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The Beneficial Responses of Sodium Butyrate against DSS-Induced Colitis Mediated by Interleukin -10 Overexpression and Gut Microbiota in Rats

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Abstract: Colitis can have a negative impact on an animal's growth and productivity as well as its health. Animals who consume dextran sulfate sodium (DSS) may develop colitis. The aim of the inquiry was to assess the beneficial effect of butyric acid as one of the gut microbiota metabolites on the inflammatory response and physiology of the colon in DSS-induced colitis. Rats were distributed equally into four distinct sets. At the final stage of the trial, blood samples were obtained to measure total white blood cells, red blood cells, and interleukin 17A, interferon-gamma in serum. Colon tissue was also gathered for histopathology. In addition, the spleen's interleukin 10 gene was identified. Following sodium butyrate treatment, data analysis revealed a considerably lower total number of white blood cells. While sodium butyrate therapy statistically raised the number of red blood cells. Sodium butyrate drastically dropped serum interleukin -17A, interferon-gamma values. Also, the Section of the colon D group showed severe thickening of mucosa-associated with the marked proliferation of submucosal solitary lymphatic follicles, with expansion into tubular glands. Further, we discovered that oral sodium butyrate treatment reduced the severity of colitis in rats, modified the diversity of the microbiota in the gastrointestinal tract so that *pseudomonas aeruginosa* predominated in the combination of drugs group, and elevated the expression of the interleukin 10 gene. These findings suggest that sodium butyrate, by reestablishing the equilibrium of gut microbiota dysbiosis, can alleviate DSS-induced colitis in rats.
Keywords: DSS-inducted colitis, Sodium Butyrate, *pseudomonas aeruginosa*, IL-10 gene, IL-17

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Introduction

Dextran sulfate sodium (DSS) delivery is a standard technique for inducing ulcer development and inflammation in animals. DSS technically has no direct impact on intestinal irritation; instead, it damages the intestinal epithelium chemically, exposing the lamina propria and submucosal section to luminal antigens and enteric microorganisms and causing inflammation to occur there. (1). Butyrate has a variety of biological effects that have been documented. For instance, butyric acid caused biochemical and morphological division

in a variety of cells, which had the simultaneous effect of suppressing neoplastic characteristics (2, 3). Recent research demonstrated that butyrate could activate colonic regulatory T cells

(Treg) which are crucial for the inhibition of inflammation and allergy reactions (4). Butyrate has been suggested as a norepinephrine reuptake inhibitor and also effectively regulates mitochondrial function, improving oxidative phosphorylation and beta-oxidation, among other things (5). Thus, we measured the sodium butyrate's ability to alleviate DSS-induced colitis by detecting cytokines and colon bacteria enrichment**.**

Materials and methods

Ethical approval

The University of Baghdad's College of Veterinary Medicine was given permission to carry out this scientific inquiry in book No. 2083/P. G

Handling of the experimental animals

Fouty mature male rats were used, weighing between 200 and 210 g and being 8 to 9 weeks old, were utilized. regulated temperature, humidity percentage, and light period were all typical in housing. The rats endured two weeks of conditioning prior to the study acclimatization. Throughout the whole experiment, the animals received free access to commercial pellets and water.

Study design

Rats were administered 200 mg/kg BW of sodium butyrate (SB) (6). DSS was given to rats at 1% in tap water (7) For nine days, both medications were taken daily. The animals were divided into four groups, each consisting of ten animals: group 1 served as the control group; Group 2 received sodium butyrate (SB) at a dose of 200 mg/kg per day; Group 3 received DSS 1% in tap water; and group 4 received both sodium butyrate and DSS in tap water.

Sample gathering and laboratory examinations

Rats were given intramuscular injections of a combination of ketamine 60 mg/kg BW and xylazine 40 mg/kg BW to put them unconscious at the conclusion of the research (26). To examine hematological parameters blood was taken from the heart (25)

Interleukin-17A -17A, interferon-gamma was quantified in accordance with the manufacturer's instructions using commercially available ELISA kits (Elabscience). After blood was drawn, colon samples were taken, preserved in buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin for examination under a microscope. To verify the expression of the interleukin -10 gene, the spleen was removed (20).

