The Herbal Extracts of Myrrh, Chamomile and Coffee Charcoal Modulate Intestinal Neurotransmission and Motility in Murine Small Intestine

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Abstract

Background: A combination of myrrh, chamomile flower and coffee charcoal has been used for decades in the treatment of functional gastrointestinal disorders and inflammatory bowel disease. However, the mechanism of action of neither the combination nor the individual components has been completely characterized.

Hypothesis/Purpose: The present study aims to investigate whether the herbal extracts have an effect on electrophysiological properties and muscular function of the smooth muscle cells of murine intestine.

Methods: Small intestinal muscular activity was elicited using electrical field stimulation (EFS). The effects of diluted herbal extracts on basal activity and EFS-induced contractility were tested in concentrations from 0.002 mg/ml - 2 mg/ml (10 mg/ml for Coffee charcoal). Intracellular electrical recordings from smooth muscular cells were performed to characterize possible effects on excitatory junction potentials (EJP) and on inhibitory junction potentials (IJP).

Results: Myrrh, Chamomile and Coffee charcoal significantly reduced the amplitudes of spontaneous contractile activity: Myrrh-ethanolic extract: 0.2 mg/mL: -50.6% ± 3.5; Myrrh-water-soluble extract: 0.1 mg/mL: -67.7% ± 3.6 Chamomile: 0.2 mg/mL: -70.3% ± 2.7; and significantly reduced EFS-induced contractile responses: Myrrh-ethanolic extract: 0.2 mg/mL: -62.9% ± 4.8; Myrrh-water-soluble extract: 2 mg/mL: -87.9% ± 2.6; Chamomile: 1 mg/mL: -53.4% ± 4.7; Coffee charcoal: 0.1 mg/mL: -89.1% ± 2.0. Chamomile: 1 mg/mL: significantly reduced sIJP (-4.4% ± 0.8), Coffee charcoal: 2 mg/mL: reduced the fIJP (-7.0% ± 1.8).

Conclusion: Myrrh, chamomile flower and coffee charcoal extracts reduce spontaneous contractility and EFS-induced contractility in the mouse small intestine in a dose-dependent manner. They also have a reducing effect on the underlying neurotransmission. Thus, the combination of myrrh, chamomile and coffee charcoal is of therapeutic use for conditions that involve intestinal hyperactivity.

Keywords: Phytotherapy; Commiphora; Chamomile; Coffee; Neurotransmission; Intestinal Electrophysiology

Abbreviations

EFS: Electrical Field Stimulation; EJP: Excitatory Junction Potential; sIJP: Slow Inhibitory Junction Potential; fIJP: Fast Inhibitory Junction Potential; IBD: Inflammatory Bowel Disease; CID: Chronic Intestinal Dysmotility; FGID: Functional Gastrointestinal Disorders; TNBS: 2,4,6-Trinitrobenzenesulfonic Acid; TNF: Tumor Necrosis Factor

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Introduction

Phytomedicine is widely spread in the supportive treatment of several gastrointestinal diseases, such as inflammatory bowel disease (IBD), chronic intestinal dysmotility (CID) and functional gastrointestinal disorders (FGID). The pathophysiology of these diseases includes disturbed gut motility, unbalanced acid production or malfunctioning enteric nervous system [1]. Among the phytopharmaceutical products used for the symptomatic treatment, Myrrhinil-Intest® is largely used in Germany. Myrrhinil-Intest® is a fixed combination of three herbal components [Myrrh (Commiphora myrrha) pulv. 100 mg, Coffee Charcoal (Carbo coffeae) 50 mg, dry-extract of Chamomile flower (Marticaria chamomilla) 70 mg (DER:4-6:1); excerpt medium: ethanol 60% (m/m)]. A randomized, double-blind study demonstrated the non-inferiority of this herbal preparation in comparison to the gold standard mesalazine in maintenance of remission in ulcerative colitis [2]. A recently published multicentric prospective observational post-marketing study showed the clinical efficacy, safety and tolerability in patients with symptoms of acute diarrhoea [3].

