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Investigation of *In Vitro* Cytotoxicity of *Hypericum Perforatum* in Pancreatic Cancer

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Abstract

Pancreatic cancer remains one of the most aggressive malignancies with poor prognosis and limited treatment options. In view of the constraints imposed by current treatment modalities, there has been an increased focus on natural products as potential complementary agents in cancer therapy. *Hypericum perforatum* (HP), commonly known as St. John's Wort, has demonstrated cytotoxic and apoptotic properties in various cancer models; however, its effects on pancreatic cancer cells are not well-studied. This study aimed to investigate the *in vitro* cytotoxic effects of *Hypericum perforatum* extract on AR42J pancreatic cancer cells. AR42J cells were cultured and treated with increasing concentrations (3.125–100 µg/mL) of HP extract for 24 and 48 hours. Cell viability was assessed using the MTT assay. Nuclear morphological changes were evaluated by DAPI staining and visualized with a Juli™ Smart Fluorescent Cell Analyzer. HP extract exhibited a dose- and time-dependent cytotoxic effect on AR42J cells. IC_{50} values were determined to be 12.5 µg/mL at 24 hours and 6.25 µg/mL at 48 hours. DAPI staining confirmed nuclear condensation and fragmentation in treated cells, supporting apoptosis as a mechanism of action. These results indicate that *H. perforatum* extract effectively reduced pancreatic cancer cell viability while inducing apoptotic cell death. These findings suggest that *H.perforatum* extract exerts significant antiproliferative and pro-apoptotic effects on AR42J pancreatic cancer cells. The study highlights its potential as a plant- based complementary therapeutic candidate for pancreatic cancer treatment.

Keywords: Pancreatic cancer, *Hypericum Perforatum*, *in vitro* study, AR42J cell, MTT assay, DAPI staining

1. Introduction

Cancer has been a leading global health issue since the beginning of human history. Concurrently, technological progress has led to significant advancements in diagnostic and treatment methodologies. A significant number of studies are underway to prevent cancer and identify a definitive cure. Pancreatic cancer is among the most aggressive and lethal malignancies, characterized by late diagnosis, limited therapeutic efficacy, and poor prognosis. Globally, its incidence is steadily increasing, with high mortality rates positioning it as a critical public health concern. Pancreatic cancer is the 13th most common cancer among both men and women and ranks seventh in cancer-related mortality. In Turkey, it stands as the fifth leading cause of cancer-related deaths. According to recent estimates, more than 510,000 individuals were diagnosed with pancreatic cancer in 2024, representing a 51% increase in incidence over the past decade. Pancreatic ductal adenocarcinoma accounts for approximately 94% of all cases, while neuroendocrine tumors comprise the remaining 6%. Despite slight differences in age of onset and prognosis between these subtypes, the overall outcome remains dismal, with a five-year survival rate below 10% [1,2].

Many approaches are used in the treatment of pancreatic cancer, including surgical resection, neoadjuvant and adjuvant therapies, chemotherapy, radiotherapy, and palliative care. Additionally, local tumor ablation techniques such as radiofrequency ablation (RFA), microwave thermotherapy, cryotherapy, and embolization are applied in selected cases [1]. Despite advances in surgical techniques, chemotherapy, and radiotherapy, the overall survival rate remains low. Consequently, there is growing interest in complementary and alternative therapeutic strategies, particularly those based on bioactive compounds derived from medicinal plants. The quest for straightforward solutions remains ongoing, with endeavours underway to evaluate both conventional and complementary medicine methodologies with a view to cancer prevention. The most natural of these methods is treatment with herbal medicines..Some plants have been used as an alternative for the treatment of cancer and other diseases for many years. Studies have focused on plants used for medicinal purposes. Among these plants, *Hypericum perforatum* (St. John's Wort) has recently become a medicinal plant whose activity has been proven as a result of clinical trials and has become widely used in the world [3-12]. developing countries and identified these plant species with anticancer properties. In a study conducted by Vlavcheski et al. (2022), the effects of the Berberine (BBR) plant on pancreatic cancer were investigated [13]. Berberine (BBR) is a naturally occurring plant-derived polyphenol that has been identified in various herbal medicines used in traditional medicine for the treatment of ulcers, infections, and jaundice. The results of *in vitro* and *in vivo* experiments have indicated that BBR can prevent and reverse tissue damage to the pancreas in cases of pancreatitis and pancreatic cancer.

