Antiproliferative and palliative activity of flavonoids in colorectal cancer

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ABSTRACT

Flavonoids are plant bioactive compounds of great interest in nutrition and pharmacology, due to their remarkable properties as antioxidant, anti-inflammatory, antibacterial, antifungal and antitumor drugs. More than 5000 different flavonoids exist in nature, with a huge structural diversity and a plethora of interesting pharmacological properties. In this work, five flavonoids were tested for their potential use as antitumor drugs against three CRC cell lines (HCT116, HT-29 and T84). These cell lines represent three different stages of this tumor, one of which is metastatic. Xanthohumol showed the best antitumor activity on the three cancer cell lines, even better than that of the clinical drug 5-fluorouracil (5-FU), although no synergistic effect was observed in the combination therapy with this drug. On the other hand, apigenin and luteolin displayed slightly lower antitumor activities on these cancer cell lines but showed a synergistic effect in combination with 5-FU in the case of HTC116, which is of potential clinical interest. Furthermore, a literature review highlighted that these flavonoids show very interesting palliative effects on clinical symptoms such as diarrhea, mucositis, neuropathic pain and others often associated with the chemotherapy treatment of CRC. Flavonoids could provide a double effect for the combination treatment, potentiating the antitumor effect of 5-FU, and simultaneously, preventing important side effects of 5-FU chemotherapy.

1. Introduction

Colorectal cancer (CRC) is the most frequent type in the world population, with 1,931,590 new cases/year in 2020, leading to important patient comorbidities and high costs for health systems [1–3]. Patients usually enter monitoring intervention programs, where short- or medium-term periodic colonoscopies are recommended, as well as modifications in their dietary habits, including lower intake of saturated fat, alcohol, barbeque foods and cured meats (which contain recognized carcinogens, such as benzopyrenes and nitrosamines) together with higher intake of calcium, vitamin D, vegetables and fruits (which provide important preventive nutraceuticals, such as flavonoids and prebiotic fibers) [2,4,5].

CRC is a complex multifactorial disease, involving genetic mutations and epigenetic alterations, causing, after several decades of life, changes in genes involved in cellular differentiation or regulatory processes in colonocytes [6]. Initial stages in the development of this cancer take place at the colon mucosa crypts, involving the stem cells placed at the

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bottom of these crypts. Their altered cellular multiplication generates aberrant crypt foci (ACF), evolving towards microadenoma, then adenoma (polyp), and finally to adenocarcinoma and metastasis [7,8].

CRC treatment depends on the development stage. Relative survival (at 5 years) in stage 1 is 92.5%, but in stage 2C is only 58.8% [9]. Current treatment for first stages is usually surgery resection, with good prognosis, but with common relapses. In advanced stages, the most common treatments are chemotherapeutic agents, such as 5-fluorouracil (5-FU), capecitabine, oxaliplatin, irinotecan, anti-VEGF (vascular endothelial growth factor) antiangiogenic drugs (bevacizumab) and anti-EGFR (epithelial growth factor receptor) antibodies (cetuximab). Drug combinations (such as 5-FU, irinotecan and oxaliplatin, FOLFOX-IRI) are very common in these cancer treatments, as this allows a reduction in drug doses and expands patient survival. However, important side effects usually arise, such as mucositis, diarrhea, neurotoxicity and neutropenia [10–12].

Flavonoids are plant secondary metabolites with widespread presence in these living organisms, where they exert antioxidant, antifungal and antibacterial bioactivities, among others. These polyphenols show a C6-C3-C6 chemical structure [13]. All of them may have usefulness in the context of CRC, as antiproliferative agents, acting as sensitizers for cancer cells or reducing the oxidative stress caused by pharmacological drugs used in these treatments [14,15]. Moreover, flavonoids have properties that also might make them useful to counteract or prevent some side effects of chemotherapy, but this has been scarcely studied [16,17].

In this work, an in vitro study on the anti-proliferative effects of 5 flavonoids (the flavones apigenin and luteolin, the flavanones naringenin and eriodictyol, and the chalcone xanthohumol) on human normal colon fibroblasts and CRC cell lines has been carried out, in comparison with 5-FU treatment. For those three flavonoids with higher anti-proliferative activity, a combination treatment with 5-FU was also performed in the cancer cell lines. Finally, a review of the antitumor effects on cancer (and specifically on CRC) of these plant flavonoids has been carried out, as well as a review on the potential palliative effects of these important plant nutraceuticals for cancer treatment side effects.

2. Materials and methods

2.1. Cell lines

The selected human CRC cell lines HCT116 (epithelial morphology, carcinoma, primary, mutated in KRAS and PIK3CA), HT-29 (epithelial morphology, adenocarcinoma, primary, mutated in APC, BRAF, PIK3CA, SMAD4 and TP53) and T84 (epithelial morphology, adenocarcinoma, metastasis in lung, mutated in APC, KRAS, PIK3CA and TP53) were maintained in DMEM/F12 1:1 supplemented with 2 mM L-glutamine, 10% FBS, penicillin (100 IU/mL) and streptomycin (100 μg/mL). Similarly, the CCD-18Co fibroblasts cell line (ATCC, crl-1459) from human normal colon was maintained in α-MEM with 2 mM L-glutamine, 10% FBS, 1.5 g/L sodium bicarbonate, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, penicillin (100 IU/mL) and streptomycin (100 μg/mL). The cells were grown at 37 °C with 5% CO_2 in a humidified CO_2 incubator (Thermo Scientific 8000DH).

2.2. Cell viability assays

Each plate contained controls, and different concentration series with three to six replicates each. Cells were grown in a 96-well plate (HCT116: 5.5×10^3 cells/well; HT-29: 8.3×10^3; T84: 1.1×10^4; CCD-18Co: 5×10^3) for 24 h to 75% confluence until complete adherence. At that point, the flavonoids were added with the corresponding dilution to the culture plate. The extracts were resuspended in H_2O/0.1% DMSO and were further incubated for 48 h. All the extracts were filtered through a 0.22 μm filter for sterility. IC50 concentrations were calculated with Quest Graph™ IC50 Calculator.

Assayed concentrations were 0, 10, 20, 30, 40 and 50 μM for all compounds (the flavonoids, i.e., xanthohumol, luteolin, naringenin, eriodictyol and apigenin, as well as 5-FU) in the four cell lines; and 0, 20, 40, 60, 80 and 100 μM in the case of T-84 cell line for apigenin, naringenin and eriodictyol.

In the case of the combined viability assays, the different flavonoids were combined with 5-FU. For these assays, a fixed flavonoid concentration was added that was able to produce 30% cell death for the different cancer cell lines. In the case of HCT116, the corresponding concentrations were 13.63 μM for apigenin, 9.6 μM for luteolin and 5.95 μM for xanthohumol. In the case of HT-29, the corresponding concentrations were 55 μM for apigenin, 44.6 μM for luteolin and 19.3 μM for xanthohumol. Flavonoids were assayed in the normal (non-tumoral) colonic cell line at a concentration of 40 μM. These flavonoids concentrations were used in combination with different 5-FU concentrations (10, 20, 30, 40, 50 μM).

Cancer cell viability assays were carried out using the Neutral Red (3-amino-7-dimethylamino-2-methyl-phenazinehydrocloride) cell uptake method. The culture medium was removed, and 100 μL of Neutral Red (40 μg/mL, dissolved in culture medium) was added to each well of the plate and incubated at 37 °C for 2 h. After the incubation time, Neutral Red was removed, and the cells washed twice with 150 μL PBS. Finally, 150 μL of Neutral Red de-stain solution (1% acetic acid-50% ethanol) was added in order to extract the dye from the cells. Finally, after 10 min of agitation, the OD was measured at 560 nm, using a microtiter plate reader spectrophotometer (GLOMAX, Promega). Viability of normal cells was determined using the MTT method. Briefly, 10 μL of MTT solution was added in each well containing 100 μL of culture medium, to achieve a final concentration of 0.45 mg/mL. Cells were then incubated for 3 h at 37 °C. After this, medium was removed, and formazan crystals were dissolved with DMSO. Absorbance was read at 570 nm using a FLUOstar Omega microplate reader (BMG Labtech).

The results were analyzed with CalcuSyn software. In each case, the combination index (CI) was obtained, allowing the effect of these combinations to be classified as synergistic (CI value below 0.85), additive (CI value between 0.85 and 1.10) or antagonistic (CI value above 1.10).

2.3. Statistical analyses

Data was expressed as the mean value ± S.E.M. Comparison between compounds in tumor cell lines with respect to the control cells was performed using two-way ANOVA test with Dunnett's multiple comparisons test. Graph Pad Prism 9 (Graph Pad Software) was used for statistical analysis and graphical representation. Differences between groups were considered significant when p-value < 0.05. The statistical significances are indicated in the figures (*$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$; ****$p < 0.0001$).

2.4. Review studies

PubMed database was analyzed for the anticancer bioactivity data (mainly on CRC) using the keywords naringenin, apigenin, eriodictyol, luteolin and xanthohumol, together with cancer or with colon cancer; a total of 32 selected publications were reviewed.

In the case of palliative effects, the search keywords were intestinal transit, diarrhea, gastric ulcer, gastrointestinal toxicity, colitis, intestinal inflammation, intestinal mucositis, oral mucositis, neurotoxicity, pain and nociception; all of them were combined with each flavonoid keyword; a total of 17 selected publications were reviewed.

3. Results

3.1. Cell viability in vitro assays with flavonoids and 5-FU

In the in vitro experiments with the HCT116 carcinoma cell line,

naringenin showed no effect as an antitumor compound (Fig. 1). Erio-dictyol had a IC50 of 54.2 μM, apigenin 21.8 μM, luteolin 14.3 μM and xanthohumol 9.4 μM, even stronger as an antitumor drug than 5-FU (IC50 14.3 μM) in this cell line (Fig. 1). In the case of xanthohumol, 30 μM caused 97.9% cancer cell death in HCT116, whereas in CCD-18CO control cells, this flavonoid caused, at 30 μM, 68.4% cell death induction. A similar effect was observed with luteolin at 30 μM (88.95% cell death induction in HCT116, in contrast with 21.92% cell death in-duction in CCD-18CO control cells) and apigenin at 30 μM (67.08% cell death induction in HCT116, in contrast with 4.55% cell death induction in control CCD-18CO cells) (Fig. 1).