Even though Myrrhinil-Intest® is popular for its beneficial effects, there is still little known regarding the underlying mechanisms on intestinal motility and electrophysiology. The gastrointestinal tract has a multitude of motility patterns which could be influenced [4-10]. Chamomile dry-extract has an inhibitory effect on the cAMP-phosphodiesterase, which would eventually lead to spasmylosis of the smooth muscle cell [11]. Myrrh works also as a muscle relaxant, this fact has been pathophysiologically explained as a result of its Calcium-antagonistic effect [12]. Existing research in this field has examined the mechanisms on healthy intestine as well as on inflamed tissue. For example, TNBS has been used to cause an inflammatory reaction on murine intestine by elevating the gene expression for TNFα [13]. In this perspective, Myrrh and chamomile have been shown to sustain the normal function of the intestine, even under the inflammatory effect of TNBS. Furthermore, Myrrh has been able to hold back the rising gene expression of TNFα and to diminish the intestinal inflammatory reaction. The herbal extracts also reduced TNFα-release from activated immune cells and the combination of myrrh, chamomile and coffee charcoal have been proven to have an effect on the innate immune system by increasing the rate of phagocytosis [14].

In-vitro experiments on intestinal tissues are needed to develop an understanding of the potential mechanisms underlying the symptoms that can be controlled with Myrrhinil-Intest®. Our project investigated the effects of myrrh, chamomile and coffee charcoal on intestinal smooth muscular contractility and muscular electrophysiological properties, both mechanisms underlying the symptoms that can be resolved by these herbal compounds.

Materials and Methods

Tissue preparations for EFS

8- to 10-week-old male Swiss mice were anaesthetized with Isofluran® and sacrificed by cervical dislocation according to the recommendations of the animal care council of the University of Munich, Germany. The proximal colon and small bowel were removed through a median incision and placed into oxygenated (95% O₂ - 5% CO₂) Krebs solution, composition as follows (mM): NaCl 120.35; KCl 5.9; MgCl₂, 2.5; NaH₂PO₄, 1.2; NaHCO₃, 15.5; CaCl₂, 2.5; Glucose 11.5; pH 7.4. The segment of the bowel was cut along the mesenteric border, washed off from remaining fecal matter and sectioned into 6 bowel pieces of 0.8 - 1.0 cm. Each segment has been tightened into individual, with Krebs solution filled organ baths. At a constant temperature of 37°C and after 90 minutes of equilibration, the electric field stimulation was started. After further 20 minutes of adaptation and at the occurrence of 3 identical contractions, the administration of cumulative concentrations of the test substances was ready to start.

Isometric contractions have been induced by EFS at 40 Volt with a stimulation duration of 10 seconds at 10 Hz in 5 minute intervals. An amplifier transformed the isometric contractions into electrical signals; these are digitalized and then recorded on a PC. The power converter had a preload of 500 mg. Following data has been taken into analysis: maximum amplitude of the spontaneous contractions and of the EFS-induced contractions.

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Tissue preparations for electrophysiological experiments

The intestine segments were retrieved using the above mentioned procedure and were pinned out in a Sylgard®-lined (Dow Corning Corp.; Midland, MI, USA) dissecting dish containing oxygenated Krebs solution. Ten millimeter wide segments of the proximal colon were separated into their corresponding layers. Mucosa, submucosa and serosa were removed, the remaining sheets of tissue consisted of circular and longitudinal muscle layers, with the attached myenteric plexus.

Intracellular electrical recordings were performed as previously described [15]. The layers of smooth muscle cells were pinned using approximately 150 - 200 Wolfram-wire micropins (15 - 25 µm thick) to the Sylgard®-lined electrophysiological chamber, with the circular muscle layer up. The chamber was constantly perfused (5 ml/min; Kwik Pump, World Precision Instruments; Sarasota, FL, USA) with preheated (37°C), oxygenated Krebs solution. The tissues had an equilibration phase for 90 - 120 min before the experiments were started. Capillary glass microelectrodes (borosilicate glass capillaries, 1.0 mm outer diameter x 0.58 mm inner diameter; Clark Electromedical Instruments; Edenbridge, UK) were shaped using a microelectrode puller (Model P-97, 3 mm wide filament, Sutter Instruments; Novato, CA, USA), filled with KCl (3M) showing resistances in the range 80 - 120 MΩ. After the equilibration phase was finished, a circular smooth muscle cell was impaled and the membrane potential was recorded against a “ground” Ag–AgCl electrode placed in the bath medium. Then the test substances were added in the perfused organ bath in cumulative concentrations. The induced potentials were amplified (Duo 733 microelectrode amplifier; World Precision Instruments; Sarasota, FL, USA) and digitalized with an analogue-to-digital converter (SCB 68 interface, National Instruments; Austin, TX, USA).

