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Pharmacological Reports

An improved rat model for chronic inflammatory bowel disease

Short title: Chronic inflammatory bowel disease in rats

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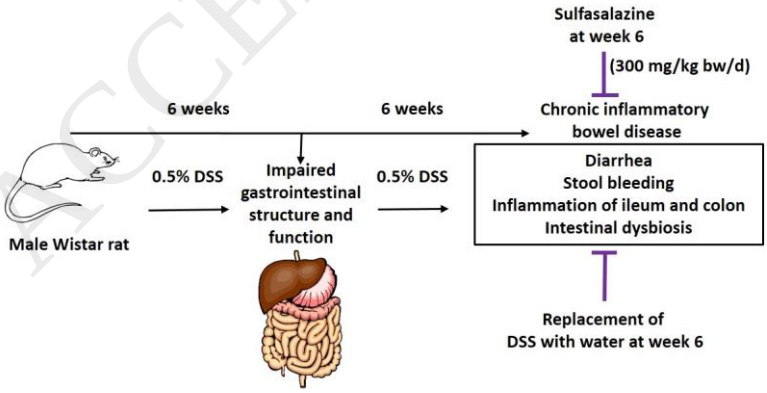
Graphical abstract



Abstract

Background: Inflammatory bowel disease (IBD) is an important cause of chronic disability in humans.

Methods: We characterized a model of chronic IBD in young male Wistar rats by administering dextran sodium sulfate (DSS: 0%, 0.25%, 0.5%, or 1% in drinking water) for six weeks, with 0.5% DSS for twelve weeks, following DSS cessation or together with treatment with sulfasalazine for the last 6 weeks. We measured gastrointestinal characteristics including stool consistency, blood in stools, small intestine and colon length, intestinal transit and permeability, and gut microbiota, as well as extra-intestinal parameters including oral glucose tolerance, systolic blood pressure, fat and lean mass, and left ventricular stiffness.

Results: At 6 weeks, 0.25-1% DSS produced gastrointestinal changes as diarrhea and blood in stools. At 12 weeks, 0.5% DSS produced chronic and sustained gastrointestinal changes, with marked infiltration of inflammatory cells throughout the gastrointestinal tract and crypt distortion. Firmicutes increased and Bacteroidetes and Actinobacteria decreased in DSS-treated rats. Changes were reversed by DSS cessation or sulfasalazine treatment. Gastrointestinal permeability and extra-intestinal parameters did not change, so DSS changes were limited to the gastrointestinal tract.

Conclusion: Chronic 0.5% DSS produces selective and reversible gastrointestinal changes, providing an improved chronic model in rats that mimics human IBD for testing new interventions.

Keywords**:** Dextran sodium sulfate; inflammatory bowel disease; rat; sulfasalazine; methods

Introduction

Inflammatory bowel disease (IBD) in humans is an uncontrolled chronic gastrointestinal inflammation that gradually worsens and can last for decades with many relapses; symptoms include weight loss, bloody diarrhea, and severe abdominal pain [1]. Animal models of IBD are generally classified as either spontaneously-induced, chemically-induced, genetically-modified, or adoptive transfer models [2]. The most commonly used rodent model for IBD is the chemical-induced model that uses dextran sodium sulfate (DSS) in the drinking water.

The advantages of DSS-induced IBD model over others are that it is inexpensive, easy to induce the disease, reproducible, and control the onset, duration, and severity of the disease can be controlled by varying the dose, duration, frequency, molecular weight of DSS, strain, and microbiome of the animal that can lead to acute, chronic, or relapsing IBD models [3, 4]. No animal model is an exact representation of the complex human IBD; however, compared to other models, DSS-induced rodent IBD closely mimics human IBD by causing bloody diarrhea, intestinal epithelial damage, increased intestinal permeability, and altered gut microbiome [3]. The limitation of DSS treatment as a model for the human disease is that this chemical is not a cause in human nor it does not require T or B cell responses [5]. DSS has been used at concentrations up to 5% for one week in acute models or 4-8 weeks in chronic models [3]. However, these protocols do not present a stable disease state, and interventions are usually for prevention rather than reversal. This differs from clinical treatment in humans, which started with sulfasalazine and now emphasizes anti-cytokine agents [6, 7].

We hypothesized that low chronic dosage of DSS in rats will mimic the symptoms, if not the causes, of human IBD. The aim of our study was to determine whether low-dose DSS produces chronic IBD in rats with stable and selective gastrointestinal changes that mimic human IBD and can be reversed by an intervention used in humans with IBD. We have

characterized treatment with different DSS doses and determined whether DSS-induced changes were restricted to the intestine by measuring gastrointestinal permeability and transit, cardiovascular, liver, and metabolic parameters. We propose that chronic low-dose DSS-induced stable IBD in rats can be used to test potential interventions for human IBD.

