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Review article

Pectin a multifaceted biopolymer in the management of cancer: A review

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ABSTRACT

This review article focuses on the multifaceted roles of pectin in cancer management, namely as an oncotherapeutic delivery vehicle and a pharmacological agent. Over the past decades, the potential of pectin as a novel therapeutical agent for the prevention and/or management of cancer has gained increasing interest. Pectin has been found to modulate different mechanisms involved in the onset and progression of carcinogenesis, such as galectin-3 inhibition, caspase-3-induced apoptosis, and autophagy. Elucidating the structure-activity relationship provides insight into the relationship between the structure of pectin and different mechanism/s. The bioactivity of pectin, with respect to its structure, was critically discussed to give a better insight of the relationship between the structure of the extracted pectin and the observed bioactive effects. The rhamnogalacturonan I part of the pectin chain was found to bind to galectin-3, associated with several cancer hallmarks. The anti-inflammatory and antioxidant potential of pectin were also described. The roles of pectin as a treatment enhancer and a drug delivery vehicle for oncotherapeutics were critically defined. The scientific findings presented in this paper are expected to highlight the potential and role of pectin recovered from various plant sources in preventing and managing cancer.

1. Introduction

Cancer also referred to as neoplasm or malignant tumour, is characterized by the abnormal and uncontrollable growth of cells. Cancer pathophysiology is complex and involves a myriad of regulatory mechanisms. Key factors contributing to cancer progression encompass the activation of oncogenes, the inactivation of tumour suppressor genes, dysregulated cell cycle control, resistance to apoptosis, promotion of angiogenesis, evasion of the immune system, and the eventual occurrence of metastasis. This intricate interplay of molecular and cellular events underpins the challenges associated to the development of effective therapeutic strategies. Cancer can manifest in nearly any organ or tissue within the body and has the potential to invade adjacent structures. Metastasis, the process by which cancer cells spread to distant sites, stands as the foremost cause of mortality associated with cancer. The most prevalent cancers are breast, lung, colorectal, and prostate cancers [1]. Every type of cancer requires a particular treatment regimen, including radiotherapy, chemotherapy or surgery, or a combination of these modalities [2]. The complexity of cancer pathophysiology, the multifactorial nature of tumour drug resistance, and the toxic effects have contributed to the lack of effective

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oncotherapeutics [3]. Hence, the quest for novel effective oncotherapeutics and the application of novel drugs in cancer treatment is ongoing.

Significant insight regarding the bio-applications of pectin has emerged over the last decades. This has created new and unforeseen opportunities for developing this uniquely complex biopolymer beyond its traditional use as a food hydrocolloid [4]. Pectin has been used to develop wound dressings, controlled drug delivery systems, and scaffolds for tissue regeneration (Table 1). The application of pectin in wound dressing is supported by its non-toxic, biocompatible, polyanionic, and hydrophilic nature [5] while its use in controlled drug delivery systems is due to its ability to evade digestion in the upper gastrointestinal tract and undergo hydrolysis by pectinolytic enzymes produced by the colonic microflora [6]. Literature has shown that over the past decades, there has been a paradigm shift towards the inherent anticancer properties of pectin [7]. A clinical study using citrus pectin modified by hydrolysation was conducted on 49 patients suffering from advanced solid tumours. The findings revealed that the condition of 34.8 % of the patients was stable following the administration of 5 g of modified (hydrolysed) citrus pectin (three times per day) for 56 days [8]. Moreover, following a 16-week treatment, a 50 % reduction in prostate-specific antigen level and a significant decrease in pain were reported in a patient with metastasized prostate carcinoma [8]. Modified citrus pectin (PectaSol®) was given to 59 patients (4.8 g thrice daily for six months) with relapsed prostate cancer. After six months, 78 % of the patients responded to the therapy and none developed grade 3 or 4 toxicity [9].

The extensive number of scientific articles reporting the recovery of pectin from different sources, including agro-industrial waste highlights that these natural resources represent potential sources of pectin which could be capitalised. Moreover, since pectin is a heterogenous polysaccharide, it has been postulated that the biological activity of pectin recovered from different sources might differ due to structural differences. This manuscript is very timely to review and consolidate the existing scientific evidence on the anticancer potential of pectin isolated from different materials to better understand the mechanisms behind these potential benefits. In this review article, pectin is presented as a "multifaceted" biopolymer in the management of cancer. The structure-activity relationship of extracted pectin with respect to specific mechanisms, such as galectin-3 inhibition, caspase-3-induced apoptosis, and autophagy, are discussed to give a better insight into the relationship between the structure of the extracted pectin molecule and the observed bioactive effects. Besides, this review aims to highlight the potential of pectin in the management of cancer and encourages future studies involving organoid tumour models, as well as preclinical and clinical investigations to confirm their relevance against human cancer.

2. Methodology

2.1. Study design

A comprehensive review study design was adopted to gather scientific information regarding the role of pectin in the management/prevention of cancer, its use as an adjuvant in cancer management, and its role as a delivery vehicle in the treatment of cancer.

2.2. Search strategy

A comprehensive search of PubMed and Scopus was done using the following key terms "pectin", "oncotherapeutics", "cancer", "antioxidant", and "anti-inflammation".

2.3. Inclusion and exclusion criteria

Studies published in languages other than English were not considered. Only articles published in the year 2000 onwards were considered. There were no restrictions on the journal impact factor.

Table 1
Pharmacological applications of pectin.

Pharmacological application	Technology implemented	References
Wound dressings	Incorporation of reactive oxygen species-responsive substance, namely zeolite imidazolate framework-8 nanoparticles, encapsulated in injectable hydrogel composed of sodium alginate and pectin, cross-linked using calcium chloride.	[10]
	Pectin-gelatin matrices loaded with aloe vera and curcumin	[11]
	Drug release profile of theophylline-loaded pectin-based hydrogels	[12]
Controlled drug delivery	Pectin/chitosan hydrochloride submicron particles prepared by spray drying coupled with continuous feeding ultrasonic atomizer.	[13]
	Delivery system for neohesperidin made from pectin-chitosan conjugated nanoliposome.	[14]
	Pectin nanoparticle loaded with doxorubincin.	[15]
	Pectin crosslinked with lactic acid while methacrylic acid for the colonic delivery of oxaliplatin.	[6]
Scaffold production	Pectin coated on titanium alloy scaffolds promoted human bone marrow stromal cell proliferation.	[16]

3. Molecular structure of pectin

In the plant cell wall, pectin is associated with hemicelluloses and cellulose microfibrils. Pectin comprises of D-galacturonic acid residues linked at α-1,4 positions, forming a backbone [17]. Different neutral sugars then branch with the D-galacturonic acid residues backbone [4]. Acetylated or methyl-esterified galacturonic acid monomers comprise 70 % of the pectin molecule [18]. The pectin chain can be divided into two regions based on its structural composition, namely, the smooth and hairy regions (Fig. 1). Homogalacturonan, consisting of approximately 100 galacturonic acid monomers, is linear and corresponds to the smooth region of the pectin molecule. Homogalacturonan, which might be partially methyl esterified at the C-6 carboxyl or acetylated at *O*-2 or *O*-3, makes up about 65 % of the pectin molecule [17]. Rhamnogalacturonan-I, xylogalacturonan, and rhamnogalacturonan-II, possessing side chain groups, are referred to as the "hairy regions". Rhamnogalacturonan-I (20–35 % of the pectin molecule) consists of a backbone of galacturonic acid units interrupted by L-rhamnose [-α-D-galacturonic acid −1,2-α- L-rhamnose -1-4-]*n*. Approximately, 20–80 % of L-rhamnose residues in the rhamnogalacturonan-I backbone have side chains composed of individual, linear, or branched α-L-arabinose and β-D-galacturonic acid residues [17]. Rhamnogalacturonan-II backbone consists of at least 8 α-1,4-linked galacturonic acid residues attached to polymeric side chains. The polymeric side chains consist of 11–12 glycosyl residues and some rare sugars, such as 2-*O*-methyl xylose, 2-*O*-methyl fucose, 2-keto-3-deoxy-D-manno octulosonic acid, 2-keto3-deoxy-D-lyxo heptulosaric acid, and acetic acid [19]. Rhamnogalacturonan-II makes up approximately 10 % of the pectin molecule and has the highest structural complexity due to the glycosyl residues linked together by, at least 22 different glycosidic bonds [20]. For xylogalacturonan or apiogalacturonan (less than 10 % of the pectin molecule), a β-glycoside bond can substitute xylose or apiose at the C-2 or C-3 of galacturonic acid backbone [18]. Usually, the proportion of xylogalacturonan is close to that of rhamnogalacturonan-II [20].

