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Effect of SKB-Gutbiotic on acetic acid induced ulcerative colitis in male Wistar rats

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Inflammatory bowel disease (IBD) is a chronic intestinal inflammation gaining increasing attention as it affects considerable number of humans. IBD is reported as ulcerative colitis (UC) and Crohn's disease (CD) Conventional therapies currently available are not satisfactory. Therefore, here, we investigated the effect of SKB-Gutbiotic on acetic acid induced ulcerative coltis (UC) in male Wistar rats. Male Wistar rats, 200-250 g were divided into six groups as follows: Gr. I (control) received 10 mL/kg of distilled water for 21 consecutive days. Gr. II received 2 mL of 4% acetic acid solution once intra rectally for induction of colitis. Gr. III received 2 mg/kg prednisolone as standard control. Groups IV, V & VI were treated with SKB-Gutbiotic @2×10^9, 20×10^9 and 50×10^9 Cfu/kg, respectively. All the animals from each group were sacrificed 24 h after the induction of colitis. Disease activity index, macroscopical damage, hematological parameters, level of superoxide dismutase (SOD), myeloperoxidase (MPO), reduced glutathione (GSH) and histopathological alterations were evaluated. Acetic acid-induced colitis significantly caused alteration in disease activity index, macroscopical damage, MPO and GSH levels (P <0.05) as compared to control group. SKB-Gutbiotic (20×10^9 and 50×10^9 Cfu/kg) administration significantly decreased disease activity index, MPO, SOD, increased GSH levels (P <0.05) as compared to colitis rats. In conclusion, SKB-Gutbiotic (20×10^9 and 50×10^9 Cfu/kg) significantly showed protective effects against acetic acid-induced colitis as a consequence of its anti-inflammatory and antioxidative properties.

Keywords: Antioxidant, Colitis, Inflammatory bowel disease (IBD), Prednisolone, Probiotic

Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory condition of multifactorial etiology, owing to complex interactions of environmental, genetic, microbial and immune factors[1]. Overall, the prevalence of IBD was found to be 26.25 per 100,000 persons[2]. This is categorized into ulcerative colitis (UC) and Crohn's disease (CD). Immune system activation is characteristic of IBD, and is accompanied by producing a wide variety of non-specific inflammatory mediators, including cytokines, chemokines, growth factors, arachidonic acid metabolites (e.g. prostaglandins and leukotrienes) and the metabolites of reactive oxygen species such as nitric oxide[3]. All of these mediators contribute to the pathogenic cascade that initiates and perpetuates the inflammatory response of the gut. In brief, the currently accepted model of pathogenesis of ulcerative colitis is an inappropriate immune response to host microorganisms in genetically susceptible people[4]. The current state of therapy for IBD is not satisfactory. About 33-50% of cases with fulminant ulcerative colitis have their colon removed because of failure of conventional medical therapy. In some animal studies, drugs such as doxepin, simvastatin, sulfasalazine, maprotiline and rosuvastatin have been shown to have positive effect in ulcerative colitis[5-8]. In an animal model of dextran sulfate sodium (DSS)-induced colitis, VSL#3 has been shown to reduce the severity of colonic damage[9]. There have been sporadic reports indicating the potential for specific probiotics to reduce the severity of DSS colitis[10-12], Also, patients with IBD frequently experience relapse, and traditional medical treatments are not potent enough to keep in remission for long-term periods[13]. However, the available drugs, such as 5-amino salicylic acid (5-ASA) derivatives, monoclonal antibiotics, steroids (glucocorticoid), anti-tumornecrosis factor (TNF)-a (infliximab) and immunosuppressive agents, have exhibited beneficial effects in the treatment of IBD[14]. However, they are not effective in all cases. Immunosuppressive and biological drugs such as azathioprine and tumor necrosis factor alpha (TNF-α) blockers, respectively,

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have been widely used as therapy for IBD[15]. Despite the proven efficacy of these pharmacological agents, they have several side effects in addition to the high cost[16]. For this reason, the development of new drug treatments is an important goal in IBD.

