2, Pankreas Kanseri, XTT.

[*] *This study was produced from the master thesis of Serkan ÖZDEMIR* *Bu çalışma Serkan ÖZDEMİR'in yüksek lisans tezinden üretilmiştir.*

INTRODUCTION

Cancer is the second leading cause of mortality worldwide, following cardiovascular diseases (WHO, 2023). It is a complex and multifactorial disease characterized by the uncontrolled proliferation of cells, with both genetic predisposition and environmental factors playing a significant role in its development (Baykara, 2016). Among various cancer types, pancreatic cancer presents a significant diagnostic and therapeutic challenge. It is the fourteenth most common malignancy globally and ranks seventh in cancer-related mortality (Modi & Shires, 2020; McGuigan et al., 2018). Notably, approximately 85-90% of pancreatic cancers are classified as ductal adenocarcinomas, which exhibit high metastatic potential, spreading to both adjacent tissues and distant organs (Wolfgang et al., 2013; Modi & Shires, 2020).

While conventional cancer treatments follow standardized protocols, therapeutic approaches vary depending on the type and stage of cancer. Current treatment modalities include chemotherapy, immunotherapy, gene therapy, radiotherapy, and surgical interventions, which are often employed individually or in combination to enhance treatment efficacy (Fitzmaurice et al., 2015; Pavlopoulou et al., 2015). However, these treatments are frequently associated with adverse side effects that can impact patient outcomes and quality of life (Bray et al., 2013). With advancements in biomedical research, novel therapeutic strategies are being explored, including the use of natural bioactive compounds with potential anticancer properties (Surh, 2003; Deng et al., 2019).

Medicinal leeches have recently attracted increasing interest in traditional medicine due to their secretion of bioactive peptides and proteins with therapeutic properties (Abdualkader et al., 2013; Ayhan & Mollahaliloğlu, 2018). The saliva of *Hirudo verbana* is secreted along the dental ridges and exits through the excretory ducts located between the teeth. These bioactive molecules not only facilitate blood-feeding but also exert diverse pharmacological effects on the host (Ayhan et al., 2021). Several key compounds found in leech saliva, including vasodilators, anticoagulants, anti-inflammatory agents, analgesics, and bacteriostatics, have been demonstrated to influence immune function, regulate blood pressure, and promote tissue and organ repair (Gödekmerdan et al., 2011; Ayhan et al., 2025). Among these components, antistasin, a protein identified in leech secretions, has been shown to inhibit cancer cell colonization and exhibit antimetastatic activity (Ammar et al., 2015; Shakouri et al., 2021). In addition to anti-proteolytic, platelet aggregation inhibitors and anticoagulant enzymes, anti-tumour activity is involved in other elements such as hyaluronidase. By eliminating the hyaluronic acid-CD44 interaction, hyaluronidase antitumour activity is thought to be mediated to some extent through pro-tumourigenic immune cell inhibition into the tumour stroma (Singh & Rajoria, 2020). Some studies have identified synthetic hirudin to be successful as an effective metastasis inhibitor of various cancer cells such as pulmonary carcinoma, osteosarcoma, leukaemia and breast carcinoma (Ammar et al., 2017). Hirudin obtained from the secretion of *Hirudo medicinalis* shows practical anti-metastatic activity in various malignancies such as pulmonary carcinoma, osteosarcoma, breast carcinoma and leukaemia (Dong et al., 2019).

Given these promising bioactive properties, this study aims to evaluate the anticancer potential of lyophilized *Hirudo verbana* secretion on the pancreatic cancer cell line MIA PaCa-2.

MATERIAL AND METHOD

Chemicals and Cell Lines: The MIA PaCa-2 cell line was obtained from the Ankara Yıldırım Beyazıt University Central Research Laboratory Application and Research Centre, where the study was conducted. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 50 ml fetal bovine serum (FBS) and 5 ml penicillin-streptomycin. All procedures were performed in a laminar flow safety cabinet to ensure sterility. The cells were subsequently seeded in fresh medium in 25 cm^2 flasks and incubated at 37°C in a humidified atmosphere containing 5% CO_2 until reaching the desired confluency.

