**Nutraceuticals for Protection and Healing of Gastrointestinal Mucosa**

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**Abstract:** Natural medicinal products have been used for millennia for the treatment of several ailments. Although many have been superseded by conventional pharmaceutical approaches, there is currently a resurgence in the interest in natural products by the general public and the use of complementary and alternative medicine is increasing rapidly in developed countries. Also, pharmaceutical industries are more and more interested in examining their potential as sources of novel medicinal compounds which may act as growth factor or show immunomodulatory or anti-microbial activity. The subgroup of natural bioactive compounds that bridge the gap between food products and drugs are termed nutraceuticals or functional foods. In contrast with most standard medicinal compounds, nutraceuticals are generally used to prevent rather than to treat disease. Many of the claims for such products are supported by very limited scientific evidence. However, there has recently been a great interest at evaluating the mechanism by which natural products exert their beneficial effects in the gastrointestinal tract. In particular, a major area of interest is for the use of biologically active chemical components of plants, i.e. phytochemicals, in a number of gastrointestinal disorders. While the major focus of phytochemical research has been on cancer prevention, several products of plant origin are being used and/or under study for a variety of other gastrointestinal problems. In this review we discuss the scientific evidence supporting the potential use of nutraceuticals as agents capable to prevent or accelerate healing of gastrointestinal mucosal damage, with a focus on polyphenol extracts obtained from apple.

**Keywords:** Apple polyphenols, gastric protection, intestinal protection, antioxidants, nutraceuticals, phytochemicals.

**INTRODUCTION**

The term “Nutraceutical” was firstly coined by DeFelice, chairman of the Foundation for Innovation in Medicine (FIM) [1, 2], and was later defined by Health Canada as, “A product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food...is demonstrated to have a physiological benefit or provide protection against chronic disease.” [3]. Nutraceuticals include a variety of dietary supplements (mineral, vitamin and antioxidant supplements) but also probiotics, herbs, essential oils, fortified foods and drinks.

For their ancient use among population as natural remedy against several diseases, phytochemicals, i.e. plant components with activities towards animal biochemistry and metabolism, are being widely studied for their ability to provide health benefits and to justify their use as nutraceuticals [4]. Phytochemicals are reported to exert several biological activities: i) participating as substrates and as cofactors or inhibitors of enzymes in biochemical reactions; ii) influencing absorption and stability of nutrients as well as scavenging and eliminating toxic compounds along the gastrointestinal tract (GIT); iii) being selective growth factors and fermentation substrates for beneficial oral and gastrointestinal bacteria as well as inhibitors of deleterious intestinal bacteria [4]. In the body phytochemicals may act at both systemic level (in organs outside the GIT) or inside the gastrointestinal tract, also if they are bound to insoluble dietary fibre [5]. In the case of benefits at systemic level (if any) phytochemicals effects are mediated by their bioavailability and/or by the activity of metabolites and biotransformation products. For long time these effects and the underlying mechanisms have been supported on the basis of in vitro studies on cell or tissue culture performed using high concentrations of polyphenol dosage that was absolutely curcumin or EGCG just after weaning was demonstrated to retard spontaneous development of multiple intestinal polyps within a few weeks of birth, the inclusion in the diet or in drinking water of curcumin or EGCG just after weaning was demonstrated to retard or reduce tumorgenesis at polyphenol dosage that was absolutely affordable by humans with diet [16-18]. Moreover animal studies...
Table 1.  Effects of Food Bioactive Polyphenols Inside the Gastrointestinal Tract (GIT)

