Pectins extracted from prunes (*Prunus domestica* L.) inhibit the invasive capacity of melanoma cells

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Received April 15, 2025; Accepted September 17, 2025

DOI: 10.3892/ijfn.2025.50

Abstract. Fruit-derived pectins exhibit notable biological effects, particularly against tumor cells. Consequently, natural products have become a major focus in the search for novel anticancer agents with fewer side-effects. The present study evaluated the effects of a pectic fraction extracted from prunes (dried fruits of *Prunus domestica*), a species cultivated worldwide, on B16F10 cells. The prune pectic fraction obtained with hot water (PWH) reduced the viability of B16F10 murine melanoma cells, while leaving normal BALB/c 3T3 cells unaffected, indicating lower cytotoxicity than the chemotherapeutic drug, doxorubicin. At concentrations of 10 and 100 μg/ml, PWH specifically inhibited B16F10 cell viability by 24 and 40%, respectively, reduced colony formation by 20 and 26%, respectively, and decreased cytoplasmic extensions by 30% at 100 μg/ml. Furthermore, FAK gene expression increased by 57 and 200%, suggesting a compensatory response to mitigate the cell area loss. Collectively, these results underscore the potential of PWH for further cancer research.

Introduction

Cancer remains one of the leading causes of mortality worldwide, with the number of new cases rising each year. Chemotherapy, although a mainstay in cancer treatment, is associated with high toxicity and significant side-effects. This approach targets not only tumor cells, but also healthy cells, resulting in severe adverse outcomes such as nausea, fatigue, organ damage and immune suppression. These effects often necessitate treatment interruption, thereby compromising therapeutic efficacy (1). Furthermore, the development of resistance to chemotherapeutic agents remains a major challenge in cancer treatment (2). These limitations underscore the need for alternative adjuvant therapies capable of exerting antitumor effects without inducing toxicity, thereby allowing for reduced dosage and/or duration of conventional chemotherapy without compromising efficacy. Natural compound-based therapies have shown promise as complementary strategies that improve the quality of life of patients and support uninterrupted treatment.

Brazil harbors a vast biodiversity of plants containing natural bioactive compounds with a high biotechnological potential for the pharmaceutical industry. Among these, a wide variety of plant-derived polysaccharides have attracted considerable attention for their immunomodulatory and antitumor properties. Pectins, a family of covalently linked D-galacturonic acid-rich polysaccharides abundant in the primary cell walls of fruits, exhibit diverse biological activities, including anti-inflammatory, antioxidant, immunoregulatory and antitumor effects, and may also serve as carriers for targeted drug delivery (3). In numerous fruits, pectic polysaccharide structures undergo chemical and enzymatic modifications during ripening, leading to substantial intramolecular changes in the pectic chain (4). Pectins derived from diverse biological sources are known to exhibit considerable structural variability, and such differences in chemical composition have been associated with distinct biological activities. Notably, accumulating evidence indicates that pectins may exert antitumor effects through the modulation of tumor cell proliferation, adhesion and apoptosis (3). Pectin derived from papaya has been shown to reduce the viability and induce the necroptosis of colon and prostate cancer cell lines (5). Previous studies have demonstrated that pectic polysaccharides extracted from potatoes (6) and sugar beet (7) significantly inhibited the proliferation of HT-29 cells *in vitro*. Furthermore, apple pectin has been reported to promote

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Key words: cancer, antitumor, pectin, *in vitro*, B16F10 cells, prunes

the apoptosis and reduce the adhesion of 4T1 breast cancer cells (8). Notably, pectins display low toxicity and minimal side-effects on normal cells compared to conventional chemotherapeutic agents (9). These properties highlight pectins as molecules with potential to serve as adjuvants in cancer therapy, exerting antitumor effects and potentially enabling a reduction in the use of chemotherapeutic agents associated with severe side effects.

Prunes are the dried fruits of *Prunus domestica* L. (European plum; *P. domestica*), a tree cultivated on all continents, with its fruits widely consumed worldwide (10). Pectins extracted from *P. domestica* fruits have been reported to exhibit antioxidant (11), gastroprotective (12,13) and anti-inflammatory activities (14). Recently, Vaz da Luz *et al* (15) demonstrated that isolated side chains of pectins (type I arabinogalactans) with different molar masses, obtained from prune tea infusions, displayed varying antitumor effects. However, unlike the study by Vaz da Luz *et al* (15), the present study aimed to evaluate the antitumor activity of pectins present in the prune pectic fraction obtained with hot water (PWH), which consists of a mixture of rhamnogalacturonans with type I arabinogalactan side chains and low-methyl-esterified homogalacturonan (13). It is well-established that a mixture of pectic polysaccharides in solution can elicit different biological effects compared to those produced by individual polysaccharide chains (4). To the best of our knowledge, the antitumor activity of pectins in the PWH fraction has not been previously investigated, which justifies the present investigation.

