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Short-Communication

Comparative evaluation of barberry-derived honey and commercial honey on HT-29 colorectal cancer cells: *In vitro* anticancer effects

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Abstract

Objective: Colorectal cancer (CRC) is a leading cause of cancer-related mortality, underscoring the need for novel therapeutic strategies. The PAD score (Phenolic compounds, protein content, Antioxidant capacity, Diastase activity) is a new biochemical index for evaluating honey quality and potential medicinal properties. This study assessed the anticancer effects of honeys with distinct PAD scores on HT-29 CRC cells.

Materials and Methods: A high-PAD score honey (HPH, barberry-derived; PAD 543) and a commercial low-PAD score honey (LPH, PAD<74) were characterized biochemically. Their effects on HT-29 cells were evaluated by MTT viability, scratch migration, RT-qPCR for apoptotic markers, and flow cytometry for cell cycle analysis.

Results: HPH showed markedly higher biochemical values than LPH. MTT assays demonstrated dose- and time-dependent cytotoxicity, with a 24 hr IC_{50} of 2.4 ± 0.2% v/v versus 10.8 ± 1.2% for LPH. Migration assays revealed that 0.65% HPH significantly suppressed cell migration, whereas LPH slightly promoted it. RT-qPCR showed HPH upregulated *Bax* (1.49-fold) and *Caspase-3* (1.41-fold) while downregulating *Bcl-2* (4-fold). Flow cytometry confirmed that HPH induced G0/G1 arrest, with 44.16% of cells in this phase compared to 35.73% in controls.

Conclusion: Honey's anticancer potential is strongly dependent on its biochemical profile. Barberry-derived HPH exhibited superior antiproliferative, anti-migratory, and pro-apoptotic effects relative to LPH. High-PAD honey may represent a promising adjuvant therapy for CRC, highlighting the need for standardized biochemical profiling in selecting natural therapeutic agents.

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Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide, accounting for approximately 9% of all diagnosed cancers (Erfanian et al. 2023; Morgan et al. 2023). Despite advancements in clinical management approaches, CRC remains a major cause of cancer-related mortality, highlighting the need for novel and effective treatment approaches (Karbasi et al. 2024b). In recent years, natural products have gained significant attention in cancer research due to their bioactive properties. Among these, honey has been recognized for its potential health-promoting benefits including antioxidant, anti-inflammatory, and anticancer effects (Al-Eisa et al. 2023). Honey anticancer activity is primarily attributed to its complex composition which includes polyphenols, flavonoids, and various bioactive compounds that can modulate multiple cellular pathways. The pharmacological potency of honey varies depending on its botanical origin and chemical composition (Bouali et al. 2022).

The PAD score (Phenolic compounds, Protein content, Antioxidant capacity, Diastase activity) is a standardized biochemical index that, for the first time, evaluates honey quality based on four key parameters: phenolic compounds, protein content, antioxidant capacity, and diastase enzymatic activity. These parameters were selected because phenolics and flavonoids contribute to antioxidant and anticancer effects, protein content reflects the presence of enzymatic and non-enzymatic bioactive proteins naturally found in honey (such as defensin-1, glucose oxidase, and catalase), antioxidant capacity mitigates oxidative stress, and diastase activity indicates honey freshness and enzymatic quality which correlates with bioactive potential (Mohammadi et al. 2024; Zamani Ranjbar Garmroodi et al. 2024). The PAD score classifies honey into six quality categories: very weak (0–99), weak (100–199), moderate (200–299), good (300–399), very good (400–499), and excellent (≥500), facilitating the identification of high-quality medicinal honeys (Karbasi, 2024 #83).

South Khorasan Province in Iran is known for producing high-quality honey due to its unique floral biodiversity. Among these, barberry honey, derived from *Berberis* plants cultivated in the region, stands out with a PAD score of 543, representing “high PAD score honey” (HPH). Preliminary biochemical analyses revealed that HPH possesses considerably higher levels of phenolic and protein compounds, antioxidant capacity, and diastase activity compared to commercially available low PAD score honey (LPH) (Afshari et al. 2022; Karbasi et al. 2024a).

Previous studies have demonstrated that honey can exert cytotoxic effects on various cancer cell lines through mechanisms such as apoptosis induction, inhibition of cell proliferation, and suppression of migration and invasion (Abu-Farich et al. 2024; Nahala et al. 2023). To investigate these effects in a relevant model, HT-29 cells were selected as a well-characterized human colorectal adenocarcinoma line, representing differentiated colon cancer and commonly used to evaluate the antiproliferative and anti-migratory effects of natural products.

Nonetheless, there is still a lack of clarity regarding comparative studies of ranked honey samples and their specific impacts on CRC cells. To address this gap, the present study aimed to compare the anticancer effects of HPH and LPH on HT-29 cells. By evaluating honeys with distinct PAD scores, this research seeks to provide mechanistic insights and support the potential translational application of high-quality medicinal honey as an adjuvant therapy for CRC, either alone or in combination with conventional treatments.

Materials and Methods

Honey sample preparation and quality assessment

Honey samples were obtained from local beekeepers in Birjand, South Khorasan

province, Iran (high PAD score honey, HPH) and a commercially available multifloral honey purchased from a local market (low PAD score honey, LPH) in January 2024. The PAD score for each honey sample was determined as an integrated biochemical index based on four key parameters: total phenolic content, protein concentration, antioxidant capacity, and diastase enzymatic activity. Each parameter contributes equally to the final score to ensure balanced representation of the honey's bioactive and nutritional profile. Total phenolic content, antioxidant capacity, and diastase enzymatic activity were quantified using the ZANTOX assay kit (ZANTOX DPPH assay, Iran), following the manufacturer's instructions. Total phenolic content was expressed as mg gallic acid equivalents (GAE) per 100 g of honey, based on a gallic acid standard curve included in the kit. Antioxidant capacity was expressed as mg Trolox equivalents (TE) per 100 g of honey, calculated according to a Trolox calibration curve generated as part of the DPPH assay.

