ORIGINAL RESEARCH

Nutrafoods (2017) 1-9
DOI 10.17470/NF-017-1027-1

Received: July 09, 2016
Accepted: December 19, 2017

Gastrointestinal activity of dietary flaxseed lignans, omega-3 fatty acids and fibres

Carmen Laurino[1,2], Beniamino Palmieri[1,2], Maria Vadalà[1,2]

Corrispondence to: Carmen Laurino - carmen.laurino@hotmail.it

ABSTRACT

Flaxseeds are a significant dietary source of α-linolenic acid, dietary fibre and lignans. The aim of this review is to describe current knowledge concerning the therapeutic and physiological effects of dietary flaxseeds on the gastrointestinal system. We reviewed in vitro, in vivo and clinical studies published between 1976 and 2016 and examining the gastrointestinal activity of lignans, omega-3 fatty acids and fibre in dietary flaxseeds. We searched PubMed/MEDLINE using the keywords 'lignans', 'flaxseeds', 'fibre', 'omega 3 fatty acids' and 'gastrointestinal' alone or combined. The results indicate that flaxseed lignans and omega-3 fatty acids may be effective for preventing and reducing colon cancer, modulating constipation and diarrhoea through the synergic activity of lignans, fibre and omega-3 fatty acids, and reducing bowel inflammation through downregulation by omega-3 fatty acids of the expression of pro-inflammatory cytokines. In addition, a positive effect on the gut microbiota has been observed both in experimental in vivo studies and in clinical trials, probably through lignan activity, although the exact mechanism of action has not been elucidated. Our review suggests dietary flaxseeds may have beneficial effects on the gastrointestinal system.

Keywords
Gastroenterology
Flaxseeds
Lignans
Omega 3
Dietary fibre

Introduction

Oestrogens bind oestrogen receptors (ERs), displaying different effects in different target cells and tissue. Oestrogen biosynthesis by the ovaries is reduced with ageing, with some withdrawal symptoms seen during menopause [1]. To reduce these side effects (hot flushes, vaginal atrophy, skin and adnexa dystrophy, bone loss and changes in cardiovascular and metabolic function), hormone replacement therapy (HRT) or botanical dietary supplements which simulate oestrogen activity are prescribed. However, HRT involves some risks such as the development and/or progression of breast and uterine cancer.

Phytoestrogens, a group of oestrogen-like naturally derived compounds [2, 3] found in animal and human food [4, 5], are becoming very popular because they bind ERs with weak oestrogenic activity potential [6], thus possibly increasing breast cancer risk. However, their agonistic activity competes with their very active oestrogenic activity to produce an antioestrogenic effect [7]. Hence, they could protect against premenopausal breast cancer but increase breast cancer risk after the menopause [8].

The two main classes of phytoestrogens found in the human diet are isoflavones (daidzein, genistein and glycitein) and lignans (enterodiol and enterolactone). The isoflavone daidzein can be metabolized by intestinal bacteria into O-desmethylangolensin (ODMA) and, in approximately 30–50% of individuals, into equol. Isoflavones are primarily found in soy and soy products, while lignans are found in cereals, flaxseeds, nuts, coffee, tea, fruit and vegetables [9, 10].

Flaxseeds (*Linum usitatissimum*), also known as linseeds, are flat, oval-shaped seeds with a pointed tip, ranging in colour from deep brown (usually of Canadian origin) to light yellow (golden; usually of US origin). Seed colour is determined by the amount of pigment in the seed coat. Brown and golden flaxseeds have a similar nutrient profile and differences between them are probably a result of differences in environmental and growing conditions rather than seed colour [11].

[1]Dipartimento Chirurgico, Medico, Odontoiatrico e di Scienze Morfologiche con Interesse Trapiantologico, Oncologico e di Medicina Rigenerativa, Università degli Studi di Modena e Reggio Emilia, Largo del Pozzo 71, 41124 Modena (MO), Italy
Tel +39 0594222483
[2]Network del Secondo Parere, Modena (MO), Italy

Flaxseeds are the main source of α-linolenic acid (ALA) and the richest dietary source of lignans [12]. The availability of a rich source of ALA has recently led to investigations of the potential value of flaxseeds in the human diet [13]. Flaxseeds are also an important source of dietary fibre (35–45%), with the proportion of soluble to insoluble fibre varying between 1:4 and 2:3 [14]. Flaxseeds are sold as whole seed, ground seed and partially defatted flaxseed meal (PDFM), with PDFM containing the highest content of dietary fibre in common forms of flaxseeds [15].

In addition, the lignans entenolactone and enterodiol are metabolized by microbiota and may have anticancer effects [16–18].

Flaxseeds contain approximately 30% dietary fibre, one third of which is water soluble. The majority of the water-soluble fibre belongs to a group of heterogenic polysaccharides consisting of neutral arabinoxylans and highly acidic rhamnose-containing polysaccharides present on the outside of the seed coat (the mucilage), which form highly viscous solutions when mixed with water [19]. Consequently, these polysaccharides are easily extractable using only water, and have been effective in reducing the digestibility of fat [20, 21] and in improving faecal fat excretion [22]. In addition, the soluble fibre, mainly in the form of mucilage, forms a gel-like substance in contact with water and may contribute to stool softening, while the insoluble fibre is assumed to be responsible for the faecal bulking effect observed with flaxseed use [11].

Aim and searching criteria

We reviewed in vitro, in vivo and clinical studies published between 1976 and 2016 on the gastrointestinal effects of lignans, omega-3 fatty acids and fibre in dietary flaxseeds. We searched PubMed/MEDLINE, using the keywords 'lignans', 'flaxseeds', 'fibre', 'omega 3 fatty acids' and 'gastrointestinal' alone or combined. This review aims to describe current knowledge concerning the therapeutic effects of flaxseeds on the gastrointestinal system, their physiological mechanism of action in specific pathological conditions, and future therapeutic perspectives.