In light of the above, the present study aimed to evaluate the antitumor activity of the PWH fraction obtained from prunes in B16F10 murine melanoma cells. The present study assessed its cytotoxicity on both tumor and normal cells, as well as its effects on tumor cell migration, colony formation and morphology.

Materials and methods

Purification, characterization and solubilization of polysaccharide fraction. The prune pectin fraction (PWH) was characterized and kindly provided by the Department of Biochemistry at the Federal University of Paraná (Paraná, Brazil). Pitted prunes (dried fruits from *P. domestica* purchased at a local market in Curitiba, Brazil; LA VIOLETERA®) were freeze-dried and milled. The extraction of pectic polysaccharides was carried out using hot water in order to obtain the molecules more tightly bound to the cell wall. Moreover, hot-water extraction results in a higher pectin yield compared to cold-water extraction. The water extract was obtained by filtration, and the polysaccharides were recovered by ethanol (3 vol.) precipitation and lyophilization, originating the fractions. A homogeneous fraction was analyzed by sugar composition, high-performance steric exclusion chromatography, methylation, and nuclear magnetic resonance spectroscopy analyses. The PWH comprises rhamnogalacturonans with type I arabinogalactans as side chains, and low-methyl esterified homogalacturonan (9). The freeze-dried pectin fraction was solubilized in the cell growth medium [RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.), supplemented with 10% fetal bovine serum (FBS, Gibco; Thermo Fisher Scientific, Inc.) and 1% antibiotic (penicillin 10,000 U and streptomycin 10 mg/l), MilliporeSigma] and stored at -20˚C until its use.

Cell groups were divided into a control group (CT) and a group treated with prune pectins (PWH). The control group received only the cell growth medium without PWH. For cytotoxicity analysis, doxorubicin (DX, Pfizer®) at a concentration of 2.5 μg/ml was used as a positive control. In this case, the same control medium was used, supplemented with the chemotherapy drug.

Cell lines and cell culture. The B16F10 murine melanoma cell line (BCRJ, 0046) and BALB/c 3T3 normal cell line (clone A31, ATCC, CCL-163) were kindly provided by the Laboratory of Inflammatory and Neoplastic Cells, Department of Cell Biology, UFPR, Curitiba, Brazil, and were initiated and maintained according to specific recommendations for each line. The BALB/c 3T3 cell line, derived from fibroblasts of BALB/c mouse embryos, is non-tumorigenic and widely employed as a representative model of normal cells in contrast to tumor cell lines. Given that fibroblasts are distributed throughout the body, this lineage provides an appropriate model for assessing normal cell function in studies of viability and cytotoxicity. Cells were cultured in growth medium and maintained in an incubator (Sanyo Scientific MCO-18AC) at 37˚C, 90% humidity, and 5% CO_2 for 72 h. All experiments were performed in biological triplicates.

Analysis of cell viability. To determine the concentrations to be used in the present study, a cell viability and cytotoxicity test was carried out to determine the concentrations that were not cytotoxic to normal cells. For this, the colorimetric tests of reduction of diphenyltetrazolium bromide (MTT) and neutral red (NR) were performed by the protocols proposed by Mosmann (16) and Repetto *et al* (17), respectively. The B16F10 cells ($5x10^2$ cells/well) and BALB/c 3T3 ($2x10^3$ cells/well) were exposed to 0, 2.5, 5, 10, 100 and 800 μg/ml PWH, and 2.5 μg/ml DX for 72 h. Briefly, the MTT cell viability assay was performed by first seeding the cells into 96-well plates and treating them as described in the experimental design. Following treatment, the wells were aspirated to remove the culture medium (RPMI-1640, MilliporeSigma; containing fetal bovine serum 10% Gibco; Thermo Fisher Scientific, Inc.), and 100 μl MTT solution [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide 5 mg/ml in PBS, MilliporeSigma, pH 7.4] were added to each well. The plates were incubated for 3.5 h at 37˚C in a humidified atmosphere with 5% CO_2 to allow the formation of formazan crystals by metabolically active cells. Following incubation, the MTT solution was discarded, and 100 μl dimethyl sulfoxide (DMSO, MilliporeSigma) were added to each well to solubilize the formazan crystals. The plates were gently shaken to ensure complete solubilization, and the absorbance was measured at 540 nm using a microplate reader (TECAN® Infinite M200; Tecan Group, Ltd.). The absorbance values obtained were directly proportional to the number of viable cells.