4. Anticancer potential of pectin

Cancer, characterized by the abnormal and uncontrollable growth of improperly differentiated cells, can occur in almost any organ or tissue of the body, and eventually spread to other parts of the body [22]. In 2020, nearly 10 million deaths related to cancer were reported, making cancer a major contributor to disease burden worldwide [23]. Despite medical advances over the last decade, cancer continues to pose significant public health and economic problems globally [1] due to its constant shape-shifting heterogeneity and adaptability [2,24]. The malignant nature of cancer cells is related to the accumulation of growth-accelerating mutations and the loss of tumour suppressor functions [24]. A typical human cancer cell contains dozens of mutations, so the notion of curing cancer with a "single bullet" is not realistic. Gene mutations in cancer are dynamic and involve complex molecular alterations of critical growth-controlling genes within each cancer cell, creating diverse, often drug-resistant, sub-populations of cells. As such, each cancer cell differs from the other since it acquires its own set of mutations. This trait underpins the malignant and highly heterogeneous behaviour of neoplastic diseases.

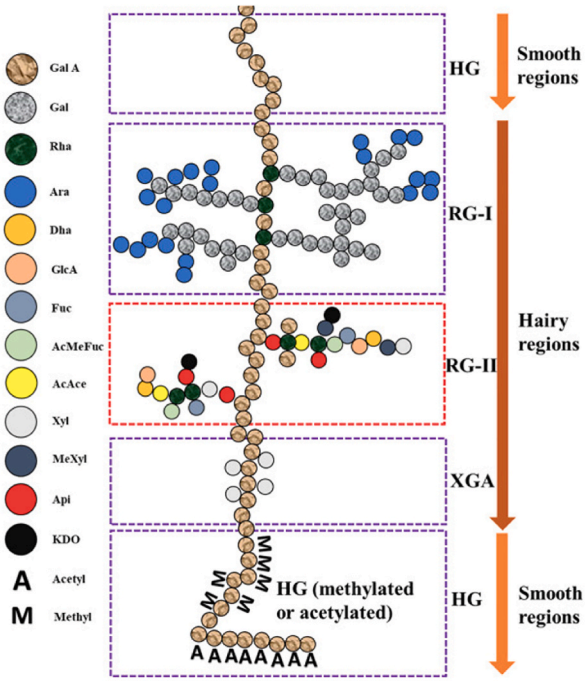

Fig. 1. Pectin molecule structure showing the galacturonic acid backbone, homogalacturonan; rhamnogalacturonan-I (RH-I); rhamnogalacturonan-II (RH-II); xylogalacturonan (XGA) [20,21].

Table 2
Anticancer activities of pectin extracted from different sources.

Pectin source	Pectin physicochemical characteristics	Cell line	Findings	Structure activity relationship	Reference
Ripe papaya pulp	Uronic fraction, Degree of esterification: 80 %, molecular weight: 127.7 kDa	Human colon cancer cell line (HCT-116)	Dose-dependent viability, galectin-3 knockout in HCT-116 cells highlighted the interaction between galectin-3 and ripe papaya pectin in reducing HCT-116 viability.	Pectin samples rich in homogalacturonan and having high degree of esterification values and lower molecular weight were the most potent in decreasing HCT-116 cell viability. Direct galectin-3 inhibition is one mechanism by which papaya pectin fractions decreased HCT-116 viability.	[49]
Pulp of papaya at intermediate ripening time point	Degree of esterification: 48 %, rich in lower molecular weight polysaccharides	Human colon cancer cell lines (HCT116 and HT29)	Dose-dependent viability of HCT116 and HT29, reduced cell proliferation, inhibited galectin-3-mediated hemagglutination at a concentration of 6 μg/mL.	Low molecular weight pectin and an increased proportion of ramifications, i.e., decreased galacturonic acid:rhamnose ratio, appear to facilitate structural interaction with both galectin-3 and cancer cells.	[50]
Citrus	Pectin was ultrasonicated Degree of methoxylation: 36.66 %, degree of acetylation: 1.56 %, molecular weight: 240.11, kDa	Human colon cancer cell line (HT-29 cell)	Dose-dependent viability, citrus pectin modified by ultrasound or pectinase improved the anticancer activity of pectin.	Higher galactose content in pectin hydrolysates improved inhibitory activity against HT-29 cells.	[89]
	Heat-fragmented citrus pectin was prepared from citrus pectin which is mainly composed of homopolygalacturonic acid	Human hepatocarcinoma (HepG2) and human lung carcinoma (A549) cells	No DNA cleavage was noted, meaning that cell death differed from classical apoptosis, heat-modified citrus pectin could have induced caspase-independent cell death since *Z*-VAD-fmk, a pan-caspase inhibitor, did not affect cell death in HepG2 and was partially protective in A549, increase in phosphatidylethanolamine-conjugated LC3 protein and a reduction in p62 protein were observed, indicating the activation of autophagy.	NR	[73]
	Irradiated and dialysed citrus pectin, Gamma irradiation: 20 kGy, molecular weight: 10 kDa, LMP (methoxy content 9.0 %)	Murine melanoma (B16F10), human lung carcinoma epithelial (A549), human colon cancer (HT29), human melanoma (SKMEL) cells	Inhibited the growth of all cell lines except A549.	NR	[90]
Orange peel	De-esterified (alkaline hydrolysis) and irradiated (25 kGy) pectin	Human epidermoid laryngeal cancer cells (HEp2) and normal African green monkey kidney cells [91]	Mortality rate of HEp2 cells reached 95 % when treated with pectin at a concentration of 5 mg/mL, the same concentration exhibited lower mortality rate (41 %) with VERO cells.	NR	[31]
Potato	Rhamnogalacturonan I Molecular weight: 42 kDa	Human colon cancer cell line (DLD1)	Dose- and time-dependent reduction in the proliferation of DLD1 cells, induced the detachment of cells, reduced expression of the adhesion molecule ICAM1.	Homogalacturonan segments in rhamnogalacturonan I and neutral sugar side-chains were essential for bioactivity.	[62]
	RG-I domain-enriched pectin	Human colon cancer cell line (HT29)	Inhibited the proliferation of HT-29 cells, induced significant G2/M cell cycle arrest, down-regulated expression of cyclin B1 and cyclin-dependent kinase 1.	NR	
Sugar beet	Pectin was subjected to an alkali treatment Molecular weight: 419 kDa, Degree of esterification: 18 %, degree of acetylation: 8.8 %,	Human colon cancer cell lines (HT29 and DLD1)	Modified pectin (1.0 mg/mL) significantly ($p < 0.001$) reduced HT29 cell proliferation (20.7 %), which was related to an induction of apoptosis without cell cycle arrest.	Enzymatic removal of galactose and arabinose (to a lesser extent) decreased the antiproliferative effect on HT29 and DLD1 cells indicating that the neutral sugar-containing RGI regions are important for pectin bioactivity.	[92]

