Effect of the standard herbal preparation, STW5, treatment on dysbiosis induced by dextran sodium sulfate in experimental colitis

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Abstract

Background: The standardized herbal preparation, STW 5, is effective clinically in functional gastrointestinal disorders and experimentally in ulcerative colitis (UC). The present study explores whether the beneficial effect of STW 5 involves influencing the intestinal microbiota.

Methods: UC was induced in Wistar rats by feeding them 5% dextran sodium sulfate (DSS) in drinking water for 7 days. Rats were treated concurrently with STW 5 and sacrificed 24 h after last drug administration. Fecal samples were used to determine changes in the abundance of selected microbial phyla and genera using real-time PCR.

Results: Induction of UC led to dysbiosis and changes in the gut microbiota. The changes included an increase in some genera of the Firmicutes, namely Enterococcus, and a decrease in others, namely Blautia, Clostridium, and Lactobacillus. DSS further induced a marked increase in the abundance of Bacteroidetes and Proteobacteria as well as in the relative abundance of Actinobacteria and its genus Bifidobacterium. Methanobrevibacter levels (phylum Euryarchaeota) were also increased. Microbial dysbiosis was associated with changes in various parameters of colonic inflammation. STW 5 effectively guarded against those changes and significantly affected the indices of edema and inflammation in the UC model. Changes in colon length, colon mass index, inflammatory and apoptotic markers, and histological changes induced by DSS were also prevented.

Conclusions: Dysbiosis plays a contributing role in the development of DSS-induced UC. Derangements in the microbial flora and associated inflammatory processes were largely prevented by STW 5, suggesting that this effect might contribute towards its beneficial usefulness in this condition.

Keywords: Dysbiosis, Gut microbiota, STW 5, Ulcerative colitis

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Background
Gastrointestinal diseases (GID), whether inflammatory or functional (FGID), affect people worldwide and impair their quality of life and work productivity [1]. Inflammatory bowel diseases (IBD) are usually manifested either as ulcerative colitis (UC) or as Crohn’s disease (CD) [2]. Colitis induced by dextran sodium sulfate (DSS) in rats mimics the clinical and histological features of UC by interfering with intestinal barrier function and stimulating local inflammatory processes [3]. Growing evidence further suggests the involvement of gut microbiota in IBD [4]. It has been postulated that the gut microbiota imbalance (dysbiosis) could initiate immune responses by compromising the mucosal barrier and stimulating local and systemic immunity [5, 6]. This fact qualifies the DSS model to be used as a dysbiosis model [7].

Furthermore, altered motility, visceral hypersensitivity, immune alterations, low-grade inflammation, dysfunctional brain-gut axis, and compromised epithelial barrier function have all been postulated to contribute to the symptoms in functional dyspepsia (FD) and irritable bowel syndrome (IBS) [8, 9]. Gut microbiota has been shown to modulate many of these physiological functions [10, 11]. Although no consistent microbial signature has been associated with FGIDs, several lines of evidence support a role for gut microbes in the development of FGID symptoms [11].

Gut microbiota is a complex ecosystem dominated by four main phyla: Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria [12, 13]. In a healthy state, the gut microbiota has a mutualistic relationship with the human host. The host intestine provides the microbes with a niche and the microbial ecosystem contributes to maintaining homeostasis by modulating several physiological functions such as nutrient digestion, immune responses, and normal perception of visceral pain [14].

STW 5 (Iberogast®) is a standardized multi-component herbal preparation consisting of a combination of nine medicinal herbal extracts, commercially available in Europe. It was shown to be effective in FD and IBS in several randomized clinical studies [15] and was previously reported to have anti-ulcerogenic and mucosal protective effects as well as potent anti-inflammatory properties [16]. The present study aimed at exploring the beneficial effect of STW 5 could also involve modulation of the intestinal microbiota.

Methods
Animals
Adult female Wistar rats, weighing 150–200 g each, were obtained from the Modern Veterinary Office for Laboratory Animals, Cairo, Egypt. Rats were provided with a standard pellet diet and were given water ad libitum. The animals were housed at a temperature of 22 ± 3 °C and a 12-h light/dark cycle as well as at a constant relative humidity throughout the experimental period. Animals were left to acclimatize for at least 7 days before subjecting them to experimentation.

