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Journal of Evidence-Based Complementary & Alternative Medicine published online 7 October 2013
DOI: 10.1177/2156587213503660

Journal of Evidence-Based Complementary & Alternative Medicine
00(0) 1-10

DOI: 10.1177/2156587213503660
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Clinical and Structural Effects of Traditional Chinese Medicine and the Herbal Preparation, Iberogast, in a Rat Model of Ulcerative Colitis

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Abstract

Plant-sourced formulations such as Iberogast and the traditional Chinese medicine formulation, Cmed, purportedly possess anti-inflammatory and radical scavenging properties. We investigated Iberogast and Cmed, independently, for their potential to decrease the severity of the large bowel inflammatory disorder, ulcerative colitis. Sprague Dawley rats (n = 8/group) received daily 1 mL gavages (days 0-13) of water, Iberogast (100 μL/200 μL), or Cmed (10 mg/20 mg). Rats ingested 2% dextran sulfate sodium or water ad libitum for 7 days commencing on day 5. Dextran sulfate sodium administration increased disease activity index scores from days 6 to 12, compared with water controls ($P < .05$). On day 10, 200 μL Iberogast decreased disease activity index scores in colitic rats compared with colitic controls ($P < .05$). Neither Iberogast nor Cmed achieved statistical significance for daily metabolic parameters or colonic crypt depth. The therapeutic effects of Iberogast and Cmed were minimal in the colitis setting. Further studies of plant extracts are required investigating greater concentrations and alternative delivery systems.

Keywords

traditional Chinese medicine, Iberogast, herbal extracts, inflammatory bowel disease, dextran sulfate sodium, colitis, rat, ulcerative colitis

Received August 4, 2013. Accepted for publication August 6, 2013.

Introduction

Inflammatory bowel disease is the term for a group of inflammatory diseases affecting the gastrointestinal tract. While its etiology is unclear, it is believed that genetically susceptible hosts are impacted by environmental triggers and antigens, thereby stimulating an abnormal immune response.[1] Ulcerative colitis is a variant of inflammatory bowel disease that primarily affects the large intestine.[2] Ulcerative colitis is characterized by acute and chronic inflammation of the mucosa, resulting in ulceration of the colon, bloody diarrhea, rectal bleeding, abdominal pain, and weight loss.[3,4] Histologically, an influx of polymorphonuclear leukocytes and mononuclear cells is usually present accompanied by red blood cell extravasation, mucin discharge, crypt abscesses, and a disruption of crypt architecture.[3] Common drug therapies such as aminosalicylates, corticosteroids, and immunomodulators are used for maintenance and remission of disease; however, when drug therapies are exhausted, invasive surgery may be required to resect the colon.[4] Currently, there are no truly effective treatment modalities for inflammatory bowel disease and alternative preventative or treatment strategies are being sought.

Oral administration of dextran sulfate sodium to rodents induces colitis with similar clinical and histopathological features to human ulcerative colitis.[5] The dextran sulfate sodium–colitis model has been used widely to test the potential

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effectiveness of newly developed ulcerative colitis treatments. Dextran sulfate sodium is a synthetic polysaccharide comprising sulfated anhydroglucose units. Its mechanism of action constitutes direct mucosal cytotoxicity; disruption of the interaction between intestinal lymphocytes, epithelial cells, and the extracellular matrix; changing expression of β_7 integrin receptors; and changes in the intestinal microflora.[5-8]

The use of natural remedies has increased in inflammatory bowel disease patients, despite reported effects being largely anecdotal or historical.[9] Indeed, up to 50% of inflammatory bowel disease patients have been reported to use some form of alternative medicine to alleviate gastrointestinal complaints.[10] Recently, protection of the stomach by naturally occurring components in plants has been described—attributed to their anti-inflammatory and antioxidant properties.[11] These components include flavonoids such as *Garcinia cambogia*, a plant extract native to Southeast Asia, and Devil's Claw (*Harpagophytum procumbens*) found in southern Africa.[12,13] In this context, a multiherbal extract known as STW 5, marketed under the name Iberogast, has been reported to possess antioxidant and anti-inflammatory properties.[14]

Iberogast is a plant-based liquid formulation comprising 9 different herbal extracts including 15% *Iberus amara* (bitter candy tuft), 10% *Angelica archangelica* (angelica root), 20% *Matricaria recutita* (chamomile), 10% *Carum carvi* (caraway), 10% *Silybum marianum* (St Mary's thistle), 10% *Melissa officinalis* (lemon balm leaf), 5% *Mentha piperita* (peppermint leaf), 10% *Chelidonium majus* (celadine herb), and 10% *Glycyrrhiza glabra* (liquorice root). Iberogast has primarily been used in the treatment of functional dyspepsia and irritable bowel syndrome,[15,16] and it has recently demonstrated promise in the treatment of chemotherapy-induced mucositis.[17] Moreover, Iberogast has a favorable safety profile with no significant toxicological or hepatotoxic data for 10-fold or higher concentrations.[18]

Traditional Chinese medicine is a decoction of various herbs that have provided medicinal aid for centuries. Traditional Chinese medicine is gaining momentum as a potential treatment strategy for ulcerative colitis, given the deficiencies in traditional Western medicines.[19] For example, maintenance of healthy digestive function has been described in Taiwanese and German inflammatory bowel disease sufferers.[20,21] However, there is currently a dearth of scientific evidence to attest the efficacy of these herbal medicines. Recently, certain traditional Chinese medicine herbs have undergone investigations for their potential therapeutic properties in the context of ulcerative colitis. Astragalus root (*Astragalus membranaceus*) and Barley (*Hordeum vulgare*) have demonstrated attenuation of diarrhea, reduction of colonic mucosal damage, and the induction of remission in colitic rats.[22,23] Lycium fruit (*Lycium barbarum*) has also been shown to reduce levels of nitric oxide in experimentally induced diabetes.[24]

The traditional Chinese medicine formulation of ChinaMed CM102 + CM132 (Cmed) is of current interest, as 2 of the active components have independently demonstrated an alleviation of colitic symptoms.[23,24] Cmed is a combination of herbal remedies CM102 and CM132. The herbal formula CM102 comprises various herbs such as Astragalus root, which is used principally to relieve digestion problems. Barley and Lycium fruit are 2 known components in the CM132 formulation, which is recommended for the reduction of nervous tension in humans.