Materials and methods

Ten groups of male Wistar rats (8-9 weeks old, 338±1 g, n=96) fed standard laboratory chow were used for three studies. Study 1: Rats were randomly divided into four groups (n=10) for six weeks and given DSS (molecular weight: 36,000-50,000 Da, MP Biomedicals) in drinking water: Group 1, no DSS; group 2, 0.25%; group 3, 0.5%; group 4, 1% DSS. Study 2: Rats in two groups (n=12) were treated for twelve weeks: Group 5, 0.5% DSS; group 6, 0.5% DSS for 6 weeks then 0% DSS for 6 weeks. Study 3: Rats in four groups (n=8) were treated for twelve weeks: Group 7, no DSS; group 8, no DSS with sulfasalazine (4.6g/kg food) for final 6 weeks; group 9, 0.5% DSS; group 10, 0.5% DSS with sulfasalazine as in group 8. Rats had free access to food and water, were individually housed with temperature control (21±2°C), 12-hour light-dark conditions, and monitored daily for body weight, and food and water intakes.

Rat stools were examined daily to assess the onset and progression of IBD. Stool consistency score was defined as 0-formed, 1-mild-soft, 2-very soft, and 3-watery soft (diarrhea). Stool bleeding score was defined as 0-normal color, 1-brown color, 2-reddish color, and 3-bloody red [8].

Dual-energy X-ray absorptiometry, non-invasive systolic blood pressure, abdominal circumference, oral glucose tolerance test, plasma measurements, isolated Langendorff heart preparation, organ weights, and organ bath study were performed [9]. Two hours before euthanasia, rats in study 3 were deprived of food and gastrointestinal transit using charcoal

solution was measured with administration of 0.1mL of a 10% charcoal solution in 5% gum arabic/10g bodyweight by oral gavage. At euthanasia, the furthermost point the charcoal had moved from the pyloric sphincter was determined. The upper gastrointestinal tract motility was estimated as a percentage of the travelled distance to the total length from the pyloric sphincter to the ileocecal junction [10]. Small and large intestinal lengths were measured. For the organ bath study, the distal ileum and distal colon (~1.5 cm) were separated and concentration-response (contraction) curves were recorded with acetylcholine (Sigma-Aldrich). Histology of ileum and colon and the gut microbiota diversity profiling were analyzed [11]. To determine gastrointestinal permeability, urine analysis for sugars was performed using GCMS Shimadzu TQ8040. The initial oven temperature was set at 200°C for 1 minute, then increased at 10°C/minute to 250°C, 1°C/minute to 260°C, 3°C/minute to 275°C and held for 2 minutes, 15°C/minute to 300°C and held for 1 minute. The MS detector ion source temperature was 250°C and the interface temperature was 280°C. Cumulative percent recovery of each sugar and intestinal permeability were calculated [12]. The detailed protocol is given in supplementary file.

Data are expressed as mean ± standard error of the mean (SEM). Results were analyzed using one-way ANOVA with Neumann-Keuls multiple comparison *post hoc* test; $p<0.05$ was considered as significant. Statistical analyses were run using GraphPad Prism version 5 for Windows (GraphPad Software, USA).

Results

Study 1: DSS dose-dependent changes over 6 weeks

DSS produced dose- and time-dependent increases in scores for stool consistency and blood in stools (Fig. 1A and 1B). Control rats maintained normal stool texture and color whereas 1% DSS rats had the most marked changes with watery bloody stool, with occasional

anal bleeding. DSS treatment had no effect on small intestinal length but there was a dose-dependent reduction of colon length in DSS groups (Table 1) with dose-dependent changes in the epithelial membrane and crypt architecture (Fig. 2). In the colon and ileum of 0.5% DSS and 1% DSS groups, there was marked loss of epithelial layer and branched crypts, atrophy of crypts and mucosa, reduction of villi length and crypt height, along with inflammatory cell infiltration (Fig. 2C, 2D, 2G, and 2H). Rats treated with 0.25% DSS had only minimal inflammation (Fig. 2B and 2F) while the control rats had healthy tissue (Fig. 2A and 2E).

DSS rats had higher intake of food, water, and energy than control rats (Table 1). However, DSS produced dose-dependent decreases in feed conversion efficiency which was reflected as reduced body weight gain in 1% DSS rats (Table 1). DSS rats showed no differences in metabolic parameters such as concentrations of fasting blood glucose, plasma triglycerides, and plasma non-esterified fatty acids (data not shown); however, plasma total cholesterol was higher in 0.5% DSS rats and blood glucose area under the curve was increased in DSS groups compared to control group (Table 1). Only 0.5% DSS rats had increased systolic blood pressure (Table 1). Heart function (diastolic stiffness) and organ weights were unchanged except for the increased spleen weight in 1% DSS rats (Table 1). Plasma alanine transaminase activity remained unchanged with DSS treatment (Table 1). As 1% DSS produced severe inflammation, a lower dose of 0.5% DSS was chosen with an extended protocol of 12 weeks to examine whether this dose produced reversible, stable, and moderate chronic IBD in rats.

Study 2: DSS-induced changes over 12 weeks

The stool consistency and bleeding scores showed similar changes in groups 3 (6 weeks) and 5 (12 weeks; Fig. S1A and S1B). Group 5 had reduced colon length (Table S1). The ileum and colon of rats of group 5 had marked mucosal inflammation, epithelial membrane loss, forked crypts, and crypt abscesses (Fig. S2A, S2C, and S2E–S2H).

