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Original Article

Dextran Sulfate Sodium Effectiveness As Inflammatory Bowel Disease Inducer In BALB/c Mice

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ABSTRACT

Background: Inflamatory Bowel Disease (IBD) in animal model could be induced by chemical agents such as dextran sulfate sodium (DSS), trinitrobenzene sulfanic acid and oxazolone. The inflammation induced by DSS gave many clinical symptoms and immnulogic reactions like in human. In the recent study, the disease activity index has been assessed on the BALB/c mice that induced by DSS 2% and 3%, the result was no significant result. Therefore, DAI score was not necessarily describe the intestinal tissue real condition, so the researcher want to continue assessing DSS influence to the microscopic features of BALB/c mice intestine and colon.

Methods: Nine male BALB/c mice 6-8 weeks, weight 25-40 g divided in 3 groups. Group I as control, while group II and III induced by 2 cycles of 2% DSS for 5 days followed by drinking water for 10 days and 3 cycles of 3% DSS for 7 days followed by drinking water for 7 days. Assesment of DSS effectiveness by microscopic examination of intestine and colon to observe inflammatory features.

Results: . The microscopic features of group II and group III mice intestine and colon has no inflammatory features as control group. Oral admission DSS 2% and DSS 3% did not establish microscopic changes in BALB/c mice intestine and colon, so it was not effective as mice IBD inducer.

INTRODUCTION

Inflammatory bowel disease (IBD) consist of two type disease that is ulcerative colitis (UC) and crohn's disease (CD).[1] IBD is an idiopathic disease and unclear pathogenesis.[2] Based on IBD molecular assessment, IBD pathogenesis related to both genetic and environmental factors.[3] Dysregulated response of innate and adaptive immune system have been proven associated with IBD. Besides that loss of tolerance to commensal bacteria, disrupted mucosal barrier, an increase in inflammatory mediators and oxidative stress were also related with IBD.[4,5]

Various animal models of IBD were used to investigate the pathogenesis and identify new drugs. Gastrointestinal inflammation experimental models have been developed and grouped into 5, namely: chemically induced experimental animal models, immunology animal models, spontaneous animal models, gene knock out animal models, and transgenic experimental animal models.[4,6] In animal models of gastrointestinal inflammation, the chemically induced experimental animal model is the most frequently used for research. The chemicals inducing gastrointestinal inflammation in experimental animal models are Dextran Sulfate Sodium (DSS), 2,4,6-Trinitro Benzene Sulfonic Acid (TNBS), Dinitro Benzene Sulfonic Acid (DNBS), Oxazolone, Acetic Acid, Carrageenan, Indomethacin, and Iodoacetamide. Chemically induced gastrointestinal inflammation in mouse models shows histopathological and immunological features of UC that are similar to humans. The proper selection of chemical induced animal models will provide the precise understanding of pathophysiology. Chemically induced gastrointestinal inflammation in animal models using DSS is widely used in research. DSS-induced IBD can mimic the clinical symptoms and immunological reactions of IBD.[6] Experimental animal modeling of gastrointestinal inflammation is influenced by many factors, including the selection of model types and research standards becoming an essential factor for interpreting the results. Until now, the experimental animal model can not provide an exact condition of the complexity of gastrointestinal inflammation in humans.[7] Administering 3-5% DSS in drinking water for 7 days and followed by drinking water for 7 days in BALB/c mice were able to induce chronic colitis after several cycles of DSS.[8]

The aim of the present study was to examine the animal model of ulcerative colitis in BALB/c mice induced by dextran sulfate sodium then assessing microscopical changes of mice intestine and colon.

METHOD

Animal Model

The male BALB/c mice were obtained from PT. Biomedical Technology Indonesia, Bogor. Experimental animals that match the inclusion criteria were male mice aged 6-8 weeks with weight of 17.2 - 23.1 g and the exclusion criteria were mice with indigestion, which was characterized by changes in the amount of eating and drinking, breathing patterns and vomiting during the acclimatization period for 14 days. Mice were placed in a cage that was maintained at a temperature of 22 ± 2 ºC and normal humidity, adequate lighting with a 12 hour light/dark cycle, providing husks as sleeping mats, and feeding and drinking tap ad libitum. All of the research procedure was done with the approval and supervision of Commission on Ethics and Animal Welfare (ACUC) No: R.04-20-IR.