Compared with the HCT116 line, the tested flavonoids showed lower antitumoral activities, generally without statistical significance, in the case of the HT-29 adenocarcinoma cell line (Fig. 1). Here, naringenin, apigenin and eriodictyol had no effect at all. Luteolin caused cell death at a similarly low level as CCD-18CO control cells (Fig. 1). However, xanthohumol (IC50 31.1 μM) caused 96.3% cell death in HT-29 at 50 μM, and affected CCD-18CO control cells in a similar manner (91.7% cell death at the same concentration), without statistically significant differences. In this case, 5-FU (Fig. 4) (IC50 73 μM) induced 47.5% cell death at 50 μM in HT-29, similar to its relatively mild effects in CCD-18CO cells (42.3% cell death induction) (Fig. 1).

Metastatic T-84 cell line is more resistant than the two other CRC cell lines used in this study. In this cell line, the resistance to 5-FU is very high, as it can be observed in Fig. 2: at 10 μM the cell death was 27.5%, and above this concentration there was no further induction of cell death (Fig. 2). Apigenin and naringenin lacked antitumor activity in this cell line, with no effect even at 50 μM concentration (Fig. 2). Eriodictyol showed no statistically significant differences with respect to CCD-18CO control cells at 40 μM, inducing 28.5% cell death in T-84 (Fig. 2).

Luteolin (IC50 35.3 μM) has a statistically significant antitumor effect, in comparison with CCD-18CO normal cells, above 30 μM, causing 44.3% cell death at this concentration (Fig. 2). Finally, xanthohumol was the most active flavonoid on T-84 cell line, with an IC50 23.2 μM, and causing 92.8% cell death at 50 μM (Fig. 2), although its effect on CCD-18CO normal cells was quite similar (91.7% cell death) (Fig. 2).

Those flavonoids showing better antitumor activities (apigenin, luteolin and xanthohumol) were used in combination with 5-FU, in order to study potential synergistic effects on the adenocarcinoma (HT-29) and carcinoma (HCT116) cancer cell lines. The flavonoid concen-tration selected for these combination antitumor assays was the one causing 30% cell death in the corresponding cell line.

In the case of HCT116 cells, xanthohumol IC50 value in combination with 5-FU was 14.5 μM, but this combined treatment did not show statistically significant differences with respect to treatment with only 5-FU (IC50 14.3 μM). The best concentration point CI for this combined treatment was 1.3 (antagonistic effect) (Fig. 3). The combination ther-apies of luteolin and 5-FU (IC50 4.8 μM) or apigenin and 5-FU (IC50 1.2 μM) showed statistically significant differences with respect to single treatment with 5-FU (Fig. 3). For these two combinations, the CI were (at the best concentration point) 0.67 and 0.29 respectively, which indicate a synergistic effect in both cases.

On the other hand, the combination antitumor assays in HT-29 cell line showed statistically significant differences with respect to single treatment with 5-FU in most cases (Fig. 3). IC50 values were 18.7 μM (combination of xanthohumol and 5-FU), 16.2 μM (combination of luteolin and 5-FU) and 15.8 μM (combination of apigenin and 5-FU), in contrast to IC50 value of 73.0 in the case of 5-FU alone. CI for these three combinations were 0.92 (xanthohumol combination), 0.99 (luteolin combination) and 0.98 (apigenin combination), indicating an additive
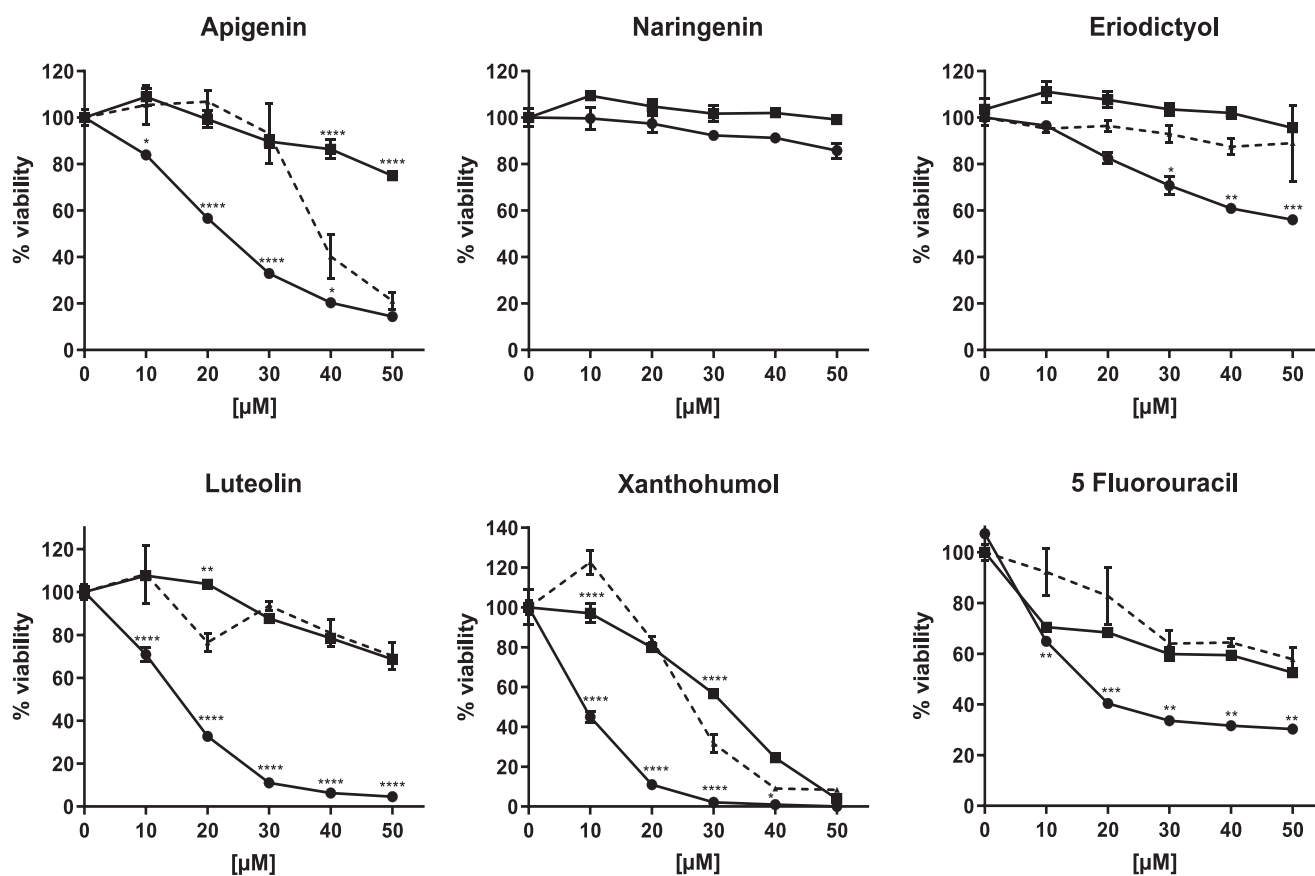

Fig. 1. Cell viability assays in the two CRC cell lines HCT116 (black circles) and HT-29 (black squares), as well as in the normal cell line CCD-18CO (dashed lines), treated with the five selected flavonoids or with the antitumoral drug 5-FU. Statistically significant results (compared with CCD-18CO control cells) for each con-centration point are indicated with asterisks.