Preparation of Medicinal Leech Secretion: Medicinal leeches (*Hirudo verbana*) were obtained from a licensed farm approved by the Ministry of Agriculture and Forestry. The leeches were maintained in glass jars containing chlorine-free tap water, which was replaced every two to three days. Species identification was performed using a Euromex NZ.1903-S trinocular stereo microscope following the taxonomic criteria established by Davies (1991), Sawyer (1986), Neubert and Nesemann (1999), and Sağlam (2004).

To optimize secretion extraction, the leeches underwent a four-month starvation period. A solution mimicking human blood fluid, composed of 0.001 M arginine and 0.07 M sodium chloride, was prepared as a stimulant for secretion induction (Abdualkader, 2011). After ingestion of the solution, the leeches were allowed to regurgitate the ingested content. The expelled colorless salivary fluids were centrifuged at 3500 rpm at +4°C for 10 minutes, followed by filtration through a 0.8 µm membrane filter. The obtained secretion was frozen at -80°C for 24

hours and subsequently lyophilized for 48 hours to obtain a solid powdered form.

Protein Analysis in Medicinal Leech Secretion Extract: The lyophilized leech secretion was dissolved in DMEM, and the total protein concentration was quantified using the Bradford Protein Assay Kit (ABP Biosciences, USA) in accordance with the Bradford method (Bradford, 1976). Based on the total protein concentration, experimental doses of 1200 µg/ml, 600 µg/ml, 300 µg/ml, 150 µg/ml, and 75 µg/ml were prepared by serial dilution for subsequent experiments.

Cytotoxic Evaluation: Cell viability, cytotoxicity, and proliferation were assessed using the XTT assay, a colorimetric method that measures the conversion of tetrazolium salt into a water-soluble orange formazan in metabolically active cells. The assay was performed in accordance with the manufacturer's protocol to evaluate the cytotoxic effects of leech secretions on the MIA PaCa-2 cell line.

Following the administration of the secretion at various concentrations, the cells were incubated under controlled conditions (95% humidity, 5% CO_2, and 37°C). After a 2.5-hour incubation period, the culture plates were removed, and absorbance was measured at 450 nm and 650 nm. To eliminate non-specific absorption, values obtained at 650 nm were subtracted from those at 450 nm. The negative control group, which was not exposed to the secretion, was considered 100% viable, and the viability rates of treated groups were calculated relative to this control. The calculation formula used was:

(Experimental group mean value / Control group mean value) × 100

The XTT assay was conducted in triplicate at dilution ratios of 1:1, 1:2, 1:4, 1:8, and 1:16 for incubation periods of 24, 48, and 72 hours. Furthermore, IC_{50} values were determined by nonlinear regression curve fitting using response data normalized against the logarithm of the secretion concentration.

Statistical Analysis: The IC_{50} values obtained at 24, 48, and 72 hours from the XTT assay were subjected to statistical analysis using the Excel power regression function. The mean, standard deviation, and percentage viability ratios were calculated using IBM SPSS Statistics 21 software (IBM SPSS Inc., Chicago, IL, USA).

RESULTS

Cytotoxic Evaluation of Medicinal Leech Secretion Extract on Cancer Cells: The percentage of cell viability was calculated using the following formula:

(Experimental group mean value / Control group mean value) × 100

The control group was considered 100% viable for cytotoxicity assessments. The power regression analysis of the XTT assay results for different concentrations of medicinal leech secretion applied to the MIA PaCa-2 cell line in Excel yielded the following IC_{50} values at the specified time points: 484.49 µg/mL at 24 hours (Figure 1), 330.92 µg/mL at 48 hours (Figure 2), and 542.75 µg/mL at 72 hours (Figure 3).

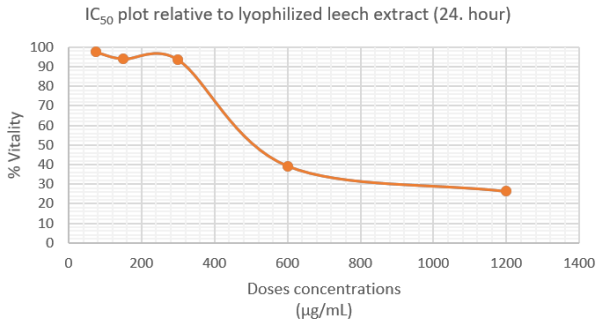

Figure 1. IC_{50} plot relative to lyophilized leech extract at 24 hours.