The study was carried out in compliance with the ARRIVE guidelines and experimental procedures were approved by the institutional Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University, Cairo, Egypt, approval number (PT 1769) following the guidelines laid out in the Guide for the Care and Use of Laboratory Animals, National Academy of Science.

Drugs
STW5 (Iberogast®) is a commercially available standardized herbal preparation that was generously provided by Bayer consumer health (Darmstadt, Germany). It consists of hydroethanolic extracts of Iberis amara L. (Brassicaceae) (15%), Melissa officinalis L. (Lamiaceae) (10%), Matricaria chamomilla (Compositae) (20%), Carum carvi L. (Apiaceae) (10%), Mentha piperita L. (Lamiaceae) (5%), Angelica archangelica L. (Apiaceae) (10%), Silybum marianum (L.) Gaertn. (Compositae) (10%), Chelidonium majus L. (Papaveraceae) (10%), and Glycyrrhiza glabra L. (Leguminosae) (10%). The preparation and every single extract were well characterized according to the guidelines of the European Medicines Agency. The extraction processes as well as the quality controls were previously described in detail [17]. Briefly, the extracts were prepared, and quality controlled according to Good Manufacturing Practice and Good Agricultural Practice of Medicinal and Aromatic Plants. The quality of each extract was tested according to individual specifications as chromatographic fingerprint [17, 18].

Induction of colitis
Colitis was induced in rats by adding DSS, molecular weight 37–40 kD, (TdB Consultancy, Uppsala, Sweden), to the drinking water in a concentration of 5% (w/v) for 1 week [19].

Experimental design
Adult rats were randomly allocated to three groups of 14–16 animals each as follows:

(a) Vehicle control group: received normal tap water (without DSS) and given 31% ethanol (STW 5 vehicle) 5 mL/kg, orally daily for 1 week.
(b) UC group: received 5% DSS in drinking water and given concurrently 31% ethanol (STW 5 vehicle) 5 mL/kg, orally daily for 1 week.
(c) UC/STW 5 group: received 5% DSS in drinking water and given concomitantly STW 5 (5 mL/kg),
orally daily for 1 week. This dose was chosen after carrying out preliminary experiments with two doses (2 ml and 5 ml /kg) of the preparation and selected on the basis that it had more consistent effects on the intestinal microbiota.

Twenty-four hours after the last drug administration, rats were euthanized using halothane anesthesia followed by cervical dislocation and the colon and caecum from all animals were excised. The colon length was measured, rinsed in ice-cold saline, cleaned of extraneous tissue, dried on filter paper, and weighed. The ratio of colon weight in milligrams to the total body weight in grams was taken as the colon mass index and was used as a measure of the degree of colonic edema and severity of inflammation. The colon was then cut longitudinally into two segments: one was fixed in 10% formalin for histological examination, and the other was homogenized in ice-cold saline to obtain a 10% homogenate for the assessment of biochemical parameters. The entire caeca were stored at -20 °C until further use. Fecal samples from each caecum were used for microbial genomic DNA isolation and further analysis.

**Determination of biochemical parameters associated with colitis**

The colon homogenate was centrifuged at 6000 rpm for 30 min at 4 °C. The supernatant was used for assaying tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NFkB), and caspase-3 using rat specific enzyme-linked immunosorbent assay (ELISA) kits from Elabscience Biotechnology Co. (Texas, USA), Hangzhou Eastbiopharm Co. (Hangzhou, China) and Cloud-Clone corp. (Texas, USA), respectively.

**Microbial genomic DNA isolation**

The excised frozen caeca were subjected to fecal genomic DNA extraction using Zymo research fecal DNA extraction kit (Zymo Research Corp., Irvine, CA, USA) following the manufacturer’s protocol. This protocol was effective in removing traces of DSS from the isolated DNA, as DSS is a PCR inhibitor [20]. DNA concentration was determined by measuring the absorbance at 260 nm using Implen nanophotometer P-330 (Implen GmbH, Munich, Germany).

