Ingredient	Study / Source	Clinical Dose	Product Dose	Study Duration	Study Type	Subjects	Results	Page#
CLEANSE AND RESTORE								
Bacillus Coagulans (probiotic) GC-30	Hun, Larysa, "*Bacillus coagulans Significantly Improved Abdominal Pain and Bloating in Patients with IBS.*" Postgraduate Medicine, 2009; 121(2), 119-124b Hun, L. 2009	1 billion cfu / day	1 billion cfu / day	8 Weeks	Randomized double-blind placebo controlled	44 Subjects	Improvements from baseline abdominal pain and bloating scores in the B coagulans GBI-30, 6086 group were statistically significant for all 7 weekly comparisons (P < 0.01) A safe and effective option for the relief of abdominal pain and bloating for patients with IBS	3-8
Bacillus Coagulans (probiotic) GC-30	Kalman, D, et al, "*A prospective, randomized, double-blind, placebo-controlled parallel-group dual site trial to evaluate the effects of a Bacillus coagulans-based product on functional intestinal gas symptoms.*" BMC Gastroenterology, 2009, 9:85	1 billion cfu / day	1 billion cfu / day	4 Weeks	Randomized double-blind placebo controlled	61 Subjects	subjects in the probiotic group achieved significant improvements in GSRS abdominal pain subscore (p = 0.046) and the GSRS total score (p = 0.048), with a strong trend for improvement on the GSRS abdominal distension subscore (p = 0.061). In conclusion, the Bacillus coagulans-based product was effective in improving the quality of life and reducing gastrointestinal symptoms in adults with post prandial intestinal gas-related symptoms and no GI diagnoses.	9-15
Bacillus Coagulans (probiotic) GC-30	Baron, M, "*A Patented Strain of Bacillus coagulans Increased Immune Response to Viral Challeng.*" Postgraduate Medicine, 2009; 121(2): 114-118, 2015	1 billion cfu / day	1 billion cfu / day	30 Days	Controlled Study	10 Subjects	The use of GanedenBC30 significantly increased T-cell production of TNF-α in response to adenovirus exposure (P = 0.027) and influenza A (H3N2 Texas strain) exposure (P = 0.004), but it did not have a significant effect on the response to other strains of influenza. No serious adverse events were reported throughout the study. Conclusions: The patented GanedenBC30 probiotic may be a safe and effective therapeutic option for enhancing T-cell response to certain viral respiratory tract infections.	16-20
Soluble Corn Fiber (Fibersol-2)	Furukawa et al 2004 J Jpn Council for Advanced Food Ingredients Res. 7(1) 55-62	4.2g / day	6g / day	2 Weeks	Placebo controlled Cross over - 1 week wash out	40 Subjects	Fecal volume and fecal frequency were significantly increased compared to placebo (p<0.05).	21, 24-27
Soluble Corn Fiber (Fibersol-2)	Kimura et al 1998. J Nutritional Food 1: 12-19	5g / day	6g / day	3 Weeks	Uncontrolled trial, compared to baseline and 1 week post consumption.	62 Subjects	In women who were classified as constipated or having moderately frequent defecations/week at baseline; there were significant (P<0.05) improvements in frequency and fecal volume. There was also significant improvement in fecal consistency in the low score subgroup.	21, 28-34
Soluble Corn Fiber (Fibersol-2)	Takagaki et al 2001 J Nutritional Food 4(4): 29-35	6g / day	6g / day	2 Weeks	Placebo controlled Cross over - 1 week wash out	71 Subjects	In the subjects who were subgrouped as having mild constipation (n=20) there was a significant increase (p<0.01) in frequency compared to control; and a significant increase in fecal amount compared to control (p<0.05). In normal subjects there was a significant increase in fecal amount compared to placebo (p<0.05).	22, 35-41
HERBAL DETOX								
Cascara Sagrada	*Drug Record. Cascara (Cascara Sagrada).* U.S. National Library Of Medicine	Approx 300 mg / day	1,152 mg Proprietary Blend	Daily	Multiple Studies	Multiple Studies	The active laxative components in cascara are anthraquinone derivatives and their glucosides, referred to as cascarosides. They appear to act locally as an irritant to the colon promoting peristalsis and stool evacuation. Anthraquinones also inhibit reabsorption of electrolytes and water from the colon.	42-46
Senna (cassia angustifolia)	Subhuti Dharmananda, Ph.D., *"Safety Issues Affecting Herbs: How Long Can Stimulant Laxatives be Used? "* Institute for Traditional Medicine, Portland, Oregon	20-30 mg / day	1,152 mg Proprietary Blend	6 Months	Multiple Studies	Multiple Studies	Laxative. Relieves constipation.	47-55
CALM AND CLEANSE TEA								
Chamomile	Amsterdam, Jay, *"Alternative Therapies."* Health Med. 2012 Sep-Oct; 18(5): 44–49, 2009	220 mg	1,152 mg Proprietary Blend	8 weeks	Randomized double-blind placebo controlled trial	57 Subjects	Determined that chamomile is not only relaxing, but it can significantly decrease anxiety and even fight depression. Clinically meaningful antidepressant activity that occurs in addition to its previously observed anxiolytic activity.	56-62

Ingredient	Study / Source	Clinical Dose	Product Dose	Study Duration	Study Type	Subjects	Results	Page#
Cascara Sagrada	Drug Record, *Cascara (Cascara Sagrada).* U.S. National Library Of Medicine	Approx 300 mg / day	1,152 mg Proprietary Blend	Daily	Multiple Studies	Multiple Studies	The active laxative components in cascara are anthraquinone derivatives and their glucosides, referred to as cascarosides. They appear to act locally as an irritant to the colon promoting peristalsis and stool evacuation. Anthraquinones also inhibit reabsorption of electrolytes and water from the colon.	42-46
Lemon Balm (Melissa officinalis)	Kennedy D, Little W, and Scholey A., *"Attenuation of Laboratory-Induced Stress in Humans After Acute Administration of Melissa officinalis (Lemon Balm)."* HumanClinicals.org, PubMed - clinical	300-600 mg	1,520 mg Proprietary Blend	7 Days	Randomized double-blind placebo controlled trial	18	lemon balm alleviated the negative mood induced by the test. These subjects' self-ratings of calmness were significantly higher than other subjects'.	63-69

Original Research: *Bacillus coagulans* Significantly Improved Abdominal Pain and Bloating in Patients with IBS

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Abstract

Background: Symptoms of irritable bowel syndrome (IBS) can have a profound impact on emotional health and quality of life, and current treatments are sometimes unsatisfactory for patients facing this lifelong disease. Probiotics, which can normalize gastrointestinal microflora, may alleviate symptoms of IBS. **Objective:** This preliminary controlled study was conducted to evaluate the effects of the probiotic *Bacillus coagulans* GBI-30, 6086 on IBS symptoms. **Methods:** This was a randomized, double-blind, parallel-group, placebo-controlled clinical trial involving 44 subjects who received either placebo or *B coagulans* GBI-30, 6086 once a day for 8 weeks. Self-assessments of the severity of IBS symptoms (abdominal pain and bloating) were recorded every day for 8 weeks. Because baseline values were significantly different between the 2 study groups, within-group analysis was conducted. **Results:** Improvements from baseline abdominal pain and bloating scores in the *B coagulans* GBI-30, 6086 group were statistically significant for all 7 weekly comparisons ($P < 0.01$). In the placebo group, only changes in abdominal pain scores at weeks 6 and 8 achieved statistical significance ($P < 0.05$). No treatment-related adverse events or serious adverse events were reported during the 8-week study period. **Conclusions:** Preliminary data suggest that the patented *B coagulans* GBI-30, 6086 probiotic may be a safe and effective option for the relief of abdominal pain and bloating for patients with IBS. Larger, extended trials are needed to verify these results.

Keywords: probiotics; irritable bowel syndrome; *Bacillus coagulans*; GanedenBC[30]; lactic acid-producing bacteria

Introduction

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder involving abdominal pain or discomfort and alterations in bowel habit (frequency, form, and passage).[1,2] The prevalence of IBS in the United States varies widely because of the use of different diagnostic criteria and survey methods. The 2 most recent surveys reported a total prevalence of 7% to 14%, with a higher prevalence in women.[3,4] Although IBS is not life threatening, the symptoms of IBS can have a profound impact on the patient's emotional health and quality of life. Irritable bowel syndrome accounts for 12% of primary care visits and is the most common complaint of patients seen by gastrointestinal (GI) specialists;[5,6] however, the majority of patients with IBS symptoms either go undiagnosed or do not seek medical care.[2,4] For those patients who are diagnosed with IBS, there are few Food and Drug Administration (FDA)-approved therapeutic options. Clinical intervention typically includes patient education, reassurance, and

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dietary modification to alleviate symptoms,[1] all of which can be unsatisfactory to patients who are facing this lifelong disease.

While the precise pathophysiology of IBS remains unclear, symptoms can be triggered by changes in GI function caused by infection, altered diet, or stress.[2] Immune activation and inflammation have also been implicated in IBS.[1] Both retrospective and prospective studies have documented the onset of IBS following bacterial gastroenteritis,[7–9] and others have provided evidence of low-grade mucosal inflammation and immune activation in patients with IBS.[10,11]

Modifications in the normal gut flora may also be a cause or a consequence of IBS. Individuals with this disease have been shown to have altered gut flora that may represent or lead to disordered GI function. The fecal flora of IBS patients differs from that of normal patients.[12] Some individuals with IBS may harbor bacterial overgrowth and their symptoms may be ameliorated by its eradication.[13,14]

Probiotics are live or attenuated bacteria or bacterial products that have been shown to re-establish balance in the gut microflora as well as modulate the mucosal immune response.[15,16] Probiotics modulate bacterial or virus-related diarrhea and could modify the course of postinfective IBS.[16] Probiotics have been demonstrated to exert anti-inflammatory effects at the mucosal surface.[17,18] By reducing mucosal inflammation, probiotics may decrease immune-mediated activation of intestinal motor and sensory neurons and modify neural traffic between the gut and the central nervous system.[17] Probiotics may also alter the volume and/or composition of stool and gas[19] or increase intestinal mucus secretion,[20] effects that may influence intestinal handling of its contents and thus modulate symptoms of IBS such as constipation and diarrhea.[18]

Bacillus coagulans GBI-30, 6086 (Ganeden Biotech, Inc., Mayfield Heights, OH) is a patented strain of lactic acid-producing bacteria that can sustain the low pH of stomach acid and become active in the intestine.[21,22] The objective of this preliminary study was to evaluate the effects of 8 weeks of *B coagulans* GBI-30, 6086 therapy on specific IBS symptoms.

Materials and Methods

Study Design

This was a randomized, double-blind, parallel-group, placebo-controlled clinical study to evaluate the effects of a marketed probiotic preparation, *B coagulans* GBI-30, 6086, on the symptoms of IBS. Fifty males and females between 23 and 70 years old were randomized to receive a probiotic preparation of GanedenBC[30] (≈800 million CFU) or placebo once a day for 8 weeks. Patients gave informed consent at study inclusion. The study protocol, informed consent, and test product(s) information received institutional review board (IRB) approval before the beginning of the study.

Patient Population

All subjects met the Rome II Criteria for IBS with diarrhea (ie, must have had, during the 12 months prior to evaluation, and for a total of at least 12 weeks [not necessarily consecutively], abdominal discomfort or pain that had 2 of 3 features: [1] relieved with defecation, [2] onset associated with a change in frequency of stool, or [3] onset associated with a change in appearance of stool). Individuals with any organic gastrointestinal conditions or diseases, previous intestinal surgery, immunodeficiency, or lactose intolerance, or who were pregnant or lactating, were excluded from the study. Patients who had taken commercially available probiotic medications within 30 days of the study were also excluded.

Fifty subjects with IBS symptoms were randomized. Six subjects did not complete the study for reasons unrelated to the study and were not included in the analysis. The majority of the subjects in the study were white (90%) and female (82%), and the average age was 48 years (Table 1). All subjects had diarrhea-prominent IBS.

Treatment and Follow-up

Subjects were required to make a total of 3 visits to the study site during the 8-week treatment period. At the first visit, subjects were screened, randomized, and provided with a sufficient supply of the assigned study product to last for the duration of the study, along with written instructions for daily product use.

Table 1. Subject Demographics

Study Population (N = 50)	
Race, n (%)	
White	45 (90%)
Asian	1 (2%)
Black	3 (6%)
Other	1 (2%)
Gender, n (%)	
Female	41 (82%)
Male	9 (18%)
Age (years)	
Average	48.36
Range	23–70

Subjects were instructed to consume 1 caplet with water once daily, at approximately the same time each day, regardless of meals. Subjects received self-assessment and product use diaries to be completed daily at home. All responses were self-reported using the following scale: 0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe, and 5 = very severe.

Self assessments of the severity of IBS symptoms (abdominal pain and bloating) were recorded in the diary each day for 8 weeks, starting at the end of the first visit (Day 0). Subjects began taking the study product the next day (Day 1). All subject responses used a 5-point scale to describe symptom severity (0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe).

Subjects returned for a second visit approximately 28 days after randomization and then for a final visit approximately 56 days after randomization. Compliance with study product use was determined by caplet count and review of product use diaries.

All adverse events were reported regardless of whether they were related to the study drug. Event duration, severity, and causal relationship to the study drug were recorded.

Statistical Analysis

Changes in symptom severity compared with baseline in the placebo and *B coagulans* GBI-30, 6086 groups were determined by Student's t-test, with $P < 0.05$ considered statistically significant.

Results

Forty-four subjects, 22 subjects in the *B coagulans* GBI-30, 6086 group and 22 subjects in the placebo group, completed the study. Average daily self-reported abdominal pain severity (Figure 1) and bloating (Figure 2) scores during the 8-week treatment period significantly improved both IBS symptoms in subjects who received *B coagulans* GBI-30, 6086, but not in subjects who received placebo.

Because baseline (Week 1) abdominal pain and bloating scores were significantly different between the 2 study groups, within-group change from baseline was used to evaluate efficacy (Tables 2, 3). Subjects who received *B coagulans* GBI-30, 6086 achieved consistently better weekly scores in change from baseline in abdominal pain and bloating during the 8-week treatment period compared with subjects who received placebo. Changes from baseline abdominal pain and bloating scores in the *B coagulans* GBI-30, 6086 group were statistically significant for each weekly comparison throughout the study ($P < 0.01$). In the placebo group, changes in abdominal pain scores at weeks 6 and 8 achieved statistical significance ($P < 0.05$).

Figure 1. Mean daily patient-reported severity scores for abdominal pain during the 8-week treatment period in 22 subjects in the *Bacillus coagulans* GBI-30, 6086 group (circles) and 22 subjects in the placebo group (squares).

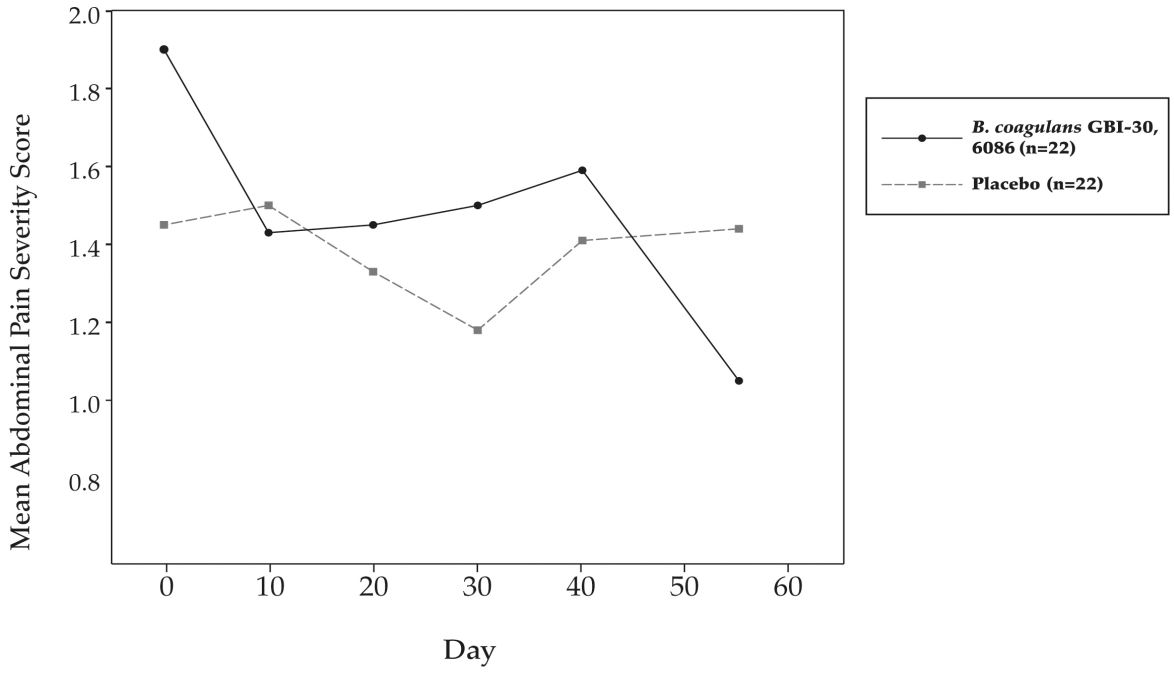
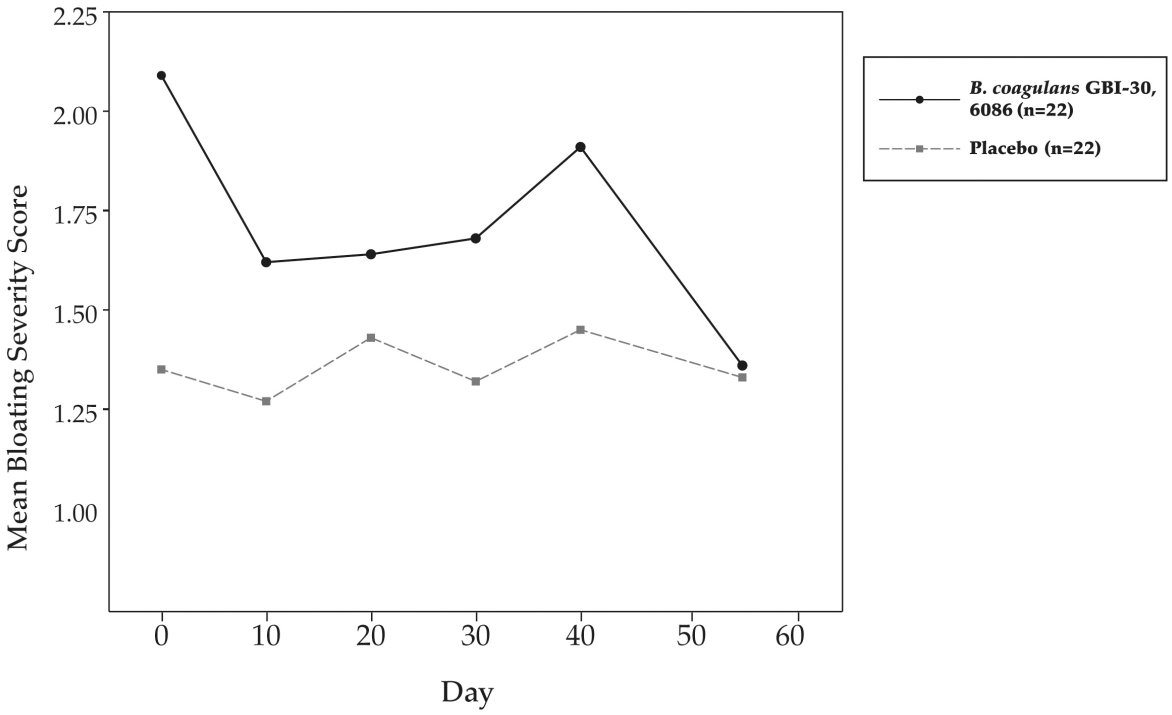


Figure 2. Mean daily patient-reported severity scores for bloating during the 8-week treatment period in 22 subjects in the *Bacillus coagulans* GBI-30, 6086 group (circles) and 22 subjects in the placebo group (squares).



There were 4 adverse events reported in the placebo group and 2 in the study group, all of which were unrelated to the treatments. No treatment-related adverse events or serious adverse events were reported during the 8-week study period.

Discussion

Studies of probiotics for the treatment of IBS have yielded contradictory results, and most studies have not offered convincing evidence that probiotics are effective for treating symptoms of IBS.[23]

A randomized study by O'Mahony et al[18] (N = 75) compared the effect of *Lactobacillus salivarius* or *Bifidobacterium infantis* on symptoms of IBS and cytokine ratios. The results showed *B infantis* was superior to *L salivarius* and placebo for relieving IBS symptoms, and the response was associated with normalization of the anti-inflammatory to proinflammatory cytokine ratio. In a large-scale, multicenter clinical trial[24] of women with IBS (N = 362), it was determined that encapsulated *B infantis* at a dose of 1×10^8 CFU was significantly superior to placebo and other doses of *B infantis* for relieving abdominal pain, bloating, bowel dysfunction,

Table 2. Average Weekly Change in Abdominal Pain Severity versus Baseline (Week 1)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
***Bacillus coagulans* GBI-30, 6086 (n = 22)**								
Mean	1.79	1.41	1.32	1.42	1.30	1.30	1.27	1.39
Change from baseline	–	–0.37	–0.46	–0.37	–0.49	–0.49	–0.52	–0.40
P value	–	0.006[a]	0.0001[b]	0.004[a]	0.0002[b]	0.0001[b]	0.0001[b]	0.007[a]
Placebo (n = 22)								
Mean	1.43	1.44	1.40	1.32	1.20	1.25	1.27	1.16
Change from baseline	–	0.00	–0.03	–0.11	–0.23	–0.18	–0.16	–0.27
P value	–	0.92	0.77	0.28	0.06	0.04[c]	0.12	0.02[c]

[a]$P \leq 0.01$; [b]$P \leq 0.001$; [c]$P \leq 0.05$.

Table 3. Average Weekly Change in Bloating Severity versus Baseline (Week 1)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
***Bacillus coagulans* GBI-30, 6086 (n = 22)**								
Mean	1.98	1.60	1.55	1.64	1.50	1.52	1.51	1.57
Change from baseline	–	–0.38	–0.43	–0.34	–0.48	–0.46	–0.46	–0.41
P value	–	0.003[a]	0.0003[b]	0.003[a]	0.0001[b]	0.0007[b]	0.0001[b]	0.002[a]
Placebo (n = 22)								
Mean	1.31	1.37	1.31	1.28	1.33	1.33	1.33	1.16
Change from baseline	–	0.06	0.00	–0.03	0.01	0.02	0.01	–0.15
P value	–	0.65	0.98	0.84	0.91	0.92	0.91	0.27

[a]$P \leq 0.01$; [b]$P \leq 0.001$.

incomplete evacuation, straining, and gas at the end of the 4-week study. The 2 other doses of *B infantis* (1×10^6 and 1×10^{10}) were not significantly different than placebo.

Finally, a 4-week, randomized study compared the composite probiotic VSL#3 containing *Bifidobacterium* (*B longum, B infantis*, and *B breve*), *Lactobacillus* (*L acidophilus, L casei, L delbrueckii* ssp. *bulgaricus*, and *L plantarum)*, and *Streptococcus salivarius* ssp. *thermophilus*) with placebo.[25] There was no statistical difference in bloating, pain, urgency, or number of stools. However, flatulence scores were reduced among patients treated with VSL#3 versus patients taking placebo.

In the present study, patients with IBS who were treated for 8 weeks with *B coagulans* GBI-30, 6086 demonstrated improvement of abdominal pain and bloating. The improvements were statistically significant at each weekly comparison. Subjects in the placebo group experienced statistically significant improvement in abdominal pain at weeks 6 and 8. According to a meta-analysis, this outcome is consistent with a number of other placebo-controlled IBS studies that have shown a high placebo effect among this patient population.[26]

Results from an earlier case-control study that investigated the efficacy of a probiotic preparation containing *L sporogenes* (*B coagulans*), *L acidophilus*, and *Streptococcus thermophilus* for alleviating the symptoms of IBS reported similar data.[27] Thirty-seven patients were given the probiotic preparation and followed for 6 months. Compared with baseline values, those who received the probiotic treatment reported significantly reduced abdominal pain ($P < 0.000001$), abdominal distention ($P = 0.003$), and constipation ($P = 0.03$), as well as reduced alternating constipation and diarrhea ($P = 0.01$).

Probiotics must survive gastric and bile acids[28] in order to reach the intestinal tract, colonize the host epithelium, and exhibit a beneficial effect.[29] Most conventional forms of lactobacilli-type probiotics are nonspore forming and, therefore, are inactivated by bile and low gastric pH.[30,31] Strains of *B coagulans* produce coagulin, which is a heat-stable, protease-sensitive, bacteriocin-like inhibitory substance with activity against gram-positive bacteria.[32] Spores of *Bacillus* are resistant to heat and hostile gastrointestinal conditions and, therefore, are able to reach the intestine where they can germinate and proliferate within the host.[21,22,30]

In addition, probiotics selected for commercial use must survive industrial manufacturing and storage to ensure long-term viability and activity.[21] Most cells of conventional lactobacilli die at 70°C, while spore-bearing lactic acid-forming bacteria do not show a decrease in viable cells even after heating in saline at 85°C for 30 minutes.[31] In addition to surviving heat and a hostile gastrointestinal environment, *B coagulans* GBI-30, 6086 maintains spore viability after 5 years of storage without the need for refrigeration (unpublished communication, Ganeden Biotech, Inc., Mayfield Heights, OH), making it particularly suitable for commercial use.

One limitation of this study was the need to use self-assessment diaries to measure outcomes, which contributed to wide variations in the average reported symptom changes during the study period. It is also likely that the small study size may have impacted the statistical outcomes. Nevertheless, the present study provides preliminary evidence that *B coagulans* GBI-30, 6086 probiotic has the ability to relieve abdominal pain and bloating in patients with IBS. No adverse events were reported with the use of this probiotic agent. Therefore, *B coagulans* GBI-30, 6086 may be a safe and effective alternative for patients with IBS who currently have limited therapeutic options. These results justify the design of larger scale, controlled clinical trials to verify our findings.

Conflict of Interest Statement

Larysa Hun, MD, FAAP discloses no conflicts of interest.

Disclosure

This study was performed at Research Testing Laboratories, an independent laboratory in Great Neck, NY.

References

1. American Gastroenterology Association. American Gastroenterological Association medical position statement: irritable bowel syndrome. *Gastroenterology*. 2002;123(6):2105–2107.
2. Drossman DA, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology*. 2002; 123(6):2108–2131.
3. Andrews EB, Eaton SC, Hollis KA, et al. Prevalence and demographics of irritable bowel syndrome: results from a large web-based survey. *Aliment Pharmacol Ther*. 2005;22(10):935–942.
4. Hungin AP, Chang L, Locke GR, Dennis EH, Barghout V. Irritable bowel syndrome in the United States: prevalence, symptom patterns and impact. *Aliment Pharmacol Ther*. 2005;21(11):1365–1375.
5. Mitchell CM, Drossman DA. Survey of the AGA membership relating to patients with functional gastrointestinal disorders. *Gastroenterology*. 1987;92(5 pt 1):1282–1284.
6. Drossman DA, Whitehead WE, Camilleri M. Irritable bowel syndrome: a technical review for practice guideline development. *Gastroenterology*. 1997;112(6):2120–2137.
7. Spiller RC, Jenkins D, Thornley JP, et al. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute *Campylobacter* enteritis and in post-dysenteric irritable bowel syndrome. *Gut*. 2000;47(6):804–811.
8. Dunlop SP, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology*. 2003;125(6):1651–1659.
9. Cumberland P, Sethi D, Roderick PJ, et al; IID Study Executive. The infectious intestinal disease study of England: a prospective evaluation of symptoms and health care use after an acute episode. *Epidemiol Infect*. 2003;130(3):453–460.
10. Collins SM. A case for an immunological basis for irritable bowel syndrome. *Gastroenterology*. 2002;122(7):2078–2080.
11. Chadwick VS, Chen W, Shu D, et al. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology*. 2002;122(7):1778–1783.
12. Si JM, Yu YC, Fan YJ, Chen SJ. Intestinal microecology and quality of life in irritable bowel syndrome patients. *World J Gastroenterol*. 2004;10(12):1802–1805.
13. Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol*. 2003;98(2):412–419.
14. O'Leary C, Quigley EM. Small bowel bacterial overgrowth, celiac disease, and IBS: what are the associations? *Am J Gastroenterol*. 2003;98(4):720–722.
15. Saggioro A. Probiotics in the treatment of irritable bowel syndrome. *J Clin Gastroenterol*. 2004;38(6 suppl):S104–S106.
16. Rolfe RD. The role of probiotic cultures in the control of gastrointestinal health. *J Nutr*. 2000;130(2S suppl):396S–402S.
17. Quigley EM. The use of probiotics in functional bowel disease. *Gastroenterol Clin North Am*. 2005;34(3):533–545.
18. O'Mahony L, McCarthy J, Kelly P, et al. *Lactobacillus* and *bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology*. 2005;128(3): 541–551.
19. Jiang T, Savaiano DA. Modification of colonic fermentation by bifidobacteria and pH in vitro. Impact on lactose metabolism, short-chain fatty acid, and lactate production. *Dig Dis Sci*. 1997;42(11): 2370–2377.
20. Ouwehand AC, Lagström H, Suomalainen T, Salminen S. Effect of probiotics on constipation, fecal azoreductase activity and fecal mucin content in the elderly. *Ann Nutr Metab*. 2002;46(3–4):159–162.
21. De Vecchi E, Drago L. *Lactobacillus sporogenes* or *Bacillus coagulans*: misidentification or mislabeling? *Int J Probiotics Prebiotics*. 2006; 1:3–10.
22. Hyronimus B, Le Marrec C, Sassi AH, Deschamps A. Acid and bile tolerance of spore-forming lactic acid bacteria. *Int J Food Microbiol*. 2000;61(2–3):193–197.
23. McFarland LV, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. *World J Gastroenterol*. 2008;14(17): 2650–2661.
24. Whorwell PJ, Altringer L, Morel J, et al. Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. *Am J Gastroenterol*. 2006;101(7):1581–1590.
25. Kim HJ, Vazquez Roque MI, Camilleri M, et al. A randomized controlled trial of a probiotic combination VSL#3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterol Motil*. 2005;17(5):687–696.
26. Patel SM, Stason WB, Legedza A, et al. The placebo effect in irritable bowel syndrome trials: a meta-analysis. *Neurogastroenterol Motil*. 2005;17(3):332–340.
27. Astegiano M, Pellicano R, Terzi E, Simondi D, Rizzetto M. Treatment of irritable bowel syndrome. A case-control experience. *Minerva Gastroenterol Dietol*. 2006;52(4):359–363.
28. Tuomola E, Crittenden R, Playne M, Isolauri E, Salminen S. Quality assurance criteria for probiotic bacteria. *Am J Clin Nutr*. 2001; 73(2 suppl):393S–398S.
29. Marco ML, Pavan S, Kleerebezem M. Towards understanding molecular modes of probiotic action. *Curr Opin Biotechnol*. 2006;17(2):204–210.
30. Casula G, Cutting SM. *Bacillus* probiotics: spore germination in the gastrointestinal tract. *Appl Environ Microbiol*. 2002;68(5):2344–2352.
31. Ara K, Meguro S, Hase T, et al. Effect of spore-bearing lactic acid-forming bacteria (*Bacillus coagulans* SANK 70258) administration on the intestinal environment, defecation frequency, fecal characteristics and dermal characteristics in humans and rats. *Microb Ecol Health Dis*. 2002;14:4–13.
32. Hyronimus B, Le Marrec C, Urdaci MC. Coagulin, a bacteriocin-like inhibitory substance produced by *Bacillus coagulans* I_4. *J Appl Microbiol*. 1998;85(1):42–50.