Results and Discussion

Flaxseeds in colon cancer

Different classes of lignans have been shown to inhibit tumorigenesis [23, 24]. Among these, nordihydroguaiaretic acid (NDGA), a well-known lignan from the resinous exudate of the creosote bush (*Larrea tridentata* (DC.) Coville, Zygophyllaceae) has been shown to prevent both activation of carcinogens and tumour promotion in mouse skin [23, 25] and the bladder [26], and it has been suggested that this effect may extend to colorectal cancer [27]. The development of colorectal cancer may be influenced by oestrogenic exposure, so phytoestrogens may act through hormonal mechanisms to reduce cancer risk by binding to ERs [28] or interacting with enzymes involved in sex steroid biosynthesis and metabolism [29]. The main in vitro and in vivo experimental studies are summarized in Table 1, while clinical studies on dietary flaxseed application and evidence are described in Table 2.

Sung *et al* [30] demonstrated that at 100 μM concentration, the lignans enterolactone and enterodiol significantly reduced the proliferation of four human colon tumour cell lines (LS174T, Caco-2, HCT-15, T-84) after 8–10 days of incubation, but with a major specificity for enterolactone, which was more than twice as effective as enterodiol at the same concentration.

Furthermore, an experimental in vivo study evaluated the effects of feeding flaxseed oil and flaxseed meal on azoxymethane (AOM)-induced aberrant crypt foci (ACF) in Fischer 344 male rats [31]. Study rats were divided into seven groups and were fed an AIN 93G diet (control, C), C+7% or 14% soybean oil, C+7% or 14% flaxseed oil, or C+10% or 20% flaxseed meal. At 7 and 8 weeks of age, all rats then received 16 mg/kg body weight of AOM. After euthanasia with CO_2 at 17 weeks of age, results indicated that flaxseed meal and flaxseed oil reduced the incidence of ACF, which are putative precursor lesions in colon cancer, in the distal colon by 88% and 77% and in the proximal colon by 86% and 87%, with total reductions of 87.5% and 84%, respectively. Glutathione-S-transferase (GST) activity was significantly ($p<0.05$) higher in rats fed C+7% and 14% flaxseed oil and C+10% and 20% flaxseed meal, as compared to rats fed C+7% and 14% soybean oil diets. These findings confirmed that flaxseed oil and flaxseed meal reduced the incidence of AOM-induced ACF formation and may therefore be effective chemopreventive agents.

Bommareddy *et al* [32] investigated the effects of dietary flaxseeds versus corn meal on the development of colon cancer. Forty-eight male Fischer rats were divided into two groups of 24 each, and their diet was supplemented with either 15% corn meal or 15% flaxseed meal. The authors induced carcinogenesis by subcutaneous injection of AOM (15 mg/kg) once a week for 3 consecutive weeks. After 35 weeks, the site, size and number of tumours were noted and the fatty acid composition of the gastrointestinal tract was analysed.