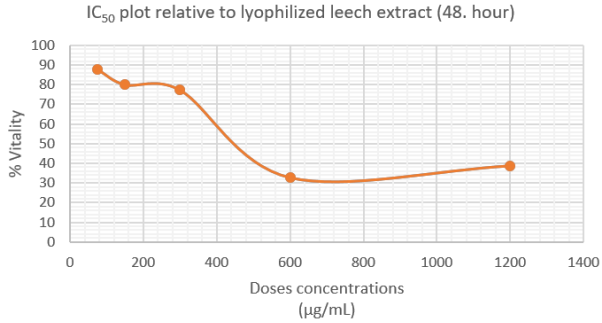

Figure 2. IC_{50} plot relative to lyophilized leech extract at 48 hours.

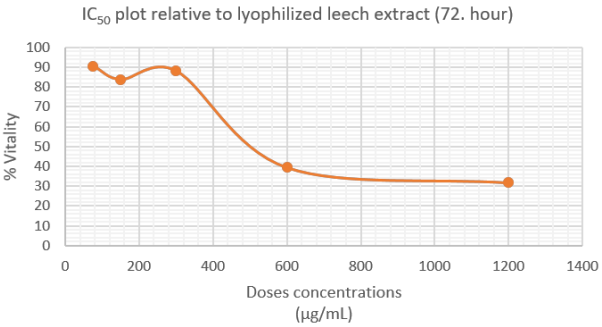

Figure 3. IC_{50} plot relative to lyophilized leech extract at 72 hours.

The viability rates of cells treated with leech secretion were statistically evaluated based on measurements taken at 24-hour intervals. The percentage of viable cells in each dose group at 24 hours was as follows: 75 µg/mL, 97.49%; 150 µg/mL, 94.06%; 300 µg/mL, 93.58%; 600 µg/mL, 39.24%; and 1200 µg/mL, 26.31%. At the 48-hour interval, the viability rates were: 75 µg/mL, 87.74%; 150 µg/mL, 80.05%; 300 µg/mL, 77.26%; 600 µg/mL, 32.63%; and 1200 µg/mL, 38.71%. At 72 hours, the viability rates were: 75 µg/mL, 90.56%; 150 µg/mL, 83.75%; 300 µg/mL, 88.04%; 600 µg/mL, 39.43%; and 1200 µg/mL, 31.79% (Table 1).

Table 1. Cell viability (%) was measured at 24, 48, and 72 hours according to the absorbances obtained as a result of the XTT test.

Times	Control	1200 µg/ml	600 µg/ml	300 µg/ml	150 µg/ml	75 µg/ml
24 hour	100	26.31	39.24	93.58	94.06	97.49
48 hour	100	38,71	32,63	77,26	80,05	87,74
72 hour	100	31,79	39,43	88,04	83,75	90,56

The cytotoxic effects of medicinal leech secretion on MIA PaCa-2 cells are illustrated in Figure 4. According to the results, a significant dose-dependent decrease in the viability of pancreatic cancer cells was observed.

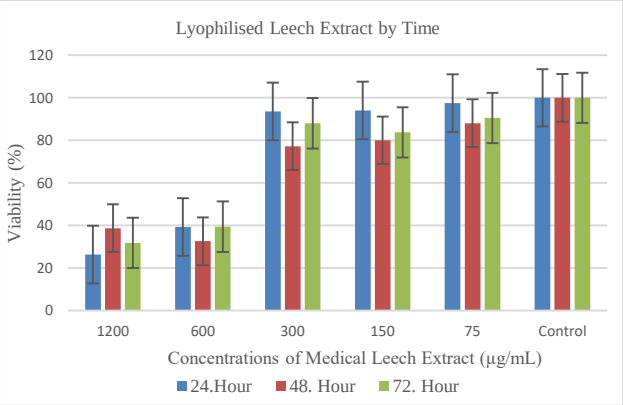

Figure 4. Effects of different concentrations of leech secretion extract on MIA PaCa-2 cell viability.