The neutral red cell viability assay was performed by first treating cells cultured in 96-well plates according to the experimental protocol. Following treatment, the medium (RPMI-1640, MilliporeSigma; containing fetal bovine serum 10% Gibco; Thermo Fisher Scientific, Inc.) was removed,

and 100 μl Neutral Red solution (MilliporeSigma, 40 μg/ml in culture medium) were added to each well. The cells were then incubated for 3 h at 37˚C in a 5% CO_2 atmosphere to allow dye uptake by viable cells. Following incubation, the dye solution was removed, and the cells were washed gently with 150 μl PBS (MilliporeSigma) to eliminate excess dye. Subsequently, 100 μl destaining solution (50% ethanol, 49% distilled water, and 1% glacial acetic acid, MilliporeSigma) was added to each well to extract the dye from the lysosomes. The plate was shaken until the complete solubilization of the dye, and the absorbance was measured at 540 nm using a microplate reader (TECAN® Infinite M200). The absorbance values obtained were directly proportional to the number of viable cells. The results obtained from the MTT and neutral red assays (measured by absorbance) were converted into percentages, using the control group (without treatment with the PWH fraction) as having 100% viability.

Cytotoxicity assay. The assay was performed through the crystal violet method, as previously described by Bonnekoh *et al* (18). The B16F10 cells ($2x10^3$ cells/well) were plated in a new 96-well plate and exposed to 0, 10 and 100 μg/ml PWH. Cell viability was measured in 24, 48 and 72 h. Following the treatment period, the medium (RPMI-1640 MilliporeSigma; containing fetal bovine serum 10% Gibco; Thermo Fisher Scientific, Inc.) was removed, and the cells were gently washed with 150 μl PBS to eliminate non-adherent cells and debris. Subsequently, 100 μl of 4% paraformaldehyde solution (MilliporeSigma) were added to each well to fix the cells for 20 min at room temperature. Following fixation, the wells were washed again with PBS, and 100 μl 0.1% crystal violet solution (prepared in 20% methanol, Merck KGaA) were added to stain the cells at room temperature for 10 min. Excess dye was removed by rinsing the plate under running distilled water until the background was clear. After drying, the bound dye was solubilized by the addition of 100 μl 10% acetic acid (MilliporeSigma) to each well. The plate was gently shaken to ensure complete solubilization. The results were obtained using a TECAN® Infinite M200 device at 590 nm. The data are presented as a percentage relative to the CT group at 24 h.

Cell migration (wound healing) assay. Following 72 h of exposure to 0, 10 and 100 μg/ml PWH, the B16F10 surviving cells were plated again ($2x10^4$ cells/well) in a new 96-well plate with culture medium (RPMI-1640 MilliporeSigma; containing fetal bovine serum 10% Gibco; Thermo Fisher Scientific, Inc.) to perform the cell migration assay (‘scratch’ method) (19). Briefly, after the cells reached full confluency, cell proliferation was inhibited with the use of RPMI growth medium lacking in FBS (1%), and a straight scratch was made in the monolayer using a sterile 200 μl pipette tip. The wells were then gently washed with PBS to remove detached cells. Images of the scratch area were captured immediately (0 h) and following 24 h of incubation at 37˚C in a 5% CO_2 atmosphere using an inverted microscope (BIOVAL® XDS-1B). The wound closure area was analyzed using ImageJ® software 1.52 (National Institutes of Health), and the migration rate was expressed as the percentage of area covered by cells after 24 h compared to time 0 (20).

Colony formation assay. Following 72 h of exposure to 0, 10 and 100 μg/ml PWH, B16F10 surviving cells were plated again at a reduced concentration ($1x10^2$ cells/well) in a 24-well plate to perform the colony formation assay analyses (21). This assay is expected to evaluate the ability of a single cell to form new colonies. For this, surviving cells were maintained only in the growth medium (RPMI-1640 MilliporeSigma; containing fetal bovine serum 10% Gibco; Thermo Fisher Scientific, Inc.) and incubated at 37˚C for 96 h. At the end of the incubation period, the colonies were fixed with 4% paraformaldehyde at room temperature for 15 min and then stained with 0.1% crystal violet solution (prepared in 20% methanol) for 10 min at room temperature. Excess dye was washed off with distilled water, and the plates were dried in air. The images were acquired using an inverted microscope (BIOVAL® XDS-1B), and colonies containing >50 cells were counted using ImageJ® software 1.52 (National Institutes of Health).

Analysis of cell morphology. This assay aimed to evaluate morphological changes in B16F10 cells following exposure to the PWH fraction. The cells ($5x10^3$ cells/well) were seeded in 24-well plates and treated with 0, 10, or 100 μg/ml PWH. Cell morphology was assessed at the start of treatment and again after 24 h. At each time point, the cells were fixed with 4% paraformaldehyde at room temperature for 10 min and stained with 0.25% crystal violet (prepared in methanol) at room temperature for 10 min (22). The area occupied by the cells in culture was quantified through digital analysis of images captured using a light microscope (BIOVAL® XDS-1B) at x400 magnification. Images were processed using ImageJ software 1.52 (National Institutes of Health), applying threshold-based segmentation to differentiate cells from the background. The total cell area was subsequently measured in pixels.