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Table 2 (*continued*)

Pectin source	Pectin physicochemical characteristics	Cell line	Findings	Structure activity relationship	Reference
Sugar beet pulp	Degree of esterification: 97 %,	Human breast cancer cell line (MCF-7)	Sugar beet pectin (12.5–25 mg/mL) killed 80 % of the breast cancer cells.	Suppression of galectin-3 activity by α,β-galactoside of sugar beet pulp pectin.	[93]
Swallow root	Low molecular weight oligosaccharide isolated pectic polysaccharide, rhamnogalacturonan I type, bearing arabinogalactan side chain with β-D-(1 → 4) galactose along with α-L-Araf (1 → 5)-α-L-Araf (1 → 3) structure on α-D-GalAOAc-(1 → 2)-α-L-Rha-(1 → 4)- linear backbone.	Mouse melanoma cells (B16F10)	Isolated oligosaccharide showed an inhibition of 34, 50, and 62 % on the proliferation of B16F10 cells at 20, 40, and 80 μg/mL, respectively. Treatment with isolated oligosaccharide showed a significant reduction ($p < 0.05$; 92 % and 59 %, respectively) in galectin-3 mRNA levels, survivin mRNA level was reduced by 78 % following treatment with 50 μg/mL oligosaccharide.	Isolated oligosaccharide inhibited galectin-3 mediated cancer progression, β-D (1 → 4) linked galactose was the specific sugar for galectin-3.	[51]
Pectasol-C modified citrus pectin	NA	Human ovarian cancer cell line (SKOV-3)	Treatment with 0.1 % PectMCP showed significant G1 arrest (74 % versus 62 % for control, $p < 0.5$) and reduced the number of cells in G2/M phase (17 % versus 27.4 % for control, $p < 0.01$), 24 h treatment with Pect-MCP led to a 37 % decrease in cell viability.	NR	[79]
Olive by-product	Uronic acid: 5.80–45.43 g/100 g sample, molecular weight: 500 and 2 kDa	Human bladder tumour cell lines (non-muscle invasive-RT112) and squamous-SCaBER)	SCaBER cells were the most sensitive to pectin samples (80 % reduction in cell proliferation) as compared to RT112 cells (20–40 % reduction in cell proliferation), slightly suppressed galectin-1 and galectin-3 protein expression, galectin inhibitory activity was evaluated using the hemagglutination assay and olive pectin samples showed potent agglutination inhibition at a minimum inhibitory concentration of 4–8 mg/mL.	The presence of phenols (16.80–72.30 g/100 g sample) could be related to the antiproliferative activity and hemagglutination inhibitory activity.	[94]
Okra pods	Molecular weight: >100 kDa, moderately methyl esterified (24 mol methyl per 100 mol of galacturonic acid), highly acetyl esterified (78 mol acetyl per 100 mol rhamnose moieties)	Metastatic mouse melanoma cell line (B16F10)	The morphology of B16F10 cells was altered, cell proliferation was significantly reduced, cell cycle was arrested in G2/M and apoptosis, *N*-cadherin and α5 integrin subunit expression were reduced.	Rhamnogalacturonan I, with very short galactan side chains, induced apoptosis by interacting with galectin-3.	[81]
Apple pomace	Molecular weight: 419–899 kDa, galacturonic acid: 61.1–74.7 %, degree of methylation: 66.3–73.4 %	Human colon cancer cell lines (HCT 116 and Caco-2)	Reduced viability of cancer cells, induced apoptosis by activating caspase-3, enhanced intracellular ROS production, increased cytotoxic and proapoptotic effects of irinotecan, showing synergistic effect.	Ester-based cross-link within pectin and the high content of rhamnogalacturonan I regions (rich in neutral sugars) might be responsible for proapoptotic activity.	[70]
		Human colon cancer cell line (HT-29) and mouse melanoma cell line (B16F10)	Exhibited anticancer potential while being non-toxic to normal fibroblast cell (L929). Pectin samples at 1 mg/mL inhibited the adhesion (23.1 %), proliferation (40.4 %), invasion (76.9 %) and anchorage-independent growth (90 %) of HT-29 cells.	Binding of galactose residues and/or phenols to galectin-3 could be one possible mechanism inhibiting tumorigenesis.	[95]
Apple	NR	Human breast cancer cell line (MDA-MD-231)	Reduced cell attachment after 24 h and inhibited cell growth, blocked sub-G1 phase, apoptosis frequency increased after 24 h as indicated by annexin/PI and DNA fragmentation, induced caspase-dependent apoptosis.	Suppression of galectin-3 activity has been proposed as a possible mechanism of action.	[72]
	NR	Murine breast cancer cell line (4T1)	Pectic acid (0.1 %) induced apoptosis, inhibited cell growth, reduced cell attachment, blocked sub-G1 phase of cell cycle.	NR	[84]

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Table 2 (*continued*)

Pectin source	Pectin physicochemical characteristics	Cell line	Findings	Structure activity relationship	Reference
Sweet potato residues	Galacturonic acid: 70.03 %, Degree of esterification: 29.45 %	Human colon cancer (HT-29) and human breast cancer (Bcap-37) cell lines	Pectin (1 mg/mL) exhibited antiproliferation effects on HT-29 and Bcap-37 b y 46.64 and 42.64 %, respectively.	NR	[96]
Jaboticaba flour	Galacturonic acid: 95 %, rich in smooth-region homogalacturonans,	Human colorectal carcinoma (HCT-116)	Inhibited recombinant galectin-3 at 30 μg/mL, inhibited HCT116 cell viability in a dose-dependent manner.	Contain a large amount of galacturonic acid in their oligosaccharides, proving that the hydrolysed forms of homogalacturonans might have inhibited galectin-3.	[97]
Polygala tenuifolia dewaxed roots	Molecular weight: 116.0 kDa, sugar composition: arabinose, galacturonic acid, galactose, and rhamnose (63.5:8.3:8.4:19.8), backbone of alternate 1, 2,4-linked α-rhamnose and 1, 4-linked α- galacturonic acid, with branches of terminal (T) -, 1, 3-,1,4-, 1, 6- and 1, 3, 6-linked β-galactose, T-, 1, 5- and 1, 3, 5-linked α-arabinose substituted at C-4 of 1, 2, 4-linked α-rhamnose	Pancreatic cancer cell line (AsPC-1 and BxPC-3)	Induced pancreatic cancer cells apoptosis, Bcl-2 down-regulation, Bax up-regulation and conversion of caspase 3 to cleaved caspase 3, induced expression of autophagy-related markers Beclin-1, Atg5, and LC3B in BxPC-3 treated cells.	NR	[77]

NR: not reported.

The hallmark of cancer, coined by Hanahan and colleagues [25–27] nevertheless provides a logical framework for understanding the complex phenotypic and genotypic diversities of cancers. The biology of tumours does not relate to the traits of cancer cells only but also to the microenvironment contributing to tumorigenesis. Originally, they proposed that most and perhaps all cancers shared six essential alterations in functional capabilities, namely, self-sufficient growth signals, insensitivity to antigrowth signals, apoptosis, unlimited replicative potential, steady angiogenesis, and tissue invasion and metastasis [26]. In the course of progress in cancer research, new hallmarks and enabling characteristics were integrated into the concept [27,28].