Body weight gain and feed efficiency were higher in rats of group 5 with no change in food, water, or energy intakes (Table S1). The abdominal fat pads decreased in these rats while fat mass, lean mass, and bone mineral density were increased (Table S1). Metabolic parameters such as concentrations of fasting blood glucose, plasma triglycerides, and non-esterified fatty acids were unchanged (data not shown) but blood glucose area under the curve and at 120 minute blood glucose were lower in rats of group 5 (Table S1). Diastolic stiffness, weights of LV, RV, and spleen were unchanged in group 5 compared to group 3 while systolic blood pressure and liver weight were decreased in group 5 (Table S1). Plasma alanine transaminase activity was unchanged in group 5 (Table S1).

The stool characteristics improved with replacement of 0.5% DSS with water for the last 6 weeks in group 6 (Fig. S1A and S1B). Group 6 had reduced colon shortening (Table S1). The inflammation of ileum and colon was reversed in group 6 (Fig. S2B and S2D). Replacement of 0.5% DSS with water did not alter the contractile responses to acetylcholine in isolated ileum and colon preparations and did not alter gastrointestinal permeability (data not shown).

Body weight gain after replacement of 0.5% DSS with water decreased with no change in food, water, or energy intakes (Table S1). Abdominal fat pads, total lean mass, and bone mineral density did not change in group 6 while decreased total fat mass was observed (Table S1). Metabolic parameters such as concentrations of fasting blood glucose, plasma triglycerides, and non-esterified fatty acids were unchanged in group 6 (data not shown). Diastolic stiffness, systolic blood pressure, weights of LV, RV, liver, and spleen were unchanged in group 6 compared to group 5 while plasma alanine transaminase activity was increased compared to group 5 (Table S1).

Study 3: Sulfasalazine on 0.5% DSS-induced changes

The doses of sulfasalazine based on food intake were 346±23mg/kg/day in group 8 rats and 350±36mg/kg/day in group 10 rats. The stool consistency and stool bleeding scores of group 8 rats were minimal and similar to group 7 rats (Fig. 1C and 1D). The small intestinal length increased in group 8 rats compared to group 7 rats, however, colon length and gastrointestinal transit were unchanged (Table 2). The ileum and colon showed healthy mucosal layer in group 8 rats as in group 7 rats (Fig. 2I, 2J, 2M, and 2N). The isolated ileum and colon preparations of groups 7 and 8 rats gave similar maximal contractile responses to acetylcholine (ileum: group 7, 27.3±4.9mN, group 8, 28.9±5.4mN; colon: group 7, 64.9±8.8mN, group 8, 63.9±15.9mN).

In group 10 rats, sulfasalazine improved stool consistency compared to group 9 rats while normalizing stool bleeding scores (Fig. 1C and 1D). The small intestinal length, colon length, and gastric transit were unchanged in group 10 rats compared to group 9 rats (Table 2). Sulfasalazine improved the ileum of group 10 rats with increased villi length and decreased inflammation compared to group 9 rats (Fig. 2K and 2L), while the colon of group 10 rats showed higher crypt numbers, increased crypt heights, and improved epithelial membrane compared to group 9 rats (Fig. 2O and 2P). The isolated ileum preparations of group 10 rats gave higher maximal contractile responses to acetylcholine than group 9 rats whereas the colon preparations showed no changes between the groups (ileum: group 9, 19.9±4.1mN; group 10, 44.5±6.1mN; colon: group 9, 68.2±7.8mN, group 10, 61.8±11.7mN). Urinary sucrose concentration was unchanged at 3 hours and 6 hours and decreased at 9 hours in all groups (Fig. S3). Mannitol and sucralose concentrations decreased at 6 hours for sulfasalazine-treated groups and at 9 hours, concentrations of both sugars further decreased with no difference between groups. Mannitol indicates small intestine permeability and sucralose indicates colon permeability so sulfasalazine improved the ileum and colon membrane permeability. However, the cumulative percent recovery of sucrose, mannitol, and sucralose at 3, 6, 9, 21, and 24 hours,

and whole gut permeability indicator ratio of sucralose/mannitol were unchanged with sulfasalazine treatment (Fig. S3).

Group 8 rats had no difference in body weight gain, intake of food, water, and energy, or feed efficiency compared to group 7 rats (Table 2). Group 8 rats had decreased abdominal fat pads compared to the group 7 rats (Table 2). There were no changes in blood glucose concentrations (data not shown) or weights of left ventricle and liver (Table 2) in group 8 rats while decreases in right ventricle and spleen weights were observed (Table 2). Sulfasalazine resulted in group 10 rats with no difference in body weight gain, intake of food, water, and energy, or feed efficiency compared to group 9 rats (Table 2). Group 10 rats had abdominal fat pads similar to rats with 0.5% DSS for 6 or 12 weeks (Table 2). Blood glucose concentrations (data not shown) and organ weights remained unchanged in group 10 rats (Table 2).

Gut microbiota

Treatment with 0.5% DSS for either 6 or 12 weeks increased abundance of Firmicutes and decreased abundance of Bacteroidetes and Actinobacteria compared to control rats (Fig. 3). Replacement of DSS with water for the last 6 weeks reversed these changes (Fig. 3). At genus level, DSS induced decreases in *Bifidobacterium* and *S24-7*, which are major representatives of phylum Actinobacteria and Bacteroidetes in this study (Fig. 4). Changes in these two genera were reversed with replacement of DSS with water for the last 6 weeks (group 6; Fig. 4). No other genus showed significant differences between control and DSS rats.