Protein concentration was measured separately using the Bradford assay kit (KalaZist, Iran). The values obtained for each parameter were normalized and incorporated into the PAD scoring formula, providing a standardized measure for comparing the overall biochemical quality of different honey samples. To prepare the samples, a stock solution was made by dissolving 1 g of honey in 1 ml of sterile distilled water to achieve a 100% concentration. The solution was then filtered through a 0.45 µm filter to remove any impurities, and subsequent dilutions were prepared as needed for experimental use.

Evaluation of protein concentration

The protein concentration of the samples was quantified using the Bradford assay. In this procedure, 20 µl of each sample and standard were mixed with 180 µl of Bradford reagent. A series of standard solutions was prepared using bovine serum albumin (BSA) at concentrations ranging from 37.25 to 1000 µg/ml. The absorbance was measured at 595 nm using a microplate reader (BioTek, USA), and a standard curve was constructed using Microsoft Excel to accurately correlate absorbance values with protein concentrations.

Cell culture

The HT-29 cell line was generously provided by Dr. Hanafi at Birjand University of Medical Sciences, Iran. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Bioidea, Iran) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). The culture medium was replaced every 48 hr, and the cells were subcultured at 80–90% confluence using trypsin-EDTA. All experiments were performed using cells at passage 4.

Anti-proliferation effect of honey on HT-29 cells

To evaluate the anti-proliferative effects of the two honey varieties on CRC cells, HT-29 cells were seeded in 96-well microplates at a density of 6×10^3 cells per well (100 µl per well) and incubated at 37°C under 5% CO_2 for 24 hr. Following incubation, the cells were treated with varying concentrations (0.65% to 20%) of the two honey samples (HPH and LPH) diluted in complete growth medium. At 24, 48, and 72 hr post-treatment, cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were incubated with MTT (5 mg/mL, Sigma, USA) for 4 hr. The resulting formazan crystals were solubilized in 10% dimethyl sulfoxide (DMSO, Sigma, USA), and absorbance was measured at 570 nm (reference wavelength: 630 nm) using a microplate reader. All experiments were conducted in triplicate and independently repeated three times.

Anti-migration effect of honey on HT-29 cells

This study employed a scratch assay to investigate the effects of HPH and LPH on the inhibition of cell migration in HT-29 cells. The cells were cultured in 12-well plates at a density of 4×10^5 cells per well. After 24 hr of treatment, uniform scratches were created using a 200 µl pipette tip, and cell migration was monitored at 0, 24, 48, and 72 hr. A sub-cytotoxic concentration (25% of IC50) was used for HPH treatment to assess its effect on migration while minimizing the influence of cytotoxicity. Images of the scratch area were analyzed using ImageJ software (version 1.52) to determine wound closure, specifically calculating the percentage of remaining open area at 72 hr relative to the initial scratch size. The experiment was performed in triplicate, with three independent biological replicates.

Effects of honey on the gene expression of HT-29 cell line

To assess the impact of honey on the expression of apoptosis-related genes (*Bax*, *Bcl-2*, and *Caspase-3*), quantitative real-time PCR (RT-qPCR) was performed. Total RNA was isolated from HPH- and LPH-treated cells (2×10^6 cells per well) using the Total RNA Extraction Kit (Parstous, Iran). Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the cDNA Synthesis Kit (Parstous, Iran). RT-qPCR was carried out in three independent biological replicates using QuantiTect™ SYBR Green PCR Master Mix (Parstous, Iran) on an ABI Step One™ Real-Time PCR System. The cycling conditions consisted of 40 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec, and data collection at 80°C for 20 sec. *GAPDH* was used as the internal control, and relative expression levels were calculated using the $2^{-}\Delta\Delta CT$ method, as described in our previous study (Erfanian et al. 2024b) (Table 1). To ensure the reliability of RT-qPCR data, both specificity and efficiency assessments were performed. Melt curve analyses were conducted for all primer sets, consistently yielding single, sharp peaks without evidence of non-specific amplification or primer–dimer formation. Furthermore, amplification efficiencies were determined from standard curves generated using serial dilutions of cDNA. All primer efficiencies fell within the acceptable range (95–100%).

Table 1. Primer sequences used for quantitative real-time PCR (RT-qPCR)

Primer	Sequence	Product size (bp)	Reference
Bax	F: TGGAGCTGCAGAGGATGATTG R: GAAGTTGCCGTCAGAAAACATG	95	(Abedini et al. 2016)
Bcl2	F: TGCACCTGACGCCCTTCACC R: CACATGACCCCACCGAACTCAAAGA	118	(Abedini et al. 2016)
Caspase-3	F: GGAAGCGAATCAATGGACTCTGG R: GCATCGACATCTGTACCAGACC	146	(Zarafu et al. 2025)
GAPDH	F: GTGATGCTGGTGCTGA R: GCTAAGCAGTTGGTGG	211	(Erfanian et al. 2024a)

Flow cytometry analysis

To evaluate the effects of honey on cell cycle distribution, HT-29 cells (1×10^5 cells/well) were treated with HPH and LPH at 50% of the HPH IC_{50} for 24 hr. Post-treatment, cells were trypsinized, washed with PBS, and fixed by adding 4.5 ml of ice-cold 70% ethanol to 500 µl of cell suspension under gentle vortexing. Fixed cells were either stored at –20°C or processed immediately. For analysis, cells were centrifuged (400 × g, 5 min), washed with PBS, and incubated with DNA extraction buffer (1:1 diluted in PBS) for 5 min. After centrifugation, cells were resuspended in 1 ml of propidium iodide (PI) staining solution and incubated for 30 min at room temperature in the dark. Flow cytometry was performed using a 488 nm excitation laser, and red fluorescence (≥600 nm) was measured. A minimum of 2×10^4 events per sample were acquired, and cell

cycle distribution was analyzed using FlowJo software (v10). All flow cytometry experiments were performed in triplicate as biological replicates.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 10.2.3). Data were first tested for normality. For experiments with two factors (MTT and scratch assays: concentration × time), two-way ANOVA was applied, followed by Tukey's post-hoc multiple comparisons. For comparisons among three groups at a single time point (RT-qPCR and flow cytometry), one-way ANOVA followed by Tukey's post-hoc test was used. All experiments were conducted in three independent biological replicates. Results are presented as mean ± SEM, and $p<0.05$ was considered statistically significant.