Pectin was found to affect multiple rate-limiting steps in cancer progression, including, interaction with cell adhesion molecules, inhibition of anti-apoptotic function, and inhibition of cancerous cell proliferation. Most *in vitro* anticancer assessments of pectin have focused on colorectal cancer, which was classified as the third most common type of cancer in 2020, with 1.93 million new cases and 916, 000 deaths [29]. The efficacy of pectin to mitigate colorectal cancer (HCT-116, Caco-2, HT29, and DLD-1) *in vitro* supports its application in food systems. For instance, the development of functional foods enriched with pectin might open new avenues regarding the management of colorectal cancer. With the progression of scientific research regarding pectin, contemporary and emerging applications have been reported, spreading the possibilities for the application of pectin as an anticancer agent. As such, the anticancer properties of pectin have been assessed against cancerous cell lines not associated with the digestive system, including, human pancreatic cancer (BxPC-3) cells, human breast cancer (Bcap-37, MDA-MD-231) cells, human liver cancer (HepG2) cells, human bladder cancer (RT-112) cells, human ovarian cancer (SKOV-3) cells (Table 2). Purified pectin (57.7 % homogalacturonan and 42.0 % rhamnogalacturonan-I, with long neutral side chains) isolated from *Campomanesia xanthocarpa* Berg, a Brazilian Myrtaceae family species, showed cytotoxic effect on glioblastoma cells, with associated increase in the cellular reactive oxygen species (ROS) levels (48 h treatment) and no cytotoxicity on normal NIH 3T3 cells [30]. Chemically modified and irradiated orange peel pectin showed cytotoxic activity on HepG2 [31]. It was found that heat-modified citrus pectin contained 4,5-dihydroxy-2-cyclopenten-1-one which showed cytotoxicity against HepG2 by forming covalent adducts on cysteines in tubulin, thereby preventing microtubule formation and cell cycle [32]. 4,5-dihydroxy-2-cyclopenten-1-one was also reported to induce the attachment of ubiquitin to proteins, forming a multiubiquitin chain which triggered the degradation of the protein by proteasome [32].

4.1. Pectin with antioxidant and anti-inflammatory properties

Oxidative stress, the physiological state in which there is an imbalance between ROS and free radicals and antioxidants, is putatively recognised as a trigger of numerous pathologies, including cancer. Oxidative stress and inflammation are implicated in the different stages of carcinogenesis, including, initiation, promotion, and progression [33]. Oxidative stress is prominently known to damage DNA and dysregulate the complex cascade of cell signalling, thereby regulating cancer progression [34]. Hydroxyl radicals have been reported to bind to the DNA molecule, damaging the deoxyribose backbone, mainly by inducing strand cleavage and oxidation of purine and pyrimidine bases [35]. 8-OH deoxyguanosine formation markedly increases during DNA oxidation/mutagenesis and is a marker for early cancer detection [36]. Sustained oxidative stress has unanimously been related to the onset of chronic inflammation by activating several transcription factors, such as activator protein 1 (AP-1), nuclear factor kappa B (NF-κB), peroxisome proliferator-activated receptor gamma (PPAR-γ), β-catenin/Wnt, nuclear factor erythroid 2-related factor 2 (Nrf 2), and hypoxia-inducible factor 1-alpha (HIF-1α) [33,37]. Considerable evidence has shown that ROS are intimately related to chronic inflammation and cancer, for instance, tumour promoters can recruit inflammatory cells and trigger them to generate ROS [33]. During the two last stages of carcinogenesis, namely, promotion and progression, inflammation increases cell proliferative and angiogenic genes, growth factors and new blood vessel formation, all of which accelerate tumour growth [34]. Moreover, chronic inflammation in the tumour microenvironment, in the form of immune cell infiltration, causes further oxidative damage [34]. The role of oxidative stress in the activation of inflammatory pathways associated with cancer cell formation, survival, proliferation, and invasion, as well as angiogenesis, chemoresistance, and radioresistance, has been described [38].

Pectin has been reported to exhibit antioxidant and anti-inflammatory properties, making it a potentially interesting candidate in the prevention and management of carcinogenesis. Pectic oligosaccharides isolated from okra were found to inhibit lipopolysaccharide (LPS)-induced nitrite and inflammatory cytokines (interleukin (IL)-1β and IL-6) and inducible nitric oxide synthase/NF-κB signalling axis in RAW 264.7 cells [39]. Moreover, water-soluble polysaccharides, including pectin, extracted from okra were reported to possess antioxidant properties [40]. Citrus pectin (degree of esterification 60 % and 90 %) attenuated inflammatory response by inducing the inhibition of pro-inflammatory cytokine, IL-1β secretion and increasing the secretion of anti-inflammatory cytokines, IL-1ra and IL-10, in human peripheral blood cells [41]. The degree of esterification was reported to be an important parameter influencing the anti-inflammatory property of pectin. Citrus pectin possessing a degree of esterification of 90 % was found to significantly inhibit protein and mRNA expressions of inducible nitric oxide synthase and cyclooxygenase-2 in LPS-activated macrophages as compared to 30 % and 60 % esterified citrus pectin [42]. The latter was found to prevent the activation of NF-κB, known to induce the expression of various pro-inflammatory genes, IκB kinase [43], which is the main activator of NF-κB, and AP-1, which regulates the expression of inflammatory gene [44–46]. Besides, it was found that 90 % esterified citrus pectin could bind with LPS, thereby decreasing LPS's ability to bind to its receptor [42]. Administration of citrus pectin solution in mice-induced endotoxin shock reduced mesenteric lymph nodes and inflammatory cytokine mRNA levels in Peyer's patches and *ex vivo* analysis revealed the suppression of IL-6 secretion from $CD11c^{+}$ cells and from RAW 264.7 cells with activated Toll-like receptor [47]. Scientific evidence highlights the influence of the pectin chemical structure, namely, the degree of esterification, on the observed pharmacological activity. It was reported that citrus pectin and even degraded pectin suppressed IL-6 secretion but no activity was observed with the main constituent of the pectin backbone only, i.e., polygalacturonic acid [47]. Treatment of LPS-stimulated RAW 264.7 cells with pectin recovered from *Rubus chingii* Hu, a plant belonging to the Rosaceae family and a functional food, was associated with a significant reduction in the expression of

inflammatory factors, namely, mRNA level of tumour necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS), IL-1β, and IL-6 [48].