DISCUSSION

Although pancreatic cancer is predominantly observed in older individuals, it is more frequently diagnosed in males and is associated with a high mortality rate (Wolfgang, 2013; Ferlay et al., 2018). The lifetime risk of developing pancreatic cancer is estimated to be approximately 1.6%. While incidence rates vary globally, an annual increase of 0.5% to 1% has been reported (Daniel, 2018; Ilic & Ilic, 2016). Pancreatic cancers can originate from both exocrine and endocrine cells, with exocrine tumors being more prevalent. Pancreatic ductal adenocarcinoma (PDAC), the most common type of exocrine tumor, accounts for over 90% of all malignant pancreatic tumors (Fesinmeyer et al., 2005). PDAC frequently metastasizes to the liver and lymph nodes (Kern et al., 2002). PDAC is often diagnosed at an advanced stage due to the absence of early symptoms, at which point metastasis may have already occurred, reducing the efficacy of available treatment options (Fesinmeyer et al., 2005). Current therapeutic options for metastatic PDAC include gemcitabine/nab-paclitaxel, modified folinic acid, irinotecan, irinotecan/fluorouracil, fluorouracil, oxaliplatin, gemcitabine/capecitabine, and gemcitabine monotherapy. Research into the cellular origin and molecular profile of PDACs has enabled oncologists to develop personalized treatment strategies (Taherian et al., 2022). However, despite extensive scientific investigation, these chemotherapy regimens remain limited in efficacy and are associated with adverse effects. Consequently, there is a need to identify natural pharmaceutical compounds with minimal adverse effects, lower cost, and reduced drug resistance for cancer treatment (Muhammad et al., 2022; Aysin et al., 2024).

Medicinal leech treatments have been utilized as a traditional and complementary therapeutic approach for centuries. Historically, leech secretions have been employed in traditional Chinese medicine for the treatment of various cancers, including esophageal, gastrointestinal, uterine, and breast cancer (Guo et al., 2006; Tural & Ayhan, 2024). In recent years, scientific studies have investigated the bioactive compounds found in leeches, leading to the development of novel therapeutic agents (Gödekmerdan et al., 2011). The United States Food and Drug Administration (FDA) approved the use of medicinal leeches for therapeutic applications in 2004. The salivary secretions of the leech contain agents that show anti-metastatic and anti-tumour activity. These secretions contain antistasin, a protein known to inhibit cancer cell colonization, and hyaluronidase, which has demonstrated antitumor activity (Baskova et al., 2008; Ammar et al., 2015; Alaama et al., 2024). Hypercoagulability is frequently observed in patients with malignant tumors. Studies have indicated that leech therapy leads to a slight prolongation of prothrombin time and activated partial thromboplastin time, alongside reductions in fibrinogen and D-dimer levels. Furthermore, when combined with conventional medical treatments, leech therapy has been found to mitigate the toxicity and side effects of interventional chemotherapy and hemorrhagic events, while also improving coagulation function in hypercoagulable patients (Tang et al., 2012). Leech therapy is a promising method clinically used for patients with different types of cancer; however, the resistance of tumour cells to this agent is still the main obstacle to effective cancer treatment.

To date, several studies have been conducted to examine the antiproliferative and cytotoxic activities of leech secretion across different cancer types. Transcriptome analysis of tumour samples showed that Leech Saliva Ekstract (LSE) had significant immunomodulatory and anti-inflammatory effects, together with significant effects on cell-cell adhesion, induction of glutathione transferase and inhibition of certain growth factors. Consequently, these effects led to significant cell cycle arrest, increase in apoptosis and decrease in proliferation (Ammar et al., 2015). A study evaluating the effects of *Hirudo medicinalis* secretion on cancer cell lines reported an antitumor activity rate increase of 97% in the liposomal form of leech secretion compared to direct application in MCF-7 breast cancer cells. Additionally, an antiproliferative effect of 10% was observed in the healthy HUVEC cell line (Shakouri, 2022). Similarly, an in vitro investigation into the effects of

Hirudo verbana secretion on a breast fibroblast cell line demonstrated significant alterations in cell viability, migration capacity, and gene expression in response to different concentrations of leech secretion (Ünal et al., 2023). The extraction yields of *H.verbana* leech saliva extracts were in line with the study by Shakouri et al. (2021), which showed the similar results with the yield of leech saliva extracts.