Neurons of the myenteric plexus were electrically stimulated via platinum electrodes, which were arranged perpendicularly to the muscle layer and connected to a Grass S11 stimulator (Grass SIU59; Grass Instruments, Quincy, Massachusetts, USA). Permanent recordings of membrane potentials were made on a PC running LABVIEW 5.0 (National Instruments; Austin, TX, USA). Nifedipine (1 mM) was present throughout the entire experiment to prevent artifactual muscular contractions.

EFS of the intrinsic neurons triggered different potentials: excitatory junction potentials (EJP) and inhibitory junction potentials (IJP, fast transient inhibitory junction potential – fIJP; slow inhibitory junction potential – sIJP).

The amplitudes of junction potentials were measured in mV compared to the resting membrane potential before applying electrical stimulation. All data were represented as mean ± SEM, N represented the number of experimental animals used.

Data analysis was performed using GraphPad Prism software (version 5.00, GraphPad Software Inc., San Diego, CA, USA). The concentration-response-curves have been created and compared using non-linear regression. A significance level of p < 0.05 was considered statistically significant.

Test substances

Individual herbal constituents contained in Myrrhinil-Intest® were used for examining their singular effect on murine intestine. Dry extracts of Myrrh (Commiphora myrrha, lot number 20772), Chamomile (Matricaria chamomilla, lot number 20935) and Coffee charcoal (Carbo coffeae, lot number 14790/20265) were kindly provided by REPHA GmBH, Biologische Arzneimittel, Langenhagen, Germany. The extracts were dissolved in ethanol or in DMSO (Dimethyl sulfoxide) and then diluted in Krebs solution as above described.

Quality and purity of myrrh, the gum resin obtained by incision of the stem of Commiphora molmol Engler (synonym Commiphora myrrha), was in accordance with all specifications of the European Pharmacopoeia monograph. Coffee charcoal was obtained by over roasting coffee beans from Coffea arabica L. and further grounding. The ethanolic myrrh extract has been prepared as previously described [14]. Briefly, myrrh powder was extracted under reflux with ethanol (96% v/v) at 60°C for 60 min. The extract was evaporated to dryness and the residue was resuspended in water and further a steam distillation was performed. The water soluble myrrh extract was prepared

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according to the European Pharmacopoeia 6.0. First myrrh was extracted with ethanol (96% v/v). Afterwards the residue was extracted with water. The water extract was evaporated to dryness and stored at room temperature. Coffee charcoal was extracted with boiling water for 15 minutes. The extract was further filtrated and the extract was evaporated to dryness. The chamomile flower dry extract (excerpt medium: ethanol 60% m/m, DER: 4-6:1) was equivalent to the active pharmaceutical ingredient of the medicinal product Myrrhinil-Intest®. All extracts were stored at room temperature.

Results

Influence of Myrrhinil-Intest® components on the amplitude of EFS-induced contractions

Ethanol Myrrh extract

The ethanol myrrh extract was gradually added to the organ baths, rising concentrations (0.002 mg/mL-2 mg/mL) were added after two successive EFS-induced contractions. This led to a reduction of the amplitude of the contractions: at 0.002 mg/mL: 98.5% ± 1.9; at 0.02 mg/mL: 90.7% ± 2.9; at 0.1 mg/mL: 86% ± 1.9; at 0.2 mg/mL: 62.9% ± 4.8; at 2 mg/mL: 28.2% ± 5.2 (Figure 1).