4.2. Pectin and galectin-3 inactivation

Several studies reporting the anticancer properties of pectin extracted from different raw materials with respect to different cancerous cell lines have highlighted the modulation of galectin activity [49–51]. The discovery of galectins in cancer development, progression and metastasis has been a major breakthrough in oncology [52]. Galectins are glycan-binding proteins, containing one or two carbohydrate domains. They mediate multiple biological functions including cell proliferation, migration, adhesion, and apoptosis [53]. The abnormal expression of galectin has been linked to the development, progression, and metastasis of cancers [54]. Girotti and colleagues [53] have scrutinised the impact of galectins 1, 3, 4, 7, 8, 9, and 12, on different cancer hallmarks and have reported that these galectins could either promote or impair cellular and molecular processes related to tumour growth and progression. Among the different galectins implicated in tumorigenesis, galectin-3 has been the most extensively investigated and reported in pectin anticancer studies. Besides, it appears that galectin-3 is associated with almost all the different cancer hallmarks [53]. Galectin-3 has been shown to upregulate the activity of the mitogen-activated protein kinase (MAPK) pathway, thus promoting tumour growth [55]. Galectin-3 was reported to induce the phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) of the MAPK pathway, which was translocated to the nucleus where it activated several genes and transcription factors related to cell proliferation, survival, and metastasis. Besides, MAPK pathway produced transforming growth factor alpha (TGF-α) which kept activating the pathway. Moreover, galectin-3 has been found to bind to αvβ3 integrins on endothelial cells, promoting focal adhesion kinase (FAK) phosphorylation which induced integrin clustering and the activation of fibroblast growth factor and vascular endothelial growth factor [56], thus promoting angiogenesis. Galectin-3 binds to integrins forming structures called focal adhesions which dynamically regulate cell migration. Since integrins have no intrinsic enzymatic activity they mediate signalling cascades by recruiting FAK, which also play a role in the assembly and disassembly of focal adhesions [57]. The destabilization of focal adhesions mediated by FAK reduces adhesion with the extracellular matrix, giving rise to nonadherent cells capable of migrating [58]. Although all galectins bind β-galactoside, they have a great ability to differentiate among carbohydrate structures due to their three-dimensional structures [59]. Therefore, binding of pectin to the carbohydrate-binding domain of galectin-3 neutralizes the latter's activity [60], thus hindering its involvement in tumour growth, angiogenesis, and metastasis (Fig. 2). Galectin-3 is involved in different steps in metastasis. For instance, it has been reported that galectin-3 protects cancer cells from anoikis (a programmed cell death that occurs upon detachment of cells from the correct extracellular matrix), mediates the adhesion of metastatic cell adhesion to the endothelium, promotes metastatic cancer cell clonogenic survival [59]. Interestingly, modified citrus pectin has been reported to modulate these rate-limiting steps in cancer metastasis by interacting with galectin-3 [61]. It has been proposed that galactan side-chains branched from rhamnogalacturonan-I (RH-I) could bind to galectin-3, implying that pectin with higher neutral sugar content could bind to galectin-3 better [62]. Moreover, modification of pectin-derived galactan side chains was found to facilitate binding with galectin-3, thereby inhibiting its role as a pro-metastatic regulatory protein [63]. As such, galectin-3 interacted specifically with β-D-galactobiose, the terminal disaccharide of the β(1 → 4)-D-galactan side chains within the RH-I regions of citrus pectin. Pectin isolated from the pulp of ripe papaya interacted with galectin-3 in human colon cancer cells and this mechanism was further confirmed upon galectin-3 gene knockout using the clustered regularly interspaced palindromic repeats (CRISPR)-Cas9 system (Table 2). Pectin samples rich in homogalacturonan and having a high degree of esterification and lower molecular weight were the most potent in decreasing human colon cancer cell viability. It was proposed that during ripening, endopolygalacturonases hydrolysed rhamnogalacturonans bound to the high molecular weighted esterified homogalacturonans, producing smaller size esterified homogalacturonans fractions [49]. Upon maturing, intrinsic enzymes act on pectin, producing lower molecular weight pectin molecules which can be absorbed by the body and the cells. Assessing the molecular structure of pectin at different maturity stages is therefore important in evaluating the ability of the pectin molecule to exhibit anticancer activity. Moreover, this might be a good strategy in order to avoid further modification of the pectin molecule.

It is noteworthy highlighting that the composition of the side chains and the degree of branching also depend on the plant source and the extraction method [64]. Substantial increase in the number of studies reporting pectin extraction from different plant sources using non-conventional extraction methods highlights the need for assessing the anticancer potential of pectin so-obtained [65–67].

4.3. Pectin and caspase 3 activation

Aberrant inactivation of cysteine-dependent aspartate proteases, notably caspases, plays a fundamental role in the onset and progression of cancer. Apoptosis initiation and execution are governed by the initiator caspases (caspase-2, -8, -9, and -10) and the effector caspases (caspase-3, -6, and -7) [68]. Caspase-3, the executioner caspase, is activated by both intrinsic and extrinsic pathways and can cleave multiple structural and regulatory proteins, critical for cell survival and maintenance [69]. Pectin has been reported to induce caspase-dependent apoptosis in cancerous cells [70–72]. Treatment of human breast cancer cells (MDA-MB-231) with pectic oligosaccharides from apples was found to cause the over-expression of caspase-3 [72]. Caspase-3 has been shown to cleave poly (ADP-ribose) polymerase (PARP), a DNA repair enzyme important in normal cell cycle (Fig. 3). As reported in Table 2, heat-modified citrus pectin cleaved PARP protein in A549 cells and HepG2 [73]. The cascade of apoptotic protein expression was observed in 1, 2-dimethylhydrazine-induced colon carcinogenesis in male Charles River-derived rats treated with citrus-pectin soluble fibre. Apoptosis in rat colonic tissue was related to the activation and initiation of protease cascade, including caspase-3 and perhaps other caspases, leading to the irreversible cleavage of downstream pro-apoptotic targets, such as PARP [74]. Pectin isolated from apple

pomace was reported to reduce the viability of cancerous cells by activating caspase-3 (Table 2). It was postulated that the ester-based cross-link within pectin and the high content of rhamnogalacturonan I regions might be responsible for proapoptotic activity [70]. However, the exact mechanism by which apple pomace pectin induced caspase-dependent apoptosis was not defined.

4.4. Pectin and autophagy

In tumorigenesis, autophagy can be regarded as a double-edged sword, inhibiting cancer-cell survival and inducing cell death, thereby suppressing tumorigenesis or promoting cancer-cell proliferation and tumour growth, thereby facilitating tumorigenesis [75]. The suppression of autophagy in cancer treatment is desired when it is associated with the deregulation of metabolism, modulation of the immune response, cancer stem cell promotion, and multidrug resistance [76]. The autophagic process is governed by a number of proteins. As described in Table 2, pectin-like polysaccharide isolated from roots of *Polygala tenuifolia* was found to suppress autophagy in pancreatic cancer cells (BxPC-3) by attenuating the expression of autophagy-related genes, namely, autophagy-related gene 5 (ATG5), Beclin-1, and microtubule-associated protein 1 light chain 3 (LC3) [77]. On the other hand, the activation of autophagy is regarded as another type of programmed cell death. Activation of autophagy in adenocarcinoma human alveolar basal epithelial cells (A549) by heat-fragmented citrus pectin corresponded to a reduction in p62 protein and an increase in phosphatidylethanolamine-conjugated LC3 protein [73]. The paradoxical dual role of autophagy in the stimulation of cell survival or the promotion of cell death necessitates further investigation. As such, further scientific studies are needed to decipher the circumstances under which autophagy will stimulate or eliminate cancer cells in order to facilitate the development of specific therapeutic strategies [76]].

4.5. Pectin and cell cycle arrest

Disruption of normal cell cycle can lead to cancer. Cell cycle consists of a highly orchestrated sequence of events that include phases associated with DNA synthesis and mitosis which are separated by gaps. As such, four discrete phases occur during cell cycle, namely, M phase or mitosis, G1 phase (gap 1) which is the interval between mitosis and initiation of DNA replication, S phase which is the period during which DNA replication takes place, G2 phase (gap 2) which is the period corresponding to the continuation of cell growth and synthesis of proteins in preparation for mitosis [22]. Agents capable of inducing cell cycle arrest have been advocated as anti-cancerous agents. Several drugs, namely, flavopiridol, indisulam, AZD5438, SNS-032, bryostatin-1, seliciclib, PD 0332991, and SCH 727965, targeting cell cycle have entered clinical trials. Administration of these drugs has produced modest results, but the combination with traditional cytotoxic chemotherapy was shown to improve cytotoxicity and suppress drug resistance. Studies summarised in Table 2 have revealed the potential of pectin to exhibit cell cycle arrest [78]. Modified citrus pectin (0.1 %) was reported to induce G1 arrest in human ovarian cancer cells (SKOV-3) [79]. In sharp contrast to apoptosis, cells which undergo growth arrest in the G1 phase remain viable with intact DNA [80]. RH-I-enriched pectin from potatoes was found to exhibit antiproliferative effect on HT-29 cells by inducing G2/M cell cycle arrest by downregulating cyclin dependent kinase 1 (CDK1) and cyclin B1 expression [62] (Fig. 4). Okra RH-I pectin containing an almost pure RH-I carrying very short galactan side chains was also shown to induce cell cycle arrest in G2/M of highly metastatic B16F10 mouse melanoma cells [81]. Treatment of human ovarian cancer cells (SKOV-3) with 0.1 % Pectasol-C modified citrus pectin revealed significant G1 arrest (74 % and control: 62 %, $p < 0.5$) and reduced the number of cells in G2/M phase (17 % and control: 27.4 %, $p < 0.01$).