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A prospective, randomized, double-blind, placebo-controlled parallel-group dual site trial to evaluate the effects of a *Bacillus coagulans*-based product on functional intestinal gas symptoms

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Abstract

Background: This randomized double blind placebo controlled dual site clinical trial compared a probiotic dietary supplement to placebo regarding effects on gastrointestinal symptoms in adults with post-prandial intestinal gas-related symptoms (abdominal pain, distention, flatulence) but no gastrointestinal (GI) diagnoses to explain the symptoms.

Methods: Sixty-one adults were enrolled (age 36.5 ± 12.6 years; height 165.1 ± 9.2 cm; weight 75.4 ± 17.3 kg) and randomized to either Digestive Advantage™ Gas Defense Formula - (GanedenBC[30] *Bacillus coagulans* GBI-30, 6086): n = 30; or Placebo: n = 31. Study subjects were evaluated every two weeks over a four-week period using validated questionnaires and standard biochemical safety testing. Outcome criteria of interest included change from baseline in Gastrointestinal Symptom Rating Scale (GSRS) abdominal pain, abdominal distention, flatus, and the Severity of Dyspepsia Assessment (SODA) bloating and gas subscores over four weeks of product use.

Results: Measured against the placebo, subjects in the probiotic group achieved significant improvements in GSRS abdominal pain subscore (p = 0.046) and the GSRS total score (p = 0.048), with a strong trend for improvement on the GSRS abdominal distension subscore (p = 0.061). A strong placebo effect was evident which could explain the lack of statistical significant differences between the groups for many of the efficacy variables.

Conclusion: In conclusion, the *Bacillus coagulans*-based product was effective in improving the quality of life and reducing gastrointestinal symptoms in adults with post prandial intestinal gas-related symptoms and no GI diagnoses.

Trial Registration: ClinicalTrials.gov Identifier: NCT00881322

Background

It is estimated that only 10% of the 10^{14} cells in the human body actually belong to the body itself. The overwhelming majority of cells consist of a diverse ecology of nonpathogenic bacteria, and 1-2 kg of them live in the gut alone, mainly in the large intestine [1]. Bengmark suggests that human beings should indeed be considered to have two separate, equally vital digestive systems: one being the organs of the gastrointestinal tract; the other being the bacteria that colonize them [2]. The bacteria have defined an ecological niche for themselves in the intestines, fermenting non-digestible dietary residue and endogenous mucus from the epithelia [3]. Though the colon contains over 500 strains of bacteria, it is generally dominated by 35-40 different types of microbes. Many disorders of the gut have been associated with a disturbance in this distribution of species. Inflammatory bowel disease, diarrhea, and even multisystem organ failure [4] are believed to be correlated with an imbalance in gut ecology favoring the growth of pathogenic strains [5].

Probiotics are nutritional supplements designed to target pathogenic microbial species distribution by augmenting the growth of nonpathogenic bacteria. A commonly accepted definition of probiotic is "a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects on the host [6]." There is strong evidence that probiotics work by helping non-pathogenic bacteria to compete with their pathogenic counterparts for nutrient availability as well as for adhesion sites along the intestinal lining, preventing both the overgrowth of pathogenic bacteria as well as their translocation through the epithelial mucosa into the rest of the body [7]. There is also evidence to suggest that intestinal flora play an important role in immune system response. Studies in humans and rodents have shown that probiotic treatment is directly correlated with an increase of salivary immunoglobin A (sIgA) production. Furthermore, exposure to luminal microbes instantly increases the number of intraepithelial lymphocytes.

In addition to overwhelming evidence in support of the effectiveness of probiotics, their lack of detrimental side effects is further reason for their growing popularity. In fact in a recent review paper, Levri et. al. suggest that physicians' advice to patients regarding a given probiotic should be a cavalier "try it [8]." It is no surprise then that there is great interest in investigating their use as an inexpensive treatment for a variety of causes of gastrointestinal discomfort.

Digestive Advantage™ Gas Defense Formula (Ganeden Biotech, Mayfield Heights, Ohio) is a probiotic supplement containing *Bacillus coagulans* as well as an enzyme blend of cellulases from *Trichoderma longibrachiatum* and *Aspergillus niger*. Studies suggest that the probiotic Bacillus coagulans decreases the symptoms of abdominal pain and bloating in subjects with inflammatory bowel disease [9]. With this in mind, we undertook a randomized, double-blind, placebo-controlled clinical trial to evaluate Gas Defense (GD). The purpose of the study was to compare its effect versus placebo on gastrointestinal quality of life in adults with intestinal symptoms but no GI diagnoses.

Methods

Experimental Design

This double-blind, placebo-controlled clinical study randomized 61 subjects at two investigative sites (Miami and the Dominican Republic). Subjects provided written informed consent prior to participating in any study procedures. Subjects were then randomized within each site in a 1:1 manner into intervention (GD) or placebo groups. Investigators and subjects were blinded to product assignment. Subjects were seen at three visits over the course of four weeks - a screening/randomization visit at Day 0, and two follow-up visits at Days 14 and 29. On Day 0, the participants were instructed to begin taking one capsule daily, at approximately the same time of day, and to continue doing so for the duration of the study. Participants were provided sufficient product at visits 1 and 2 to cover the time between visits. Compliance with product use was measured via the pill counting method. During each visit, the participants were evaluated with a series of questionnaires in addition to hemodynamics and adverse event monitoring. The research was in compliance with the Helsinki Declaration and approved by the Aspire Independent Review Board San Diego, California (approved May 13, 2008) and Consejo Nacional de Bioetica en Salud (Conabios), Santo Domingo, Dominican Republic (approved June 23, 2008).

Subject Population

Subjects were drawn from the Greater Miami area and the Dominican Republic. All were between 20-68 years of age and had self-reported post-meal intestinal gas-related symptoms including abdominal pain, cramps, distended feeling/bloating, and flatulence. Out of a total of 98 subjects interviewed by phone, 64 attended the screening evaluation. Three of those subjects did not meet entry criteria. In the final study population, seven subjects came from Miami and 54 came from the Dominican Republic. Sixty subjects began the study but one was terminated at the discretion of the investigator after a single dose. An additional subject was subsequently enrolled with IRB notification and approval. All subjects were in otherwise good health, willing and able to comply with the protocol, and, if female, neither pregnant nor lactating and willing to use a reliable method of birth control. All subjects

signed the IRB-approved Informed Consent prior to any procedures being conducted.

Exclusion criteria for entering this study included; active heart disease, uncontrolled high blood pressure, renal or hepatic impairment, Type I or II diabetes, psychiatric and immune disorders, unstable thyroid disease, Parkinson's disease, a history of cancer, previous stomach or intestinal surgery, the consumption of medication or supplements that would interfere with the natural flora of the gut such as antibiotics, probiotics, or prebiotics within the last 30 days prior to screening. Subjects with gastrointestinal disorders or other digestive problems such as Crohn's disease, short bowel, ulcerative colitis, Irritable Bowel Syndrome, constipation, or lactose intolerance were also excluded. Lactose intolerance was excluded as per subject profession or previous diagnosis. Similarly, subjects using GI medications to control the function of the gut, such as anti-spasmodics, motility agents, pro-kinetic agents, or laxatives were excluded. Subjects were only permitted to use over-the-counter gas relief products as rescue treatment during the study. Only one subject reported having done so. Subjects allergic to wheat, fish, or any other ingredients in GD or the placebo were excluded.

Intervention

The active product tested is a probiotic supplement containing *Bacillus coagulans* (specifically *Bacillus coagulans* GBI-30, 6086, also known as GanedenBC[30]). The product specifically contained *B. coagulans*, Enzyme Blend (cellulase - *Trichoderma longibrachiatum*, cellulase - *Aspergillus niger*, hemicellulase, α-galactosidase, invertase) with the inactive ingredients of a vegetarian capsule, magnesium stearate, silicon dioxide, and maltodextrin. There were 2.0×10^9 colony forming units per capsule.

The placebo was provided by the manufacturer and matched in size and color to the active product. Independent product analysis for content was carried out to confirm label content claim (ULTRAtab Labs, Highland, New York). All subjects were instructed to take one tablet daily for the duration of the study.

Assessment

During the study, subjects were asked to complete several questionnaires, each targeting a different symptom. Distension, pain, and flatus were tracked using the corresponding subsections of the GI Symptoms Rating Scale (GSRS) [10]. Bloating and gas were measured with the Severity of Dyspepsia Assessment (SODA) [11]. Other assessments included the GSRS overall score and the SODA Non-Pain Symptoms (NPS) subscore, as well as the SODA subscore for satisfaction with dyspepsia-related health, SF-36v2 quality of life physical and mental component summaries, and 7-point anchored Visual Analog Scale (VAS-Gas) assessment of gas symptoms.

All questionnaires were completed by the study subjects at every visit, except for VAS-Gas, which was administered only at the second and third visits because it asks for a consideration of relative change from baseline. Blood pressure and heart rate were measured at each visit, and study compliance was monitored by the pill count method.

Statistical Methods

The two primary endpoints for analysis in this study were the GSRS subscores for abdominal pain, distension, and flatus; and the SODA subscores for bloating and gas. Other endpoints included the GSRS overall score, SODA-NPS score, SODA subscore for satisfaction with dyspepsia-related health, the SF-36v2 summaries, and the VAS-Gas assessment.

The formal efficacy analysis consisted of a set of analyses of covariance (ANCOVAs), one for each efficacy endpoint. The value of the efficacy variable at Visit 3 (end of study) was the dependent variable, the product group (GD or placebo) was the variable of interest, and the value of the efficacy variable at Visit 1 (baseline) was a covariate. Investigative site (US or DR) was also included in the model. Only p-values less than or equal to 0.05 were considered significant.

Other descriptive (non-inferential) summaries and comparisons were carried out - mean changes from baseline to each subsequent time point were tested by the paired Student t test or Wilcoxon signed-ranks test, and mean differences between product groups were tested by the unpaired Student t test or Mann-Whitney U test. Differences in the distribution of categorical variables between the product groups were tested by the Fisher Exact test.

Sample size was determined on the basis of time, cost, and the ability to detect a clinically important effect size. It was determined that 25 analyzable subjects per group would provide 80% power to obtain a significant result for a 0.8-sigma effect size. To allow for a possible 15% attrition from the study, 30 subjects were enrolled per group. No adjustment for multiple testing was applied in the analysis of data from this study. Each test was evaluated at the 0.05 alpha level ($p \leq 0.05$ considered significant).

Results and Discussion

Most subject characteristics at baseline (the screening/randomization) were evenly matched between the two product groups (Table 1). The placebo group was, on average, four years older and eight kilograms heavier than the GD

Table 1: Baseline and Descriptive Characteristics

Group	Gas Defense (n = 30)	Placebo (n = 31)
Site		
Dominican Republic	27 (90%)	27 (87%)
Miami, FL	3 (10%)	4 (4%)
Age, Years	34.8 ± 12.5	38.2 ± 12.6
Gender		
Female	16 (53%)	17 (55%)
Male	14 (47%)	14 (45%)
Ethnicity		
Hispanic	27 (90%)	28 (90%)
Non-Hispanic	3 (10%)	3 (10%)
Race		
Black/AA	8 (27%)	7 (23%)
Caucasian	9 (30%)	9 (29%)
Other	12 (43%)	15 (48%)
Height, cm	164.2 ± 8.6	165.8 ± 9.8
Weight, kg	71.4 ± 14.1	79.2 ± 19.3
Status		
Completed Protocol	30 (100%)	30 (97%)
Early Termination	0 (0%)	1 (3%)
Heart Rate, beats/minute	69.9 ± 12.1	70.7 ± 10.3
Systolic Blood Pressure, mm Hg	121.2 ± 17.0	122.2 ± 10.9
Diastolic Blood Pressure, mm Hg	75.1 ± 9.0	76.0 ± 7.2
GSRS - Abdominal Pain Subscore	3.17 ± 1.85	3.14 ± 1.48
GSRS - Abdominal Distension Subscore	3.38 ± 2.13	4.14 ± 1.43
GSRS - Increased Flatus Subscore	3.86 ± 1.92	4.07 ± 1.53
GSRS - Total GI Symptom Score	40.8 ± 19.8	39.4 ± 12.1
SODA - Bloating Subscore	2.52 ± 1.48	2.93 ± 1.25
SODA - Gas Subscore	3.28 ± 0.96	3.28 ± 0.84
SODA - Non-pain Symptoms Score	16.83 ± 3.35	17.00 ± 2.09
SODA - Satisfaction Score	8.3 ± 3.4	9.2 ± 3.5
SF-36v2 - Physical Component Summary	49.9 ± 8.5	49.0 ± 9.9
SF-36v2 - Mental Component Summary	51.3 ± 10.1	51.3 ± 9.9

Values are expressed as mean ± standard deviation.

group. Most of the endpoints tracked did not show a significantly different response between GD and placebo. These included the GSRS increased flatus subscore, the SODA bloating subscore, the SODA non-pain symptoms and satisfaction scores, and the SF-36v2 physical and mental component summaries. However, all but the SF-36v2 MCS showed differences in the direction that indicated a larger beneficial effect for GD than the placebo.

Table 2 shows the ANCOVA coefficient of the product group - an estimate of the amount by which the four-week improvement in the GD group exceeds that of the placebo group, along with its standard error and p-value indicating whether or not there is a statistically significant difference between the product and placebo. GD performed significantly or nearly significantly better than placebo for the following endpoints (Tables 3, 4 and 5): GSRS: abdominal pain subscore (p = 0.046), GSRS: abdominal distension subscore (p = 0.061), and GSRS total score (p = 0.048).

While other efficacy endpoints do not indicate statistical significance for GD relative to placebo, all but the SF-36v2 MCS and the VAS-Gas score showed differences in the direction that indicates a larger beneficial effect for GD than for placebo.

The lack of significance for many of the efficacy endpoints can be attributed to several factors. First, as seen in the descriptive summary tables for each endpoint, a very strong placebo effect was evident in this study. Subjects generally liked the product they were taking and tended to report substantial improvement regardless of which product they were taking. All endpoints showed large four-week improvement in both product groups. This may be partly cultural, with people wanting to demonstrate what they considered to be the "expected" improvement, although this cannot be established from the available data. Whatever the cause, this kind of phenomenon is quite common in studies involving subjective endpoints (especially discomfort-related endpoints). With the pla-

Table 2: Efficacy Analysis (ANCOVA)

Endpoint	Coefficient ± Std Err	p-value
GSRS: Abdominal Pain Subscore (lower is better)	-0.627 ± 0.307	0.046 †
GSRS: Abdominal Distension Subscore (lower is better)	-0.572 ± 0.299	0.061 ‡
GSRS: Increased Flatus Subscore (lower is better)	-0.511 ± 0.353	0.154
GSRS: Total Score (lower is better)	-4.806 ± 2.381	0.048 †
SODA: Bloating Subscore (lower is better)	-0.229 ± 0.216	0.294
SODA: Gas Subscore (lower is better)	-0.348 ± 0.219	0.118
SODA: Non-Pain Symptoms Score (lower is better)	-1.025 ± 0.870	0.244
SODA: Satisfaction Score (lower is better)	-0.058 ± 1.358	0.966
SF-36v2: Physical Component Summary (higher is better)	0.941 ± 1.118	0.403
SF-36v2: Mental Component Summary (higher is better)	-2.400 ± 2.010	0.238

† Significant ($p \leq 0.05$)
‡Approaches significance ($p \sim 0.05$)

Table 3: Gastrointestinal Symptom Rating Scale Abdominal Pain Subscore

Visit	Gas Defense	Placebo
Day 0 Screen/Rand	3.17 ± 1.85 (29)	3.14 ± 1.48 (29)
Day 14 Mid-Study	2.10 ± 1.29 (29)	2.28 ± 1.51 (29)
Day 29 End-of-Study	1.59 ± 0.95 (29)	2.21 ± 1.45 (29)
Change from Day 0 to Day 14	-1.07 ± 1.70 (29)	-0.86 ± 1.81 (29)
Change from Day 0 to Day 29	-1.59 ± 1.70 (29)	-0.93 ± 1.67 (29)

Values are expressed as mean ± standard deviation.

cebo group showing such a large improvement, there was not much "room for improvement" for the GD group over placebo.

Also, there was a considerable amount of random variability in most of the efficacy endpoints. That is, most of the efficacy variables had large within-group standard deviations for four-week changes from baseline. This is quite common with subjective, semi-quantitative endpoints like the GSRS and SODA questionnaire scales, and it has the effect of reducing the power to detect significance. This study was powered to provide a good chance of getting a significant result for an endpoint if the average amount of improvement (for GD, compared to Placebo) was at least 4/5 as large as the within-group standard deviation for that endpoint. In this study, the magnitude of the improvements tended to be less than that.

Table 4: Gastrointestinal Symptom Rating Scale Abdominal Distension Subscore

Visit	Gas Defense	Placebo
Day 0 Screen/Rand	3.38 ± 2.13 (29)	4.14 ± 1.43 (29)
Day 14 Mid-Study	1.83 ± 1.04 (29)	2.48 ± 1.38 (29)
Day 29 End-of-Study	1.66 ± 1.08 (29)	2.38 ± 1.21 (29)
Change from Day 0 to Day 14	-1.55 ± 1.88 (29)	-1.66 ± 1.70 (29)
Change from Day 0 to Day 29	-1.72 ± 2.02 (29)	-1.74 ± 1.68 (29)

Values are expressed as mean ± standard deviation.

Table 5: Gastrointestinal Symptom Rating Scale Total Score

Visit	Gas Defense	Placebo
Day 0 Screen/Rand	40.8 ± 19.8 (29)	39.4 ± 12.1 (29)
Day 14 Mid-Study	29.0 ± 8.7 (29)	31.6 ± 11.4 (29)
Day 29 End-of-Study	25.2 ± 10.0 (29)	29.4 ± 9.7 (29)
Change from Day 0 to Day 14	-11.9 ± 16.4 (29)	-7.8 ± 11.7 (29)
Change from Day 0 to Day 29	-15.6 ± 17.4 (29)	-9.9 ± 12.3 (29)

Values are expressed as mean ± standard deviation.

Conclusion

The *Bacillus coagulans*-based probiotic product showed superior numerical scores to placebo in 10 of 12 efficacy variables, and the differences were significant or nearly significant in three of the 12 variables. Within this study population, the *Bacillus coagulans*-based probiotic product was effective and safe for abating symptoms of gastrointestinal distress, particularly abdominal pain and distention in the post-prandial period.

Competing interests

The authors received funding for this study from Ganeden Biotech.

Authors' contributions

DSK participated in the design of the study and drafting of the manuscript. HIS participated in the design of the study and served as a Sub Investigator. PA participated in the design of the study, served as co-Principal Investigator and contributed to the manuscript. SF participated in the design of the study, data collection and manuscript preparation. JCP participated in the study design, data collection and performed the statistical analysis. DRK participated in the design of the study, served as co-Principal Investigator and contributed to the manuscript. All authors read and approved the final manuscript.

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References

1. Savage DC: **Microbial ecology of the human gastrointestinal tract.** *Annual Review of Microbiology* 1977, **31:**107-133.
2. Bengmark S: **Pre-, pro-, and synbiotics.** *Current Opinion in Clinical Nutrition and Metabolic Care* 2001, **4:**571-579.
3. Roberfroid MB, Bornet F, Boulet C, Cummings JH: **Colonic microflora: nutrition and healthL summary and conclusions of an**

International Life Sciences Institute (ILSI) Europe workshop held in Barcelona, Spain. *Nutrition Reviews* 1995, **53:**127-130.
4. Lichman SM: **Bacterial translocation in humans.** *Journal of Pediatric Gastroenterol Nutrition* 2001, **33:**1-10.
5. Guarner F, Malagelada JR: **Gut flora in health and disease.** *The Lacent* 2003, **360:**512-519.
6. de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J: **Probiotics - compensation for lactase insufficiency.** *American Journal of Clinical Nutrition* 2001, **73(Suppl):**421-429.
7. Bernet MF, Brassart D, Neeser JR, Servin AL: ***Lactobacillus acidophilus* LA 2 binds to culture human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria.** *Gut* 1994, **35:**483-489.
8. Levri KM, Ketvertis K, Deramo M, Merestein JH, D'Amico F: **Do probiotics reduce adult lactose intolerance?** *The Journal of Family Practice* 2005, **54:**613-620.
9. Hun L: **Original research: *Bacillus coagulans* significantly improved abdominal pain and bloating in patients with IBS.** *Postgraduate Medicine* 2009, **121(2):**119-124.
10. Dimenas E, Glise H, Hallerback B, Hernqvist H, Svedlund J, Wiklund I: **Quality of life of patients with upper gastrointestinal symptoms: An improved evaluation of treatment regimens?** *Scandinavian Journal of Gastroenterology* 1993, **28:**681-687.
11. Rabeneck L, Cook KF, Wristers K, Souchek J, Menke T, Wray NP: **Soda (severity of dyspepsia assessment): A new effective outcome measure for dyspepsia-related health.** *Journal of Clinical Epidemiology* 2001, **54:**755-765.

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Original Research: A Patented Strain of *Bacillus coagulans* Increased Immune Response to Viral Challenge

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Abstract

Background: Viral respiratory tract infection is the most common illness among humans. Probiotics have been known to enhance the immune system and, therefore, may represent a significant therapeutic advancement for treating viral respiratory tract infections. **Objective:** A controlled study was conducted to evaluate the effects of the patented GanedenBC^{30} probiotic (*Bacillus coagulans* GBI-30, 6086, marketed as Sustenex® [Ganeden Biotech, Inc., Mayfield Heights, OH]) on the immune system when exposed to adenovirus and influenza in otherwise healthy adults. **Methods:** Ten healthy men and women (average age, 44 years) were instructed to consume 1 capsule of GanedenBC^{30} with water once a day for 30 days. At baseline and after completion of the 30-day treatment, blood levels of cytokines were measured in vitro after T-cell exposure to adenovirus and influenza A. Each participant served as his/her own control with baseline blood draw. **Results:** The use of GanedenBC^{30} significantly increased T-cell production of TNF-α in response to adenovirus exposure ($P = 0.027$) and influenza A (H3N2 Texas strain) exposure ($P = 0.004$), but it did not have a significant effect on the response to other strains of influenza. No serious adverse events were reported throughout the study. **Conclusions:** The patented GanedenBC^{30} probiotic may be a safe and effective therapeutic option for enhancing T-cell response to certain viral respiratory tract infections.

Keywords: probiotics; immune response; respiratory tract infection; *Bacillus coagulans*; GanedenBC^{30}; lactic acid-producing bacteria

Introduction

Viral respiratory tract infections (eg, common cold, influenza) are the most common illnesses among humans.[1] They have significant health and economic consequences, especially among young children, the elderly, and people with underlying or chronic conditions. Every year, an average of 5% to 20% of people in the United States contract influenza, more than 200 000 people are hospitalized with influenza-related complications, and approximately 36 000 people die from influenza.[2] It is also estimated that 1 billion colds occur annually in the United States.[3] To date, only symptomatic medications and homeopathic remedies are available.[4] With such high incidence rates, a safe and effective alternative is clearly needed to reduce the burden of illness.

Probiotics are live microbial preparations that have the ability to modulate host physiological systems.[5] There are several probiotic strains, particularly *Lactobacillus* sp., available in commercial products today. Gram-positive, lactic acid-producing bacteria have received major consideration in the past decade.[6] They exhibit a variety

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of effects, including enhancement of the systemic immune response and mucosal immunity to defend against viral respiratory tract infection.[7,8]

Bacillus coagulans is a gram-positive, spore-forming rod, 0.9 μm by 3 μm to 5 μm in size, and is aerobic to microaerophilic. Because of forming spores, these bacilli can withstand the acidic environment of the stomach to reach the intestine where they germinate and proliferate, producing the favored L (+) optical isomer of lactic acid.[9] Traditional lactobacilli and bifidobacteria are much less likely to tolerate gastric and bile acid.[10]

GanedenBC^{30} (*B coagulans* GBI-30, 6086, marketed as Sustenex® [Ganeden Biotech, Inc., Mayfield Heights, OH]) is a patented strain of *B coagulans* that has the potential to improve the immune response to various pathogens. The aim of the present study was to evaluate the effects of GanedenBC^{30} on T-cell immune response after exposure to adenovirus and influenza in otherwise healthy adults.

Materials and Methods

This study was a controlled trial including 10 healthy volunteers. All participants gave signed informed consent before inclusion in the study. The study was approved by the institutional review board (IRB). It was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and was consistent with Good Clinical Practice and applicable regulatory requirements. The study was also conducted in accordance with the regulations of the US Food and Drug Administration as described in 21 CFR 50 and 56, applicable laws, and the IRB requirements.

Participants

A total of 10 participants between the ages of 18 and 75 years were recruited for the study.

The exclusion criteria included the following: any chronic or current illness, pregnancy or breastfeeding, allergies to the test products or their ingredients, alcohol or drug abuse, immunization within 2 months of screening, and taking any investigational drug within 30 days of enrollment.

Participants were required to make 2 visits to the clinic during the study. At the first visit, an initial blood draw was taken. Participants were then instructed to consume 1 capsule of GanedenBC^{30} with water once daily for 30 days, at approximately the same time each day, regardless of meals. After 30 days, participants returned to the study site for a second blood draw. Compliance with the study treatment regimen was determined by caplet count.

Treatment

Participants were given capsules containing 2 billion CFU of GanedenBC^{30}. The probiotic bacteria used during the study period was tested by the manufacturer to ensure bacteria counts prior to shipping.

Laboratory Evaluation

In this study, each participant served as his/her own control, with 30-day results compared with baseline results. Blood drawn at baseline and after the 30-day treatment period was used in 2 separate assays to measure immune response.

For the first assay, 5 aliquots of whole blood were prepared in sterile microtubes. Antigens were added to the whole blood and a saline control. The antigens included adenovirus (Fitzgerald, 30-AA02; stock, 2.8 mg/mL) at a concentration of 10 μg/mL, influenza A Texas 1/77 (H3N2) (Fitzgerald, 30-A150; stock, 1 mg/mL) at a concentration of 50 μg/mL, influenza A New Caledonia/20/99 IVR 116 (H1N1) (ProSpec-Tany TechnoGene, IHA-003; stock, 1 mg/mL) at a concentration of 10 μg/mL, and lipopolysaccharide at a concentration of 10 ng/mL. After a 24 ± 2-hour incubation period at 37°C and 5% CO_2, supernatants were collected and cytokine concentrations were measured using the TNF-α and IFN-γ Luminex® cytokine assay per the manufacturer's instructions (Luminex Corp., Austin, TX).

For the second assay, whole blood was stimulated with phytohemagglutinin overnight. A CD4 test was conducted at baseline to screen for immunocompromised subjects. CD4 count was evaluated again at 30 days to ensure that the study treatment did not induce immunosuppression. CD4 cells were separated and immune response was measured using the ImmuKnow® immune function assay (Cylex® Inc., Columbia, MD) according to manufacturer's instructions.

Statistical Analysis

For each marker, the difference in viral-induced cytokine production at baseline and after 30 days of GanedenBC^{30} treatment was calculated to obtain a difference score. Wilcoxon signed-rank tests were performed to evaluate whether there was a change in marker levels at 30 days versus baseline. A P value < 0.05 was considered significant and indicated 95% confidence that the true parameter value was different than the null value. Analyses were performed using SAS Version 9.1 (SAS System, SAS Institute, Cary, NC) and StatXact Version 4.0 (Cytel Software Corporation, Cambridge, MA) statistical software.

Results

A total of 10 adults were screened for inclusion in the study. One participant was excluded from analysis because baseline values indicated a possible underlying infection. The remaining 9 subjects completed the study and were compliant with the treatment regimen. There was an even distribution of men and women participants. Ninety percent of participants were white and 10% were black. The average age was 44 years (range, 33–63 years) (Table 1).

Results showed a significant increase (250%) in the TNF-α response to adenovirus after 30 days of treatment with GanedenBC^{30} versus baseline (P = 0.027) (Table 2). There was also a significant increase (1709%) in the TNF-α response to influenza A (H3N2 Texas strain) after 30 days of treatment with GanedenBC^{30} versus baseline (P = 0.004) (Table 2). Treatment with GanedenBC^{30} did not have a significant effect on plasma TNF-α levels upon exposure to other strains of influenza, nor was there any effect on plasma IFN-γ production after exposure to any viral strains. No serious adverse events were reported throughout the study.

Discussion

The intestine is the largest immunological organ in the body and contains 70% to 80% of all IgA-producing cells.[11] It is known that gram-positive, lactic acid-producing bacteria improve the balance in the composition of the gut microflora and potentially modulate immune responses.[8]

Although the mechanism of action is not fully understood, it is postulated that probiotics may stimulate an immune response by increasing the number of IgA-secreting cells, which migrate from the Peyer's patches to distant mucosal sites such as the respiratory glands.[11]

In the present study, we investigated the effects of the patented GanedenBC^{30} probiotic on immune response after exposure to adenovirus and influenza. The use of GanedenBC^{30} significantly increased the production of TNF-α in response to exposure to adenovirus, which indicates a heightened immunological effect. Likewise, the use of GanedenBC^{30} significantly increased the production of TNF-α in response to exposure to influenza A (H3N2 Texas strain).

Among subjects treated with GanedenBC^{30}, there were no serious adverse events reported throughout this study. Studies have shown that lactobacilli and bifidobacteria may be associated with opportunistic infections such as bacteremia, sepsis, or endocarditis among immunocompromised patients.[12,13] The transience of GanedenBC^{30} in the gut reduces the risk of developing pathogenesis-conferring mutations and causing infection compared with other probiotics that colonize and adhere to the gut epithelium (eg, lactobacilli, bifidobacteria).

Studies investigating the effects of gram-positive, lactic acid-producing bacteria on viral respiratory tract infections are limited and results are conflicting. A double-blind, placebo-controlled intervention study by Winkler et al[14] found that probiotics including *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, and *B bifidum* MF 20/5 plus vitamins and minerals did not significantly reduce the incidence of the common cold and did not affect the duration of infection. An earlier study found significantly fewer respiratory infections in children attending day care who consumed milk containing *L rhamnosus* GG.[8] Likewise, elderly subjects who consumed milk with *L casei* showed a 20% reduction in the duration of gastrointestinal and respiratory infections during winter months.[15] A more recent study investigating the effects of probiotic bacteria on viral respiratory tract infections found that lactobacilli and bifidobacteria in tablet form had no effect on the incidence of colds, but did reduce the duration of episodes by almost 2 days.[16]

It is known that probiotics tend to exhibit various overlapping mechanisms, such as the regulation of intestinal microbial homeostasis, the stimulation of local and systemic immune responses, the prevention of pathogens infecting the mucosa, the stabilization or maintenance of the gastrointestinal barrier function, the inhibition of procarcinogenic enzymatic activity, and the competition for limited nutrients.[17,18] Given the diversity of effects, it is unlikely that any 1 probiotic strain can accomplish all of these functions.[17] Rather, probiotic effects appear to be strain specific.[6]

Regardless of the strain and its potential effect, probiotics must survive gastric and bile acids[19] in order to reach that intestinal tract, colonize the host epithelium, and exhibit a beneficial effect.[20] Most conventional forms of lactobacilli probiotics are inactivated by bile and low gastric pH.[10]

Table 1. Participant Demographics

Participants (N = 10)	
Race	**n (%)**
White	9 (90%)
Black	1 (10%)
Gender	**n (%)**
Women	5 (50%)
Men	5 (50%)
Age	**Years**
Average	44
Range	33–63

Table 2. Means and Standard Errors for the Marker Difference Scores (day 30 minus day 0) and *P* Values for the (exact) Wilcoxon Signed-Rank Test

Marker	Mean (Standard Error)	*P* Value (Wilcoxon)
TNF-α		
Adenovirus VI	245.3 (107.6)	0.027
Influenza A (H3N2 Texas strain)	304.3 (142.2)	0.004
Influenza A (H1N1 New Caledonia)	−262.9 (330.1)	0.50
INF-γ		
Adenovirus VI	1.52 (4.10)	0.81
Influenza A (H3N2 Texas strain)	2.84 (2.21)	0.38
Influenza A (H1N1 New Caledonia)	−3.96 (2.85)	0.062
CD4[a]	−5.56 (5.22)	0.44
PHA CD4	15.6 (33.7)	0.65

Abbreviation: PHA, phytohemagglutinin.
[a]CD4 was evaluated at baseline to screen for immunocompromised subjects and at 30 days to ensure that the study treatment did not induce immunosuppression.