Results

Comparison of physicochemical properties between high and low PAD score honey

The two honey samples (HPH and LPH) exhibited distinct physicochemical profiles, with significant differences in phenolic content, antioxidant capacity, and diastatic activity.

High PAD score honey exhibited substantially higher phenolic content (208.7 ± 18.5 vs. 31.0 ± 6.0 mg GAE/100 g), antioxidant activity (235.0 ± 25.0 vs. 25.0 ± 5.0 mg TE/100 g), diastatic activity (29.0 ± 4.0 vs. 3.0 ± 1.0 IU), and protein content (70.0 ± 10.0 vs. 12.3 ± 2.5 mg/100 g) compared to low PAD score honey, with all differences being statistically significant ($p<0.001$), resulting in a PAD score of 543 versus <74 for HPH and LPH, respectively (Table 2).

Table 2. Comparison of key biochemical properties between high PAD score honey and low PAD score honey samples.

Property	HPH (Mean ± SD)	LPH (Mean ± SD)	t-stat	p-value
Phenolic Content (mg GAE/100 g honey)	208.7 ± 18.5	31.0 ± 6.0	15.82	< 0.001
Antioxidant Activity (mg TE/100 g honey)	235.0 ± 25.0	25.0 ± 5.0	14.27	< 0.001
Diastatic Activity (IU)	29.0 ± 4.0	3.0 ± 1.0	10.92	< 0.001
Protein Content (mg/100g)	70.0 ± 10.0	12.3 ± 2.5	9.69	< 0.001
PAD score	543	<74		

GAE: Gallic acid equivalents; TE: Trolox equivalents; IU: International Units. PAD score is a composite index derived from phenolic content, antioxidant activity, diastatic activity, and protein content to assess the overall biochemical potency of honey samples.

Anti-proliferation effects of honey samples on HT-29 cell line

The HT-29 cell line was treated with varying concentrations of honey samples (0.65–20% v/v) for different durations (0–72 hr). High-PAD score honey (HPH) suppressed cell proliferation in a dose- and time-dependent manner compared to low-PAD score honey (LPH), with notable reductions observed at 24, 48, and 72 hr. The 24-hr IC50 value for HPH was 2.4 ± 0.2% v/v, whereas LPH exhibited a weaker inhibitory effect, with an IC50 of 10.8 ± 1.2% v/v. Although LPH also reduced cell viability dose- and time-dependently, its potency was markedly lower than that of HPH (Figure 1).

HPH inhibited migration in HT-29 cell line

The scratch assay was performed to evaluate the inhibitory effects of different concentrations of HPH and LPH on the migration of HT-29 cells. The results demonstrated that treatment with 0.65% honey (equivalent to 25% of the IC50 determined for HPH in the MTT assay) effectively reduced cell migration compared to the control group after 48 hr. In contrast, LPH at the same concentration (0.65%) not only failed to inhibit migration but also slightly enhanced cell migration compared to the control group (Figure 2 and Table 3).

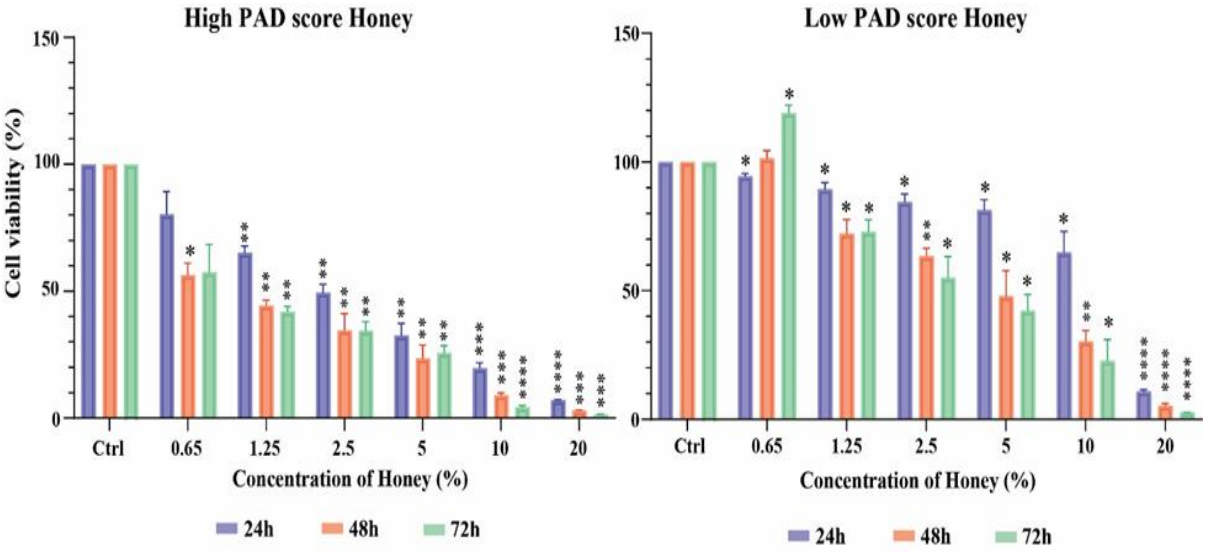

Figure 1. MTT assay showing the effects of high PAD score honey (HPH) and low PAD score honey (LPH) on HT-29 cell viability (%). HT-29 cells were treated with various concentrations of HPH and LPH for 24, 48, and 72 hr. Cell viability was assessed by MTT assay. Data were obtained from three replicates. HPH exhibited marked anti-proliferative effects (IC50 = 2.4 ± 0.2% v/v) compared to LPH (IC50 = 10.8 ± 1.2% v/v). At each time point, treated cells were compared with the corresponding control (*p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001).