Results and discussion

A thorough measurement of blood

Figure 1-A is a bar graph representing the WBC count due to the inflammatory upshot caused by DSS. As shown in the bar graph. Group C and SB are relative in count, showing insignificant variation thus labeled as normal, and contradictory, Group D showed intense elevation $P<0.05$ in number, as disclosed. Group SB+D shows a slight increase in statistics; however, it is prodigious to know the noxious effect of DSS in vivo.

Figure 1-B in relation to groups C, and SB similar to WBC count shows proportionate results, Group D showed a distinct decline in RBC dramatically $P<0.05$, due to the pernicious DSS substance. Conceivably group SB+B shows an inconsequential decrease in RBC, alike and in comparison, to groups C, and SB.

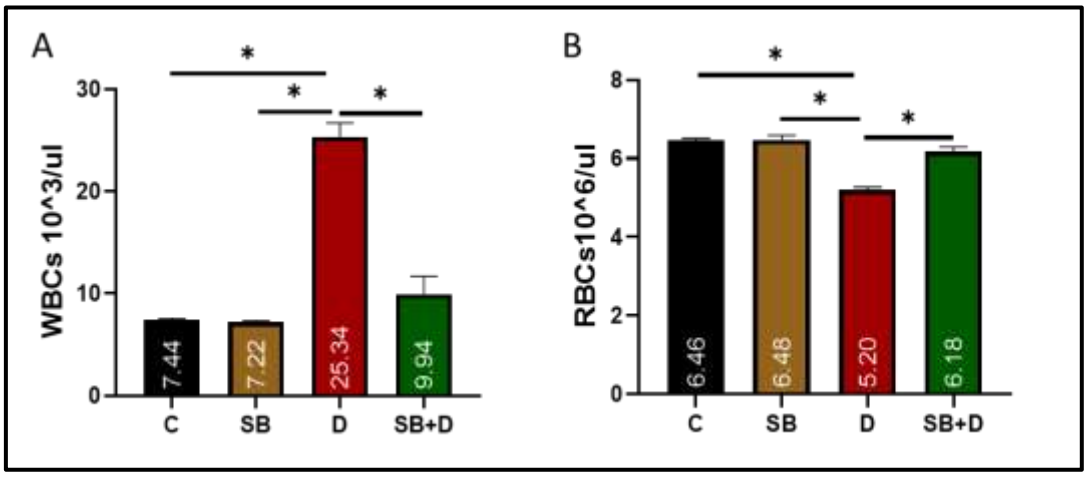

Figure (1): Sodium butyrate's impact, DSS, and the two coupled on
(A) total white blood cell count((×10^3µL)
(B) total red blood cell count (×10^6µL), in male rats. Mean±SEM, n=10. C, naïve rats; SB, oral administration to rats sodium butyrate; D, rats received DSS in tap water; SB+D, oral administration to rats sodium butyrate and DSS in tap water. *P<0.05.

Detection interleukin -17A and IFN-γ in serum

When looking at group C in Figure 2 A, a miniature amount of interleukin -17A is observed, the same goes for the SB group. Whilst looking at the D group; a towering increase is recorded statistically P0.05. Chiefly group SB+D shows a typical uplift, inconsiderable to be of any danger; and could be perceived as an average uprise. In panel 2 B IFN-γ level was elevated dramatically in group D related to SB+D, SB, and C groups. Group SB+D showed a significant rise compared to group SB and C concentration.