Fig. 2. Cell viability assays in the metastatic T-84 (black circle) CRC cell line, as well as in the normal cell line CCD-18CO (dashed lines), treated with the five selected flavonoids or with the antitumoral drug 5-FU. Statistically significant results (compared with CCD-18CO control cells) for each concentration point are indicated with asterisks.
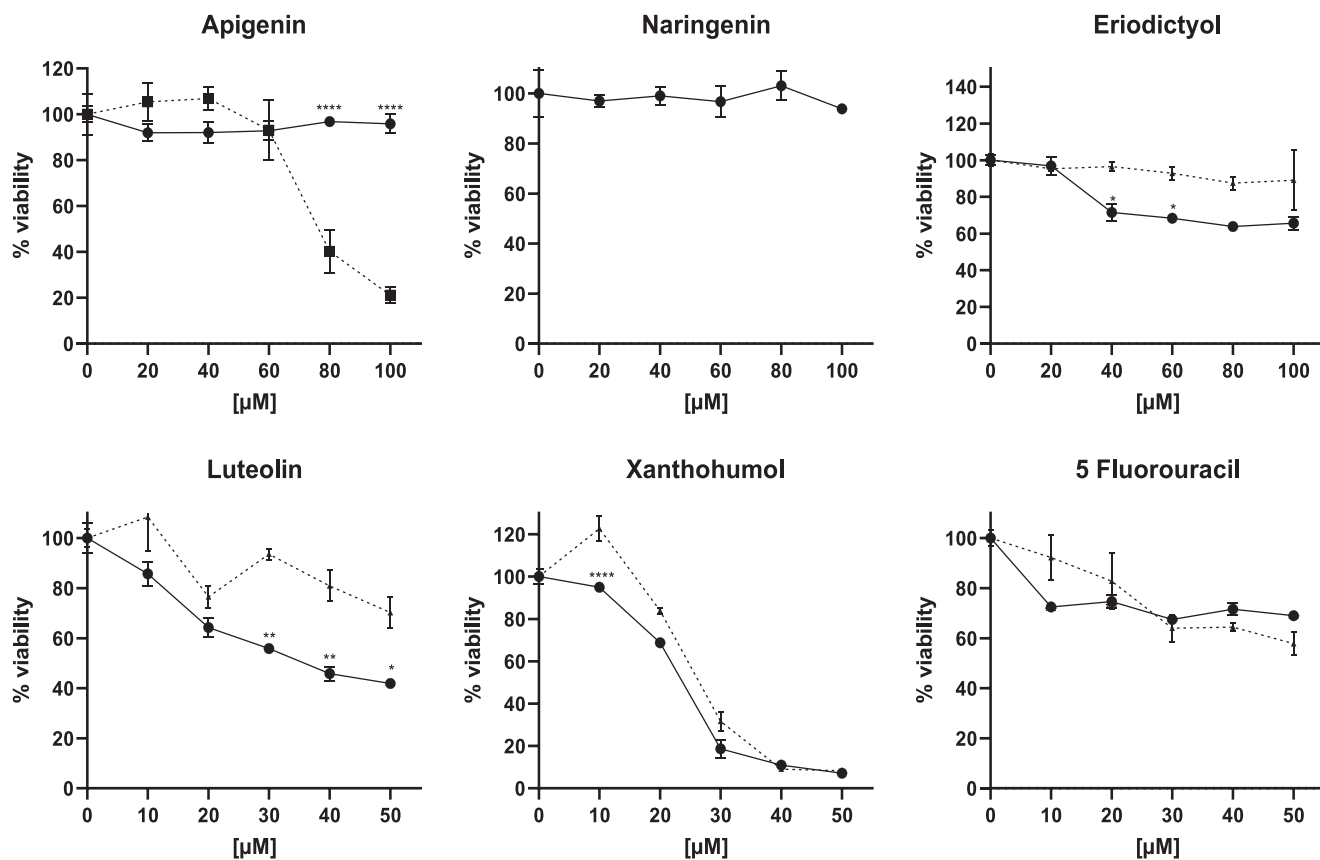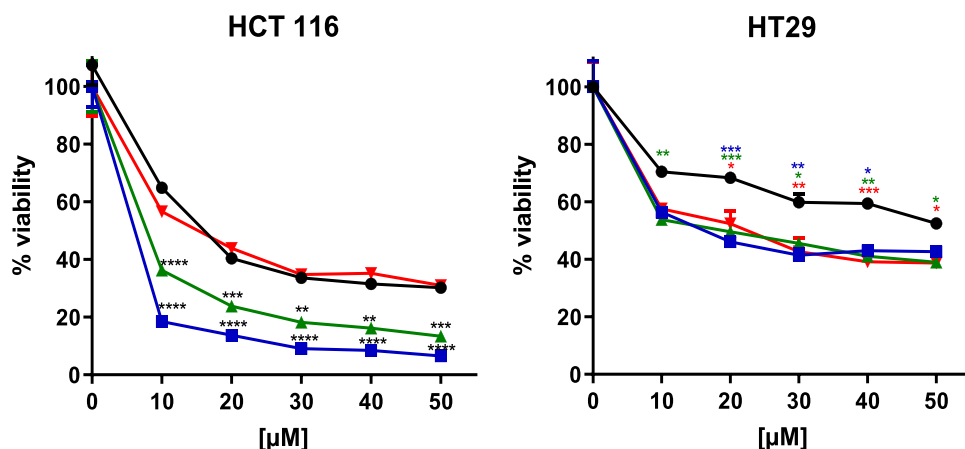

Fig. 3. Combinatorial therapy assays with only 5-FU (at 10, 20, 30, 40 and 50 μM, black circles), or with this compound plus the corresponding flavonoid concentration causing 30% cell viability reduction in two cancer cell lines: apigenin plus 5-FU (blue squares), luteolin plus 5-FU (green triangles), xanthohumol plus 5-FU (red inverted triangles). Statistically significant results (in comparison with 5-FU treatment alone) for each concentration point are indicated with asterisks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

effect in all three cases.

3.2. Antitumor effects of flavonoids in cancer

Apigenin (Fig. 4A) is a flavone present in parsley, oranges, olive oil, pistachios and several herbs (onion, rosemary, sage, oregano) [18]. In SKOV3 ovarian cancer cells, apigenin downregulates the expression of Mcl-1, inducing apoptosis in these tumor cells [19]. This plant metabolite is able to inhibit p38 activation (phosphorylation) in HCT116 CRC cell line, inhibiting the multiplication of these cells; and its inhibitory effects are also associated to the inhibition of the expression of NF-κB and Snail transcription factors, blocking the epithelial to mesenchymal

transition [20–22]. In a mouse model for CRC using azoxymethane as tumor inducer, apigenin antitumor effects were dependent on the presence of gut microbiota, in particular of several *Bacteroides* and *Actinobacteria* taxons, as depleting mice gut microbiota by using antibiotic therapy in these animals reduced the antitumor effects of this flavonoid [23].

Luteolin (Fig. 4B) is another flavone whose biological activities have been attributed to the hydroxyl moieties in rings A and B, and the 2,3 double bond in ring C [24]. Luteolin is present in human diet from different sources, such as olive oil, herbs (sage, thyme, rosemary) and other foods (lentils, pistachio, olives, artichokes) and has shown antineoplastic effects against multiple cancers, including leukemia,
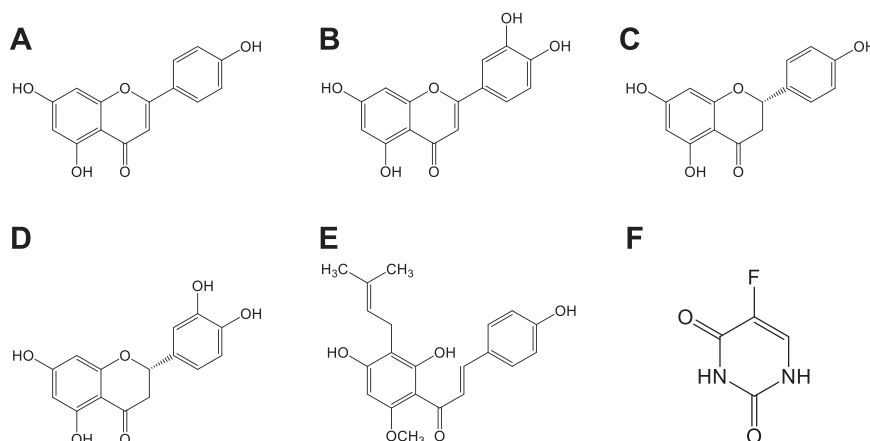
Fig. 4. Chemical structures of the flavonoids apigenin (A), luteolin (B), naringenin (C), eriodictyol (D), xanthohumol (E) and the chemotherapy drug 5-fluoruracil (F).

melanoma, as well as pancreatic, prostate, liver, breast, colorectal, lung, brain, and gastric cancer [18,25]. One of the mechanisms found to be involved in this antitumor role is its effect as pro-oxidant, sensitizing lung cancer cells to tumor necrosis factor (TNF)-triggered apoptosis, which is exerted by luteolin through reactive oxygen species (ROS)-mediated NF-κB suppression [26].

In a mouse model of liver cancer, the antineoplastic effect of luteolin has been attributed to its antiinflammatory function. In this animal model, intraperitoneal administration of luteolin decreased the levels of glutathione as well as inflammatory cytokines, including IFN-γ and IL-2 [27,28]. In HT-29 human CRC cell line, luteolin anti-oxidant effect reduced the mitochondrial membrane action potential, increasing Bax expression, inhibiting Bcl-2 titers and causing cytochrome C release to the cytoplasm and triggering apoptosis via caspase-9 and caspase-3. In HT-29 CRC cell line, luteolin reduced intracellular ROS through the activation of antioxidant enzymes like SOD (superoxide dismutase) and CAT (catalase), as well as through increasing levels of reduced glutathione. In this cell line, the apoptosis induction was due to activation of the AKT signaling pathway via JNK and p38 induction [29,30].

Naringenin (Fig. 4C) is a flavanone present in wine, grapefruit and almonds, among other common foods [18]. This flavonoid is able to induce apoptosis in LNCaP prostate cancer cells by activating AKT phosphorylation and increasing their sensitivity to ROS [31]. Its antitumor effects are also associated to the expression of estrogen receptors (ER) α or β in tumor cells, such as DLD-1 CRC cell line. This flavonoid induces apoptosis in these CRC cells via p38 activation (MAPK signaling pathway) and further stimulation of ATF3 transcription factor [32,33].

Eriodictyol (Fig. 4D), another flavanone present in diverse herbs (such as marjoram) and in foods (such as almonds and pistachios), possesses an extra hydroxyl group in B ring (in comparison with naringenin) and a 2,3-unsaturation in the C-ring, which are important for its beneficial biological properties [18,34]. Eriodictyol exerts multiple therapeutic effects including anti-oxidant, anti-inflammatory, anti-diabetic, anti-obesity, hepatoprotection, neuroprotection and cardioprotection [35]. This flavanone exerts antioxidant ability through the upregulation of antioxidative defenses. For example, this flavonoid is able to preserve mitochondrial function of human hepatocellular cancer cells (HepG2) by significantly decreasing the intracellular ROS and lipid peroxidation [36]. A different anticancer mechanism in another human lung cancer cell line has been also described, where induction of mitochondrial-mediated apoptosis and G2/M cell cycle arrest in addition with inhibition of m-TOR/PI3K/Akt signaling pathway were observed after treatment with eriodictyol [37]. Lipid peroxidation inhibition mediated by eriodictyol antioxidant activity was also observed accompanying a significant reduction in the number of polyps and ACF in a rat model of colon carcinogenesis, with enhanced SOD, CAT and reduced glutathione activities in the animals treated with eriodictyol [38].

Xanthohumol (Fig. 4E) is a chalcone found in the female inflorescences of hops (*Humulus lupulus*). About 4–14% of the dry weight of hops are polyphenols, xanthohumol being the most abundant compound among the prenylated flavonoids (85% of total content) [39]. The presence of xanthohumol in diet is mainly due to beer consumption, as hops are one of the main ingredients in beer brewing. The diet intake of this type of prenylated flavonoid is very low because xanthohumol is converted into the flavonone isoxanthohumol by thermal processes during brewing [40]. Therefore, dietary intake of xanthohumol would not be enough to support appropriate body levels for its bioactivity [39]. Pharmacokinetic studies have shown that after oral administration of xanthohumol, bioavailability of this compound is very low because about 80% of the xanthohumol that is consumed in the diet is excreted in the feces and urine [41,42]. Anti-inflammatory effects of xanthohumol have been described as being produced by the inhibition of cyclooxygenase 2 (Cox-2), which is responsible for the synthesis of prostanoids, involved in cellular inflammation processes. This inhibition is mediated by iNOS suppression, induced by LPS (lipopolysaccharide) [43]. In addition, antiangiogenic effects, which are essential to limit tumor metastasis, have been corroborated in in vitro assays. Xanthohumol is able to suppress the formation of microvasculature and reduce tumor extension [44].