The current study compared the potential for Iberogast and ChinaMed CM102 + CM132 to independently ameliorate features of dextran sulfate sodium–induced ulcerative colitis in rats.

Materials and Methods

General Experimental Procedures

All animal experiments were performed with ethical approval from the animal ethics committees of the Women's and Children's Hospital and The University of Adelaide, in compliance with The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Male Sprague Dawley rats (135-150 g) were housed individually in metabolism cages (Tecniplast, Exton, PA) throughout acclimatization (2 days prior to experimentation) and the experimental period with ad libitum access to an 18% casein-based diet and water.

The dose of the combined Cmed preparation (ChinaMed, Sun Herbal Pty Ltd, New South Wales, Australia) was prepared for a human equivalent (Cmed1, 10 mg/mL; Cmed2, 20 mg/mL) in distilled water. Iberogast (IBE, Flordis Herbal Medicines, Crow's Nest, New South Wales, Australia) was prepared at 100 µL/mL (Ibe1) or 200 µL/mL (Ibe2).

Rats were randomly assigned to 10 groups (n = 8): Groups 1-5 ingested water ad libitum and were gavaged daily for days 0 to 12 with 1 mL of either water (Group 1), Cmed1 (Group 2), Cmed2 (Group 3), Ibe1 (Group 4), or Ibe2 (Group 5). Between days 5 and 13, drinking water was substituted for a 2% dextran sulfate sodium drinking solution in Groups 6 to 10 (ICN Biomedicals, Inc, Aurora, OH) to induce experimental colitis. The dextran sulfate sodium–treated rats were gavaged with water (Group 6), Cmed1 (Group 7), Cmed2 (Group 8), Ibe1 (Group 9), or Ibe2 (Group 10).

Disease Activity Index and Daily Metabolic Data

Body weight, food and water intake, and fecal and urine output were monitored and measured daily. The severity of colitis was assessed daily, using a disease activity index, which scored body weight loss, stool consistency, rectal bleeding, and overall general condition of the animal, increasing in severity on a scale of 0 to 3 for each parameter. These scores were totaled to achieve an overall maximum disease activity index score of 12.

^{13}C-Sucrose Breath Test

The ^{13}C-sucrose breath test provides a noninvasive measurement of small intestinal function.[25] The ^{13}C-sucrose breath test involves measuring $^{13}CO_2$ levels in expired breath as an indirect measure of small intestinal brush-border activity. The breath test was performed on days 0, 5, and 13. The ^{13}C-sucrose breath test was performed at approximately 9 AM on designated days. After an overnight fast, rats were placed in a perspex breath collection container 5 minutes prior to the ^{13}C-sucrose breath test for acclimatization. The containers were closed for a period of 2 minutes to obtain a baseline breath sample into an evacuated tube. Once baseline data were collected, the rats were orally gavaged with 1 mL of 25% ^{13}C-labelled sucrose solution (MERCK Pty Ltd, Victoria, Australia). Following administration of

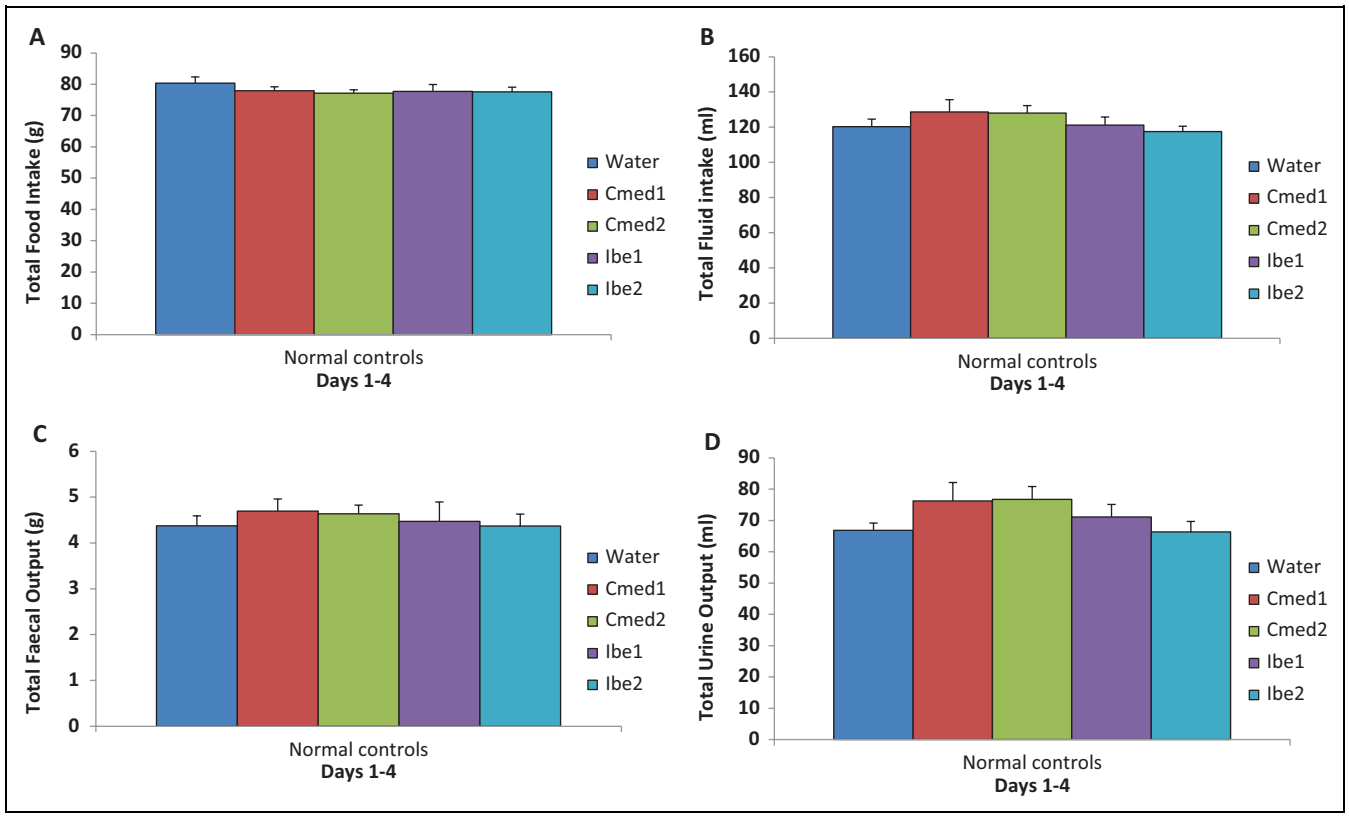

Figure 1. Total food (A) and fluid (B) intake and fecal (C) and urine (D) output, prior to dextran sulfate sodium consumption (days 1-4) in rats (n = 8/group). Day 0 data was excluded due to rats fasting. Data are expressed as mean (g or mL) ± standard error of the mean.

the sucrose solution, breath samples were collected every 15 minutes for a period of 2 hours. Breath samples were then analyzed for $^{13}CO_2$ content using an isotope ratio mass spectrometer (Europa Scientific, ABCA 20/20, Crewe, United Kingdom). Data were expressed as percentage cumulative dose at 90 minutes.