DSS-induced IBD in Animal Model

Nine male BALB/c mice 6-8 weeks, weight 25-40 g divided in 3 groups. Group I (control) was given drinking water only and group II induced by 2 cycles of 2% DSS for 5 days followed by drinking water for 10 days, and group III induced by 3 cycles of 3% DSS for 7 days followed by drinking water for 7 days. Fresh DSS (Sigma-Aldrich code 42867, Mr~40,000) was made every 2 days, administered to groups II and III ad libitum. The induction of intestinal inflammation in this study was carried out to form chronic inflammation.

Assessment of Intestine and Colon Microscopic Features of Animal Model

Mice intestine and colon tissue was taken at the termination of the experimental animals and placed in 10% solution formalin. The tissue is assessed macroscopically and microscopically by making it paraffin embedded, and cutting tissue with a thickness of 4 microns.

The preparations were stained with Hematoxyllin and Eosin (HE) and evaluated sequentially histology, and the parameters assessed were inflammatory cell infiltration, crypt depletion, reduction of goblet cells, ulcers and edema formation. This study used an assessment scheme for the chemical induction of colonic inflammation appears in table 1 for the DSS group.[9,10]

Histomorphological assessment of experimental animal models of IBD is diffuse inflammation in the sub/mucosa colon, aggregation of lymphocytes and plasma cells in basal crypts (basal plasmacytosis), flat mucosa, erosion and ulceration, goblet cells disappear, crypts distortion, crypts disappear and wall thickening. For optimal diagnosis andclassification of IBD, a histology examination of the colon is carried out in the intestinal section namely: 1 part of the rectum, 4 parts of the colon (descending colon, transverse colon, ascenden, and cecum), and 1 section of terminal ileum and each section consists of 2 biopsies

Terminating Animal Model

The termination of experimental animals was carried out in group II on day 31, while groups I and III on day 42 by cervical dislocation.

Table 1. Frequency distribution of Stage I UTB values with the national average

Inflammatory cell infiltration		**Score 1**	**Colon Appearance**		**Score 2**
Severity	**Extent**		**Epithelial changes**	**Mucosa appearance**	
Mild	Mucosa	1	Focal erosion		1
Moderate	Mucosa and submucosa	2	Erosion	Focal ulceration	2
Marked	Transmural	3		Extended ulceration + granulation tissue + pseudopolyps	3

RESULT AND DISCUSSION

In the study, the experimental animal group was evaluated for microscopic changes of intestine and colon after day 31 on group II and day 42 on group I and III. The results showed that there was no inflammatory cell infiltration, epithelial or mucosal changes in the form of erosion or ulceration. (Figure 1-3)**.**