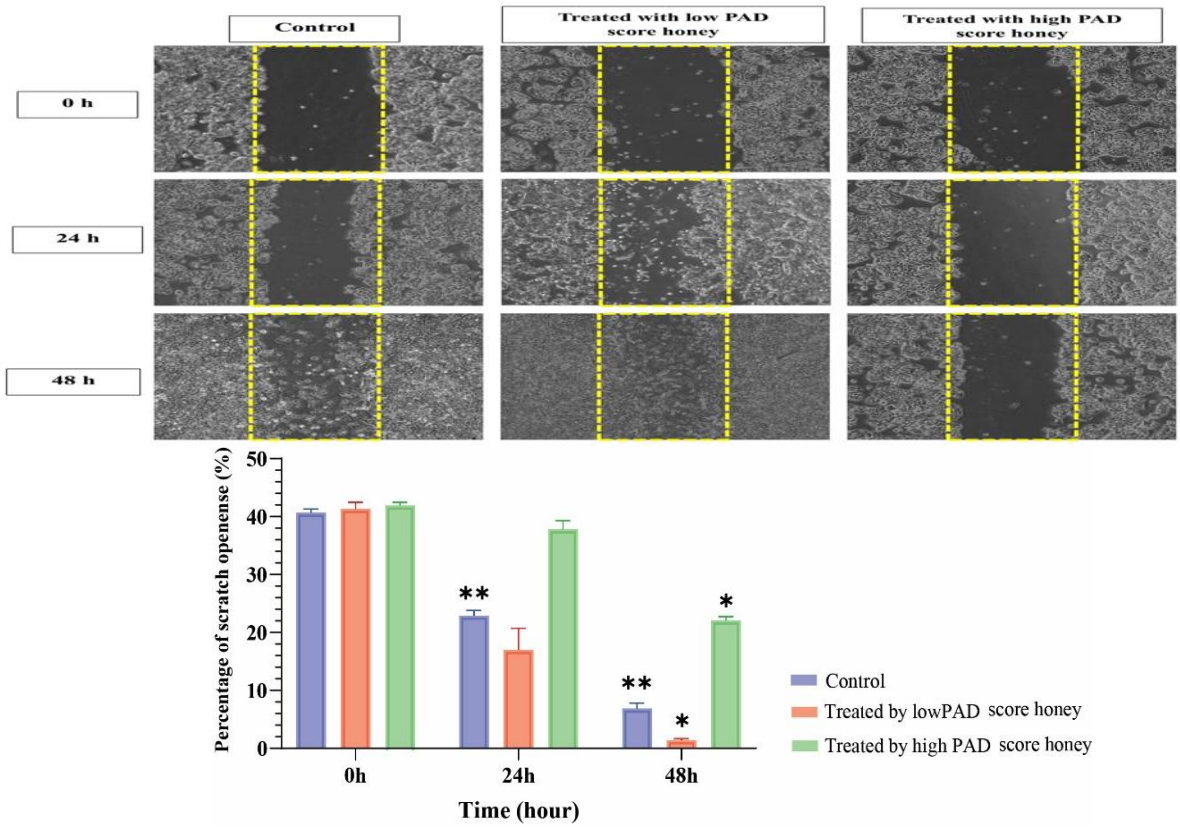

Figure 2. Scratch test showing the effects of low PAD score (LPH) and high PAD score (HPH) honey on reducing the migration of the HT-29 cell line. Data were obtained from three replicates. Under 80% confluence, a scratch was made on wells using a sterile yellow-colored pipette tip. Subsequently, 25% IC50 of HPH was added to the cells. Pictures were taken at 0 and 24 and 48 hr, and the percentage of scratch open area was calculated. Statistical comparisons were made between each group (control, LPH, and HPH) at 24 and 48 hr with its own baseline at 0 hr (*p<0.05 and **p<0.01).

Table 3. Wound closure (%) at 24 and 48 hr in different treatment groups.

	Control	Low PAD score Honey	High PAD score Honey
24 hr	43.6±2.1	58.9±9.1	9.8±3.6
48 hr	83.0±2.3	96.6±0.6	47.3±1.4

Note: Data represent mean ± SD of three replicates. Closure was calculated relative to the open area at 0 hr.

HPH Triggers Apoptosis pathway

To characterize the apoptosis-related effects of HPH and LPH, their impacts on the expression of key apoptosis regulatory genes assessed: *Bax*, *Bcl-2*, and *Caspase-3* in HT-29 cells. Data revealed that HPH significantly elevated the expression of *Bax* (1.49-fold, $p<0.05$) and *Caspase-3* (1.41-fold, $p<0.05$), while simultaneously reducing *Bcl-2* expression by 4-fold ($p<0.01$). Conversely, LPH treatment led to a significant downregulation of all three genes *Bax* (1.9-fold, $p<0.05$), *Caspase-3* (8.5-fold, $p<0.01$), and *Bcl-2* (2.43-fold, $p<0.05$), indicating a broader suppression of apoptotic signaling (Figure 3).

HPH arrest cells in G0/G1

Cell cycle analysis was performed to evaluate the impact of HPH and LPH honey samples on the progression of HT-29 cells using flow cytometry. The results showed variations in the distribution of cells in the G0/G1 phase following treatment with honey samples of different PAD scores. In the control group, 35.73% of cells were in the G0/G1 phase. Treatment with HPH increased this percentage to 44.16%, while LPH resulted in 42.06% of cells in G0/G1 (Figure 4).