Tissue Collection

On day 13, rats were sacrificed by CO_2 asphyxiation followed by cervical dislocation. Following sacrifice, weights and lengths of all visceral organs were recorded and discarded. Two-centimeter segments of colon were removed and placed in 10% buffered formalin for histological analyses.

Histological Analysis

Proximal and distal colonic samples were routinely processed, embedded in paraffin wax, sectioned (4 μm), and stained with hemotoxylin and eosin. Histological analysis was performed using a light microscope (Nikon Corporation, Tokyo, Japan) with digital camera (Progres C5, Jenoptik, Germany) and Image Pro Plus Software Package Version 5.1 (Media Cybernetics, Rockville, MD). Measurements of crypt depth were determined from 40 crypts over 4 cross sections for both proximal and distal colonic samples per rat and a mean value was then obtained.[26,27]

Statistical Analysis

Bodyweight data prior to (days 1-4) and during (days 6-12) dextran sulfate sodium consumption comparisons were performed using a 2-way ANOVA test with Tukey's post hoc test and expressed as mean ± standard error of the mean. Disease activity index data were compared using a Kruskal–Wallis 1-way ANOVA based on ranks test with pairwise multiple comparison procedures (Dunn's method) and expressed as median [range]. Data from all other analyses were compared using a 1-way ANOVA with a Tukey's post hoc test and expressed as mean ± standard error of the mean. Statistical comparisons were performed using SPSS version 16.0 for Windows (SPSS Inc, Chicago, IL). For all analyses, $P < .05$ was considered significant.

Results

Daily Metabolic Data

Total food and fluid intake and urine and fecal output of all groups remained unchanged by ingestion of traditional Chinese medicine or Iberogast during each experimental period, prior to (days 1-4; Figure 1) and during (days 6-12; Figure 2) dextran sulfate sodium consumption ($P > .05$). Body weight was determined as a mean percentage change from the beginning of each period, prior to (days 1-4) and during (days 6-12) dextran sulfate sodium consumption. Bodyweight remained unchanged for all treatment groups prior to and during dextran sulfate sodium consumption. Furthermore, there were no significant differences in body weight change between any groups during either period (Table 1).

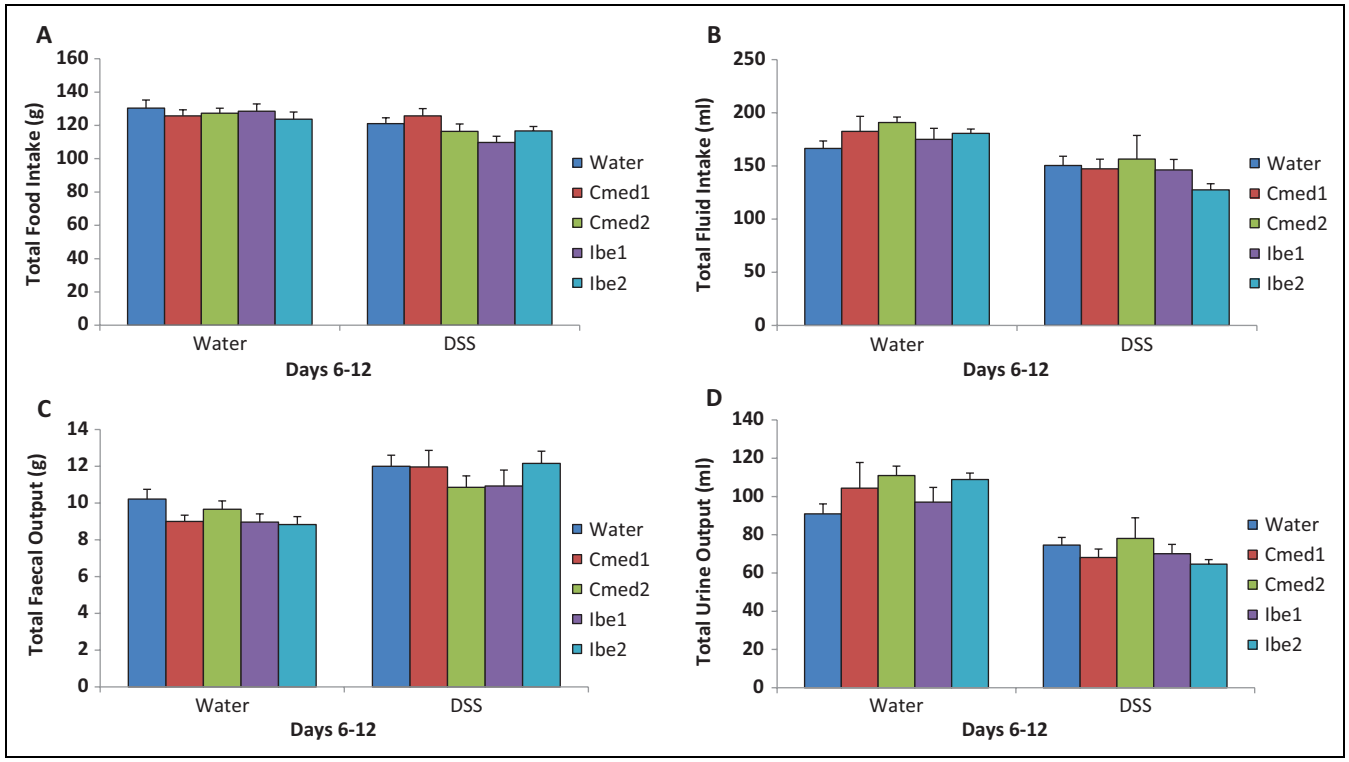

Figure 2. Total food (A) and fluid (B) intake and fecal (C) and urine (D) output, during dextran sulfate sodium consumption (days 6-12) in rats (n = 8/group). Days 5 and 13 data were excluded due to rats fasting. Data are expressed as mean (g or mL) ± standard error of the mean.