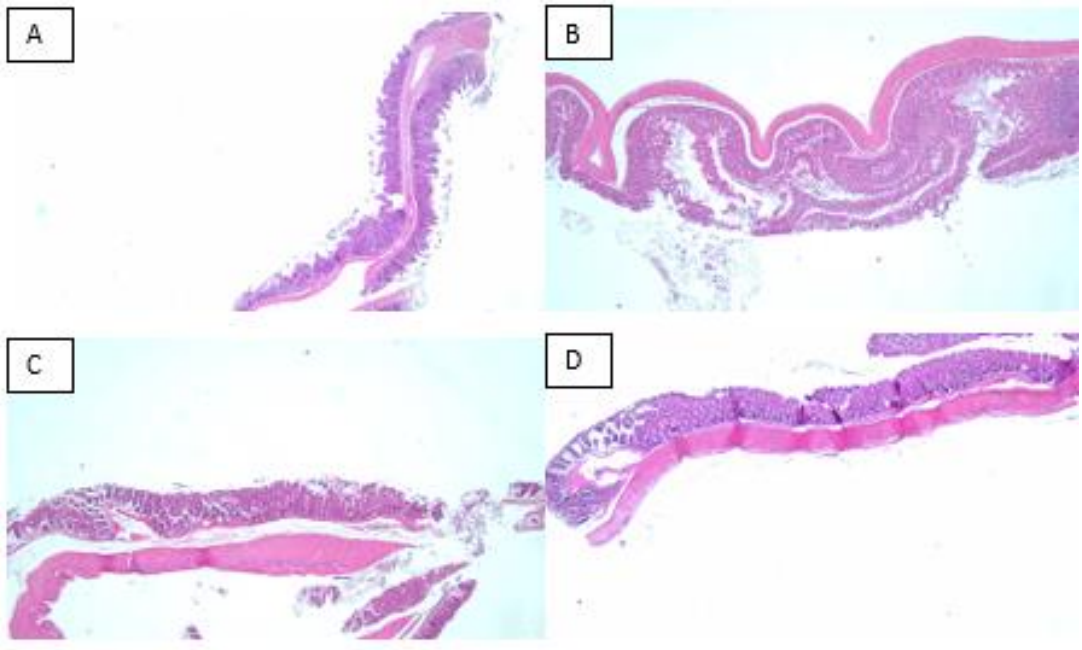

Figure 1. Representative images of H&E-stained colon sections of Group I BALB/c mice (control group) illustrate normal histological appearance. (A) terminal ileum (×400). (B) transverse colon (×400). (C) descending colon (×400) and (D) rectum (×400).

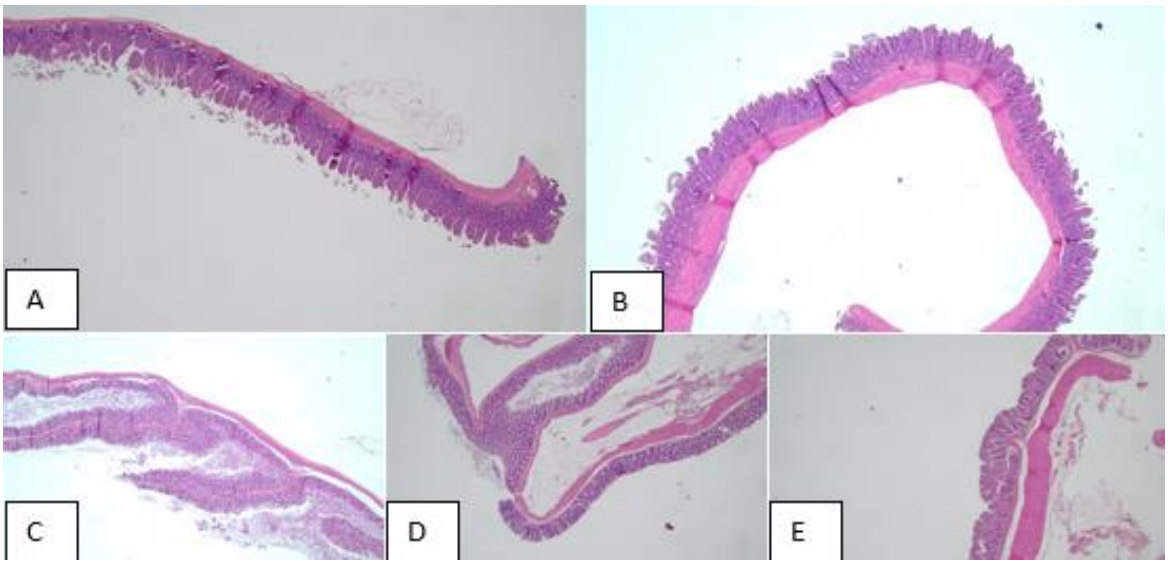

Figure 2. Representative images of H&E-stained colon sections of Group II BALB/c mice DSS 2%-induced illustrate normal histological appearance. (A) terminal ileum (×400). (B) ascenden colon (×400). (C) transverse colon (×400). (D) descending colon (×400) and (E) rectum (×400).