Hypericum perforatum L. (St. John's Wort) is a well-known medicinal plant traditionally used for its antidepressant, wound-healing, anti-inflammatory, and antimicrobial properties. More recently, attention has turned toward its potential anticancer effects. The plant's main bioactive constituents *hyperforin* and *hypericin* have been shown to exhibit cytotoxic, apoptotic, and anti- proliferative activities in various cancer cell lines, including prostate, colorectal, and skin cancers. However, scientific evidence regarding its efficacy against pancreatic cancer remains scarce.

With developing technologies, new studies are carried out on plants every day and the treatment of many diseases becomes possible. These plants may contain not just one but dozens of active substances. Although it has been known for many years that plants of the genus *Hypericum*, especially *H. perforatum*, contain active compounds, the bioactive structures of many species in this genus have not been fully discovered. The active ingredient *Hypericin* in the *H. perforatum* plant has recently attracted attention in the scientific community as a promising substance in the treatment of cancer. According to the literature, the antitumor and antiviral effects of *Hypericin*, the most important component of the *H. perforatum* plant, are scientific facts. The antitumor effect of *hypericin* has been studied *in vivo* and *in vitro* in melanoma, breast, colon, glioma, prostate, pituitary, nasopharynx, and esophagus cancers, and its mechanism of action has been investigated. Its antiviral effects have been studied extensively since 1988, and research has shown that *Hypericin* only affects enveloped viruses and retroviruses. It has been stated that the active ingredient in *H. perforatum, Hypericin*, breaks down and accumulates within the cell [13,14]. Our results were found to agree with these studies.

While previous studies have demonstrated the anti-proliferative and pro-apoptotic effects of *H. perforatum* in several cancer models including breast (MCF-7), glioblastoma (U87), prostate, and colon cancer cell lines there is limited literature examining its impact on pancreatic cancer cells [15.16.17]. Schepp and friends' studies was stated that *hypericin* and *hyperforin*, metabolites contained in *Hypericum* species, prevented cancer formation and development by causing programmed cell death in different cancer cell lines. It has also been reported that *hypericin* has a strong cytotoxic effect on three different cancer cell lines [8]. In the study conducted by Mirmalek et al. (2016), the active component of *Hypericum perforatum*, known as *hypericin*, was examined for its potential cytotoxic and apoptotic effects on the MCF-7 breast cancer cell line. It was reported that *hypericin* exhibited cytotoxic effects by inducing apoptosis in MCF-7 cells [17].

Notably, to the best of our knowledge, no comprehensive *in vitro* studies have assessed the cytotoxic effect of *H. perforatum* extract on the AR42J pancreatic cell line. Our study addresses this gap by evaluating the dose- dependent cytotoxic potential of *H. perforatum* and observing cell viability and nuclear morphology using MTT and DAPI assays, respectively. This investigation provides a novel contribution to the field of plant-based adjunct cancer therapies, particularly in the context of pancreatic cancer, which currently lacks effective early interventions.

Considering the urgent need for novel therapeutic approaches and the promising pharmacological profile of *H. perforatum*, this study aims to investigate the cytotoxic effects of *H. perforatum* liquid extract on AR42J pancreatic cancer cells. By addressing a significant gap in literature, our research explores the feasibility of this medicinal plant as a potential complementary agent in pancreatic cancer treatment.

2. Material

2.1 Characteristics of *Hypericum Perforatum* Plant

The *Hypericum* genus belongs to the Clusiaceae family and the *Hypericoideae* subfamily, comprising approximately 400 species worldwide. The *Hypericum* genus, which is distributed across Europe, Asia, Australia, and certain regions of America, comprises 10 species in Europe and 70 species in Turkey. *Hypericum perforatum*, commonly known as St John's wort, is distributed extensively throughout the regions of Marmara, the Black Sea, the Aegean, Central and Eastern Anatolia. [18,19].