Table 1. Percentage Change of Bodyweight in Rats[a].

	Bodyweight (Mean % Change ± SEM)		
	Days 1-4	Days 6-12	
	Normal Controls	Water	DSS
Water	29.97 ± 0.92	33.66 ± 0.77	29.52 ± 1.00
Cmed1	29.21 ± 0.79	31.30 ± 1.36	29.59 ± 1.16
Cmed2	28.07 ± 0.64	32.23 ± 1.94	28.24 ± 2.10
Ibe1	28.39 ± 0.85	34.37 ± 0.88	26.30 ± 2.43
Ibe2	27.99 ± 0.69	31.64 ± 1.31	27.96 ± 1.37

Abbreviations: DSS, dextran sulfate sodium; SEM, standard error of the mean.
[a] Percentage change of bodyweight in rats (n = 8/group) from the beginning of periods before DSS (days 1-4) and during DSS consumption (days 6-12). Days 0, 5, and 13 data were excluded due to rats fasting. Data are expressed as mean (%) ± SEM.

Disease Activity Index

An increase in disease activity index, a quantitative measurement of the progression and resolution of disease, is a common feature of the dextran sulfate sodium–induced model of colitis. Disease activity index score was significantly greater in colitic rats on days 6 to 12 compared to healthy controls ($P < .01$). On day 10, Iberogast (200 µL/mL) administered to dextran sulfate sodium–treated rats resulted in a significantly lower disease activity index score compared to dextran sulfate sodium–treated control rats ($P < .05$). Otherwise, neither the lower dose of Iberogast nor Cmed significantly affected colitic disease activity (Table 2).

^{13}C-Sucrose Breath Test

Activity of the small intestinal brush border enzyme, sucrase, provides an indication of the functional integrity of the small intestine. On days 5 and 13, the absorptive capacity of the small intestine was not significantly affected by dextran sulfate sodium, Cmed, or Iberogast, as indicated by the percentage cumulative dose at 90 minutes ($P > .05$; Figure 3). Furthermore, the percentage cumulative dose at 90 minutes coefficient of variation of healthy rats on day 0 was 32%.

Visceral and Gastrointestinal Organ Weights and Lengths

Dextran sulfate sodium consumption, Cmed, or Iberogast did not significantly affect visceral and gastrointestinal organ weights (expressed as a percentage of body weight; $P > .05$; Tables 3 and 4). Although dextran sulfate sodium decreased mean colon length by 16% (12.24 ± 0.63 cm) compared to its water control group (14.84 ± 0.53 cm), this just failed to attain statistical significance (Table 5). Dextran sulfate sodium administration did not affect duodenum or jejunum-ileum length, and there were no additional effects of either Cmed or Iberogast on these parameters (Table 5).

Colonic Histology

Crypt depths in the proximal and distal colon were unaffected in rats receiving dextran sulfate sodium compared to healthy controls ($P > .05$). Cmed and Iberogast did not significantly affect colonic architecture (Figure 4).

Table 2. Disease Activity Index[a].

	Treatment Groups									
	Water					DSS				
Day of Trial	Water	Cmed1	Cmed2	Ibe1	Ibe2	Water	Cmed1	Cmed2	Ibe1	Ibe2
Day 5	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]
Day 6	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0-1]*	0 [0]	0 [0-1]	0 [0]	0 [0-1]
Day 7	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0-1]*	1 [0-2]	1 [0]	0 [0-1]	0 [0]
Day 8	0 [0]	0 [0]	0 [0-1]	0 [0]	0 [0]	0 [0-1]*	0.5 [0-1]	1 [1-2]	1 [0-1]	0 [0]
Day 9	0 [0]	0 [0-1]	0 [0-1]	0 [0]	0 [0]	1 [0-1]*	1 [0-2]	1 [1-2]	1 [0-2]	1 [0-1]
Day 10	0 [0-1]	0 [0-1]	0 [0]	0 [0]	0 [0-1]	1 [0-4]*	0.5 [0-3]	1.5 [1-3]	2 [0-4]	0 [0-1]^
Day 11	0 [0-1]	0 [0-1]	0 [0]	0 [0]	0 [0-1]	1 [0-3]*	1 [0-2]	2 [1-4]	1 [0-5]	1 [0-2]
Day 12	0 [0-1]	0 [0]	0 [0]	0 [0]	0 [0]	2 [0-5]*	1 [0-2]	2 [1-4]	3 [0-5]	1 [0-3]

Abbreviations: DAI, disease activity index; DSS, dextran sulfate sodium.
[a] DAI between days 5 and 12 during the development of DSS-induced colitis in rats (n = 8/group). Data are expressed as median DAI score [range].
*$P < .05$ compared with water + water. ^$P < .05$ compared with DSS + water.

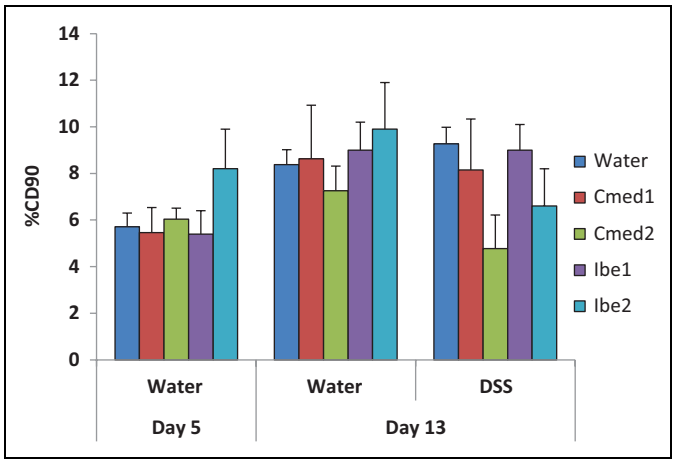

Figure 3. Overall small intestinal functional health status in rats assessed using the ^{13}C-sucrose breath test immediately prior to dextran sulfate sodium (DSS) consumption (day 5) and on day of sacrifice (day 13) (n = 8/group). Data are expressed as mean (% cumulative dose of ^{13}C at 90 minutes; %CD90) ± standard error of the mean.