Reference	Application	Type of the study	Type of control	Dose and time of treatment	Outcomes
Sung *et al* (1998) [30]	Antitumour activity	Experimental in vitro study; colon tumour cell lines (LS174T, Caco-2, HCT-15, T-84)	Not available	100 μM concentration of the lignans enterolactone and enterodiol; 8 to 10- day incubation period	Significant reduction in proliferation of the four human colon tumour cell lines, but with a major specificity for enterolactone, which was more than twice as effective as enterodiol at the same concentration
Williams *et al* (2007) [31]	Colon cancer prevention	Experimental in vivo study; Fischer 344 male rats; C+7% and 14% flaxseed oil group and C+10% and 20% flaxseed meal group	AIN 93G diet (control, C) group, C+7% and 14% soybean oil group	All rats received 16 mg/kg body weight of azoxymethane (AOM) at 7 and 8 weeks of age to induce carcinogenesis. Supplementation for 17 weeks (see details for each group)	Flaxseed meal and flaxseed oil reduced the incidence of aberrant crypt foci (ACF), which are putative precursor lesions in the development of colon cancer in the distal colon by 88% and 77%, in the proximal colon by 86% and 87% with a total reduction of 87.5% and 84%, respectively. Glutathione-S-transferase (GST) activities were significantly ($p<0.05$) higher in rats fed C+7% and 14% flaxseed oil and C+10% and 20% flaxseed meal, as compared to rats fed C+ soybean oil diets.
Bommareddy *et al* (2006) [32]	Colon cancer prevention	Experimental in vivo study; 48 male Fischer rats; experimental group supplemented with 15% flaxseed meal diet (24 rats)	Control group supplemented with 15% corn meal diet (24 rats)	All rats received subcutaneous injections of AOM (15 mg/kg) once a week for 3 consecutive weeks to induce carcinogenesis; supplementation for 35 weeks	Colon cancer incidence, multiplicity and tumour size were 82.6% and 29.4%, 1.3 and 0.3, and 44.4 and 5.3 mm^2 in the corn and flaxseed meal groups, respectively. The flaxseed meal group exhibited higher levels of omega-3 fatty acids. COX-1 and COX-2 expression in the flaxseed group was significantly lower ($p<0.05$) as compared to the corn group
Hernandez-Salazar *et al* (2013) [33]	Colon cancer prevention	Experimental in vivo study; Sprague-Dawley rats; experimental groups: flaxseed group and total non-digestible fraction (TNDF) group	Non-supplemented group (AOM group);	Carcinogenesis was induced by injections of AOM; 2 tablespoons of flaxseed daily and 2 tablespoons of TNDF	Flaxseed significantly reduced crypt multiplicity (10.50±3.5) compared with TNDF treatment (34.00±11.0); flaxseed induced the expression of p53 and p21, whereas TNDF triggered the p21-independent expression of p53; TNDF induced mitochondrial apoptosis because the TNDF+AOM group exhibited increased caspase-3 expression, decreased bcl-2 expression and increased bax expression
Bommareddy *et al* (2009) [34]	Colon cancer prevention	Experimental in vivo study; male in Apc(Min) mice; experimental groups: flaxseed meal and flaxseed oil supplemented diets	Control groups: control (AIN-93M meal), corn meal and corn oil supplemented diets	Not available	Dietary flaxseed significantly decreased ($p<0.05$) colon cancer size and number of foci compared to controls; lignans were detected in the serum and colon samples in the flaxseed meal group, and COX-1 and COX-2 expression in the gut biopsies were significantly lower ($p<0.05$) as compared to the corn meal group
Jenab *et al* (1999) [35]	Colon cancer prevention	Experimental in vivo study; seven groups of 6 female rats	Control group: basal high-fat (20%) diet (BD) group	4 Weeks; BD, BD supplemented with 2.5%, 5.0% or 10.0% flaxseed, or BD with daily gavage of 0.75, 1.5 or 3.0 mg of secoisolariciresinol diglycoside (SDG)	Specific and total activities of beta-glucuronidase in the caecum were significantly related to the levels of flaxseed ($r=0.539$, $p<0.008$ and $r=0.599$, $p<0.002$, respectively) and SDG ($r=0.567$, $p<0.007$ and $r=0.435$, $p<0.04$, respectively). Urinary mammalian lignan excretion also increased with increasing flaxseed or SDG levels and thus was significantly related to the specific activity ($r=0.38$, $p<0.017$) and total activity ($r=0.429$, $p<0.007$) of beta-glucuronidase
Xu *et al* (2012) [12]	Constipation	Experimental in vivo study; constipated mouse model (induced by atropine-diphenoxylate)	Normal mouse group	Supplementation with 2.5%, 5% and 10% (w/w) partially defatted flaxseeds meal (PDFM (L-, M- and H-PDFM)) for 14 days	M- and H-PDFM significantly increased small intestinal transit rates in the constipation model mice; the overall amount of administered PDFM markedly shortened the time to defecation; M- and H-PDFM significantly increased stool frequency and weight in both normal and constipation model mice
Palla *et al* (2015) [44]	Diarrhoea	Experimental in vivo study; mice	Normal mice	100, 300 and 500 mg/kg	Flaxseeds reduced the diarrhoeal score in mice by 39%, 63.9% and 68.34% at doses of 100, 300 and 500 mg/kg, respectively, the intestinal secretions from insulated gut loops by 24.12%, 28.09% and 38.8%, and intestinal motility by 31.66%, 46.98% and 56.2%
Cheshmehkani *et al* (2015) [48]	Colon inflammation	Experimental, in vivo study; Sprague-Dawley rats	Control diet	7 Weeks	Increased colon free-fatty acid receptor-4 (FFAR4) expression; reduction of TNF-α expression
Martin and Bolling (2015) [49], Zarepoor *et al* (2014) [50]	Colon inflammation	Experimental, in vivo study; C57BL/6 mice	Control diet	10% flaxseed diet	Increased inflammatory bowel disease activity index
Jonecova *et al* (2015) [54]	Turnover of both epithelial and lamina propria cells of the colon mucosa	Experimental, in vivo study; piglets fed with flaxseed diet and a flaxseed+sunflower oil diet	Normal diet+ sunflower oil	Flaxseed diet; from 10 days before to 21 days after weaning	Increased crypt depth in comparison with both groups supplemented with sunflower oil ($p<0.05$ and $p<0.001$, respectively) on the weaning day. Significant decrease in villus height ($p<0.01$) and crypt depth ($p<0.01$) 21 days after weaning in comparison with the sunflower oil group. Significantly higher proliferative activity in the mucosal connective tissue, in the group with flaxseed supplementation in comparison with the sunflower oil group was observed on the day of weaning, as well as 3 days later (both $p<0.05$)

Table 1 - In vitro and in vivo studies of dietary flaxseed supplementation

Reference	Application	Type of the study	Type of control	Dose and time of treatment	Outcomes
Cockerell *et al* (2012) [11]	Constipation	Open randomized controlled trial; 40 patients affected by irritable bowel syndrome (IBS)	No flaxseed diet	4 Weeks; 14 patients received two tablespoons of whole flaxseeds daily; 13 patients received two tablespoons of ground flaxseeds daily;13 subjects did not receive any flaxseeds as control	Patients fed whole flaxseeds did not reach statistical significance versus ground flaxseeds (p=0.62), whole flaxseeds only (p=0.12) and ground flaxseeds only (p=0.10) regarding improvement in symptom severity and changes in stool frequency or consistency
Pruthi *et al* (2012) [47]	Postmenopausal hot flashes	Phase III, randomized, placebo-controlled, double-blind trial; 188 postmenopausal women with or without breast cancer affected by hot flashes	Placebo group	A flaxseed bar (providing 410 mg of lignans) or placebo bar for 6 weeks	Significant reduction in hot flashes of 50% in patients treated with flaxseed bar; gastrointestinal side effects were probably due to the high fibre content (20%) of the flaxseed bar
Brahe *et al* (2015) [53]	Gut microbiota modulation	Randomized controlled trial; 58 obese postmenopausal women	*Lactobacillus paracesei* F19 group; placebo group	6 Weeks; daily intake of either *L. paracasei* F19 (9.4×10^{10} colony-forming units), flaxseed mucilage (10 g) or placebo	Reduction in serum C-peptide and insulin release during an oral glucose tolerance test (p<0.05) and improved insulin sensitivity measured by the Matsuda index (p<0.05) in the flaxseed group. Quantitative modifications of 33 metagenomics species (p<0.01), including decreased relative abundance of eight Faecalibacterium species in the flaxseed group
Lagkouvardos *et al* (2015) [55]	Modulation of gut faecal species and blood metabolites	Observational non-controlled study	No control group	1 Week of flaxseed supplementation (0.3 g/kg/day flaxseeds)	Increase in blood enterolignans; significant increase in faecal excretion of propionate and glycerol; diversity and composition of dominant faecal bacteria remained individual specific throughout the study. Enterolactone production was linked to the abundance of two molecular species (*Ruminococcus bromii* and *Ruminococcus lactaris*); the relative sequence abundance of one Gemmiger species (Ruminococcaceae) and of *Coprococcus comes* (Lachnospiraceae) correlated positively with blood levels of LDL cholesterol and triglycerides, respectively