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Phenolic compound and source</th>
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<td>Colorectal cancer patients</td>
<td>Pure resveratrol in capsules 2.2 – 4.4 mmol/d x 8 days</td>
<td>Colon cancer</td>
<td>Tumor cell proliferation is 5% reduced</td>
<td>Resveratrol (0.5-1.0 g/d) produces levels in the human GIT sufficient to elicit antitumorogenic effects.</td>
<td>[8]</td>
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<td>87</td>
<td>Patients with resected colon cancer or polyps</td>
<td>Mixture of flavonoids in tablets (apigenin and EGCG) 40 mg/d x 2.5 years</td>
<td>Colorectal cancer</td>
<td>No significant effect in cancer and polyp recurrence rates</td>
<td>Sustained long-term treatment with a flavonoid mixture could reduce the recurrence rate of neoplasia in patients with resected colon cancer.</td>
<td>[15]</td>
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<tr>
<td>15</td>
<td>colorectal cancer patients</td>
<td>Curcuminoids in capsules Curcuminoids from 0.5 to 4 g/d x 4 months</td>
<td>Colorectal cancer</td>
<td>No partial responses to treatment. Reduction by ~60% of inducible PGE2 production in blood 1 h after higher dose</td>
<td>Systemic pharmacological properties with 4g curcuminoids / die</td>
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<td>Chronic gastritis H. pylori patients</td>
<td>Curcumin tablets vs Antibiotics</td>
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<td>It is unlikely that curcumin alone has antibacterial effect on H. pylori.</td>
<td>[13]</td>
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<td>Subjects with proctitis 5 with Crohn’s</td>
<td>Curcumin in capsules 1.1.1-1.7 g/d x 1 month 1.1 g/d x 3 months</td>
<td>Inflammatory bowel disease</td>
<td>Amelioration of proctitis Reduction of Crohn’s Disease Activity Index</td>
<td>Curcumin at dosage of 1.1 g/d can improve inflammatory bowel disease</td>
<td>[14]</td>
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<td>150</td>
<td>Patients with adenomatos polyps</td>
<td>Soy-drink powder Isoflavones 83 mg/d vs 3 mg/d (control) x 12 months</td>
<td>Colorectal epithelial cell proliferation</td>
<td>In colon: ↑ cell proliferation and number of crypt labeled nuclei In cecum/colon: ↑ proliferation count. No effect on proliferation distribution and crypt height</td>
<td>Soy isoflavones has no effect on some markers of colorectal cancer</td>
<td>[12]</td>
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<tr>
<td>35</td>
<td>Apc^Min/+ mice</td>
<td>Curcumin 150-300-750 mg/kg pd Vs Control diet x 8 weeks</td>
<td>Intestinal tumorigenesis</td>
<td>Curcumin 300 mg/kg pd prevented/retarded adenoma formation</td>
<td>Curcumin is efficacy at a dose that in humans would be of 1.6 g per 70 kg person</td>
<td>[16]</td>
</tr>
<tr>
<td>141</td>
<td>Apc^Min/+ mice</td>
<td>Pure EGCG Caffeine 0.02-0.32% Vs Caffeine 0.044% Vs control in drinking water x 6-7 weeks</td>
<td>Intestinal tumorigenesis</td>
<td>EGCG decreases small intestinal tumorigenesis in a dose-dependent manner In small intestinal tumors increases levels of E-cadherin and decreases several trascriptor factors of oncogenic genes Caffeine has no effect</td>
<td>EGCG inhibits intestinal tumorigenesis in Apc^Min/+ mice</td>
<td>[17]</td>
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<tr>
<td>27</td>
<td>Apc^Min/+ mice</td>
<td>EGCG or ECG 0.01% Vs vehicle in drinking water x 2 months</td>
<td>Intestinal tumorigenesis</td>
<td>Only EGCG reduces the size and number of tumors and the total number of polyps and tumor load compared with controls. suppresses bFGF in tissue</td>
<td>EGCG may suppress intestinal tumorigenesis in vivo by reducing bFGF expression and angiogenesis</td>
<td>[18]</td>
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<td>78</td>
<td>piglets</td>
<td>Apple pomace diet (AP) Red-wine pomace diet (RWP) Vs Control diet (CD)</td>
<td>Total polyphenols ingestion (mg/d): AP 26.0 – 110 RW 57.2 – 242 CD 16.9 – 71.5 x 4 weeks</td>
<td>Morphology of GI tract</td>
<td>Villus length of jejunum and ileum in: CD decrease after weaning AP and RWP never decrease Villus breadth of jejunum in: CD increase by 73% AP and RWP increase by 10% Peyer’s patches area: CD increase AP and RWP no effect</td>
<td>RWP inhibitory effect on the jejunum villi growth AP and RWP stimulating effect on crypt size in piglet colon can reduce the GALT activation via the Peyer’s patches in the ileum.</td>
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<td>32</td>
<td>rats</td>
<td>Apple polyphenol extracts (APE) in drinking water 10 mL APE 10^4 M/d (phenols 0.9 mg/kg) x 10 days before indomethacin treatment</td>
<td>Injury of gastric mucosa</td>
<td>APE pretreatment decreased the extent of macroscopic and microscopic injury vs control.</td>
<td>APE prevents indomethacin injury on gastric mucosa in rats.</td>
<td>[20]</td>
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<tr>
<td>98</td>
<td>Apc^Min/+ mice Wild type mice</td>
<td>Apple polyphenol extracts (APE) in drinking water APE 8 μM catechin eq/kg b.w. Vs water x 12 weeks + Western Diet (WD) + Balanced Diet (BD)</td>
<td>Familial adenomatous polyposis (FAP)</td>
<td>APE prevents cachexia in Apc^Min/+ WD reduces polyp number and eliminates high-grade dysplasia in Apc^Min/+ mice influences polyp growth has antioxidant and anti-lipoperoxidation properties protects against DNA hypomethylation</td>
<td>APE is a candidate chemopreventive agent for coloorectal cancer, particularly for high risk populations (such as FAP) eating a WD</td>
<td>[22]</td>
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</table>
demonstrated, accordingly with epidemiological evidences, that mixture of phytochemicals, as found in natural products, may function even better than single pure compounds, due to synergisms among individual molecules and/or nutrients which can lead to modification of GIT morphology, as found by [19] in piglets administered with apple or red wine pomace. This fact together with biochemical and epigenetic activities of compounds in the GI environment and mucosa can explain the ability of phytochemicals to counteract dietary chemical toxins or the detrimental long term effect of nutritionally imbalanced diet, as demonstrated in studies relative to stomach [20, 21] and colorectal [22] mucosa protection by apple polyphenols (APE), respectively.