However, spore-bearing, lactic acid-forming bacteria, such as GanedenBC[30], are protected by a hardened spore coating that can withstand gastric and bile acid for delivery to the small and large intestines.[10] This may partly explain why GanedenBC[30] stimulated a significant immune response while other forms of probiotics in the studies mentioned above have not.

In addition, probiotics selected for commercial use must survive industrial manufacturing and storage to ensure long-term viability and activity.[6] Most cells of conventional lactobacilli die at 70°C, while spore-bearing, lactic acid-forming bacteria do not show a decrease in viable cells even after heating in saline at 85°C for 30 minutes.[10] In addition to surviving heat, gastric acidity, and bile, GanedenBC[30] maintains spore viability without the need for refrigeration, making it an ideal product for commercial use.

The present study provides evidence that the patented GanedenBC[30] probiotic has the ability to boost the immune response upon exposure to adenovirus and a strain of influenza A. Therefore, in addition to its other therapeutic effects, GanedenBC[30] may be considered a safe and effective option for enhancing immune defense against certain viral infections of the respiratory tract. Only TNF-α and IFN-γ responses were measured because of financial limitations. Larger clinical studies that include additional markers are warranted.

Acknowledgments

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Conflict of Interest Statement

Mira Baron, MD discloses no conflicts of interest.

References

1. Fendrick AM, Monto AS, Nightengale B, Sarnes M. The economic burden of non-influenza-related viral respiratory tract infection in the United States. *Arch Intern Med*. 2003;163(4):487–494.
2. Centers for Disease Control and Prevention. Influenza: the disease. http://www.cdc.gov/flu/about/disease/index.htm. Accessed July 28, 2008.
3. National Institute of Allergy and Infectious Diseases of the National Institutes of Health. Common cold. http://www3.niaid.nih.gov/healthscience/healthtopics/colds/overview.htm. Accessed July 30, 2008.
4. Fendrick AM. Viral respiratory infections due to rhinoviruses: current knowledge, new developments. *Am J Ther*. 2003;10(3):193–202.
5. Cross ML, Ganner A, Teilab D, Fray LM. Patterns of cytokine induction by gram-positive and gram-negative probiotic bacteria. *FEMS Immunol Med Microbiol*. 2004;42(2):173–190.
6. De Vecchi E, Drago L. *Lactobacillus sporogenes* or *bacillus coagulans*: misidentification or mislabeling? *Int J Probiotics and Prebiotics*. 2006;1:3–10.
7. Yasui H, Kiyoshima J, Hori T. Reduction of influenza virus titer and protection against influenza virus infection in infant mice fed *Lactobacillus casei* Shirota. *Clin Diagn Lab Immunol*. 2004;11(4):675–679.
8. Hatakka K, Savilahti E, Pönkä A, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. *BMJ*. 2001;322(7298):1–5.
9. Breed RS, Murray EGD, Smith NR, et al. *Bergey's Manual of Determinative Bacteriology*. 7th ed. Baltimore, MD: The Williams and Wilkins Co; 1957.
10. Ara K, Meguro S, Hase T, et al. Effect of spore-bearing lactic acid-forming bacteria (*Bacillus coagulans* SANK 70258) administration on the intestinal environment, defection frequency, fecal characteristics and dermal characteristics in humans and rats. *Microb Ecol Health Dis*. 2002;14:4–13.
11. Perdigón G, Fuller R, Raya R. Lactic acid bacteria and their effect on the immune system. *Curr Issues Intest Microbiol*. 2001;2(1):27–42.
12. Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. *Lactobacillus* sepsis associated with probiotic therapy. *Pediatrics*. 2005;115(1):178–181.
13. Ishibashi N, Yamazaki S. Probiotics and safety. *Am J Clin Nutr*. 2001;73(2 suppl):465S–470S.

14. Winkler P, de Vrese M, Laue Ch, Schrezenmeir J. Effect of a dietary supplement containing probiotic bacteria plus vitamins and minerals on common cold infections and cellular immune parameters. *Int J Clin Pharmacol Ther*. 2005;43(7):318–326.
15. Turchet P, Laurenzano M, Auboiron S, Antoine JM. Effect of fermented milk containing the probiotic *Lactobacillus casei* DN-114001 on winter infections in free-living elderly subjects: a randomised, controlled pilot study. *J Nutr Health Aging*. 2003;7(2):75–77.
16. de Vrese M, Winkler P, Rautenberg P, et al. Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized, controlled trial. *Vaccine*. 2006;24(44–46):6670–6674.
17. Boirivant M, Strober W. The mechanism of action of probiotics. *Curr Opin Gastroenterol*. 2007;23(6):679–692.
18. Rolfe RD. The role of probiotic cultures in the control of gastrointestinal health. *J Nutr*. 2000;130(2 suppl):396S–402S.
19. Tuomola E, Crittenden R, Playne M, Isolauri E, Salminen S. Quality assurance criteria for probiotic bacteria. *Am J Clin Nutr*. 2001;73(2 suppl): 393S–398S.
20. Marco ML, Pavan S, Kleerebezem M. Towards understanding molecular modes of probiotic action. *Curr Opin Biotechnol*. 2006;17(2):204–210.

I = intervention * = undescribed starch source

	Type	Reference	Title	Fibersol dose (source)	Treatment Duration/grp	Age of Participants	Qualifying Parameters	Study Design	N/group	Observed variables	Result(s) (significance)	Comments
1	I	Furukawa et al 2004 J Jpn Council for Advanced Food Ingredients Res. 7(1) 55-62.	Effects Prepared Cocoa Powder Containing Indigestible Dextrin on Human Defecation.	**4.2 g indigestible dextrin*** added to prepared cocoa in test drink (100 ml), equal amount of maltodextrin added in control	2 weeks	?	healthy adult subjects	**Placebo controlled Cross over** - 1 week wash out	40 (7 men; 33 women)	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, feeling post defecation and gastrointestinal symptoms).	**Fecal volume and fecal frequency were significantly increased compared to placebo (p<0.05).** There appeared to be non-significant improvements in fecal conditions but the trend was minor.	
2	I	Inaki et al 1999 J Nutritional Food	Effects of the administration of soft drink containing indigestible dextrin on defecation frequency and fecal characteristics of Japanese healthy female volunteers.	**4.6 g/d** (2 servings per day of 2.3 g indigestible dextrin (PF-C *) in a pulverized fruit juice drink dissolved in 150 ml water) -control was same drink, only without PF-C	2 weeks	21.3 ± 0.7 yrs	healthy women subjects	**Cross over design** - 2 week non treated wash out period	47	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, and feeling post defecation).	Significant increase in number of days per week of defecation frequency druing treatment compared to non-treatment phase (p<0.05). However this was not significantly different from the control treatment phase (juice drink only). Effect could be due to juice rather than fiber addition. Similar effect in stool volume, NS from control drink (w/o fiber) but different from non treatment washout phase. **Stool consistancy was significiantly improved compared to juice w/o fiber (p< 0.05).**	Study complicated by use of a juice drink to deliver the fibersol. Short treatment period.
3	I	Kimura et al 1998. J Nutritional Food 1: 12-19	Effect of Jelly Drink Containing Dietary Fiber on Human Defecation.	**5 g** indigestible dextrin * in one serving (160 g) of "jelly drink" which contained 10% prune juice	3 weeks	?	healthy women subjects	**Uncontrolled trial**, compared to baseline and 1 week post consumption.	62	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, and feeling post defecation).	In women who were classified as constipated or having moderately frequent defecations/week at baseline; there were **significant (P<0.05) improvments in frequency and fecal volume**. There was also significant improvement in fecal consistency in the low score subgroup.	Sub group analysis based on frequency. Also, secondary subgrouping post-hoc based on scoring for consistency. **All groups experienced abdominal distension and flatus.**
4	I	Ogiso et al 1999 J Jpn Assoc Dietary Fiber Res	Effects of cookies containing indigestible dextrin on defecation and fecal conditions in human subjects.	**5g/d** indigestible dextrin (PF-C)* contained in 26 g of cookie (divided in 3 cookies/d)	20 days	36.4 ± 7.0 yrs (M) 31.8 ± 8.4 yrs (W)	healthy adult subjects	**Uncontrolled trial,** compared to baseline (10 d period wihtout cookies).	39 (12 men and 27 women)	Gastric conditions assessed by questionnaire with each bowel movement (frequency, volume, conditions, fecal odor, feeling post defecation and gastrointestinal symptoms).	Subjects in Group A (n=12) (who had basal frequency of 8 times or less in 10 d) had significant improvement in frequency and volume (p<0.05). There was no significant impact on frequency or volume in the other two groups. There was a tendency of decreases in hard, pasty and muddy stools, and increases in the frequency of banana shaped stools after ingestion of the cookies.	Subjects were divided into 3 groups based on defecation frequency in the 10 d baseline period: group A (8 times or less), Group B (9-11 times), and Group C (>12 times).
5	I	Sato et al 2000. J Nutritional Food 3(4): 47-54	Effect of cooked and cured loin-roll ham containing indigestible dextrin on fecal amount and defecation frequency.	**5.9 g/d** indigestible dextrin (PF-C*), contained in ham compared to ham without PF-C	2 weeks	23.7 yrs	healthy women subjects	**Cross over** - 1 week wash out	28 women	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, and feeling post defecation).	**Frequency: there was no significant effect on stool frequency in the whole group of subjects.** People with mild constipation (n=8) there was a significant increase in frequency compared to the non consumption and placebo periods (p<0.05). There were similar findings for volume: in the subgroup which tended to be constipated, (n=8) there was a significant increase (p<0.05) in amount compared to the ham not containing fiber. **There was no overall influence of the test food on stool form.**	Ham was the delivery food, but the placebo was simply the food lacking addition of PF-C. Subgroup analysis of individuals: <5 defecations/wk were classified as mildly consiptated; 5 or more were considered normal. This provided a separate group of 8 women which produced data which were analyzed separately, the sub group analysis was only a small number of subjects compared to the whole group (less than 1/3)
6	I	Sato et al 2000. J Nutritional Food 3(4): 55-62	Effect of sausage containing indigestible dextrin on fecal amount and defecation frequency.	**5.9 g/d** indigestible dextrin contained in a sausage (65 g, 153 kcal) control was sausage without PF-C	2 weeks	24.8 yrs	healthy women subjects	**Cross over** - 1 week wash out	29 women	Gastric conditions assessed by questionnair with each bowel movement (frequency, volume, conditions, fecal odor, feeling post defecation and gastrointestinal symptoms).	**Frequency: there were no statistically significant effects of the treatment in the whole group.** However, post-hoc analysis of people who were classified as mildly constipated (<5 defecations/wk during non treatment run-in) n=11, there was a significant increase in the frequency (p<0.05) compared to control sausage. **Volume: average stool volume increased for all subjects compared to the control (p<0.05).** No impact on stool characteristics observed, even after classifying subjects according to bowel frequency.	Post hoc analysis based on stool frequency, lessened the number of subjects. There was a tendency for the total subjects to ave an increase in frequency, but this did not become significant. Statistics could be improved.
7	I	Satouchi et al 1993 Jpn. J. Nutr. 51: 31-37	Effects of Indigestible Dextrin on Bowel Movements.	**10, 20, 40 g** indigestible dextrin (potato based) dissolved in 100 ml of water	acute test, drink containing PF consumed after breakfast	?	healthy adult subjects	**Cross over** with 1 week washout. But no control beverage	74 (53 Men and 21 women)	48 hour observation of stool and gastrointestinal conditions, compared with observations before intake of treatment beverage.	Stool conditions: feces become softer at 40 g intake. **"Muddy feces" were found at 40 g intake**, but not seen at 10 or 20 g intake. Diarrhea occurred at 40 g (2.5% in men and 5.3% in women). No statistics noted.	9 male subjects also consumed 60 g dose to further the experiment- no diarrhea noted in these few subjects. There was occurrence of flatulence, but not severe and no clinical concern.

8	I	Satouchi et al 1993 Jpn. J. Nutr. 51: 31-37	Effects of Indigestible Dextrin on Bowel Movements.	**35 g/d** indigestible dextrin (PF- potato based)	PF consumed daily for **5 days** (M-F) compared to non treated time period	?	healthy male subjects	**Cross over** - 1 week wash out	8 men	Wet and dry weight of stool, moisture content. Defecation time observed and recorded.	**Wet and dry weight of stool increased significantly (p<0.05)** due to treatment. No significant changes in moisture. **Stool frequency increased with treatment (p<0.05).**	Non treated control, and short duration of treatment. Small number of subjects.
9	I	Satouchi et al 1993 Jpn. J. Nutr. 51: 31-37	Effects of Indigestible Dextrin on Bowel Movements.	**5 or 10 g/d** indigestible dextrin (PF - potato based)	PF consumed daily for 5 days (M-F) after breakfast, dissolved in 100 ml water	?	healthy adult subjects	**Uncontrolled trial,** compared to baseline.	30 total Group 1 n=13 (3 M, 10W) Group 2 n=17 (4M, 13W)	Gastric conditions assessed by questionnair (frequency, volume, conditions, feeling post defecation).	Significant increase between before and after administration with 10 g (p<0.01) for frequency, volume, stool conditions, and feeling after defecation. 5 g intake group only had a significant increase in the score for feeling after defecation.	Non treated control, and short duration of treatment.
10	I	Shi et al 2000 J Nutritional Food 3(2): 37-44	The effects of rice crackers containing indigestible dextrin on female defecation.	**5g/d** PF-C* contained one serving (40g) of either Arare rice crackers (test A) or Kakinotane (test B) rice crackers; control crackers contained rice starch	20 days per test period	43-45 yrs on average (per group)	healthy women subjects	**Placebo controlled cross over trial,** compared to baseline and control (two different test foods).	40 women (in two groups) **Subgroups**: Group I and Group II	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, and feeling post defecation).	Fecal frequency was significantly greater than control in the group which consumed the Arare rice crackers containing PF-C. This did not hold true for the Kakinotane rice cracker, which showed no increase in frequency compared to either baseline or control cracker, in fact the fecal frequency increased with the control cracker in the second half of the control phase. Fecal volume in the subjects consuming the Arare crackers was increased singificantly compared to the control, but only in the first half of the testing phase, but fecal volume in the control phase was also increased over baseline to a slimilar level as the test meal.	Subjects were subgrouped (Group I or II) based on defecation frequency and fecal amount. Group I was predisposed to constipation and Group II was not. Group I showed the greatest improvment in fequency and amounts in both test foods. The two different types of crackers were very similar in protein/fat/carbohydrate contents. Uncertain why there was disagreement in the results between the two crackers considering they had equivalent composition and same amount of fibersol in each. Results are confounding and significant results, if any, were transient and not always compared to the most appropriate control.
11	I	Takagaki et al 2001 J Nutritional Food 4(4): 29-35	The effect of AOJIRU drink powder containing indigestible dextrin on defecation frequency and fecal characteristics.	**6 g/d indigestible dextrin** PF-C* consumed in 3 - 2 g servings, in a drink powder. Control drink powder containined maltodextrin.	2 weeks	28.2 yrs	healthy adult subjects	**Placebo controlled Cross over** - 1 week wash out	71 (38 men, 33 women)	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, feeling post defecation and gastrointestinal symptoms).	Fecal frequency and volume did not improve in the total subject group. **In the subjects who were subgrouped as having mild constipation (n=20) there was a significant increase (p<0.01) in frequency compared to control; and a signficant increase in fecal amount compared to control (p<0.05)**. In normal subjects there was a significant increase in fecal amount compared to placebo (p<0.05).	Subgrouping based on baseline fecal frequency: mild constipation <7/wk; normal 7-8/wk; frequent >9/wk.
12	I	Tanaka et al 2000 J Nutritional Food 3(4): 39-45	Beneficial effect of a vegetable drink containing indigestible dextrin on defecation in women with constipation	**7.5 g/d** indigestible dextrin (PF-C*) contained in vegetable drink. Placebo drink contained 7.5 g of maltodextrin.	2 weeks	23 ± 4.3 yrs	healthy women subjects	**Placebo controlled cross over study -** 1 week wash out period	22 women	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, feeling post defecation and gastrointestinal symptoms).	**There were no significant differences between the test beverage compared to placebo control.** There was an increase in frequency and volume compared to the non-ingestion periods for the test meal, but there was also an increase in voume with the control meal compared to the non-treated baseline (p<0.05). No significant differences for stool characteristics between the test drink and control or baseline non treated periods.	Vegetable drink (160 ml) containined 60% vegetable juice (spinach, celery, cabbage, lettuce, broccoli, green pepper, kale, watercress, parsley, radish, and honewart) and 40% fruit juice (apple, grapefruit, grape, pineapple, banana, and lemon. 52 kcal, 0.96g prot, 0 g fat, 12 g carb, and 1.1 g fiber without treatment addition.
13	I	Umekawa et al 1999 J Nutritional Food 2(2): 52-57	Effect of drinks supplemented with indigestible-dextrin on fecal amount.	**5g/d** indigestible dextrin (PF-C)* or digestible dextrin (control) contained in 500 ml/d grapefruit juice drink, once a day	10 days	30.1 ± 7.9 yrs	healthy adult subjects	**Placebo controlled cross over study** - 10 d non-treated wash out period	27 (6 M, 21 W)	Gastric conditions assessed by questionnaire with each bowel movement (frequency, volume, conditions, fecal odor, and feeling post defecation).	Significant increases in stool frequency (p<0.005) and stool volume (p<0.001) compared to control drink. The ratio of generally ideal "banana-shaped" and "semi-pasty" stools increased from 73.4% in the non-consumption (baseline) phase to 80.8% in the test food consumption phase (no stats).	Well designed study, with an appropriate placebo control. Would have benefited from a longer study duration.
14	I	Unno et al 2000 J Nutr Food 3 (4): 31-38.	Effect of drinks supplemented with indigestible dextrin on defecation in human.	**4.9 g/d** PF-C * in one serving (280g) of test drink, control drink contained 2.3 g of digestible maltodextrin. Each test drink contained 134 kcal; 0 g prot, 0 g fat, and 33.5 g carb, and 34 mg sodium.	2 weeks	?	healthy adult subjects	**Placebo controlled cross over study -** 1 week wash out period	84 (58 men, 26 women)	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, feeling post defecation and gastrointestinal symptoms).	In the subgroup with stool frequencies below 7 /week (n=27) during the run-in phase; there were statistically significant increases in both stool frequency and volume during the treatment period compared to baseline (non ingestion period), and different from the control treatment period (p<0.05). Neither of the other subgroups (7-8x's / wk or >9x/wk) had significant improvements in frequency or volume. No significant changes in stool consistancy noted.	Subjects were further subdivided into 3 groups based on stool frequencies at baseline (under 7 times, 7-8 times and 9 times or more).

15	I	Unno et al 2000 J Nutr Food 4(4): 21-27.	Effect of a vegetable drink supplemented with indigestible dextrin on defecation in females.	**4.8 g/d Fibersol-2*** contained in vegetable drink (control was drink w/o Fibersol).	2 weeks	21-41 yrs	healthy women subjects	**Cross over** - 1 week wash out	42 women	Gastric conditions assessed by questionnaire daily (frequency, volume, conditions, fecal odor, and feeling post defecation).	Fecal frequency in the total subject group was not significantly effected. Stool amount was increased compared to the non treatment baseline period, however so was the "control beverage" period. There was no difference compared to the control beverage. In subjects with "small stool frequencies" (<6/wk) (n=20), there was significant improvement ($p<0.05$) compared to the control drink period in both frequency and amount. There was a significant improvemetn in fecal shape ($p<0.05$) compared to control in all subjects.	Beverage contained 80% vegetable jice, and 20% fruit juices. Short treatment period.
16	I	Yamamoto et al 2000 J Nutritional Food 3(2): 29-36	The effect of ingestion of beverage supplemented with indigestible dextrin on human defecation	**8 g/d** indigestible dextrin in test drink or 8 g of digestible dextrin (control).	1 week	Men: 44.7 $\pm$ 8.3 Women 30.1 $\pm$ 2.3	healthy adult subjects	**Cross over** - 1 week wash out	29 (6 men, 23 women)	Gastric conditions assessed by questionnaire with each bowel movement (frequency, volume, conditions, fecal odor, feeling post defecation).	**Frequency and volume increased significantly compared to baseline and control periods (P=0.05) Significant increase in "banana-shaped" feces during treatment ($p<0.01$).**	Very short time for the study, but fair design, with proper placebo control, and subject numbers. Studies, of this nature need to be carried for at minimum 2 weeks.

Translation: B-16

Title: Effects Prepared Cocoa Powder Containing Indigestible Dextrin on Human Defecation

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Abstract

In order to find out the possible effect on human defecation by the ingestion of prepared cocoa powder which contains indigestible dextrin (a water-soluble dietary fiber), we have conducted a test with a total of 40 volunteers of healthy adult male and female subjects. The required period for the text was 6 weeks (42 days) and it was divided into the following 4 periods: non-intake period (7 days) and intake period–I (14 days), intermediate non-intake period (7 days) and intake period–II (14 days).

The subjects were divided into two groups and a single blind cross-over test was carried out. Each of the subjects was requested to ingest a test drink which was prepared cocoa powder containing 4.2 g of indigestible dextrin (3.8 g as dietary fiber) per cup of 14.2 g, and dissolved in 100 ml of hot water, as well as a placebo which was also a prepared cocoa powder drink but contained malt-dextrin instead of the indigestible dextrin. They were requested to drink both the test drink and placebo once a day at any time.

As a result, we have observed a significant increase in defecation frequency and fecal amount in the case of intake of prepared cocoa powder which contained indigestible dextrin during the above-mentioned intake periods, compared to those of placebo intake periods, non-intake period and intermediate no-intake period. ($p<0.05$)

Key words: indigestible dextrin, dietary fiber, prepared cocoa powder, defecation frequency, fecal amount, bowel movement

Summary

1. Testing Method

1) Test Meal

Test meal: 4.2 g of indigestible dextrin-added prepared cocoa containing cocoa powder, creaming powder (glucose syrup, fat, etc.), salt, flavors, and sweeteners. Nutrient content-51.3kcal, water 0.5 g, protein 0.9 g, fat 2.8 g, ash 0.5 g, saccharide 3.6 g, and dietary fiber 5.4 g per serving (14.2 g).

Placebo: Maltodextrin was added to the above test meal instead of indigestible dextrin.

1) Subjects

7 male and 33 female subjects.

3) Time table for the study

Figure 1 shows the schedule for the study. The subjects were randomly assigned to either Group I or II for a single-blind crossover study using a placebo.

The study period consisted of a non-consumption period (A, 7 days), consumption period-I (B, 14 days), non-consumption period (C, 7 days) and consumption period-2 (D, 14 days). Group I consumed the test meal in period B and the placebo in period D while Group II consumed the placebo in period B and the test meal in period D.

The test meal and placebo were dissolved in 100ml of hot water and 1 packet per day was consumed.

4) Survey method using the questionnaire

Subjects were required to record information on fecal characteristics on a questionnaire. Daily records including food consumption were mandatory regardless of any or no bowel movement.

3. Results

1) Effects on defecation frequency and stool volume

Table 1, Figure 2, and Figure 3.

2) Effects on form, color and odor of stool and on feeling after defecation

Figure 4, Figure 5, Figure 6, Figure 7, and Table 2.

3) Gastrointestinal symptoms

Gastrointestinal symptoms reported on the questionnaire during the study period were summarized in Table 2. There were no significant differences by the periods.

4. Conclusion

The study results confirm that 1 packet per day of consumption of cocoa powder containing indigestible dextrin can improve bowel movement of healthy subjects.

Figure & Tables

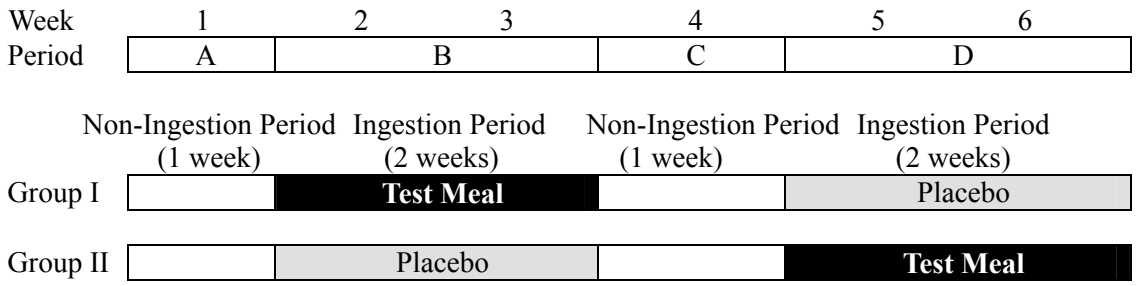

Figure 1 Study schedule

Table 1 Fecal frequencies and fecal amount by visual measurement during each period

		Non Ingestion Period A	Interval Period B	Placebo Meal	Test Meal
Fecal frequencies (times/ 7 days)	Average n=40	7.3±2.7	6.4±3.0	7.1±2.9b	8.2±3.1abc
Fecal amount -visually measured (times/ 7 days)	Average n=40	12.5±7.5	12.2±9.3	12.9±9.7	15.9±12.1abc

*Mean±SEM
*Fecal amount was converted into the number of L-size eggs (3.5 cmφ×5 cm).
a: statistically significant differences to Non-Ingestion Period at $p<0.05$
b: statistically significant differences to Interval Period at $p<0.05$
c: statistically significant differences to Placebo Meal Period at $p<0.05$

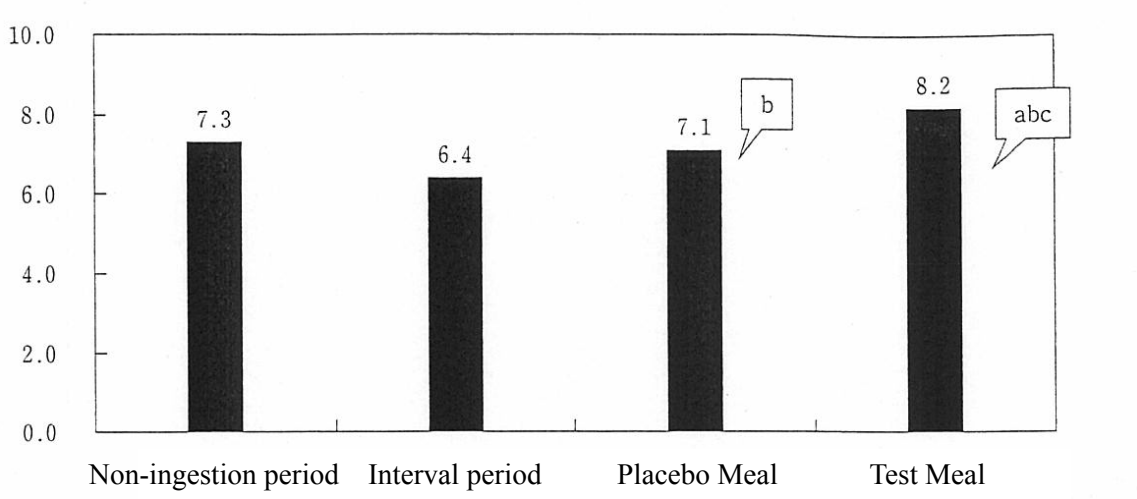

Figure 2 Fecal frequencies during each period (times/ 7 days)
a: statistically significant differences to Non-Ingestion Period at $p<0.05$
b: statistically significant differences to Interval Period at $p<0.05$
c: statistically significant differences to Placebo Meal Period at $p<0.05$

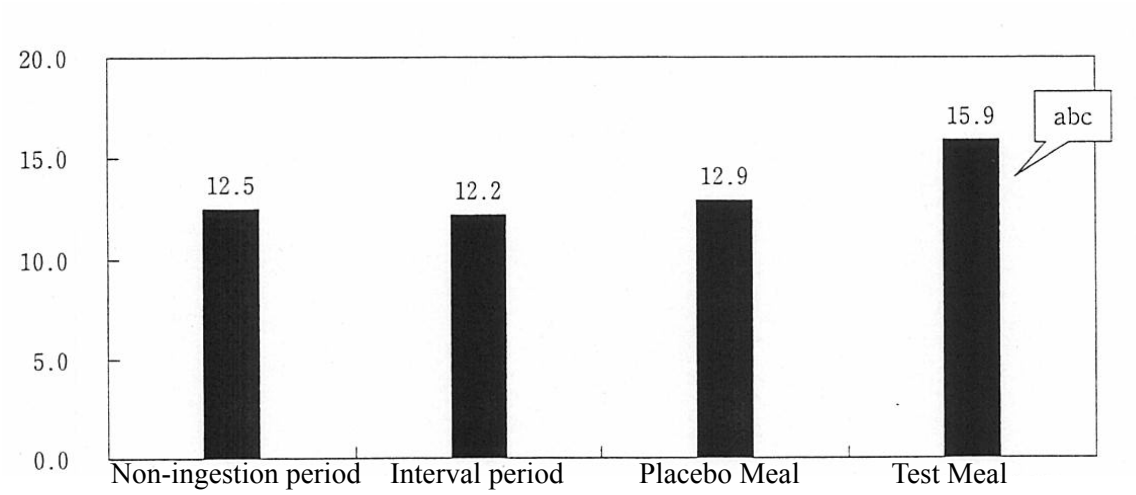

Figure 3 Fecal amount by visual measurement during each period
a: statistically significant differences to Non-Ingestion Period at $p<0.05$
b: statistically significant differences to Interval Period at $p<0.05$
c: statistically significant differences to Placebo Meal Period at $p<0.05$
Fecal amount was converted into the number of L-size eggs (3.5 cmφ×5 cm).