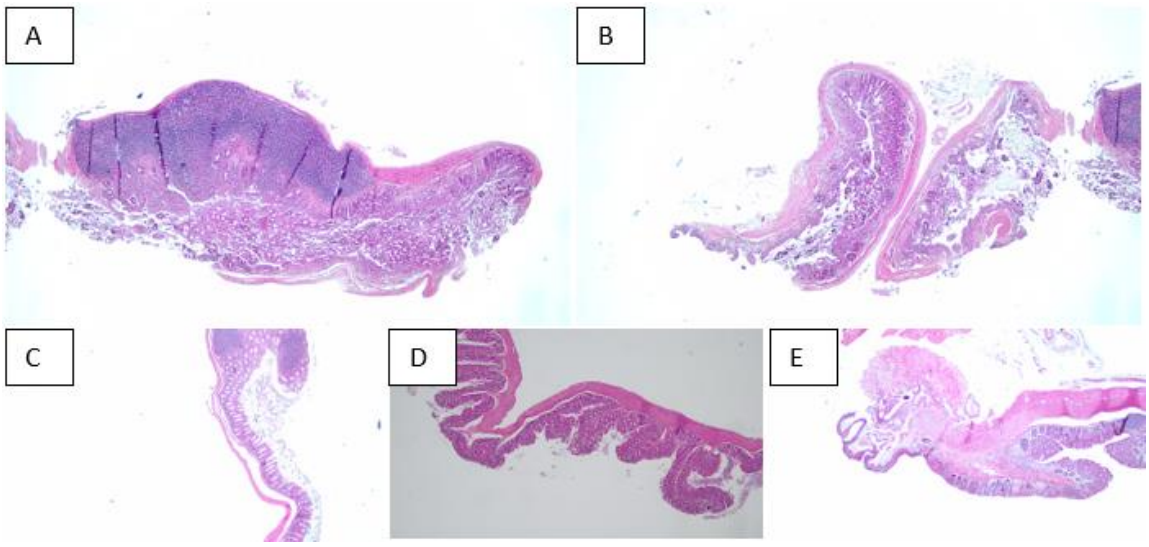

Figure 3. Representative images of H&E-stained colon sections of Group III BALB/c mice DSS 3%-induced illustrate normal histological appearance. (A) terminal ileum (×400). (B) ascenden colon (×400). (C) transverse colon (×400). (D) descending colon (×400) and (E) rectum (×400)

There is a graph showing data on food and drink consumption. The food consumption in groups I, II, and III were 5.79 g/day, 6.58 g/day, and 5.21 g/day, respectively (Figure 4). Drinking water consumption in groups I, II, and III was 5.97 ml/day, 5.70 ml/day, and 5.22 ml/day, respectively (Figure 5)**.**