In the present study, *Hirudo verbana* secretion, one of the most commonly studied medicinal leech species, was investigated for the first time on the MIA PaCa-2 pancreatic cancer cell line. The findings reveal the selective and dose-dependent anticancer effects of leech secretion on MIA PaCa-2 cells, corroborating earlier reports of lower IC_{50} values in cancer cells. It is hypothesized that bioactive compounds in leech secretion, including hirudin, hyaluronidase, and antistasin, may exert cytotoxic and apoptotic effects. The effect of leech secretion on cellular viability was found to be dependent on both concentration and exposure duration. Notably, following treatment with 1200 µg/mL of leech secretion for 24 to 72 hours, cell growth inhibition reached up to 75%. Lower concentrations exhibited a more pronounced effect on cell viability at 48 and 72 hours, while minimal effects were observed at 24 hours. In conclusion, leech secretion has the potential to serve as a natural and effective anticancer agent. Further studies are required to elucidate the mechanisms of action and in vivo efficacy.

CONCLUSION

The results presented in this study make a novel contribution to the existing literature on the mechanism of action of medicinal leech secretions and provide a foundation for further research. The findings have significant implications for the development of drugs that can effectively target different aspects of cancer treatment. The IC_{50} value of the medicinal leech secretion decreased at 48 hours and increased at 72 hours. Additional studies are still needed to elucidate the reason for this, to separate and determine the active principle, to check the mechanism of action and to evaluate the effect on different cell line types. Despite increasing interest in the bioactive compounds of leeches, further in vitro and in vivo studies are necessary to fully establish their potential anticancer properties. The anti-cancer effect of leech saliva can be further improved by the use of combined therapy and thus becomes a promising remedy for the application of pancreatic cancer patients.

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STATEMENTS AND DECLARATIONS

Ethical Approval Certificate: *Ethical approval is not required since the medicinal leeches secreted in our study are invertebrate animals.*

Author Contribution Statement: *Serkan ÖZDEMİR: Investigation, formal analysis; writing-original draft; methodology; validation.*

Hüseyin AYHAN: Investigation; writing-original draft; Supervision; methodology; validation; writing-review & editing; software; formal analysis; data curation.

Conflict of Interest: *The authors declare no conflict of interest.*

REFERENCES

Abdualkader, A.M., Merzouk, A., Ghawi, A.M. & Alaama, M. (2011). Some biological activities of Malaysian leech saliva extract. *IIUM Engineering Journal*, ***12***(4). DOI: 10.31436/iiumej.v12i4.156

Abdualkader, A.M., Ghawi, A.M., Alaama, M., Awang, M. & Merzouk, A. (2013). Leech therapeutic applications. *Indian journal of pharmaceutical sciences*, **75**(2), 127-137.

Alaama, M., Kucuk, O., Bilir, B., Merzouk, A., Ghawi, A. M., Yerer, M. B., Ahmado, M. A., Abdualkader, A. M. & Helaluddin, A. B. M. (2024). Development of Leech extract as a therapeutic agent: A chronological review. *Pharmacological Research-Modern Chinese Medicine*, 10, 100355. DOI: 10.1016/j.prmcm.2023.100355

Ammar, A.E., Hassona, M.H., Meckling, G.R., Chan, L.G., Chin, M.Y., Abdualkader, A., Alaama, M., Merzouk, A., Helaluddin, A., Ghawi, A., Kucuk, O. & Guns, E.S. (2015). Assessment of the antitumor activity of leech (*huridinaria manillensis*) saliva extract in prostate cancer. *Cancer Research*, ***75***(15_Supplement), 5130-5130. DOI:10.1158/1538-7445.AM2015-5130

Ammar, A., Guns, E., Kucuk, O., Abdualkader, A., Alaama, M., Uddin, A.H., ... & Hassona, M. (2017). Mechanism of anticancer activity of BPS-001 (lyophilized leech saliva extract). Cancer Research, ***77*** (13_Supplement), 107-107. DOI: 10.1158/1538-7445.AM2017-107

Ayhan, H. & Mollahaliloğlu, S. (2018). Tıbbi sülük tedavisi: Hirudoterapi. *Ankara Medical Journal*, ***18***(1), 141-148. DOI: 10.17098/amj.409057

Ayhan, H., Özyurt Koçakoğlu, N. & Candan, S. (2021). Functional morphology of the suckers and teeth of the medicinal leech *Hirudo verbana* Carena, 1820 (Annelida; Clitellata; Hirudinida): A scanning electron microscope study. *Microscopy Research and Technique*, ***84***(12), 2930-2935. DOI: 10.1002/jemt.23851