Discussion

This study showed that 0.5% DSS for 6 or 12 weeks in male Wistar rats produced stable, moderate, chronic, and reversible IBD symptoms based on stool characteristics and histological

examination. The intestinal permeability was not affected by 0.5% DSS for 6 or 12 weeks. However, dysbiosis was observed with increases in Firmicutes and decreases in Actinobacteria and Bacteroidetes, mainly due to decreases in *Bifidobacteria* and *S24-7*. Extra-intestinal effects on glucose tolerance, blood pressure, cardiac and liver function, body weight, and bone mineral parameters were minimal. To further characterize our model, we demonstrated that a reversal protocol with removal of DSS or treatment with sulfasalazine normalized the stool characteristics, allowed repair of the gut epithelial membrane with reduced inflammation and reversed changes in gut microbiota. We propose that 0.5% DSS for 12 weeks produces a chronic IBD model in male rats with clinical, physiological, morphological, histological, and dysbiosis symptoms similar to chronic human IBD that can be reversed by treatment with sulfasalazine.

The concentration and duration of DSS administration determine the severity of the symptoms [5]. An improved model of low-dose DSS-induced IBD in rats was necessary because high-dose DSS at concentrations of 2% and greater in the drinking water for short periods of 5-7 days induced acute severe IBD in mice and rats [5, 13-15]. Further, most studies tested prevention of IBD by interventions rather than reversal of symptoms [3]. These studies may not be relevant to IBD patients who have existing chronic disease which requires long-term treatment to increase remission times. The advantage of our model is that it produced stable gastrointestinal inflammation allowing the testing of potential therapeutic agents such as nutraceuticals [7] in a reversal protocol for comparison with standard drugs such as sulfasalazine. DSS models have advantages over genetically modified or spontaneously induced models as these models are suitable for the small group of IBD patients with a similar susceptible gene as in the genetically modified models. Other chemical models of IBD include intervention with 2,4,6-trinitrobenzene sulfonic acid (TNBS)/ethanol, oxazolone/ethanol or acetic acid which involve intrarectal administration of the chemical agents as enemas and result

in acute IBD in 2-6 days, with TNBS-induced changes resembling Crohn's disease and oxazolone or acetic acid-induced changes resembling ulcerative colitis affecting distal colon [16]. However, in our model non-invasive administration of non-lethal dose of DSS in drinking water was less stressful and produced chronic IBD affecting both ileum and colon closely representing human IBD.

DSS causes intestinal inflammation extending from the rectum towards the distal colon and further to the rest of the colon [17]. There was colon repair and re-epithelization with squamous epithelium following replacement of 0.5% DSS in drinking water with normal water as observed in mice [4]. Though the exact mechanism of action of DSS-induced gut inflammation is unclear, DSS causes loss of epithelial barrier integrity and leads to disruption of epithelial membrane which is further aggravated by apoptosis and reduced rate of cell renewal thereby leading to reduced colon length [18]. This compromised epithelial barrier in DSS rats is consistent with the changes in gut structure and function in our study. DSS decreases the stool consistency leading to diarrhea and hematochezia due to the disruption of intestinal membrane [3]. This leads to movement of gut microbiota from the intestinal lumen to the crypts causing inflammation due to excess immune reactions; these symptoms in rats mimic human IBD [19].

Increased intestinal permeability is observed in IBD patients due to the disruption of tight junctions in the intestinal epithelial membrane, and there is leakage of LPS or its binding protein into the systemic circulation triggering the immune system to secrete inflammatory cytokines leading to systemic inflammation [20]. However, we did not observe changes in intestinal permeability in our model. We consider that the DSS-induced insult is not severe making this a suitable model for testing reversal interventions. There is increased epithelial cell apoptosis and redistribution of tight junctions in DSS-induced IBD rats and the increased

intestinal permeability could be a cause or effect of inflammation and therefore dependent on the severity and extent of inflammation [21, 22]. Decreased intestinal motility is observed in severely inflamed intestine of IBD patients and also DSS-induced colitis mice model with the presence of macrophages or mast cells in the intestinal muscular layer [23] but was not observed after 12 weeks of 0.5% DSS, suggesting that this change occurs later in the disease progression. Sulfasalazine-treated DSS-induced colitis rats had normal colonic contractility and this could be due to inhibition of NF-κB that causes dampened activity of L-type calcium channels leading to gut inflammation [24]. However, there was no change in colonic contractility in our study but there was increased ileal contractility in DS rats and L-type calcium channels are involved in rat ileum contraction as well [25]. In contrast, an *in vitro* study on guinea pig ileum showed that sulfasalazine inhibited contractile response due to bacterial N-formyl-methionyl oligopeptides by antagonism of enteric cholinergic (M1) receptors [26].