Table 2 - Clinical studies of dietary flaxseed supplementation

Expression of cyclooxygenase (COX)-1 and COX-2 and serum and colon concentrations of lignans were also investigated. Tumours were found in 82.6% and 29.4% of the corn meal and flaxseed groups, respectively. There were an average of 1.3 and 0.3 tumours with an average size of 44.4 and 5.3 mm^2, respectively, in the two groups. Higher levels of omega-6 fatty acids were found in colon and serum samples from the corn meal group, while higher levels of omega-3 were found in the flaxseed group, in addition to a significant reduction in COX-1 and COX-2 expression (p<0.05). Augmentation of omega-3 fatty acids and a reduction in COX-1 and COX-2 levels by lignans and omega-3 fatty acids in dietary flaxseed meal may be effective for preventing colon cancer.

A further study investigated the effect of two tablespoons of flaxseed daily and its total non-digestible fraction (TNDF) on the expression of genes involved in AOM-induced colon cancer in Sprague–Dawley rats [33]. Results suggest an association between cell cycle gene expression and the antioxidant activity of dietary flaxseed in colon cancer prevention. Specifically, flaxseed was more effective for preventing colon cancer by reducing crypt multiplicity compared to TDNF treatment (10.50±3.5 and 34.00±11.0, respectively). The flaxseed and TNDF diets induced cell cycle arrest but in different ways: while flaxseed activated the expression of p53 and p21, the TNDF diet induced p21 independently of p53 expression. Moreover, the unusual expression of apoptosis-related genes (e.g., an increase in caspase-3 and a reduction in bcl-2) found in the TNDF+AOM-treated group suggested mitochondrial apoptosis was induced by the TNDF diet.

A review by Bommareddy *et al* [34] confirmed colorectal cancer was prevented by dietary flaxseed in the AOM mouse model by augmenting colon levels of lignans and reducing expression of COX-1 and COX-2 in the colon. Apc(Min) mice were divided into five groups and fed a control diet (AIN-93M meal), corn meal, flaxseed meal, corn oil or flaxseed oil. At the end of the study, the group fed the flaxseed meal showed significantly reduced (p<0.05) colon cancer size and number of foci compared to controls. Higher levels of lignans were also found in the serum and colon of the flaxseed meal group in addition to minor expression of COX-1 and COX-2 enzymes in colon biopsies, compared to the group fed the corn meal diet (p<0.05).

Flaxseeds may also prevent colon cancer by reducing bacterial beta-glucuronidase activity, which increases colon cancer risk [35, 36]. An in vivo experimental study conducted by Jenab *et al* [35] considered a basal hyper lipid concentrated diet (20%) (HLCD), HLCD supplemented with 2.5%,

5.0% or 10.0% flaxseed, or HLCD supplemented with 0.75, 1.5 or 3.0 mg of secoisolariciresinol diglucoside (SDG), which is the main lignan precursor in mammals. The diets were administered daily for 4 weeks to seven groups of six female rats each. At the end of the study, caecum beta-glucuronidase enzyme activity was significantly higher in mice fed flaxseed and SDG, while urinary levels of lignans were positively correlated with the specific activity of the beta-glucuronidase enzyme. These findings support the hypothesis that flaxseeds prevent colon cancer by promoting the intestinal absorption of lignans.

Flaxseeds and functional gastrointestinal diseases

Flaxseeds display beneficial effects in functional gastrointestinal diseases such as irritable bowel syndrome (IBS) and colitis [37], and have a laxative effect in both healthy [37, 38] and constipated individuals [39], but there are only a small number of controlled trials. Xu *et al* [12] assessed whether PDFM facilitated faecal output in normal mice and in an experimental constipated mice model (induced by atropine-diphenoxylate) after supplementation with 2.5%, 5% and 10% (w/w) PDFM (L-, M- and H-PDFM) for 14 days. Results showed that M- and H-PDFM significantly increased small intestinal transit times in the constipated mice, while the overall amount of administered PDFM markedly shortened time to defecation, and M- and H-PDFM significantly increased stool frequency and weight in both the normal mice and the constipated mice. These findings suggest that the high concentration of fibre in PDFM has a role in increasing small intestinal transit times. In addition, bacterial fermentation of dietary fibre in the intestine also produces short-chain fatty acids such as acetate, propionate and butyrate [40]. There is a large body of evidence that suggests that physiological concentrations of these short-chain fatty acids significantly increase colonic motility and stimulate colonic transit through various mechanisms [41, 42]. Finally, an additional laxative effect might be due to oil remnants in the flaxseed meal [14].

An open randomized controlled trial [11] compared the clinical effectiveness of whole flaxseeds to ground flaxseeds for the treatment of 40 patients affected by IBS. For 4 weeks, 14 patients received two tablespoons of whole flaxseeds daily, 13 patients received two tablespoons of ground flaxseeds daily, and 13 subjects did not receive any flaxseeds as control. Symptom severity and bowel habit were assessed before and at the end of the study. Results showed that improvements in symptom severity and changes in stool frequency or consistency did not reach statistical significance in patients administered whole flaxseeds versus ground flaxseeds (p=0.62), whole flaxseeds versus control (p=0.12) or ground flaxseeds versus control (p=0.10). These findings did not show any substantial difference in clinical effectiveness between whole flaxseeds and ground flaxseeds for the treatment of patients with IBS.

The mechanisms by which flaxseeds improve symptoms of wind and bloating in IBS are not well understood, but the improvement is presumed to be due to effects exerted not by fibre and omega-3 fatty acids but by ALA and lignans. The laxative activity of flaxseeds is also controlled by a weak histaminergic effect activating the cholinergic pathway which also shows antidiarrhoeal activity, putatively through $K^{(+)}$ channel activation [43].