In cancer cells, glycolysis (Warburg effect) is preferred as an energy source. The initial reaction is carried out by the mitochondrial HK (hexokinase) enzymes. Among them, the HK2 isoform is overexpressed in several cancers such as CRC, leading to higher resistance to apoptosis [45]. Xanthohumol is able to reduce HK2 protein levels in vitro. This decrease appears to be mediated by AKT inhibition, which is part of the epidermal growth factor receptor (EGFR) signaling pathway. Therefore, xanthohumol may act on the EGFR pathway by directly inhibiting AKT protein, leading to a reduction in HK2 levels that will block glycolysis and induce apoptosis in tumor cells [46]. In addition to this pathway, xanthohumol can act at different levels, either by overexpressing pro-apoptotic proteins such as Bax and different caspases (3, 6, 9) or by blocking anti-apoptotic proteins such as Bcl-2. It can also inhibit different proliferative proteins such as Notch-1 or mTOR and stop the cell cycle mediated by p53 or p21 [47–49].

Several studies in animal models show that xanthohumol does not cause toxicity, even at concentrations of 500 mg/kg. Furthermore, at sub-chronic toxicity doses (above 1000 mg/kg), only a slight hepatotoxicity has been observed in these animals, which may be reversible after decreasing the dose administered. This high concentration could be toxic due to direct cytotoxic processes, mitochondrial damage or oxidative stress. In spite of this, the concentrations to produce this liver

damage are very high and could hardly be reached into the bloodstream with a normal diet [50]. On the other hand, administration of xanthohumol in animals at earlier stages of CRC significantly reduces preneoplastic lesions (ACF). In addition, this flavonoid reduced iNOS and Cox-2 levels, an inhibited the Wnt/β-catenin pathway, suggesting it is able to reduce inflammatory and cell proliferation processes. Moreover, an increase in apoptosis processes induced by the caspase 3 and Bax pathway was observed. Therefore, in vivo studies have shown that xanthohumol can act as an antitumor agent at all levels, reducing inflammation, cell proliferation and increasing apoptosis, which led to a decrease in preneoplastic lesions in CRC [51], without relevant toxic effects [49].

3.3. Flavonoids as palliative bioactive agents of side effects associated to cancer chemotherapy

Chemotherapy-induced side effects are an important drawback of conventional (cytotoxic) cancer treatment that worry both patients and clinicians [52,53]. Among them, those affecting the gastrointestinal system (nausea, vomiting, mucositis, diarrhea, constipation) mainly occur during treatment and are reasonably well managed in most patients. However, in some cases these adverse effects may be severe, or patients may be refractory to prophylactic treatment, and quality of life is greatly reduced [54]. Even fatalities may occur due to dehydration and electrolyte imbalance in case of heavy uncontrolled vomiting and diarrhea [55]. On the other hand, neuropathic pain due to neurotoxic effects on the peripheral nervous system may develop during treatment [56,57]. Remarkably, this adverse effect is a frequent sequel of cancer treatment that may remain long after treatment has ceased, imposing a substantial decrease in the quality of life of cancer survivors [58,59].

The flavonoids selected for the present study have been shown to

exert antioxidant and anti-inflammatory properties that might be beneficial to prevent or palliate some of the chemotherapy-induced side effects mentioned above. However, they have been only scarcely studied in the context of chemotherapy. Here, a specific literature search was performed in Pubmed database using the following keywords and their combinations (flavonoid AND adverse effect): apigenin, eriodictyol, luteolin, naringenin, xanthohumol (flavonoids); nausea, vomiting, emesis, mucositis, diarrhea, neuropathy, neurotoxicity and pain (adverse effects). This allowed us to identify 17 reports dealing with all the flavonoids studied here, except xanthohumol. As shown in Table 1, only two studies were identified that evaluated the potential benefits of these flavonoids to prevent or alleviate chemotherapy-induced adverse effects affecting the gastrointestinal tract. One of them evaluated the effect of apigenin on the oral mucositis induced by 5-FU in the hamster model [60]. In this case, apigenin accelerated healing of the mucosal lesions, probably through the inhibition of the expression of different inflammatory factors [61]. The second study used irinotecan in the mouse to induce intestinal mucositis and tested the effects of luteolin administered in a preventive fashion (before the antineoplastic drug was injected). In this case, the flavonoid prevented intestinal damage and reduced the consequent occurrence of diarrhea and weight loss through the partial agonism of peroxisome proliferator-activated receptor-γ (PPAR-γ) [62], an intracellular receptor whose activation displays anti-inflammatory actions [63]. In other gastrointestinal inflammation and diarrhea models, unrelated with the use of antineoplastic drugs, not only apigenin [64] and luteolin [65,66], but also naringenin [67] and eriodictyol [68] were able to prevent the development of damage and symptoms in the gastrointestinal tract, through anti-inflammatory and antioxidant effects that protected the mucosa. In addition, naringenin [69] and apigenin [64] were also able to reduce motility and secretion,

Table 1

Effects of the flavonoids of interest (naringenin, apigenin, luteolin, eriodictyol and xanthohumol[a]) on the gastrointestinal system with potential usefulness in the context of prevention or palliation of chemotherapy-induced side effects.

Condition	Associated to	Model	Treatment	Flavonoid	Effect	Mechanism	Reference
Intestinal transit	–	Mouse	–	Naringenin	Inhibition of gastrointestinal motility and secretion	α_2-adrenergic agonism and calcium channel blockade	[69]
Diarrhea	Castor oil or MgSO$_4$ (oral)	Mouse	Preventive and palliative	Apigenin	Antidiarrheal and antispasmodic effect: inhibition of intestinal motility and reduction of water absorption	Opioid receptor agonism	[72,73]
Diarrhea	Castor oil (oral)	Rat and mouse	Preventive	Luteolin	Antidiarrheal effect	Na$^+$ reabsorption through enterocyte Na$^+$/K$^+$-ATPase	[66]
Gastric ulcer	Ethanol/HCl (oral) and indomethacin (i.p.)	Mouse	Preventive	Eriodictyol	Gastroprotective effect (reduction of the number of gastric lesions)	Reduction of oxidative stress	[68]
Gastrointestinal toxicity (diarrhea and bleeding)	Diclofenac (oral) + NaF (oral)	Rat	Preventive	Luteolin	Reduction of hemorrhages, mucosal erosions, and cell necrosis	Reduced levels of MDA, AOPP, protein carbonyls, H$_2$O$_2$, and NO, enhanced GSH-Px, GST and SOD activities, and increased GSH and protein levels.	[65]
Colitis	Acetic acid (intrarectal)	Rat	Preventive	Apigenin	Anti-inflammatory effect: reduction of MPO and macroscopic and histological inflammatory alterations	Suppression of NOS and COX-2 enzyme induction and inhibition of IL-4	[64]
Intestinal inflammation	DSS (oral)	Mouse	Preventive	Naringenin	Anti-inflammatory effect	Inhibition of NF-kB activity, reduction of inflammatory cytokine production and negative regulation of inflammatory mediators	[74]
Intestinal mucositis	Irinotecan (i.p.)	Mouse	Preventive	Luteolin	Prevention of intestinal damage and reduction of diarrhea and weight loss	Partial agonism of PPARγ (anti-inflammatory effects)	[62]
Oral mucositis	5-FU (i.p.)	Hamster	Palliative	Apigenin	Reduction of healing time and number of inflammatory cells	Inhibition of the expression of inflammatory factors (IL-6, IL-8 and ICAM-1)	[60,61]

Abbreviations: AOPP, advanced oxidation protein products; COX-2, cyclooxygenase 2; DSS, dextran sodium sulfate; GSH, glutathione; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; ICAM-1, intercellular adhesion molecule 1; IL-4, interleukin 4; IL-6, interleukin 6; IL-8, interleukin 8; i.p., intraperitoneal injection; MDA, malondialdehyde; MPO, myeloperoxidase; Na$^+$/K$^+$-ATPase, sodium/potassium pump; NF-kB, nuclear factor kappa B; NO, nitric oxide; NOS, nitric oxide synthase; PPARγ, peroxisome proliferator-activated receptor gamma; SOD, superoxide dismutase.

[a] No result was found for this compound in the literature review.

through the inhibition of calcium channels (naringenin) and activation of the presynaptic α$_2$-adrenergic (naringenin) and opioid (apigenin) receptors, which inhibit neurotransmitter release in the gastrointestinal tract [70,71].

Importantly, the potential beneficial effect of the selected flavonoids in chemotherapy-induced neuropathic pain has not been directly evaluated [16,17]. However, it has been proven in preclinical models of neuropathic pain of other etiologies. Thus, in the mouse model of sciatic nerve ligation (a surgical model of asymmetric neuropathic pain [75]), luteolin, given before the surgery, was able to reduce pain and potentiate morphine-induced analgesia [76]. This effect is probably involved in the reduction of both pro-inflammatory cytokine expression and nitric oxide (NO) release [77]. In another neuropathic model, induced by the injection of streptozotocin (STZ, a toxin that specifically destroys pancreatic β cells, leading to hyperglycemia and type 1 diabetes mellitus [78,79]), naringenin (combined with insulin) reduced both hyperglycemia and the associated neuropathic pain in the rat, through antioxidant and anti-inflammatory mechanisms [80]. Like chemotherapy-induced neuropathy, diabetic neuropathy is also symmetric (affects both sides of the body) and develops in a "glove-and-stocking" fashion [81]. Therefore, these results suggest that at least naringenin could be useful to reduce the impact of chemotherapy on the peripheral nervous system through neuroprotective effects, but this warrants further investigation. Interestingly, in models of acute and inflammatory pain, naringenin [82], as well as apigenin [83] and eriodictyol [84], showed antinociceptive effects. Moreover, in some models of neurotoxicity, eriodictyol (in vitro) [85,86] and apigenin (in vivo) [87] were able to exert specific neuroprotective effects, further supporting the possibility that these flavonoids may be useful neuroprotectants also applicable to cancer chemotherapy, as found for other flavonoids [16,17]. These results are summarized in Table 2.