Discussion

The use of plant and herbal extracts to ameliorate inflammatory disorders has been practiced for centuries. However, only recently have well-controlled studies of specific extracts been conducted in preclinical (animal model) systems. Previous in vitro studies have demonstrated antioxidant properties of Iberogast and its constituents.[28,29] Lemon balm and peppermint leaves have been reported to possess potent radical scavenging capabilities in a xanthine oxidase test reaction that investigated Iberogast and its components for responses to oxidative stress.[14] Furthermore, Iberogast decreased myeloperoxidase-driven reactions by 33% in vitro by inducing reactive oxygen species.[30] Previous experiments have investigated the effects of Iberogast on inflammatory models including mucositis and Crohn's disease, demonstrating reduced inflammation in different regions of the gastrointestinal tract.[17,31,32] Indeed, recently, efficacy has been reported against experimental colitis in a rat model,[33] although therapeutic benefit was only evident at very high dose rates (5 mL/kg) that would equate to several hundred milliliters per day for human subjects. Several herbal remedies have improved parameters associated with colitic damage including the *Garcinia cambogia* extract. Jackson et al[19] reported a significant decrease in the disease activity index and a decrease in myeloperoxidase activity in mice with dextran sulfate sodium–induced colitis following administration of a combination of herbal extracts.

Five main substance classes comprise Iberogast: terpenes, volatile oil, coumarines, flavonoids, and phenol carboxylic acids.[18] Flavonoids and phenol carboxylic acid contain radical scavenging properties that target reactive oxygen species including peroxynitrate and hydrogen peroxide. The current study demonstrated that the highest concentration of Iberogast tested (200 µL/mL) only minimally improved colitic disease activity on day 10 and did not improve any of the histologically assessed parameters of colonic integrity in the dextran sulfate sodium–induced model of ulcerative colitis in rats. Presumably, small intestinal degradation and metabolism of Iberogast during its transit through the bowel may have decreased its bioavailability and hence its bioactivity at the mucosal interface of the more distal bowel regions. This would account for its lack of effectiveness in the distal colon compared to its previously reported efficacy in the more proximal intestinal regions. Microencapsulation or enema-based routes of Iberogast administration could therefore be indicated in an attempt to combat inflammatory disorders affecting the more distal regions of the alimentary tract.

In the current study, Cmed only minimally improved crypt depth, just failing to attain statistical significance. In previous studies, Astragalus root, one of the constituents of Cmed, has been shown to downregulate inducible nitric oxide synthase in dinitro benzene sulfonic acid–induced colitic rats.[23] Histological damage and elevated colonic myeloperoxidase activity by dinitro benzene sulfonic acid was reduced by Astragalus root treatment.[23] Also there was a marked reduction in the

Table 3. Visceral Organ Weights[a].

	Treatment Groups									
	Water					DSS				
Visceral Organs	Water	Cmed1	Cmed2	Ibe1	Ibe2	Water	Cmed1	Cmed2	Ibe1	Ibe2
Heart	0.40 ± 0.01	0.41 ± 0.01	0.4 ± 0.020	0.41 ± 0.01	0.41 ± 0.02	0.41 ± 0.01	0.40 ± 0.01	0.39 ± 0.01	0.40 ± 0.02	0.37 ± 0.01
Lungs	0.63 ± 0.02	0.69 ± 0.05	0.64 ± 0.02	0.62 ± 0.03	0.54 ± 0.06	0.63 ± 0.02	0.67 ± 0.09	0.58 ± 0.03	0.58 ± 0.05	0.59 ± 0.02
Left kidney	0.42 ± 0.02	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.02	0.42 ± 0.01	0.42 ± 0.01	0.41 ± 0.01	0.43 ± 0.01	0.43 ± 0.01
Right kidney	0.43 ± 0.02	0.44 ± 0.01	0.44 ± 0.02	0.43 ± 0.01	0.44 ± 0.02	0.43 ± 0.01	0.44 ± 0.01	0.42 ± 0.01	0.44 ± 0.01	0.43 ± 0.02
Liver	3.39 ± 0.08	3.32 ± 0.09	3.33 ± 0.05	3.49 ± 0.11	3.33 ± 0.19	3.54 ± 0.11	3.83 ± 0.01	3.60 ± 0.11	3.56 ± 0.24	3.53 ± 0.06
Thymus	0.31 ± 0.02	0.30 ± 0.02	0.32 ± 0.02	0.31 ± 0.02	0.26 ± 0.02	0.27 ± 0.02	0.29 ± 0.27	0.30 ± 0.02	0.29 ± 0.04	0.30 ± 0.02
Spleen	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.26 ± 0.02	0.30 ± 0.01	0.28 ± 0.01	0.30 ± 0.01	0.30 ± 0.03	0.29 ± 0.02
Stomach	0.57 ± 0.02	0.59 ± 0.02	0.56 ± 0.01	0.59 ± 0.01	0.55 ± 0.03	0.60 ± 0.02	0.62 ± 0.02	0.57 ± 0.02	0.61 ± 0.03	0.59 ± 0.03
Cecum	0.29 ± 0.01	0.29 ± 0.01	0.3 ± 0.01	0.30 ± 0.01	0.24 ± 0.05	0.37 ± 0.04	0.36 ± 0.03	0.38 ± 0.03	0.39 ± 0.05	0.34 ± 0.01

Abbreviations: DSS, dextran sulfate sodium; SEM, standard error of the mean.
[a] Visceral organ weights in rats following adjustment for body weight (n = 8/group). DSS was administered between days 5 and 13 and rats were sacrificed on day 13. Data are expressed as mean (% relative to body weight) ± SEM.

Table 4. Gastrointestinal Organ Weights[a].