Another hypothesis suggests that the antidiarrhoeal effect of flaxseeds is mediated by inhibition of calcium channels, displaying antimotility and antisecretory activity [44]. In fact, since calcium channel blockers are known to have a non-specific spasmolytic effect, they are expected to block the effect of all agonists including acetylcholine, histamine and 5-HT [45].

An experimental in vivo study investigated the antidiarrhoeal and antimicrobial activity of flaxseeds in infectious and non-infectious diarrhoea in mice [44]. At doses of 100, 300 and 500 mg/kg, flaxseed extract reduced the diarrhoeal score in mice by 39%, 63.9% and 68.34%, intestinal secretions from insulated gut loops by 24.12%, 28.09% and 38.8%, and intestinal motility by 31.66%, 46.98% and 56.2%, respectively.

Flaxseed extract, when tested on isolated rabbit jejunum preparations, caused a dose-dependent inhibition of both spontaneous and high $K^{(+)}$ (80 mM)-induced contractions, and shifted the concentration–response curves of calcium to the right with suppression of the maximum response.

In another study, flaxseed consumption in rats caused inhibition of some proteolytic digestive enzymes, such as trypsin and chymotrypsin, and a reduction in protein utilization, in addition to negative effects on rat growth and a reduction in intestinal villi height, indicating flaxseeds should not be considered a complete source of protein [46].

Flaxseed extract administered at 12.5 mg/ml shows antimicrobial activity towards different bacterial species, including vancomycin-resistant *Enterococcus faecalis*, *Escherichia coli* K1, methicillin-resistant *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Salmonella typhi*, with efficacy ranging from 100% to 26% [44]. At the lower concentration of 10 mg/ml, the extract was not effective in suppressing *E. coli* K1, exhibiting only bacteriostatic activity against *P. aeruginosa* and *S. typhi*. The effectiveness of

flaxseed extract against Gram positive and Gram negative bacteria, including drug-resistant pathogens, suggests the presence of constituents that could serve as a source of new and better antimicrobial compounds.

Controversially, abdominal distension, flatulence, diarrhoea and nausea were reported in a phase III, randomized, placebo-controlled, double-blind trial with flaxseed administration in 188 postmenopausal women with or without breast cancer affected by hot flashes [47]. Consumption of a flaxseed bar providing 410 mg of lignans or a placebo bar for 6 weeks significantly reduced hot flashes by 50%, but gastrointestinal side effects were also recorded, probably due to the high fibre content (20%) of the flaxseed bar.

Flaxseeds and bowel inflammation

It has also been reported that flaxseeds have intestinal anti-inflammatory activity. Cheshmehkani *et al* [48] identified free-fatty acid receptor-4 (FFAR4), one the receptors of omega-3 fatty acids, on the colon mucosa of Sprague–Dawley rats, as a modulator of anti-inflammatory and insulin-sensitizing effects in response to polyunsaturated fatty acids such as ALA which occur naturally in flaxseeds. The mechanism involves stimulation by FFAR4 of secretion of the insulin secretagogue glucagon-like peptide-1 (GLP-1) from the gastrointestinal tract, which acts as a dietary sensor to regulate energy availability. The administration of a flaxseed diet for 7 weeks results in increased colon FFAR4 expression, and in reduced expression of the pro-inflammatory cytokine TNF-α compared to animals fed a control diet.

Controversially, an experimental in vivo study reported that a 10% flaxseed diet increased the inflammatory bowel disease activity index in 2% dextran sulphate sodium (DSS)-treated C57BL/6 mice [49, 50].

Despite the well-documented anti-inflammatory properties of omega-3 polyunsaturated fatty acids, supplementation with flaxseed abolished the anti-inflammatory effect of *Lactobacillus plantarum*, except for IL-2 levels in rats treated with the pro-carcinogen N,N-dimethylhydrazine (DMH) [51]. This effect probably depends on the high content of ALA (40–60%) in flaxseed and their metabolites. Nemcova *et al* [52] reported that flaxseeds rich in polyunsaturated fatty acids may support the immunomodulatory effects of *L. plantarum* specifically through the stimulatory effect of the probiotic on adhesion of *E. coli* K88 to the intestinal mucosa of piglets.

Flaxseeds and gut microbiota

Flaxseeds modulate gut microbiota. A randomized controlled trial evaluated the effect of interventions with *Lactobacillus paracasei* F19 or flaxseed mucilage on the gut microbiota and metabolic risk markers in 58 obese postmenopausal women [53]. *L. paracasei* F19 (9.4×10^{10} colony-forming units), flaxseed mucilage (10 g) or placebo was administered daily for 6 weeks. Quantitative metagenomics analysis of faecal DNA was performed to identify changes in the gut microbiota. Results showed that dietary flaxseed mucilage consumed over 6 weeks reduced serum C-peptide and insulin release during an oral glucose tolerance test ($p<0.05$) and improved insulin sensitivity measured by the Matsuda index ($p<0.05$). Comparison of gut microbiota composition at baseline and after 6 weeks of treatment with flaxseed mucilage showed quantitative modifications of 33 metagenomics species ($p<0.01$), including decreased relative abundance of eight Faecalibacterium species, but this effect was not seen in patients supplemented with *L. paracasei* F19. Gut microbiota modulation and insulin sensitivity improvement might be a good therapeutic option in the clinical setting.

A further study in piglets investigated the effect of 30-day supplementation with flaxseed and lactobacilli compared with control supplementation based on sunflower oil [54]. Analyses of jejunal mucosa after the study showed that flaxseed and lactobacilli supplementation significantly reduced villus height and crypt depth but resulted in increased cell proliferation in the epithelial and lamina propria as compared with control piglets ($p<0.01$ and $p<0.05$, respectively).