In this framework and starting from the ancient proverb that “an apple a day takes the doctor away”, in this review scientific evidence supporting the potential use of nutraceuticals as agents capable to prevent or accelerate healing of gastrointestinal mucosal damage will be discussed majorly focusing on apple polyphenols.

NUTRACEUTICALS IN THE PROTECTION AND HEALING OF GASTRIC MUCOSA DAMAGE

In the stomach there is a massive production of reactive oxygen species (ROS), their concentration being 1000-fold higher than that in other tissues or plasma [23]. Generation of ROS contributes to exogenous injury to the gastric mucosa, including damage brought about by ethanol or nonsteroidal anti-inflammatory drugs (NSAIDs) [24,25]. Moreover, ROS play a major role in the multistep process leading to the development of gastric cancer [26].

Naturally occurring anti-oxidants exert protective biochemical effects in a number of biological experimental systems and are known to scavenge oxygen and nitrogen free radicals breaking lipid chain peroxidation reaction. In particular, phenolic compounds, which are widely distributed in vegetable foods, are considered to play an important role in the prevention of oxidative damage in living systems [27]. Beside their action as radical scavengers, phenolic compounds also have several indirect effects, in fact they are able to inhibit lipoxygenase [28], to reduce platelet aggregation [29] and to reduce the bioavailability of food carcinogens [30]. Certain flavonoids or compounds with flavonoid-like properties have anti-ulcer activity and prevent gastric mucosal lesions brought about by a number of ulcerogens [31-33]. This effect has been related to their ability to inhibit lipid peroxidation, to scavenge ROS, and/or to modulate leukocyte function [34,35]. Singh and colleagues [36] demonstrated that melatonin and β-carotene were protective against indomethacin-induced gastric injury in the rat and that this effect was mediated by scavenging of oxygen-derived free radicals. Similarly, Alarcon de la Lastra et al. [31] showed that extra-virgin olive oil-enriched diets prevented indomethacin-induced gastric damage in rats. Finally, the synthetic flavonoid mecinanolad has been shown to prevent ethanol- and aspirin-induced gastric injury in the rat [37].

A long list of phytochemicals has been demonstrated to exert gastroprotective effects in \textit{in vivo} experimental model. Aloe vera, a medicine since before Roman times, contains several potentially bioactive compounds, including salicylates, lupeol, campesterol, β-sitosterol, γ-linolenic acid, aloechinA, and andraquiones. Acemannan, a component of \textit{Aloe vera}, has been reported to prevent stress-induced gastric ulceration in the rats [38]. \textit{In vivo} and \textit{in vitro} studies have provided evidence that the wound healing properties of \textit{Aloe vera} depend on its ability to stimulate collagen synthesis and fibroblast activity, thus enhancing the remodeling phase of the ulcer healing process [39,40].