In normal gut, Firmicutes and Bacteroidetes are the most abundant phyla followed by Actinobacteria and Proteobacteria [27]. During dysbiosis in IBD, decreases in Firmicutes and Bacteroidetes are observed with increases in Proteobacteria and Actinobacteria [27]. *B. pseudolongum* has high adherence to human intestinal cells with gut protective effects due to its anti-oxidant and anti-inflammatory activity [28-30]. Decreases in *S24-7* were responsible for changes in the gut function in a colitis model [31]. In our study, *Bifidobacterium* species and *S24-7* species were decreased with DSS intervention while these changes were reversed with replacement of DSS with water. In previous studies, decline of *Lactobacillus* impaired the gut barrier leading to invasion of bacteria and inflammation [32]. Further, *Streptococcus* species increased in Iranian IBD patients in active and remission stages [33]. The mucolytic bacteria *Ruminococcus gnavus* predominated in Crohn's disease and ulcerative colitis patients compared to healthy individuals [34]. We did not see major changes in these species in our

model. Sulfasalazine modulated gut microbiota in rats with TNBS-induced colitis with decreased Proteobacteria and Enterococcus, and increased bacteria that produce short-chain fatty acids [35].

Extra-intestinal parameters changed to a minor extent in our IBD model but obesity could aggravate IBD [36]. Hyperplasia of fat around inflamed intestines in Crohn's disease may allow colonization and translocation of intestinal bacteria but we found reduced omental fat in DSS rats [37]. This topic needs further research as few have studied the link between visceral adipose tissue and IBD. The plasma lipid inflammatory markers were normal in IBD children unlike in IBD adults [38]. Further, IBD patients with prolonged disease may be prone to arthropathies and osteoporosis [39]. Increased cardiovascular disease, especially during IBD episodes, has been reported [40], but IBD patients did not show hypertension and diabetes [41]. Abnormality of liver functional tests was transient in IBD patients and returned to normal [42], similar to our study. Absence of extra-intestinal abnormalities in our model indicates that there is no systemic inflammation, which may only occur after a much longer period of disease progression.

The major limitations of our study are firstly, that while DSS is the causative agent in this model, the cause of IBD in humans is unknown and therefore the causative factor was not mimicked in this study. Secondly, while we have characterized functional and structural changes, the molecular changes were not investigated. Thirdly, we have not investigated all possible extra-intestinal changes that occur in IBD patients, for example arthropathies or ankylosing spondylitis [39]. Fourthly, the increased risk of cardiovascular disease and endothelial dysfunction in humans with IBD [40], possibly due to chronic systemic inflammation following increases in intestinal permeability, was not shown in this 12 week intervention in rats.

Thus, treatment with 0.5% DSS in this study produced a relatively stable gastrointestinal disease state that can be tested for possible reversal interventions such as nutraceuticals [7], functional foods or new drugs, which could then be further tested in human clinical trials. Positive trials may eventually lead to an enhanced life-style for IBD patients.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

N.K.R.G., S.K.P., and L.B. developed the original study aims. N.K.R.G. conducted the experiments. N.K.R.G., S.K.P., and L.B. analyzed and interpreted the data; N.K.R.G. and S.K.P. prepared manuscript drafts. L.B. has been the corresponding author throughout the writing process. All authors read and approved the final manuscript.

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Figure legends

Fig. 1. Effect of dextran sodium sulfate (DSS) and sulfasalazine on stool characteristics. Stool consistency (**A**) and bleeding (**B**) in groups 1-4. Stool consistency (**C**) and bleeding (**D**) in rats from groups 7-10 as C, CS, D, and DS, respectively. Values are mean±SEM, n=8-10. Endpoint means without a common letter differ, $p < 0.05$.