![Figure 1: Influence of an ethanol myrrh extract (0.002 - 2 mg/mL) on the amplitude of the EFS-induced contraction of untreated small murine intestine. Evaluation: *=low significant; **=average significant; ***=highly significant; N.S. (not significant) from p > 0.05.](image)

Water-soluble Myrrh extract

This extract also showed a significant decrease in the amplitude of EFS-induced contractions: at 0.002 mg/mL: 96.4% ± 1.8; at 0.02 mg/mL: 95.3% ± 1.4; at 0.1 mg/mL: 93.3% ± 2.3; at 0.2 mg/mL: 89.9% ± 2.7; at 2 mg/mL: 87.9% ± 2.6; at 10 mg/mL: 85% ± 3 (Figure 2).

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**Figure 2:** Influence of a water-soluble myrrh extract (0.002 - 10 mg/mL) on the amplitude of the EFS-induced contraction of untreated small murine intestine. Evaluation: * = low significant; ** = average significant; *** = highly significant; N.S. (not significant) from p > 0.05.

**Chamomile extract**

Rising concentrations of dry Chamomile extracts were added to the organ baths, showing dose-dependent significant drops of amplitudes: at 0.002 mg/mL: 96.7% ± 1.7; at 0.02 mg/mL: 97.7% ± 1.1; at 0.2 mg/mL: 79.7% ± 3.8; at 1 mg/mL: 53.4% ± 4.7; at 2 mg/mL: 13% ± 1.6 (Figure 3).

**Figure 3:** Influence of a chamomile extract (0.002 - 2 mg/mL) on the amplitude of the EFS-induced contraction of untreated small murine intestine. Evaluation: * = low significant; ** = average significant; *** = highly significant; N.S. (not significant) from p > 0.05.
Coffee charcoal extract

Compared to the other investigated plant extracts, the Coffee charcoal extract showed a slower effect in reducing the contraction amplitude: at 0.002 mg/mL: 94.2% ± 1.6; at 0.02 mg/mL: 92.1% ± 1.5; at 0.1 mg/mL: 89.1% ± 2; at 0.2 mg/mL: 85% ± 2.5; at 1 mg/mL: 72% ± 3.1; at 2 mg/mL: 40.6% ± 9.2 (Figure 4 and 5).

**Figure 4:** Influence of an Carbo coffeae extract (0.002 - 1 mg/mL) on the amplitude of the EFS-induced contraction of untreated small murine intestine. Evaluation: * = low significant; ** = average significant; *** = highly significant; N.S. (not significant) from p > 0.05.

**Figure 5:** Original recording of isometric contractions induced by EFS under the influence of: a. ethanolic Myrrh extract (0.002 - 2 mg/mL); b. water-soluble Myrrh extract (0.002 - 10 mg/mL); c. chamomile (0.002 - 2 mg/mL); d. Carbo coffeae extract (0.002 - 1 mg/mL).
Influence of *Myrrhinil-Intest*® components on the amplitude of the basal tonus

**Ethanolic Myrrh extract**

The amplitudes of spontaneous contractions showed significant decreases under rising concentrations of ethanolic Myrrh extract: at 0.002 mg/mL: 83.1% ± 4.2; at 0.02 mg/mL: 65.2% ± 3.7; at 0.1 mg/mL: 62.4% ± 6.4; at 0.2 mg/mL: 50.6% ± 3.5; at 2 mg/mL: 40.6% ± 4.9 (Figure 6).

![Figure 6: Influence of an ethanolic myrrh extract (0.002 - 2 mg/mL) on the amplitude of the basal tonus of untreated small murine intestine. Evaluation: * = low significant; ** = average significant; *** = highly significant; N.S. (not significant) from p > 0.05.

**Water-soluble Myrrh extract**

The water-soluble myrrh extract was added in increasing concentrations, starting with 0.002 mg/mL up to 10 mg/mL. This extract showed a significant decrease of amplitude in the basal tonus of murine intestine: 86.1% ± 3.3; at 0.02 mg/mL: 79.4% ± 3.3; at 0.1 mg/mL: 67.7% ± 3.6; at 0.2 mg/mL: 58.9% ± 3; at 2 mg/mL: 47.7% ± 3.1; at 10 mg/mL: 43.3% ± 2.9 (Figure 7).