In contrast, a further study investigated the impact of flaxseed supplementation (0.3 g/kg/day flaxseeds for 1 week) on faecal bacterial species and their associations with faecal and blood metabolites in nine healthy subjects [55]. An increase in blood enterolignans and a significant increase in faecal excretion of propionate and glycerol was noted. The diversity and composition of microbial species in individual stool was generally conserved during the study period. Furthermore, the presence of *Ruminococcus bromii* and *Ruminococcus lactaris* was mostly correlated with enterolactone production, while the presence of faecal Bacteroidales was associated with an increased concentration of some acids, such as acetic, isovaleric and isobutyric, in stool, and a reduced concentration of serum triglycerides. Finally, some bacterial species, such as Ruminococcaceae and Lachnospiraceae, were directly correlated with elevated serum levels of LDL cholesterol and triglycerides, respectively. These findings do not support the hypothesis that flaxseed supplementation alters the faecal metabolome and dominant bacterial communities, but do suggest that Ruminococcaceae may be involved in the regulation of enterolignan production and blood lipids.

Dietary flaxseed as functional food

Flaxseed is becoming important as a functional food, defined as a food or food ingredient that may provide physiological benefits and help prevent and/or cure disease [56], based on in vitro studies on cell lines, in vivo studies on animal models and human clinical studies [57]. Consequently, it is necessary to examine the bioactive ingredients of each nutritional component in functional foods and the biochemical and pathophysiological basis of metabolic imbalance or diseases that these natural compounds can modify.

Flaxseed currently has new prospects as a functional food because of the consumer's growing interest in foods with health benefits. Owing to its excellent nutritional profile and potential health benefits, it has become an attractive ingredient in diets specifically designed for particular health benefits [58]. Flaxseed is considered to be a functional food owing to the presence of three main active constituents: ALA, lignans and dietary fibre [59]. ALA is one of the essential polyunsaturated fatty acids and is reported to exhibit anti-inflammatory, antithrombotic and anti-arrhythmic properties [60]. In addition, public health organizations recommend that omega-3 fatty acids be incorporated into the diet. In this context, flaxseed is the best omega-3 fatty acid source for non-fish eaters. Consequently, flaxseed has been proposed as a nutritional additive for different foods, such as baked cereal products, ready-to-eat cereals, fibre bars, salad toppings, meat extenders, bread, muffins and spaghetti [61, 62]. Lignans contain phytoestrogens which have been reported to reduce the risk of hormone-dependent cancers, heart disease and osteoporosis [63, 64]. Finally, the water-binding capacity of the insoluble fibre in flaxseed increases intestinal bulk, which is effective for the treatment of bowel inflammation and functional gastrointestinal diseases. Soluble fibre from flaxseed mucilage increases the viscosity of intestinal contents and delays gastric emptying and nutrient absorption [65].

Clinical trials have confirmed that flaxseed contains important quantities of compounds with functional and bioactive properties which are effective for disease prevention and have therapeutic benefits. This encourages the development of new branded healthy and functional foods using flaxseeds, flaxseed oil and flaxseed cake. However, more in vivo studies are required to validate the health benefits of flaxseed constituents and to determine the minimum amount of flaxseed required for its therapeutic potential to be realised in various population groups, including pregnant and lactating women, and to determine possible side effects. Rapid, reproducible and economic techniques for the analysis of nutraceuticals from flaxseed need to be developed.

Conclusions

Our review suggests a potential beneficial effect of flaxseeds in gastroenterology. For instance, flaxseed lignans and omega-3 fatty acids may be effective for preventing or reducing colon cancer, for modulating constipation and diarrhoea by the synergic activity of lignans, fibre and omega-3 fatty acids, and for reducing bowel inflammation through down-regulation by omega-3 fatty acids of the expression of pro-inflammatory cytokines.

Finally, positive modulation of the gut microbiota has been observed both in experimental in vivo studies and in clinical trials, probably due to lignan activity, although the exact mechanism of action has not yet been elucidated. The large numbers of experimental in vitro and in vivo studies in animal models suggest that dietary flaxseed may be used in human clinical practice, for instance for gastrointestinal diseases, although the number of clinical randomized controlled studies is very small. However, the results discussed in this review have confirmed the clinical importance of dietary flaxseed in cancer, in constipation and in modulation of the gut microbiota for relieving symptoms and improving quality of life. Nutraceuticals are increasing being administered in combination with normal drug treatment for gastrointestinal disease and can provide significant symptomatic improvement. Regular daily flaxseed intake is very effective due to its reported benefits, particularly its preventive action against bowel cancer.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Boonmuen N, Gong P, Ali Z, Chittiboyina AG, Khan I, Doerge DR, *et al*. (2016) Licorice root components in dietary supplements are selective estrogen receptor modulators with a spectrum of estrogenic and anti-estrogenic activities. Steroids 105:42-49
2. Taxvig C, Elleby A, Sonne-Hansen K, Bonefeld-Jorgensen EC, Vinggaard AM, Lykkesfeldt AE, *et al*. (2010) Effects of nutrition relevant mixtures of phytoestrogens on steroidogenesis, aromatase, estrogen, and androgen activity. Nutr Cancer 62(1):122-131
3. Adlercreutz H, Bannwart C, Wahala K, Makela T, Brunow G, Hase T, *et al*. (1993) Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J Steroid Biochem Mol Biol 44(2):147-153
4. Adlercreutz H, Mazur W (1977) Phyto-oestrogens and Western diseases. Ann Med 29(2):95-120
5. Ingram D, Sanders K, Kolybaba M, Lopez D (1997) Case-control study of phyto-oestrogens and breast cancer. Lancet 4;350(9083):990-994

6. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, *et al*. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139(10):4252-4263
7. Miksicek RJ (1994) Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. J Steroid Biochem Mol Biol 49(2-3):153-160
8. Glazier MG, Bowman MA (2001) A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy. Arch Intern Med 14;161(9):1161-1172
9. Duncan AM, Phipps WR, Kurzer MS (2003) Phyto-oestrogens. Best Pract Res Clin Endocrinol Metab. 17(2):253-271
10. Magee PJ, Rowland IR (2004) Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. Br J Nutr 91(4):513-531
11. Cockerell KM, Watkins AS, Reeves LB, Goddard L, Lomer MC (2012) Effects of linseeds on the symptoms of irritable bowel syndrome: a pilot randomised controlled trial. J Hum Nutr Diet 25(5):435-443
12. Xu J, Zhou X, Chen C, Deng Q, Huang Q, Yang J, *et al*. (2012) Laxative effects of partially defatted flaxseed meal on normal and experimental constipated mice. BMC Complement Altern Med 12:14
13. Martinchik AN, Baturin AK, Zubtsov VV, Molofeev V (2012) Nutritional value and functional properties of flaxseed. Vopr Pitan 81(3):4-10
14. Tarpila A, Wennberg T, Tarpila S (2005) Flaxseed as a functional food. Curr Top Nutraceut Res 3:167-188
15. Bassett CM, Rodriguez-Leyva D, Pierce GN. (2009) Experimental and clinical research findings on the cardiovascular benefits of consuming flaxseed. Appl Physiol Nutr Metab 34(5):965-974
16. Adlercreutz H, Mousavi Y, Hockerstedt K (1992) Diet and breast cancer. Acta Oncol 31(2):175-181
17. Serraino M, Thompson LU (1992) The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis. Nutr Cancer 17(2):153-159
18. Serraino M, Thompson LU (1991) The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis. Cancer Lett 60(2):135-142
19. Warrand J, Michaud P, Picton L, Muller G, Courtois B, Ralainirina R, *et al*. (2005) Structural investigations of the neutral polysaccharide of *Linum usitatissimum* L. seeds mucilage. Int J Biol Macromol 35(3-4):121-125
20. Kristensen M, Damgaard TW, Sorensen AD, Raben A, Lindelov TS, Thomsen AD, *et al*. (2008) Whole flaxseeds but not sunflower seeds in rye bread reduce apparent digestibility of fat in healthy volunteers. Eur J Clin Nutr 62(8):961-967
21. Kristensen M, Savorani F, Christensen S, Engelsen SB, Bugel S, Toubro S, *et al*. (2013) Flaxseed dietary fibers suppress postprandial lipemia and appetite sensation in young men. Nutr Metab Cardiovasc Dis 23(2):136-143
22. Kristensen M, Jensen MG, Aarestrup J, Petersen KE, Sondergaard L, Mikkelsen MS, *et al*. (2012) Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depend on food type. Nutr Metab (Lond) 9:8
23. Wang ZY, Agarwal R, Zhou ZC, Bickers DR, Mukhtar H (1991) Antimutagenic and antitumorigenic activities of nordihydroguaiaretic acid. Mutat Res 261(3):153-162
24. Yang K, Lamprecht SA, Liu Y, Shinozaki H, Fan K, Leung D, *et al*. (2000) Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. Carcinogenesis 21(9):1655-1660
25. Belman S, Solomon J, Segal A, Block E, Barany G (1989) Inhibition of soybean lipoxygenase and mouse skin tumor promotion by onion and garlic components. J Biochem Toxicol 4(3):151-160
26. Yu A, Hashimura T, Nishio Y, Kanamaru H, Fukuzawa S, Yoshida O (1992) Anti-promoting effect of nordihydroguaiaretic acid on N-butyl-N-(4-hydroxybutyl)nitrosamine and sodium saccharin-induced rat urinary bladder carcinogenesis. Jpn J Cancer Res 83(9):944-948
27. Hoshiyama Y, Sekine T, Sasaba T (1993) A case-control study of colorectal cancer and its relation to diet, cigarettes, and alcohol consumption in Saitama Prefecture, Japan. Tohoku J Exp Med 171(2):153-165
28. Setchell KD (1998) Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. Am J Clin Nutr 68(6 Suppl):1333S-1346S
29. Krazeisen A, Breitling R, Moller G, Adamski J (2001) Phytoestrogens inhibit human 17beta-hydroxysteroid dehydrogenase type 5. Mol Cell Endocrinol 171(1-2):151-162
30. Sung MK, Lautens M, Thompson LU (1998) Mammalian lignans inhibit the growth of estrogen-independent human colon tumor cells. Anticancer Res 18(3A):1405-1408
31. Williams D, Verghese M, Walker LT, Boateng J, Shackelford L, Chawan CB (2007) Flax seed oil and flax seed meal reduce the formation of aberrant crypt foci (ACF) in azoxymethane-induced colon cancer in Fisher 344 male rats. Food Chem Toxicol 45(1):153-159
32. Bommareddy A, Arasada BL, Mathees DP, Dwivedi C (2006) Chemopreventive effects of dietary flaxseed on colon tumor development. Nutr Cancer 54(2):216-222
33. Hernandez-Salazar M, Guevara-Gonzalez RG, Cruz-Hernandez A, Guevara-Olvera L, Bello-Perez LA, Castano-Tostado E, *et al*. (2013) Flaxseed (*Linum usitatissimum* L.) and its total non-digestible fraction influence the expression of genes involved in azoxymethane-induced colon cancer in rats. Plant Foods Hum Nutr 68(3):259-267
34. Bommareddy A, Zhang X, Schrader D, Kaushik RS, Zeman D, Mathees DP, *et al*. (2009) Effects of dietary flaxseed on intestinal tumorigenesis in Apc(Min) mouse. Nutr Cancer 61(2):276-283
35. Jenab M, Rickard SE, Orcheson LJ, Thompson LU (1999) Flaxseed and lignans increase cecal beta-glucuronidase activity in rats. Nutr Cancer 33(2):154-158