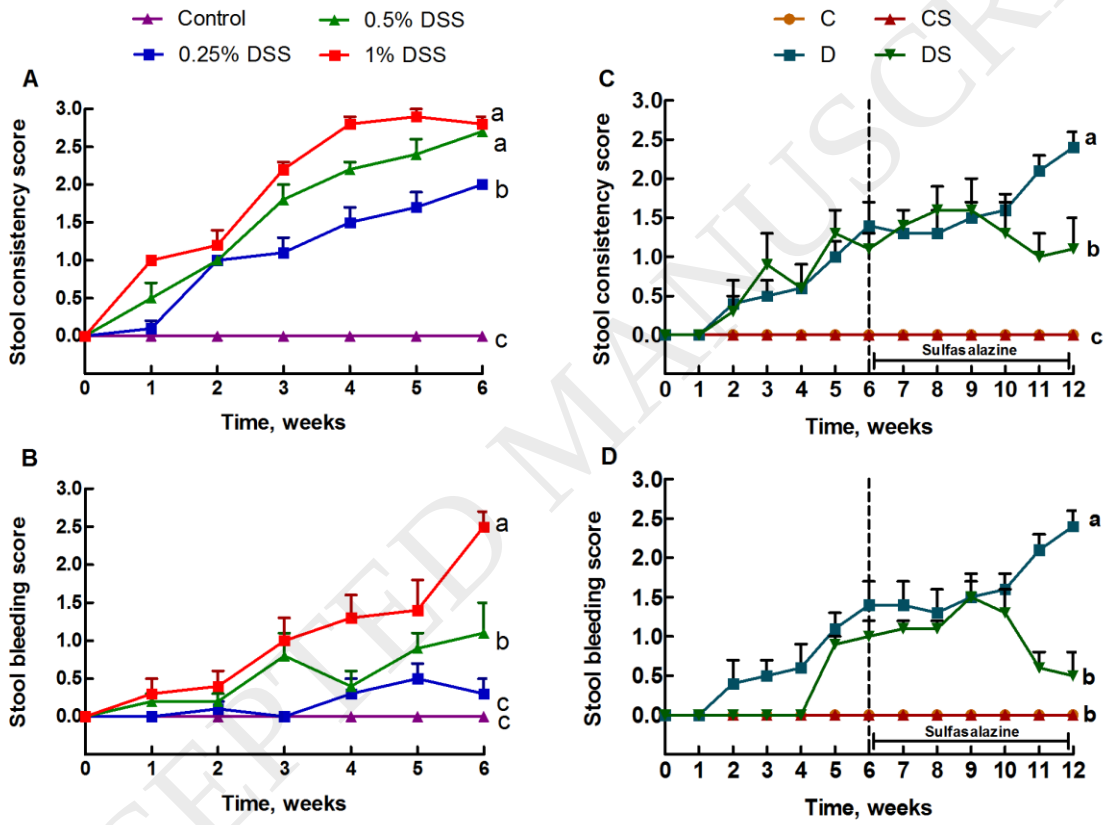

Fig. 2. Effect of dextran sodium sulfate (DSS) and sulfasalazine on inflammation in the intestine. Hematoxylin and eosin staining of ileum and distal colon showing infiltration of inflammatory cells "in" (**C, D, G, H, J,** and **N**), epithelial disruption "ed" (**C, D, G, H, J,** and **N**), crypt distortion "cd" (**C, D, G, H, J,** and **N**), branched crypt "bc" (**H**), and mucosal atrophy "ma" (**C, D, G, H, J,** and **N**) (×20) (n=4). Ileum of rats of group 1 (**A**), group 2 (**B**), group 3 (**C**), and group 4 (**D**). Colon of rats of group 1 (**E**), group 2 (**F**), group 3 (**G**), and group 4 (**H**).

Ileum of rats of group 7 **(I)**, group 8 **(J)**, group 9 **(K)**, and group 10 **(L)**. Colon of rats of group 7 **(M),** group 8 **(N)**, group 9 **(O)**, and group 10 **(P).**

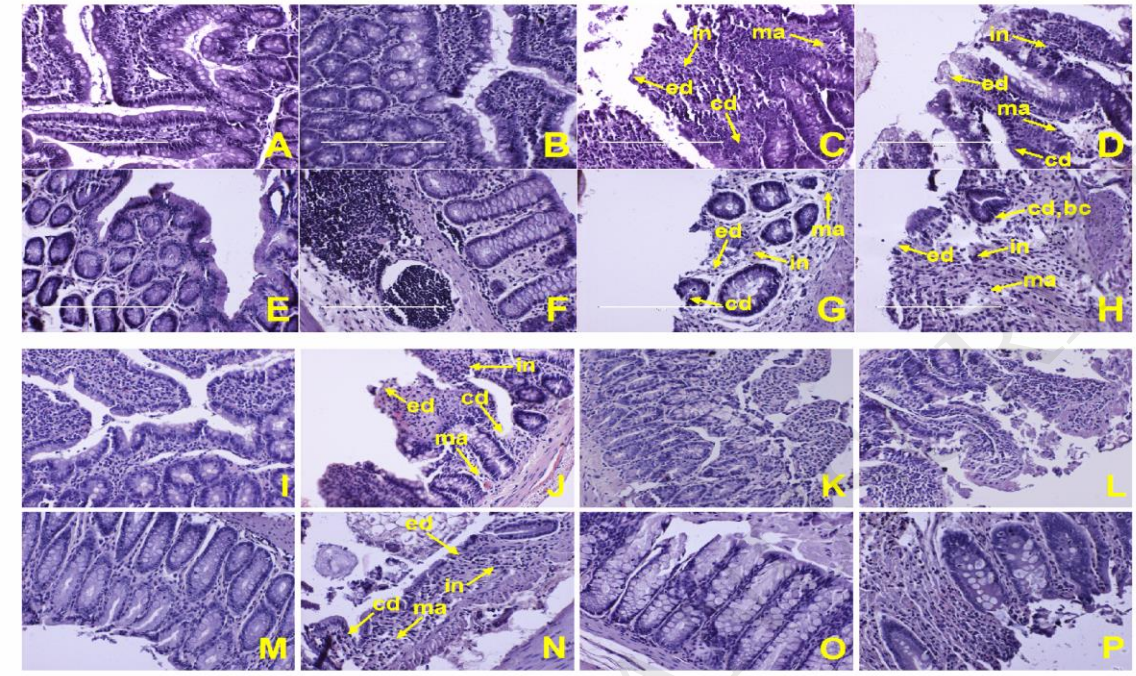

Fig. 3. Effects of DSS on relative abundance of gut microbiota at phylum level in groups 1, 3, 5, and 6, n=6/group.