4. Discussion

Diet is a very important factor in the development of some cancer types, such as CRC. This neoplasia is the most common type in Western populations, with 35 new cases per 100,000 habitants, mainly due to the high intake of saturated fat and processed meat products (a source of carcinogens such as benzopyrenes, nitrosamines, etc.) [90]. CRC initiates in the colon mucosa, showing development of ACF, due to mutations in stem cells (which are placed at the bottom of each colon mucosa crypt), which after several decades evolves towards polyps (microadenomas) of small diameter (mm), then to adenomas, and finally to adenocarcinomas with eventual metastasis to distant organs (liver, lung). In CRC cells, one of the most frequent alterations involves β-catenin cytosolic degradation (Wnt pathway), which is reduced, and therefore allows this protein, normally with structural functions, to translocate in higher amounts to the cell nucleus, where it acts as a transcriptional factor, enhancing the expression of genes involved in cellular proliferation. This slow degradation of β-catenin is usually caused by a malfunction of the Wnt signaling pathway, due to a mutation in the *apc* gene (sporadic, during the patient's life, or hereditary at birth) [91].

From the different flavonoids assayed in this work, the naringenin flavanone did not show any antitumor activity in any of the three different CRC cell lines (including a metastatic one, T-84) (Figs. 1 and 2), so it was not assayed on normal cells. The absence of antitumor activity can be explained, in the cases of HT-29 and T-84 cell lines, due to the mutations they possess in *TP53* gene, a key tumor suppressor gene which induces apoptosis and cellular cycle arrest, after activation by upstream factors, such as p38 (MAPK pathway) [92,93].

In these in vitro experiments, apigenin blocked cellular proliferation only in HCT116 in a statistically significant manner between 10 μM and 40 μM, inhibiting by 79.7% its proliferation at 40 μM (Fig. 1). This cell line possesses a functional p38 protein kinase (a known target for apigenin downregulation) and its downstream p53 transcriptional factor.

Table 2
Effects of the flavonoids of interest (naringenin, apigenin, luteolin, eriodictyol and xanthohumol[a]) on the peripheral nervous system with potential usefulness in the context of prevention or palliation of chemotherapy-induced side effects.

Condition	Associated to	Model	Treatment	Flavonoid	Effect	Mechanism	Reference
Neurotoxicity	Glutamate (in vitro)	HT22 and BV2 neuronal cell lines	Preventive	Eriodictyol	Neuroprotective effect and low cytotoxicity per se	Restoration of antioxidant enzyme activities (SOD, GSH-R and GSH-Px) and inhibition of NO production by microglial cells	[85]
Neurotoxicity	H$_2$O$_2$ (in vitro)	PC12 cell line	Preventive	Eriodictyol	Inhibition of cell death associated with H$_2$O$_2$-induced oxidative stress.	Regulation of HO-1 expression and γ-GCS activation of the Nrf2/ARE pathway	[86]
Acute pain	Capsaicin (i.pl.)	Rat and mouse	Preventive	Eriodictyol	Antinociceptive effect	TRPV1 antagonism and antioxidant activity	[84]
Nociception	Acetic acid (i.p.), formalin (i.pl.) or hot plate	Mouse	Preventive	Apigenin	Reduction of abdominal contortions (acetic acid) and paw-licking (formalin and hot plate)	Cholinergic receptor activation and opioid agonism	[83]
Nociception and inflammation	Hot plate, acetic acid (i.p.) and carrageenan (i.pl.)	Rat and mouse	Preventive	Naringenin	Antinociceptive and anti-inflammatory effect	Suppression of inflammatory cytokines and possible COX inhibition	[82,88]
Neurotoxicity	Acrylonitrile (intragastric)	Rat	Preventive	Apigenin	Protection against neurotoxicity, inhibition of neuroinflammation, and suppression of neuronal apoptosis	Reduction of oxidative stress and inhibition of the TLR4 / NF-kB signaling pathway	[87]
Neuropathic pain	Sciatic nerve ligation	Mouse	Preventive	Luteolin	Antinociceptive and morphine potentiating effect	Reduction of TNF-α, IL-1, IL-6 and NO and IL-1β expression	[76,77,89]
Neuropathic pain associated to DM	Streptozotocin (i.p.)	Rat	Palliative	Naringenin	Reduction of hyperglycemia and reversal of neuropathic pain in conjunction with insulin	Modulation of oxidative stress, inflammatory cytokine release and MMP inhibition	[80]

Abbreviations: COX, cyclooxygenase; DM, diabetes mellitus; GSH-Px, glutathione peroxidase; GSH-R, glutathione reductase; HO-1, hemooxygenase; IL-1, interleukin 1; IL-1β, interleukin 1 beta; IL-6, interleukin 6; i.p., intraperitoneal injection; i.pl., intraplantar injection; MMP, matrix metalloproteinases; Nrf2/ARE, nuclear erythroid factor 2/antioxidant response element; SOD, superoxide dismutase; TLR4/NF-kB, toll like receptor 4/nuclear factor kappa B; γ-GCS, γ-glutamylcysteine synthetase.

[a] No result was found for this compound in the literature review.

However, p53 is mutated in HT-29 and T-84 cell lines, as well as *apc* (generating a constitutive activation of the cell cycle via β-catenin). It has been recently described that at least three p38 isoforms are present in HCT116 cell line, the activation of p38α and p38β being necessary for triggering apoptosis (probably due to p53 downstream activation). In contrast, the activation of p38δ isoform is necessary for activating cell survival. According to this, the apoptosis induction after apigenin treatment in HCT116 could rely on the presence of p53, a transcriptional factor which is actually mutated in HT-29 and T-84, therefore protecting these two cancer cell lines from the apigenin antitumor effect [23,94]. In CCD-18CO normal cells apigenin did not cause growth inhibition at the tested concentrations (Figs. 1 and 2).

The ring B hydroxylation of naringenin generates eriodictyol, another flavanone that produced a moderate inhibition of cell growth in HCT116 and T-84 cell lines, and almost no inhibition on normal cells nor in HT-29 cells (Figs. 1 and 2), which share as a common cancer promoting feature a mutation in $KRAS^{G13D}$ gene, which generates, in turn, a constitutively activated RAS GTPase [95,96]. Eriodictyol has been described, in other tumor cell lines (such as skin fibroblasts), to inhibit downstream cell cycle signal transducers depending on RAS, such as RSK2 serine/threonine kinase [97]. Therefore, the antitumor effect of eriodictyol on HCT116 and T84 could rely on its inhibition of RSK2, which blocks upstream cellular multiplication signals from constitutively activated RAS.

In a similar way to apigenin, luteolin is another flavone, but with an extra hydroxylation in ring B. Luteolin antitumor effect on the three CRC cell lines studied here showed a similar pattern to apigenin, acting mostly on HCT116, but with stronger inhibition on this cell line than apigenin (Fig. 1). Luteolin does show some inhibition on T-84 cells, an effect which was absent in the case of apigenin, and also shows less inhibition of normal CCD-18CO cells (Fig. 2). The antitumor effect of luteolin could be based, as in the case of apigenin, on an inhibition of p38 isoforms activation, blocking the cell survival signaling in these cell lines, or alternatively, to its direct interaction with p53, a key factor for apoptosis induction in cancer cells, which is present in HCT116, but mutated in the other two cell lines [94,98].

From the different flavonoids tested in this work, xanthohumol is the one that shows a better cancer cell inhibition, with statistical significance in HCT116 CRC cell line (Figs. 1 and 2). Xanthohumol induced over 92% cell viability in all three cell lines. This general antitumor effect, independently from the cell line, may be due to a global inhibitory effect of this flavonoid on cancer cell glycolysis, a key metabolic process, as xanthohumol is a known inhibitor of hexokinase 2 (HK2), and downregulation of this key enzyme generates liberation of mitochondrial cytochrome C and apoptosis induction [46]. Although xanthohumol also exerted high cytotoxicity in CCD-18CO, in vivo animal studies with this chalcone have shown a lack of side effects, even at high concentrations (500 mg/kg) [50]. Interestingly, xanthohumol antitumor effects are better than the commercial drug 5-FU, at the same concentrations, for the three CRC cell lines (Figs. 1 and 2).

Different signaling pathways involved in CRC development may be tackled by using combination therapy involving nutraceutical compounds (such as flavonoids and other polyphenols) together with conventional antitumor drugs (such as 5-FU). These combination therapies may induce chemosensitization in cancer cells, avoiding resistance mechanisms commonly present in cancer cells (such as ROS resistance, apoptosis inhibition, alterations in mitochondrial function, multidrug transport systems activation, etc.) [99]. In this work, another alternative was used to test if there was a synergistic effect of the tested flavonoids, on CRC cell lines, in combination with 5-FU. For this, those flavonoid concentrations causing a 30% cell viability inhibition (apigenin, luteolin and xanthohumol in HCT116 and HT-29 cell lines) were used in combination with 10–50 μM 5-FU (Fig. 3).

In the case of the combination treatment in HCT116, the strong antitumor effect of xanthohumol showed an antagonistic effect when combined with the pharmaceutical drug. However, the combination of 5-FU with apigenin or luteolin showed a synergistic effect, which could be explored in the future using an appropriate animal model for this cancer type.