Probiotics are defined as live microorganisms that confer human health benefits when consumed in an adequate quantity[17]. Probiotic bacteria have beneficial effects on the intestinal epithelium by diverse mechanisms such as enhanced barrier function, modulation of the mucosal immune system, production of antimicrobials and alteration of the intestinal micro-flora[18]. Reports are available on the potential of probiotics in reducing the severity of a number of inflammatory conditions including pouchitis[19] and ulcerative colitis[20]. SKB-Gutbiotic is a consortium of a rod shaped, Gram-positive, spore forming bacteria (*Bacillus subtilis* SKB2074, *Bacillus vallismortis* SKB1701 and *Bacillus licheniformis* SKB 1809). It is non-pathogenic and safe for consumption. Microorganisms utilized in SKB-Gutbiotic is very tough and stable making it the probiotic of choice in the animal feed, food and aquaculture industry. The microbes in the consortia form endospores to survive extreme conditions of temperature, pH and desiccation. All microorganisms used in the formulation are facultative anaerobes. The microbial strains in the consortium are capable of producing 12 different enzymes, vitamins, and secondary metabolites which confer specific probiotic properties to SKB-Gutbiotic

Some features of SKB-Gutbiotic are better tolerance to stress conditions and can with stand pH fluctuations from 3.5 to 9.5. Their spores were found to be stable in presence of bile salts and acids. Their spores are stable to high temperatures and can be easily pelletized at high temperature. It can be incorporated into different formulations to improve digestibility of the feed. It is compatible with vitamins, enzymes, electrolytes, proteins and carbohydrates. They adapt to intestinal tract and maintain intestinal health. SKB-Gutbiotic has proven compatibility with antibiotics, antimicrobial growth promoters, coccidiostats and acidifiers, and did not show any adverse effect. It is found to be susceptible to most of the antibiotics. SKB-Gutbiotic also showed non-haemolytic activity hence showed non-toxic nature. It has zero withdrawal period and can be administered throughout the life cycle of animals[21]. Withdrawal period refers to the minimum period of time from administering the last dose of medication and the production of meat or other animal-derived products for food.

In the present study, we assessed the protective effect of SKB-Gutbiotic in the acetic acid model of rat colitis. Furthermore, we studied disease activity index, macroscopic and histological parameters and analyzed some hematological parameters, oxidative stress markers and inflammatory markers such as MPO activity.

Materials and Methods

Animals

Male Wistar rats (200-250 g) were purchased from Bombay Veterinary College, Mumbai. The animals were kept under normal environmental conditions with a 12 h light/dark cycle and had free access to standard pellet diet and water. Animals were separately housed in their cages and were acclimatized for at least one week before behavioral studies. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. The Institutional Animal Ethical Committee (IAEC) approved protocol of this study (MGV/PC/CPCSEA/XXXVI/01/ 2018/03).

Chemicals

SKB-Gutbitotic (Gift sample from SK BiobizPvt Ltd, Jaulke, Nashik)[21]. Prednisolone (Interved India Pvt. Ltd. Pune), acetic acid (Sigma-Aldrich, Mumbai), DTNB (Modern-Science Apparatus Pvt. Ltd.Nasik), TCA (Modern-Science Apparatus Pvt. Ltd. Nasik), HTAB (Modern-Science Apparatus Pvt. Ltd. Nasik), O-dianisidine (Modern-Science Apparatus Pvt. Ltd. Nasik), Epinephrine (Sigma-Aldrich, Mumbai), sodium hydroxide (Sigma-Aldrich, Mumbai) were used for the study.