Curcumin, the yellow pigment of turmeric (\textit{Curcuma longa}), a widely used spice in Indian and Thai cuisine, has been shown to improve endoscopic healing of peptic ulcers as well as symptoms of patients with non-ulcer dyspepsia [41]. A new zinc(II)-curcumin complex has been demonstrated to prevent cold-restraint stress-induced gastric ulcers in mice and this gastroprotective effect was associated to increased mRNA expression of HSP70 and attenuation of increased iNOS mRNA expression in the mucosa [42] (ok). Also, curcumin prevents indomethacin-induced gastric ulcerations in the rat and this effect is mediated by preventing peroxidase inactivation and by enhancing ROS scavenging [43].

The Bael (\textit{Aegle marmelos}) tree grows in tropical and subtropical countries. Luvangetin, a pyranocoumarin isolated from the seed of Aegle marmelos, has been shown to protect rodents gastric mucosa in multiple model of gastric ulceration through a nonidentified prostaglandin-independent pathway [44].

Garlic derivatives are commonly used by the public for a variety of condition including hypercholesterolemia. Also, ingestion of garlic results in the formation of diallyl disulphide from its organosulphur constituents, thus causing an increase in the tissue activities of phase II detoxification enzymes quinine reductase and glutathione transferase [45]. Garlic oil has been shown capable of preventing ethanol-induced gastric injury in rats and this protective effect has been attributed to its anti-oxidant activity [46]. Infact, garlic oil decreased lipid peroxidation and ameliorated the decrease in anti-oxidant enzyme levels brought about by ethanol [46].

Several other plant-derived extracts are of potential interest in the protection of gastric mucosa against axogenous injury. Often these extracts derive from sources such as South America folklore products. One example is provided by Dragon’s blood (i.e. Sangre de grado). This viscous red tree sap is used by indigenous cultures of the Amazon river basin because of its healing properties. Sangre de grado derives from several \textit{Croton} species and is used orally for gastritis and gastric ulcer [47]. In particular, sangre de grado was able to accelerate the healing of acetic acid-induced gastric ulcers in rats, reducing myeloperoxidase activity, ulcer size and bacterial content of the ulcer. Also, sangre de grado attenuated the ulcer-induced overexpression of proinflammatory genes such as TNF, iNOS, IL-1β, IL-6, and COX2 [47].

Natural honey and \textit{Nigella sativa} have been in use as a natural remedy for over thousand years in various part of the world. In particular, honey has been used to advantage as a topical preparation for wound healing due to its capacity to stimulate tissue growth, enhance re-epithelialization and minimize scar formation [48]. These effects are ascribed to honey’s acidity, hydrogen peroxide content, osmotic effect, antioxidant and immuno-stimulatory effect [48]. A study by Bukhari and coworkers has recently shown that both natural honey and \textit{Nigella sativa} seeds were equally effective in healing gastric ulcers induced by acetylsalicylic acid in rats at the macroscopic and microscopic level [49]. Phytochemicals have shown great promise in the treatment of several intractable infectious diseases, including opportunistic AIDS infections. In particular, honey can inhibit the growth of \textit{H. pylori}, the main etiologic agent of gastritis, peptic ulcer and gastric adenocarcinoma [50], thus raising the possibility of using honey orally for the treatment of \textit{H. pylori} infection.

In the past few years, we have been interested in evaluating the potential gastroprotective effect of a polyphenolic extract obtained from a variety of apple (i.e. \textit{Malus} Apple) tipical of Southern Italy. We have shown that this apple polyphenol extract (APE), rich in catechin and epicatechin, is able to prevent ROS- or indomethacin-induced injury to gastric epithelial cells \textit{in vitro} [19]. Also, APE is able to prevent indomethacin- or aspirin-induced injury to the rat stomach \textit{in vivo} both macroscopically and at the histological level [19,20]. This effect is associated with a significant increase in the intracellular anti-oxidant activity and to a decrease in drug-induced lipid peroxidation as assessed by determination of malondialdehyde, thus suggesting that the APE protective effect is mainly contributed by its anti-oxidant activity. Moreover, APE
counteracted the increased expression both at the mRNA and protein level, of COX-2 and HB-EGF, observed following administration of a damaging dose of aspirin [20]. More interestingly, the gastroprotective effect exerted by APE was not associated to a decrease in gastric acid secretion [20] (Fig. 1). This has potential clinical relevance, because prolonged acid suppression, which is the currently adopted method to prevent gastric damage against NSAIDs or aspirin, has been reported to be associated with increased susceptibility to infections, spontaneous hip fracture and formation of gastric polyps mainly in the fundus of the stomach [51-53].