4.6. In vivo studies

Isolated pectin has demonstrated anti-proliferative activity in experimental animal models. Adult male Sprague-Dawley rats administered modified citrus pectin (400 or 1200 mg/kg) on a daily basis in drinking water for 4 weeks showed an elevated level of caspase-3 and reduced glutathione and superoxide dismutase as well as a reduced expression of galectin-3 [82]. Findings gathered from this study substantiate previous *in vitro* studies, which suggested the possible induction of caspase-3-dependent cell death. On the other hand, six-week-old female C57BL/6 mice fed with 10 mg pectin/kg body weight daily, following the incubation of MC38 colon adenocarcinoma cells, indicated that pectin significantly enhanced the efficacy of monoclonal antibody targeting the programmed cell death protein 1, a novel immunotherapy drug [7]. Lewis lung carcinoma tumour-bearing mice (male C57BL/6 mice, 6–8 weeks old) were intragastrically treated with homogalacturonan (degree of esterification: 85.16 %, molecular weight: 4992 Da) isolated from *Hippophae rhamnoides* berry (200 mg/kg body weight) for 14 consecutive days. A significant decrease in the tumour volumes was observed. Treatment with homogalacturonan isolated from *Hippophae rhamnoides* berry augmented macrophage cytotoxic activity against tumour cells (48.67 %) and increased the macrophage secretion of NO (51.0 μM) [83]. Female BALB/c mice (4–5 weeks old) with 4T1 breast cancer tumours were treated with apple pectin solution (1 %) for three weeks. It was found that the apple pectin inhibited tumour progression by activating p53 which, in turn, activated apoptosis pathways and cell growth arrest [84]. The p53 tumour suppressor gene is central to activating apoptosis, and so is referred to as the guardian of the genome. Modified citrus pectin inhibited breast cancer development in 4T1-luc orthotopic and metastasis models by decreasing tumour-associated macrophages, found in hypoxia area of the tumour tissue and involved in metastasis [85]. Tumour-associated macrophages have been associated with the immunosuppressive tumour microenvironment by producing cytokines, chemokines, and growth factors [86]. Oral administration of a xylorhamnoarabinogalactan I pectic polysaccharide isolated from Bael (*Aegle marmelos* L.) to UV/DMBA induced skin carcinogenic mice downregulated galectin-3, ~5.6 fold, NF-κB, ~5.7 fold, vascular endothelial growth factor, ~4.1 fold and inflammatory markers - IL 10, ~5.7 fold, IL 17, ~6.6 fold [87]. An inhibition in growth was observed in sarcoma 180 tumours implanted

in Swiss female mice (25–30 g) following administration of pectin extracted from passion fruit for 7 days (10 or 25 mg/kg, intraperitoneally, and 50 or 100 mg/kg, orally). It was observed that passion fruit pectin treatment normalized the number of lymphocytes and neutrophils and did not affect the kidney and liver [88].

5. Pectin as a treatment enhancer

A number of studies have revealed the synergistic benefits of pectin, particularly modified citrus pectin, in cancer management [98–100]. Irinotecan, a widely used drug to treat colon cancer is rapidly transformed into an active metabolite, SN-38 [101], which is glucuronidated for excretion [102]. Gut bacterial β-glucuronidase removes glucuronic acid from glucuronidated SN-38, producing SN-38 which induces epithelial damage, resulting in bleeding diarrhoea and acute weight loss [103]. Apple pectin was found to enhance the cytostatic action of irinotecan and reduced the activity of β-glucuronidase, highlighting that apple pectin constitutes a promising candidate as an adjunct to irinotecan therapy that might alleviate its side effects, increasing its therapeutic efficacy [104].

The broad anticancer activity of honokiol, a lignan from *Magnolia officinalis* or magnolia bark, has been proven. Honokiol acts by regulating several signalling pathways, such as the induction of G0/G1 and G2/M cell cycle arrest by regulating cyclin-dependent kinase (CDK) and cyclin proteins, epithelial-mesenchymal transition inhibition by downregulating mesenchymal markers and upregulating epithelial markers, cell migration suppression and invasion by downregulating several matrix-metalloproteinases (activation of 5′ AMP-activated protein kinase (AMPK) and kisspeptin (KISS)1/KISS1R signalling), cell migration, invasion, and metastasis inhibition, anti-angiogenesis activity induction by downregulating vascular endothelial growth factor [98]. A combination of modified citrus pectin and honokiol (9:1) was found to produce a greater reduction in inflammation as compared to these two agents individually and the formulation has been patented [99,105]. The combination of honokiol and modified citrus pectin caused a synergistic effect corresponding to the enhanced inhibition of tumour necrosis-α, NF-κB activity, cyclooxygenase-II activity, and lipid peroxidation in LPS-induced mouse monocytes [100].

Human SKOV-3 ovarian cancer cells treated with a combination of paclitaxel (100 nM), an anticancer drug originally isolated from the bark of the Pacific yew, and PectaSol-C modified citrus pectin (0.1 %), revealed synergistic cytotoxic effects. The combined formulation reduced cell viability (75 %) and increased caspase-3 activity by 3.9-fold [79]. The enhancing cytotoxic effect of PectaSol-C modified citrus pectin, as a specific competitive inhibitor of galectin-3 on paclitaxel was confirmed in another study. Later Hossein and colleagues [106] described the role of galectin-3 in paclitaxel resistance through signal transducer and activator of transcription 3 (STAT3) activation in monolayer ovarian cancer cells and multicellular tumour spheroid ovarian cancer cells (SKOV-3). PectaSol-C modified citrus pectin combined with paclitaxel killed multicellular tumour spheroid ovarian cancer cells through the