Reverse transcription-quantitative PCR (RT-qPCR). RNA isolation from the B16F10 cell samples following 72 h of PWH treatment was performed using TRIzol® reagent (Bio-Rad Laboratories, Inc.) according to the manufacturer's recommendations. The extracted RNA samples were then converted to cDNA using the iScript Reverse Transcription Supermix for RT-qPCR kit (cat. no. 1708841, Bio-Rad Laboratories, Inc.). The reactions were taken to the thermocycler (Applied Biosystems; Thermo Fisher Scientific, Inc.), and the samples were incubated in three cycles: 1st cycle of 5 min at 25˚C, 2nd cycle of 20 min at 46˚C, and 3rd cycle of 1 min at 95˚C. The RT-qPCR reaction was performed using the one-step RT-PCR SYBR®-Green kit (cat. no. 1725270, Bio-Rad Laboratories, Inc.) and the appropriate primers for each reaction, as follows: Focal adhesion kinase (FAK) forward, 5'-TCTGTGGAATTGGCAATCGG-3' and reverse, 5'-TGGATGGTCTGCACTTGGTT-3'; beta actin (ACTB) forward, 5'-CTGTATTCCCCTCCATCGTG-3' and reverse, 5'-GGGTCAGGATACCTCTCTTGC-3'; hypoxanthine-guanine phosphoribosyltransferase (HPRT1) forward, 5'-GTTGGGCTTACCTCACTGCT-3' and reverse, 5'-TAATCACGACGCTGGGACTG-3'. The samples were analyzed in a QuantStudioTM 5 device (Applied Biosystems; Thermo Fisher Scientific, Inc.). For each reaction, a control RT (without conversion of RNA to cDNA) was performed.