Ayhan, H., Sevin, S., Karaaslan, S. & Ayaz, F. (2025). Immunomodulatory effects of medicinal leech saliva extract on in vitro activated macrophages. *Immunologic Research*, ***73***(1), 1-8. DOI: 10.1007/s12026-024-09575-5

Aysin, F., Özek, N.Ş., Acet, N. & Koc, K. (2024). The Anti-Proliferative Effects of Ficus carica Latex on Cancer and Normal Cells. *Journal of Anatolian Environmental and Animal Sciences*, ***9***(2), 145-151. DOI: 10.35229/jaes.1412816

Baskova, I.P., Kostrjukova, E.S., Vlasova, M.A., Kharitonova, O.V., Levitskiy, S.A., Zavalova, L.L., Moshkovskii, S.A. & Lazarev, V.N. (2008). Proteins and peptides of the salivary gland secretion of medicinal leeches *Hirudo verbana*, *H. medicinalis*, and *H. orientalis*. *Biochemistry (Moscow)*, ***73***, 315-320. DOI: 10.1134/S0006297908030127

Baykara, O. (2016). Kanser Tedavisinde Güncel Yaklaşımlar. *Balıkesir Sağlık Bilimleri Dergisi*, ***5***(3), 154-165.

Bray, F., Ren, J.S., Masuyer, E. & Ferlay, J. (2013). Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *International journal of cancer*, 132(5), 1133-1145. DOI: 10.1002/ijc.27711

Daniel D. Von Hoff. (2018). Pancreatic Cancer: Harrison's Principles of Internal Medicine 20th ed., Mc Graw Hill Education.

Davies, R.W. (1991). *Annelida: Leeches, Polychaetes and Acanthobdellids.* In: Ecology and Classification of North American Freshwater Invertabrates. In J. H., Thorp, A. P., Covich, (Eds.) Academic Press, New York, 437-79.

Deng, S., Shanmugam, M.K., Kumar, A.P., Yap, C.T., Sethi, G. & Bishayee, A. (2019). Targeting autophagy using natural compounds for cancer prevention and therapy. *Cancer*, ***125***(8), 1228-1246. DOI: 10.1002/cncr.31978

Dong, P., Rakesh, K.P., Manukumar, H.M., Mohammed, Y.H.E., Karthik, C.S., Sumathi, S., ... & Qin, H.L. (2019). Innovative nano-carriers in anticancer drug delivery-a comprehensive review. *Bioorganic chemistry*, ***85***, 325-336. DOI:10.1016/j.bioorg.2019.01.019

Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D.M., Piñeros, M., Znaor, A. & Bray, F. (2019). Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International journal of cancer*, ***144***(8), 1941-1953. DOI: 10.1002/ijc.31937

Fesinmeyer, M.D., Austin, M.A., Li, C.I., De Roos, A.J. & Bowen, D.J. (2005). Differences in survival by histologic type of pancreatic cancer. *Cancer Epidemiology Biomarkers & Prevention*, ***14***(7), 1766-1773. DOI: 10.1158/1055-9965.EPI-05-0120

Fitzmaurice, C., Dicker, D., Pain, A., Hamavid, H., Moradi-Lakeh, M., MacIntyre, M.F. & Naghavi, M. (2015). The global burden of cancer 2013. Global Burden of Disease Cancer Collaboration. *JAMA oncology*, ***1***(4), 505-527. DOI: 10.1001/jamaoncol.2015.0735

Gödekmerdan, A., Arusan, S., Bayar, B. & Sağlam, N. (2011). Tıbbi sülükler ve hirudoterapi. *Turkish Journal of Parasitology*, ***35***(4), 234-239.

Guo, Y., Tian, X. & Xiao, Z. (2006). Study on inhibition effects of freeze-thawing leech extract on HepG2 cells. *Chinese Journal of Information on Traditional Chinese Medicine*, (12).