**Relative abundance of microbial phyla using quantitative real-time PCR**

The changes in the main gut-associated microbiota were quantified using specific primers targeting different microbial genera 16S ribosomal ribonucleic acid (rRNA) gene by Real Time-PCR (qPCR) as described in Table 1. qPCR experiments were performed using Quantifast SYBR Green PCR Kit (Qiagen, Hilden, Germany) on a Rotor-Gene Real-Time PCR machine (Qiagen, Hilden, Germany). The thermal cycling conditions were optimized as follows: an initial DNA denaturation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, primer annealing at optimal temperature for 20 s, extension at 72 °C for 15 s. Melt curve analysis was performed by slowly cooling from 95 °C to 60 °C (0.05 °C per cycle) with simultaneous measurement of the SYBR Green I signal intensity. All PCR tests were carried out in duplicates of each group pool.

**Microbial genomic DNA standard curves**

Microbial genomic DNAs used for the construction of the standard curves were extracted from *Lactobacillus acidophilus* (ATCC 4356) and *Enterococcus faecalis* (ATCC 19433) obtained from the American type culture collection (Virginia, USA). The genomic DNA of *Escherichia coli* (DSM 498), *Prevotella intermedia* (DSM 20706), *Blaubrio producta* (DSM 2950), *Bacteroides vulgatus* (DSM 1447), *Methanobrevibacter smithii* (DSM 861), *Hyobacter polytropus* (DSM 2926), *Clostridium leptum* (DSM 753) and *Bifidobacterium bifidum* (DSM 20456) were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. Standard curves were constructed for each experiment using serial ten-fold dilution of microbial genomic DNA of the mentioned standard cultures of microbes, corresponding to 30 to 3 × 10^9 16S rRNA gene copies. The mass for one microbial genome was calculated by using Avogadro’s number and assuming the mean molecular weight of a base pair to be 660 g/mol. Standard curves were normalized to the copy number of the 16S rRNA gene for each microbial species. The microbial concentration from each fecal sample was calculated by comparing the cycle threshold (Ct) values obtained from the standard curves and expressed as gene copies/gram of feces.

**Histopathological examination of the colon**

Transverse sections, 4–6 μm thin, were prepared from paraffin-embedded colon segments from each animal of all experimental groups. The sections were stained with hematoxylin and eosin (H&E) and examined under 200 magnification using a light microscope by a pathologist blinded to the treatment regimens.

**Statistical analysis**

All data obtained were presented as means ± SEM. Results were analyzed using a one-way analysis of variance test (one-way ANOVA) followed by Tukey’s multiple comparison test. Statistical analysis was performed using Graph Pad Prism software (version 6.04). For all the
statistical tests, the level of significance was taken at $p \leq 0.05$.

**Results**

**Effect on colon length, colon mass index, colon histology, inflammatory and apoptotic biomarkers**

Induction of colitis led to a reduction in rat colon length by approximately 20% (Fig. 1a). This was associated with an 18% increase in colon mass index (Fig. 1b). Histological photomicrographs of control colons showed well-defined crypt lengths and no edema in the mucosa and submucosa (Fig. 1c). However, DSS-treated animals (Fig. 1d) showed loss of epithelial cell and crypt architecture, inflammatory cell infiltration with marked necrosis of the epithelium, and submucosal edema. Treatment with STW 5 (Fig. 1e) largely protected against these histological changes. Furthermore, DSS induced changes in various parameters indicative of inflammation and apoptosis as evidenced by a marked elevation in the colonic content of TNF-α, NFκB (Fig. 2a and b), and caspase-3 level (Fig. 2c). These changes tended to be prevented by treatment with STW 5.

**Effect on intestinal microbiota**

DSS administration led to a 15-fold increase in the Enterococcus population of the Firmicutes phylum whereas the other three representatives of the phylum, namely, Clostridium, Lactobacillus, and Blautia, showed a decrease by 23, 73, and 28% respectively (Fig. 3). All the studied representative members of the Bacteroidetes phylum showed an increase in the UC model, an effect that was largely prevented by the herbal preparation STW 5 except for Bacteroides (Fig. 4). Furthermore, DSS led to a nearly 3-fold increase in the Actinobacteria phylum (Fig. 5), but a 500-fold increase in Bifidobacterium (Fig. 5). DSS administration led to a 20-fold increase in the relative abundance of Proteobacteria, an effect which was significantly reduced to only 3% after STW 5 treatment (Fig. 6). Changes in 16S rRNA DNA showed an increased level of *Methanobrevibacter* by 2.5-fold.