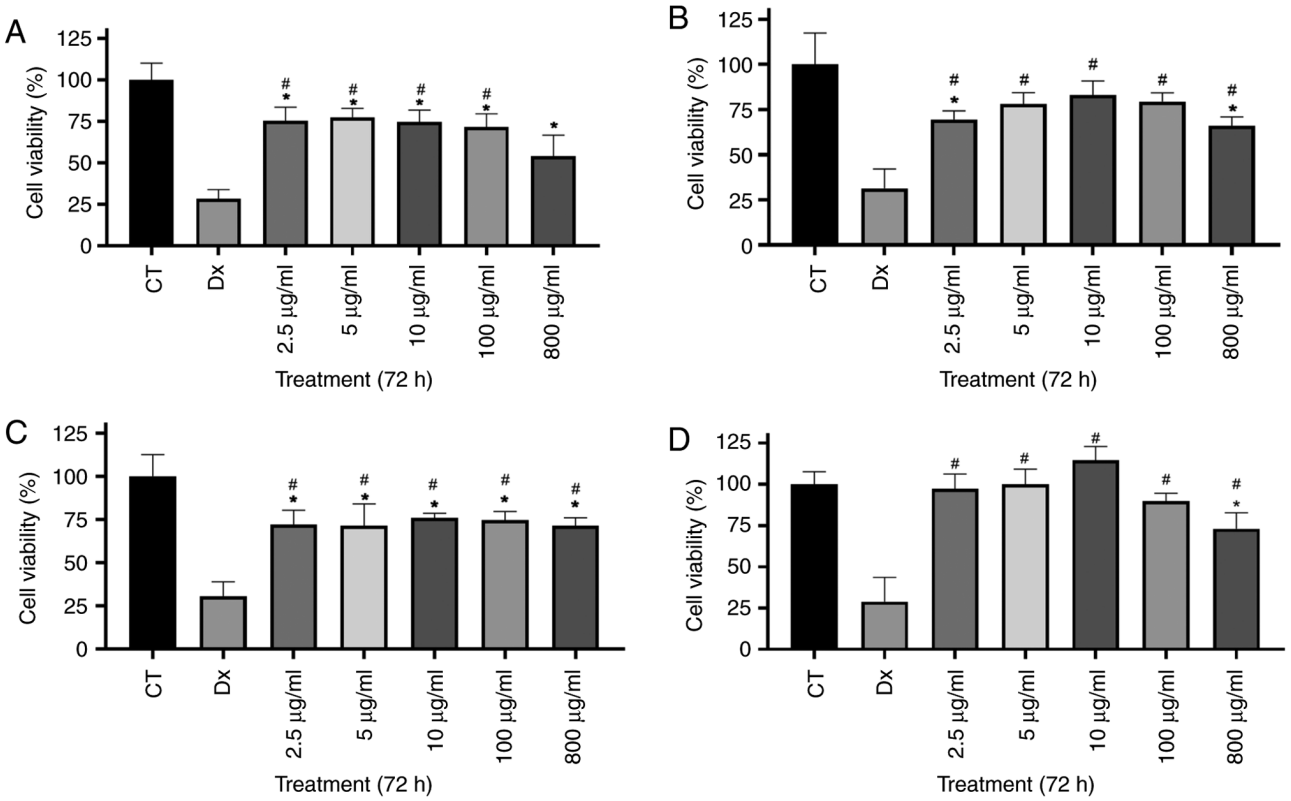

Figure 1. Cell viability of tumor (B16F10) and normal (BALB/c 3T3) cells, expressed as a percentage relative to the control group (CT) and positive control group (DX) after 72 h of polysaccharide treatment. (A) B16F10 cells assessed using the neutral red method; (B) BALB/c 3T3 cells assessed using the neutral red method; (C) B16F10 cells assessed using the MTT method; (D) BALB/c 3T3 cells assessed using the MTT method. Results represent at least three biologically independent experiments analyzed using one-way ANOVA followed by Tukey's test. Data are presented as the mean ± SD. *P<0.05, significant differences relative to the CT group; $^{\#}$P<0.05, significant differences relative to the DX group. CT, control; DX, doxorubicin.

The relative quantification was measured according to the pre-set threshold fluorescence level of the target gene (FAK) compared to the endogenous controls used (ACTB and HPRT1). ACTB and HPRT1 were utilized for normalization. The quality and purity of total RNA were evaluated using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc.). Samples exhibited A260/A280 ratios between 1.8 and 2.0 and A260/A230 ratios >1.8, indicating minimal contamination by proteins or phenolic compounds and falling within commonly accepted parameters for gene expression analysis. For each RT-qPCR reaction, 4.5 μl cDNA, synthesized from a standardized RNA concentration of 10 ng/μl, was used, corresponding to 45 ng total RNA per reaction. The assay performance was monitored using two endogenous reference genes (ACTB and HPRT1), selected for their stability across the analyzed *Mus musculus* samples. No-reverse transcriptase (RT-) controls were included to confirm the absence of genomic DNA contamination. Each sample was tested in triplicate, and the relative gene levels were normalized using the $2^{-\Delta\Delta Cq}$ method (23).

Statistical analysis. The normal distribution of values was verified using the Shapiro-Wilk test. One-way ANOVA followed by Tukey's test was performed in all analyses, apart from cell proliferation, which was analyzed using two-way ANOVA (mixed model) followed by the Bonferroni post hoc test, both through GraphPad Prism8 Software® (Dotmatics). P<0.05 was considered to indicate a statistically significant difference. Tukey's test was used as a post hoc test. Data are presented as the mean ± SD of at least three independent experiments.

Results

Cell viability. In the NR assay, all PWH concentrations significantly reduced the viability of the B16F10 cells compared with the CT group (Fig. 1A). In the BALB/c 3T3 cells, a significant reduction was observed only following treatment with 2.5 and 800 μg/ml PWH in relation to the CT group (Fig. 1B). The MTT assay revealed a similar viability profile for the B16F10 cells as in the NR assay (Fig. 1C). In the BALB/c 3T3 cells, cytotoxicity was detected only following treatment with 800 μg/ml PWH, although it remained lower than that induced by DX (Fig. 1D). Overall, PWH was less cytotoxic than DX across all concentrations and assays (Fig. 1A-C), apart from the concentration of 800 μg/ml in the NR assay (Fig. 1A). These assays suggest that the PWH fraction exhibits greater cytotoxicity toward tumor cells than toward normal cells.

Cytotoxicity assay. Based on the cell viability data, concentrations were selected to avoid cytotoxic effects on normal cells while effectively reducing tumor cell viability. Subsequent experiments were performed using 10 and 100 μg/ml PWH, with the control (CT) group remaining polysaccharide-free (0 μg/ml). The results demonstrated the cytotoxicity of the

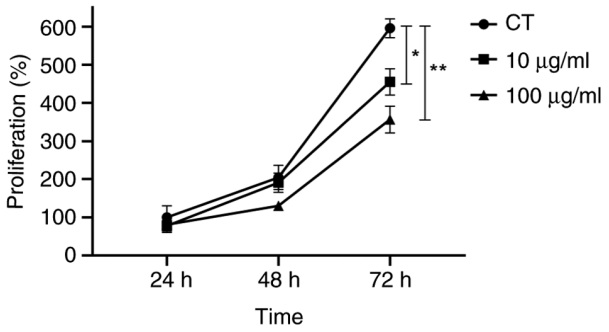

Figure 2. B16F10 cell proliferation over 72 h of treatment. The percentage of proliferation was calculated relative to the 24-h results. Data represent at least three biologically independent experiments analyzed using two-way ANOVA (mixed model) followed by the Bonferroni post hoc test, and are expressed as the mean ± SD. *P<0.05 and **P<0.01.