Hypericum species are generally perennial and have a fringe root system. The leaves are in full leaf form and are arranged opposite each other on the stem (Figure 1). There are transparent pores on the leaves that can be seen when held up to light and make the leaves look like holes (Figure 2). The name "*perforatum*", which means porous in Latin, originates from these pores on the leaf. Flowers occur in groups of 5-10 at the ends of the main and side branches. When the black glands, which are densely found on the edges of plant and flower leaves, are rubbed by hand, a red liquid is released, and one of its Turkish names, bloodwort, comes from this feature (Figure 3). This red liquid consists of a substance called *hypericin*, and when the plant is consumed in excess, it causes photosensitivity on hairless and light-colored skin surfaces. This sensitivity caused by *hypericin* causes serious poisoning every year, especially in small ruminants, and that is why *Hypericum* species are also called sorrel among the public. The substance *hypericin* is found only in species bearing these black glands. As the weather begins to warm up in March in Turkey, plants begin to grow on the soil. Flowering begins in May, reaches its peak in the second half of June, and continues until August. Fruits in capsule form from the beginning of July. These capsules, which are brown when mature, mature from green at first the beginning of August and crack and shed seeds. Seeds that fall into the ground can remain viable for more than 10 years, but their germination rate is extremely low. The plant prefers vegetative reproduction. However, apomixis reproduction is also quite common [20,21].

Hypericum species are generally considered to be perennial plants, characterised by a fibrous root system (Figure 1). The leaves are distinguished by the presence of transparent pores, which become discernible when the leaves are held up to the light, thereby creating a perforated appearance (see Figure 2). The Latin name *"perforatum",* meaning "perforated", is derived from these pores on the leaves. The black glands, which are found in abundance along the margins of the plant and flower leaves, secrete a red liquid when manually rubbed. This distinctive property has contributed to the formation of one of its Turkish names, "kan otu" (blood grass) (Figure 3). The *hypericin* compound has been found to be present exclusively in species that possess black glands. In Turkey, the onset of plant growth in the soil is observed in March, coinciding with the warming of the weather. The onset of flowering occurs in May, reaching its zenith in the latter half of June and persisting until August. Capsule-shaped fruits begin to form in early July. These capsules, which are brown when ripe, undergo a chromatic shift to green by early August, subsequently split open, and disperse their seeds. The plant demonstrates a clear preference for vegetative reproduction. However, apomixis reproduction is also a prevalent phenomenon [20,21]. The present study is investigating its usability.

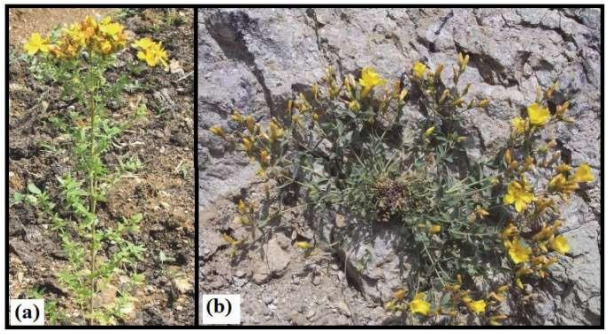

Figure 1. General view of *H. perfatum* (a), the most common *H. perfatum* wort species, and *H. avicullariifolium* (b), an endemic species specific to our country [20].

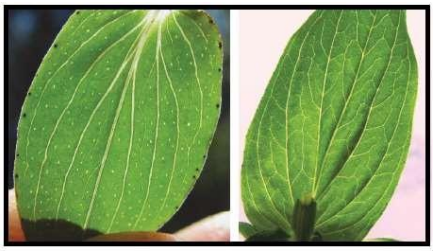

Figure 2. Transparent pores on the leaf surface [20].

Figure 3. Black glands and red liquid containing *hypericin* in the flowers [20].