Cordyceps sinensis (Cordyceps caterpillar mushroom) is a traditional Chinese medicine and health food used to support many organ systems. It is commercially produced in a liquid medium or on a solid (grain/potato) phase. Marchbank et al. have recently demonstrated that Cordyceps sinensis extract is able to prevent indomethacin –induced injury to the rat stomach, the results being similar to those obtained with the potent gastroprotective agent EGF [54].

Fermentation is a commonly used process in the standard food industry as well as in the bioactive food field. Fermentation of food products has many effects including partial degradation of protein constituents which, as well as aiding absorption from the gut, may also influence its biological activity. Recently, Fitzgerald et al. have demonstrated that a commercial fish protein hydrolysate preparation significantly prevents indomethacin-induced gastric injury in the rat and accelerates epithelial cell migration and proliferation (i.e. the main events leading to gastrointestinal mucosal repair) [55].

In conclusion, the use of food bioactive compounds to reduce drug-induced gastric injury is a reliable approach well acceptable by the general public and that can be particularly effective for stomach disorders, as food bioactive compounds are present in the stomach at the same concentration as in the original foods without uncertainties related to the bioavailability and biotransformation. In particular, the use of NSAID, including low-dose aspirin, continues to be associated with an unacceptable risk for GI ulceration and bleeding. The most effective strategy to reduce NSAID-associated gastric toxicity consists of the use of anti-secretory agents, such as H2-receptor antagonists or proton pump inhibitors (PPI). In particular, the use of NSAID combined with PPI has been adopted widely in clinical practice and it is estimated that PPI use could significantly reduce the rate of endoscopic NSAID-related ulcers. However, prolonged use of PPI is costly and might carry some risk (51-53). That dietary polyphenols, such as those extracted from apple, are able to prevent NSAID injury to the stomach without altering gastric homeostasis, indicates that there might be low cost, effective and safe means of addressing the issue of the prevention of NSAID gastropathy.

### ROLE OF NUTRACEUTICALS IN THE LOWER GASTROINTESTINAL TRACT

Foods contain complex mixtures of components [56]; to understand their impact on human health, their nature, origin, amount in the diet, bioavailability and microbial metabolism in the colon need to be investigated. In this respect, gaining understanding of the metabolism pathways of polyphenols and dietary fibre by the microbiota and the kind of bioactive metabolites that are formed during this process is of great interest. Moreover, the effects of such metabolites on the composition of the microbiota itself might be investigated to prepare new strategies that could be an important tool for future therapeutic approaches.

Several studies in the past decade have supported evidence for an alternative approach to the treatment of inflammatory bowel disease (IBD) via manipulation of the resident enteric microflora. This may be achieved with pre- and pro-biotics, and with exogenous butyrate. Increasing appreciation of the pivotal role of the enteric microflora in the maintenance of a healthy gut, and in the pathogenesis of IBD, [57,58] has supported the interest in this area. IBD, mainly ulcerative colitis (UC) and Crohn’s disease (CD), is a chronic relapsing disorder associated with uncontrolled inflammation within the gastrointestinal tract [59], which has been shown to predispose to the development of colorectal cancer later in life [60]. Recently, it has been estimated that IBD affects

![Control](image1.png)

![Esomeprazole](image2.png)

![APE](image3.png)