PWH fraction on tumor cells over time. PWH did not significantly affect cell viability at 24 or 48 h compared to the CT group. However, after 72 h, viability was markedly reduced at both concentrations, with decreases of 23.6 and 40.3%, respectively (Fig. 2).

Cell migration and colony formation capacity. In the migration assay, tumor cells treated with the PWH fraction closed the scratch to a similar extent as untreated cells, indicating that PWH did not significantly impair cell migration at either concentration within 24 h (Fig. 3A).

By contrast, PWH effectively reduced colony formation at both concentrations. Compared to the CT group, the number of new colonies decreased by 19.6% in the cells treated with 10 μg/ml PWH and by 25.9% in those exposed to 100 μg/ml PWH (Fig. 3B). These data indicated that tumor cells exhibited a reduced proliferation and diminished colony formation capacity following treatment with PWH.

Area occupied by cultured cells. Morphological changes were observed in the cells exposed to PWH under an inverted microscope. To confirm and further investigate these changes, a detailed morphological analysis was performed. Immediately after treatment (0 h), no noticeable differences were detected between the treated and control cells (Fig. 4A). However, following 24 h of exposure, the cells treated with 100 μg/ml PWH exhibited a 30% reduction in the area occupied by cultured cells compared to the control group (Fig. 4B), suggesting that contact with the polysaccharides in the PWH fraction interfered with the cytoskeletal organization of tumor cells.

RT-qPCR. Based on the observed effects on cell proliferation, colony formation and morphology, it was hypothesized that PWH may influence the focal adhesion points of cells. To determine this, FAK gene expression was quantified. FAK is a central regulator that promotes focal adhesion to the extracellular matrix and cytoskeletal remodeling. The results revealed the altered expression of the FAK gene in the B16F10 cells treated with PWH, with a 57% increase at the concentration of 10 μg/ml and a 3-fold increase at the concentration of 100 μg/ml (Fig. 5).

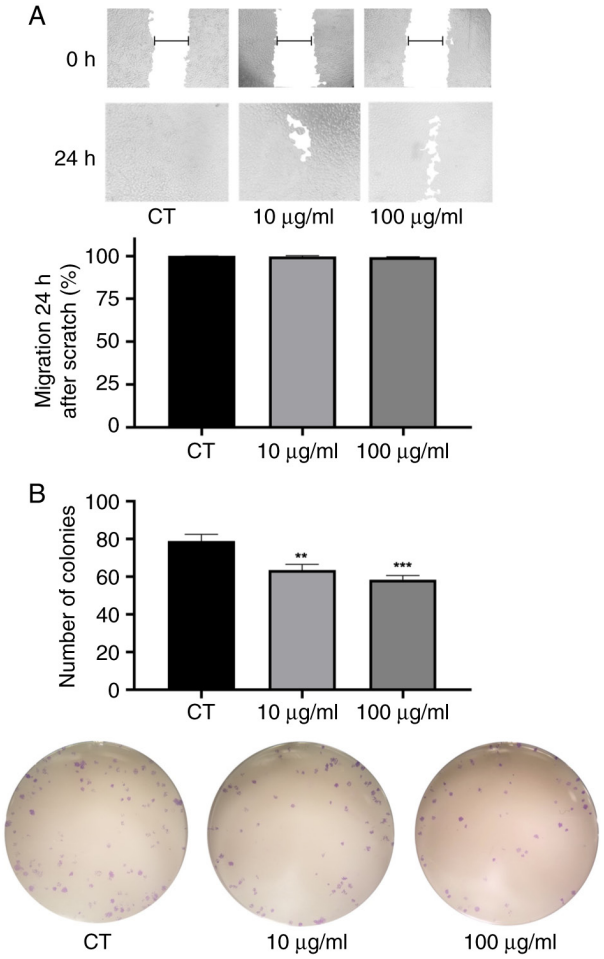

Figure 3. B16F10 cell migration and colony formation capacity. (A) The graph represents the percentage of slot invasion by cells previously treated with polysaccharide for 72 h, with corresponding images captured immediately after the scratch and 24 h later. (B) The number of colonies formed after 72 h of polysaccharide treatment and 96 h post-replating. Results are based on at least three biologically independent experiments analyzed using one-way ANOVA followed by Tukey's test. Data are presented as the mean ± SD. **P<0.01 and ***P<0.001, significant differences compared to the CT. CT, control.