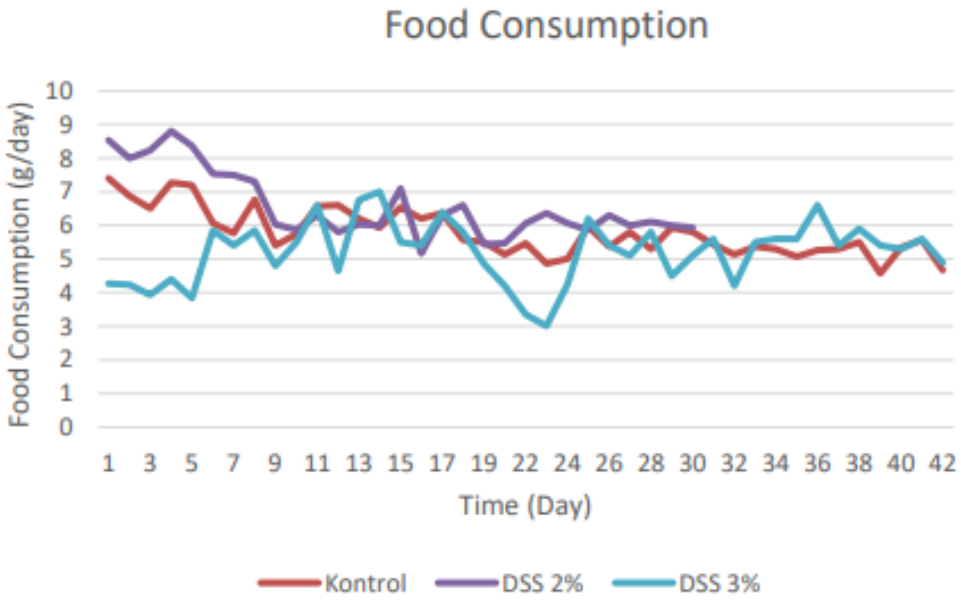

Figure 4. Food consumption in 3 groups, measuring everyday

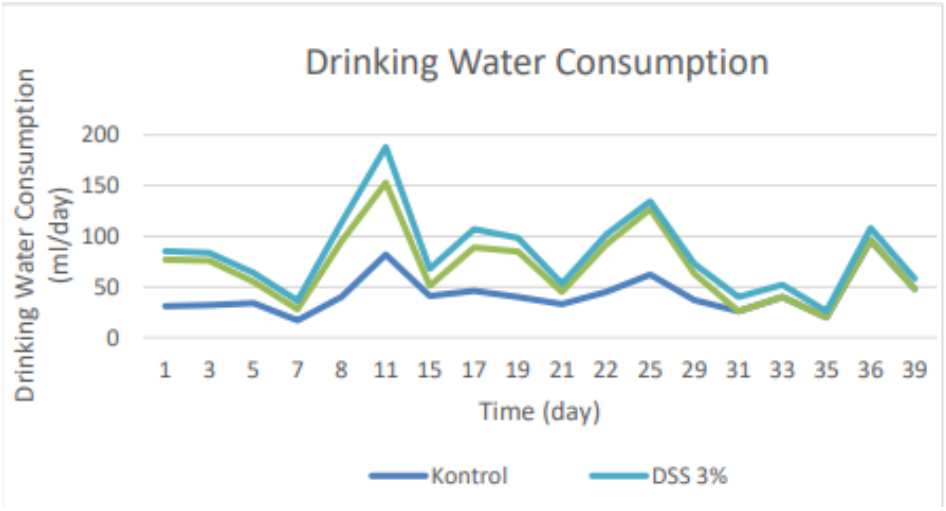

Figure 5. Drinking water consumption in 3 groups, measuring every 2-3 days

DISCUSSION

Inflammatory cell infiltrate means the density of leukocyte in the lamina propria. The severity divided in to 4 gropus: minimal (less then 10%), mild (10-25%), moderate (26-50%) and marked (more than 50%). Extent of inflammatory cell infiltrate means expansion of leukocyte infiltration in the mucosal, sub mucosal and transmural. In this study, there are no inflammatory cell infiltrate appearance in the group II and III after 31 and 42 days of treatment, even though the eating and drinking charts for the three groups did not show any differences.

Epithelial changes consist of hyperplasia, goblet cell loss, cryptitis, crypt abcesses and erosion. This changes also not found in the group II and III after 31 and 42 days of treatment, even though the eating and drinking charts for the three groups did not show any differences.

Mucosal appearance means ulceration, granulation tissue, irregular crypt, crypt loss and villous blunting. This changes also not found in the group II and III after 31 and 42 days of treatment, even though the eating and drinking charts for the three groups did not show any differences.

In the group of BALB/c mice induced by 2-3% DSS, there was no microscopic feature of inflammation. In this group, there was also no decrease in the consumption of food and drinking water. The results of this study did not match the research question posed because administering 2-3% DSS did not show symptoms of UC in BALB/c mice experimental animals. In mice given DSS with BM 40,000 kDa caused colitis in the middle and distal colon, weight loss occur on the 3rd day and reaches a peak on the 5th day. Based on the literature regarding the protocol for experimental colitis animals, administering DSS 3- 10% for 7-10 days induce acute inflammation, while for chronic colitis conditions, DSS is administered for 3-5 cycles followed by 1-2 weeks of drinking water.[11,12]

This study was administered DSS 2-3 cycles without clinical symptoms of IBD. The difference in susceptibility to DSS is not related to differences in DSS consumption in drinking water but depends on the concentration of DSS in drinking water. This means that the DSS 2-3% in this study is not sufficient to induce colitis. The limitation of this study is small sample size and small range of DSS concentration.

CONCLUSION

In this study, the administration of 2% DSS and 3% DSS did not provide microscopic changes of IBD in BALB/c mice, so it was not effective as mice IBD inducer**.**

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