	Treatment Groups									
	Water					DSS				
Gastrointestinal Organ	Water	Cmed1	Cmed2	Ibe1	Ibe2	Water	Cmed1	Cmed2	Ibe1	Ibe2
Duodenum	0.22 ± 0.01	0.18 ± 0.03	0.21 ± 0.02	0.22 ± 0.01	0.21 ± 0.02	0.22 ± 0.02	0.23 ± 0.02	0.21 ± 0.01	0.21 ± 0.02	0.20 ± 0.01
Jejunum-ileum	1.95 ± 0.05	1.84 ± 0.05	1.93 ± 0.05	1.96 ± 0.03	1.85 ± 0.08	2.06 ± 0.03	1.95 ± 0.07	2.00 ± 0.06	1.95 ± 0.05	1.94 ± 0.06
Colon	0.43 ± 0.05	0.40 ± 0.020	0.39 ± 0.01	0.39 ± 0.02	0.38 ± 0.02	0.52 ± 0.03	0.45 ± 0.02	0.45 ± 0.02	0.50 ± 0.06	0.45 ± 0.01

Abbreviations: DSS, dextran sulfate sodium; SEM, standard error of the mean.
[a] Gastrointestinal organ weights in rats following adjustment for body weight (n = 8/group). DSS was administered between days 5 and 13 and rats were sacrificed on day 13. Data are expressed as mean (% relative to body weight) ± SEM.

Table 5. Gastrointestinal Organ Lengths[a].

	Treatment Groups									
	Water					DSS				
Gastrointestinal Lengths (cm)	Water	Cmed1	Cmed2	Ibe1	Ibe2	Water	Cmed1	Cmed2	Ibe1	Ibe2
Duodenum	6.88 ± 0.29	6.85 ± 0.38	7.35 ± 0.24	7.04 ± 0.38	6.74 ± 0.23	6.66 ± 0.22	7.61 ± 0.78	6.86 ± 0.30	6.80 ± 0.17	6.34 ± 0.25
Jejunum-ileum	79.13 ± 3.44	77.50 ± 3.38	77 ± 20.38	81.57 ± 3.94	76.88 ± 3.51	75.71 ± 4.06	78.25 ± 3.12	77 ± 2.24	76.71 ± 2.24	75.88 ± 1.65
Colon	14.84 ± 0.53	15 ± 0.85	15.11 ± 0.52	14.67 ± 0.5	14.73 ± 0.52	12.24 ± 0.63	12.45 ± 0.88	11.39 ± 0.57	11.51 ± 0.44	12.21 ± 0.74

Abbreviations: DSS, dextran sulfate sodium; SEM, standard error of the mean.

[a] Gastrointestinal organ lengths of rats (n = 8/group). DSS was administered between days 5 and 13 and rats were sacrificed on day 13. Data are expressed as mean (cm) ± SEM.

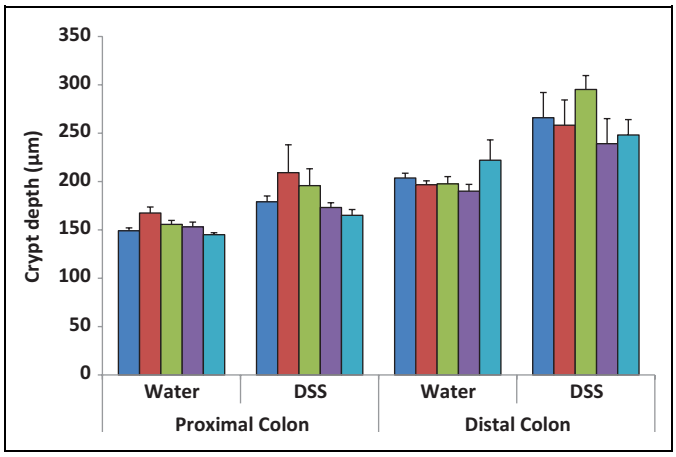

Figure 4. Crypt depth in the proximal and distal colon of rats (n = 8/group). Dextran sulfate sodium (DSS) was administered between days 5 and 13 and rats were sacrificed on day 13. Data are expressed as mean (μm) ± standard error of the mean.

expression of interleukin-1β, interleukin-6, and tumor necrosis factor-α in zymosan-induced air pouches in mice treated with astragalus, although the precise mechanisms remain undefined.[34-36]

Barley, incorporated in many traditional Chinese medicine preparations, contains insoluble protein and dietary fiber that has demonstrated attenuation of diarrhea, reduction of colonic mucosal damage, and induction of remission in colitic rats.[22] Germinated barley foodstuff reduced the clinical activity of ulcerative colitis in both short-term and long-term administration studies, with no apparent side-effect.[37-39] The potential mechanism for germinated barley foodstuff action is believed to be the ability to maintain epithelial cells, facilitate epithelial repair, and suppress epithelial nuclear factor kappa B-DNA binding activity.[40] 3,4-Dihydroxybenzaldehyde derived from purified barley attenuated cell death and apoptosis induced by hydrogen peroxide when evaluated by the 1,1-diphenyl-2-picryl hydrazyl free radical scavenging activity assay.[41]

Lycium fruit (*Lycium barbarum*), a further constituent of Cmed, has also been demonstrated to decrease inducible nitric oxide synthase levels and glutathione peroxidise activity in streptozotocin-induced diabetic rats.[24] Polysaccharides are major constituents of Lycium fruit, believed to be responsible for the antioxidant effects,[24,42] possibly due to its ability to increase the activity of superoxide dismutase and protect against lipid peroxidation in biological membranes.[24,42] Changtai granule, a traditional compound of traditional Chinese medicine, has been reported to attenuate macroscopic and histological colon injury in the 2,4,6-trinitrobenzene sulfonic acid model of colitis in rats.[43,44] Indeed, these investigators reported a decrease in mucosal mRNA levels for several inflammatory mediators, including inducible nitric oxide synthase and tumor necrosis factorα.[43,44] Similarly, Si-Ni-San, a traditional Chinese medicine formulation, resulted in a decreased severity score and myeloperoxidase activity in a mouse model of 2,4,6-trinitrobenzene sulfonic acid–induced colitis.[41] Furthermore, Si-Ni-S caused a decrease in interferon-γ, interleukin-12, tumor necrosis factor-α, and interleukin-17 levels.[45] Similarly, Yun Nan BaiYao, a traditional Chinese herbal remedy used for treating hemorrhages and wounds, decreased the levels of several pro-inflammatory cytokines in the colonic mucosa, including tumor necrosis factor-α, interleukin-12, and interleukin-17, in both the dextran sulfate sodium–induced and 2,4,6-trinitrobenzene sulfonic acid–induced models of colitis.[46] The recent finding that the natural plant product sophocarpine ameliorates dextran sodium sulfate–induced colitis in mice by regulating cytokine balance[47] indicates that future studies of traditional Chinese medicine preparations in models of experimentally induced ulcerative colitis should consider effects on systemic and mucosal cytokine profiles as a firstline analytical measure.