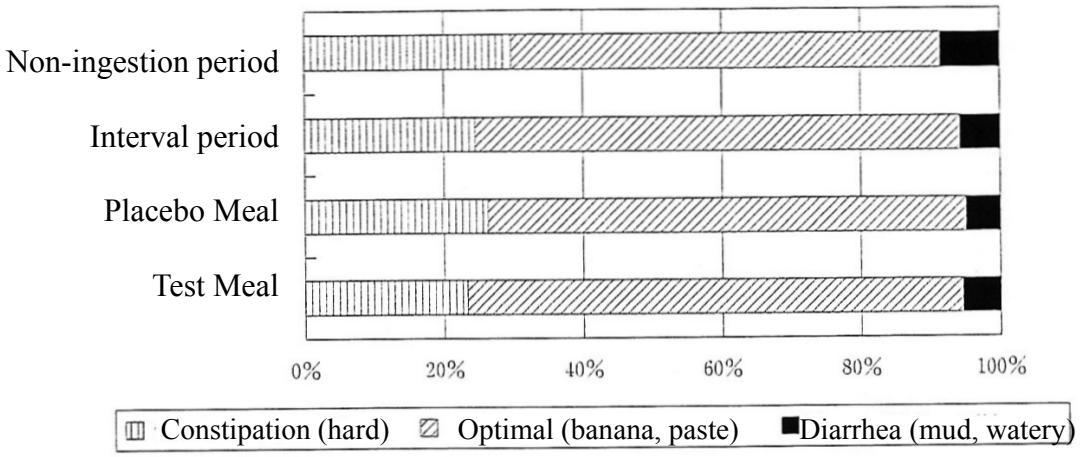

Figure 4 Fecal conditions during each period (appearance ratio)

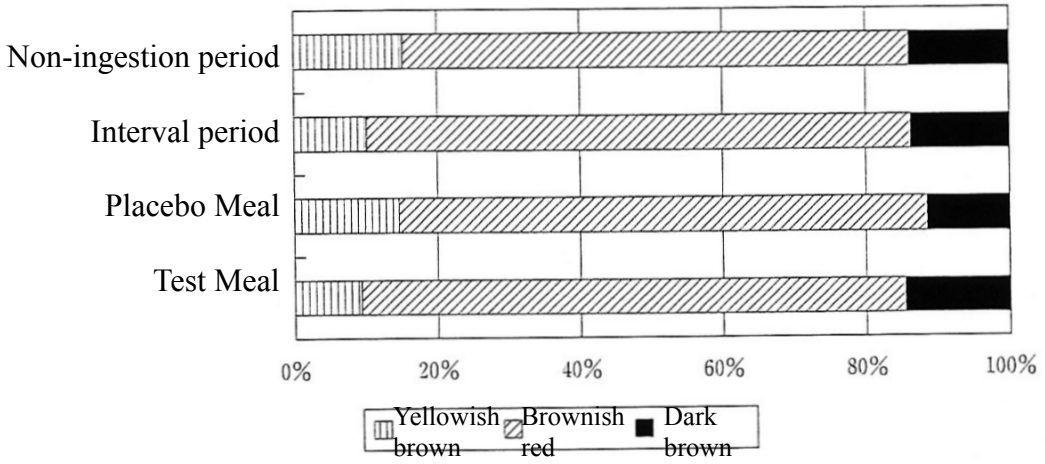

Figure 5 Fecal colors during each period (appearance ratio)

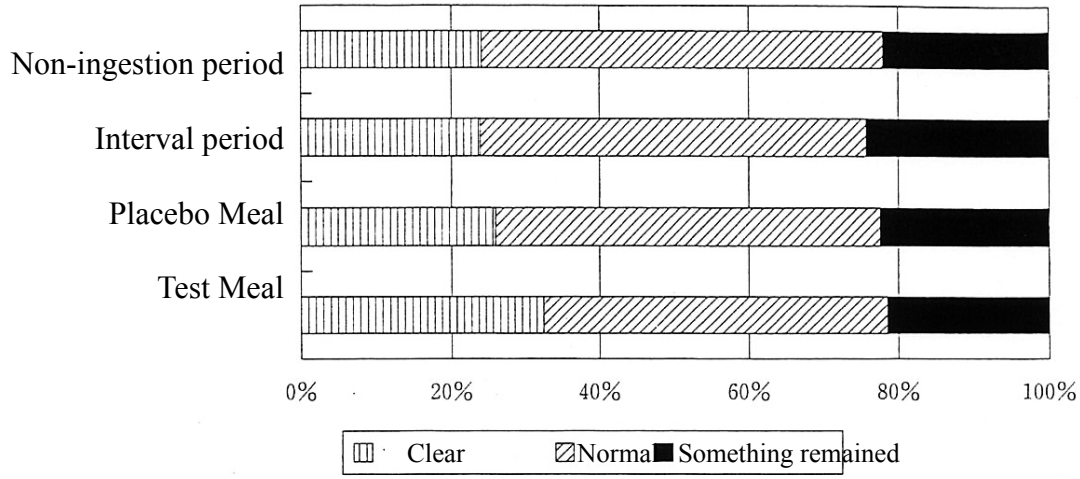

Figure 6 Feeling after evacuation during each period (appearance ratio)

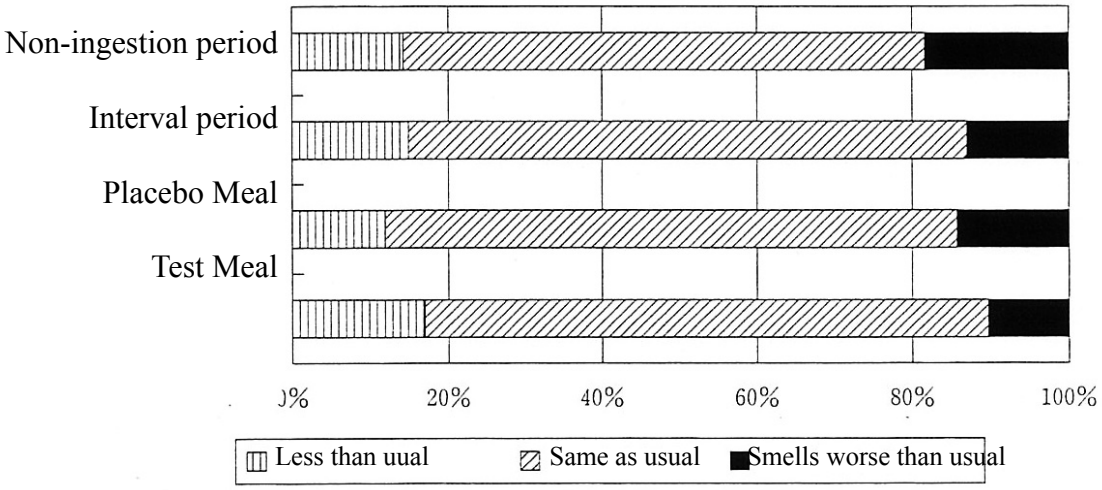

Figure 7 Fecal odor during each period (appearance ratio)

Table 2 Numbers of people who had gastrointestinal symptoms

	Abdominal Pain	Desire but no defecation	Sounds	Bloating	Gas	Nausea
Non-ingestion & Interval	8	1	2	5	3	2
Placebo Meal	4	3	6	5	6	1
Test meal	5	2	6	5	6	2

Non-ingestion period and interval period were combined to align all the periods to 14 days.

B-2

Title: Effect of Jelly Drink Containing Dietary Fiber on Human Defecation

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[Abstract]

An investigation was made on the effect of the administration of a jelly drink supplemented with indigestible dextrin, a water-soluble dietary fiber, on the defecation of 62 healthy female subjects. The efficacy of this drink was demonstrated in the subjects who had less than 3 defecations/ week. When a bottle of the drink (content : 160 g) containing 5 g of the indigestible dextrin was given daily to each subject, a significant increase was found in the number of defecation/ week, the number of days/ week with defecation and fecal amount. In addition, there were some improvements in fecal properties including state, color and odor; and the physiological feeling after defecation.

1. Introduction

Dietary fiber is a food constituent that is hardly hydrolyzed by human digestive enzymes or absorbed[1)], which has been regarded as useless. However, epidemiological reevaluation of the relationship of dietary fiber with diseases epidemiologically has been revealed physiological significance of dietary fiber[2)]; dietary fiber consumption is closely associated with alimentary disease, such as constipation, colorectal cancer, and diverticulosis, probably because of its actions on the digestive tract, for instance, retardation of discharge of stomach content, shortening of bowel transit time, and increasing of stool volume.

Dietary fiber exhibiting such health-promoting physiological effects via various mechanisms during bowel transit has been establishing the status of the sixth essential nutrient[3)]. Japan Association of Prefectural and Municipal Public Health Institute[4)] and the Resource Council of the Science and Technology Agency[5)] published the Japanese Standard Tables of Dietary Fibers in Foods. In March 1994, the Recommended Dietary Allowances for the Japanese－5th Revision, set the target daily intake of dietary fiber for adults at 20-25 g (10 g/1,000 kcal) for the first time[6)]. However, in the 1990 Report on Dietary Fiber Determination reviewing results of determination of dietary fiber in 252 major foods by local institutes of hygiene sciences, the former Health Promotion Department of the Ministry of Health and Welfare (the present Life-related Disease Control Task Force, Community Health, Health Promotion and Nutrition Division of the Health Service Bureau, the Ministry of Labor, Health and Welfare) reported that the daily dietary fiber consumption by adults ranged from about 17 g to 20 g[7)], being 3 g to 5 g below the target level.

In this context, developing a food product supplemented with dietary fiber is valuable in supplement of dietary fiber and its beneficial physiological actions. The present study investigated effects of jelly drink containing dietary fiber on human defecation. The dietary fiber used was indigestible dextrin (Matsutani Chemical Industry, Co. Ltd.), which is produced by hydrolysis of potato starch-based pyrodextrin with α-amylase, demineralization and bleaching with ion exchange resin, purification, and drying. It is manufactured on the industrial scale and food products supplemented with it are already commercially available. Its safety has been confirmed by an established analysis method[8)]. As its physiological effects, regulation of intestinal functions (relief of constipation)[9)], suppression of postprandial insulin secretion[10)], and improvement of serum lipid metabolism[11)] have been reported, but no inhibitory effect on absorption of trace metals has been observed[12)]. With reference to the first two effects, food products for specified health use that contain indigestible dextrin as the relevant ingredient have been approved.

The present study investigated changes in defecation conditions, using 5 g of indigestible

dextrin per serving of jelly drink (160 g net weight), which healthy adult women consumed for three consecutive weeks.

The present study was designed and conducted in conformity to the Declaration of Helsinki.

2. Materials and Methods

1) Subjects

Subjects were recruited in conformity to Basic Principles 1-12 of the Declaration of Helsinki. Candidates were adequately informed of the methods, anticipated benefits and potential hazards of the study and the discomfort it might entail. They were also informed that they were at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. They then received a written study protocol. Eventually 62 adult female subjects participated in the study, all of who understood the above-described issues and gave informed consent to the participation. They consisted of 47 students of the Faculty of Human Life and Environment, Nara Women's University (age 19-25), and 15 married employees working at a food processing factory of MEIDI-YA Co., Ltd. (age 33-54).

2) Materials

Jelly drinks containing 10% prune juice were used, one serving of which (160 g) contained 5 g of indigestible dextrin. The indigestible dextrin content (5 g) was determined as indigestible fraction (dietary fiber) by the enzyme-HPLC method[13]. One serving (160 g) contained 74.6 g of moisture, 0.2 g of protein, 0.0 g of fat, 20.0 g of saccharide, 5.0 g of dietary fiber, 2.0 g of ash, 77 mg of sodium, 2.5 mg of iron, and 87 kcal of energy.

3) Methods

Healthy adult women (n=62) consumed one serving daily for three consecutive weeks (Study Phases 2-4).

Study Phase	1	2	3	4	5
	1 wk	2 wk	3 wk	4 wk	5wk
Test Material	None	1 botl/ day	1 botl/ day	1 botl/ day	None

They had to consume one serving of jelly drink daily but could do it at any time of the day. There was no special dietary restriction other than two requirements: consuming one serving of jelly drink daily and not having any other dietary fiber-added processed food.

Defecation frequency and fecal amount, fecal consistency, color and odor, feeling after defecation, and intestinal symptoms were surveyed by a questionnaire method, as summarized in Table 1.

Table 1. Surveyed Items on Defecation/ Fecal Conditions

Items	Description
Defecation Frequencies	Write the times of defecation of the day
Fecal Amount	Write the amount in numbers of eggs (1 = one L-size egg: ~50 grams, ~3.5 cm diameter, ~5cm length)
Fecal Conditions	1: Banana-shaped or pastry dough 2: Like a stick 3: Hard or dry solid 4: Muddy or watery Choose one from 1-4.
Fecal Color	1: Yellow Brown 2: Brown 3: Dark Brown Choose one from 1-3.
Fecal Odor	1: Not like feces, hardly smells 2: Less smells than usual 3: Smells as usual 4: Badly smells Choose one from 1-4.
Feeling after Defecation	1: Feel good, empty 2: Same as usual 3: Feces still remain but soft 4: Feces still remain but hard Choose one from 1-4.
Intestinal Symptoms	a) Abdominal pains b) Feel demands but cannot evacuate due to pain c) Abdominal sounds d) Getting gas, flatulence e) Getting gas, fart f) Feel vomiting Choose appropriate items from a)-f)

A self-administered questionnaire was prepared according to Satouchi *et al*[9]. The subjects had to fill out the questionnaire every day to report their own observation, whether or not bowel movements occurred.

Defecation frequency and fecal amount, fecal consistency, color and odor, and feeling after defecation were also surveyed in the week before the consumption period (Phase 1) and the week after the consumption period (Phase 5).

Fecal consistency was evaluated by selecting one

from examples illustrated in a reference guide distributed: banana-shaped or semi-pasty, rod-shaped, rigid or lumpy, and muddy or watery. Fecal color was also evaluated by selecting the most similar one from brown (the color of milk chocolate, standard), tan (paler than the standard) and dark brown (darker than the standard). Fecal odor and feeling after defecation were evaluated by selecting one of examples in the same way. Intestinal and general conditions during the study period were also surveyed; the subjects were asked to report any change.

4) Statistical analysis

Both defecation frequency and number of days on which defecation occurred were presented as weekly figures, of which mean±standard deviation was calculated by phase and group. Fecal consistency, color and odor, and feeling after defecation were converted to numerical data, of which mean±standard deviation was calculated by phase and group. Table 2 shows the conversion table for fecal conditions.

Wilcoxon signed rank test was carried out to test significance, using data in Phase 1 (the week before drink consumption) as the control, with a statistical analysis package Stat123/win according to *Igaku Seibutsugaku Tokei* (Medical/ Biological Statistic) Manual[14].

Table 2. Scoring of Defecation/Fecal Conditions

Items		Score
Fecal Conditions	1: Banana-shaped or pastry dough	3
	2: Like a stick	2
	3: Hard or dry solid	1
	4: Muddy or watery	1
Fecal Color	1: Yellow Brown	3
	2: Brown	2
	3: Dark Brown	1
Fecal Odor	1: Not like feces, hardly smells	4
	2: Less smells than usual	3
	3: Smells as usual	2
	4: Badly smells	1
Feeling after Defecation	1: Feel good, empty	3
	2: Same as usual	2
	3: Feces still remain but soft	1
	4: Feces still remain but hard	1

5) Diet survey

Subjects were asked to record diets every day during the study to investigate dietary fiber consumption. They were adequately informed of the procedure for recording before the study started and asked to describe the menu and record the name and quantity of foodstuffs used as specifically as possible. However, analysis of the records revealed that the diet survey could not provide satisfactorily specific information on the type and quantity of foodstuffs. Dietary fiber consumption was thus calculated by rough estimate.

Upon estimating dietary fiber consumption, commercially available cookbooks were referred to, to learn general quantity of foodstuffs used in diets, when the detail was unclear. Standard Tables of Food Composition in Japan - Fifth Revision - New Foods[15], the Japanese Standard Tables of Dietary Fibers in Foods[5], and the 1990 Report on Dietary Fiber Determination[7] were used to determine weekly dietary fiber consumptions during the study.

3. Results

The questionnaire survey on 62 subjects indicated that jelly drink consumption improved defecation of costive subjects with a smaller number of days on which defecation occurred and lower defecation frequency. Subjects were classified into three groups according to Takezoe *et al*.[16], who defined three or more defecations per week as constipation, four to six defecations per week as moderately frequent, and seven or more defecations per week as frequent, based on the weekly defecation frequency in Phase 1 without jelly drink consumption: Group A (defecation frequency≤3; n=13), Group B (4-6; n=22), and Group C (≥7; n=27). Table 3 shows changes in defecation frequency and fecal amount by jelly drink consumption.

Group A showed a significant increase in defecation frequency and fecal amount in Phase 2 (Week 1 of jelly drink consumption), relative to Phase 1 (no consumption). Significant increases were also found in Phases 3 and 4 (Weeks 2 and 3 of consumption). Group B also showed significant increases in these parameters in Phase 2-4 (consumption) and Phase 5 (no consumption), although the difference was not significant in Phase 4 (Week 3 of consumption). Group C showed no significant difference in either defecation frequency or fecal amount.

Subjects were also classified into three different groups, based on the number of days on which defecation occurred in Phase 1 (the week before

Table 3. Effect of Jelly Drink Containing Dietary Fiber on Fecal Frequencies, Days, and Amount

		Changes in Defecation Frequencies (times) and Fecal Amount (Divided by Defecation Numbers)				Changes in Defecation Frequencies (days) and Fecal Amount (Divided by Days with Defecation)		
	(/week)	Group A ≤3 times	Group B 4-6 times	Group C ≥7 times		Group a ≤3 days	Group b 4-6 days	Group c ≥7 days
1 week without consumption	Defecation Times	2.5 ± 0.9	5.0 ± 0.6	8.7 ± 2.3	Defecation Days	2.6 ± 0.8	4.8 ± 0.9	7.0 ± 0.0
	Fecal amount	28.8 ± 12.2	57.0 ± 21.6	88.1 ± 40.3	Fecal amount	34.3 ± 19.5	70.9 ± 39.0	84.0 ± 31.4
Week 1 of consumption	Defecation Times	4.5 ± 2.1*	6.6 ± 2.3*	7.9 ± 2.4	Defecation Days	3.6 ± 1.7*	5.0 ± 1.0	6.5 ± 0.5
	Fecal amount	54.6 ± 29.3*	75.9 ± 28.7*	94.4 ± 44.6	Fecal amount	56.2 ± 31.2*	77.9 ± 28.2	107.6 ± 49.7*
Week 2 of consumption	Defecation Times	4.2 ± 2.0*	6.3 ± 2.9*	7.7 ± 2.4	Defecation Days	4.0 ± 1.7*	5.2 ± 1.1	6.3 ± 1.1
	Fecal amount	48.0 ± 21.4*	77.5 ± 37.0*	104.8 ± 71.9	Fecal amount	56.5 ± 38.1*	81.7 ± 43.6	114.6 ± 81.5
Week 3 of consumption	Defecation Times	4.6 ± 1.9*	5.8 ± 2.2	8.3 ± 2.9	Defecation Days	4.4 ± 1.5*	4.9 ± 1.6	6.2 ± 0.9
	Fecal amount	49.2 ± 19.7*	64.3 ± 25.5	105.0 ± 61.5	Fecal amount	57.8 ± 26.3*	75.0 ± 48.7	109.3 ± 58.7*
The week after consumption	Defecation Times	3.5 ± 1.8*	6.0 ± 2.0*	7.0 ± 2.4	Defecation Days	3.3 ± 1.5*	4.8 ± 1.5	6.3 ± 0.9
	Fecal amount	37.6 ± 21.0	75.0 ± 30.7*	93.5 ± 61.9	Fecal amount	41.2 ± 21.4	77.2 ± 41.7	107.3 ± 65.0

·Significantly different at *p<5%, compared with Phase I, '1 week without consumption'.
·Fecal amount recorded as the numbers of L-size eggs (~50 grams) were converted into weight, and shown in this table as Mean ± SEM (grams) per day.

jelly drink consumption): Group a (< 3 days; n=16), Group b (4-6 days; n=31), and Group c (7 days or more; n=15). Group a showed a significant increase in the number of days on which defecation occurred in Phase 2 (Week 1 of consumption). Significant increases were also found in both number of days per week on which defecation occurred and defecation frequency in Phases 3 and 4 (Weeks 2 and 3 of consumption). Group a also showed a significant increase in fecal amount in Phase 2. Significant increases were also found in Phases 3 and 4 (Weeks 2 and 3 of consumption). Groups b and c showed no significant increase in the number of days on which defecation occurred even in Phase 4 (Week 3 of consumption), although Group c showed a significant increase in fecal amount occasionally.

Fecal consistency, color and odor, and feeling after defecation were converted to numerical data as shown in Table 2. Table 4 shows statistical analysis results. Subjects were classified into two groups, based on the score in Phase 1 before jelly drink consumption: Low Score Group (<2) and High Score Group (≥2). The Lower Score Group showed significant improvement of fecal consistency, color and odor, and feeling after defecation in Phase 2 (Week 1 of consumption), which lasted to the end of Phase 5 (the week after consumption). Both groups had abdominal distension and flatus as intestinal symptoms but no serious general symptoms that create health problems.

4. Discussion

Recently, decreasing consumption of dietary fiber due to westernization of diets has been often indicated in Japan. Since dietary fiber has effects on the intestinal tracts and is associated with colorectal cancer, development of food products containing dietary fiber can be expected to promote improvement of dietary fiber consumption in modern dietary habits.

The present study demonstrated that defecation frequency, the number of days on which defecation occurred, and fecal amount of subjects with a weekly defecation frequency of three times or below was increased by consuming one serving (160 g) of jelly drink containing 5 g of indigestible dextrin (water-soluble dietary fiber) daily.

One indigestible dextrin administration study reported that dietary fiber consumption required to relieve constipation was 2.9 g to 5.8 g as indigestible fraction[9]. As the single-administration effective dose (ED_{50}) so far reported for diarrhea is 1.4 g/kg body weight in both sexes[9], the content of

Table 4. Effect of Jelly Drink Containing Dietary Fiber on Fecal Conditions

	Fecal Condition		Color		Odor		Feeling after defecation	
	<2 points	≥2 points	<2 points	≥2 points	<2 points	≥2 points	<2 points	≥2 points
1 week without consumption	1.4 ± 0.3	2.5 ± 0.4	1.6 ± 0.2	2.2 ± 0.3	1.6 ± 0.2	2.3 ± 0.4	1.5 ± 0.3	2.1 ± 0.2
Week 1 of consumption	1.8 ± 0.6*	2.5 ± 0.4	1.8 ± 0.4*	2.1 ± 0.3	2.2 ± 0.3*	2.2 ± 0.5	1.8 ± 0.4*	2.0 ± 0.4
Week 2 of consumption	1.7 ± 0.5*	2.5 ± 0.5	1.9 ± 0.5*	2.1 ± 0.3	2.2 ± 0.3*	2.2 ± 0.4	1.8 ± 0.5*	2.1 ± 0.5
Week 3 of consumption	2.1 ± 0.9*	2.4 ± 0.8	1.7 ± 0.4	2.0 ± 0.6	1.9 ± 0.3*	2.1 ± 0.5	1.8 ± 0.5*	2.0 ± 0.6
The week after consumption	1.7 ± 0.6*	2.3 ± 0.6	1.9 ± 0.4*	2.0 ± 0.4	2.2 ± 0.3*	2.1 ± 0.4	1.7 ± 0.5*	2.0 ± 0.3

·Significantly different at *p<5%, compared with Phase I, '1 week without consumption' of Fecal Condition, Color, Odor, and Feeling after defecation..
·The scores (Mean ± SEM) were calculated by dividing the total scores of the week by numbers of defecation.

indigestible dextrin in jelly drink used in this study (5 g) is unlikely to cause diarrhea. Estimated dietary fiber consumption of the subjects in this study was about 54±17 g/week to 66±20 g/week or about 8±2 g/week to 9±3 g/day (Table 5). This level was much lower than the mean Japanese consumption per person in the 1980s (17 g/day to 20 g/day). By defecation frequency group, the dietary fiber consumption was about 54±17 g to 61±18 g in Group A (weekly defecation frequency≤3), about 55±15 g to 64±16 g in Group B (defecation frequency=4-6), and about 60±17 g to 66±20 g in Group C (defection frequency≥7); thus, the gradually increasing differences in defecation frequency among these groups reflect the difference in dietary fiber consumption.

The mean daily dietary fiber consumption was 8.8 g in a questionnaire survey by Kumemura *et al*. on diet and lifestyle in 119 female students[17] and 6.7±2.0g to 8.2±2.6 g in a four-week diet survey by Nakanaga *et al*. in 15 junior high school students[18], which is consistent with the estimate of the present study.

By age (students or married employees), the estimated dietary fiber consumption in the present study was about 69±8 g/week to 76±11 g/week in married employees and about 53±15 g/week to 60±17 g/week in students (Table 5); thus, the younger women consumed less dietary fiber.

The subjects with a weekly defecation frequency of three times or below consumed less dietary fiber consumption than those with a frequency of seven times or above. As dietary fiber consumption is known to correlate with fecal amount in general[19], the present study again indicates that higher dietary fiber consumption from diet is associated with higher defecation frequency and also higher fecal amount.

Consuming jelly drinks containing dietary fiber of the present study increased the defecation frequency and fecal amount of the subjects who had the aforementioned dietary habits with a weekly defecation frequency of three times or below, or four to six times, which supports that dietary fiber consumption may play a significant role in bowel movement improvement.

The results in Phase 5 (the week after jelly drink consumption) showed a decreasing tendency in defecation frequency and fecal amount, which was especially marked in the subjects with a lower defecation frequency. Thus, consuming jelly drinks of the present study is expected to help dietary habit improvement as primary prophylaxis of constipation, and thus health promotion and maintenance.

5. Conclusion

Effects of consumption of jelly drink (160 g) containing 5 g of water-soluble indigestible dextrin on bowel movements of healthy adult women were investigated.

The results showed that, in subjects with a weekly defecation frequency of three times or below and number of days on which defecation occurred of three or below, consuming one serving of jelly drink daily increased significantly weekly defecation frequency, number of days on which defecation occurred and fecal amount and also

Table 5 Total Amount of Dietary Fiber Intake

	DF Intake of Groups Divided by Defecation Frequencies			DF Intake of Groups Divided by Ages	
	Group A ≤3 times per week	Group B 4-6 times per week	Group C ≥7 times per week	Students' Group (19-25 yrs old)	Adult females' Group (33-54 yrs old)
1 week without consumption	56.3 ± 15.9	64.0 ± 15.9	64.7 ± 16.9	60.0 ± 16.8	71.1 ± 12.2
Week 1 of consumption	61.4 ± 17.7	59.7 ± 16.4	66.1 ± 19.9	58.5 ± 18.0	76.4 ± 11.1
Week 2 of consumption	56.7 ± 16.5	58.6 ± 13.8	60.9 ± 13.6	56.1 ± 14.4	69.0 ± 8.0
Week 3 of consumption	56.2 ± 16.4	56.4 ± 15.7	60.7 ± 16.6	54.5 ± 16.1	70.0 ± 9.4
The week after consumption	53.6 ± 17.5	55.0 ± 14.9	60.4 ± 17.4	52.8 ± 15.3	70.6 ± 12.4

Total Amount of Dietary Fiber Intake was the estimated total amount of the week (Mean ± SEM, grams).

improved fecal consistency, color and odor, and feeling after defecation. Consuming jelly drink containing 5 g of indigestible dextrin was shown to be suitable for healthy persons who have inclination to constipation, relieving constipation. Consuming the jelly drink continuously also resulted in increases in fecal amounts of healthy subjects without costiveness.

(Received on February 27, 1998)

[References] All 19 references are in Japanese.

厚生労働省許可 特定保健用食品

Category	: **FOSHU** approved products /Beverage (Jelly-type drink) **(Fo**od for **S**pecified **H**ealth **U**se**)**
Country	: JAPAN
Product Name	: **Kaiteki Senn-i Prune ‹Jelly›** **(Well-fitted Fiber Prune ‹Jelly›)**
Manufacturer	: Meidi-ya Co., Ltd.
Net Weight	: 160 g
Retail Price	: J¥ 200
Product Launch	: March, 2000
Our Product Employed	: Fibersol-2 (5.0 g/ container)
Labeling of Our Product	: Soluble dietary fiber (Indigestible dextrin)
Claims on Package	: “It is a jelly-type beverage by which you can take easily 5 g dietary fiber and 2.5 mg iron, both of which most people tend to lack.” / “Prune Juice 10%”

Ingredients:

Prune Juice, Fructose syrup, *Soluble dietary fiber (Indigestible dextrin)*, High viscosity polysaccharides, Acids, Coloring (caramel), Flavors, Ferrous sodium citrate, Sweetener (stevioside)

Nutrition Facts (per 1 bag, 160 g):

Calories	60	Sodium (mg)	69
Protein (g)	0.0	Dietary fiber (g)	5.3
Fat (g)	0.0	Iron (mg)	2.5
Saccharides (g)	15.0		
The component which is related to the promotion of health:	**Indigestible Dextrin**		5.0 g

Approved FOSHU Health Claim:

“Kaiteki Senn-i Prune” is a dietary-fiber drink which supplies dietary fiber easily and maintains the intestinal regularity. For the people who concern about their intestinal regularity.

Recommendation for Daily Intake Amount:

RDI of dietary fiber is 20-25 g. Considering the total amount of dietary fiber intake with other foods, one bag per day is recommended. Too much intake may cause the loose bowels.

April 19, 2000

B-14

Title: The effect of AOJIRU drink powder containing indigestible dextrin on defecation frequency and fecal characteristics

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Journal: *J. Nutritional Food*, 2001, **4** (4), 29-35 (Original paper is in Japanese)

[Abstract]

The effects of AOJIRU drink powder containing indigestible dextrin on defecation frequency and fecal characteristics

Kinya Takagaki*, Motoya Ikeguchi*, Yuki Ariura*, Natsuko Fujinaga**, Yukitaka Ishibashi***, Yohko Sugawa-Katayama**

Effects of AOJIRU drink powder (ADP) containing indigestible dextrin (ID) on defecation frequency and fecal characteristics were studied in 71 healthy volunteers (male : 38, female : 33, average age : 28.2 years). The subjects were divided into two groups. A cross-over test was performed in a test period for 6 weeks including a week of pre-test period. Three packages a day of the ADP (2.0 g ID/package) or the placebo powder including maltodextrin instead of ID were given to the subjects for 2 weeks each with a week interval (washing out period). The subjects filled out a questionnaire about the defecation frequency and fecal characteristics (quantity, appearance, color, odor, physical sensation after defecation) during the examination period. The results showed that the administration of the test AOJIRU drink powder was effective in increasing the fecal frequency and the fecal quantity significantly compared to those in the control period.

Key words : indigestible dextrin, defecation frequency, fecal quantity, AOJIRU drink powder

Journal of Nutritional Food, **4** (4), 29−35, 2001

1. Introduction

Since the 1970s, the importance of dietary fiber in the daily diet has been recognized[1)] and it has been suggested that there is a link between dietary fiber and colon disorders, ischemic heart disease and diabetes[2)]. The intake of dietary fiber by the Japanese is declining and it has been indicated that average diet does not usually contain sufficient amounts of dietary fiber[3)].

Indigestible dextrin contains digestion resistant material which can be measured as a dietary fiber by enzyme-HPLC method[4)]. Its recognized properties include improving bowel movement and fecal characteristics[5)] as well as enhancing the metabolism of fat[6)] and glucose[7)]. Currently, there are products in the market such as soft drinks[8)], rice biscuits[9)] and processed meat[10) 11)] which contain indigestible dextrin and their properties to improve fecal characteristics are recognized. However, the existing products are not convenient to consume and are not portable.

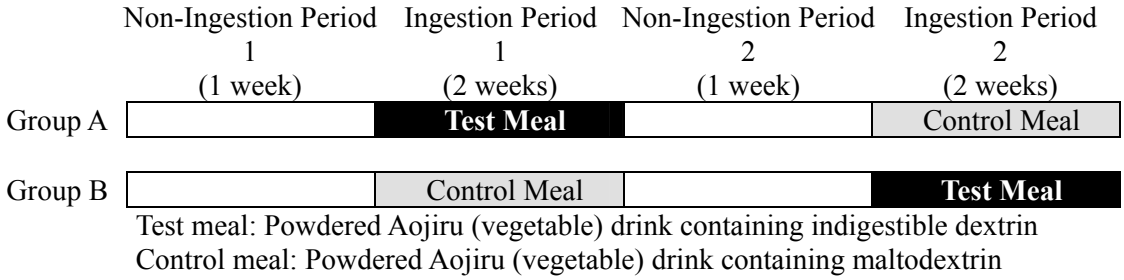
Therefore, an *Aojiru* drink powder containing indigestible dextrin was developed. The product was designed as individual packets of 4.3g for easy handling and consumption.

The study examined the impact on defecation frequency, stool volume and fecal characteristics on healthy individuals to determine the effects of the newly developed *Aojiru* drink powder containing indigestible dextrin.

Table 1 Profiles of subjects

		Male/ Female	Age (years old)	Body height (cm)	Body weight (kg)
Total of subjects	(n=71)	38/33	28.2 ± 8.1	165.5 ± 9.6	58.7 ± 12.6
Group A	(n=33)	16/17	28.7 ± 7.3	164.6 ± 8.8	56.5 ± 10.2
Group B	(n=38)	22/16	27.7 ± 8.7	166.2 ± 10.2	60.6 ± 14.3

Mean±SEM



Test meal: Powdered Aojiru (vegetable) drink containing indigestible dextrin
Control meal: Powdered Aojiru (vegetable) drink containing maltodextrin

Figure 1 Study schedule

2. Testing Method

1) Subjects

Healthy volunteers were recruited from employees of Toyo Shinyaku Co., Ltd. and students and staff of Fukuoka Women's University. Healthy individuals were selected through a preliminary questionnaire. Respondents who gave replies such as "No problems in physical condition" and "Not currently undergoing treatment for any disorders" as well as determined by a physician to be appropriate for the study were chosen as subjects. Based on the principle of the Declaration of Helsinki, 78 subjects signed a letter of consent after receiving an explanation on the objective and method of the study. The following subjects were eliminated from the study: 3 individuals who claimed poor health such as colds, 3 individuals who submitted incomplete responses on their questionnaires and an individual who was unable to follow the consumption regimen due to a business trip. The final number of subjects was 71 (38 men, 33 women: average age 28.2) (table 1).