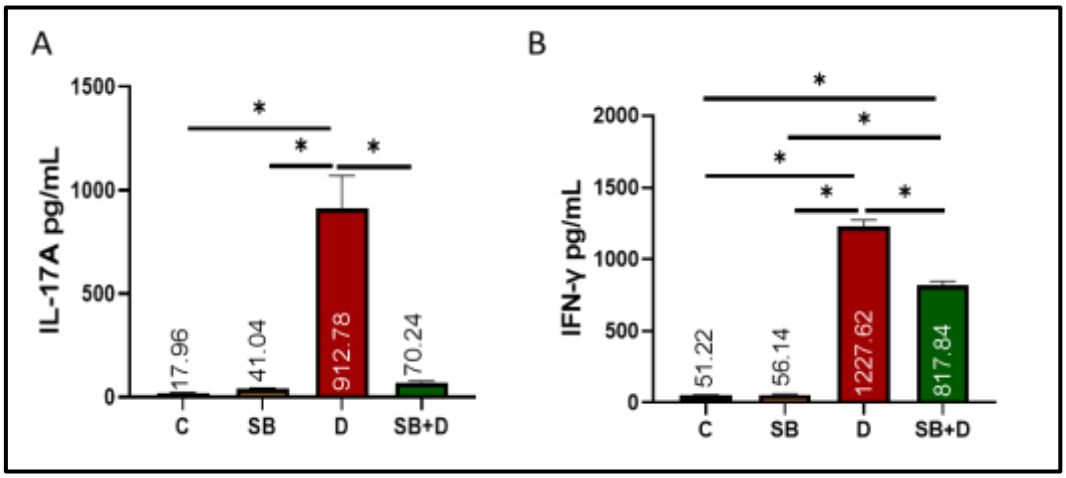

Figure (2): Sodium butyrate's impact, DSS, and the two coupled on
(A) Interleukin -17A in male rats.
(B) interferon-gamma. Mean±SEM, n=10. C, naïve rats; SB, oral administration to rats sodium butyrate; D, rats received DSS in tap water; SB+D, oral administration to rats sodium butyrate and DSS in tap water. *P<0.05.

Measuring gene expression of interleukin -10

Figure (3) is a bar graph representing interleukin-10 Inwardly, Group C has the average expression as to what is expected, undoubtedly the more interleukin -10 present the more control the Immune reaction are qualified, and groups SB and SB+D show an elevated enumeration $P<0.05$, with or without the detrimental induction of DSS the SB elevates the interleukin -10 above the C group making the colon less exposed to the damaging outcome of the DSS, proven when observed in group D, DSS governance shows a low and decreasing value of interleukin -10 making the body immunity more viable and accessible to inflammation and essentially colitis.

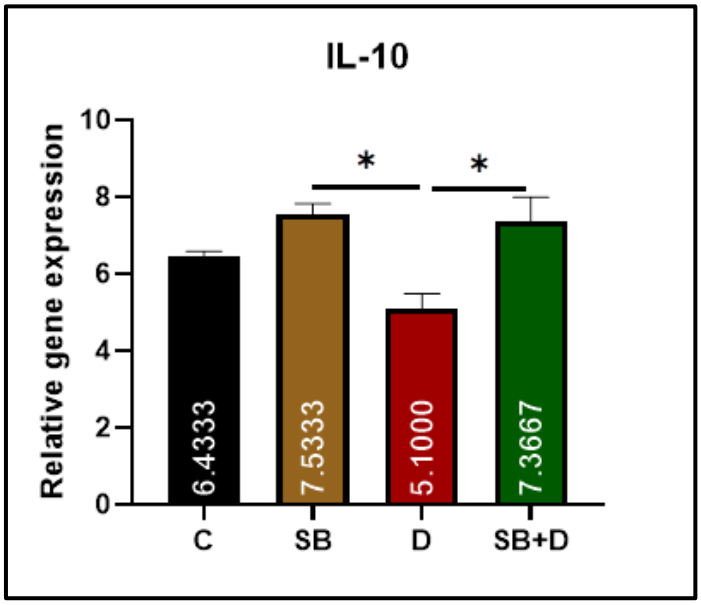

Figure (3) Sodium butyrate's impact, DSS, and the two coupled on IL-10 gene expression in male rats. Mean±SEM, n=10. C, naïve rats; SB, oral administration to rats sodium butyrate; D, rats received DSS in tap water; SB+D, oral administration to rats sodium butyrate and DSS in tap water. *P<0.05.