Conclusions

In conclusion, although there was only minimal improvement in colonic structure in colitic rats following administration of Iberogast and Cmed, the findings of the current study support further preclinical studies in which other plant-sourced formulations could be tested for the potential treatment of colitis. Combining histological and immunological (cytokine profile) end-points is recommended for future studies. Although the mechanism of action of these extracts is complex, further studies could address the potential for combinations of plant extracts such as Iberogast and Cmed to increase clinical efficacy, either by additive or synergistic means. Maximizing bioavailability of these formulations, either by encapsulation or enema administration, could represent a future strategy to target the specific site of injury in inflammatory disorders affecting the distal bowel.

Acknowledgments

The authors would like to thank Esther Burt and Betty Zacharakis for analysis of breath samples.

Author Contributions

SM significantly contributed toward animal trials, experiments and data analyses, and prepared the final version of the article. BVH and MV conducted all animal trials, experiments and data analyses, and contributed toward the preparation of the article. KAL significantly contributed toward the animal trials. CF-B assisted with experimental planning and analysis of data. GSH contributed to the experimental design, analysis of data, and preparation of the article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship and/or publication of this article: Professor Gordon S. Howarth is supported by the Sally Birch Cancer Council Australia Senior Research Fellowship in Cancer Control.

Ethical Approval

All animal experiments were performed with ethical approval from the animal ethics committees of the Women's and Children's Hospital and The University of Adelaide, in compliance with The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

References

1. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol*. 2006;3:390-407.
2. Gionchetti P, Rizzello F, Habal F, et al. Standard treatment of ulcerative colitis. *Dig Dis*. 2003;21:157-167.
3. Hendrickson BA, Gokhale R, Cho JH. Clinical aspects and pathophysiology of inflammatory bowel disease. *Clin Microbiol Rev*. 2002;15:79-94.
4. Rutgeerts P, Vermeire S, Van Assche G. Biological therapies for inflammatory bowel diseases. *Gastroenterology*. 2009; 136:1182-1197.
5. Gaudio E, Taddei G, Vetuschi A, et al. Dextran sulfate sodium (DSS) colitis in rats: clinical, structural, and ultrastructural aspects. *Dig Dis Sci*. 1999;44:1458-1475.
6. Damiani CR, Benetton CA, Stoffel C, et al. Oxidative stress and metabolism in animal model of colitis induced by dextran sulfate sodium. *J Gastroenterol Hepatol*. 2007;22:1846-1851
7. Ni J, Chen SF, Hollander D. Effects of dextran sulphate sodium on intestinal epithelial cells and intestinal lymphocytes. *Gut*. 1996;39:234-241.
8. Kullmann F, Messmann H, Alt M, et al. Clinical and histopathological features of dextran sulfate sodium induced acute and chronic colitis associated with dysplasia in rats. *Int J Colorectal Dis*. 2001;16:238-246.
9. Langmead L, Rampton DS. Review article: complementary and alternative therapies for inflammatory bowel disease. *Aliment Pharmacol Ther*. 2006;23:341-349.
10. Rawsthorne P, Shanahan F, Cronin NC, et al. An international survey of the use and attitudes regarding alternative medicine by patients with inflammatory bowel disease. *Am J Gastroenterol*. 1999;94:1298-1303.
11. Geronikaki AA, Gavalas AM. Antioxidants and inflammatory disease: synthetic and natural antioxidants with anti-inflammatory activity. *Comb Chem High Throughput Screen*. 2006;9:425-442.
12. Langmead L, Dawson C, Hawkins C, Banna N, Loo S, Rampton DS. Antioxidant effects of herbal therapies used by patients with inflammatory bowel disease: an in vitro study. *Aliment Pharmacol Ther*. 2002;16:197-205.
13. dos Reis SB, de Oliveira CC, Acedo SC, et al. Attenuation of colitis injury in rats using *Garcinia cambogia* extract. *Phytother Res*. 2009;23:324-329.
14. Schempp H, Weiser D, Kelber O, Elstner EF. Radical scavenging and anti-inflammatory properties of STW 5 (Iberogast) and its components. *Phytomedicine*. 2006;(suppl 5):36-44.
15. Braden B, Caspary W, Borner N, Vinson B, Schneider AR. Clinical effects of STW 5 (Iberogast) are not based on acceleration of gastric emptying in patients with functional dyspepsia and gastroparesis. *Neurogastroenterol Motil*. 2009;21: 632-638.
16. Allescher HD, Wagner H. STW 5/Iberogast: multi-target-action for treatment of functional dyspepsia and irritable bowel syndrome. *Wien Med Wochenschr*. 2007;157:301-307.
17. Wright TH, Yabeck R, Lymn KA, et al. The herbal extract, Iberogast, improves jejunal integrity in rats with 5-fluorouracil (5-FU)-induced mucositis. *Cancer Biol Ther*. 2009;8:923-929.
18. Wegener T, Wagner H. The active components and the pharmacological multi-target principle of STW 5 (Iberogast). *Phytomedicine*. 2006;13(suppl 5):20-35.
19. Jackson LN, Zhou Y, Qiu S, Wang Q, Evers BM. Alternative medicine products as a novel treatment strategy for inflammatory bowel disease. *Am J Chin Med*. 2008;36:953-965.
20. Chen YC, Chen FP, Chen TJ, Chou LF, Hwang SJ. Patterns of traditional Chinese medicine use in patients with inflammatory bowel disease: a population study in Taiwan. *Hepatogastroenterology*. 2008;55:467-470.
21. Joos S, Rosemann T, Szecseny J, Hahn EG, Willich SN, Brinkhaus B. Use of complementary and alternative medicine in Germany—a survey of patients with inflammatory bowel disease. *BMC Complem Altern Med*. 2006;6:19.
22. Kanauchi O, Serizawa I, Matsumura T, Fukuda Y, Satomi M. Evaluation of antigenicity of germinated barley foodstuff for the treatment of ulcerative colitis in a chronic murine colitis model. *Int J Mol Med*. 2001;7:143-147.
23. Ko JK, Lam FY, Cheung AP. Amelioration of experimental colitis by *Astragalus membranaceus* through anti-oxidation and inhibition of adhesion molecule synthesis. *World J Gastroenterol*. 2005;11:5787-5794.
24. Li XM. Protective effect of *Lycium barbarum* polysaccharides on streptozotocin-induced oxidative stress in rats. *Int J Biol Macromol*. 2007;40:461-465.
25. Pelton NS, Tivey DR, Howarth GS, Davidson GP, Butler RN. A novel breath test for the non-invasive assessment of small intestinal mucosal injury following methotrexate administration in the rat. *Scand J Gastroenterol*. 2004;39:1015-1016.
26. Geier MS, Tivey DR, Yazbeck R, McCaughan GW, Abbott CA, Howarth GS. Development and resolution of experimental colitis in mice with targeted deletion of dipeptidyl peptidase IV. *J Cell Physiol*. 2005;204:687-692.
27. Abimosleh SM, Lindsay RJ, Butler RN, Cummins AG, Howarth GS. Emu oil increases colonic crypt depth in a rat model of ulcerative colitis. *Dig Dis Sci*. 2012;57:887-896.
28. Germann I, Hagelauer D, Kelber O, et al. Antioxidative properties of the gastrointestinal phytopharmaceutical remedy STW 5 (Iberogast). *Phytomedicine*. 2006;13(suppl 5):45-50.
29. Schemann M, Michel K, Zeller F, Hohenester B, Ruhl A. Region-specific effects of STW 5 (Iberogast) and its components in gastric fundus, corpus and antrum. *Phytomedicine*. 2006;13(suppl 5):90-99.
30. Schempp H, Hippeli S, Weiser D, Kelber O, Elstner EF. Comparison of the inhibition of myeloperoxidase-catalyzed hypochlorite formation in vitro and in whole blood by different plant extracts contained in a phytopharmacon treating functional dyspepsia. *Arzneimittelforschung*. 2004;54:389-395.
31. Michael S, Kelber O, Hauschildt S, Spanel-Borowski K, Nieber K. Inhibition of inflammation-induced alterations in rat small