### Table 1 Primer sequences and annealing temperatures for selected analyzed microbial phyla and genera 16S rRNA gene

| Microbial phyla and genera       | 16S rRNA Gene Primer Sequence 5′–3′ | Annealing Temperature (°C) | Reference |
|----------------------------------|-------------------------------------|-----------------------------|-----------|
| **Firmicutes phylum**            | ATG TGG TTT AAT TCG AAG CA          | 60                          | [21]      |
|                                  | AGC TGA CGA CAA CCA TGC AC          |                             |           |
| **Lactobacillus genus**          | GAG GCA GCA GTA GGG AAT CTT C       | 53                          | [22]      |
|                                  | GGC CAG TTA CTA CCT TCA TCC TTC TTC|                             |           |
| **Clostridium genus**            | GCA CAA GCA GTG GAG T               | 50                          | [23]      |
|                                  | CTT CCT CCG TTT TGT CAA             |                             |           |
| **Blautia genus**                | CGG TAC CTG ACT AAG AAG C            | 55                          | [24]      |
|                                  | AGT TTC ATT CTT GCG AAC G            |                             |           |
| **Enterococcus genus**           | CCC TTA TTG TTA GTT GCC ATC ATT     | 61                          | [24]      |
|                                  | ACT CGT TCT TCC CAT GT              |                             |           |
| **Bacteroidetes phylum**         | CAT GTG GTT TAA TTC GAT GAT         | 60                          | [21]      |
|                                  | AGC TGA CGA CAA CCA TGC AG          |                             |           |
| **Bacteroides genus**            | GAG AGG AAG GTC CCC CAC             | 60                          | [21]      |
|                                  | CGC TAC GTT GCT GGT TCA G            |                             |           |
| **Prevotella genus**             | GGT TCT GAG AAG GGT GGC CCCC       | 55                          | [25]      |
|                                  | TCC TGC ACG CTA CCT GGC TG          |                             |           |
| **Actinobacteria phylum**        | CGC GGC CTA TCA GCT TGT TG          | 57                          | [26]      |
| **Bifidobacterium genus**        | CTG CTG GAA AGG GGT GG              | 55                          | [23]      |
|                                  | GGT GTT CTG CCC GAT ATC TAC A       |                             |           |
| **Proteobacteria phylum**        | CAT GAC GTT ACC CGG AGA AGA AG      | 63                          | [27]      |
|                                  | CTC TAC GAG ACT CAA GCT TGC         |                             |           |
| **Fusobacteria phylum**          | CCC TTC AGT GCC GCA GT              | 51                          | [27]      |
|                                  | GTC GCA GGA TGT CAA GAC             |                             |           |
| **Euryarchaeota phylum**         | CCG GGT ATC TAA TCC GGT TC          | 50                          | [28]      |
| Methanobrevibacter genus         | CTC CCA GGG TAG AGG TGA AA          |                             |           |
Fig. 1 Effect of STW 5 treatment on colon length, colon mass index, and histopathological changes, in colonic tissue of rats with DSS-induced colitis. Induction of colitis led to a reduction in colon length A as compared to the vehicle control group and this was associated with an increase in colon mass index B. Normal histological structure of colonic mucosa in normal control rats C. Colon of rats with DSS-induced colitis showing necrosis of epithelium, distortion of crypts, inflammatory infiltrate in lamina propria as well as sub-mucosal edema D. Apparently normal mucosa in the colon of rats with DSS-induced colitis after treatment with STW 5 which tended to prevent these changes E. Data represented as means ± standard deviation of at least two independent experiments with number of animals of at least 14 animals per group.

Fig. 2 Effect of STW 5 treatment on inflammatory and apoptotic biomarkers in colonic tissue of rats with DSS induced UC. TNF-α, A, NFκB B, and caspase-3 C measured by ELISA were significantly elevated in DSS-induced colitis, but this rise was prevented by STW 5 treatment. Data represented as means ± standard deviation of at least two independent experiments with number of animals of 6 animals per group.
(Fig. 7 a) while levels of *Fusobacterium* phylum were not significantly affected by DSS (Fig. 7 b). STW 5 treatment guarded against all the changes induced by DSS.