The subjects were requested to refrain from excessive consumption of food and drink including alcohol as well as restricted from consuming dietary fiber enriched food/drink, and from using laxatives, purgatives or medication for intestinal problems. No other restrictions were imposed on diet or activities.

2) Beverage for the study

The test drink for the study was an *Aojiru* drink in powder form which contained 2.0g of indigestible dextrin (Pine Fibre C, Manufacturer: Matsutani Chemical Industry Co., Ltd.) in one packet (4.3g). The subject consumed three packets (12.9g) per day. 85% to 95% of Pine Fibre C is dietary fiber and its moisture and ash contents are equal or less than 5% and 0.2% respectively. It is a pale yellow amorphous powder which is soluble in cold water and has a slightly sweet taste. Apart from indigestible dextrin, the *Aojiru* drink powder contained powdered kale, sucrose, maltose, powdered green tea and powdered spirulina. The calories and nutritional data for 3 packets (12.9g) of the test drink were; calories 24kcal (calculated by multiplying the amount of protein, fat and saccharide with their respective calories of 4kcal, 9kcal and 4kcal), moisture 0.2g, protein 0.8g, fat 0.2g, saccharide 4.8g, dietary fiber 6.5g (5.1g of dietary fiber from indigestible dextrin) and ash 0.4g.

An *Aojiru* drink powder containing maltodextrin instead of indigestible dextrin was prepared as a placebo. The test drink and placebo were indistinguishable by flavor, color, appearance or other factors.

3) Time table for the study

Figure 1 shows the schedule for the study. The subjects were randomly assigned to either Group A or B for a single-blind crossover study using a

placebo.
The study period consisted of a non-consumption period-1 (1 week), consumption period-1 (2 weeks), non-consumption period-2 (1 week) and consumption period-2 (2 weeks). Group A consumed the test drink in consumption period-1 and the placebo in consumption period-2 while Group B consumed the placebo in consumption period-1 and the test drink in consumption period-2.

The test drink and placebo were dissolved in 100ml of water and consumed. The subjects consumed 3 packets (12.9g) per day. The timing of consumption was not specified.

4) Survey method using the questionnaire
Subjects were required to record information on fecal characteristics on a questionnaire. Daily records were mandatory regardless of any or no bowel movement.

The six items surveyed were; 1. Defecation frequency, 2. Stool volume, 3. Stool form, 4. Stool color, 5. Odor and 6. Feeling after defecation. Data was recorded for each bowel movement.

Stool volume was visually estimated as the amount equivalent to a ping-pong ball (40mm in diameter). Ping-pong balls were shown to the subjects in advance to confirm the actual size. A selection of six stool forms was provided; lumpy (1 point), hard (2 points), banana-shape (3), paste (4), sludge (5) and watery (6). Stool color was selected from 3 options which were tan (3 points), brown (2) and dark brown (1 point). Subjects were provided with a colored illustrative guide on stool shape and color. There were 4 grades for odor of the stool based on the relative strength compared with the odor of usual stool: Sour-sweet (3 points), weaker (3), the same (2) and stronger (1 point). Subjects were asked to rate the feeling after defecation using the following 3 grades: Feel purged (3 points), normal (2) and retained stool (1 point). Subjects were also asked to report in the remarks column any incidence of gastrointestinal symptoms such as abdominal pain, tenesmus, flatulence, bloating and rumbling as well as any other changes in physical condition.

5) Statistical analysis
Defecation frequency was indicated as the number of bowel movements per week. Stool volume per week was indicated as the equivalent number of ping-pong balls. Form, color, and odor of the stool as well the feeling after defection were converted into scores using the assigned points and average values (per defecation) were calculated. Incidence rates for each week were calculated for gastrointestinal symptoms. The figures indicated are average values plus/minus standard deviation. Since no significant differences between Group A and B were identified using the Mann-Whitney U test, analysis was conducted with the data of the two groups combined. Wilcoxon signed rank test with a 5% significance level was used for the analysis. The software used for the statistical analysis was StatView version 5.0 (SAS Institute Inc.).

3. Results
1) Effects on defecation frequency and stool volume
Table 2 shows the defecation frequency and stool volume per week for the non-consumption period-1, test drink consumption period, non-consumption period-2 and placebo consumption period. Subjects who reported less than 7 defecations in non-consumption period-1 were classified as those with mild constipation. Similarly, those with 7 to 8 defecations were classified as subjects with normal bowel movements and those with 9 defecations or more were classified as subjects with frequent bowel movements[12].

For subjects with mild constipation, defecation frequency increased significantly to 5.8 times per week for the test drink consumption period compared with 4.2 and 4.6 times per week for the non-consumption period-1 and placebo consumption period respectively ($p<0.01$). In addition, there was a significant difference between the defecation frequency for the non-consumption period-2 and non-consumption period-1 (table 2) ($p<0.05$). Stool volume which was visually estimated using the size of a ping-pong ball (40mm diameter) also increased significantly to 18.4/week (ping-pong ball equivalent) for the test drink consumption period compared with 13.4 and 14.5 for the non-consumption period-1 and placebo consumption period respectively ($p<0.01$ and $p<0.05$ respectively) (table 2).

For subjects with normal bowel movements, stool volume increased significantly to 27.4/week for the test drink consumption period compared with 22.2 and 24.5 for the non-consumption period-1 and placebo consumption period respectively ($p<0.01$ and $p<0.05$ respectively).

Table 2 Changes in fecal frequencies and fecal amount per week by intake of powdered Aojiru drink containing indigestible dextrin

	Groups		Non-Ingestion 1	Test Meal	No-Ingestion 2	Control Meal
Fecal frequencies (times/ week)	Constipation*	(n=20)	4.2 ± 1.5	5.8 ± 2.0[ac]	5.1 ± 2.0[b]	4.6±1.6
	Normal**	(n=25)	7.4 ± 0.5	8.0 ± 1.9	8.2 ± 2.2	7.8±1.3
	Frequent***	(n=26)	11.9 ± 3.6	12.1 ± 5.0	11.9 ± 5.5	11.3±4.7
Fecal amount (numbers/ week)	Constipation*	(n=20)	13.4 ± 7.0	18.4 ± 9.8[ad]	14.3 ± 6.1	14.5±5.0
	Normal**	(n=25)	22.2 ± 11.2	27.4 ± 13.2[ad]	25.5 ± 13.4	24.5±12.8
	Frequent***	(n=26)	33.9 ± 17.4	36.4 ± 21.6	35.9 ± 21.8	36.9±23.0

Mean±SEM
a: statistically significant differences to Non-Ingestion Period 1 at p<1%
b: statistically significant differences to Non-Ingestion Period 1 at p<5%
c: statistically significant differences to Control Meal Period at p<1%
d: statistically significant differences to Control Meal Period at p<5%
*: the group of subjects who have ≤6 fecal frequencies (times/ week)
**: the group of subjects who have 7~8 fecal frequencies (times/ week)
***: the group of subjects who have ≥9 fecal frequencies (times/ week)

Table 3 Changes in fecal shape and other fecal conditions by intake of powdered Aojiru drink containing indigestible dextrin

	Groups		Non-Ingestion 1	Test Meal	No-Ingestion 2	Control Meal
Fecal shapes	Constipation*	(n=20)	3.0 ± 0.8	3.0 ± 0.5	2.9 ± 0.8	3.2 ± 0.7
	Normal**	(n=25)	3.2 ± 0.5	3.2 ± 0.4	3.2 ± 0.6	3.1 ± 0.6
	Frequent***	(n=26)	3.6 ± 0.5	3.5 ± 0.5	3.6 ± 0.5	3.6 ± 0.4
Fecal colors	Constipation*	(n=20)	2.2 ± 0.4	2.2 ± 0.3	2.2 ± 0.5	2.1 ± 0.3
	Normal**	(n=25)	2.1 ± 0.3	2.0 ± 0.2	2.0 ± 0.1	2.0 ± 0.2
	Frequent***	(n=26)	2.1 ± 0.2	2.0 ± 0.3	2.1 ± 0.3	2.1 ± 0.2
Fecal odor	Constipation*	(n=20)	2.0 ± 0.5	2.0 ± 0.3	1.9 ± 0.3	1.9 ± 0.2
	Normal**	(n=25)	2.0 ± 0.1	1.9 ± 0.2	2.0 ± 0.2	2.0 ± 0.1
	Frequent***	(n=26)	2.1 ± 0.3	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.3
Feelings after evacuation	Constipation*	(n=20)	2.0 ± 0.5	2.0 ± 0.4	1.9 ± 0.5	2.0 ± 0.4
	Normal**	(n=25)	2.0 ± 0.5	2.0 ± 0.4	2.0 ± 0.4	2.0 ± 0.4
	Frequent***	(n=26)	2.2 ± 0.3	2.1 ± 0.3	2.1 ± 0.4	2.1 ± 0.3

Mean±SEM
Total scores during each period were divided by the fecal frequencies (times) of the period.
*: the group of subjects who have ≤6 fecal frequencies (times/ week)
**: the group of subjects who have 7~8 fecal frequencies (times/ week)
***: the group of subjects who have ≥9 fecal frequencies (times/ week)

For subjects with frequent bowel movements, there were no significant differences in defecation frequency and stool volume for all periods.

2) Effects on form, color and odor of stool and on feeling after defecation

With regard to form, color and odor of stool and feeling after defecation, the average values for each period are shown in table 3. There were no specific changes in any of the items for the test drink consumption period for all groups (mild constipation, normal bowel movements and frequent bowel movements).

3) Gastrointestinal symptoms

Gastrointestinal symptoms reported on the questionnaire during the study period were summarized as the number of incidents per week

Table 4 Changes in appearance ratios of gastrointestinal symptoms by intake of powdered Aojiru drink containing indigestible dextrin

Total subjects (n=71)	Non-Ingestion 1	Test Meal	No-Ingestion 2	Control Meal
abdominal pain	0.4 ± 0.8	0.3 ± 0.9	0.2 ± 0.9	0.2 ± 0.5
tenesmus	0.3 ± 0.8	0.3 ± 0.8	0.3 ± 0.7	0.2 ± 0.5
abdominal growl	0.5 ± 1.4	1.0 ± 1.8[ace]	0.6 ± 1.2	0.7 ± 1.3
abdominal distention	0.3 ± 0.8	0.6 ± 1.2[bde]	0.4 ± 1.1	0.4 ± 1.0
abdominal wind	0.3 ± 0.8	0.2 ± 0.6	0.2 ± 0.7	0.2 ± 0.6

Mean±SEM
a: statistically significant differences to Non-Ingestion Period 1 at p<1%
b: statistically significant differences to Non-Ingestion Period 1 at p<5%
c: statistically significant differences to Non-Ingestion Period 2 at p<1%
d: statistically significant differences to Non-Ingestion Period 2 at p<5%
e: statistically significant differences to Control Meal Period at p<5%

and the results are shown in Table 4. There was a significant increase in the incidence of "flatulence" in the test drink consumption period compared with the non-consumption period-1, non-consumption period-2 and placebo consumption period ($p<0.01$, $p<0.01$ and $p<0.05$ respectively). In addition, the incidence of "bloating" also significantly increased in the test drink consumption period compared with the non-consumption period-1, non-consumption period-2 and placebo consumption period ($p<0.05$). However, all symptoms were transient and lasted only for 3 to 4 days. There were no incidences of gastrointestinal symptoms in the placebo consumption period for which the consumption of the placebo was indicated as a cause.

4. Discussion

In this study, it was confirmed that the consumption of the test drink was effective in increasing the defecation frequency and stool volume for individuals with mild constipation. These results support the existing reports on the effect of indigestible dextrin on improving bowel movement in people with mild constipation[9)12)13)]. In addition, the fact that defecation frequency and stool volume did not change for subjects with frequent bowel movements is a favorable aspect of the test drink concerning its safety.

Since indigestible dextrin is not digested and absorbed in the upper gastrointestinal tract, it reaches the large intestine and is exposed to intestinal bacteria. Short-chain fatty acids, which are produced by the bacteria, act to lower the pH of intestinal contents which in turn activates peristaltic motion and stimulates defecation or improves the condition of intestinal flora to enhance bowel movement[5)]. As a result, the properties of indigestible dextrin on improving bowel movement act more gradually compared with medication such as laxatives[14)]. Some of the subjects may have been still under the effect of the test drink during the non-consumption period-2 (the first 2 days), and for this reason defecation frequency for the non-consumption period-2 was higher than the non-consumption period-1 for subjects with mild constipation. The figures for the non-consumption period-1 and non-consumption period-2 (latter 4 days) were compared for both Group A and B but the differences were not statistically significant. The significant increase in the symptoms of "flatulence" and "bloating" for the test drink consumption period compared with other periods suggests a change in the intestinal environment.

There is a concern that excessive consumption of ingestible dextrin may have negative effects on the gastrointestinal system and cause symptoms such as diarrhea. However, the ED50 for diarrhea (the dosage necessary to produce diarrhea in 50% of the subjects) is reported to be 1.4g per one kg of body weight for both sexes[5)]. For an individual weighing 50kg, this amounts to 70g of indigestible dextrin. Therefore, more than 40 packets of the test drink must be consumed per day to induce diarrhea in half of the subjects. Since the subjects consumed 3 packets (5.1g of dietary fiber from indigestible dextrin) per day, the test drink was unlikely to cause diarrhea.

The results suggest that continued daily

consumption of 3 packets of powdered *Aojiru* drink containing indigestible dextrin used in this study is beneficial in maintaining and promoting good health in the aspect of preventing constipation.

5. Conclusion

The study examined the effects of an *Aojiru* drink powder containing indigestible dextrin on bowel movement using healthy individuals as subjects. The study results confirm that the consumption of 3 packets of *Aojiru* drink powder containing indigestible dextrin (4.3g/packet) significantly increases the defecation frequency and stool volume for subjects with mild constipation.

References

All 14 references are in Japanese.

(October 22, 2001 received)

Category	:	FOSHU Product (Kale product)
Country	:	JAPAN
Product Name	:	***Sukitto Kaitsu Aojiru***
Manufacturer	:	The Nisshin OilliO, Ltd.
Retail Price/ Packaging	:	JP¥3,200 (4.3 grams×30 packets=129 grams)
Product Launch	:	April 2003
Our Product Employed	:	Fibersol-2
Labeling of Our Product	:	Soluble Dietary Fiber (Indigestible Dextrin)

Ingredients:

Soluble dietary fiber (indigestible dextrin), Powdered kale (a kind of green vegetables), Sucrose, Maltose, Powdered green tea, Powdered spirulina (a kind of blue-green algae)

Directions:

Dissolve one packet into ~100 ml of cold/ hot water. Taking 3 packets per day is recommended.

Nutrition Facts (per 4.3 g, per packet):

Calories: 17, Protein: 0.3 gram, Fat: 0.1 gram, Saccharides: 1.5 gram, Sodium: 1.5 mg, Fiber: 2.3 gram

Ingredient involved in the health claim: Indigestible Dextrin (1.7grams as fiber)

Approved FOSHU Health Claim:

This product contains dietary fiber (indigestible dextrin) and improves the bowel movements.

April 17, 2003

DRUG RECORD

CASCARA *(CASCARA SAGRADA)*

OVERVIEW
Cascara

Introduction

Cascara is a popular herbal medication and over-the-counter therapy of constipation. Cascara is generally safe and well tolerated, but can cause adverse events including clinically apparent liver injury when used in high doses for longer than recommended periods.

Background

Cascara sagrada is an herbal medication used for centuries as a laxative which is now available in the United States without prescription for short term treatment of constipation. Cascara is typically an extract from the dried, aged bark of Rhamnus purshiana, a species of buckthorn tree or shrub native to North America. Cascara sagrada is Spanish for "sacred bark" and was used for centuries by Native Americans as a laxative. Cascara became accepted in western medical practice in the 19th century and is still used in over-the-counter laxative preparations, often in combination with other herbals such as aloe vera. The active laxative components in cascara are anthraquinone derivatives and their glucosides, referred to as cascarosides. They appear to act locally as an irritant to the colon promoting peristalsis and stool evacuation. Anthraquinones also inhibit reabsorption of electrolytes and water from the colon. Cascara is minimally absorbed. The typical dose is 300 mg once daily, but it is recommended for short term use only (less than one week). Side effects include abdominal cramps and electrolyte imbalance. Long term use or abuse can lead to "cathartic" colon with diarrhea, cramps, weight loss and darkened pigmentation of the colonic mucosa.

Hepatotoxicity

Use of cascara in the recommended doses for a limited period of time has been associated with few side effects, most of which are mild and transient. With longer term use of high doses of cascara, however, adverse events have been described including several cases of clinically apparent liver injury. The time to onset of liver injury has varied from a few days to 2 months of use, and the pattern of serum enzyme elevations was typically hepatocellular. The liver injury ranged from mild to severe, but usually resolved rapidly with discontinuation. Immunoallergic features and autoimmune markers were not prominent or consistently present in the published cases.

Mechanism of Injury

The liver injury due to cascara has been attributed to the direct toxicity of anthraquinone derivatives in the herbal extract; however, the clinical characteristics of the published cases

suggest an idiosyncratic rather than direct hepatotoxic etiology. Other anthraquinones used to treat constipation have been implicated in causing liver injury with long term use, including sennosides and hydroxyanthraquinone.

Outcome and Management

Liver injury from long term cascara use is rare and most cases have been self-limited and rapidly reversible upon stopping the laxative. However, severe cases with acute liver failure and development of ascites and portal hypertension have been described. There is no evidence of cross sensitivity to hepatic damage with other laxatives. Restarting cascara has been associated with recurrence of liver injury and should be avoided.

Drug Class: Herbals and Dietary Supplements

Other drugs in the Anthraquinone Subclass: Senna

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PRODUCT INFORMATION
Cascara

REPRESENTATIVE TRADE NAMES

Cascara – Generic

DRUG CLASS

Herbals and Dietary Supplements

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CHEMICAL FORMULA AND STRUCTURE
Cascara

DRUG	CAS REGISTRY NUMBER	MOLECULAR FORMULA	STRUCTURE
Cascara	8047-27-6	Herbal mixture	Not applicable

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REFERENCES
Cascara and Senna

References updated: 05 May 2014

1. Zimmerman HJ. Unconventional drugs. Miscellaneous drugs and diagnostic chemicals. In, Zimmerman, HJ. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. 2nd ed. Philadelphia: Lippincott,1999: pp. 731-4. (Expert review of hepatotoxicity published in 1999; cascara is not discussed).
2. Seeff L, Stickel F, Navarro VJ. Hepatotoxicity of herbals and dietary supplements. In, Kaplowitz N, DeLeve LD, eds. Drug-induced liver disease. 3rd ed. Amsterdam: Elsevier, 2013, pp. 631-58. (Review of hepatotoxicity of herbal and dietary supplements [HDS]; cascara is listed as having been linked to cases of cholestatic hepatitis).
3. Cascara sagrada. In, PDR for Herbal Medicines. 4th ed. Montvale, New Jersey: Thomson Healthcare Inc. 2007: pp. 161-4. (Compilation of short monographs on herbal medications and dietary supplements).
4. Tolman KG, Hammar S, Sannella JJ. Possible hepatotoxicity of Doxidan. Ann Intern Med 1976; 84: 290-2. PubMed Citation (24 year old man developed fatigue 6 months after starting Doxidan [containing hydroxyanthraquinone] for chronic constipation and at 12 months developed nausea and syncope [bilirubin 0.8 mg/dL, AST 560 U/L, Alk P 339 U/L, ANA negative, slight neutropenia], liver biopsy showing chronic hepatitis, resolving within 1 month of stopping, recurring upon rechallenge).
5. Beuers U, Spengler U, Pape GR. Hepatitis after chronic abuse of senna. Lancet 1991; 337:

372-3. PubMed Citation (26 year old nurse taking high doses of senna alkaloids developed jaundice and pruritus [bilirubin not given, ALT 303 U/L, Alk P 227 U/L], resolving within a few weeks of stopping and recurring upon restarting [ALT >280 U/L]).

6. Franz G. The senna drug and its chemistry. Pharmacol 1993; 47(Suppl 1): 2-6. PubMed Citation (Senna is dried leaflets or fruits of Cassia senna, either acutifolia [Alexandrian] or angustifolia [Indian senna], native to Northern Africa, middle East and India, similar constituents including anthraquinone glycosides, called sennosides).
7. Woolf GM. Senna-induced hepatotoxicity. Hepatology 1999; 550A: 1560. Not in PubMed (81 year old woman developed recurrent jaundice after taking senna for one month [bilirubin 3.2 rising to 29.6 mg/dL, peak ALT 1455 U/L, Alk P 168 U/L], recurring more rapidly and recovering more slowly after each of 3 episodes after reexposure).
8. Nadir A, Reddy D, Van Thiel DH. Cascara sagrada-induced intrahepatic cholestasis causing portal hypertension: case report and review of herbal hepatotoxicity. Am J Gastroenterol 2000; 95: 3653-7. PubMed Citation (48 year old man developed jaundice 3 days after starting cascara sagrada [bilirubin 11.8 mg/dL, ALT 999 U/L, Alk P 309 U/L, ANA 1: 640], developing ascites, but recovering within 3 months of stopping).
9. Wurtz AS, Vial T, Isoard B, Saillard E. Possible hepatotoxicity from Copaltra, an herbal medicine. Ann Pharmacother 2002; 36: 941-2. PubMed Citation (49 year old woman developed jaundice 3 months after starting Copaltra tea [Coutarea latiflora and Centaurium erythreae] for diabetes [bilirubin 20.8 mg/dL, ALT 3010 U/L, Alk P 132 U/L], with recovery in 3 months).
10. De Smet PAGM. Herbal remedies. N Engl J Med 2002; 347: 2046-56. PubMed Citation (Review of status and difficulties of herbal medications including lack of standardization, federal regulation, contamination, safety, hepatotoxicity and drug-herb interactions; specific discussion of 4 herbs with therapeutic promise: ginkgo, hawthorn, saw palmetto and St. John's wort).
11. Stedman C. Herbal hepatotoxicity. Semin Liver Dis 2002; 22: 195-206. PubMed Citation (Review and description of patterns of liver injury, including discussion of potential risk factors, and herb-drug interactions; cascara is listed as causing liver injury).
12. Schiano TD. Hepatotoxicity and complementary and alternative medicines. Clin Liver Dis 2003; 7: 453-73. PubMed Citation (Comprehensive review of herbal associated hepatotoxicity; cascara is listed as a potential hepatotoxin).
13. Ernst E. Risks of herbal medicinal products. Pharmacoepidemiol Drug Saf 2004; 13: 767-71. PubMed Citation (Review of the adverse effects of over-the-counter herbal medications, focusing on the hepatotoxicity of kava, drug interactions with St. John's wort, and contamination of traditional Chinese medications with heavy metals [arsenic, lead, mercury, thallium] and conventional western medications).
14. Russo MW, Galanko JA, Shrestha R, Fried MW, Watkins P. Liver transplantation for acute liver failure from drug-induced liver injury in the United States. Liver Transpl 2004; 10: 1018-23. PubMed Citation (Among ~50,000 liver transplants reported to UNOS between 1990 and 2002, 270 [0.5%] were done for drug induced acute liver failure, including 7 [5%] for herbal medications, none attributed to cascara).
15. Seybold U, Landauer N, Hillebrand S, Goebel FD. Senna-induced hepatitis in a poor metabolizer. Ann Inter Med 2004; 141: 650-1. PubMed Citation (28 year old woman developed jaundice after drinking herbal tea containing senna leaves with positive rechallenge [ALT ~1390 U/L, GGT ~190 U/L], with Rhein anthrone detectable in serum).
16. Vanderperren B, Rizzo M, Angenot L, Haufroid V, Jadoul M, Hantson P. Acute liver failure with renal impairment related to the abuse of senna anthraquinone glycosides. Ann Pharmacother 2005; 39: 1353-7. PubMed Citation (52 year old woman developed jaundice, acute liver failure, lactic acidosis, and phosphate wasting nephropathy after ingesting herbal tea made from senna fruits for 3 years [bilirubin 6.2 mg/dL, ALT 9160 U/L, INR 5.3], requiring ventilator support, ultimately resolving).
17. Sonmez A, Yilmaz MI, Mas R, Ozcan A, Celasun B, Dogru T, Taslipinar A, Kocar IH. Subacute cholestatic hepatitis likely related to the use of senna for chronic constipation. Acta Gastroenterol Belg 2005; 68: 385-7. PubMed Citation (77 year old male developed jaundice after taking a senna preparation for constipation for 3 months [bilirubin 4.9 rising to 16.9 mg/dL, ALT 657 U/L, Alk P 160 U/L], resolving within 1 month of stopping the herbal).

18. Bruguera M, Herrera S, Lazaro E, Madurga M, Navarro M, De Abajo FJ. [Acute hepatitis associated with consumption of Copalchi: a summary of 5 cases]. Gastroenterol Hepatol 2007; 30: 66-8. PubMed Citation (Five cases of hepatotoxicity of Copalchi in 4 men and 1 woman ages 59 to 77 years, taking herb for 2 to 13 months [bilirubin 0.7-13 mg/dL, ALT 403-865 U/L, GGT 54-116 U/L], resolving in all).

19. García-Cortés M, Borraz Y, Lucena MI, Peláez G, Salmerón J, Diago M, Martínez-Sierra MC, et al. Liver injury induced by "natural remedies": an analysis of cases submitted to the Spanish Liver Toxicity Registry. Rev Esp Enferm Dig 2008; 100: 688-95. PubMed Citation (Among 521 cases of drug induced liver injury submitted to Spanish registry, 13 [2%] were due to herbals, including one due to Rhamnus purshianus [cascara], with onset after 2 months [bilirubin 7.1 mg/dL, ALT 56 times ULN, Alk P normal], resolving in 3 months).

20. Chalasani N, Fontana RJ, Bonkovsky HL, Watkins PB, Davern T, Serrano J, Yang H, Rochon J; Drug Induced Liver Injury Network (DILIN). Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. Gastroenterology 2008; 135: 1924-34. PubMed Citation (Among 300 cases of drug induced liver disease in the US collected between 2004 and 2008, 9% of cases were attributed to herbal medications, but none were specifically linked to cascara).

21. Freeman HJ. "Melanosis" in the small and large intestine. World J Gastroenterol 2008; 14: 4296-9. PubMed Citation (Pigment found in small and large intestine with long term anthracene laxative [cascara, senna, aloes and rhubarb], use is usually lipofuscin rather than hemosiderin or melanin and is present in macrophages in the lamina propria).

22. Jacobsen C, Semb S, Kromann-Andersen H. [Toxic hepatitis following consumption of the herbal medicinal product Cascara Sagrada]. Ugeskr Laeger 2009; 171: 3367-9. Danish. PubMed Citation (49 year old woman developed jaundice 4 weeks after starting daily cascara sagrada for constipation [bilirubin 8.4 rising to 24.6 mg/dL, ALT 944 U/L, ascites], resolving within 4 months of stopping).

23. Jacobsson I, Jönsson AK, Gerdén B, Hägg S. Spontaneously reported adverse reactions in association with complementary and alternative medicine substances in Sweden. Pharmacoepidemiol Drug Saf 2009; 18: 1039-47. PubMed Citation (Review of 778 spontaneous reports of adverse reactions to herbals to Swedish Registry does not list or mention senna or cascara).

24. Vitalone A, Menniti-Ippolito F, Raschetti R, Renda F, Tartaglia L, Mazzanti G. Surveillance of suspected adverse reactions to herbal products used as laxatives. Eur J Clin Pharmacol 2012; 68: 231-8. PubMed Citation (Among 519 adverse events attributed to herbals reported to an Italian registry between 2002 and 2011, 26 were related to herbal laxatives, including 3 cases of liver injury, 1 due to cascara and 2 to senna).

25. Teschke R, Wolff A, Frenzel C, Schulze J, Eickhoff A. Herbal hepatotoxicity: a tabular compilation of reported cases. Liver Int 2012; 32: 1543-56. PubMed Citation (A systematic compilation of all publications on the hepatotoxicity of specific herbals identified 185 publications on 60 different herbs, herbal drugs and supplements, including 1 implicating cascara [Nadir 2000] and 3 senna [Beuers 1991, Seybold 2004, Vanderperren 2005]).

26. Bunchorntavakul C, Reddy KR. Review article: herbal and dietary supplement hepatotoxicity. Aliment Pharmacol Ther 2013; 37: 3-17. PubMed Citation (Systematic review of the literature on HDS associated hepatotoxicity mentions that cascara has been implicated in causing clinically apparent acute liver injury).

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SAFETY ISSUES AFFECTING HERBS:

How Long can Stimulant Laxatives be Used?

by Subhuti Dharmananda, Ph.D., Director, Institute for Traditional Medicine, Portland, Oregon

BACKGROUND

Ingestion of laxatives is one of the primary self-treatment methods in the West. Laxatives are readily available as over-the-counter drug products and as dietary supplements. They are used for two purposes: to treat constipation and to aid in weight loss. Most health authorities discourage the use of laxatives for weight loss, saying that they do not significantly reduce absorption of food calories (26). Even so, senna leaf teas (or preparations in capsule or tablet form) are widely used for this purpose and are a major import from China and Europe. In the treatment of constipation, there are three basic types of laxatives:

- stimulants: these induce intestinal peristalsis and are the ones taken for weight control;
- bulking agents, mainly fiber, but also other polymers; and
- softeners (emollient laxatives): they either add oily material, increase water retention in the intestine, or aid mixing of water and oil components in the fecal material.

With the exception of taking fiber supplements to replace dietary fiber that is consumed in insufficient quantities, laxatives are indicated for short-term use. Nonetheless, many people with chronic constipation use them routinely.

Abuse of laxatives, including misuse in treatment of obesity and persistent use in treatment of chronic constipation, is a significant problem that has been mentioned in the medical literature. Laxative abuse is particularly prevalent with stimulant purgatives, for which several concerns have been raised: laxative dependence (that is, stimulated peristalsis begins to replace natural peristalsis), potassium imbalance, and potential damage to the intestinal tract after years of relying on them. The California Department of Health, Food and Drug Branch, now requires products with stimulant laxatives to warn about taking them during pregnancy, nursing, while taking medications, or if the user has a "medical condition," as well as warning about potential diarrhea, loose stools, or abdominal pain (21, 27).

The nature of laxative dependence is a controversial matter (1). It may result, in part, from degeneration of the nerves in the intestines, dulling the natural responses that stimulate peristalsis; however, laxative dependence may simply be a psychological dependence. The link between use of stimulant laxatives and colon nerve damage or other structural changes is not well established but is an active area of investigations (25). Potassium imbalance, from long-term use of laxatives, especially at excessive dosage, has been blamed for deaths of apparently otherwise healthy women (21). Laxatives are always mentioned in discussions of drug interactions because of the concern that they will exacerbate potassium losses that may be an otherwise minor side effect of drug therapies.

ANTHRAQUINONES

The naturally occurring stimulant laxatives that are sufficiently safe and gentle for general use involve one basic category of chemical compounds, known as anthraquinones (see Figure 1). These include both the simple anthrones and the bianthrones, also called dianthrones, comprised of two anthrones linked together (see Figure 2). The anthraquinones often occur in plants in the form of glycosides. These compounds are found in rhubarb root (the prominent Chinese laxative), senna leaf and pod (a Middle Eastern laxative), cascara sagrada (a North American laxative), buckthorn (also known as frangula; a

European laxative), and aloe (known worldwide). The anthraquinones are colorful, in the spectrum of yellow to orange to red, evident in certain varieties of rhubarb, sometimes called "painted rhubarb" because the colors appear as beautiful striations across the sliced root surface. The colors of rhubarb result mainly from its content of emodin (orange), chrysophanol (yellow), rhein (yellow-orange), and physcion (brick-red).