Finally, in the case of the combined treatment in HT-29, the three tested combinations are additive. This is also of interest, as the necessary treatment concentration for 5-FU could be reduced in patients, when using combination therapy with flavonoids. This strategy might eventually reduce in a proportional way the appearance of adverse side effects in these patients, an alternative which may be worth exploring in the future. Regarding this, luteolin has shown potential as a palliative agent, preventing neuropathic pain in an animal model for sciatic nerve ligation, as well as diarrhea, bleeding, and mucositis (even in combination with the antitumor irinotecan, one of the usual treatments in combined therapy with 5-FU, such as FOLFIRI) (see Section 3.3, Tables 1 and 2). As a future prospect, luteolin could be used in vivo alone, or in combination with 5-FU, in order to check if under these conditions, it exerts palliative effects against this antitumor drug, as well as to eventually demonstrate that its combination with the drug shows a synergistic effect in vivo, as it has demonstrated in vitro in the case of HCT116 cell line. Similarly, apigenin was demonstrated to exert protective effects against 5-FU-induced oral mucositis [60,61] as well as diarrhea (Table 1) and neurotoxicity (Table 2) of non-chemotherapy etiologies, suggesting that this compound might also be promising in this context.

5. Conclusions

Xanthohumol, as a single treatment, acts with high efficiency against all three stages of CRC represented by the three cell lines tested in this work (adenocarcinoma and metastatic stage), its antitumor effect being better than that of current pharmacological drugs in use for chemotherapy of this cancer, such as 5-FU. However, this chalcone does not provide an extra synergistic effect in combination treatment. Furthermore, it lacks specificity against tumoral cells, as normal fibroblasts were also, to a great extent, inhibited by this chalcone.

On the other hand, the structurally related flavones apigenin and luteolin, show an interesting antitumor effect in HCT116 cell line, especially luteolin. Moreover, these two flavonoids show a synergistic effect in combination treatment with 5-FU, which is a novel interesting feature and paves the way towards their future application in preclinical and clinical uses, especially in the case of the luteolin, which lacks toxicity against normal fibroblasts.

Also, importantly, of all these flavonoids, apigenin, luteolin, naringenin and eriodictiol (xanthohumol has not yet been specifically evaluated) show interesting palliative effects on symptoms typically associated to CRC chemotherapy (diarrhea, ulcers, gastric bleeding, colitis, mucositis, pain, etc.), which is of great interest as an additional property to their antitumor activities and a potential added advantage in their use as combination therapy with canonical antitumor drugs, such as 5-FU, for preventing or reducing the important adverse side effects (both digestive and neurologic) of these CRC treatment.

CRediT authorship contribution statement

Javier Fernández: Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Blanca Silván:** Methodology, Investigation, Writing – original draft. **Rodrigo Entrialgo-Cadierno:** Investigation. **Claudio J. Villar:** Methodology, Writing – original draft. **Raffaele Capasso:** Writing – review & editing. **José Antonio Uranga:** Methodology, Writing – original draft, Writing – review & editing, Funding acquisition. **Felipe Lombó:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition. **Raquel Abalo:** Methodology, Writing – original draft, Writing – review & editing, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of interest

The authors declare no conflict of interest.

References

[1] R.M. Merrill, A.E. Anderson, Risk-adjusted colon and rectal cancer incidence rates in the United States, Dis. Colon Rectum 54 (2011) 1301–1306.

[2] F. Bray, J.-S. Ren, E. Masuyer, J. Ferlay, Global estimates of cancer prevalence for 27 sites in the adult population in 2008, Int. J. Cancer 132 (2013) 1133–1145.

[3] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, CA Cancer J. Clin. 71 (2021) 209–249.

[4] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, CA Cancer J. Clin. 61 (2011) 69–90.

[5] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (2015) E359–E386.

[6] A. Humphries, N.A. Wright, Colonic crypt organization and tumorigenesis, Nat. Rev. Cancer 8 (2008) 415–424.

[7] W.R. Bruce, D.E. Corpet, The colonic protein fermentation and insulin resistance hypotheses for colon cancer etiology: experimental tests using precursor lesions, Eur. J. Cancer Prev. 5 (Suppl 2) (1996) 41–47.

[8] K.W. Kinzler, B. Vogelstein, Lessons from hereditary colorectal cancer, Cell 87 (1996) 159–170.

[9] T. Eom, Y. Lee, J. Kim, I. Park, G. Gwak, H. Cho, K. Yang, K. Kim, B.-N. Bae, Prognostic factors affecting disease-free survival and overall survival in T4 colon cancer, Ann. Coloproctol. 37 (2021) 259–265.

[10] H.Q. Xiong, J.A Ajani, Treatment of colorectal cancer metastasis: the role of chemotherapy, Cancer Metastasis Rev. 23 (2004) 145–163.

[11] B. Gustavsson, G. Carlsson, D. Machover, N. Petrelli, A. Roth, H.-J. Schmoll, K.-M. Tveit, F. Gibson, A review of the evolution of systemic chemotherapy in the management of colorectal cancer, Clin. Colorectal Cancer 14 (2015) 1–10.

[12] A. Mahipal, A. Grothey, Role of biologics in first-line treatment of colorectal cancer, J. Oncol. Pract. 12 (2016) 1219–1228.

[13] L. Marín, I. Gutiérrez-Del-Río, P. Yagüe, Á. Manteca, C.J. Villar, F. Lombó, De novo biosynthesis of apigenin, luteolin, and eriodictyol in the Actinomycete Streptomyces albus and production improvement by feeding and spore conditioning, Front. Microbiol. 8 (2017) 921.

[14] S. Redondo-Blanco, J. Fernández, I. Gutiérrez-del-Río, C.J. Villar, F. Lombó, New insights toward colorectal cancer chemotherapy using natural bioactive compounds, Front. Pharmacol. 8 (2017) 109.

[15] R. Hossain, M.T. Islam, M.S. Mubarak, D. Jain, R. Khan, A.S. Saikat, Natural-derived molecules as a potential adjuvant in chemotherapy: normal cell protectors and cancer cell sensitizers, Anticancer Agents Med. Chem. (2021).

[16] P. Basu, A. Basu, In vitro and in vivo effects of flavonoids on peripheral neuropathic pain, Molecules 25 (2020) 1171.

[17] M. Siddiqui, B. Abdellatif, K. Zhai, A. Liskova, P. Kubatka, D. Büsselberg, Flavonoids alleviate peripheral neuropathy induced by anticancer drugs, Cancers (2021) 13.

[18] V. Neveu, J. Perez-Jiménez, F. Vos, V. Crespy, L. du Chaffaut, L. Mennen, C. Knox, R. Eisner, J. Cruz, D. Wishart, A. Scalbert, Phenol-Explorer: an online comprehensive database on polyphenol contents in foods, Database: J. Biol. Databases Curation 2010 (2010) 24, bap024–bap024.

[19] Y. Qi, Z. Ding, Y. Yao, F. Ren, M. Yin, S. Yang, A. Chen, Apigenin induces apoptosis and counteracts cisplatin-induced chemoresistance via Mcl-1 in ovarian cancer cells, Exp. Ther. Med. 20 (2020) 1329–1336.

[20] X. Zhang, W. Zhang, F. Chen, Z. Lu, Combined effect of chrysin and apigenin on inhibiting the development and progression of colorectal cancer by suppressing the activity of P38-MAPK/AKT pathway, IUBMB Life 73 (2021) 774–783.

[21] J. Tong, Y. Shen, Z. Zhang, Y. Hu, X. Zhang, L. Han, Apigenin inhibits epithelial-mesenchymal transition of human colon cancer cells through NF-κB/Snail signaling pathway, Biosci. Rep. 39 (2019) 39.

[22] X.-Y. Ai, Y. Qin, H.-J. Liu, Z.-H. Cui, M. Li, J.-H. Yang, W.-L. Zhong, Y.-R. Liu, S. Chen, T. Sun, H.G. Zhou, C. Yang, Apigenin inhibits colonic inflammation and tumorigenesis by suppressing STAT3-NF-κB signaling, Oncotarget 8 (2017) 100216–100226.

[23] S. Bian, H. Wan, X. Liao, W. Wang, Inhibitory effects of apigenin on tumor carcinogenesis by altering the gut microbiota, Mediat. Inflamm. 2020 (2020), 7141970.

[24] M. Imran, A. Rauf, T. Abu-Izneid, M. Nadeem, M.A. Shariati, I.A. Khan, A. Imran, I. E. Orhan, M. Rizwan, M. Atif, T.A. Gondal, M.S. Mubarak, Luteolin, a flavonoid, as an anticancer agent: a review, Biomed. Pharmacother. 112 (2019), 108612.

[25] S.A. Ganai, F.A. Sheikh, Z.A. Baba, M.A. Mir, M.A. Mantoo, M.A. Yatoo, Anticancer activity of the plant flavonoid luteolin against preclinical models of various cancers and insights on different signalling mechanisms modulated, Phytother. Res. 35 (2021) 3509–3532.

[26] W. Ju, X. Wang, H. Shi, W. Chen, S.A. Belinsky, Y. Lin, A critical role of luteolin-induced reactive oxygen species in blockage of tumor necrosis factor-activated nuclear factor-kappaB pathway and sensitization of apoptosis in lung cancer cells, Mol. Pharmacol. 71 (2007) 1381–1388.

[27] Q. Zhang, J. Yang, J. Wang, Modulatory effect of luteolin on redox homeostasis and inflammatory cytokines in a mouse model of liver cancer, Oncol. Lett. 12 (2016) 4767–4772.

[28] J. Baby, A.R. Devan, A.R. Kumar, J.N. Gorantla, B. Nair, T.S. Aishwarya, L.R. Nath, Cogent role of flavonoids as key orchestrators of chemoprevention of hepatocellular carcinoma: a review, J. Food Biochem. 45 (2021) e13761.

[29] K.A. Kang, M.J. Piao, Y.S. Ryu, Y.J. Hyun, J.E. Park, K. Shilnikova, A.X. Zhen, H. K. Kang, Y.S. Koh, Y.J. Jeong, J.W. Hyun, Luteolin induces apoptotic cell death via antioxidant activity in human colon cancer cells, Int. J. Oncol. 51 (2017) 1169–1178.