**Fig. (1).** Effects of APE or esomeprazole, a proton pump inhibitor, on gastric mucosal damage induced by aspirin (panel A) and on gastric acid secretion (panel B) in rats. APE or esomeprazole were equally effective in preventing aspirin-induced gastric damage (panel A). This was at the expenses of profound acid inhibition with esomeprazole, whereas the protective dose of APE did not alter gastric acid secretion (panel B).
approximately one million people in USA alone [61]. An
underlying factor in the development of these conditions appears
to be a dysregulated immune response to the host microbiota in
genetically susceptible individuals [62]. Whilst CD and UC both
fall under the collective term IBD, these conditions can be quite
distinct, with different pathogenesis, underlying inflammatory
profiles, symptoms and treatment strategies. CD is predominantly a
Th1-driven immune response, characterised initially by increased
interleukin (IL)-12 expression, followed by interferon (IFN)-γ and
tumour necrosis factor (TNF-α) [63]. CD can occur in any region of
the gastrointestinal tract and is characterised by transmural,
granulomatous inflammation. In contrast, UC is believed to be a
Th2 immune response, leading to increased production of
proinflammatory cytokines. UC is restricted to the colon and
generally begins in the rectum and spreads proximally, dependent
upon disease severity [63]. To further complicate IBD diagnosis,
approximately 10% of IBD patients present with symptoms that
cannot be categorized as typical of UC or CD. These conditions are
referred to as indeterminate colitis, and may develop into UC or CD
as the disease progresses [59]. Whilst the aetiology of IBD is not
well understood, environmental, genetic and immunological factors
appear to play a role in the development of both diseases [64].

As the intestinal microbiota has been linked to the pathogenesis
of IBD, probiotic treatment is a consequent choice for therapeutic
intervention. An alternative is via the administration of prebiotics.
Prebiotics are indigestible dietary fibres producing: short-chain fatty
acids, including butyrate, propionate and acetate in the colon as a
consequence of fermentation by luminal bacteria. Short-chain fatty
acids are readily absorbed by the intestinal mucosa and are an
important source of substrate for metabolism of colonocytes; they
are trophic to the intestinal mucosa, stimulate water and sodium
absorption in the colon, and induce enzymes that promote mucosal
restoration [65,66]. A direct anti-inflammatory role for butyrate, the
most extensively studied of the short-chain fatty acids, has been
suggested [67]; butyrate enemas have been shown to be of benefit
in the management of ulcerative colitis [68] and in animal models
of colon inflammation. Germinated barley foodstuff (GBF), a
dietary component high in glutamine-rich protein and hemi-
cellulose-rich dietary fibre, has demonstrated prebiotic
characteristics in the DSS model of rat colitis, as it decreased the
incidence of bloody diarrhoea and mucosal injury [69]. In animal
and human studies, ingestion of resistant fibre has resulted in an
increase in the population of Bifidobacillus and Lactobacillus in the
colon and an increase in faecal butyrate concentration; GBF has been
shown to attenuate inflammation in dextran sodium sulphate,
trinitrobenzene sulfonic acid (TNBS) and HLA transgenic animal
models of colitis [70-73]. Improvement in clinical and endoscopic
indices has been reported in a pilot study of 10 patients with active
ulcerative colitis with 4 weeks of treatment with GBF that was well
tolerated and did not show significant adverse events [74].
Furthermore, the fibre also contributes to increase stool consistency
due to its high avidity for water and possibly its adsorption of
luminal bile salts [72,75]. An open-label, parallel-group, randomized
study of another fibre, Platago ovata seeds, also reported equal
efficacy to mesalazine in maintaining remission in patients with
ulcerative colitis [76]. GBF was also shown to display a
greater capacity to reduce the symptoms of DSS colitis than a
probiotic mixture of lactobacilli and C. butyricum [77]. More
recently, it was reported that a mixture of long-chain inulin and
oligosaccharide was able to reduce macroscopic scores and
inflammatory histological scores in the colon [78].
Oligosaccharides are other prebiotics which have shown a capacity
to reduce DSS colitis in rats [79]. There is definitive evidence from
animal models that prebiotic supplementation of the diet may
provide a therapeutic option in the treatment of IBD. The rationale
behind prebiotic use is to elevate the endogenous numbers of
beneficial bacterial strains including lactobacillus and
bifidobacterium [80]. This increase leads to the beneficial effects
seen by probiotic administration, including an increase in short
chain fatty acids production, particularly butyrate, which can
provide fuel for colonocytes, production of anti-bacterial
substances, and decreased luminal pH [62]. Our previous study [81]
demonstrated that serum and tissue transglutaminase (TG) activity
correlate with the severity of inflammation in the TNBS-model
of colitis; moreover butyrate stimulates TG activity in several cultured
cell lines [82]. Based on these evidences, we treated TNBS rats
with butyrate, mesalamine, or butyrate in combination with
mesalamine enemas, obtaining an improvement of histology more
marked in the presence of butyrate that also increases TG activity
in the colon. Further evidences have shown that different isoforms
of TG play a key role in tissue healing in UC [83] (Fig. 2).