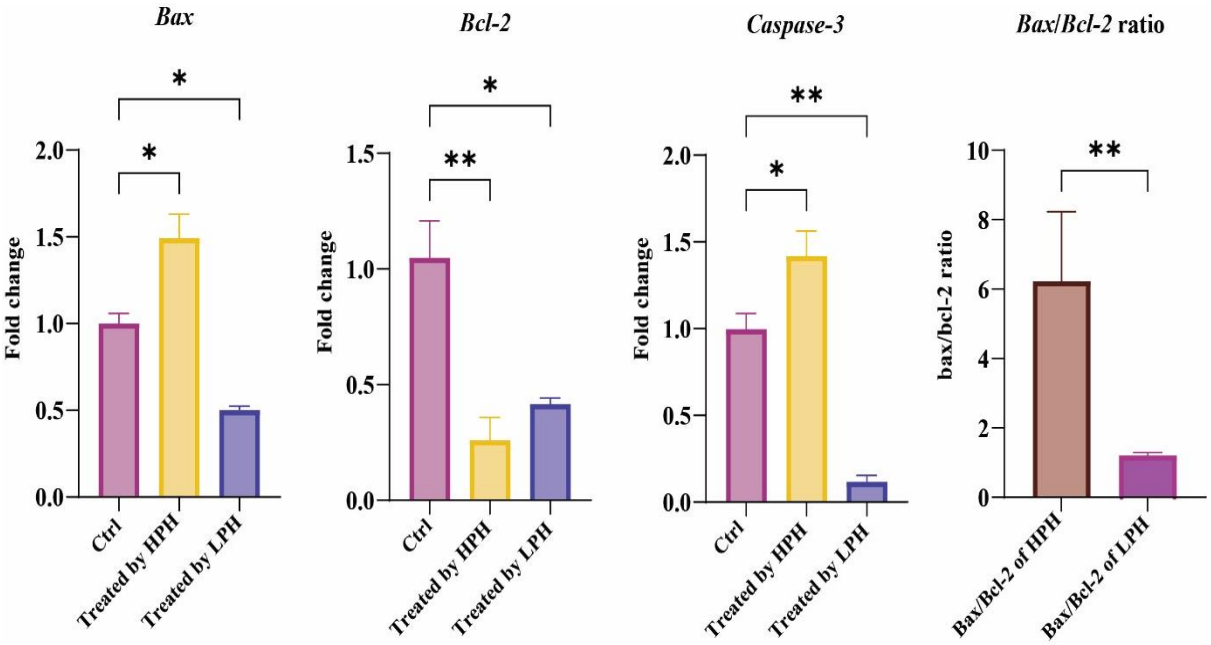

Figure 3. Gene expression levels of *Bax*, *Bcl-2*, and *Caspase-3* in HT-29 cell line in response to high PAD score (HPH) and low PAD score honey (LPH) treatment. Y-axis represents the fold change in the expression of the target gene compared with the untreated group. Data were obtained from three replicates. Statistical comparisons were performed between HPH vs. control and LPH vs. control ($2^{-\Delta\Delta ct}$ = 1 in untreated group), (*$p<0.05$ and **$p<0.01$).

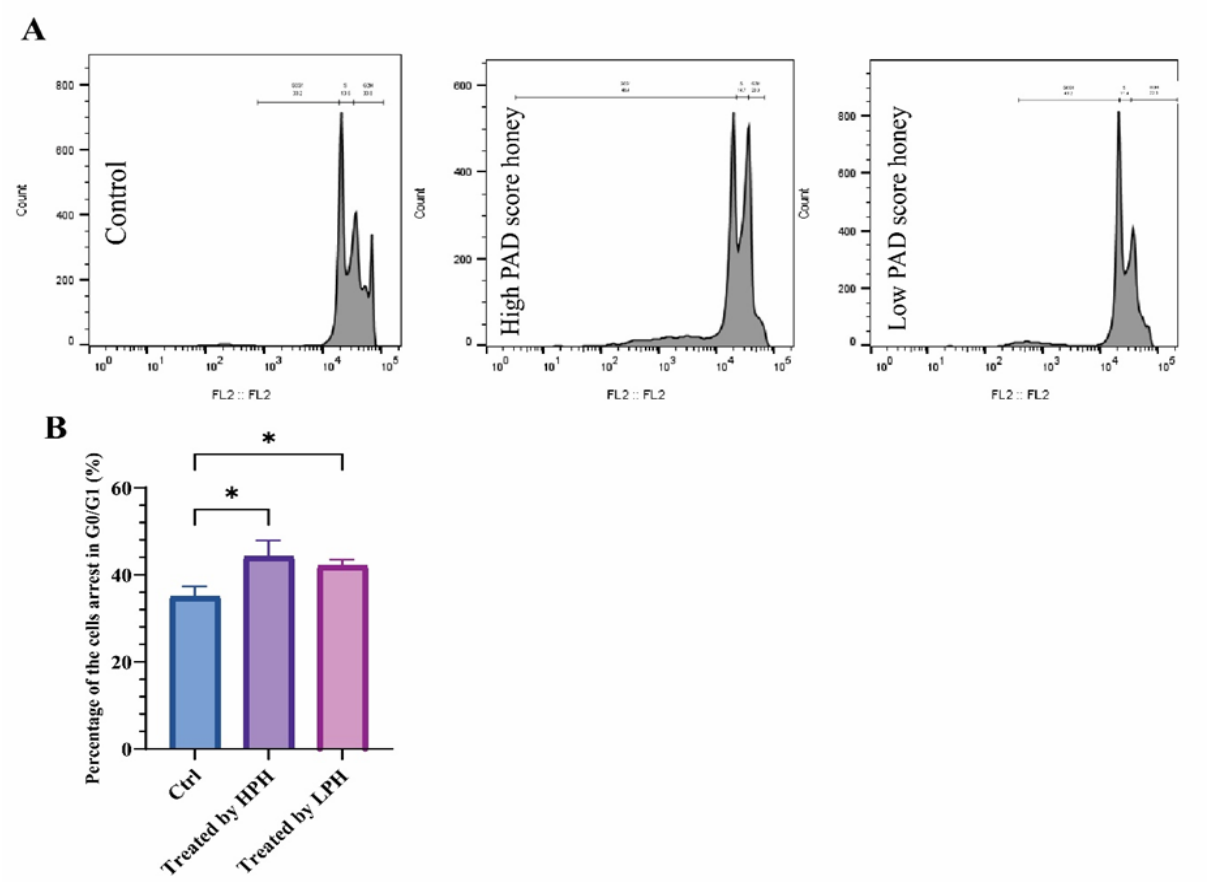

Figure 4. Flow cytometric analysis using propidium iodide (PI) staining reveals the cell cycle distribution in HT-29 cells across high PAD score honey (HPH)-treated, low PAD score honey (LPH)-treated, and control groups. Data were obtained from three replicates. A marked accumulation of cells in the G0/G1 phase was observed in the HPH-treated group, indicating a stronger G0/G1 cell cycle arrest compared to the control and LPH-treated groups. Statistical comparisons were performed between HPH and control, and between LPH and control (*$p<0.05$).