Emodin is the most widely occurring anthraquinone in medicinal herbs. Among Chinese herbs, it is found in several species of *Rheum*, *Rumex*, *Polygonum*, and *Cassia*. Valuable medicinal aspects of emodin have been proclaimed on the basis of laboratory research, including: potential cancer prevention activity (blocking the growth of newly transformed cells); antiviral activity; antioxidant properties; gastric ulcer protection; liver protection; promoting blood circulation; inhibiting autoimmune attack; and benefiting the kidneys (inhibiting undesired proliferation of cells). Rhubarb root is a dominant herbal therapy in China for treatment of nephritis and prevention of renal failure.

Anthraquinones are also found in other herbs, usually in small quantities, most notably in species of *Polgyonum*. There are anthraquinones in vegetables, such as cabbage and lettuce, being particularly high in beans (36 mg/kg fresh weight). Physcion is the dominant anthraquinone in foods. Purpurin, an anthraquinone from madder root (*Rubia tinctorium*), is a component of an approved red food coloring.

In relation to the laxative effects, it is understood that anthraquinones act directly on the intestinal wall (in the colon region) to produce the desired result. They are poorly absorbed, and bianthrones are virtually unabsorbed; rather, they are degraded in the colon to produce more active metabolites, mainly anthrones (2). Anthraquinone laxatives increase fluid electrolyte accumulation in the distal ileum and colon (change in absorption and secretion of water; retention of potassium) through unknown actions, possibly via an irritation of the intestinal mucosa and endothelial cells. There may also be a direct stimulation of peristaltic activity. The bianthrones, especially sennosides, as found in rhubarb and senna, appear to be more active as laxatives than the simple anthraquinones. In single-dose treatment of constipation, the effects of the anthraquinones are noted in about 6-8 hours, the time it takes for them to reach the colon.

TRADITIONAL USE OF PURGATIVE THERAPY

The use of laxatives to treat acute constipation has been mentioned throughout the literature of the world's various herbal traditions. Acute constipation may occur as the result of a disease or a change of diet. It is usually resolved by taking the laxative preparation once or twice; perhaps the treatment will need repeating for a few days. In the history of Chinese medicine, use of rhubarb for acute constipation is firmly established in relation to the "*yangming*" phase of a disease; this is where the body fluids become dry as the result of a feverish condition; the intestines become dry, and the person suffers from constipation. Mirabilitum (Epsom salt; magnesium sulfate) is often used along with rhubarb in the Chinese treatments for acute constipation. Compared to rhubarb, it causes peristalsis higher in the intestinal tract (small intestine region), produces a quicker action (within 2-3 hours, even more quickly with higher dosage), and retains more water in the intestinal tract.

Purgation was also adopted worldwide as a therapy for diseases. The idea behind this broad application was that something in the body, the entity or toxin that caused the disease, needed to be flushed out. The elimination of this pathological influence was thought to be accomplished by either sweating it out through the pores, vomiting it out through the mouth, or disposing of it through the intestines or urinary tract. In the ancient Chinese system of therapy prevalent during the Han Dynasty, the three methods of diaphoresis, emesis, and purgation were widely used for this purpose. Although the reliance on such approaches to treating diseases faded at times (some medical authorities considered the methods too drastic, debilitating the patient), they were later revived. For example, these methods were again promoted during the Jin-Yuan medical reform (12th-13th century) by proponents of the Purgation

school (or Attacking school). In America, treatment of numerous diseases by elimination techniques, especially purgation, has became a hallmark of the natural healing profession since the 19th century and is still deemed an important component by its practitioners, including use of such questionable therapies as colonics (intestinal cleansing with water).

CHRONIC CONSTIPATION AND ITS RESOLUTION

During the 20th century, chronic constipation had become a common health problem, particularly of the elderly. This disorder, known also as idiopathic constipation, is not widely discussed in the medical literature. Rather, the main focus of medical investigations and discussions has been on constipation that is secondary to a specific disease process, bowel malformation, or a side effect of drugs. While commercial advertisements and labeling for over-the-counter laxative products mention that the products are intended for treatment of "occasional irregularity," the fact is that many people experience constipation that is so regular that they treat it persistently. Constipation does not necessarily involve infrequent or irregular elimination; the term has been used by people to describe difficult bowel movements (straining) or a sensation of incomplete bowel movements.

Studies on the etiology of chronic constipation have not been reported, but several lines of information converge on two key contributors. One is the sedentary lifestyle that has become increasingly prevalent. The absence of physical labor, or compensating vigorous exercise, results in laxness of the abdominal muscles, as well as other muscles. Current recommendations for exercise that are given to those who do little or no exercise are rarely helpful for overcoming the abdominal weakness. For example, it is commonly stated in the popular literature that walking just 20 minutes per day will improve health. While this is undoubtedly a benefit compared to no exercise, unless the walking is on an uneven path (e.g., a hilly natural path with numerous irregularities), there is essentially no exercise of the abdominal muscles involved. Further, 20 minutes of exercise represents activity during only 2% of the waking day, with 98% of the daily routine potentially being virtually inactive. Such a low level of activity was probably never experienced before in human history, except by the few who held the highest offices of the land and by those debilitated from disease or injury. Abdominal muscle laxity not only contributes to inability to move the bowels, but also may lead to prolapse and expansion of the lower intestinal tract, making it possible for the fecal matter to stagnate in masses that are difficult to pass. Lack of exercise also contributes to obesity, osteoporosis, heart disease, and depression, to mention a few well-known adverse consequences; it is associated with higher rates of colon cancer.

A second contributor to chronic constipation is insufficient bulk in the intestines as the result of low intake of fiber and water. The pressure of this bulk of material against the intestinal walls is a stimulus to natural peristalsis. Health authorities repeatedly emphasize the value of fruits and vegetables, many of which are high in fiber and water, and they recommend consumption of large amounts of additional fluids, mostly in the form of water. A standard recommendation is to consume two quarts of water (equivalent to 8 servings of one cup each) per day. Fiber is also obtained via ingestion of whole grains and fiber supplements; however, this dry fiber is not a satisfactory solution in the absence of adequate water ingestion. Even with a diet that emphasizes fibrous foods, inadequate total intake of food can contribute to constipation. In particular, those who are sedentary may consume less than the 2,000-calorie daily intake that is normally expected, and the amount of food material may be low enough, despite its fiber content, that the intestines are not stimulated to move it through in a normal manner.

Practitioners involved with natural health-care therapies often recommend various dietary supplements, including herbs, which will have a laxative effect. While these supplements can be quite helpful, it is important to put them into proper context and to be aware of potential problems from extended use of stimulant purgatives. Relying on laxatives to permanently replace exercise, fibrous foods, and water is inappropriate except in extreme cases where there are no other options (such as elderly patients with limited capabilities). While use of the laxatives as part of a changing lifestyle may

be a valuable component of therapy leading towards alleviation of chronic constipation, it is inconsistent with the tenets of natural healing to recommend use of supplements only, particularly without a reasonable limit of duration.

BLACK INTESTINES

Colonoscopy was developed as a routine diagnostic tool during the 1970s and it was soon found that many people had a black or dark brown intestinal wall; the condition was named melanosis coli. Normally, the intestinal wall has a healthy pink appearance. This darkened intestinal wall was found to be associated with chronic use of anthraquinone laxatives. Histological study of colon biopsies in chronic laxative users showed an increased number of macrophages in the connective tissue of the colon mucosa (3). The anthraquinones and their metabolites are retained in the macrophages, where they yield the dark color. Melanosis coli could also arise more rarely from serious diseases of the intestine, the blackening coming from areas of cell necrosis. Thus, the laxative-related condition is sometimes called pseudomelanosis coli, to indicate that the darkening of the intestinal wall is believed to be due only to discoloration rather than a physiological disorder.

A debate over the significance of melanosis coli in relation to anthraquinone use soon arose, and it persists to this day. On the one side, many doctors have interpreted this condition as one of simple staining of the intestinal wall by anthraquinone residues, and viewed it as a harmless and reversible condition (1, 4, 16, 17). Other doctors have seen it as more than just staining, as possibly involving significant damage to the colon wall, and considered that it might be a precursor to more serious intestinal problems, such as colon cancer (15, 18). The majority of articles written about melanosis coli indicate that the condition arises after years of frequent use of anthraquinone laxatives and is not associated with harm. Nonetheless, there are worries that the changes in colon macrophage content and possible changes in colon wall structure that appear to accompany persistent anthraquinone use represent potential harm.

Some patients with melanosis coli have obvious damage to the colon wall (epithelium plus mucosa), which may or may not have been induced by the persistent ingestion of anthraquinones. It is possible that the laxative ingredients have caused cumulative damage in persons whose intestinal repair mechanisms are weak (the intestinal wall cells are among the fastest replicating in the body). In one study (5), 45 patients with prolonged use of anthraquinone laxatives were found to have abnormalities in the absorptive epithelial cells of the colon and autonomic nerve fibers were in various stages of degeneration. A Scandinavian study indicated the presence of nerve damage that might correlate with reduced motility of the intestines (10) in chronic laxative users. In a recent study carried out in Argentina (6), it was reported that the colon cells in patients with melanosis coli had a larger proportion of dead cells, and that the discoloration may have been a combination of anthraquinone residues plus saccharides from the dying cells. After administration of a single dose of sennosides, it was shown that there was an increase in apoptosis (cell death) of colon epithelial cells (18). One implication is that the anthraquinones may have been inducing premature cell death, with potential for colon wall damage.

On the other hand, the chronic constipation may have been associated with or a causative factor in intestinal damage, and the anthraquinones may have simply darkened the cells but not contributed to the damage nor provided any additional threat to the health of the colon. Repeated damage to cells, especially rapidly replicating cells as found in the intestinal wall, might be expected to increase the rate of cancer formation. A retrospective study in Germany with more than 2,200 patients suggested that there was no increase in colorectal cancer incidence in persons with melanosis coli compared to those without the condition (4). There was a more frequent finding of adenomas in these patients, but this was attributed to easier detection not higher incidence, since the adenomas do not incorporate the pigment and show up as white spots on a black colon wall background. A case report of a woman with melanosis coli after 20 years of laxative use showed no colon abnormalities (16). In a series of over 1,000

rectoscopies conducted in Germany, 10% of the patients were found to have melanosis coli; accompanying inflammation of the colon mucosa was seldom found (17); mild inflammation was attributed to an increased turnover of mucosa cells, which is not necessarily harmful.

In relation to potential carcinogenic action, anthraquinones have been tested for mutagenic potential. Purpurin (7) and the anthraquinones of rhubarb (8) were shown to have antimutagenic effects in laboratory studies. The anthraquinone chrysophanol did not cause chromosomal aberrations when tested in laboratory animals (9). While some laboratory screening tests indicate a genotoxic activity of emodin and aloe-emodin, human clinical trials and animal studies do not support concerns that senna laxatives (the ones most commonly used) pose a genotoxic risk to humans when consumed as normally prescribed (19).

RECOMMENDATIONS: DOSAGE AND DURATION OF USE

Use of anthraquinones should probably be limited in dosage and duration to avoid any potential adverse health consequences related to melanosis coli. In one study of colon submucosal nerves in patients with chronic abuse of laxatives, it appeared that nerve fiber damage was related to both dosage and duration of laxative use (10). Pseudomelanosis coli is usually found after a minimum of 9-12 months of regular stimulant laxative use (15). Presumably, after a break in the use of anthraquinones for several weeks, the colon will return to normal and a course of laxative therapy could be safely repeated if deemed necessary. During the break from use of the anthraquinone-containing preparations, lactulose preparations or polyethylene glycol preparations (the newest therapies to show good results) may be used instead. These bulking agents are taken in a dose of about 10 grams each time, twice daily, and have relatively quick effects: normal stool within one to four weeks (11, 12). Their use is becoming prevalent; in a study of more than 3,200 elderly patients in Italy, it was found that lactulose was the most frequently used laxative, followed by anthraquinone laxatives; the use of anthraquinones declined, in favor of lactulose, during hospitalization (13).

In a small study in France of elderly patients (average age 81 years) with chronic constipation (14), 20 mg sennosides daily was administered for six months. This treatment was shown to be without adverse effects: there were no abnormal losses of either protein or potassium; no testing was done for melanosis coli, which would not be expected to occur in six months. Little is known about the dosage that causes melanosis coli or that might cause a more severe form of the condition with intestinal damage. A limiting daily dosage corresponding to 20-30 mg of anthraquinones from senna leaf has been recommended in the herbal literature based on European suggestions for safe use (20). Over-the-counter stimulant laxative drug products are deemed safe and effective when administered in amounts of 12 to 50 mg of sennosides per dose, once or twice per day (21).

In order to evaluate the dosage of various herbal preparations, it is necessary to know the content of anthraquinones in the crude dried herbs and in the prepared teas. A study of rhubarb constituents revealed that the dried root contained 1.2% anthraquinones for *R. tanguticum* and 3.4% for *R. palmatum*, with lower amounts in processed roots (22). To get a 20-mg daily dose of anthraquinones (comparable to the dose of sennosides used in the above study), one would consume about 600 mg of rhubarb root derived from *R. palmatum*. Senna leaf (from *S. alexandrina*) is reported to contain a similar level of 1.5-3.0% anthraquinones and senna pod contains about 1.4-3.5% (20). Therefore, daily doses of about 500 to 1,000 mg of senna leaf appear to be in the safe range. In a study carried out in California (21), senna teas were tested for sennoside content: teas (in teabag form) labeled as laxatives contained 7-10 mg of sennosides per cup; a dieter's tea yielded 19 mg per cup.

In a report from China, patients with addiction to senna leaf tea as a laxative, were reported to suffer from symptoms of fidgetiness, sleeplessness, dilated pupils, and loss of appetite when consuming 5-9 grams of senna daily (24). This is 10 times the range mentioned above that would be deemed safe (based

on an estimate of sennoside content for Chinese senna leaf at 1%). About half the people who showed these symptoms of regular senna ingestion had lost weight, indicating the potential modest success rate for long-term overdose of laxatives in a weight loss regimen.

Cases of rare hepatic inflammation possibly induced by anthraquinone derivatives have been reported (28, 29, 30, 31) and may be dose related. It is suggested that the anthraquinone may be metabolized in the intestines to form a hepatotoxic compound that some people are sensitive to, resulting in reversible liver damage. Herbs that have been associated with the liver reaction include senna, ho-shou-wu (*Polygonum multiflorum*; being used as a blood tonic to prevent graying of hair, not as a laxative), and cascara sagrada. In the reported case involving senna ingestion, the dosage was very high, corresponding to 100 mg sennosides per day in addition to twice per week ingestion of a tea made with 10 grams of senna leaves (31). These reactions appear to be so rare that they are not considered cause for warnings or alerts.

Limiting the daily intake of anthraquinones to 20-30 mg per day, and limiting duration of continual use to 9-12 months may be a reasonable means of avoiding any of the potential problems associated with stimulant laxatives. If, after this course of therapy, constipation cannot be alleviated by exercise, diet, and physical therapies (e.g., acupuncture, abdominal massage), then anthraquinone use could be continued after an interval of a few weeks while relying on bulking agents as a substitute.

REFERENCES

1. Muller-Lissner SA, *Adverse effects of laxatives: fact and fiction*, Pharmacology 1993; 47 (Supplement 1): 138-145.
2. de Witte P, *Metabolism and pharmacokinetics of antrhanoids*, Pharmacology 1993; 47 (Supplement 1): 86-97.
3. Steer HW and Colin-Jones DG, *Melanosis coli: studies on the toxic effects of irritant purgatives*, Journal of Pathology 1975; 115(4): 199-205.
4. Nusko G, et al., *Melanosis coli-a harmless pigmentation or a precancerous condition?*, Zeitschrift Gatroenterology 1997; 35(5): 313-318.
5. Balazs M, *Melanosis coli: Ultrastructure study of 45 patients*, Diseases of Colon and Rectum 1986; 29(12): 839-844.
6. Benavides SH, et al., *The pigment of melanosis coli: A lectin histochemical study*, Gastrointestinal Endoscopy 1997; 46(2): 131-138.
7. Takahashi E, et al., *Preventive effects of anthraquinone food pigments on the DNA damage induced by carcinogens in Drosophila*, Mutation Research 2001; 480-481, 139-145.
8. Sun M, et al., *Cytochrome P4501A1-inhibitory action of antimutagenic anthraquinones in medicinal plants and the structure-activity relationship*, Bioscience, Biotechnology, and Biochemistry 2000; 64(7): 1373-1378.
9. Mengs U, Schuler D, and Marshall RR, *No induction of chromosomal aberrations in Chinese hamster ovary cells by chrysophanol*, Mutation Research 2001; 492 (1-2): 69-72.
10. Reimann JF, Schmidt H, and Zimmermann W, *The fine structure of colonic submucosal nerves in patients with chronic laxative abuse*, Scandinavian Journal of Gastroenterology 1980; 15(6): 761-768.
11. Connolly P, Huges IW, Ryan G, *Comparison of Duphalac and irritant laxatives during and after treatment of chronic constipation: a preliminary study*, Current Medical Research and Opinion, 1974-1975; 2(10): 620-625.

12. Attar A, et al., *Comparison of low dose polyethylene glycol electrolyte solution with lactulose for treatment of chronic constipation*, Gut 1999; 44(2): 226-230.

13. Pahor M, et al., *Age and laxative use in hospitalized patients*, Aging (Milan); 1995; 7(2): 128-135.

14. Emeriau JP, et al., *Measurement of the intestinal clearance of alpha 1-antitrypsin and the exchangeable potassium pool in elderly patients treated with anthraquinone glycosides*, Gastroenterology Clinical Biology; 1983, 7(10): 799-801.

15. Siegers CP, et al., *Anthranoid laxative abuse-a risk for colorectal cancer?* Gut 1993; 34(8): 1099-1101.

16. Fleischer I and Bryant D, *Melanosis coli or mucosa ischemia? A case report*, Ostomy Wound Management, 1995; 41(4): 46-47.

17. Gobel D, *Melanosis coli*, Clinical Medicine 1978; 73(14): 519-523.

18. van Gorkom BA, et al., *Apoptosis induction by sennoside laxatives in man; escape from a protective mechanism during chronic sennoside use?* Journal of Pathology 2001; 194(4): 493-499.

19. Brusick D and Mengs U, *Assessment of the genotoxic risk from laxative senna products*, Environmental Molecular Mutagens 1997; 29(1): 1-9.

20. Blumenthal M (senior editor), **Herbal Medicine: Expanded Commission E Monographs**, 2000 American Botanical Council, Austin, TX.

21. Loscutoff S, *Adverse reactions cause the department of health services to require a label notice on foods and dietary supplements containing ingredients with stimulant laxative effects*, California Morbidity, 1998 (September): 1-2.

22. Tang W and Eisenbrand G, *Chinese Drugs of Plant Origin*, 1992 Springer-Verlag, Berlin.

23. Xing JH and Soffer EE, *Adverse effects of laxatives*, Diseases of the Colon and Rectum 2001; 44(8): 1201-1209.

24. Yang Yufu, *A report on 21 cases of addiction to senna induced by chronic use*, Chinese Journal of Chinese Materia Medica 1992; 17(3): 184-185.

25. Huang ZS and Wang ZW, *Study on emodin in plant distribution and pharmacological research*, International Journal of Oriental Medicine 1998; 23(4): 177-182.

26. Kurtzweil P, *Dieter's brews make tea time a dangerous affair*, FDA Consumer 1997: 6-11.

27. Blumenthal M, *California requires warning labels for products containing stimulant laxative herbs*, HerbalGram 1997; (39): 26-27.

28. But PP, Tomlinson B, Lee KL, *Hepatitis related to the Chinese medicine Shouwu Pian, manufactured from Polygonum multiflorum*, Veterinary and Human Toxicology 1996; 38(4): 280-282.

29. Park GJ, Mann SP, Ngu MC, *Acute hepatitis induced by Shouwu Pian, a herbal product derived from Polygonum multiflorum*, Journal of Gastroenterology and Hepatology 2001; 16(1); 115-117.

30. Nair A, Reddy D, and Van Thiel DH, *Cascara sagrada induced intrahepatic cholestasis causing portal hypertension: case report and review of herbal hepatotoxicity*, American Journal of Gastroenterology 2000; 95(12): 3634-3637.

31. D'Arcy PF, *Adverse reactions and interactions with herbal medicines*, Part 1, Adverse Drug

Reactions and Toxicology Reviews 1991; 10(4): 189-208.

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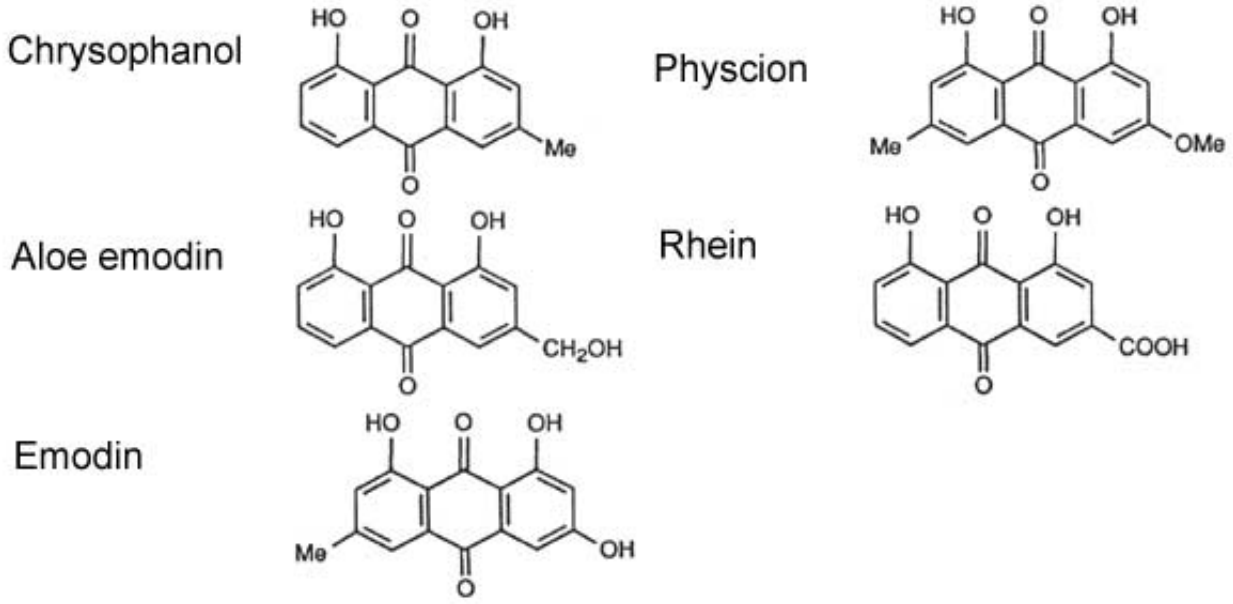

Figure 1: Anthraquinones.

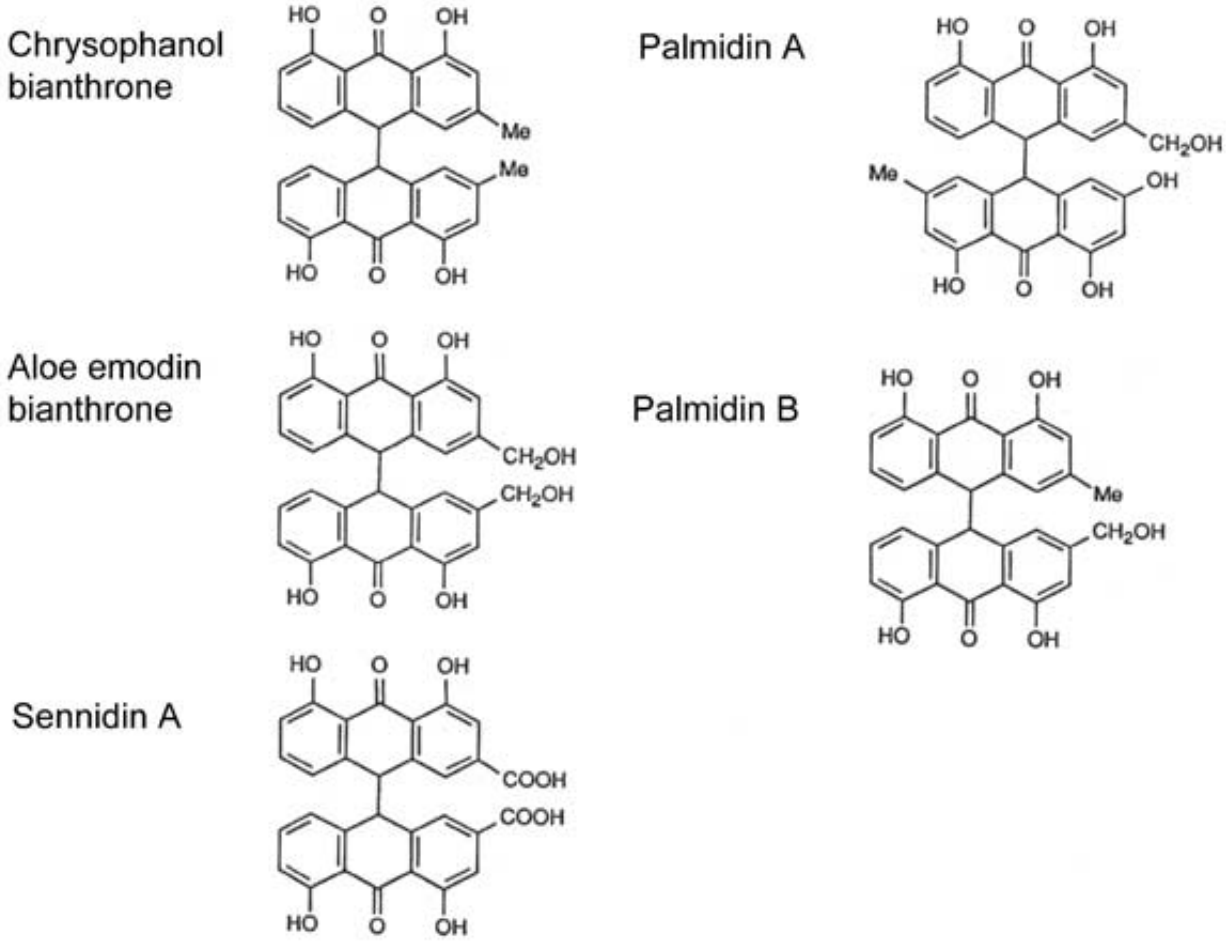

Figure 2: Dianthrones.

ORIGINAL RESEARCH

Chamomile (*Matricaria recutita*) May Provide Antidepressant Activity in Anxious, Depressed Humans: An Exploratory Study

Jay D. Amsterdam, MD; Justine Shults, PhD; Irene Soeller, MSN, CRNP; Jun James Mao, MD, MSCE; Kenneth Rockwell, MS, PharmD; Andrew B. Newberg, MD

ABSTRACT

Context • Anxiety and depression are the most commonly reported psychiatric conditions and frequently occur as comorbid disorders. While the advent of conventional drug therapies has simplified treatment, a large segment of the population goes untreated or declines conventional therapy for financial, cultural, or personal reasons. Therefore, the identification of inexpensive and effective alternative therapies for anxiety and depression is of relevance to public health.

Objective • The current study explores data from a 2009 clinical chamomile trial in humans to determine if chamomile provides clinically meaningful antidepressant activity versus a placebo.

Design: • In the 2009 randomized, double-blind, placebo-controlled study, the research team examined the antianxiety and antidepressant action of oral chamomile (*Matricaria recutita*) extract in participants with symptoms of comorbid anxiety and depression.

Setting • In the 2009 study, all of participants' evaluations took place at the Depression Research Unit at the University of Pennsylvania. The study drew participants from patients at the Department of Family Medicine and Community Health's primary care clinic at the University of Pennsylvania, Philadelphia.

Participants • Of the 57 participants in the 2009 trial, 19 had anxiety with comorbid depression; 16 had anxiety with a past history of depression; and 22 had anxiety with no current or past depression.

Intervention • The intervention and placebo groups in the 2009 trial received identically appearing 220-mg capsules containing either pharmaceutical-grade chamomile extract standardized to a content of 1.2% apigenin or a placebo (ie, lactose monohydrate NF), respectively.

Outcome Measures • In the current study, the research team used generalized estimating equations analysis to identify clinically meaningful changes over time in scores from the Hamilton Depression Rating (HAM-D) questionnaire among treatment groups.

Results • In the current study, the research team observed a significantly greater reduction over time in total HAM-D scores for chamomile vs placebo in all participants ($P < .05$). The team also observed a clinically meaningful but nonsignificant trend for a greater reduction in total HAM-D scores for chamomile vs placebo in participants with current comorbid depression ($P = .062$). When the team examined the HAM-D core mood item scores, it observed a significantly greater reduction over time for chamomile vs placebo in all participants ($P < .05$) and a clinically meaningful but nonsignificant trend for a greater reduction over time for chamomile vs placebo in participants without current or past depression ($P = .06$).

Conclusion • Chamomile may provide clinically meaningful antidepressant activity that occurs in addition to its previously observed anxiolytic activity. (*Altern Ther Health Med.* 2012;18(5):44-49.)

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Anxiety and depression are the most commonly reported psychiatric conditions[1-3] and frequently occur as comorbid disorders.[4-7] Both conditions can be chronic or recurrent[5,8] and can frequently require long-term therapy.[9] While the advent of conventional drug therapies has simplified treatment, a large segment of the population goes untreated or declines conventional therapy for financial, cultural, or personal reasons.[10] Many of these individuals seek complementary and alternative medicine (CAM) remedies for their symptoms.[11] Therefore, the identification of inexpensive and effective alternative therapies for anxiety and depression is of relevance to public health.[12,13] Researchers need to perform rigorous testing of candidate CAM therapies to expand available therapeutic options for anxiety and depression.

The use of chamomile as an herbal remedy dates back to ancient Greece and Rome. Practitioners have used chamomile (*Matricaria recutita*) as a traditional herbal remedy for its calming effect. While many varieties of chamomile exist, Roman (*Anthemis nobilis*) and German (*M recutita*) are the most widely used. These types are members of the Compositae (Asteracae) family. Practitioners consider *M recutita* to be the more potent variety and use it widely for medicinal purposes. Researchers have documented use of *M recutita* for relief of depressive and anxiety symptoms in a number of regions in southern Italy,[14] Sardinia,[15] Morocco,[16] and Brazil.[17] *M recutita* is grown as a cash crop in Argentina, Egypt, Hungary, Slovakia, and Germany.[18] In addition, practitioners have used other varieties of chamomile to treat the symptoms of depression and anxiety, including *Anthemis arvensis* and *Tanacetum parthenium* in Tuscany[19] and *Chamaemelum fuscatum* in Spain.[20] In spite of these uses, only one randomized controlled study has explored the effects of chamomile on mood in the past. This randomized, double-blind, placebo-controlled trial of oral chamomile extract for generalized anxiety disorder (GAD) found a significantly greater reduction in mean ratings for anxiety symptoms for chamomile vs placebo ($P = .047$) and a nonsignificant (albeit clinically meaningful) reduction in depression ratings[21] with chamomile vs placebo ($P = .136$).[22]

Based upon prior observations from in vivo and in vitro animal studies suggesting that chamomile may possess antidepressant activity,[23-27] the research team conducted the current secondary, exploratory analysis of its prior, clinical chamomile trial in humans[22] to examine whether chamomile demonstrated antidepressant activity in conjunction with its antianxiety effects. The team hypothesized that chamomile would show clinically meaningful, antidepressant activity (vs placebo) as measured by change over time in ratings for depression symptoms.

METHODS

Participants

The Department of Family Medicine and Community Health's primary care clinic at the University of Pennsylvania, Philadelphia referred patients to the study. These individuals were ≥18 years old and had a primary *DSM IV* Axis I diagnosis of GAD that the research team confirmed using the *Structured Diagnostic Interview for DSM IV* (SCID).[28] Participants had mild-to-moderate symptoms of depression and a minimum baseline score ≥9 on the Hamilton Anxiety Rating (HAM-A)[29] questionnaire.