Ilic, M. & Ilic, I. (2016). Epidemiology of pancreatic cancer. *World journal of gastroenterology*, ***22***(44), 9694. DOI: 10.3748/wjg.v22.i44.9694

Kern, S.E., Hruban, R.H., Hidalgo, M. & Yeo, C.J. (2002). An introduction to pancreatic adenocarcinoma genetics, pathology and therapy. *Cancer Biology & Therapy*, ***1***(6), 607-613. DOI: 10.4161/cbt.307

McGuigan, A., Kelly, P., Turkington, R.C., Jones, C., Coleman, H.G. & McCain, R.S. (2018). Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. *World Journal of Gastroenterology*, ***24***(43), 4846. DOI: 10.3748/wjg.v24.i43.4846

Modi B. & Shires G.T. (2020). Pancreatic cancer, cystic pancreatic neoplasms, and other nonendocrine pancreatic tumor. In: Feldman M, Friedman L, Brandt L; (Eds.) *Sleisenger and Fordtran's Gastrointestinal and Liver Disease*, 11th edition. Philadelphia: Elsevier, 947-965.

Muhammad, N., Usmani, D., Tarique, M., Naz, H., Ashraf, M., Raliya, R., Tabrez S., Zughaibi, T. A., Alsaieedi A., Hakeem, I.J. & Suhail, M. (2022). The role of natural products and their multitargeted approach to treat solid cancer. *Cells*, *11*(14), 2209. DOI: 10.3390/cells11142209

Neubert, E. & Nesemann H. (1999). Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea. *Süßwasserfauna von Mitteleuropa 6/2*. Spektrum Akademischer Verlag, 176.

Pavlopoulou, A., Spandidos, D.A. & Michalopoulos, I. (2015). Human cancer databases. *Oncology Reports*, ***33***(1), 3-18. DOI: 10.3892/or.2014.3579

Sağlam, N. (2004). *Tatlı Su ve Deniz Sülükleri Tanı Anahtarı*. Fırat Üniversitesi Basım Evi.

Sawyer, R.T. (1986). *Leech Biology and Behaviour*. Clarendon Press, Oxford.

Shakouri, A. & Wollina, U. (2021). Time to change theory; medical leech from a molecular medicine perspective leech salivary proteins playing a

potential role in medicine. *Advanced Pharmaceutical Bulletin*, ***11***(2), 261. DOI: 10.34172/apb.2021.038

Shakouri, A., Kahroba, H., Hamishekar, H. & Abdolalizadeh, J. (2021). Nanoencapsulation of *Hirudo medicinalis* proteins in liposomes as a nanocarrier for inhibiting angiogenesis through targeting VEGFA in the Breast cancer cell line (MCF-7). *BioImpacts: BI*, ***12***(2), 115. DOI: 10.34172/bi.2021.39

Singh, S.K. & Rajoria, K. (2020). Medical leech therapy in Ayurveda and biomedicine–A review. *Journal of Ayurveda and Integrative Medicine*, ***11***(4), 554-564. DOI: 10.1016/j.jaim.2018.09.003

Surh, Y.J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer*, ***3***(10), 768-780.

Taherian, M., Wang, H. & Wang, H. (2022). Pancreatic ductal adenocarcinoma: molecular pathology and predictive biomarkers. *Cells*, ***11***(19), 3068. DOI: 10.3390/cells11193068

Tang, L., Duan, Q.H. & Fan, P.S. (2012). Clinical study on leech in treating hypercoagulable state of malignant tumors. *Clinical Journal of Traditional Chinese Medicine*, ***24***, 871-872.

Tural, Ö. & Ayhan, H. (2024). The Anti-Proliferative Activity of Lyophilised Medicinal Leech (*Hirudo Verbana*) Saliva Extract on Breast Cancer Cell Line (MCF-7). *Journal of Anatolian Environmental and Animal Sciences*, ***9***(4), 508-513. DOI: 10.35229/jaes.1541179

Ünal, K., Tırık, N., Erol, M., İbrahimkhanlı, L., Elçi, M. & Ayhan, H. (2023). The Investigation of Effects of Medicinal Leech Saliva Extract on the Breast Fibroblast Cell Line In Vitro: An Experimental Study. *Geleneksel ve Tamamlayıcı Tıp Dergisi*, ***6***(2). DOI: 10.5336/jtracom.2022-92875

Wolfgang, C.L., Herman, J.M., Laheru, D.A., Klein, A.P., Erdek, M.A., Fishman, E.K. & Hruban, R.H. (2013). Recent progress in pancreatic cancer. *CA: A Cancer Journal for Clinicians*, ***63***(5), 318-348. DOI: 10.3322/caac.21190

World Health Organization (WHO). (2023). Noncommunicable disease. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases Access date: 28.07.2024