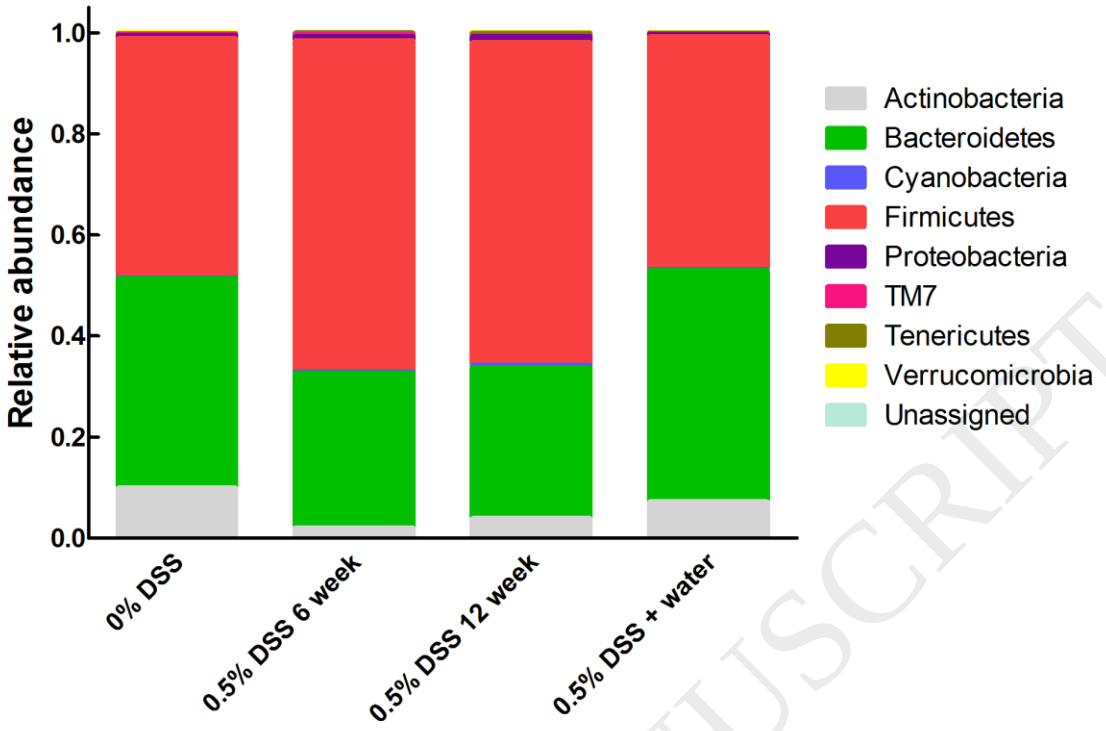

Fig. 4. Effects of DSS on relative abundance of gut microbiota at genus level in groups 1, 3, 5, and 6, n=6/group.

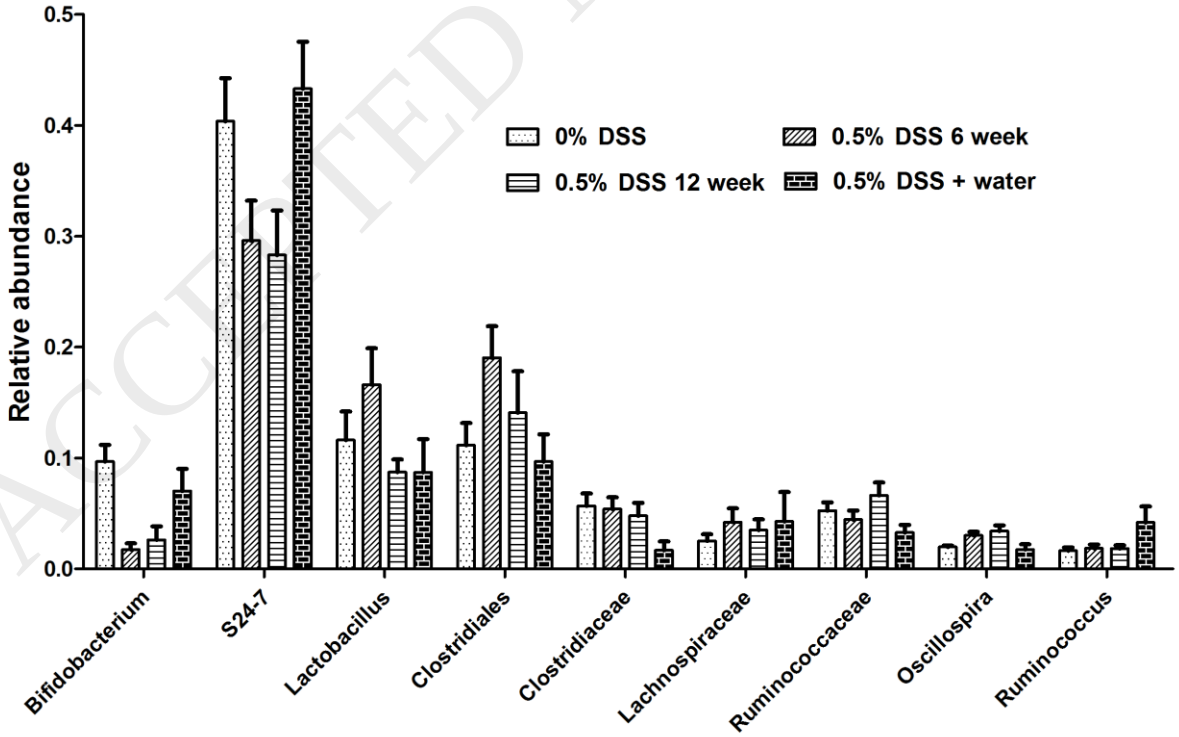

Table 1. *Metabolic, cardiovascular, hepatic, and gastrointestinal parameters in rats treated with dextran sodium sulfate (DSS) (0%, 0.25%, 0.5%, or 1%) for 6 weeks*.