2.2. Pharmacological properties of *H. perforatum*

H. perforatum, fresh plants in the flowering phase or dried stem parts (*Hyperici* herba) are generally used as the base material. Factors such as where the plant used grows, the climate of this region, the time of collection, and the drying process directly affect the quality of the drug. Tinctures used in homeopathy and plant juices or oils obtained by pressing the plant are prepared from the fresh plant [22]. The traditional uses of the plant are much wider.

Oil-based preparations are preferred for dyspepsia complaints. Externally, it is used to heal wounds, myalgia, and burns. The red oil formed when the fresh plant is crushed and left in sunlight with olive oil or another oil can be used externally, directly, or mixed into ointments or in capsule form. Dry drug is obtained by drying the fresh plant. The liquid extract resulting from the extraction of the dry drug in powder form with alcohol is included in this way or the composition of some drops, ointments, or tinctures. The product, which is formed by concentrating the alcohol extract and then turning it into dry powder with a lyophilizer, is used internally by entering into the composition of tablets or capsules after the standardization stage [23]. The chemical composition of the H. *perforatum* species has been elucidated by many studies. Accordingly, compounds with naphthodiantrone structure (*hypericin, pseudohypericin, etc.),* phloroglucinols *(hyperforin, adhiperforin*, etc.), flavonoids (hyperoside, routine, quercetin, etc.), biflavones (biapigenin, amentoflavone), phenolic acids (ferulic acid, caffeic acid, etc.), proanthocyanidins. Essential oils, and some other chemical compounds. These substances are responsible for the important pharmacological effects that the plant has [24,25].

Herbal medicines have been used for the treatment of cancer and other diseases for many years. Researchers have identified these plant species with anticancer properties by focusing on plants used for medicinal purposes in developing countries [26]. Some medicinal plants are grown on a large scale to meet the demand for alternative natural medicines. In a study, the beneficial effects of Aloe vera on the skin were investigated. Study has demonstrated the cytotoxic effects of various leaf extracts and aloe emodin (AE) on a type of skin cancer and as potential for anticancer drug research [27,28].

3. Methods

3.1. H. perforatum extract density

In this study, liquid extract of *H. perforatum* was used as the test material. The extract was supplied by the Düzce University Research Laboratory. The density of the extract was measured as 1.01 g/mL using a digital density (Anton Paar). (Figure 4)

Figure 4. Measurement of *H. perforatum* extract liquid density

3.2. Evaluation of *H. perforatum in vitro* cytotoxicity in AR42J cell line

The present study aims to evaluate the *in vitro* cytotoxic potential of *H. perforatum* in the AR42J cell line. The cytotoxic effects of *H. perforatum* extract were evaluated on the AR42J pancreatic cancer cell line using the MTT colorimetric assay. AR42J cells were cultivated in DMEM/F-12 medium, which was enriched with 20% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin-streptomycin. Cells were cultivated at 37°C in a humidified atmosphere containing 5% CO_2. After reaching approximately 80% confluence, the cells were subcultured. As demonstrated in Figure 5(a), upon reaching 80% confluence within the plates, the medium was replaced and harvesting of AR42J cells. (Figure 5 a). Once enough cells had been obtained, MTT assays were conducted in order to determine the half-maximal inhibitory concentration (IC_{50}) of the extract. The cells were then seeded into 96-well plates at a density of 0.5×10^4 cells per 100 µL per well (Figure 5b). Following a 24-hour incubation period, the culture medium was removed and substituted with fresh medium containing various concentrations of H. perforatum extract (100, 50, 25, 12.5, 6.25, and 3.125 µM). Application of *H. Perforatum* extract to AR42J pancreatic cancer cells demonstrated in Figure 5c and 5d.