Histopathological examination of the colon

Figure (4) Panel A: section of colon (c) showed normal mucosa l fold (F), normal tubular glands (Arrows), and muscularis (Asterisk). panel B: section of colon (SB) showed normal mucosal folds with normal glands (Red arrows) and normal epithelial crypts (Black arrows). Panel C: section of colon (D) showed severe thickening of mucosa-associated with the marked proliferation of submucosal solitary lymphatic follicles (Asterisk) with expansion into tubular glands (Arrows). Panel D: The section of the colon (SB+B) showed moderate degeneration with necrosis and tissue depletion of some mucosal folds (Asterisk).

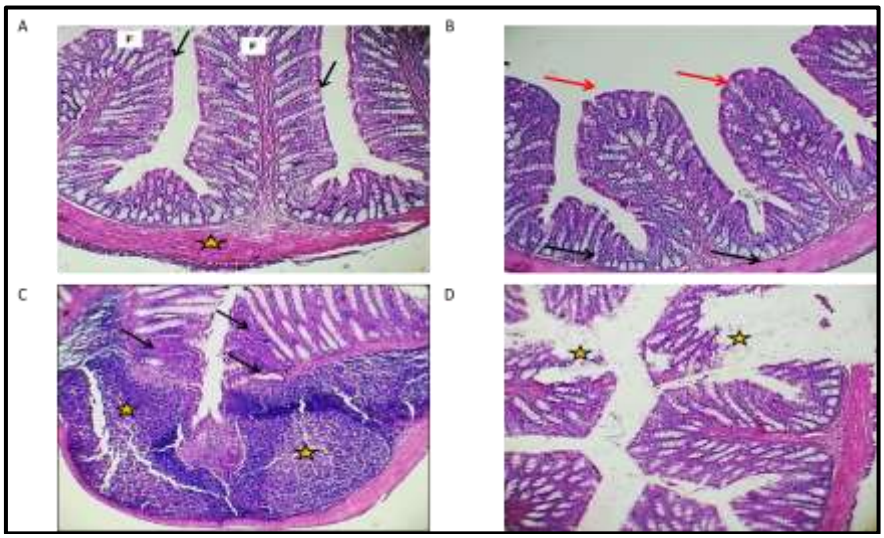

Figure (4): Examples of histopathological pictures of the colon (A) Control animal exhibited no obvious damage in the colon's cellular structure. (B) When sodium butyrate is administered orally, rats' colonocytes are normal and exhibit no pathogenic alterations, (C) administration to rats 1% of DSS severe thickening of mucosa-associated with the marked proliferation of submucosal solitary lymphatic follicles, (D) rats orally given rats sodium butyrate at 200 and DSS 1% in tap water showing showed moderate degeneration. H&E, 40×

Bacterial additional activities in the colon

Data in (Figure 5) represents the percentage of bacteria in different groups of the experiment in group C the percentage of *Serratia fonticola* is 40% and *Kocuria kristinae* is 40%. In SB group *Pseudomonas aeruginosa* is 50% and *Kocuria kristinae* is 20% and, *Serratia fonticola is 20%*. In the D group, *Klebsiella pneumonia* is dominant. The percentage of Pseudomonas aeruginosa is 60% and the percentage of Actinobacter baumannii is 30%. Unidentified bacteria had a very low percentage in the C, SB, and SB+D groups.

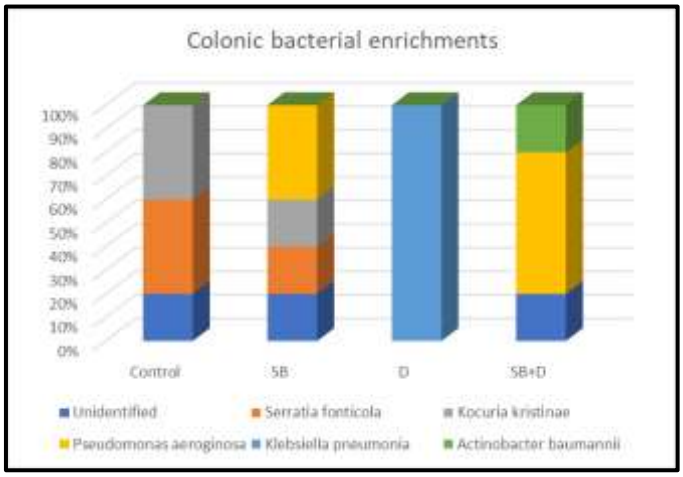

Figure (5): Sodium butyrate's impact, DSS, and the two coupled on bacterial enrichment in male rats. Mean±SEM, n=10. C, naïve rats; SB, oral administration to rats sodium butyrate; D, rats received DSS in tap water; SB+D, oral administration to rats sodium butyrate and DSS in tap water