Discussion

The findings of this study demonstrate that both HPH and LPH reduced the viability of HT-29 cells in a dose- and time-dependent manner, with HPH exhibiting meaningfully greater cytotoxicity than LPH. This study introduces a novel classification system for the first time, based on medicinal properties, using total phenolic and protein content, antioxidant capacity, and diastase enzyme activity to classify samples into high and low PAD (Phenolic Acidity Degree) score honey. The *Berberis* (Zereshk) honey used in our study had a PAD score of 543, qualifying it as high PAD, while the commercial honey scored less than 74.

CRC remains one of the most common malignancies and a leading cause of cancer-related mortality worldwide. The rising incidence of CRC, which is expected to reach 3.2 million cases by 2040, underscores the need for novel treatment strategies (Cavic et al. 2024). Natural honey has garnered significant interest for its potential anticancer properties, primarily attributed to its rich content of bioactive compounds, including phenolics, total antioxidant activity, and diastatic activity (Usman et al. 2025).

The enhanced anti-proliferative effect of HPH can be attributed to its higher concentrations of bioactive molecules such as phenols, proteins, and antioxidants, which may increase resistance to oxidative stress, induce apoptosis, and inhibit cancer

cell survival. In contrast, while LPH contains some bioactive compounds, its weaker effects suggest that industrial processing or the presence of artificial additives may diminish its biological efficacy potential.

Phenolic compounds are primarily responsible for honey bioactivity, contributing to its antioxidant, anti-inflammatory, and anticancer effects (Iosageanu et al. 2024). These compounds include phenolic acids (such as gallic, caffeic, and ferulic acids) and flavonoids (such as quercetin and rutin), which have been widely identified and quantified in various types of monofloral honeys. They play a critical role in neutralizing free radicals, modulating cell signaling pathways, and protecting cells from oxidative stress, thereby enhancing honey bioactive efficacy (Purgatorio et al. 2024).

For instance, quercetin, one of the most abundant flavonoids in honey, has been reported to inhibit proliferation and induce apoptosis in CRC cells by activating caspase cascades, upregulating *Bax*, and suppressing the anti-apoptotic protein *Bcl-2* (Li and Gao 2013). Similarly, gallic acid, a representative phenolic acid, induces apoptosis by generating reactive oxygen species (Purgatorio et al.), disrupting mitochondrial membrane potential, and enhancing pro-apoptotic signaling pathways (Shao et al. 2024). The combined actions of quercetin and gallic acid may therefore explain the stronger apoptotic induction and cell cycle arrest observed in the HPH-treated cells compared to LPH.

Recent reviews have reinforced these mechanisms, showing that high-quality honeys can selectively induce apoptosis, regulate oxidative stress, suppress cell proliferation, and exert anti-inflammatory effects, often through the synergistic action of multiple phenolic compounds (Martinotti et al. 2024). Antioxidant capacity refers to honey ability to scavenge ROS and prevent oxidative damage to cellular components, including lipids, proteins, and DNA (Ahmed et al. 2018). This property is largely attributed to phenolic compounds but is also supported by other bioactive components such as enzymes (e.g. glucose oxidase), organic acids, vitamins (e.g. vitamin C), and trace elements. Various assays, including 2,2-Diphenyl-1-picrylhydrazyl radical scavenging (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization (ABTS), and Ferric Reducing Antioxidant Power (FRAP), are commonly employed to quantify antioxidant capacity, which correlates directly with honey potential to inhibit cancer development and progression (Wilczyńska and Żak 2024).

Diastase activity serves as an enzymatic marker of honey's freshness and biological quality. Active diastase (or amylase) is a natural enzyme in honey that breaks down starch into simpler sugars. Its activity decreases with prolonged storage or exposure to high temperatures; consequently, high diastase activity indicates minimal processing and better preservation of honey's natural properties, which may enhance its therapeutic effectiveness. Measuring diastase activity is part of standard honey quality assessments (Gil et al. 2024; Razali et al. 2019).

Consistent with our findings, Saudi Sidr Honey exhibited significant antiproliferative effects on HCT-116 CRC cells, with an IC50 of 61.89 µg/mL, inducing cell cycle arrest in the G1 phase and increasing apoptosis rates (Qanash et al. 2023).

In addition, multiple studies on Manuka honey have confirmed its anticancer potential. Masad et al. demonstrated that oral administration of Manuka honey not only inhibited tumor growth but also enhanced immune responses through Interferon gamma (IFNγ)-dependent pathways, increased tumor infiltration of $CD4^+$ and $CD8^+$ T cells, and upregulated MHC class I and II expression. Importantly, these effects were linked to modulation of gut microbiota composition, highlighting a dual mechanism involving both immune

stimulation and microbial regulation. While our study did not directly address immune responses, the strong cytotoxic and pro-apoptotic effects of HPH suggest that *Berberis* honey may act primarily through intrinsic apoptotic mechanisms, potentially complementing the immune-modulating role of Manuka honey (Masad et al. 2024a; Masad et al. 2024b).