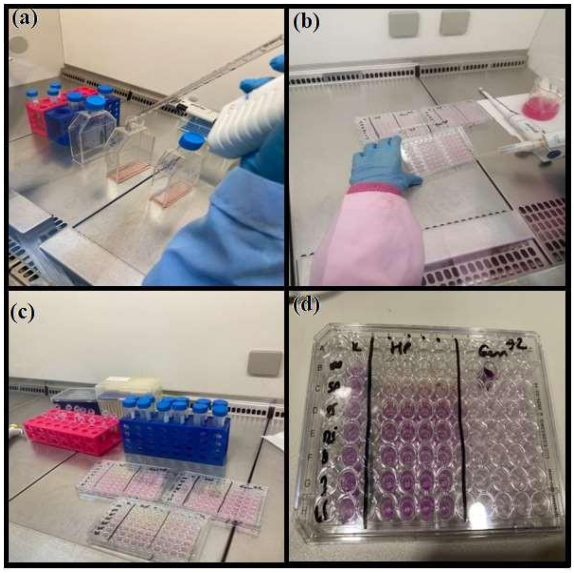

Figure 5. The following steps in the cell culture experiment a) Medium replacement and harvesting of AR42J cells at 80% confluency, b) Seeding of AR42J cells into 96-well plates and c–d) Application of *H. perforatum* extract to AR42J cells and performance of the MTT assay to evaluate cytotoxic effects.

The cells were then subjected to an incubation period of 24 or 48 hours. At the end of each designed period, 10 µL of MTT reagent (final concentration: 0.5 mg/mL) was added to each well and subsequently incubated for a further 4 hours at 37°C and 5% CO_2 in incubator. After the process of incubation, the medium containing MTT was aspirated and substituted with 150 µL of dimethyl sulfoxide (DMSO) in order to facilitate the dissolution of the resulting formazan crystals. Following a 15-minute incubation period, the absorbances were measured at a wavelength of 570 nm, employing a microplate reader. Cell viability percentages were calculated accordingly

3.3. Imaging Cells

After the cells grew in the medium, their images were obtained under an inverted microscope (Figure 6).

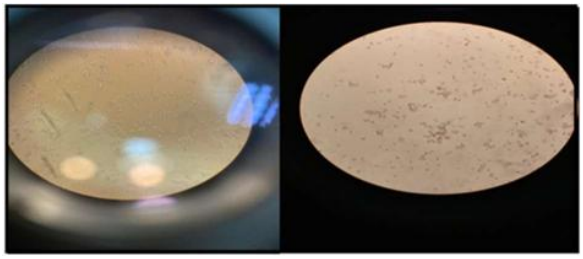

Figure 6. Microscope images of lanted cells

3.4. Imaging of the AR42J Cell Line with DAPI Cell Dye

In order to visualise the nuclear morphological changes associated with cell death, AR42J cells were treated with the IC_{50} concentration of *H. perforatum* extract and stained with DAPI (4′,6-diamidino-2-phenylindole). AR42J cells were seeded into 6-well plates and allowed to reach 80% confluency. The medium was then removed, and the cells were washed twice with PBS. Subsequently, 2 mL of medium containing the IC_{50} dose of *H. perforatum* extract was added to each well and incubated for 24 hours. Following the incubation period, the medium was removed and the wells were washed 2–3 times with PBS. The DAPI solution (300nM) was added to each well, after which the cells were left to incubate for 10 minutes at 37°C under 5% CO_2. The nuclear morphology was examined using a Juli™ Smart Fluorescent Cell Analyzer.

4. Results and Discussion

4.1. The cytotoxicity results of *H. perforatum* extract on AR42J pancreas cell line

The cytotoxic effect of *H. perforatum* extract on the AR42J pancreatic cancer cell line was evaluated using the MTT assay at 24 and 48 hours after treatment with various concentrations (100, 50, 25, 12.5, 6.25, and 3.12 µg/mL). The graph depicting the percentage viability as determined by the MTT assay, conducted at the 24- and 48-hour intervals following the measurement of absorbances at 570 nm using a microplate reader, is presented in Figure 7. As demonstrated in Figure 7, a dose-dependent and time-dependent decline in cell viability was observed. At the highest concentration (100 µg/mL), both 24-hour and 48-hour treatments significantly reduced cell viability to below 10%, indicating strong toxicity. Conversely, as the concentration decreased, cell viability increased. For instance, at a concentration of 25 µg/mL, the cell viability was approximately 40% after 24 hours and had decreased to nearly 25% after 48 hours. At the lowest concentration that was examined in this study (3.12 µg/mL), cell viability remained above 90% at 24 hours and around 60% at 48 hours. The findings indicate that *H. perforatum* extract exhibits significant antiproliferative effects on pancreatic cancer cells, especially at higher concentrations and extended exposure durations. The IC_{50} doses that were determined to be effective were ascertained to be 12.5 µg at the 24th hour and 6.25 µg at the 48th hour