To gain more insight into the beneficial effects of STW 5 in the DSS model of colitis, it was necessary to show whether the herbal preparation has any effect on the normal microbiota flora or not on its own. The dose of STW 5 (5 ml/kg) have been tested on the microbiota flora in normal rats and was found to be largely insignificant (Supplementary Data).

**Relative abundance of bacterial and archaeal phyla**

To better understand how STW 5 affects the unbalanced microbial community induced by DSS colitis, the relative abundance of bacterial and archaeal phyla was compared between the groups. The graphs illustrate the changes in the relative abundance of different bacterial phyla, such as *Firmicutes*, *Enterococcus*, *Clostridium*, *Lactobacillus*, and *Blautia*, in rats with DSS-induced UC. STW 5 treatment showed a significant normalization of the *Firmicutes* phylum, with a 15-fold increase in the *Enterococcus* population compared to the vehicle control group. On the other hand, *Clostridium*, *Lactobacillus*, and *Blautia* decreased by 1.3, 3.7, and 1.4 folds (23, 73, and 28%), respectively. STW 5 tended to slightly increase these levels. The microbial concentration expressed as ng per gram of feces was calculated taking 16S rRNA gene copy number into consideration as detailed in the Methods section. Data represented as means ± standard deviation of at least two independent experiments with number of animals of at least 14 animals per group.
Fig. 4 Effect of STW 5 treatment on the relative abundance of Bacteriodetes phylum A, Bacteroides B, Prevotella C in rats with DSS induced UC. All members of Bacteriodetes phylum showed a significant increase in DSS-induced colitis model. STW 5 succeeded to significantly reverse these changes. The microbial concentration expressed as ng per gram of feces was calculated taking 16S rRNA gene copy number into consideration as detailed in methods section. Data represented as means ± standard deviation of at least two independent experiments with number of animals of at least 14 animals per group.

Fig. 5 Effect of STW 5 treatment on the relative abundance of Actinobacteria A and Bifidobacterium B in rats with DSS induced UC. Actinobacteria and Bifidobacterium displayed the same pattern of change. DSS induced colitis led to a 2.8 and 486-fold increase in Actinobacteria and Bifidobacterium respectively, an effect which was significantly reversed by STW 5 treatment. The microbial concentration expressed as ng per gram of feces was calculated taking 16S rRNA gene copy number into consideration as detailed in the Methods section. Data represented as means ± standard deviation of at least two independent experiments with number of animals of at least 14 animals per group.
abundance of measured phyla and genera was analyzed as shown in Fig. 8. DSS-induced dysbiosis was evident by changes in the relative abundance of Firmicutes, Fusobacterium and Methanobrevibacter phyla and Lactobacillus, Blautia, and Bacteroides genera. Most of these changes have been resolved by STW 5 treatment, particularly those of Blautia and Methanobrevibacter.

Discussion

Gut microbiota is known to maintain a balance between its members to preserve intestinal integrity by preventing pathogen colonization and initiation of inflammation. Derangement of this balance is associated with the development of ulcerative colitis in man [29]. Furthermore, alterations in function and relative abundance of intestinal bacteria belonging to the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria phyla have been implicated in experimental colitis [30]. As well as changes in Fusobacteria and members of Methanobrevibacter genus [31–33].

In the present study, DSS-induced colitis was associated with variable changes in the different phyla and genera examined. The genera belonging to the Firmicutes showed a five-fold increase in the relative abundance of Enterococcus but a significant decrease in that of some commensal microbiota including Clostridium cluster IV, Blautia, and Lactobacilli. These species owe their commensality to the fact that they produce short-chain fatty acids (acetic, butyric, and lactic acids) as fermentation end-products that have an essential role in the metabolic welfare of colonocytes [34–36]. A decrease
in *Lactobacillus* has been reported in colonic biopsies from patients with active UC [37, 38].

DSS also induced a dramatic increase in *Prevotella* (phylum *Bacteroidetes*), conforming with earlier reports [39]. Another relevant bacterial phylum in IBD is *Actinobacteria* with its genus *Bifidobacterium*. In the present study, *Actinobacteria* and *Bifidobacterium* levels were dramatically increased by DSS similar to previous studies showing increased proportions of *Bifidobacterium* in UC patients [40]. Furthermore, earlier studies showed an increase in the levels of *Poteobacteria* in DSS-induced colitis [7] as well as in *Fusobacterium varium* in the colonic mucosa of UC patients [31]. Our experimental findings showed similar effects regarding *Proteobacteria* but failed to show significant changes in the *Fusobacterium* population.