The research involving the use of prebiotics to treat IBD is not
currently as extensive as that regarding probiotic therapy. Probiotics
are provided in processed foods or in dietary supplements as live
bacteria. Yogurt is the most common probiotic-carrying food;
however, cheese, fermented and unfermented milks, juices,
smoothies, cereal, nutrition bars, and infant/toddler formula all are
vehicles for probiotic delivery. The main probiotic supplements
on the market utilize lactobacilli, streptococci and bifidobacteria,
which are normal constituents of the human gastrointestinal
microflora. However, studies are also investigating potential
probioic roles of other microbes such as yeast (Saccharomyces
boulardii), which are not normally found in the gastrointestinal
tract [84,85]. Probiotic microorganisms do not act exclusively in
the large intestine by affecting the intestinal flora but also affect
other organs, either by modulating immunological parameters, intestinal
permeability and bacterial translocation, or by providing bioactive
metabolites [86]. As the microbial environment has been shown to
play a role in the development of IBD, targeting of the microbiota
presents an option for therapeutic intervention [87]. One potential
method to manipulate the intestinal microbiota in an attempt to
reduce the inflammatory response is via the administration of
probiotics. Probiotics have been used in the treatment of a number

Fig. (2). Role of different transglutaminases in the colon: in the normal
colon, Keratinocyte transglutaminase (TGk) contribute to intracellular tight
junction formation while tissue transglutaminase (tTG) is preferentially
localised within the cell cytosol. In the presence of pathogens, TGk is
downregulated allowing cell separation and antigens to penetrate and
perpetrate the mucosal damage. At that stage, tTG is upregulated and
massively released in the inflamed area together with the circulating
transglutaminase factor XIIIa; they promote cross linking of proteins in the
extracellular matrix (ECM) initiating the healing process.
of inflammatory conditions including arthritis [88], atopic eczema [89], pouchitis [90], radiationinduced [91], and NSAID-induced enteropathy [92], chemotherapy-induced mucositis [93], ulcerative colitis [94], Crohn's disease [95], antibiotic-induced diarrhoea [96] and experimental colitis [97]. The mode of action of probiotics is complex and not completely understood; there have been a large number of known probiotic species, most of which show differing mechanisms of action. However there are a number of common mechanisms present in a wide variety of probiotic strains. One such mechanism is adherence to the intestinal mucosal surface which prevents colonisation of pathogenic bacteria through a form of competition between the two species [98]. Evidence for this mode of action has been shown in numerous in vitro model systems for example, pre-incubation with Lactobacillus rhamnosus GG (LGG) has been shown to prevent the adherence of B. vulgatus to mouse epithelial IEC-6 cells [99]. A further common mode of action is via stimulation of the intestinal immune system. Probiotics are believed to be involved in the modulation of cytokine levels by inhibiting production of pro-inflammatory cytokines (including TNF-α and IL-1β) and promoting production of anti-inflammatory cytokines (including IL-10) [99]. Probiotics are also believed to function via the modulation of cell proliferation and apoptosis [100]. The increase in epithelial cell proliferation is believed to be due to the ability of probiotic strains to produce short chain fatty acids via the fermentation of polysaccharides. The antiapoptotic effects of probiotics have been shown in human and mouse colon cells by the activation of the anti-apoptotic Akt pathway and by the inhibition of the proapoptotic p38MAPK pathway [101]. Therefore, probiotics have the potential to be beneficial in the treatment of IBD due to their capacity to prevent the colonisation of pathogenic bacteria [98], reduce inflammatory cytokine expression [99], enhance epithelial cell proliferation [100], inhibit apoptosis [101] and provide metabolic energy for colonocytes [98].