Chamomile extract

Amplitudes of spontaneous contractions were influenced by Chamomile dry-extract, showing significant decreases: at 0.002 mg/mL: 86.1% ± 3.3; at 0.02 mg/mL: 79.1% ± 3.1; at 0.2 mg/mL: 70.3% ± 2.7; at 1 mg/mL: 58.9% ± 3.9; at 2 mg/mL: 44.5% ± 3.6 (Figure 8).
Coffee charcoal extract

Compared to the other investigated plant extracts, the Coffee charcoal extract showed a rather slow effect in reducing the contraction amplitudes. A decrease in the amplitudes occurred with increasing extract concentration: at 0.002 mg/mL: 84.2% ± 1.9; at 0.02 mg/mL: 71.5% ± 2.3; at 0.1 mg/mL: 77.2% ± 8.1; at 0.2 mg/mL: 63.1% ± 6.6; at 1 mg/mL: 56.9% ± 7.1; at 2 mg/mL: 25.8% ± 5.6 (Figure 9). This fact was significant starting from 1 mg/mL. An evident foam formation in the organ bath became visible simultaneously to the application of higher extract concentration.

Influence of Myrrhinil-Intest® components on the amplitude of junction potentials

Ethanolic Myrrh extract

Increasing concentrations of ethanolic Myrrh extract showed inhibitory effects on the level of intracellular neurotransmission. The reduced amplitude was most significant regarding the EJP: at 0.2 mg/mL: 23.7 ± 1.5 mV; at 1.0 mg/mL: 15.4 ± 0.2 mV; at 2.0 mg/mL: 12.8 ± 0.7 mV. The effect on the fIJP and sIJP was of lower significance. For fIJP: at 0.2 mg/mL: 17.7 ± 0.7 mV; at 1.0 mg/mL: 16.2 ± 0.8 mV; at 2.0 mg/mL: 10.4 ± 0.3 mV. For sIJP: at 0.2 mg/mL: 9.3 ± 0.4 mV; at 1.0 mg/mL: 6.0 ± 0.0 mV; at 2.0 mg/mL: 5.8 ± 0.4 mV (Figure 10).
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**Figure 10:** Influence of an ethanolic myrrh extract (0.2 - 2.0 mg/mL) on the amplitude of the cholinergic excitatory, the peptide-ergic and the nitrergic inhibitory neurotransmission in the proximal murine colon. Evaluation: *=low significant; ** =average significant; *** =highly significant; N.S. (not significant) from p > 0.05.

**Water-soluble Myrrh extract**

This dry extract showed significant amplitude reductions only in the maximal test concentration and affected only the EJP and sIJP. For EJP: at 0.2 mg/mL: 16.3 ± 0.2; at 1.0 mg/mL: 10.9 ± 0.6 mV; at 2.0 mg/mL: 11.5 ± 2.4 mV. For fIJP: at 0.2 mg/mL: 12.6 ± 0.6 mV; at 1.0 mg/mL: 23.1 ± 0.2 mV; at 2.0 mg/mL: 18.2 ± 3.0 mV. For sIJP: at 0.2 mg/mL: 4.9 ± 0.3 mV; at 1.0 mg/mL: 10.6 ± 0.2 mV; at 2.0 mg/mL: 10.2 ± 1.8 mV (Figure 11).
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**Figure 11**: Influence of an water-soluble myrrh extract (0.2 - 2.0 mg/mL) on the amplitude of the cholinergic excitatory, the peptidergic and the nitrergic inhibitory neurotransmission in the proximal murine colon. Evaluation: * = low significant; ** = average significant; *** = highly significant; N.S. (not significant) from p > 0.05.

**Chamomile extract**

Low concentrations of this dry extract showed minimal effect, whereas maximal tested doses started decreasing amplitudes in every potential. For EJP: at 0,1 mg/mL: 18,6 ± 2,0 mV; at 0,2 mg/mL: 22,5 ± 2,7 mV; at 1 mg/mL: 8,2 ± 0,8 mV. For flJP: at 0,1 mg/mL: 21,9 ± 0,2 mV; at 0,2 mg/mL: 22,0 ± 1,5 mV; at 1 mg/mL: 12,1 ± 0,1 mV. For slJP: at 0,1 mg/mL: 10,0 ± 0,7 mV; at 0,2 mg/mL: 10,3 ± 0,6 mV; at 1 mg/mL: 4,4 ± 0,8 mV (Figure 12).
**Coffee charcoal extract**