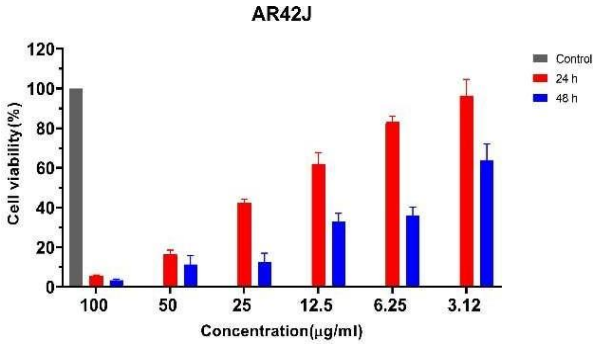

Figure 7. MTT Analysis Results

When compared with the existing literature, our findings are in agreement with previous studies demonstrating the anticancer potential of HP and its constituents in other cancer models. Mirmalek et al. (2016) reported that hypericin, a key active compound of HP, induced apoptosis and exhibited cytotoxic effects in MCF-7 breast cancer cells [17]. Furthermore, Deng et al. (2020) isolated novel compounds from the endophytic fungus *Aspergillus terreus* associated with HP and demonstrated their cytotoxic activity against pancreatic cancer cell lines AsPC-1, SW1990, and PANC-1 [29]

Hypericum perforatum (HP) extract on AR42J pancreatic cancer cells, thus corroborating an increasing

number of recent studies. For instance, Gökçek-Saraç et al. (2025) reported that methanolic HP extracts exhibited significant antiproliferative and apoptotic effects on TT thyroid cancer cells, reduced Bcl-2 expression, and increased Bax and caspase-3/12 expression [30]. In a similar vein, Haake et al. (2025) discovered that HP extract (HP01) functions as a radiosensitiser, reducing cell viability in MCF-7 and HT-29 cells. It has been reported that HP has an inhibitory and cytotoxic effect on the growth of both tumour cell lines at concentrations of 10 µg/mL and above. In our study, IC_{50} values of 12.5 µg/mL were found, which are similar to those reported in the literature. [31]. Moreover, Wen et al. (2025) recently reported that hypericin, one of the main active components of HP, inhibits M2 macrophage polarization in the tumor microenvironment and enhances antitumor immune responses [32]. In addition, Mete et al. (2024) investigated the effects of a methanolic extract of *H. perforatum* on the U87 glioblastoma cell line and found that it reduced cell proliferation by inducing apoptosis [16]. These comparable outcomes across distinct cancer models support the hypothesis that HP possesses broad-spectrum anticancer properties mediated by apoptotic pathways.

The present study contributes to this growing body of evidence by highlighting, for to our knowledge, the dose- dependent cytotoxic potential of *HP* extract on the AR42J pancreatic cell line, underscoring its promise as a complementary therapeutic candidate in the treatment of pancreatic cancer.

4.2. Nuclear Morphology Analysis with DAPI Staining

In order to assess nuclear changes and cell viability after treatment with *H. perforatum* extract, DAPI staining was performed on AR42J cells. The results were then visualised using a Juli™ Smart Fluorescent Cell Analyzer. The DAPI (staining) results of *H. perforatum* extract on the AR42J cell line were observed as shown in Figures 8 and 9. As demonstrated in Figure 8, cells exposed to the IC_{50} dose of *H. perforatum* extract manifested condensed and fragmented nuclei, which are hallmarks of apoptotic cell death. Conversely, Figure 9, representing the control group, exhibited a higher number of intact and uniformly stained nuclei, indicative of normal nuclear morphology. The observed morphological differences provide a robust evidential basis for the cytotoxic findings derived from the MTT assay. A decline in viability was observed when the *H. perforatum*-treated AR42J cell line group was compared with the control group. The *H. perforatum* extract was found to be effective on the AR42J cells