Sodium butyrate (SB) is an ameliorative agent, and as per (the American Chemical Society) (8). SB is purported to enhance gut health in humans and many other animal species. Dextran sodium sulfate (DSS) is a potent polymer of sulfated polysaccharides, continuous administration of DSS to study the induction (acute) phase of intestinal injury, which in vivo causes colitis (21). In addition, WBC, which corresponds with the subject matter, is compatible. To determine the mechanism by which dextran sulfate sodium (DSS) causes colitis and tumors in animals, the effects of DSS on intestinal epithelial cells and intraepithelial lymphocytes were examined (29). Greater quantities and timeframes result in enhanced cytotoxicity targeting intestinal epithelial cells, and virgin gastrointestinal lymph nodes (9). DSS lethality tends to be focused attention, time frame, and cell type-based (28). DSS was infused into the drinking water of wild-type and knockout mice, and a considerable rise in CD45+ leukocyte infiltration into the colon was seen (10). This is referred to as accelerated leukocyte infiltration in knockout mice after DSS administration (11). The research found that people with ulcerative colitis frequently suffer from bleeding, such as bloody stools, and as a result, they are losing these red blood cells (22). Hemoglobin stimulates the differentiation of anti-inflammatory macrophages with reparative function when hemorrhage occurs in injured tissues (12). Others have found that commonly in colitis Inflammation may contribute to increased red cell distribution width levels not only by disrupting the iron metabolism, but also by shortening the life of red blood cells, inhibiting the erythropoietin response, or inhibiting erythropoietin production(24). Mucosal bleeding is one of the pathological features of inflammatory bowel diseases. IL (interleukin), a cytokine that is commonly understood, is best known for its ability to induce immunological and inflammatory responses (13, 27). The interleukin 17A gene generates the interleukin-17A protein. Interleukin-17A used to be referred to as CTLA8 in rodents because of its resemblance to a viral gene (14) assessed that; The activation of the NFκ-B and mitogen-activated protein kinase signaling pathways by the microbial peptidoglycan binding to MDP results in the production of number of cytokines, including TNF and interleukin-1 (30). TNF-α, a marker of DSS-induced colitis, IL-6, IL-17, IL-1, TGF-α, mucin, TLR2/4 gene expression, and MPO activity are just a few of the several inflammatory mediators assessed. There were variations in inflammatory profiles between the acute and chronic DSS stages. Additionally, compared to individuals on day 7, the levels of inflammation and damage in pathological portions decreased after 14 days. The levels of interleukin, interleukin -17A, interleukin -6, and C-reactive protein (CRP) varied daily and peaked in the mornings. Interleukin-10 is an anti-inflammatory cytokine that keeps the immune system in check and promotes infection clearance with the least amount of host-damaging effects (15) (23). Detecting whether interleukin -10 could leave an immune cell's DNA permanently altered, offering persistent protection against colitis, because our mouse model causes transcription of the IL-10 transgene in the bone marrow and higher service interleukin -10 content(16). Demonstrate that this was untrue and that the sole time interleukin -10

induction was associated with protection against DSS-mediated colitis. Thus, new approaches are needed, especially to offer long-lasting protection, regardless of the protection provided by interleukin -10 in colitis. In our results, we identified Pseudomonas aeruginosa as the dominant bacteria in the combination group, In addition to a class of peptides known as pyocins and other heterocyclic substances like quinolines, phenylpyrroles, and phenazines, *Pseudomonas aeruginosa* strains also create substances having antibacterial characteristics (17). In the disease group, *Klebsiella pneumoniae* is dominant. *Klebsiella pneumoniae* and ulcerative colitis have a synergistic connection. *Klebsiella pneumoniae* can cause colitis by releasing interleukin 18 in gut epithelial cells and urinary tract (18, 19).

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