DSS further raised the relative abundance of *Methanobrevibacter* (phylum *Euryarchaeota*) in accordance with reports that its levels are increased in UC patients and responsible for the bloating and decreased intestinal motility symptoms [41, 42]. STW 5 tended to normalize *Methanobrevibacter* abundance, a fact that might explain the clinically proven efficacy of STW 5 in bloating.

Gut microbiota has been shown to play an important role in intestinal inflammatory conditions, some in initiation and progression of the inflammatory process and some in having an anti-inflammatory effect. For example, *Proteobacteria* has been associated with inflammation in different models of colitis [43, 44] while *Bacteroides vulgatus* has been shown to activate the signaling of NF-κB in the gut epithelial cell culture [45]. However, butyrate which is produced by commensal *Clostridia* inhibits NF-κB activation in gut cells leading to an intestinal anti-inflammatory effect [35]. Furthermore, various strains of *Bifidobacteria* have been shown to exert an anti-inflammatory effect through induction of intestinal IL-10 [40] and treatment with *Bifidobacterium bifidum* was shown to partially protect mice from Th1-driven inflammation in a chemically induced model of colitis [46]. The present findings show indeed that DSS-induced colitis was associated with a marked increase in inflammatory and apoptotic markers such as TNF-α, NFκB, and caspase-3.

STW 5 administration significantly decreased colon inflammation and apoptosis. The anti-inflammatory effect of STW 5 might be attributed in part to decreasing *Proteobacteria* and *Enterococcus* levels and increasing...
Clostridia population, a fact that might help to explain the reduced inflammation and maintenance of the normal bacterial ecosystem. While the administration of STW 5 itself in normal rats had largely insignificant effects on the tested microbiota (Supplementary Data), yet when given to animals with DSS-induced colitis, it significantly decreased the relative abundance of Bacteroidetes and Prevotella and tended to normalize the abundance of both Actinobacteria and Bifidobacterium populations. Furthermore, STW 5 increased the abundance of Bacteroides, Lactobacillus, Clostridium, Blautia as well as Fusobacterium and succeeded to normalize Methanobrevibacter abundance, a fact that might explain the clinically proven efficacy of STW 5 in bloating. It is difficult to ascribe the beneficial effect of STW 5 to any one or more of its active constituents. Earlier studies on its gastroprotective effects showed that each individual component contributes to the overall efficacy, but optimal activity was exerted by their combined effects [47, 48]. The preparation as a whole was also shown to be effective in experimental models of functional dyspepsia [49] and colitis [19] as well as clinically in IBS and FD [15, 16]. It would therefore be reasonable to assume that the effects obtained in the present study are the result of the combined activity of the individual components of the standardized preparation.

Conclusion
DSS-induced colitis was associated with gut microbial dysbiosis, an effect that tends to create a pro-inflammatory milieu, initiating intestinal inflammation. This shift in gut microbial composition included reduced beneficial indigenous microbiota that acts to maintain epithelial health. Treatment with STW 5 showed anti-inflammatory and antiapoptotic effects and favorably affected the intestinal microbiota by decreasing bacteria that contribute to intestinal inflammation as Proteobacteria and Prevotella and increasing bacteria with anti-inflammatory properties as Bifidobacteria and Lactobacilli. The results provide an additional novel mechanism of action underlying the beneficial effect of using STW 5 in gastrointestinal disorders.

Abbreviations
CD: Crohn’s disease; DSS: Dextran sodium sulfate; FD: Functional dyspepsia; FGID: Functional gastrointestinal diseases; GID: Gastrointestinal diseases; IBD: Inflammatory bowel diseases; IBS: Irritable bowel syndrome; NFkB: Nuclear factor kappa B; TNF-alpha: Tumor necrosis factor-alpha; UC: Ulcerative colitis

Supplementary Information
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Authors’ contributions
NFA, WW, HA, SR, RMA, LAA, and MTK contributed conception and design of the study; SSM, NFA, and WW performed the experiments; SSM and NFA performed the statistical analysis; SSM, NFA and MTK wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Availability of data and materials
The raw data generated during the current study are available from the corresponding author upon request.