Discussion

The results of the present study demonstrated that PWH exerted specific effects on B16F10 cells, including reduced viability, decreased colony formation, fewer cytoplasmic extensions and an altered FAK gene expression. These changes are closely linked to melanoma differentiation and invasiveness (24-26). Even the lowest concentration of PWH (2.5 μg/ml) was sufficient to reduce B16F10 cell viability following 72 h of treatment. From a pharmacological perspective, the ability to decrease cancer cell viability at low concentrations are highly desirable (27). It was also observed that the reduction in cell viability was similar at both low and high concentrations, a phenomenon previously reported in studies involving pectins (28). Notably, the effect of PWH on normal cell viability was substantially lower than that of DX, consistent with other reports highlighting the low toxicity of pectins (29,30).

Cell cytotoxicity assays over time are essential for understanding the mechanisms of action of any proposed treatment.

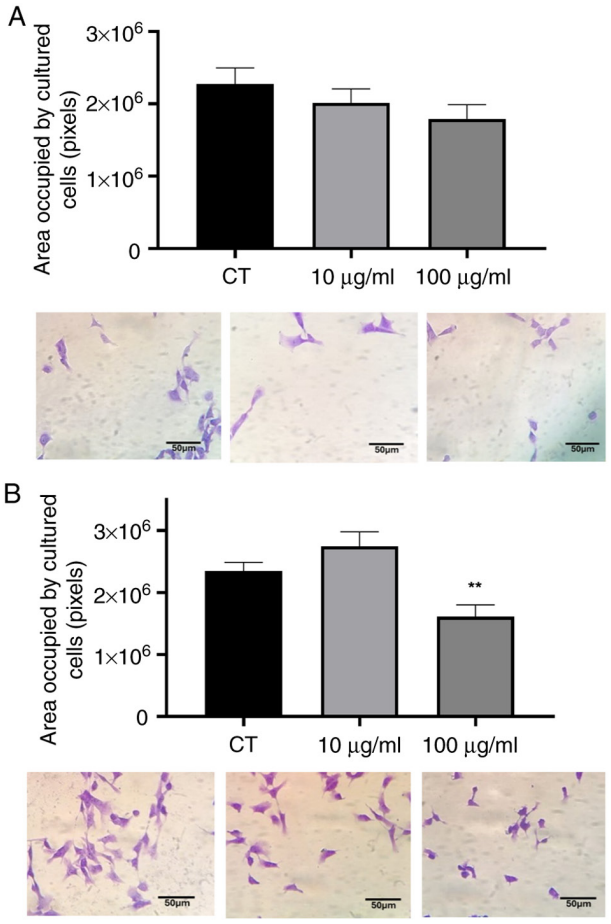

Figure 4. (A) Area occupied by cultured cells (pixels) at the time of PWH exposure. (B) Area occupied by cultured cells (pixels) 24 h after PWH exposure. Results represent at least three biologically independent experiments analyzed using one-way ANOVA followed by Tukey's test. Data are presented as the mean ± SD. **P<0.01, significant differences compared to the CT. CT, control; PWH, prune pectic fraction obtained with hot water.

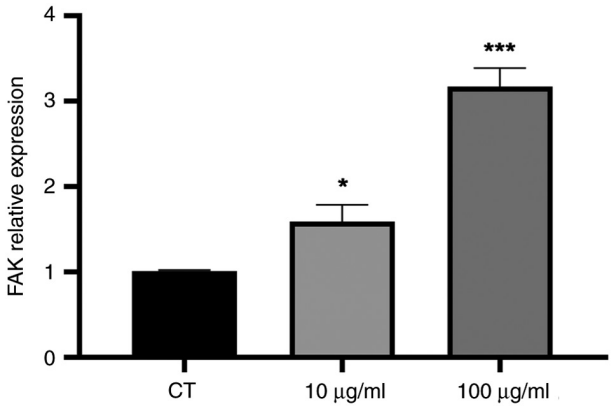

Figure 5. FAK relative gene expression in B16F10 cells over 72 h of treatment. RT-qPCR analysis was performed on at least three biologically independent experiments and evaluated using one-way ANOVA. Data are presented as the mean ± SD. *P<0.05 and ***P<0.001, significant differences compared to the CT. CT, control; FAK, focal adhesion kinase.

In the present study, a concentration-dependent reduction in tumor cell viability was observed after 72 h of exposure to PWH. Similar effects of pectins on tumor cell cultures have been reported in other studies (5,31,32). Of note, despite the 10-fold difference between the lowest and highest PWH concentrations (10 and 100 μg/ml), the effect on B16F10 cell viability was not proportionally greater at the higher concentration. This suggests that the impact of the PWH fraction on cell viability may reach a plateau beyond a certain concentration, indicating that the cellular pathways involved are already fully modulated, and increasing the concentration to 100 μg/ml does not further enhance the response. Indeed, a similar non-proportional reduction in viability with increasing concentrations of a comparable polysaccharide fraction has been reported previously (33).