In our study, HPH inhibited the migration of HT-29 cells, whereas LPH showed no such effect. This observation suggests that LPH may contain components that promote cancer cell motility, possibly due to industrial processing, adulteration, or added sugars that could serve as energy sources for tumor cells. It should be noted that sugars present in processed honey may also interfere with cellular metabolic assays such as MTT, potentially influencing observed proliferation and migration outcomes. Future studies should control for sugar content to isolate the specific effects of bioactive compounds. These findings highlight the critical importance of honey quality in determining its biological activity and raise concerns regarding the use of processed or commercially honeys in clinical or medicinal applications, particularly for cancer treatment (Bose et al. 2024). This is in agreement with reports showing that Manuka honey can suppress epithelial–mesenchymal transition (EMT) and angiogenesis in colon cancer stem cells, further confirming that high-quality honeys are capable of interfering with metastatic pathways (Cianciosi et al. 2024).

Gene expression analysis revealed that potent honey influenced the expression of genes involved in the apoptosis process in favor of activating this pathway in HT-29 cells. Specifically, HPH upregulated the pro-apoptotic genes *Bax* and *Caspase-3* while downregulating the anti-apoptotic gene *Bcl-2*. In contrast, LPH treatment resulted in the downregulation of all three genes, indicating a lack of apoptotic induction. The concurrent suppression of *Bax*, *Caspase-3*, and *Bcl-2* in LPH-treated cells suggests that this lower-quality honey fails to initiate apoptosis, reinforcing the idea that honey quality, defined by its phenolic content, antioxidant capacity, and diastase activity, directly influences its anti-cancer efficacy (Yusof et al. 2021). In alignment with the present study, a recent report on *Ziziphus jujube* (Bodnár et al.) honey also indicated its ability to induce a pro-apoptotic gene profile in MCF-7 breast cancer cells, including the upregulation of *Bax*, *p53*, and *p21*, alongside downregulation of *Bcl-2* genes (Karbasi et al. 2024a). Similarly, Das et al. showed that Indian honeys exert anticancer activity by inducing both intrinsic and extrinsic apoptotic pathways and suppressing the β-catenin/Wnt signaling cascade in CRC cells, suggesting that honeys may act on multiple molecular targets beyond apoptosis alone (Das et al. 2022).

Finally, HPH influenced cell cycle progression. Flow cytometric analysis showed that treatment of HT-29 cells with HPH resulted in a considerable accumulation of cancer cells in the G0/G1 cell cycle phase, with 44.16% of the cell population arrested at this checkpoint compared to 35.73% in the untreated control group. LPH treatment also increased G0/G1 phase arrest, but to a lesser extent (42.06%). This cell cycle arrest at the G0/G1 phase suggests that HPH may interfere with the transition to the S phase, thereby limiting DNA synthesis and proliferation. These findings further support the notion that HPH exerts its anti-proliferative effects not only through cytotoxicity and apoptotic induction but also by modulating cell cycle machinery, highlighting its multifaceted role in inhibiting cancer cell growth (Bose et al. 2024). Overall, *Berberis* and other high-quality honeys share core anticancer actions such as apoptosis induction and cell cycle arrest, but differ in their complementary mechanisms. These variations suggest that combining honeys of different origins may enhance their anti-cancer efficacy against CRC.

Our findings suggest that honey quality, as captured by the PAD scoring system, strongly influences its biological activity against colorectal cancer cells. High PAD score honey demonstrated multifaceted anticancer effects, whereas low PAD score honey lacked such efficacy, highlighting the importance of composition and authenticity. PAD scoring may therefore serve as a practical framework for distinguishing honeys with therapeutic promise. While these results are preliminary and derived from *in vitro* experiments, they provide a rationale for further *in vivo* and clinical studies to validate the translational potential of high-quality honeys in cancer research.

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Conflicts of interest

The authors declare that they have no conflict of interest**.**

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Ethical considerations

In accordance with ethical guidelines, this research was carried out under the approved ethics ID IR.BUMS.REC.1402.226, granted by the Ethics Committee of Birjand University of Medical Science, Iran.

Code of ethics

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Authors' contributions

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References

Abedini MR, Erfanian N, Nazem H, Jamali S, Hoshyar R (2016) Anti-proliferative and apoptotic effects of Ziziphus Jujube on cervical and breast cancer cells. Avicenna J Phytomed 6(2):142

Abu-Farich B, Hamarshi H, Masalha M, et al. (2024) Polyphenol contents, antibacterial and antioxidant effects of four palestinian honey samples, and their anticancer effects on human breast cancer cells. J Pure Appl Microbiol 18(2):1372–1385

Afshari A, Ram M, Mohamadi S (2022) Quality evaluation of Iranian honey collected from Khorasan province, Iran. Int J Food Sci 2022(1):3827742

Ahmed S, Sulaiman SA, Baig AA, et al. (2018) Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxid Med Cell Longev 2018(1):8367846

Al-Eisa RA, Ashour AA, Helal M, et al. (2023) Anticancer effects of honey varieties on human cells by studying some physical parameters, hydrogen peroxide content, catalase, glucose oxidase, and microbial activities. J Biobased Mater Bioenergy 17(2):160–166

Bodnár K, Fehér P, Ujhelyi Z, Bácskay I, Józsa L (2024) Recent approaches for the topical treatment of psoriasis using nanoparticles. Pharmaceutics 16(4):449

Bose D, Famurewa AC, Akash A, Othman EM (2024) The Therapeutic Mechanisms of Honey in Mitigating Toxicity from Anticancer Chemotherapy Toxicity: A Review. J Xenobiot 14(3):1109–1129

Bouali N, Hamadou WS, Badraoui R, et al. (2022) Phytochemical composition, antioxidant, and anticancer activities of Sidr Honey: In vitro and in silico computational investigation. Life 13(1):35

Cavic M, Nikolic N, Marinkovic M, et al. (2024) Two Decades of Progress in Personalized Medicine of Colorectal Cancer in Serbia—Insights from the Institute for Oncology and Radiology of Serbia. Biomedicines 12(10):2278

Cianciosi D, Forbes-Hernández TY, Armas Diaz Y, et al. (2024) The anti-metastatic effect of Manuka honey on colonspheres enriched with cancer stem cells: how does it influence the epithelial-mesenchymal transition process, angiogenesis, and telomere length? Food Funct 15(13):7200–13