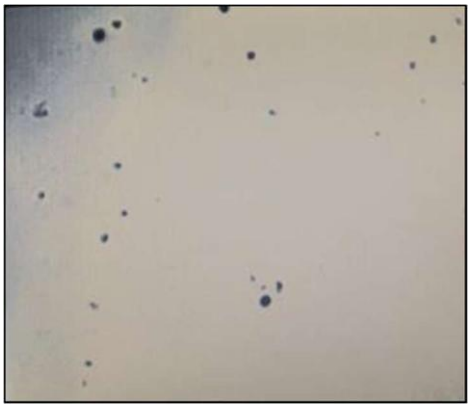

Figure 8. Nuclear morphology of AR42J cells after treatment with *H. perforatum* extract (IC_{50} dose) stained with DAPI.

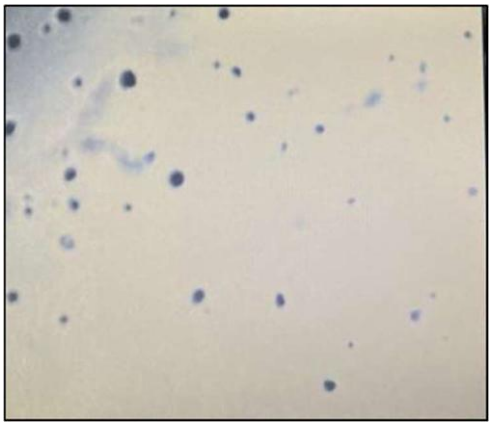

Figure 9. DAPI-stained AR42J control cells exhibiting normal nuclear morphology without extract treatment
As a result of our study on the AR42J cell line, it is observed that the retention rate of HP in the cell is lower in the HP control groups in which 24 hours of incubation is performed compared to 48 minutes of incubation. However, when the viability rates of the *H. perforatum* groups were compared, it was observed that *HP* therapy applied after 48 hours of incubation reduced the viability more.

1. 5. Conclusion

In our study, the effect of *Hypericum perforatum* extract on pancreatic cancer cells was investigated. To our knowledge, there are limited studies in the literature directly addressing the impact of this plant on pancreatic cancer models.

The present study demonstrated that *H.perforatum* extract exerts significant cytotoxic and pro-apoptotic effects on AR42J pancreatic cancer cells *in vitro*. The observed decrease in cell viability, both dose- and time-dependently, coupled with nuclear morphological alterations detected by DAPI staining, suggests that *H. perforatum* has potential as a complementary therapeutic agent in pancreatic cancer treatment. These findings are consistent with previous research reporting the anticancer properties of *H. perforatum* and its bioactive constituents in various cancer cell lines, including breast, glioblastoma, and pancreatic models.
In view of the limited effectiveness of conventional therapies for pancreatic cancer and the urgent need for novel treatment strategies, the findings of this study support the further investigation of *H. perforatum* in preclinical animal models and clinical trials. It is expected that these studies will be supported by animal experiments on organic environments and positive results can be obtained. This study contributes to the

growing body of evidence supporting the anticancer potential of medicinal plants and highlights *H. perforatum* as a promising candidate for plant-based therapeutic development in oncology.

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Derya Özel: Conception, Design Study,Data Acquisition Data Analysis Interpretation, Drafting Manuscript Critical Revision of Manuscript

Şeyma Karagül: Conception, Design Study,Data Acquisition Data Analysis Interpretation, Drafting Manuscript

Fatma Yurt: Conception, Design Study,Data Acquisition, Interpretation, Drafting Manuscript Critical Revision of Manuscript, Final Approval and Accountability, Supervision

Ethics

There are no ethical issues after the publication of this manuscript.

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