Pectins from various sources have been reported to reduce tumor cell migration (34,35), including the migration of B16F10 cells (36). In contrast, in the present study, treatment with PWH did not produce significant changes in the migration rate of B16F10 cells. Nevertheless, the potential effect of the polysaccharides on cell migration cannot be ruled out, as the present study focused solely on migration at 0 and 24 h after scratch creation, which may have overlooked any early delays in movement immediately following the 'wound'. Additionally, employing alternative techniques to evaluate migratory capacity could provide further insights.

The process of metastasis involves not only the ability of cells to migrate, but also the capacity of a single cell to proliferate and form a new colony in a tissue different from its primary origin. Studies investigating the effects of polysaccharides on tumor cell colony formation have reported promising results (37,38). These findings are consistent with those of the present study, in which PWH significantly reduced colony formation at its highest concentration (100 μg/ml).

In the present study, PWH at 100 μg/ml reduced the cytoplasmic area of B16F10 cells. Since cell-cell contact is a key mechanism for proliferation, the reduction in cell area and dendritic projections may lead to decreased release of critical growth factors necessary for sustaining cell proliferation (39). Furthermore, the data presented herein indicated that exposure to PWH was associated with a reduced cell area alongside increased FAK gene expression in B16F10 murine melanoma cells. Cytoskeletal reorganization plays a crucial role in the adaptation of a cell to specific exogenous stimuli or inhibitors present in the surrounding microenvironment. Consequently, the signaling proteins involved in this reorganization are essential for maintaining cell morphology and regulating biophysical dynamics (40). Focal adhesion sites are specialized regions where the cytoskeleton connects with the extracellular matrix (ECM). These sites rely on the coordinated activity of integrins, the cytoskeleton and signaling proteins, such as FAK and Src. They are essential for maintaining cellular architecture and sensing mechanical cues from the environment. The FAK-Src pathway can be activated by various signals involved in cell survival, invasion and adhesion. FAK is a central regulator of tumor cell motility and invasiveness. Upon activation, via integrin-ECM interactions or growth factor signaling, FAK autophosphorylates at Tyr397 and recruits Src kinases, promoting focal adhesion turnover and cytoskeletal remodeling through targets such as paxillin, p130Cas and Rho GTPases. FAK signaling also enhances MMP-2 and MMP-9 expression, supporting extracellular matrix degradation and invasion, and contributes to

invadopodia formation and EMT-like phenotypes in melanoma (25,41). Notably, research has shown that an increased FAK expression is directly associated with heightened cancer aggressiveness (42), which is in contrast to the findings of the present study. However, it was hypothesized that the pectins present in PWH may impair cell adhesion to the surrounding environment. This could explain why, unable to adhere efficiently to their microenvironment, the cells remodel their cytoskeleton and display a reduced morphology compared to the control group. Consequently, in the absence of optimal adhesion and spreading, a compensatory mechanism may be triggered, leading cells to upregulate FAK in an attempt to form additional focal adhesion points for survival. Further detailed investigations of the mechanisms involved in the FAK-Src signaling pathway are required to clarify these findings.

The present study has certain limitations which should be mentioned. The analysis of additional genes related to cell proliferation and migration/invasion, as well as their corresponding protein expression, was not performed here and should be addressed in future work.

In conclusion, the pectins from prunes present in the PWH fraction reduced the viability of B16F10 murine melanoma cells, while exhibiting minimal toxicity toward normal BALB/c 3T3 cells, exhibiting lower cytotoxicity than the chemotherapeutic agent DX. Additionally, PWH inhibited malignant colony formation and appeared to affect focal adhesion in the B16F10 cell line. These results provide preliminary evidence supporting PWH as a potential compound for future cancer research.

Acknowledgements

The authors are grateful to Dr Edvaldo da Silva Trindade and Dr Fernanda Fogagnoli Simas from the Laboratory of Inflammatory and Neoplastic Cells - Federal University of Paraná, Curitiba, Brazil, for kindly providing the cell lines.

Funding

The present study was funded by the Araucaria Foundation (Official Agreement no. 051/2017), CNPq (grant nos. 404717/2026-0, 310731/2021-6 and 403295/2021-1), and fellowships from CAPES (Funding code 001, PROEX - Grant no. 88881.924191/2023-01).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

APB, SCSB and RFA conducted the assays. GDOF and FFW analyzed the photo-derived data. LMCC isolated and characterized the polysaccharides. MHA contributed to the experimental design. KN and LCF assisted in the analysis and discussion of the results. FI was involved in the experimental design, and data analysis and interpretation. APB and FI confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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