This substance had an inhibitory effect on the fIJP. The amplitude of the EJP and sIJP remained unaffected by coffee charcoal extracts. For EJP: at 0.2 mg/mL: 18.3 ± 1.7 mV; at 1.0 mg/mL: 17.0 ± 2.9 mV; at 2.0: 11.0 ± 3.7 mV. For fIJP: at 0.2 mg/mL: 11.8 ± 0.2 mV; at 1.0 mg/mL: 13.3 ± 2.5 mV; at 2.0 mg/mL: 10.4 ± 2.8 mV. For sIJP: at 0.2 mg/mL: 6.4 ± 0.1 mV; at 1.0 mg/mL: 9.4 ± 1.2 mV, at 2.0 mg/mL: 7.0 ± 1.8 mV (Figure 13).

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**Figure 12:** Influence of an chamomile extract (0.1 - 1.0 mg/mL) on the amplitude of the cholinergic excitatory, the peptidergic and the nitrergic inhibitory neurotransmission in the proximal murine colon. Evaluation: *=low significant; ** =average significant; *** =highly significant; N.S. (not significant) from p > 0.05.

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Discussion

Functional gastrointestinal disorders compile a number of complex pathophysiological pathways which are still widely unexplored. Therefore it is difficult to gain full understanding regarding the pharmacological effect of symptom-relieving substances or drugs. Even though research has been undertaken in this field [16-22], there is still a lot of missing information regarding the underlying mechanisms.

Our research shows that the individual components, myrrh, chamomile and coffee charcoal have significant effects on the activity of smooth muscle cells. We have tested effects on several levels and conditions of murine intestine. First, we have examined individual effects of the dry extracts on the basal tonus of the smooth muscle cell. Myrrh, both in ethanolic and water-soluble extracts, has had a reducing
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effect over the amplitude of basal contractions, where no EFS-induced contractions had been triggered. The same reaction has been observed in case of chamomile and coffee charcoal extracts, whereas the latter had a more significant effect on the basal tonus compared to the one measured at the EFS-induced contraction. These facts imply that the constituents of Myrrhinil-Intest® contribute significantly to the muscle relaxant effects.

Of a higher interest is the reaction of the intestine after electric field stimulation, which induces distressing contractions resembling to muscle-spasms. Expected was decreasing amplitude of these contractions after applying cumulative concentrations of individual dry extracts. In this regard we have identified significant reductions of EFS-induced contractions in all the tested substances.

Another important aspect of our research was the investigation of the neurotransmission in the murine intestine, respectively in the myenteric plexus. Neurons have been activated through EFS and the response has been measured as an EJP and as a biphasic IJP (fIJP and sIJP). The EJP represents the cholinergic excitatory neuronal pathways [23] and it has been not significantly influenced by the herbal extracts.

In contrast, the IJP, fIJP for peptidergic inhibitory neurotransmission [24] and sIJP for nitrergic inhibitory neurotransmission have been influenced by the herbal extracts, particularly by Chamomile (sIJP) and Coffee charcoal (fIJP). This shows that the electrophysiology underlying gastrointestinal motility is attenuated by the herbal extracts. The achieved reduction of the amplitude is concentration-dependent. These changes involve both peptidergic and nitrergic neuronal mechanisms and the fact that they can be securely attributed to chamomile and coffee charcoal is of major interest for the perspective of further investigations. However, the dose-response curve is a nonlinear one and significant effects appear mostly at higher doses, a noticeable threshold value would be of 1 mg at the majority of the tested substances.

In summary our study demonstrates that Myrrhinil-Intest® and its individual components are able to modulate multiple basic parameters such as the basal tonus of the smooth muscle cells, the EFS-induced contractions and the inhibitory neurotransmission which lies at the electrophysiological ground of smooth muscle contractility and motility of the bowel. Therefore, this unique herbal preparation positively influences the contractility and motility, which are generally accepted as the prime parameters for functional bowel disorders.

Conflict of Interest

This study has been supported by REPHA GmbH Biologische Arzneimittel, Langenhagen, Germany. The company had no influence and never tried to influence data acquisition, analysis or interpretation.

Bibliography