intestine by the herbal preparations STW 5 and STW 6. *Phytomedicine*. 2009;16:161-171.
32. Michael S, Abdel-Aziz H, Weiser D, Müller CE, Kelber O, Nieber K. Adenosine A2A receptor contributes to the anti-inflammatory effect of the fixed herbal combination STW 5 (Iberogast) in rat small intestinal preparations. *Naunyn Schmiedebergs Arch Pharmacol*. 2012;385:411-421.
33. Wadie W, Abdul-Aziz H, Zaki HF, Kelber O, Weiser D, Khayyal MT. STW 5 is effective in dextran sulfate sodium-induced colitis in rats. *Int J Colorectal Dis*. 2012;27:1445-1453.
34. Hei ZQ, Huang HQ, Zhang JJ, Chen BX, Li XY. Protective effect of *Astragalus membranaceus* on intestinal mucosa reperfusion injury after hemorrhagic shock in rats. *World J Gastroenterol*. 2005;11:4986-4991.
35. Ryu M, Kim EH, Chun M, et al. Astragali Radix elicits anti-inflammation via activation of MKP-1, concomitant with attenuation of p38 and Erk. *J Ethnopharmacol*. 2008;115:184-193.
36. Sun WY, Wei W, Gui SY, Wu L, Wang H. Protective effect of extract from *Paeonia lactiflora* and *Astragalus membranaceus* against liver injury induced by bacillus Calmette-Guérin and lipopolysaccharide in mice. *Basic Clin Pharmacol*. 2008;103:143-149.
37. Hanai H, Kanauchi O, Mitsuyama K, et al. Germinated barley foodstuff prolongs remission in patients with ulcerative colitis. *Int J Mol Med*. 2004;13:643-647.
38. Kanauchi O, Mitsuyama K, Homma T, et al. Treatment of ulcerative colitis patients by long-term administration of germinated barley foodstuff: multi-center open trial. *Int J Mol Med*. 2003; 12:701-704.
39. Kanauchi O, Suga T, Tochihara M, et al. Treatment of ulcerative colitis by feeding with germinated barley foodstuff: first report of a multicenter open control trial. *J Gastroenterol*. 2002;37(suppl 14):67-72.
40. Kanauchi O, Iwanaga T, Mitsuyama K. Germinated barley foodstuff feeding. A novel neutraceutical therapeutic strategy for ulcerative colitis. *Digestion*. 2001;63(suppl 1):60-67.
41. Jeong JB, Hong SC, Jeong HJ. 3,4-Dihydroxybenzaldehyde purified from the barley seeds (*Hordeum vulgare*) inhibits oxidative DNA damage and apoptosis via its antioxidant activity. *Phytomedicine*. 2009;16:85-94.
42. Niu AJ, Wu JM, Yu DH, Wang R. Protective effect of *Lycium barbarum* polysaccharides on oxidative damage in skeletal muscle of exhaustive exercise rats. *Int J Biol Macromol*. 2008;42:447-449.
43. Cao YB, Zhang JD, Diao YY, et al. Effects of Changtai granules, a traditional compound Chinese medicine, on chronic trinitrobenzene sulfonic acid-induced colitis in rats. *World J Gastroenterol*. 2005;11:3539-3543.
44. Liu BG, Jia XM, Cao YY, et al. Changtai granule, a traditional Chinese drug, protects hapten-induced colitis by attenuating inflammatory and immune dysfunctions. *J Ethnopharmacol*. 2007;4:1-8.
45. Sun Y, Cai TT, Shen Y, Zhou XB, Chen T, Xu Q. Si-Ni-San, a traditional Chinese prescription, and its active ingredient glycyrrhizin ameliorate experimental colitis through regulating cytokine balance. *Int Immunopharmacol*. 2009;9:1437-1443.
46. Li R, Alex P, Ye M, Zhang T, Liu L, Li X. An old herbal medicine with a potentially new therapeutic application in inflammatory bowel disease. *Int J Clin Exp Med*. 2011;4:309-319.
47. Wang XJ, Deng HZ, Jiang B, Yao H. The natural plant product sophocarpine ameliorates dextran sodium sulfate-induced colitis in mice by regulating cytokine balance. *Int J Colorectal Dis*. 2012;27:575-581.