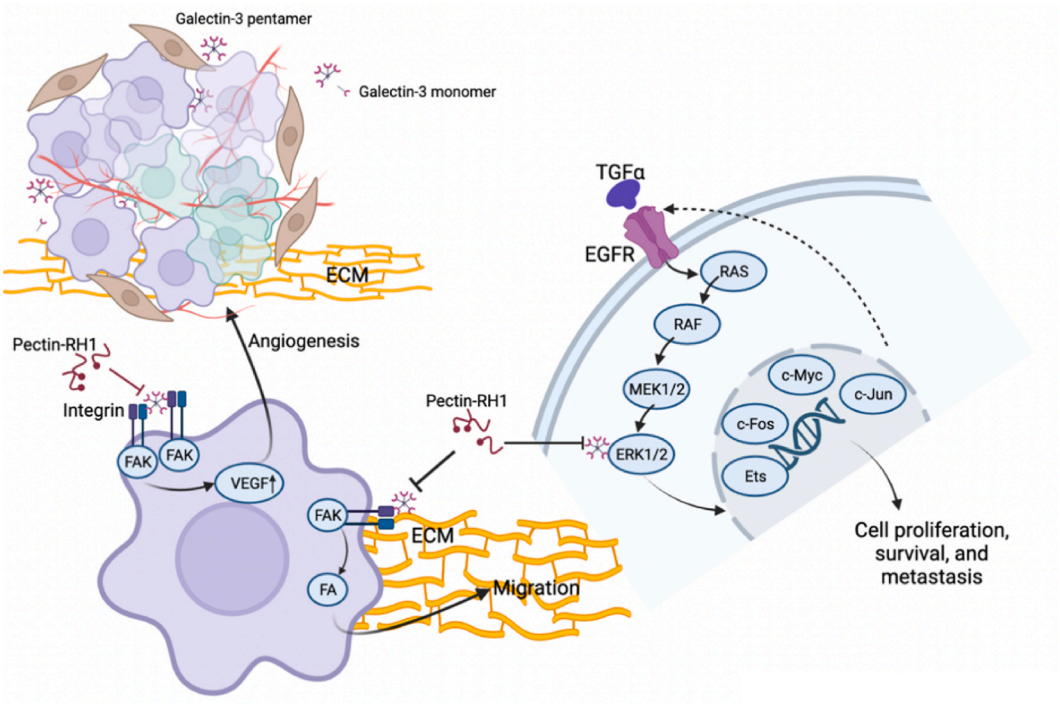

Fig. 2. *The role of galectin-3 in tumour growth, angiogenesis, and metastasis and its inhibition by pectin.* This figure represents the role of galectin-3 in the phosphorylation of ERK1/2 of the MAPK pathway which is involved in cell proliferation, survival, and metastasis; how the MAPK pathway produces TGF-α which keeps activating the pathway; binding of galectin-3 to αvβ3 integrins on endothelial cells, which promotes FAK phosphorylation, inducing integrin clustering and activation of the vascular endothelial growth factor (VEGF), promoting angiogenesis; binding of galectin-3 to integrins forming structures called focal adhesions (FA) which reduce adhesion with the extracellular matrix, promoting metastasis.

abrogation of STAT3 activity [106].

The combined effect PectaSol-C modified citrus pectin and polybotanical compounds, namely, BreastDefend and ProstaCaid, was found to inhibit the adhesion and migration of highly invasive human breast cancer cells (MDA-MB-231) and prostate cancer cells (PC-3) and suppressed secretion of urokinase plasminogen activator, which is associated with cancer metastasis by inducing cancer cell adhesion, migration, and invasion [107]. The synergistic effect of PectaSol-C modified citrus pectin and doxorubicin, a broad-spectrum anticancer agent, was reported. The combination of PectaSol-C modified citrus pectin and doxorubicin resulted in a steep decrease in IC_{50} values for prostate cancer cell lines (DU-145: 1.5-fold and LNCaP: 1.3-fold). The combination treatment led to an increased in G_2/M arrest in LNCaP cells and increased p53, p27 and B-cell lymphoma-2 (Bcl-2) expression while an increase in sub-G_1 arrest and a reduction in p27 gene and protein expression were observed in DU-145 cells [108]. PectaSol-C modified citrus pectin was combined with radiotherapy, a conventional treatment modality used for the localized treatment of cancers, and was found to sensitize human prostate cancer cells (PC-3, DU-145, and Cl-1) to ionizing radiation by downregulating galectin-3, modulating DNA repair pathways, and increasing ROS production [109].

Chemoresistance remains a major clinical hurdle in cancer treatment. The combination of modified pectin with different anticancer agents represents an interesting strategy to overcome drug resistance in cancer patients. It has been postulated that the overexpression of galectin-3 decreased the sensitivity of cancer cells to chemotherapeutic drugs, thereby suppressing apoptosis. By acting on galectin-3, modified citrus pectin has been found to sensitize malignant endothelial cells to doxorubicin and decrease their proliferation [110]. Modified citrus pectin (GCS-100) reversed the resistance of multiple myeloma cells to bortezomib and enhanced their response to dexamethasone by inhibiting the anti-apoptotic function of galectin-3 [111]. Activation of calpain, a calcium-dependent protease, has been found to mediate apoptosis in prostate cancer cells by cleaving androgen receptors into androgen-independent isoforms. Inhibition of galectin-3 by modified citrus pectin enhanced calpain activation. Wang and colleagues demonstrated that calpain activation through galectin-3 inhibition using modified citrus pectin sensitized prostate cancer cells to cisplatin [112].

6. Pectin in anticancer drug delivery

Conventional administration of chemours by intravenous injections was reported to provoke severe side effects in cancer patients. An alternative to reduce systemic adverse effects as well as increase drug availability at tumour site is the development of targeted drug delivery systems, such as pectin-based drug delivery systems. The use of pectin as a drug delivery vehicle to treat colon cancer appears to be a promising strategy since pectin is hydrolysed at the level of the colon by colonic microflora but is not degraded by upper gastrointestinal enzymes [113]. A number of studies have demonstrated that pectin alone might be used to develop delayed-release formulations but on the other hand, the combination of pectin with other materials/polymers has shown successful colon-specific delivery of therapeutic agents [114]. Das [114] has published a comprehensive review on the ability of pectin-based multi--particulate carriers, including, calcium-pectinate, zinc-pectinate, pectin-polyethyleneimine, pectin-glutaraldehyde, pectin-chitosan, pectin-alginate, among other, to exhibit microbial/enzyme-triggered release of bioactive agents for the treatment of colon cancer. A number of patents have also been filled on pectin-based multi-particulate colon-specific delivery systems for colon cancer treatment, such as particles comprising a core (drug) and a coating for the core, where the coating comprises a mixture of polysaccharide (e.g., amylopectin) and a film-forming polymeric material (e.g., acrylate polymer) [114].

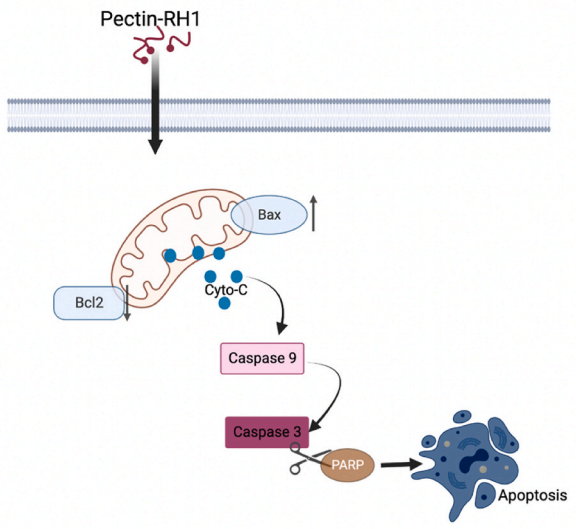

Fig. 3. *Pectin-induced caspase-3 dependent cell death.* Pectin has been reported to increase Bax/Bcl 2 ratio, and thus might decrease cellular resistance to apoptotic stimuli and enhance the release of cytochrome-C, necessary for the activation of caspases-dependent apoptosis. *In vitro* studies have revealed that heat-modified citrus pectin could cleave PARP protein, favouring apoptosis.

Nanoengineering of pectin gels has become an attractive domain of biomedical research to develop bioactive nanomaterials with innovative features for therapeutic applications. Pectin has gained enormous attention among other biopolymers due to its high availability in nature, non-toxic nature, and its mucoadhesive properties together with resistance to degradation by proteases and amylases.

With the advancement in nanotechnology, nanostructures, such as nanoparticles, nanocomplexes, nanocomposites, and nanogels, have gained increasing popularity as drug delivery carriers. These different nanostructures have been used in conjunction with pectin for the delivery of cancer drugs in the management of different cancers. For instance, the use of pectin as delivery vehicle in the treatment of pancreatic cancer has been reported. Modified pectin and tannic acid nanocomplexes were used to encapsulate anticancer drugs, namely, 5-fluorouracil, gemcitabine, and irinotecan. Cellular uptake studies demonstrated that modified pectin and tannic acid nanocomplexes successfully penetrated human pancreatic adenocarcinoma (HPAF-II) and carried the molecules of interest in a time and dose-dependent manner. No toxicity was observed for the blank nanocomplexes, implying that the modified pectin and tannic acid nanocomplexes were safe drug delivery vehicles [115]. Proliferation and colony formation assays revealed the anticancer potential of modified pectin and tannic acid nanocomplexes loaded with anticancer drugs against cancerous cells as compared to free drug treatments [115].