[30] Q. Zuo, R. Wu, X. Xiao, C. Yang, Y. Yang, C. Wang, L. Lin, A.-N. Kong, The dietary flavone luteolin epigenetically activates the Nrf2 pathway and blocks cell transformation in human colorectal cancer HCT116 cells, J. Cell. Biochem. 119 (2018) 9573–9582.

[31] W. Lim, S. Park, F.W. Bazer, G. Song, Naringenin-induced apoptotic cell death in prostate cancer cells is mediated via the PI3K/AKT and MAPK signaling pathways, J. Cell. Biochem. 118 (2017) 1118–1131.

[32] P. Totta, F. Acconcia, S. Leone, I. Cardillo, M. Marino, Mechanisms of Naringenin-induced Apoptotic Cascade in cancer cells: involvement of estrogen receptor α and β signalling. IUBMB Life (Int. Union Biochem. Mol. Biol. Life) 56 (2004) 491–499.

[33] H.M. Song, G.H. Park, H.J. Eo, J.B. Jeong, Naringenin-mediated ATF3 expression contributes to apoptosis in human colon cancer, Biomol. Ther. 24 (2016) 140–146.

[34] J.A. Manthey, K. Grohmann, N. Guthrie, Biological properties of citrus flavonoids pertaining to cancer and inflammation, Curr. Med. Chem. 8 (2001) 135–153.

[35] A. Islam, M.S. Islam, M.K. Rahman, M.N. Uddin, M.R. Akanda, The pharmacological and biological roles of eriodictyol, Arch. Pharm. Res. 43 (2020) 582–592.

[36] Y. Liang, H. Niu, L. Ma, D. Du, L. Wen, Q. Xia, W. Huang, Eriodictyol 7–O-β-D glucopyranoside from Coreopsis tinctoria Nutt. ameliorates lipid disorders via protecting mitochondrial function and suppressing lipogenesis, Mol. Med. Rep. 16 (2017) 1298–1306.

[37] Y. Zhang, R. Zhang, H. Ni, Eriodictyol exerts potent anticancer activity against A549 human lung cancer cell line by inducing mitochondrial-mediated apoptosis, G2/M cell cycle arrest and inhibition of m-TOR/PI3K/Akt signalling pathway, Arch. Med. Sci. 16 (2020) 446–452.

[38] P. Mariyappan, T. Kalaiyarasu, V. Manju, Effect of eriodictyol on preneoplastic lesions, oxidative stress and bacterial enzymes in 1,2-dimethyl hydrazine-induced colon carcinogenesis, Toxicol. Res. 6 (2017) 678–692.

[39] J.F. Stevens, J.E. Page, Xanthohumol and related prenylflavonoids from hops and beer: to your good health!, Phytochemistry 65 (2004) 1317–1330.

[40] J.F. Stevens, A.W. Taylor, J.E. Clawson, M.L. Deinzer, Fate of xanthohumol and related prenylflavonoids from hops to beer, J. Agric. Food Chem. 47 (1999) 2421–2428.

[41] A. Nookandeh, N. Frank, F. Steiner, R. Ellinger, B. Schneider, C. Gerhäuser, H. Becker, Xanthohumol metabolites in faeces of rats, Phytochemistry 65 (2004) 561–570.

[42] B. Nowak, B. Poźniak, J. Popłoński, Ł.; Bobak, A.; Matuszewska, J.; Kwiatkowska, W.; Dziewiszek, E.; Huszcza, A. Szelag, Pharmacokinetics of xanthohumol in rats of both sexes after oral and intravenous administration of pure xanthohumol and prenylflavonoid extract, Adv. Clin. Exp. Med. 29 (2020) 1101–1109.

[43] F. Zhao, Y. Watanabe, H. Nozawa, A. Daikonnya, K. Kondo, S. Kitanaka, Prenylflavonoids and phloroglucinol derivatives from hops (Humulus lupulus), J. Nat. Prod. 68 (2005) 43–49.

[44] J. Sridhar, N. Akula, D. Sivanesan, M. Narasimhan, A. Rathinavelu, N. Pattabiraman, Identification of novel angiogenesis inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 4125–4129.

[45] J.E. Wilson, Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function, J. Exp. Biol. 206 (2003) 2049–2057.

[46] W. Liu, W. Li, H. Liu, X. Yu, Xanthohumol inhibits colorectal cancer cells via downregulation of hexokinases ii-mediated glycolysis, Int. J. Biol. Sci. 15 (2019) 2497–2508.

[47] C.H. Jiang, T.L. Sun, D.X. Xiang, S.S. Wei, W.Q. Li, Anticancer activity and mechanism of xanthohumol: a prenylated flavonoid from hops (Humulus lupulus L.), Front. Pharmacol. 9 (2018) 1–13.

[48] X. Liu, L.J. An, Y. Li, Y. Wang, L. Zhao, X. Lv, J. Guo, A.L. Song, Xanthohumol chalcone acts as a powerful inhibitor of carcinogenesis in drug-resistant human colon carcinoma and these effects are mediated via G2/M phase cell cycle arrest, activation of apoptotic pathways, caspase activation and targeting Ras /MEK/ERK pathway, JBUON 24 (2019) 2442–2447.

[49] M. Festa, A. Capasso, C.W. D'Acunto, M. Masullo, A.G. Rossi, C. Pizza, S. Piacente, Xanthohumol induces apoptosis in human malignant glioblastoma cells by increasing reactive oxygen species and activating MAPK pathways, J. Nat. Prod. 74 (2011) 2505–2513.

[50] R. Hussong, N. Frank, J. Knauft, C. Ittrich, R. Owen, H. Becker, C. Gerhäuser, A safety study of oral xanthohumol administration and its influence on fertility in Sprague Dawley rats, Mol. Nutr. Food Res. 49 (2005) 861–867.

[51] H. Liu, L. Zhang, G. Li, Z. Gao, Xanthohumol protects against Azoxymethane-induced colorectal cancer in Sprague-Dawley rats, Environ. Toxicol. 35 (2020) 136–144.

[52] K. Nurgali, R.T. Jagoe, R. Abalo, Editorial: adverse effects of cancer chemotherapy: anything new to improve tolerance and reduce sequelae? Front. Pharmacol. 9 (2018) 245.

[53] L.L. Ngai, E. Ter Veer, H.G. van den Boorn, E.H. van Herk, J.J. van Kleef, M.G. H. van Oijen, H.W.M. van Laarhoven, TOXview: a novel graphical presentation of cancer treatment toxicity profiles, Acta Oncol. 58 (2019) 1138–1148.

[54] A. Davies, C. Lum, R. Raju, E. Ansell, K. Webber, E. Segelov, Anti-cancer therapy made easier: a 25-year update, Intern. Med. J. 51 (2021) 473–480.

[55] P. Smith, A. Lavery, R.C. Turkington, An overview of acute gastrointestinal side effects of systemic anti-cancer therapy and their management, Best Pract. Res. Clin. Gastroenterol. 48–49 (2020) 101691.

[56] Y. Li, M.B. Lustberg, S. Hu, Emerging pharmacological and non-pharmacological therapeutics for prevention and treatment of chemotherapy-induced peripheral neuropathy, Cancers (2021) 13.

[57] M. Laforgia, C. Laface, C. Calabrò, S. Ferraiuolo, V. Ungaro, D. Tricarico, C. D. Gadaleta, P. Nardulli, G. Ranieri, Peripheral neuropathy under oncologic therapies: a literature review on pathogenetic mechanisms, Int. J. Mol. Sci. (2021) 22.

[58] M.A.L. Tanay, J. Armes, R. Moss-Morris, A.M. Rafferty, G. Robert, A systematic review of behavioural and exercise interventions for the prevention and management of chemotherapy-induced peripheral neuropathy symptoms, J. Cancer Surviv. (2021).

[59] G. Cavaletti, P. Alberti, A.A. Argyriou, M. Lustberg, N.P. Staff, S. Tamburin, Toxic neuropathy consortium of the peripheral nerve society chemotherapy-induced peripheral neurotoxicity: a multifaceted, still unsolved issue, J. Peripher. Nerv. Syst. 24 (Suppl 2) (2019) S6–S12.

[60] P. Molina Prats, F. Gómez Garcia, F.; Martinez Diaz, R.; Amaral Mendes, P. Lopez-Jornet, The therapeutic effects of apigenin and dexamethasone on 5-fluorouracil-induced oral mucositis - a pilot study using a Syrian hamster model, J. Oral. Pathol. Med. 46 (2017) 142–147.

[61] A. García-Lafuente, E. Guillamón, A. Villares, M.A. Rostagno, J.A. Martínez, Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease, Inflamm. Res. 58 (2009) 537–552.

[62] T. Boeing, P. de Souza, S. Speca, L.B. Somensi, L.N.B. Mariano, B.J. Cury, M. Ferreira Dos Anjos, N.L.M. Quintão, L. Dubuqoy, P. Desreumax, L.M. da Silva, S. F. de Andrade, Luteolin prevents irinotecan-induced intestinal mucositis in mice through antioxidant and anti-inflammatory properties, Br. J. Pharmacol. 177 (2020) 2393–2408.

[63] A. Vetuschi, S. Pompili, E. Gaudio, G. Latella, R. Sferra, PPAR-γ with its anti-inflammatory and anti-fibrotic action could be an effective therapeutic target in IBD, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 8839–8848.

[64] H. Sadraei, G. Asghari, M. Khanabadi, M. Minaiyan, Anti-inflammatory effect of apigenin and hydroalcoholic extract of Dracocephalum kotschyi on acetic acid-induced colitis in rats, Res. Pharm. Sci. 12 (2017) 322–329.

[65] A.S. Akinrinde, K.O. Soetan, M.O. Tijani, Exacerbation of diclofenac-induced gastroenterohepatic damage by concomitant exposure to sodium fluoride in rats: protective role of luteolin, Drug Chem. Toxicol. (2020) 1–13.

[66] C.-L. Dong, Y. Qin, J.-X. Ma, W.-Q. Cui, X.-R. Chen, L.-Y. Hou, X.-Y. Chen, B.-O. God'spower, N. Eliphaz, J.-J. Qin, W.X. Guo, W.Y. Ding, Y.H. Li, The active ingredients identification and antidiarrheal mechanism analysis of Plantago asiatica L. superfine powder, Front. Pharmacol. 11 (2021), 612478.