The research team did not exclude individuals from the trial if they had a comorbid DSM IV Axis I dysthymic disorder nor if they had a depressive disorder not otherwise specified (NOS) where the comorbid condition did not constitute the primary disorder. The team did exclude individuals from the trial if they had a current diagnosis of major depressive disorder, bipolar disorder, panic disorder, phobic disorder, obsessive-compulsive disorder, posttraumatic stress disorder, acute stress disorder, substance-induced anxiety disorder, psychosis, dementia, or substance abuse or dependence within the preceding 3 months. Other exclusion criteria were (1) an unstable medical condition, (2) hepatic or renal insufficiency, (3) malignancy, or (4) known sensitivity to chamomile, plants of the asteraceae family, mugwort, or birch pollen. The study did not permit concurrent use of anxiolytics, antidepressants, mood stabilizers, sedatives, or herbal remedies (including chamomile preparations). Women with childbearing potential had to employ a medically proven form of contraception and had to have a negative pregnancy test before starting therapy.

To assign participants to groups, the research team performed blocked randomization with varying block sizes. First, the team randomly selected a block size from among a small set of block sizes and randomly permuted the group numbers within that block. The team continued the procedure until it had randomized all participants into the groups. The team permuted the random numbers within each block using the random number generator and user code in Stata 10.0 software (StataCorp LP, College Station, Texas).

Evaluation Procedures

Participants provided informed consent in accordance with the ethical standards of the Institutional Review Board of the University of Pennsylvania. The research team conducted the study using the *Principles of Good Clinical Practice Guidelines* with oversight by the local office of human research (OHR) and by an independent data and safety monitoring board. At the screen and baseline visits for the study, the research team obtained a participant's psychiatric

history using the SCID format.[28] The team performed a medical history, physical examination, and laboratory evaluation that included a complete blood count; a test of electrolyte levels; hepatic, renal, and thyroid panels; a pregnancy test (in women with childbearing potential); urinalysis; and a urine drug screen. At weeks 2, 4, 6, and 8 during the study, a research doctor or nurse obtained structured outcome ratings using the Hamilton Depression Rating Scale (HAM-D) questionnaire[29] and a treatment-emergent, side-effects profile (TESS).[30] The data on side effects included the date of onset and cessation of any adverse event, its severity, its relationship to treatment or the study's procedure, and the outcome.[30] The research team obtained sitting and standing blood pressure, pulse, and weight at each of the study's visits. All of participants' evaluations took place at the Depression Research Unit at the University of Pennsylvania.

Materials

The research team dispensed chamomile product and lactose monohydrate (placebo) under an Investigational New Drug (IND) exemption. The team prepared identically appearing 220-mg capsules containing either pharmaceutical-grade chamomile extract standardized to a content of 1.2% apigenin (Spectrum Pharmacy Products, New Brunswick, New Jersey) or placebo (ie, lactose monohydrate NF, Spectrum Pharmacy Products, New Brunswick, New Jersey).

Treatment Procedures

The research team initiated chamomile or placebo therapy at one capsule daily for the first week and increased it to two capsules daily during the second week of therapy. For participants with a ≤50% reduction in total HAM-A scores vs the baseline, the team increased the dosage to three capsules daily during week 3 of therapy and then to four capsules daily during week 4. For participants who continued to have a ≤50% reduction in baseline HAM-A scores at week 4, the team increased the dosage to five capsules daily during weeks 5 through 8 of the study. Dose reductions could occur at any time based upon drug tolerability.

Outcome Measurement

The research team obtained additional outcome measurements at baseline and after 2, 4, 6, and 8 weeks of treatment, including structured Hamilton Depression Rating (HAM-D)[21] scores for the test's total of 17 items, the HAM-D core mood items score (ie, depressed mood, guilt, suicidal ideation), and individual HAM-D symptom item scores. An experienced research doctor or research nurse from the Depression Research Unit assigned outcome ratings. The team analyzed results under blinded conditions.

Statistical Procedures

The research team conducted analyses using the xtgee procedure for Stata 10.0.[31] The team implemented generalized estimating equations (GEE) with 2-sided tests of hypotheses and a P-value $< .05$ as the criteria for statistical significance. Exploratory analysis examined the subgroups of those subjects in the chamomile and placebo treatment arms to see whether the impact of chamomile therapy was dependent upon group status (ie, current comorbid depression, past history of depression, or no current or past depression).

Given the available sample size, the team fit GEE models for all participants individually and for individual participants in the subgroups to identify trends that may inform future hypotheses. The GEE models included the total HAM-D score, HAM-D core depression items scores, and individual HAM-D item scores as the main outcome variables. The GEE models also included the covariates of time, baseline value for each HAM-D outcome measure, an indicator variable for chamomile, and a chamomile x-time interaction term. If the chamomile x-time interaction was significant, this finding indicated that the change over time with chamomile differed from placebo. The GEE models allowed for a variable number of measurements per participant so that information on all participants was available for the analysis. Finally, given the exploratory nature of this study, the team did not control for multiple comparisons.

The research team used the lincom procedure in Stata 10.0 to estimate (with 95% CI) the difference in overall changes between groups. In addition, the team calculated effect sizes as the absolute value of the estimated difference between groups divided by the standard deviation (SD) of the outcome variable under consideration.

RESULTS

Enrollment

Sixty-one participants enrolled in the trial: 73.7% Caucasian, 12.3% African American, and 14.0% other. The mean (SD) age of the chamomile participants was 45.5 (14.53) years and the mean (SD) age of the placebo participants was 45.9 (10.88) years ($P = .98$). Table 1 gives the full descriptions of participants. Fifty-seven participants had a baseline visit plus at least one post-baseline measurement: chamomile (n = 28) and placebo (n = 29).

The research team performed exploratory analyses on the entire group of participants and on subgroups that included participants with current comorbid depression (n = 19), with a history of depression but no current depression (n = 16), and with no past or current depression (n = 22). Table 1 displays clinical and demographic variables for each subgroup. Participants with current comorbid depression had a secondary diagnosis of depressive disorder NOS (n = 15) or dysthymic disorder (n = 4). Participants with a past history of depression had a prior diagnosis of major depressive disorder (n = 2), dysthymic disorder (n = 2), depressive disorder NOS (n = 11), or postpartum depression (n = 1). Of the 57 randomized participants whom the team evaluated, eight (14.03%) discontinued treatment prematurely: two for adverse events (one for allergic reaction from placebo and one for abdominal discomfort from chamomile), three for withdrawn consent, two lost to follow-up,

Table 1. Clinical and Demographic Characteristics of Participants' Subgroups[b]

	Comorbid Depression(n = 19)	Past History of Depression (n = 16)	No Depression (n = 22)
Chamomile/placebo	7/12	8/8	13/9
Gender, men/women	9/10	8/8	6/16
Age at consent (y)[a]	43.7 (16.5)	48.6 (12.5)	42.2 (9.8)
Age at consent, range (y)	29-78	22-70	25-62
Age GAD onset (y)[a]	23.0 (14.9)	24.9 (8.3)	30.8 (11.9)
Age GAD onset, range (y)	12-75	14-47	18-58
Illness length (y)[a]	19.9 (14.7)	23.5 (14.3)	11.8 (11.3)
Illness length, range (y)	0.5-51	3-54	0.3-33
Episode length (mo)[a]	56.2 (67.9)	41.3 (64.8)	47.2 (53.7)
Episode length, range (mo)	2-240	6-256	3-240
Prior episodes (no.)[a]	4.7 (7.0)	7.2 (11.3)	1.4 (2.0)
Prior episodes, range	0-10	0-43	0-6
Baseline HAM-A[a]	16.1 (4.1)	13.7 (3.1)	14.5 (3.3)
Baseline HAM-A, range	11-26	10-21	9-22
Baseline HAM-D[a]	12.2 (3.5)	10.13 (3.3)	9.8 (3.5)
Baseline HAM-D, range	5-19	5-18	3-15

[a]Mean ± standard deviation
[b]All scores come from data obtained in a 2009 study.[22]
Abbreviations: GAD, generalized anxiety disorder; HAM-A, Hamilton Rating Scale for Anxiety; HAM-D, Hamilton Rating Scale for Depression.

and one for noncompliance. Please note that one subject withdrew consent after taking a single dose of the study's drug. To be conservative, however, in the interpretation of its observations, the team retained all efficacy data for this participant in its analyses. The average number of adverse events per participant was greater with placebo (0.77) vs chamomile (0.39) ($P = .26$). Amsterdam et all have provided a detailed description of the safety profile of chamomile vs placebo previously.[22]

Antidepressant Activity

Table 2 displays quasi-least squares (QLS) analyses of individual HAM-D symptom scores, HAM-D core mood scores, and total HAM-D scores. After controlling for baseline values, the research team observed a significantly greater reduction over time in total HAM-D scores for chamomile vs placebo in all participants ($P < .05$). The team also observed a clinically meaningful but nonsignificant trend for a greater reduction in total HAM-D scores for chamomile vs placebo in participants with current comorbid depression ($P = .062$). When the team examined the HAM-D core mood item scores, it observed a significantly greater reduction over time for chamomile vs placebo in all participants ($P < .05$) and a clinically meaningful but nonsignificant trend for a greater reduction over time for chamomile vs placebo in participants without current or past depression ($P = .06$).

DISCUSSION

The observation of a significantly greater reduction in total HAM-D scores with chamomile (vs placebo) in all participants ($P < .05$) and a clinically meaningful trend for a greater reduction in total HAM-D scores for chamomile (vs placebo) in participants with current comorbid anxiety and depression ($P = .062$), suggests that chamomile may exert an antidepressant effect in conjunction with its previously reported antianxiety effects in the same population. While the research team did not power this secondary, exploratory study specifically to detect statistically significant differences between treatment conditions for HAM-D outcome measures or between participants' subgroups, it did expect to find clinically meaningful changes over time in HAM-D outcome measures that would favor chamomile vs placebo.

Chamomile's mode of antidepressant action is unknown, although it may be independent of its anxiolytic activity.[22] Several lines of evidence suggest that one or more of chamomile's flavanoid constituents may exert an antidepressant effect via modulation of central noradrenalin (NA), dopamine (DA), serotonin (5-HT), and γ-amino butyric acid neurotransmission.[23-27] In addition, chamomile also appears to modulate hypothalamic-pituitary-adrenocortical (HPA) axis activity.[32,33] For example, Lorenzo et al[34] found that apigenin increased NA activity in an isolated rat atria model and inhibited monoamine-oxidase activity in rat atria homoge-

Table 2. Differences in Change in Hamilton Depression Rating Symptoms With 95% Confidence Interval and Effect Size for Chamomile vs Placebo

HAM-D Item	All Participants (n=57)	Comorbid Depression (n=19)	Pas Depression (n=16)	No Depression (n=22)
Depressed mood (#1)	−0.13 (−0.44, 0.18)	−0.09 (−0.75, 0.58)	−0.10 (−0.56, 0.35)	−0.11 (−0.62, 0.39)
	ES=0.18	ES=0.11	ES=0.19	ES=0.17
Guilt (#2)	−0.55 (-0.85, −0.25)[a]	−0.29 (−0.88, 0.29)	−0.78 (−1.36, −0.18)[a]	−0.62 (−1.03, −0.21)[a]
	ES=0.72	ES=0.36	ES=1.08	ES=1.07
Suicide ideation (#3)	−0.12 (−0.27, 0.03)	−0.25 (−0.66, 0.16)	−0.10 (−0.30, 0.11)	−0.04 (−0.14, 0.06)
	ES=0.26	ES=0.35	ES=0.33	ES=0.33
Insomnia, early (#4)	0.03 (−0.26, 0.32)	0.21 (−0.34, 0.75)	−0.70 (−1.29, −0.12)[a]	0.49 (0.10, 0.88)[a]
	ES=0.04	ES=0.25	ES=0.86	ES=0.66
Insomnia, middle (#5)	−0.09 (−0.40, 0.21)	−0.29 (−0.86, 0.28)	0.27 (−0.34, 0.89)	−0.33 (−0.77, 0.12)
	ES=0.12	ES=0.41	ES=0.34	ES=0.42
Insomnia, late (#6)	−0.53 (−0.86, −0.20)[a]	−0.90 (−1.4, −0.41)[a]	0.10 (−0.61, 0.80)	−0.83 (−1.32, −0.33)[a]
	ES=0.69	ES=1.16	ES=0.12	ES=1.10
Work/activities (#7)	−0.03 (−0.40, 0.30)	−0.22 (−0.90, 0.47)	−0.34 (−1.0, 0.36)	0.32 (−0.21, 0.84)
	ES=0.04	ES=0.24	ES=0.46	ES=0.42
Retardation (#8)	0.02 (−0.14, 0.17)	0.07 (−0.28, 0.43)	−0.38 (−0.61, −0.15)[a]	0.16 (−0.04, 0.37)
	ES=0.04	ES=0.15	ES=1.02	ES=0.60
Agitation (#9)	0.06 (−0.22, 0.34)	−0.08 (−0.47, 0.31)	0.12 (−0.40, 0.64)	−0.19 (−0.64, 0.27)
	ES=0.09	ES=0.12	ES=0.17	ES=0.31
Anxiety, psychic (#10)	−0.30 (−0.62, 0.01)	−0.23 (−0.86, 0.40)	−0.25 (−0.74, 0.25)	−0.32 (−0.85, 0.21)
	ES=0.42	ES=0.31	ES=0.40	ES=0.43
Anxiety, somatic (#11)	−0.07 (−0.36, 0.23)	−0.39 (−0.95, 0.17)	0.37 (−0.19, 0.93)	−0.05 (−0.49, 0.38)
	ES=0.10	ES=0.54	ES=0.50	ES=0.08
Gastrointestinal (#12)	−0.01 (−0.20, 0.19)	0.03 (−0.44, 0.50)	0.26 (0.02, 0.51)[a]	−0.27(−0.52, −0.01)[a]
	ES=0.01	ES=0.05	ES=0.71	ES=0.70
Somatic, general (#13)	−0.32 (−0.59, −0.05)[a]	−0.34 (−0.79, 0.11)	−0.55 (−1.02, −0.09)[a]	−0.10 (−0.58, 0.37)
	ES=0.52	ES = 0.53	ES=0.94	ES=0.16
Somatic, libido (#14)	−0.21 (−0.42, −0.002)[a]	−0.53 (−0.94, −0.12)[a]	−0.43 (−0.67, −0.20)[a]	0.15 (−0.21, 0.51)
	ES=0.33	ES = 0.72	ES=0.96	ES=0.22
HAM-D core (#1, #2, #3)	−0.71 (−1.33, −0.10)[a]	−0.25 (−1.54, 1.04)	−0.98 (−2.02, 0.06)	−0.78 (−1.60, 0.03)[c]
	ES=0.47	ES = 0.14	ES=0.78	ES=0.71
HAM-D total	−2.11(−4.17, −0.06)[a]	−3.74 (−7.7, 0.19)[b]	−2.03 (−5.62, 1.56)	−1.47 (−4.68, 1.73)
	ES=0.42	ES = 0.65	ES=0.47	ES=0.32

[a]($P < .05$); [b]($P = .062$); [c]($P = .06$)
Abbreviations: Ham-D, Hamilton Rating Scale for Depression; ES, effect size.

nates. Morita et al[23] found that apigenin stimulated the uptake of L-[^{14}C]-tyrosine (a DA precursor) into cultured adrenal chromaffin cells, and flavone produced an increase in [^{14}C]-catecholamine production without altering [^{14}C]-tyrosine turnover. Nakazawa et al (2003)[24] found an antidepressant-like activity of apigenin on NA and DA turnover in the amygdala and hypothalamus in mice exposed to the forced swim test (FST), while Anjaneyulu et al[25] found that quercetin reduced the immobility of mice during the FST in a dose-dependent fashion comparable to fluoxetine and imipramine. Yi et al[27] found that apigenin reduced immobility during the FST in mice; reversed FST-induced reduction in sucrose intake in rats; lowered stress-induced alterations in 5-HT, DA, and their metabolites; and reversed FST-induced increases in HPA-axis activity.

Researchers should consider several caveats in the interpretation of the current findings. The research team did not power the study to detect statistically significant differences between treatment conditions for HAM-D outcome measures or between participants' subgroups. The small sample size necessarily limited the team's ability to identify small-to-moderate differences in HAM-D outcome measures between treatment conditions.

The post hoc division of participants into subgroups necessarily resulted in an unbalanced distribution of baseline clinical and demographic variables that could have increased the likelihood of a type 1 or type 2 error. Similarly, given the exploratory design of the study, the research team did not control for multiple comparisons. It is possible that the reduction in HAM-D outcome scores was not the result of an antidepressant action per se but may have resulted from chamomile's anxiolytic activity as previously described.[22] A future study could evaluate this possibility. It is possible that the team would have found a different antidepressant outcome if the primary diagnosis in these participants had been depression rather than anxiety or if the baseline HAM-D scores had been higher. It is also possible that the antidepressant outcome may have been different if the team had employed a greater chamomile dose or a longer treatment duration. It is also possible that another chamomile species or chamomile extract with a different standardization may have produced different results.

Finally, the research team notes that the current analyses were exploratory and only suggest the possibility of an antidepressant activity for chamomile. Researchers will need to conduct future prospective trials in participants with primary depression to confirm the putative antidepressant activity of chamomile.

CONCLUSION

The identification of safe and effective CAM therapies for depression would be of public-health relevance for many individuals unable or unwilling to use conventional antidepressant therapy. The observation of a significant reduction over time in total HAM-D scores ($P < .05$) and a reduction in HAM-D core mood symptom scores ($P < .05$) for chamomile vs placebo in all participants and of a clinically meaningful trend for a reduction in total HAM-D scores for chamomile vs placebo in anxious participants with current comorbid depression ($P = .062$), suggests that chamomile may produce a clinically meaningful antidepressant effect in humans. Researchers will need to conduct future controlled clinical trials in patients who have depression as their primary diagnosis to confirm these exploratory findings.

REFERENCES

1. Wittchen HU. Generalized anxiety disorder: prevalence, burden, and cost to society. *Depress Anxiety.* 2002;16(4):162-171.
2. Kessler RC, McGonagle KA, Zhao S, et al. Lifetime and 12-month prevalence of *DSM-III-R* psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry.* 1994;51(1):8-19.
3. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of *DSM-IV* disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry.* 2005;62(6):593-602.
4. Ballenger JC. Anxiety and depression: optimizing treatments. *Prim Care Companion J Clin Psychiatry.* 2000;2(3):71-79.
5. Brown TA, Campbell LA, Lehman CL, Grisham J, Mancill R. Current and lifetime comorbidity of the *DSM-IV* anxiety and mood disorders in a large clinical sample. *J Abnorm Psychol.* 2001;110(4):585-599.
6. Schoevers RA, Deeg DJ, van Tilburg W, Beekman AT. Depression and generalized anxiety disorder: co-occurrence and longitudinal patterns in elderly patients. *Am J Geriatr Psychiatry.* 2005;13(1):31-39.
7. Dunlop BW, Davis PG. Combination treatment with benzodiazepines and SSRIs for comorbid anxiety and depression: a review. *Prim Care Companion J Clin Psychiatry.* 2008;10(3):222-228.
8. Bruce SE, Yonkers KA, Otto MW, et al. Influence of psychiatric comorbidity on recovery and recurrence in generalized anxiety disorder, social phobia, and panic disorder: a 12-year prospective study. *Am J Psychiatry.* 2005;162(6):1179-1187.
9. Allgulander C, Bandelow B, Hollander E, et al. WCA recommendations for the long-term treatment of generalized anxiety disorder. *CNS Spectr.* 2003;8(8 Suppl 1):53-61.
10. Wang PS, Lane M, Olfson M, Pincus HA, Wells KB, Kessler RC. Twelve-month use of mental health services in the United States: results from the National Comorbidity Survey Replication. *Arch Gen Psychiatry.* 2005;62(6):629-640.
11. Barnes PM, Powell-Griner E, McFann K, Nahin RL. Complementary and alternative medicine use among adults: United States, 2002. *Adv Data.* 2004;(343):1-19.
12. Givens JL, Houston TK, Van Voorhees BW, Ford DE, Cooper LA. Ethnicity and preferences for depression treatment. *Gen Hosp Psychiatry.* 2007;29(3):182-191.
13. Givens JL, Katz IR, Bellamy S, Holmes WC. Stigma and the acceptability of depression treatments among african americans and whites. *J Gen Intern Med.* 2007;22(9):1292-1297.
14. Pieroni A, Quave C, Nebel S, Heinrich M. Ethnopharmacology of the ethnic Albanians (Arbëreshë) of northern Basilicata, Italy. *Fitoterapia.* 2002;73(3):217-241.
15. Bruni A, Ballero M, Poli F. Quantitative ethnopharmacological study of the Campidano Valley and Urzulei district, Sardinia, Italy. *J Ethnopharmacol.* 1997;57(2):97-124.
16. Merzouki A, Ed-derfoufi F, Molero Mesa J. Contribution to the knowledge of Rifian traditional medicine. II: Folk medicine in Ksar Lakbir district (NW Morocco). *Fitoterapia.* 2000;71(3):278-307.
17. Di Stasi LC, Oliveira GP, Carvalhaes MA, et al. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. *Fitoterapia.* 2002;73(1):69-91.
18. Böttcher H, Günther I, Franke R, Warnstorff K. Physiological postharvest responses of Matricaria (*Matricaria recutita* L.) flowers. *Postharvest Biol Technol.* 2001;22(13):39-51.
19. Uncini Manganelli RE, Tomei PE. Ethnopharmacobotanical studies of the Tuscan Archipeligo. *J Ethnopharmacol.* 1999;65(3):181-202.
20. Vazquez FM, Suarez MA, Pérez A. Medicinal plants used in the Barros Area, Badajoz Province, Spain. *J Ethnopharmacol.* 1997;55(2):81-85.
21. Williams JB. A structured interview guide for the Hamilton Depression Rating Scale. *Arch Gen Psychiatry.* 1988;45(8):742-747.
22. Amsterdam JD, Li Y, Soeller I, Rockwell K, Mao JJ, Shults J. A randomized, double-blind, placebo-controlled, trial of oral *Matricaria recutita* (Chamomile) extract therapy of generalized anxiety disorder. *J Clin Psychopharmacol.* 2009;29(4):378-382.
23. Morita K, Hamano S, Oka M, Teraoka K. Stimulatory actions of bioflavonoids on tyrosine uptake into cultured bovine adrenal chromaffin cells. *Biochem Biophys Res Comm.* 1990;171(3):1199-1204.
24. Nakazawa T, Yasuda T, Ueda J, Ohsawa K. Antidepressant-like effects of apigenin and 2,4,5-trimethoxycinnamic acid from *Perilla frutescens* in the forced swimming test. *Biol Pharm Bull.* 2003;26(4):474-480.
25. Anjaneyulu M, Chopra K, Kaur. Antidepressant activity of quercetin, a bioflavonoid, in streptozotocin-induced diabetic mice. *J Med Food.* 2003;6(4):391-395.
26. Pinto SA, Bohland E, Coelho Cde P, Morgulis MS, Bonamin LV. An animal model for the study of Chamomilla in stress and depression: pilot study. *J Homeopathy.* 2008;97(3):141-144.
27. Yi LT, Li JM, Li YC, Pan Y, Xu Q, Kong LD. Antidepressant-like behavioral and neurochemical effects of the citrus-associated chemical apigenin. *Life Sci.* 2008;82(13-14):741-751.
28. First MB, Spitzer RL, Gibbon M, Williams JB. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition With Psychotic Screen (SCID-I/P W/ PSY SCREEN). New York, NY: Biometrics Research, New York State Psychiatric Institute, 2002.
29. Hamilton M. The assessment of anxiety status by rating. *Br J Med Psychol.* 1959;32(1):50-55.
30. National Institute of Mental Health. Treatment Emergent Symptoms Scale (TESS). *Psychopharmacol Bull.* 1985;21:1069-1073.
31. Shults J, Ratcliffe SJ, Leonard M. Improved generalized estimating equation analysis via xtqls for quasi-least squares in Stata. *Stata J.* 2007;7(2):147-166.
32. Yamada K, Miura T, Mimaki Y, Sashida Y. Effect of inhalation of chamomile oil vapour on plasma ACTH level in ovariectomized rat under restriction stress. *Biol Pharmacol Bull.* 1996;19(9):1244-1246.
33. Reis LS, Pardo PE, Oba E, Kronka Sdo N, Frazatti-Gallina NM. *Matricaria chamomilla* CH12 decreases handling stress in Nelore calves. *J Vet Sci.* 2006;7(2):189-192.
34. Lorenzo PS, Rubio MC, Medina JH, Adler-Graschinsky E. Involvement of monoamine oxidase and noradrenaline uptake in the positive chronotropic effects of apigenin in rat atria. *Eur J Pharmacol.* 1996;312(2):203-207.

Attenuation of Laboratory-Induced Stress in Humans After Acute Administration of *Melissa officinalis* (Lemon Balm)

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Objective: *Melissa officinalis* (lemon balm) is contemporaneously used as a mild sedative and/or calming agent. Although recent research has demonstrated modulation of mood in keeping with these roles, no studies to date have directly investigated the effects of this herbal medication on laboratory-induced psychological stress. **Methods:** In this double-blind, placebo-controlled, randomized, balanced crossover experiment, 18 healthy volunteers received two separate single doses of a standardized *M. officinalis* extract (300 mg, 600 mg) and a placebo, on separate days separated by a 7-day washout period. Modulation of mood was assessed during predose and 1-hour postdose completions of a 20-minute version of the Defined Intensity Stressor Simulation (DISS) battery. Cognitive performance on the four concurrent tasks of the battery was also assessed. **Results:** The results showed that the 600-mg dose of Melissa ameliorated the negative mood effects of the DISS, with significantly increased self-ratings of calmness and reduced self-ratings of alertness. In addition, a significant increase in the speed of mathematical processing, with no reduction in accuracy, was observed after ingestion of the 300-mg dose. **Conclusion:** These results suggest that the potential for *M. officinalis* to mitigate the effects of stress deserves further investigation. **Key words:** acute effects, *Melissa officinalis*, lemon balm, stress, mood.

DISS = Defined Intensity Stressor Simulation.

INTRODUCTION

The perennial lemon scented herb *Melissa officinalis* (lemon balm) has been in use as a pancultural medicinal treatment for more than 2 millennia. Its traditional indications have included administration for its general beneficial effects on the brain, as a treatment for memory disorders (1), and for "all complaints supposed to proceed from a disordered state of the nervous system'' (2). Contemporary reports emphasize the sedative, spasmolytic, and antibacterial effects of *M. officinalis*, with indications encompassing nervous disorders, gastrointestinal disorders, and sleep disturbance (3–5).

Melissa is most commonly sold over the counter in combination with other herbs, most notably *Valeriana officinalis* (valerian) (6,7). This combination has been shown to improve the sleep quality of healthy normal sleepers (8), and to have an effect on sleep parameters in poor sleepers similar to that of 0.125 mg of triazolam (9). Several studies in rodents have also suggested a mildly sedative effect of *M. officinalis* alone, with observations of a reduction in spontaneous movement in mice after administration of both the volatile oil of *M. officinalis* as well as the isolated terpenes (10) and a reduction in behavioral parameters in mice after the administration of a hydroalcoholic extract of *M. officinalis* (11). A single, double-blind, placebo-controlled study also assessed the behavioral effects of *M. officinalis* aromatherapy in a group of patients suffering from severe dementia. In comparison to placebo a significant reduction in agitation and social withdrawal, and an increase in constructive activities resulted from the 4-week treatment with essential oil (12).

Although the mechanisms of action of Melissa are poorly understood, it has been suggested that the active components of extracts made from the leaves include monoterpenoid aldehydes, flavonoids, polyphenolic compounds including rosmarinic acid (13), and monoterpene glycosides (14). These components may well underlie a number of effects seen in vitro, which include potent antioxidant properties (15,16) and an affinity for binding to both nicotinic and muscarinic receptors in human brain cortex tissue (17). The latter mechanism is of specific interest as modulation of the cholinergic system may well be beneficial to cognitive function, most particularly in conditions, such as Alzheimer's disease, that feature cholinergic dysregulation.

Given the potential for extracts of *M. officinalis* to interact with the cholinergic system, two recent studies from our own laboratories have assessed both cholinergic receptor binding and the cognitive and mood effects of single doses of *M. officinalis* in healthy humans. In the first of these double-blind, placebo-controlled, balanced-crossover studies (18), three separate single doses of a concentrated commercial *M. officinalis* extract (300 mg, 600 mg, 900 mg; Pharmaton S.A., Lugano, Switzerland) plus a placebo were administered in a counterbalanced manner to 20 participants, with a 7-day washout period between testing days. The most notable result of this experiment was a striking dose-dependent impairment in accuracy across a number of timed, computerized memory tasks. Mood was also modulated, with participants' self-ratings of calmness, as assessed with Bond-Lader mood scales, increasing for the lowest dose (300 mg), whereas "alertness" was decreased for the highest, and cognitively most deleterious, dose (900 mg). Although this pattern of results is broadly in line with the contemporary role of *M. officinalis* as a mild sedative, it is not in keeping with beneficial modulation of cholinergic activity. Indeed, the subsequent in vitro analysis of the extract showed that it did not exhibit the expected cholinergic receptor binding properties, with negligible displacement of [^{3}H]-(*N*)-nicotine from nicotinic receptors, and comparatively low displacement of [^{3}H]-(*N*)-scopolamine from muscarinic receptors in human brain tissue. The second investigation (19), therefore, extended this line of research by initially screening a number of dried leaf samples for cholinergic binding, with a dried leaf with both substantial nicotinic and muscarinic binding properties being taken forward into

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the behavioral assessment. In this instance, decrements were seen on the same memory tasks as the previous experiment, but these reduced with increasing dose of dried leaf. The highest dose (1,600 mg) was overwhelmingly beneficial, with improved accuracy seen on immediate and delayed word recall tasks. Both the highest and middle (1,000 mg) doses also again led to significantly increased self-ratings of calmness. Although cognitive (and particularly memory) improvement was restricted to the *M. officinalis* with cholinergic receptor binding properties, both the methanolic extract (18) and the dried leaf (19) led to increased calmness. This suggests that this robust modulation reflects the working of another, as yet unidentified, mechanism.

In this preliminary, double-blind, placebo-controlled, balanced-crossover study, the calming properties of *M. officinalis* were examined further with an assessment of its ability to modulate performance and mood during mild, laboratory-induced, psychological stress at 1 hour after treatment (mood effects were detected at this time point in both of the previous investigations). The effects of single doses (300 mg and 600 mg) of the previously utilized (18) noncholinergic, methanolic *M. officinalis* extract on mood and cognitive performance were assessed while participants completed the Defined Intensity Stressor Simulation (DISS) computerized battery. The DISS has previously been shown to increase negative ratings of mood and engender physiological responses concomitant with increased stress (20,21).

MATERIALS AND METHODS

Participants

Ten males and eight females (mean age = 29.11 years, SD = 6.81) took part in the study, which was approved by the Ethics Committee of the Psychology Division of Northumbria University and was carried out in accordance with the Declaration of Helsinki. Participants comprised an unpaid opportunity sample of undergraduates from the University of Northumbria. Before taking part in the study, participants signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, were not taking any recreational or prescription drugs, with the exception of oral contraceptive pills, and were nonsmokers. Participants refrained from consuming any caffeine-containing products for a minimum of 2 hours, and alcohol for a minimum of 12 hours before each testing session. Participants were tested individually in laboratory conditions.

Before the experiment, a power analysis (22) suggested that, for the intended within-subjects analysis and alpha level of 0.05, the sample size of 18 would have an 80% chance of detecting an effect size (0.6) similar to that seen in the previous investigations of the mood effects of *M. officinalis* (18,19).