Pectin nanoparticles loaded with 5-fluorouracil, one of the most significant antineoplastic agents used to treat a variety of solid tumours, showed higher cytotoxicity on human liver cancer cells (HepG2) as compared to free 5-fluorouracil. *In vivo* evaluation of the pharmacokinetics on Sprague–Dawley rats showed that pectin nanoparticles loaded with 5-fluorouracil had a longer half-life in the circulation fluids as compared to free drug and biodistribution in healthy Kunming mice demonstrated that the loaded pectin nanoparticles possessed long circulation effect [116]. A core-shell structure pectin-eight-arm polyethylene glycol-ursolic acid/-hydrooxycampothecin nanoparticle was designed. The nanoparticle enhanced cytotoxicity and cellular uptake as compared to the free drugs. A higher survival was observed in 4T1 tumour-bearing mice when using this novel drug delivery system compared to rodents treated with free drugs [117]. Piperine-loaded nanostructured lipid carriers coated with pectin (citrus pectin, degree of esterification 35 %, galacturonic acid content 86 %, molecular weight 80 kDa) were found to exhibit enhanced cytotoxicity and cellular uptake in HepG2 cells. Pectin acted as an active targeting ligand selectively binding to asialoglycoprotein receptors which are overexpressed on the surface of hepatocellular carcinoma cells, thereby enhancing clathrin-mediated endocytosis and cellular uptake [118]. Moreover, *in vivo* assessment of liver tissues of mice bearing diethylnitrosamine-induced hepatocellular carcinoma confirmed that treatment with free piperine showed mild restoration of hepatic architecture, while encapsulated piperine showed noticeable restoration of normal hepatic architecture, reversion of dysplastic changes, and normalization of hepatocytes and nuclear size [119].

Nanogels are three-dimensional modifiable porous hydrogels consisting of nanoparticles [120]. Apart from enhancing drug solubility, absorption, controlled/sustained release, and drug accumulation, pectin-based nano-gels are biodegradable, biocompatible, water-soluble, and non-toxic [121]. Nano-gel loaded with methotrexate, an inhibitor of dihydrofolate reductase clinically used for the management of cancer, was developed from lysozyme-pectin nanoparticles. *In vitro* assessment revealed that the nano-gel showed enhanced apoptosis in HepG2 cells compared to free methotrexate due to more effective endocytosis [122].

Nanocomposite pectin scaffolds loaded with gemcitabine, a chemotherapeutic drug used in the management of ovarian cancer,

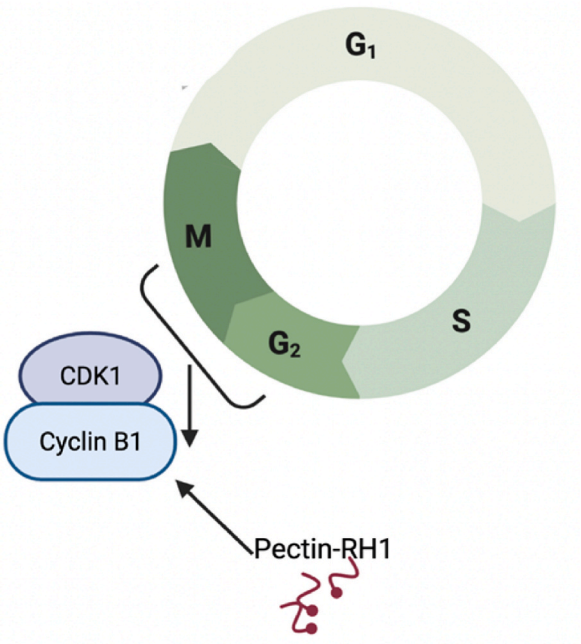

Fig. 4. *Pectin-induced cell cycle arrest*. RH-I-enriched pectin downregulated CDK1 and cyclin B1 expression, inducing G2/M cell cycle arrest.

were biocompatible with mammalian cell lines, showed cytotoxic and apoptotic responses against ovarian cancer cells *in vitro*, exhibited controlled and sustained release of gemcitabine [123]. The conjugation of pectin with graphene oxide nanomaterial has been advocated. The numerous oxygen holding functional groups of graphene oxide, such as carboxyl, hydroxyl, and epoxy are responsible for its enhanced drug-loading capacity and water-solubility. However, their poor dispersity in electrolyte solutions, agglomeration, and low colloidal stability limit their application in cancer therapy. Conjugation of graphene oxide nanocarrier with pectin was reported to enhance biocompatibility, drug loading and release performance [124].

The degree of esterification is an important physicochemical characteristic of pectin which might dictate its effectiveness to act as a delivery vehicle. As such, pectin can be classified as low methoxyl (degree of esterification $<$50 %) or high methoxyl (degree of esterification $>$50 %) depending on the ratio of carboxyl groups being in the methyl ester form. The degree of esterification of pectin will ultimately affect the development of gel delivery system. Low methoxyl pectin readily forms cross-linkages in the presence of divalent ions, such as calcium, resulting in the "egg-box" model which has a more stable structure and is commonly used for the development of pectin-based delivery system. The conversion of high methoxyl pectin to low methoxyl pectin using chemical, physical and enzymatic methods alone or in combination is possible. However, the conversion method might affect their performance as targeted drug delivery vehicles. Cai and coworkers [125] reported the difference in the release rate of curcumin-pectin calcium gel beads prepared from pectin which has been de-esterified by alkaline, enzymatic, and high hydrostatic pressure assisted enzymatic method.

7. Concluding remarks

Pectin isolated from natural resources, such as fruits, vegetables, and tubers can have multiple applications in cancer management. Since the focus has been on citrus pectin, particularly derived from orange, lime and lemon, there is a need for bioprospecting other agro-products as potential sources of pectin. The anticancer properties of pectin appear to be related to its molecular structure and more specifically to the RH-I region of the biopolymer chain. Considering that pectin is a highly heterogeneous biopolymer, perhaps the most heterogeneous one, assessing the anticancer properties of pectin extracted from different sources is crucial. The fast-growing number of scientific studies reporting the extraction of pectin from natural sources, particularly from agro-industrial waste, does not match the progress made on the assessment of the anticancer potential of recovered pectin. Indeed, it was reported that the structure of pectin, which depends on the extraction method, was decisive in its capacity to induce apoptosis [93]. The emergence of green chemistry and technologies adds another dimension to the extraction of pectin since the extraction technique might affect the molecular structure of recovered pectin, thereby influencing its anticancer properties. Therefore, reporting the extraction procedure is fundamental. Moreover, the purification of recovered pectin to reduce the levels of proteins and polyphenols effectively should be considered. To elucidate the structure-activity relationship, assessment of the physico-chemical properties, such as the degree of esterification, the molecular weight, and the structure of pectin, are important. Besides, the determination of the bioavailability of extracted pectin should be considered in future studies.

The complexity of cancer is undeniable. Cancer will be conquered only if cancers and their metastatic sub-populations receive individualised attention. Powerful technological innovations, that are just now emerging, are contributing to providing better insights into cancer pathobiology [24]. However, more studies assessing the anticancer effects of pectin extracted from different sources, three-dimensional cell culture models improving pre-clinical studies and bridging the gap between conventional cell culture and animal studies, are required to bring this idea from bench to bedside.

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Declaration of competing interest

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