[67] W. Dou, J. Zhang, A. Sun, E. Zhang, L. Ding, S. Mukherjee, X. Wei, G. Chou, Z.-T. Wang, S. Mani, Protective effect of naringenin against experimental colitis via suppression of Toll-like receptor 4/NF-κB signalling, Br. J. Nutr. 110 (2013) 599–608.

[68] A.C.H. Pereira, D. Lenz, B.V. Nogueira, R. Scherer, T.U. Andrade, H.B. Costa, W. da; Romão, T.M.C. Pereira, D.C. Endringer, Gastroprotective activity of the resin from Virola oleifera, Pharm. Biol. 55 (2017) 472–480.

[69] G. Di Carlo, G. Autore, A.A. Izzo, P. Maiolino, N. Mascolo, P. Viola, M.V. Diurno, F. Capasso, Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationships, J. Pharm. Pharmacol. 45 (1993) 1054–1059.

[70] C. Blandizzi, Enteric alpha-2 adrenoceptors: pathophysiological implications in functional and inflammatory bowel disorders, Neurochem. Int. 51 (2007) 282–288.

[71] K. Chamie, V. Golla, A.T. Lenis, P.M. Lec, S. Rahman, E.R. Viscusi, Peripherally acting μ-opioid receptor antagonists in the management of postoperative ileus: a clinical review, J. Gastrointest. Surg. 25 (2021) 293–302.

[72] H. Sadraei, G. Asghari, F. Shahverdi, Antidiarrhoeal assessment of hydroalcoholic and hexane extracts of Dracocephalum kotschyi Boiss. and apigenin in mice, Res. Pharm. Sci. 11 (2016) 200–209.

[73] P.L. Katavic, K. Lamb, H. Navarro, T.E. Prisinzano, Flavonoids as opioid receptor ligands: identification and preliminary structure-activity relationships, J. Nat. Prod. 70 (2007) 1278–1282.

[74] W. Dou, J. Zhang, A. Sun, E. Zhang, L. Ding, S. Mukherjee, X. Wei, G. Chou, Z.-T. Wang, S. Mani, Protective effect of naringenin against experimental colitis via suppression of Toll-like receptor 4/NF-κB signalling, Br. J. Nutr. 110 (2013) 599–608.

[75] S.R. Challa, Surgical animal models of neuropathic pain: pros and cons, Int. J. Neurosci. 125 (2015) 170–174.

[76] M. Hashemzaei, M. Abdollahzadeh, M. Iranshahi, E. Golmakani, R. Rezaee, K. Tabrizian, Effects of luteolin and luteolin-morphine co-administration on acute and chronic pain and sciatic nerve ligated-induced neuropathy in mice, J. Complement. Integr. Med. 14 (2017) 20160066.

[77] J. Wu, X. Xu, Y. Li, J. Kou, F. Huang, B. Liu, K. Liu, Quercetin, luteolin and epigallocatechin gallate alleviate TXNIP and NLRP3-mediated inflammation and apoptosis with regulation of AMPK in endothelial cells, Eur. J. Pharmacol. 745 (2014) 59–68.

[78] G.J. Biessels, V. Bril, N.A. Calcutt, N.E. Cameron, M.A. Cotter, R. Dobrowsky, E. L. Feldman, P. Fernyhough, J. Jakobsen, R.A. Malik, A.P. Mizisin, P.J. Oates, I. G. Obrosova, R. Pop-Busui, J.W. Russell, A.A. Sima, M.J. Stevens, R.E. Schmidt, S. Tesfaye, A. Veves, A.I. Vinik, D.E. Wright, S. Yagihashi, M.A. Yorek, D. Ziegler, D.W. Zochodne, Phenotyping animal models of diabetic neuropathy: a consensus statement of the diabetic neuropathy study group of the EASD (Neurodiab), J. Peripher. Nerv. Syst. 19 (2014) 77–87.

[79] M. Kitada, Y. Ogura, D. Koya, Rodent models of diabetic nephropathy: their utility and limitations, Int. J. Nephrol. Renov. Dis. 9 (2016) 279–290.

[80] P. Singh, P. Bansal, A. Kuhad, A. Kumar, K. Chopra, Naringenin ameliorates diabetic neuropathic pain by modulation of oxidative-nitrosative stress, cytokines and MMP-9 levels, Food Funct. 11 (2020) 4548–4560.

[81] E.L. Feldman, B.C. Callaghan, R. Pop-Busui, D.W. Zochodne, D.E. Wright, D. L. Bennett, V. Bril, J.W. Russell, V. Viswanathan, Diabetic neuropathy, Nat. Rv. Dis. Primers. 5 (2019) 41.

[82] T.-W. Chung, S. Li, C.-C. Lin, S.-W. Tsai, Antinociceptive and anti-inflammatory effects of the citrus flavanone naringenin, Ci ji yi xue za zhi = Tzu-chi Med. J. 31 (2019) 81–85.

[83] M.M.G. Pinheiro, F. Boylan, P.D. Fernandes, Antinociceptive effect of the Orbignya speciosa Mart. (Babassu) leaves: evidence for the involvement of apigenin, Life Sci. 91 (2012) 293–300.

[84] M.F. Rossato, G. Trevisan, C.I. Walker, J.Z. Klafke, A.P. de Oliveira, J.G. Villarinho, R.B. Zanon, L.F. Royes, M.L. Athayde, M.V. Gomez, J. Ferreira, Eriodictyol: a flavonoid antagonist of the TRPV1 receptor with antioxidant activity, Biochem. Pharmacol. 81 (2011) 544–551.

[85] N. Cho, J.H. Choi, H. Yang, E.J. Jeong, K.Y. Lee, Y.C. Kim, S.H. Sung, Neuroprotective and anti-inflammatory effects of flavonoids isolated from Rhus verniciflua in neuronal HT22 and microglial BV2 cell lines, Food Chem. Toxicol. 50 (2012) 1940–1945.

[86] H. Lou, X. Jing, D. Ren, X. Wei, X. Zhang, Eriodictyol protects against H(2)O(2)-induced neuron-like PC12 cell death through activation of Nrf2/ARE signaling pathway, Neurochem. Int. 61 (2012) 251–257.

[87] F. Zhao, Y. Dang, R. Zhang, G. Jing, W. Liang, L. Xie, Z. Li, Apigenin attenuates acrylonitrile-induced neuro-inflammation in rats: involved of inactivation of the TLR4/NF-κB signaling pathway, Int. Immunopharmacol. 75 (2019) 105697.

[88] Y.-R. Li, D.-Y. Chen, C.-L. Chu, S. Li, Y.-K. Chen, C.-L. Wu, C.-C. Lin, Naringenin inhibits dendritic cell maturation and has therapeutic effects in a murine model of collagen-induced arthritis, J. Nutr. Biochem. 26 (2015) 1467–1478.

[89] F. Shi, F. Zhou, Z. Ji, Z. Xu, H. Yang, Anti-arthritic activity of luteolin in Freund's complete adjuvant-induced arthritis in rats by suppressing P2X4 pathway, Chem. Biol. Interact. 226 (2015) 82–87.

[90] J. Fernández, S. Redondo-Blanco, I. Gutiérrez-del-Río, E.M. Miguélez, C.J. Villar, F. Lombó, Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: a review, J. Funct. Foods 25 (2016) 511–522.

[91] J. Fernández, S. Redondo-Blanco, E.M. Miguélez, C.J. Villar, A. Clemente, F. Lombó, Healthy effects of prebiotics and their metabolites against intestinal diseases and colorectal cancer, AIMS Microbiol. 1 (2015) 48–71.

[92] Y. Liu, W.F. Bodmer, Analysis of P53 mutations and their expression in 56 colorectal cancer cell lines, Proc. Natl. Acad. Sci. USA 103 (2006) 976–981.

[93] X. He, J. Liao, F. Liu, J. Yan, J. Yan, H. Shang, Q. Dou, Y. Chang, J. Lin, Y. Song, Functional repair of p53 mutation in colorectal cancer cells using trans-splicing, Oncotarget 6 (2015) 2034–2045.

[94] A. Pranteda, V. Piastra, L. Stramucci, D. Fratantonio, G. Bossi, The p38 MAPK signaling activation in colorectal cancer upon therapeutic treatments, Int. J. Mol. Sci. 21 (2020) 2773.

[95] S. Alves, L. Castro, M.S. Fernandes, R. Francisco, P. Castro, M. Priault, S.R. Chaves, M.P. Moyer, C. Oliveira, R. Seruca, M. Côrte-Real, M.J. Sousa, A. Preto, Colorectal cancer-related mutant KRAS alleles function as positive regulators of autophagy, Oncotarget 6 (2015) 30787–30802.

[96] S.S. Kumar, T.J. Price, O. Mohyieldin, M. Borg, A. Townsend, J.E. Hardingham, KRAS G13D mutation and sensitivity to cetuximab or panitumumab in a colorectal cancer cell line model, Gastrointest. Cancer Res. 7 (2014) 23–26.

[97] K. Liu, Y.-Y. Cho, K. Yao, J. Nadas, D.J. Kim, E.-J. Cho, M.-H. Lee, A. Pugliese, J. Zhang, A.M. Bode, Z. Dong, Z. Dong, Eriodictyol inhibits RSK2-ATF1 signaling and suppresses EGF-induced neoplastic cell transformation, J. Biol. Chem. 286 (2011) 2057–2066.

[98] C. Jang, N. Moon, J. Oh, J.-S. Kim, Luteolin shifts oxaliplatin-induced cell cycle arrest at G_0/G_1 to apoptosis in HCT116 human colorectal carcinoma cells, Nutrients 11 (2019) 770.

[99] S. Redondo-Blanco, J. Fernández, I. Gutiérrez-del-Río, C.J. Villar, F. Lombó, New insights toward colorectal cancer chemotherapy using natural bioactive compounds, Front. Pharmacol. 8 (2017) 109.