Declarations
Ethics approval and consent to participate
Animal experimental procedures were approved by the institutional Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University, Cairo, Egypt, approval number (PT 1769) following the guidelines laid out in the Guide for the Care and Use of Laboratory Animals, National Academy of Science.

Consent for publication
Not applicable.

Competing interests
The authors RMA, SR and HA were employed by Bayer Consumer Health, Darmstadt, Germany. They were kind enough to consent to the proposed study presented to the company and to help in revising the manuscript. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References
1. M’koma AE. Inflammatory bowel disease: an expanding global health problem. Clin Med Insights Gastroenterol. 2013;6:33–47. https://doi.org/10.4137/CGast.S12731.
2. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. World J Gastroenterol. 2014;20(1):91–9. https://doi.org/10.3748/wjg.v20.i1.91.
3. H. Laroui, S. A. Ingersoll, H. C. Liu, M. T. Baker, S. Ayyadurai, M. A. Charania, F. Larou, Y. Yan, S.V. Sitaraman, D. Merlin, Dextran sodium sulfate (DSS) induces colitis in mice by forming nano-lipocomplexes with medium-chain-length fatty acids in the colon, PLoS One (2012), 7, e32084, 3, doi:https://doi.org/10.1371/journal.pone.0032084.
4. Cammarota G, Ianiro G, Cianci R, Bibbò S, Gasbarrini A, Currò D. The involvement of gut microbiota in inflammatory bowel disease