Treatments

An encapsulated, standardized, commercial extract of *M. officinalis* prepared by Pharmaton SA (Lugano, Switzerland) was utilized in the current study. The production method involves dried leaves of *M. officinalis* being reduced to fragments and extracted up to exhaustion in a 30:70 methanol–water mixture. The resultant liquid extract is evaporated and homogenized to yield a soft extract, to which inert processing agents (dried glucose syrup and colloidal anhydrous silicon dioxide to 7% and 3% of the final dried weight respectively) are added. This mixture is homogenized and taken to dryness, ground, mixed, and sieved.

On each study day, participants received four capsules of identical appearance, each containing either an inert placebo or 150 mg of *M. officinalis* extract. Depending on the condition to which they were allocated on that particular day the combination corresponded to a dose of either 0 mg (placebo), 300 mg, or 600 mg of *M. officinalis* extract. Blinding of the treatments for the study was undertaken by a disinterested third party. After completion of each testing session participants were asked whether they had formed any opinion as to the nature of the day's treatment.

Materials

The Defined Intensity Stressor Simulation (DISS) Computerized Battery

The DISS computerized battery (Stress-Sim Ltd, The Coach House, Plymouth, www.stress-sim.co.uk) comprises a set of four concurrent cognitive and psychomotor tasks presented via a split screen. This newly developed instrument was chosen for several reasons. It has the advantage over other laboratory stressors of being both automated (thus essentially eliminating experimenter effects) and drawing on random stimuli for each test, allowing for multiple testing sessions of the same participant. All responses are made with an external mouse. In this instance, a 20-minute version of the DISS was employed. The modules selected were the mathematical processing, visual monitoring, auditory monitoring, and memory search tasks. Participants were instructed via on screen standard instructions to attend simultaneously to all four tasks, while monitoring the central counter displaying their accumulated aggregate score. Accuracy and speed of response dictate the score, with failure to respond resulting in negative scoring. Previous research has shown that simultaneous performance of these four tasks engenders increases in subjective stress and frustration and stress-related physiological responses, including an increase in salivary IgA (20,21).

In the current study, all four tasks were set at a medium difficulty/intensity level and were performed for 20 minutes. The on-screen layout of the battery is shown in Figure 1. The four tasks are described below.

Mathematical Processing Task

A series of calculations are presented. The participant adds two numbers, entering the three-figure answer via an onscreen number pad. On completion of each calculation, the participant clicks on the "done" button, which cues the next calculation. Ten points are awarded for each correct answer and 10 points are deducted from the running total for each incorrect answer.

Auditory Monitoring Task

One of two tones of different pitches is sounded approximately every 5 seconds throughout the session in a random order. Participants are instructed to click on the box labeled "incoming mail" every time they hear the higher pitched of the two tones. Ten points are awarded for correctly identifying the higher pitched tone and 10 points are deducted for an incorrect response or for no response.

Visual Monitoring Task

A small dot drifts outwards from the center of a target comprising five concentric circles. The participant is instructed to allow the dot to travel as far out of the center as possible, without letting it hit the edge of the target, before clicking on the "reset" button. Two points are added to the running total for every circle that the dot passes through (with a maximum of 10 points), with a penalty of 10 points for every half second that passes between the dot hitting the outer edge and the participant clicking on the "reset" button.

Memory Search Task

Four letters appear for the participants to remember. After 4 seconds, the letters disappear but can be viewed again by clicking on "retrieve list" button. Approximately every 10 seconds, a single target letter appears. Participants are instructed to indicate whether the target letter had appeared in the original list of four letters by clicking on the "yes" or "no" buttons. Ten points are awarded for a correct answer, 10 points deducted for an incorrect answer or no response, and 5 points are deducted every time the list was retrieved.

Bond-Lader Visual Analogue Mood Scales (23)

Mood was assessed before and after each completion of the DISS battery using the visual analogue scales of Bond and Lader (23). These scales were

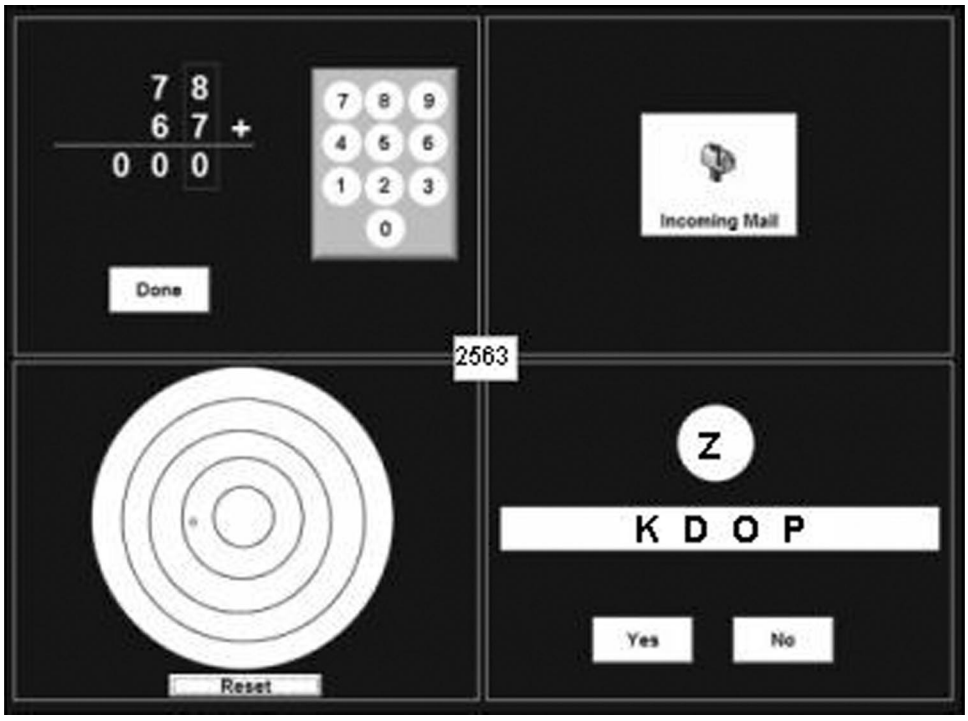

Figure 1. The on-screen layout of the Defined Intensity Stressor Simulation battery. The concurrent tasks comprise (clockwise from top left) "mathematical processing," "auditory monitoring," "memory search," and "visual monitoring." The participant's aggregate score for the four tasks is shown at the center of the display. All responses are made via a standard computer mouse.

originally designed for assessing the mood effects of anxiolytics (23) and have been subsequently utilized in numerous pharmacological, psychopharmacological, and medical trials. As with other mood visual analogue scales, high reliability and validity have been demonstrated (24).

The Bond and Lader scales comprise a total of 16 100-mm lines anchored at either end by antonyms. Participants mark their current subjective state between the antonyms on the line. Each line is scored as millimeters to the mark from the negative antonym. From the resultant scores, three measures derived by factor analysis can be isolated (23). These have been described by Bond and Lader as representing the following: "alertness" (represented by lines anchored by alert–drowsy, attentive–dreamy, lethargic–energetic, muzzy–clearheaded, well-coordinated–clumsy, mentally slow–quick witted, strong–feeble, interested–bored, incompetent–proficient); "calmness" (calm–excited, tense–relaxed); and "contentedness" (contented–discontented, troubled–tranquil, happy–sad, antagonistic–friendly, withdrawn–sociable). Scores for each factor represent the unweighted average number of millimeters (maximum 100 mm) from the negative antonym for the individual scales contributing to the factor.

In the current study, raw scores (pre- and post-DISS, at both pre- and posttreatment) were analyzed in the initial three-way analysis of variance (ANOVA) (see below) to assess the main effect of the stressor battery and interactions with treatment. For the primary statistical analysis (planned comparisons), for each completion of the DISS, mood scores before completion were subtracted from mood scores after completion. This provided a single score representing the change in each mood factor engendered by the stressor battery. This score for the predose completion of the battery was then subtracted from the same score 1 hour postdose to generate a single "change from baseline" score representing the differential effects of treatment on the two battery completions.

Procedure

Before the first study day, a third party, using random number tables, allocated participants to a treatment regimen dictated by a Latin square, which counterbalanced the order of treatments across the 3 days of the study. Each of the 3 days was separated by a 7-day "washout" period, with testing taking place in dedicated laboratory facilities at the same time on each day.

Immediately before and after each completion of the DISS battery, participants filled out Bond-Lader mood scales.

Each day of the study comprised an initial predose completion of the 20-minute DISS battery (plus mood scales before and after), followed by ingestion of the day's treatment. One hour postdose, participants completed the DISS battery (plus mood scales before and after).

The running order of each testing session (plus mean scores at each mood scale completion) is represented in Figure 2.

Statistics

Initial Analysis

Before the primary analysis of mood and performance data (planned comparisons; see below) an initial three-way repeated measures ANOVA (pre/post DISS mood scores × pretreatment/posttreatment × condition) was carried out on the raw Bond-Lader mood scores (alertness, contentedness, calmness) to establish the following: the main effects of the DISS battery on mood; any main effects of treatment group or interaction between treatment and pre/posttreatment; and any interaction between treatment group and the change in mood scores (pre/post DISS) pre and posttreatment.

Only planned comparisons of those mood measures that reached significance on the initial ANOVA are reported below. Significant results from the initial analyses are reported with the relevant measure below.

Mood Effects of DISS

In the case of mood measures generating a significant main effect on the initial three-way ANOVA a further confirmatory analysis assessed the mood effects of the DISS in the absence of treatment. This was accomplished by submitting pre- and post-DISS data from all three baseline completions (ie, data from the mood assessments before and after the DISS before all three condition treatments) to a two-way repeated-measures ANOVA (Condition X mood scores before and after predose DISS) with planned comparisons

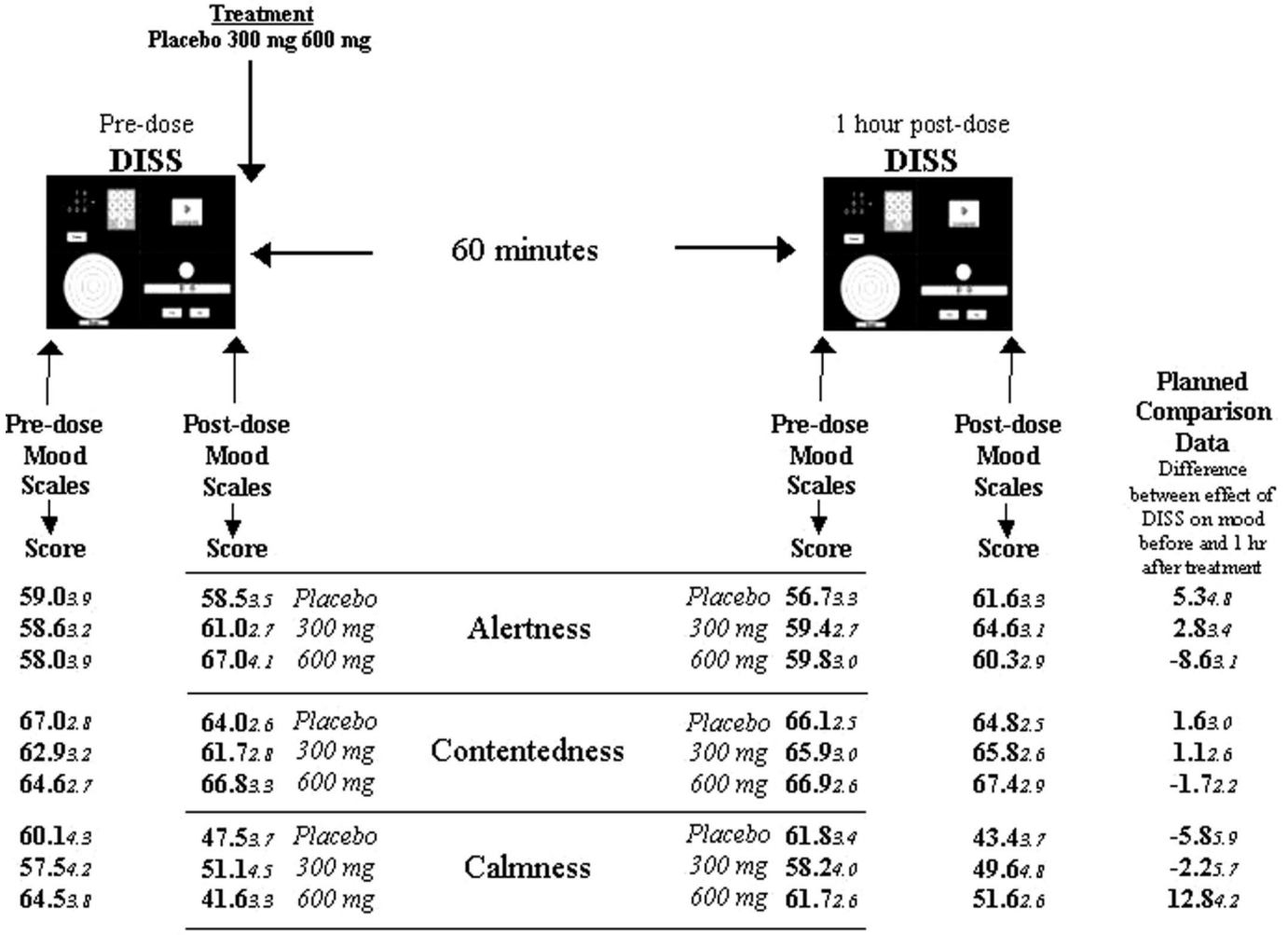

Figure 2. Schematic representation of the running order of the experiment, with mean scores (with standard errors) for each of the mood assessments, and "change from baseline" data (change in mood score during predose Defined Intensity Stressor Simulation (DISS) *minus* change in mood scores during 1 hour posttreatment DISS).

(utilizing MSError) of pre- vs. post data for "alertness," "contentedness," and "calmness" as described below.

Primary Analysis of Treatment Effects on Mood and DISS Performance

Scores for treatment-related changes in the participants' "alertness," "contentedness," and "calmness" mood factor scores during pre- and posttreatment DISS completion were analyzed as "change from baseline." To accomplish this, the change in mood score during the DISS before treatment was subtracted from the change in mood score during the DISS 1 hour after treatment.

An initial, one-way, repeated-measures ANOVA was then carried out to establish MSError for each measure. The primary statistical analysis of the "change from baseline" data followed the recommendations of Keppel (25) and was undertaken using a priori planned comparisons, utilizing *t* tests with the MSError from the one-way ANOVA as an error term, with the resulting *t* score evaluated on the degrees of freedom for MSError. For each measure, data from the placebo condition were compared with that for each of the two doses of *M. officinalis* (300 mg, and 600 mg).

To ensure the overall Type I error protection level, only those planned comparisons associated with measures that generated a significant main effect or interaction effect, or a trend toward the same, on the initial ANOVA are reported. Furthermore, all testing was two-tailed, comparisons were strictly planned before the study and were restricted to the number of conditions − 1 (25).

RESULTS

No adverse side effects were reported for any of the treatments, and all the participants completed the study.

Mood Effects of DISS

The initial three-way ANOVAs showed that there was a significant main effect ($F(1,17) = 4.92$, $p = .04$) of DISS completion on "alert" scores from the Bond-Lader visual analogue scales with scores rising from 58.6 (average millimeters) pre- DISS to 62.2 mm post-DISS. Similarly "calm" was significantly reduced ($F(1,17) = 39.27$, $p < .001$) with average ratings reducing from 60.6 mm to 47.5 mm.

Confirmatory planned comparisons of the pretreatment baseline data (for all three conditions; see Statistics) showed that completion of the DISS in the absence of treatment led to a significant reduction in subjective ratings of "calmness" (60.7 mm reducing to 46.7 mm) on the Bond-Lader visual analogue scales ($t(34) = 3.86$, $p < .001$). However, battery completion in the absence of treatment narrowly failed to have a significant effect on ratings of "alertness" ($t(34) = 1.97$, $p =$

.06) with scores rising from 58.5 mm to 62.2 mm. There was no effect detected by either analysis on contentedness.

Effects of Treatment on Mood Change During the DISS

Calmness

The initial three-way ANOVA showed a significant ($F(2,34) = 3.42$, $p = .04$) three-way interaction for calmness (pre/post DISS mood scores × pretreatment/posttreatment × condition, ie, the difference between how participants' mood was affected by completing the pre- and posttreatment DISSs was modulated by the treatment). Planned comparisons of the Bond-Lader factor "change from baseline" scores revealed that, in comparison to placebo, the 600-mg dose of *M. officinalis* led to significantly increased self-ratings of calmness after completion of the DISS battery ($t(34) = 2.47$, $p = .02$).

Alertness

The initial three-way ANOVA revealed a similar three-way interaction for the alertness factor ($F(2,34) = 4.97$, $p = .01$). Planned comparisons showed that the same 600-mg dose also led to a significant reduction in alertness ($t(34) = 2.96$, $p = .006$) during the posttreatment DISS.

The lower (300-mg) dose did not lead to any significant modulation of mood. Contentedness was also not significantly affected. Change from baseline scores for the mood factors are shown in Figure 3.

Effects of Treatment on Cognitive Measures During the DISS

Planned comparisons revealed that, in comparison to placebo, the 300-mg dose of *M. officinalis* led to a marginally significant increase in the number of calculations completed on the mathematical processing task ($t(34) = 2.03$, $p = .05$). The same dose also led to a significantly greater number of correctly answered calculations ($t(34) = 2.07$, $p = .04$). No significant effects of treatment were revealed either for the higher dose of *M. officinalis* or on the performance of the memory search task or the visual/auditory monitoring tasks. Treatment effects on performance outcomes are presented in Figure 4.

With regard to identification of the days' treatments, on questioning, too few participants had formed an opinion as to the nature of the respective days treatments to allow any meaningful statistical analysis. Reference to the data from those who believed that they could identify the respective treatments suggested that their ability to detect the treatment was at the level of chance.

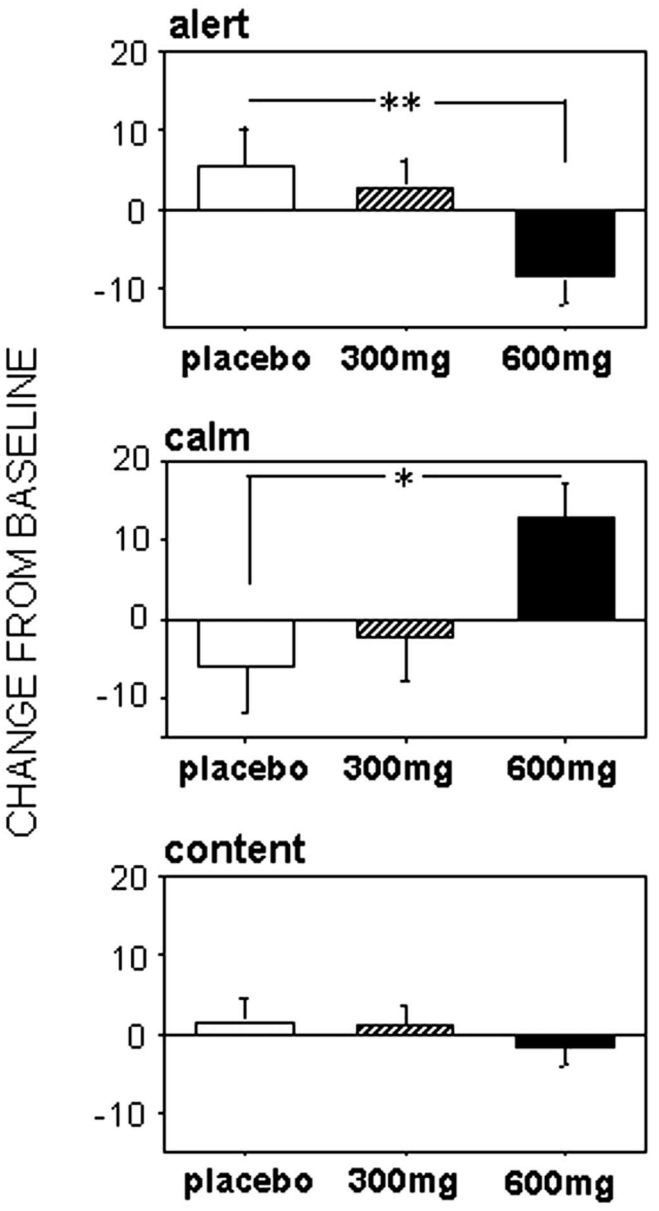

Figure 3. Effects of treatment (300 mg, 600 mg *Melissa officinalis*) on modulation of mood during the Defined Intensity Stressor Simulation battery (*$p < .05$; **$p < .001$ from planned comparisons).

DISCUSSION

The results of the current study confirm that acute administration of *M. officinalis* can ameliorate the negative change in mood associated with a 20-minute psychological stressor battery. While the completion of the DISS battery led to significant reductions in calmness, and a trend toward increased alertness, the 600-mg dose of *M. officinalis* directly ameliorated these mood effects. This dose was associated both with significantly improved calmness and significantly decreased alertness in comparison to placebo during the postdose completion of the DISS. This direct improvement in mood was seen in the absence of any detrimental effects on performance of the DISS tasks. Although no modulation of the mood effects of the stressor battery were seen after the 300-mg dose, this dose was associated with increased speed and accuracy of mathematical processing. The initial analysis of variance suggested that there was no significant effect on mood predose and 1 hour postdose in the absence of the stressor.

The mood effects associated with the 600-mg dose of *M. officinalis* in the present study are consistent with this herb's

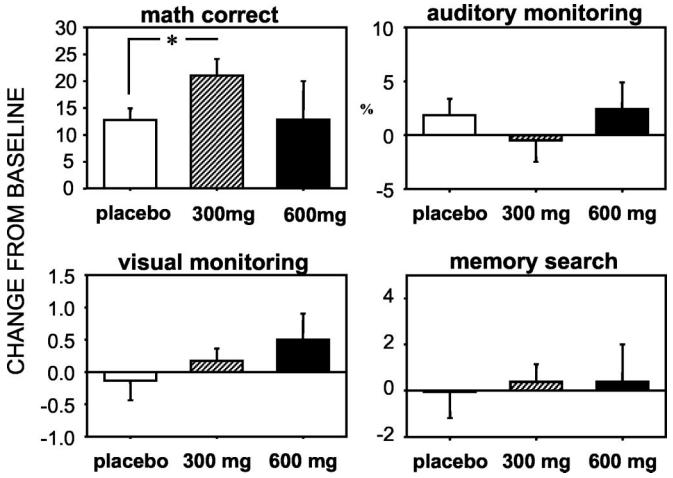

Figure 4. Effects of treatment (300 mg, 600 mg *Melissa officinalis*) on performance accuracy of the four concurrent tasks making up the Defined Intensity Stressor Simulation battery. Scores are change in number of correct answers for "math correct" and percentage change for the other three tasks (*$p < .05$ from planned comparisons).

traditional reputation as a calming agent and mild sedative (4). They also support contemporary reports of sedative effects in mice (10,11), reduced agitation in severe dementia patients after chronic consumption (12), and modulated self-ratings of mood after acute administration to healthy young volunteers (18,19).

The current study utilized single doses of the same standardized extract of *M. officinalis* as a previous study (18) that reported both significantly increased calmness, most notably after the lowest dose (300 mg), and significantly reduced alertness after the highest dose (900 mg). In contrast to the present study, no changes in mood were reported after administration of the 600-mg dose. Although the reason for this inconsistency is unclear, it is notable that in the previous study the mood scale data were collected on each occasion before completion of a 25 minute computerized cognitive battery, and therefore reflected "resting" mood rather than that after a potential psychological stressor. Alternatively, the discrepancy in dose–response may more parsimoniously be simply seen as reflecting the differences between the two experimental situations.

In both of the previous single-dose studies (18,19), cognitive decrements in terms of either reduced accuracy or reduced speed were evident on several tasks. In the current study, no cognitive decrements were evident, and the lowest dose led to improved mathematical processing. In the previous studies, the decrements were specific to the most difficult tasks (ie, those with the longest response latencies), with no effect seen on the performance of easier tasks. It is possible that the tasks utilized in the multitasking battery here would individually fall into the "easier" category. It may also be relevant that cognitive improvements evinced in the current study were restricted to the mathematical processing task, which was the only self-paced task within the DISS. All other tasks provoked responses continuously throughout the session.

The mechanism by which Melissa increased subjective ratings of calmness, reduced alertness, and improved some aspects of performance is currently unknown. Previous investigations have demonstrated negligible nicotinic and low muscarinic binding properties for this particular extract (18). The effects seen here are therefore unlikely to be attributable to direct interactions within the cholinergic system. It seems plausible to suggest that interactions may take place with other neurotransmitters. Given the pattern of mood modulation, the GABAergic system would seem to be an obvious potential target, with the sedative properties of *M. officinalis* potentially being elicited through the inhibitory action of GABA within the central nervous system. In this respect, it is particularly noteworthy that the pattern of mood modulation evinced here is identical to that previously seen after administration of benzodiazepines (ie, decreased alertness, increased calmness, no effect on contentedness) utilizing Bond-Lader mood scales (26). It is also possible, given the wide range of potentially active components, that the effects of *M. officinalis* are mediated through a combination of mechanisms, with potential interactions with a number of neurotransmitter systems. The potential for receptor binding across neurotransmitter systems by this species deserves further attention.

Because *M. officinalis* is rarely sold by itself, the effects of herbal combinations might usefully be investigated utilizing the same paradigm. Of particular interest here, *Valeriana officinalis* (valerian) is known for its sedative effects and anxiolytic properties (27) and is the most common herb to be sold commercially in combination with *M. officinalis* (see the German pharmaceutical industry's current "Rote Liste" for details). Although research into the effects of valerian on stress is limited, a recent study suggested that its ingestion could ameliorate participants' subjective ratings of "pressure" and reduce systolic blood pressure during laboratory induced stress (28).

Although the present study only investigated the effects of single doses of *M. officinalis*, the results suggest that, taken at a moderate dose, extracts from this plant may be beneficial in moderating subjective feelings of stress, without impairing cognitive performance. Because this was intended as a preliminary study in healthy humans, it will be necessary to confirm the clinical significance of the stress reducing effects of this herb both in pathologically stressed groups and in volunteers suffering natural "day to day" stress. Furthermore, it is important that future studies incorporate physiological measurements of stress indicators. The results here, together with those from previous studies of this herb in humans (12,18,19), certainly suggest a robust effect on mood. Nevertheless, it has to be acknowledged that the sample size here was relatively small ($n = 18$, repeated measures), and the treatment was administered immediately after an acute stressor.

In conclusion, the results of the current study suggest that extracts of *M. officinalis* can attenuate the subjective effects of laboratory-induced stress. Because the ingestion of *M. officinalis* appears to be well tolerated (8) with no reported side effects (29), and has now been shown to have robust effects on

mood (12,18,19), further research might well be directed to the question of whether either acute or chronic regimens of *M. officinalis* (or combinations including *V. officinalis*) might provide a safer alternative to prescribed drugs such as benzodiazepines in the mitigation of the effects of mild psychological stress.

REFERENCES

1. Perry EK, Pickering AT, Wang WW, Houghton PJ, Perry NSL. Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. J Pharm Pharmacol 1999;51:527–34.
2. Grieve M. A modern herbal. London: Johnathan Cape; 1931.
3. Bartram T. Encyclopaedia of herbal medicine. London: Grace Publishers; 1995.
4. Bisset NG, Wichtl M. Herbal drugs. Stuttgart: Medpharm; 1994.
5. Kommission E. Monograph. Melissenbla'tter, Bundesanzeiger, 1984; 05.12.
6. Weiss RF. Herbal medicine. Sweden: Arcanum; 1988.
7. Rote liste CMDI/Boehringer Ingelheim. 2001.
8. Cerny A, Schmid K. Tolerability and efficacy of valerian/lemon balm in healthy volunteers: a double-blind, placebo-controlled, multicentre study. Fitoterapia 1999;70:221–8.
9. Dressing H, Riemann D, Low H, Schredl M, Reh C, Laux P, Muller WE. Insomnia: are valerian/balm combination of equal value to benzodiazepine? Therapiewoche 1992;42:726–36.
10. Wagner H, Sprinkmeyer L. Uber die pharmakologische Wirkung von Melissengeist. Dtsch Apoth Ztg 1973;113:1159–66.
11. Souliman R, Fleurentin J, Mortier F, Misslin R, Derrieu G, Pelt JM. Neurotropic action of the hydroalcoholic extract of Melissa officinalis in the mouse. Planta Medica 1991;57:105–9.
12. Ballard C, O'Brien J, Reichelt K, Perry E. Aromatherapy as a safe and effective treatment for the management of agitation in severe dementia: the results of a double blind, placebo controlled trial. J Clin Psychiatry 2002;63:553–8.
13. Carnat AP, Carnat A, Fraisse D, Lamaison JL. The aromatic and polyphenolic composition of lemon balm (Melissa officinalis L. subsp. officinalis) tea. Pharm Acta Helv 1998;72:301–5.
14. Mulkens A, Stephanou E, Kapetenadis I. Heterosides a genines volatiles dans les feuilles de Melissa officinalis L. (lamiaceae). Pharm Acta Helv 1985;60:276–8.
15. Mantle D, Eddeb F, Pickering AT. Comparison of relative antioxidant activities of British medicinal plant species in vitro. J Ethnopharmacol 2000;72:47–51.
16. Hohmann J, Zupko I, Redei D, Csanyi M, Falkay G, Manthe I, Janicsak G. Protective effects of the aerial parts of Salvia officinalis, Melissa officinalis and Lavandula angustifolia and their constituents against enzyme-dependent and enzyme independent lipid peroxidation. Planta Med 1999;65:576–8.
17. Wake G, Court J, Pickering A, Lewis R, Wilkins R, Perry E. CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory. J Ethnopharmacol 2000;69:105–14.
18. Kennedy DO, Scholey AB, Tildesley NTJ, Perry EK, Wesnes KA. Modulation of mood and cognitive performance following acute administration of Melissa officinalis (lemon balm). Pharmacol Biochem Behav 2002;72:953–64.
19. Kennedy DO, Wake G, Savelev S, Tildesley NTJ, Perry EK, Wake G, Wesnes KA, Scholey AB. Modulation of mood and cognitive performance following administration of single doses of Melissa officinalis (Lemon balm) with human CNS nicotinic and muscarinic receptor binding properties. Neuropsychopharmacology 2003;28:1871–81.
20. Wetherell MA, Sidgreaves MC. The Defined Intensity Stressor Simulation (DISS): the effects of increasing intensity on S-IgA reactivity and perceptions of workload, stress and arousal. Proc Br Psychol Soc 12:74.
21. Wetherell MA, Hyland ME, Harris JE. Secretory immunoglobulin A reactivity to acute and cumulative acute multi-tasking stress: relationships between reactivity and perceived workload. Biol Psychol 2004;66: 257–70.
22. Faul F, Erdfelder E. A priori, post-hoc and compromise power analyses for MS-DOS (computer programme) Bonn, FRG, University of Bonn. Dept of Psychology. 1992.
23. Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Psychol 1974;47:211–8.
24. Ahearn EP. The use of visual analog scales in mood-disorders: a critical review. J Psychiatr Res 1997;31:569–79.
25. Kepple G. Design and analysis. Englewood Cliffs, NJ: Prentice Hall; 1991.
26. Thompson JM, Neave N, Moss MC, Scholey AB, Wesnes K, Girdler NM. Cognitive properties of sedation agents: comparison of the effects of nitrous oxide and midazolam on memory and mood. Br Dent J 1999;187: 557–62.
27. Kohnen R, Oswald WD. The effects of valerian, propranolol, and their combination on activation, performance and mood of healthy volunteers under social stress conditions. Pharmacopsychiatry 1988;21:447–8.
28. Cropley M, Cave Z, Ellis J, Middleton RW. Effects of kava and valerian on human physiological and psychological responses to mental stress assessed under laboratory conditions. Phytother Res 2002;16:23–7.
29. Wong AHC, Smith M, Boon HS. Herbal remedies in psychiatric practice. Arch Gen Psychiatry 1998;55:1033–44.