A number of clinical trials have focused on the use of probiotics in IBD, however, there is a deficiency of large, randomised, double-blind, placebo-controlled clinical trials investigating the efficacy of candidate probiotic species or combinations thereof. One promising study involving the use of probiotics in IBD treatment was in the setting of pouchitis, an inflammatory condition which often arises following surgical resection as a treatment for UC. The study involved patients deemed to be in clinical remission from pouchitis following surgical resection for UC. Patients received daily treatment with probiotics or placebo and were periodically assessed for signs of relapse. Only 15% of treated patients showed signs of relapse compared to 100% of the placebo group [90]. This study indicated that probiotics have the potential to prevent inflammatory conditions in humans. Based on the results from studies in both animal models and clinical trials, there is evidence that a number of probiotic species assist in the reduction of inflammation and intestinal damage whilst others have no effect, depending on the disease setting. For probiotics to become a legitimate therapeutic option for the treatment of IBD there needs to be more focus on the determination of which probiotic strains have the greatest efficacy in a specific disease setting and whether these candidate probiotics are more effective alone, or in conjunction with other pro- or prebiotics. Moreover more consideration of possible adverse side effects and knowledge of the effect of the probiotics on immune regulation in the intestinal mucosa is claimed. An important point is the time that the species remains in the gastrointestinal tract, as this will determine the frequency and dose requirements [102]. It may also be the case that our genetic profile may predispose our responsiveness to probiotic treatment, as is the case with chemotherapy [103]. Identification of these “probiotic responsiveness genes” may lead to screening to determine whether a patient will be responsive to probiotic therapy, and to which probiotics they would respond more efficiently.

There is currently a growing interest in the use of natural bioactive products by the general public, with many healthy subjects and patients taking them for the prevention and treatment of multiple conditions, including gastrointestinal disorders. The gastroprotective effect of a polyphenolic extract (APE) obtained from a variety of apple prompted us to test the possibility that APE may be effective in the treatment of experimental colitis. In TNBS rats APE reduces macroscopic and microscopic disease activity and reduced the expression of pro-inflammatory cytokine [104]. As the long lasting inflammation has been shown to predispose to the development of colorectal cancer, the chemopreventive effect of APE in intestinal polyps formation in ApcMin/+ Mice [22] warrants future investigations for APE in IBD. Furthermore, in a recent article, Shapiro et al. [105], suggest that the addition of polyphenols to artificial nutritional formulas would improve the outcome of patients with IBD and acute pancreatitis in need of enteral or parenteral nutrition.

Zinc carnosine (ZnC), is a health food product claimed to possess health-promoting and gastrointestinal supportive activity. A recent study [106] demonstrated that ZnC stimulated cell migration and proliferation, and that oral ZnC decreased gastric and small intestinal injury in animals. No significant increase in gut permeability was seen in volunteers treated with indomethacin when ZnC was co-administered.

Pharmacological options to reduce problems related to gastrointestinal symptoms including cramps, diarrhoea, nausea, and bleeding are limited, particularly in competitive athletics. One product that is attracting great interest is bovine colostrum. Colostrum is the first milk produced after birth and is particularly rich in immunoglobulins, antimicrobial peptides and other bioactive molecules including growth factors [107]. Some studies suggest it may be of value in eliminating infection and stimulating growth of the neonatal gastrointestinal tract [108,109]. Its value in the prevention and treatment of adult gastrointestinal injury has now been suggested [110]. Using a combination of a clinical trial and in vitro experiments, this study has shown that bovine colostrum reduces the exercise-induced increase in gut permeability, possibly through mechanisms including reducing temperature-induced apoptosis and induction of heat shock protein.

CONCLUSIONS

Many healthy subjects and patients are taking potentially bioactive products for the prevention and treatment of multiple conditions, including gastrointestinal disorders. This forms the basis of a world-wide, multi-million dollar major commercial industry. While the scientific validity of the use of a number of these products is lacking, in the past few years much effort has been made to provide solid knowledge of the mechanisms underlying the beneficial effects of nutraceuticals. Scientifically rigorous research is warranted in order to identify novel compounds to be used alone or in combination with standard drugs in gastrointestinal disorders. Also, future research should be aimed at further increasing the efficacy of a promising nutraceutical, trying to use it as a chemical template for combinatorial synthesis. Finally, researchers in this area should focus on the understanding of the molecular action of each nutraceutical and test the possible synergistic effects with other nutraceuticals and or derivatives, food components, or conventional drug. However, one must keep in mind that just because isolated compounds start from a natural food, they are not necessarily safe and natural and, therefore strict quality control and regulatory issues are mandatory. Future clinical studies are necessary to determine whether these compounds will be as interesting as preventive or therapeutic agents as they are in preclinical studies.

REFERENCES


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