36. Jenab M, Thompson LU (1996) The influence of flaxseed and lignans on colon carcinogenesis and beta-glucuronidase activity. Carcinogenesis 17(6):1343-1348
37. Cunnane SC, Hamadeh MJ, Liede AC, Thompson LU, Wolever TM, Jenkins DJ (1995) Nutritional attributes of traditional flaxseed in healthy young adults. Am J Clin Nutr 61(1):62-68
38. Dahl WJ, Lockert EA, Cammer AL, Whiting SJ (2005) Effects of flax fiber on laxation and glycemic response in healthy volunteers. J Med Food 8(4):508-511
39. Tarpila S TA, Grohn P, Silvennoinen T, Linderberg L (2004) Efficacy of ground flaxseed on constipation in patients with irritable bowel syndrome. Curr Top Nutraceut Res (2):7
40. Stewart ML, Savarino V, Slavin JL (2009) Assessment of dietary fiber fermentation: effect of *Lactobacillus reuteri* and reproducibility of short-chain fatty acid concentrations. Mol Nutr Food Res 53(Suppl 1)
41. Grider JR, Piland BE (2007) The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. Am J Physiol Gastrointest Liver Physiol 292(1):G429-G437
42. Soret R, Chevalier J, De Coppet P, Poupeau G, Derkinderen P, Segain JP, *et al*. (2010) Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. Gastroenterology 138(5):1772-1782
43. Hanif Palla A, Gilani AH (2015) Dual effectiveness of Flaxseed in constipation and diarrhea: possible mechanism. J Ethnopharmacol 169:60-68.
44. Palla AH, Khan NA, Bashir S, Ur-Rehman N, Iqbal J, Gilani AH (2015) Pharmacological basis for the medicinal use of *Linum usitatissimum* (flaxseed) in infectious and non-infectious diarrhea. J Ethnopharmacol 160:61-68
45. Gilani AH, Khan AU, Ali T, Ajmal S (2008) Mechanisms underlying the antispasmodic and bronchodilatory properties of *Terminalia bellerica* fruit. J Ethnopharmacol 116(3):528-538
46. Anaya K, Cruz AC, Cunha DC, Monteiro SM, Dos Santos EA (2015) Growth impairment caused by raw linseed consumption: can trypsin inhibitors be harmful for health? Plant Foods Hum Nutr 70(3):338-343
47. Pruthi S, Qin R, Terstreip SA, Liu H, Loprinzi CL, Shah TR, *et al*. (2012) A phase III, randomized, placebo-controlled, double-blind trial of flaxseed for the treatment of hot flashes: North Central Cancer Treatment Group N08C7. Menopause 19(1):48-53
48. Cheshmehkani A, Senatorov IS, Kandi P, Singh M, Britt A, Hayslett R, *et al*. (2015) Fish oil and flax seed oil supplemented diets increase FFAR4 expression in the rat colon. Inflamm Res 64(10):809-815
49. Martin DA, Bolling BW (2015) A review of the efficacy of dietary polyphenols in experimental models of inflammatory bowel diseases. Food Funct 6(6):1773-1786
50. Zarepoor L, Lu JT, Zhang C, Wu W, Lepp D, Robinson L, *et al*. (2014) Dietary flaxseed intake exacerbates acute colonic mucosal injury and inflammation induced by dextran sodium sulfate. Am J Physiol Gastrointest Liver Physiol 306(12):G1042-G1055
51. Stofilova J, Szabadosova V, Hrckova G, Salaj R, Bertkova I, Hijova E, *et al*. (2015) Co-administration of a probiotic strain *Lactobacillus plantarum* LS/07 CCM7766 with prebiotic inulin alleviates the intestinal inflammation in rats exposed to N,N-dimethylhydrazine. Int Immunopharmacol 24(2):361-368
52. Nemcova R, Borovska D, Koscova J, Gancarcikova S, Mudronova D, Buleca V, *et al*. (2012) The effect of supplementation of flax-seed oil on interaction of *Lactobacillus plantarum*--Biocenol LP96 and *Escherichia coli* O8:K88ab:H9 in the gut of germ-free piglets. Res Vet Sci 93(1):39-41
53. Brahe LK, Le Chatelier E, Prifti E, Pons N, Kennedy S, Blaedel T, *et al*. (2015) Dietary modulation of the gut microbiota--a randomised controlled trial in obese postmenopausal women. Br J Nutr 114(3):406-417
54. Jonecova Z, Toth S, Ciccocioppo R, Rodrigo L, Kruzliak P, Nemcova R (2015) Influence of dietary supplementation with flaxseed and lactobacilli on the mucosal morphology and proliferative cell rate in the jejunal mucosa of piglets after weaning. Int J Exp Pathol 96(3):163-171
55. Lagkouvardos I, Klaring K, Heinzmann SS, Platz S, Scholz B, Engel KH, *et al*. (2015) Gut metabolites and bacterial community networks during a pilot intervention study with flaxseeds in healthy adult men. Mol Nutr Food Res 59(8):1614-1628
56. SY A-O (2005) Highlights on functional foods, with special reference to flaxseed. Nat Fibers 2(3):5
57. Vadala M, Palmieri B (2015) [From algae to "functional foods"]. Clin Ter 166(4):e281-300
58. Oomah BD (2001) Flaxseed as a functional food source. J Sci FoodAgric 8:5
59. Kajla P, Sharma A, Sood DR (2015) Flaxseed-a potential functional food source. J Food Sci Technol 52(4):1857-1871
60. Simopoulos AP (1999) Essential fatty acids in health and chronic disease. Am J Clin Nutr 70(3 Suppl):560S-569S
61. Singh KK, Mridula D, Rehal J, Barnwal P (2011) Flaxseed: a potential source of food, feed and fiber. Crit Rev Food Sci Nutr 51(3):210-222
62. Singh KK, Jhamb S, Kumar R (2011) Effect of pretreatments on performance of screw pressing for flaxseed. J Food Process Eng 35(4)
63. Krajcova A, Schulsova V, Hajslova J, Bjelkova M (2009) Lignans in flaxseed. Czech J Food Sci 27:3
64. Toure A, Xu X (2010) Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components and health benefits. Compr Rev Food Sci Food Saf 9(8):261
65. Rebole A, Rodriguez ML, Ortiz LT, Alzueta C, Centeno C, Trevino J (2002) Mucilage in linseed: effects on the intestinal viscosity and nutrient digestion in broiler chicks. J Sci Food Agric 82:5