Das N, Ray N, Patil AR, et al. (2022) Inhibitory effect of selected Indian honey on colon cancer cell growth by inducing apoptosis and targeting the β-catenin/Wnt pathway. Food Funct 13(15):8283–8303

Erfanian N, Nasseri S, Miraki Feriz A, Safarpour H, Namaei MH (2023) Characterization of Wnt signaling pathway under treatment of Lactobacillus acidophilus postbiotic in colorectal cancer using an integrated in silico and in vitro analysis. Sci Rep 13(1):22988

Erfanian N, Safarpour H, Tavakoli T, Mahdiabadi MA, Nasseri S, Namaei MH (2024a) Investigating the therapeutic potential of Bifidobacterium breve and Lactobacillus rhamnosus postbiotics through apoptosis induction in colorectal HT-29 cancer cells. Iran J Microbiol 16(1):68

Erfanian N, Saffar M, Khatami S, et al. (2024b) Modulating the Wnt Signaling Pathway in Wound Healing: The Potential of L-Carnosine, Zinc Sulfate, and B-Complex Vitamins Cocktail. Jundishapur J Nat Pharm Prod 19(2)

Gil J, Lee WY, Kim H-k (2024) Physicochemical and Diastase Activity Properties for Domestic and Imported Honey. J Pract Agric Fish Res 26(3):64–71

Iosageanu A, Stefan LM, Craciunescu O, Cimpean A (2024) Anti-Inflammatory and Wound Healing Properties of Different Honey Varieties from Romania and Correlations to Their Composition. Life 14(9):1187

Karbasi S, Asadian AH, Azaryan E, Naseri M, Zarban A (2024a) Quantitative analysis of biochemical characteristics and anti-cancer properties in MCF-7 breast cancer cell line: a comparative study between Ziziphus jujube honey and commercial honey. Mol Biol Rep 51(1):344

Karbasi S, Erfanian N, Dehghan H, Zarban A, Hanafi-Bojd MY (2024b) Assessment of the anti-cancer effects of Camel milk exosomes (CMEXOs) on murine colorectal cancer cell line (CT-26). Iran J Allergy Asthma Immunol 23(3):321–329

Li Z, Gao Q (2013) Induction of apoptosis in HT-29 cells by quercetin through mitochondria-mediated apoptotic pathway. Anim Cells Syst 17(3):147–153

Martinotti S, Bonsignore G, Ranzato E (2024) Understanding the anticancer properties of honey. Int J Mol Sci 25(21):11724

Masad RJ, Bashir G, Al-Sbiei A, et al. (2024a) Oral administration of Manuka honey modulates gut microbiota composition and enhances anti-tumor immunity in a preclinical model of colorectal cancer. Front Immunol 15:1354297

Masad RJ, Idriss I, Mohamed YA, et al. (2024b) Oral administration of Manuka honey induces IFNγ-dependent resistance to tumor growth that correlates with beneficial modulation of gut microbiota composition. Front Immunol 15:1354297

Mohammadi Y, Tahergorabi Z, Sharifzadeh GR, Rajabi Moghadam M, Zarban A (2024) Protective Effects of Some Graded Iranian Honey Samples Against Cold Water Immersion-Induced Gastric Ulcers in Rats. Food Sci Nutr 12(12):10211–10222

Morgan E, Arnold M, Gini A, et al. (2023) Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Gut 72(2):338–344

Nahala NM, Pareeth CM, Hussan KS, Babu TD, Soni K, Amritha V (2023) Anticancerous Properties of Tetragonula travancorica (stingless bee) Honey on

Reproductive Cancer Cells. Int J Agro Nutr Pract 3(4):1–9

Purgatorio R, Boccarelli A, Pisani L, De Candia M, Catto M, Altomare CD (2024) A critical appraisal of the protective activity of polyphenolic antioxidants against iatrogenic effects of anticancer chemotherapeutics. Antioxidants 13(1):133

Qanash H, Bazaid AS, Binsaleh NK, Patel M, Althomali OW, Sheeha BB (2023) In vitro antiproliferative apoptosis induction and cell cycle arrest potential of Saudi Sidr honey against colorectal cancer. Nutrients 15(15):3448

Razali MF, Fauzi NAM, Sulaiman A, Rahman NAA (2019) Effect of high-pressure processing (hpp) on antioxidant, diastase activity and colour for Kelulut (stingless bee) honey. J Teknol 81(3)

Shao J, Yang L, Jin Z, et al. (2024) Antiproliferative Effect of Gallic Acid is Mediated via Mitochondrial-or ER-Stress-Induced Apoptosis and Canonical Autophagy in HT-29 Cells. J Chem 2024(1):7139556

Usman AN, Fendi F, Nulandari Z, Agustin DI (2025) Trends, key contributors, and emerging issues in honey and breast cancer: A bibliometric analysis from 2014 to 2024. F1000Res 14:17

Wilczyńska A, Żak N (2024) Polyphenols as the Main Compounds Influencing the Antioxidant Effect of Honey—A Review. Int J Mol Sci 25(19):10606

Yusof H, Zulpa AK, Isa NSM, Ahmad FT, Kassim MNI (2021) Quality characteristics, antioxidant and anticancer potential of stingless bee honey and honeybee honey from similar environmental conditions. IIUM 20(4)

Zamani Ranjbar Garmroodi B, Rajabi Moghadam M, Zarban A, Bideh M (2024) Hepatoprotective Effects of Medicinal Honey: Introducing a New Classification Based on Phenolic Content and Antioxidant Capacity. J Food Biochem 2024(1):4475104

Zarafu I, Mușat I, Limban C, et al. (2025) Multifunctional Evaluation of Graphene Oxide–Sulfonamide Nanoconjugates: Antimicrobial, Antibiofilm, Cytocompatibility and Xenobiotic Metabolism Gene Expression Insight. Molecules 30(12):2585