Additional file 1: Supplementary data. Effect of STW 5 (5 mL/Kg) on the microbiome in healthy rats. Microbial population concentrations are expressed as g, mg, μg, ng, or pg per gram of feces (indicated for each microbial population) and calculated taking 16S rRNA gene copy number into consideration as detailed in methods section. Data represented as means 5 ± standard deviation.
pathogenetic potential for therapy. Pharmacol. Ther. 2015;149:191–212. https://doi.org/10.1016/j.pharmthera.2014.12.006.
5. Du Z, Hudcovic T, Mazej K, Kozakova H, Sruclkova D, Schwarzer M, et al. Development of gut inflammation in mice colonized with mucosa-associated bacteria from patients with ulcerative colitis. Gut Pathog. 2015;7(1):32. https://doi.org/10.1186/s13099-015-0080-2.
6. Tannock G. Molecular analysis of the intestinal microflora in IBD. Mucosal Immunol. 2008;1(5):515–8. https://doi.org/10.1038/mi.2008.54.
7. Munyaka PM, Rabbi MF, Khafipour E, Ghia JE. Acute dextran sulfate sodium (DSS)-induced colitis promotes gut microbial dysbiosis in mice. J Basic Microbiol. 2016;56(9):986–98. https://doi.org/10.1002/jobm.201500726.
8. Stanghellini V, Chan KL, Hasler WL, Malagelada JR, Suzuki H, Tack J, et al. Gastrointestinal Disorders. Gastroenterology. 2016;150(6):1380–92. https://doi.org/10.1053/j.gastro.2016.02.011.
9. Quigley EM. Bugs on the brain; brain in the gut–seeking explanations for common gastrointestinal symptoms. Ir J Med Sci. 2013;182:1–6.
10. Shin A, Preidis GA, Shulman R, Kashyap PC. The gut microbiome in adult and pediatric functional gastrointestinal disorders. Clin Gastroenterol Hepatol. 2019;17:256–74.
11. Tap J, Derrien M, Törnblom H, Brazeilles R, Cools-Portier S, Doré J, et al. Identification of an intestinal microbiota signature associated with severity of irritable bowel syndrome. Gastroenterology. 2017;152(1):111–23. https://doi.org/10.1016/j.gastro.2016.09.049.
12. Hold GL, Pryde SE, Russell VI, Furrie E, Flint HJ. Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. FEMS Microbiol Ecol. 2002;39:133–5. https://doi.org/10.1111/j.1574-6941.2002.tb00904.x.
13. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7401):207–14. https://doi.org/10.1038/nature11234.
14. Collins SM. A role for the gut microbiota in IBD. Nat Rev Gastroenterol Hepatol. 2014;11(8):497–505. https://doi.org/10.1038/nrgastro.2014.40.
15. Allescher HD, Wagner H, STW Silbergost: multi-target-action for treatment of functional dyspepsia and irritable bowel syndrome. Wien. Med. Wochenschr. 2007;157(13–14):301–7. https://doi.org/10.1007/s10055-007-0429-3.
16. Oettlinger B, Storr M, Malfertheiner P, Allescher HD. STW 5 (Iberogast®) — a safe and effective standard in the treatment of functional gastrointestinal disorders. Wien Med Wochenschr. 2013;163:65–72.
17. Kroll UJ, Cordes C. Pharmaceutical prerequisites for a multi-target therapy. Phytomedicine. 2006;13:12–9. https://doi.org/10.1016/j.phymed.2006.03.016.
18. Wegener T, Wagner H. The active components and the pharmaceutical multi-target principle of STW 5 (Iberogast®). Phytomedicine. 2006;13:20–35. https://doi.org/10.1016/j.phymed.2007.06.001.
19. Wedde W, Abdel-Aziz H, Zaki HF, Kelber O, Weiser D, Khayyal MT. STW 5 is effective in dextran sulfate sodium-induced colitis in rats. Int'l Colorectal Dis. 2012;27(11):1443–9. https://doi.org/10.1111/j.1472-4732.2012.02432.x.
20. Viennois E, Chen F, Larou H, Baker MT, Merlin D. Dextran sodium sulfate inhibits the activities of both polymerase and reverse transcriptase: lithium chloride purification, a rapid and efficient technique to purify RNA. BMC Res Notes. 2013;6:360.
21. Guo X, Xia X, Tang R, Zhou J, Zhao H, Wang K. Development of a real-time PCR method for Firmicutes and Bacteroidetes in faeces and its application to quantify intestinal population of obese and lean pigs. Lett App Microbiol. 2008;37(5):367–73. https://doi.org/10.1111/j.1365-2765.2008.02048.x.
22. Delcroix JM, Boulvin AI, Parminter M, Dauphin RD, Vandenbol M, Portetelle D. Quantification of Bifidobacterium spp. and Lactobacillus spp. in rat fecal samples by real-time. PCR Res. 2008;16:663–70.
23. Matsuki T, Watanabe K, Fujimoto J, Takada T, Tanaka R. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. Appl Environ Microbiol. 2004;70(12): 7220–8. https://doi.org/10.1128/AEM.70.12.7220-7228.2004.
24. Rinttilä T, Kaisinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rRNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol. 2004;97(6):1166–77. https://doi.org/10.1111/j.1365-2672.2004.02409.x.
25. Bekele AZ, Koike S, Kobayashi Y. Genetic diversity and diet specificity of ruminal Prevotella revealed by 16S rRNA gene-based analysis. FEMS Microbiol Lett. 2010;30:49–57.
dependent manner. Anaerobe. 2017;47:209–17. https://doi.org/10.1016/j.anaerobe.2017.06.002.

46. Philippe D, Heupel E, Blum-Sperisen S, Riedel CU. Treatment with
Bifidobacterium bifidum 17 partially protects mice from Th1-driven
inflammation in a chemically induced model of colitis. Int J Food Microbiol.
2011;149(1):45–9. https://doi.org/10.1016/j.ijfoodmicro.2010.12.020.

47. Khayyal MT, El-Ghazaly MA, Kenawy SA, Sel-h-Nasr M, Mahran LG, Kafafi YA,
et al. Antiulcerogenic effect of some gastrointestinaly acting plant extracts
and their combination. Arzneimittelkorsch. 2001;51(7):545–53. https://doi.
org/10.1055/s-0031-1300078.

48. Khayyal MT, Seif-El-Nasr M, El-Ghazaly MA, Okpanyi SN, Kelber O, Weiser D.
Mechanisms involved in the gastro-protective effect of STW 5 (Iberogast)
and its components against ulcers and rebound acidity. Phytomedicine.
2006;13(Suppl 5):56–66.

49. Abdel-Aziz H, Wadie W, Zaki HF, Müller J, Kelber O, Efferth T, et al. Novel
sequential stress model for functional dyspepsia: Efficacy of the herbal
preparation STW5. Phytomedicine. 2015;22(5):588–95.

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