Variables	***0% DSS***	***0.25% DSS***	***0.5% DSS***	***1% DSS***
Small intestinal length, cm	124±1	114±6	126±3	124±2
Colon length, cm	24.4±0.5^{a}	19.7±0.6^{b}	20.2±0.5^{b}	18.1±0.6^{c}
Body weight gain, g	155±6^{a}	166±7^{a}	168±8^{a}	133±7^{b}
Food intake, g/d	31.9±0.4^{b}	39.5±1^{a}	39.5±1.3^{a}	38.2±1^{a}
Water intake, g/d	49.1±3.2^{b}	53.7±2.8^{ab}	64.1±4.0^{a}	62.1±4.8^{ab}
Energy intake, kJ/d	440±5^{b}	544±14^{a}	545±18^{a}	528±13^{a}
Feed efficiency, g/KJ	0.35±0.01^{a}	0.31±0.01^{a}	0.31±0.02^{a}	0.25±0.01^{b}
Abdominal fat pads, mg/mm tibial length	575±36	636±51	704±68	536±40
Total fat mass, g	137±8	158±18	152±16	111±11
Total lean mass, g	332±10	318±10	308±16	325±12
Bone mineral density, g/cm^{2}	0.168±0.003	0.172±0.003	0.165±0.003	0.165±0.003
Blood glucose 120 minutes, mmol/L	5.5±0.2	6.1±0.3	6.1±0.3	5.6±0.2
Area under the curve, mmol/L×minutes	721±15^{b}	860±29^{a}	810±25^{a}	804±30^{a}
Plasma total cholesterol, mmol/L	1.1±0.1^{b}	1.2±0.1^{b}	1.6±0.1^{a}	1.4±0.1^{ab}
Systolic blood pressure, mmHg	124±3^{b}	126±1^{b}	136±3^{a}	133±2^{ab}
LV wet weight, mg/mm tibial length	22.0±0.8	22.7±1.1	20.5±3.5	21.9±0.5
RV wet weight, mg/mm tibial length	4.75±0.31	4.78±0.35	4.62±0.17	4.12±0.14
Diastolic stiffness (κ)	26.8±1.8	25.7±1.3	22.8±2.1	26.7±1.5
Liver wet weight, mg/mm tibial length	352±17	357±22	390±11	369±8
Plasma alanine transaminase activity, U/L	26.1±6.2	20.5±2.6	19.1±1.9	22.1±2.2
Spleen wet weight, mg/mm tibial length	23.9±0.8^{b}	23.8±1.0^{b}	25.0±1.1^{b}	30.0±2.1^{a}

All values are mean ± SEM, n = 6-10. Mean values within a row with a different superscript are significantly different, $p<0.05$.

Table 2. *Metabolic and gastrointestinal parameters in rats treated with sulfasalazine*

Variables	***C***	***CS***	***D-6***	***D***	***DS***
Small intestine length, cm	114±3^{b}	129±2^{a}	129±2^{a}	120±2^{ab}	127±4^{a}
Colon length, cm	22.6±1.4^{a}	22.9±0.3^{a}	22.9±0.3^{ab}	18.9±0.9^{b}	18.4±1.0^{b}
Gastrointestinal transit, %	76.8±5.2	86.5±3.5	-	86.6±3.2	87.6±4.4

Body weight gain, g*	92±7.9	68.1±6.4	-	88.9±5.7	71.8±5.5
Food intake, g/d*	36.8±1.4	38.9±1.7	-	36.7±2.1	38.7±2.3
Water intake, g/d*	59.9±5.9	53.1±4.3	-	53.1±3.2	66.2±6.5
Energy intake, kJ/d*	511±30	534±34	-	503±36	542±54
Feed efficiency, g/kJ*	0.18±0.01	0.13±0.01	-	0.18±0.02	0.14±0.01
Abdominal fat pads, mg/mm	944±76^{a}	567±72^{b}	704±69^{b}	775±44^{ab}	675±66^{b}
Blood glucose 120 minutes, mmol/L	5.4±0.1	5.2±0.3	6.1±0.3	5.7±0.2	5.8±0.2
Area under the curve, mmol/L×minutes	727±18	725±21	810±25	740±9	767±31
LV wet weight, mg/mm tibial length	23.5±0.9	21.7±0.8	20.5±3.5	22.4±0.7	21.4±0.7
RV wet weight, mg/mm tibial length	6.53±0.61^{a}	4.53±0.3^{b}	4.62±0.17^{b}	5.56±0.41^{ab}	4.74±0.42^{b}
Liver, mg/mm tibial length	403±19	347±17	390±11	379±12	347±7
Spleen, mg/mm tibial length	27.3±1.3^{a}	22.0±1.4^{b}	25.0±1.1^{ab}	25.5±1.3^{ab}	24.2±0.7^{ab}

All values are mean ± SEM, n = 6-8. Mean values within a row with a different superscript are significantly different, $p<0.05$. C, group 7; CS, group 8; D-6, group 3; D, group 9; DS, group 10; * mean values calculated for the intervention period with sulfasalazine (weeks 7-12).