Induction of ulcerative colitis

Colitis was induced by the method originally described by Millar *et al*.[22]. The animals were fasted 24 h and then anaesthetized with isoflurane. They were rectally administered acetic acid (2 mL 4%, v/v,) by 2 mm diameter cannula 8 cm deep. The animals were then kept in a head-down position for 30 s and then returned to their cages to recover from anesthesia. The rats in the non-colitic group (Gr. I Control) received 10 mL/kg distilled water. Two days later, the animals were sacrificed by euthanasia, and

blood and colon tissue were removed to assess the macroscopic damage, histological and antioxidant parameters.

Experimental design

Animals were divided into seven groups of six animals each. Gr. I served as normal control and received vehicle (10 mL/kg, p.o.) for 21 days. Gr. II served as colitis control and received acetic acid (4% 2 mL p.r. once) for induction of ulcerative colitis. Gr. III received prednisolone (2 mg/kg, p.o.). Groups IV, V and VI received SKB-Gutbiotic (2×10^9, 20×10^9 and 50×10^9 Cfu/kg, p.o., respectively) for 21 days. Induction of colitis was done on 19th day. Twenty-four hours later, the animals were sacrificed under euthanasia. The blood samples from all the rats were drawn via cardiac puncture and collected into EDTA tubes for hematological analysis (H S Pathology Private Limited, Nasik; Equipment- Mindray BC 3000 PLUS-AGAPPE). Colon of the rats was removed for macroscopical, antioxidant and histological study.

Disease Activity Index (DAI)

A disease activity index based on bodyweight change, stool consistency, rectal bleeding and overall condition of the animal[23] was calculated daily from the start of acetic acid treatment to the end of the experiment.Scores were recorded.

Measurement weight, length and macroscopical score of colons

On 22nd day, all rats were sacrificed by euthanasia. The gastrointestinal tract was removed and the colon (up to 10 cm from rectum) was obtained, fat and mesentery removed, weighed and its length measured under a constant load. To evaluate macroscopical damage, colon was excised and opened longitudinally, rinsed with ice-cold normal saline and colonic damage was evaluated according to scale ranging from 0 to 4 as follows[22]: 0-normal appearance; 1-mucosal erythema only; 2-mild edema, slight bleeding or small erosions; 3-moderate edema, bleeding, ulcers; and 4-severe ulcerations, erosions, edema and tissue necrosis.

Biochemical assay

The colon tissue was washed with ice-cold saline and homogenized (Remi motors ltd. RQ- 127A) with 0.1 M tris buffer (pH 7.5) using Remi homogenizer (Remi instruments ltd. VCAC-62) to give 10% homogenate. The homogenate was centrifuged at 8000 rpm for 20 min and supernatant was used for estimation of antioxidant enzyme levels.

Superoxide Dismutase (*SOD*)

The supernatant was collected for the estimation of SOD and was assayed by the method described by Saggu *et al*.[24]. Briefly, 0.05 mL of supernatant was added to 2.0 mL of carbonate buffer and 0.5 mL of 0.01 Mm EDTA solution. The reaction was initiated by addition of 0.5 mL of epinephrine and autoxidation of adrenaline to adrenochrome was measured at 480 nm. The change in absorbance for every minute was measured against blank. The results were expressed as unit of SOD activity (mg/wet tissue).

Reduced Glutathione (*GSH*)

Homogenate (1 mL) was added to 1 mL of 10% TCA and centrifuged. One mL of supernatant was treated with 0.5 mL of Ellman's reagent [19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 mL of 1% of sodium citrate] and 3mL of phosphate buffer (pH-8). The colour developed was measured at 412 nm[25].

Myeloperoxidase Activity (*MPO*)

The supernatant (0.1 mL) was suspended in hexadecyltrimethylammoniumbromide (HTAB) buffer (0.5% HTAB in 50 mM phosphate buffer, pH 6.0). HTAB is a detergent that releases MPO from the primary granules of the neutrophil. The tissue homogenate was sonicated on ice for 10 s and centrifuged at 40,000 g for 15 min. The supernatant was assayed for MPO activity. About 0.1 mL of supernatant was combined with 2.9 mL of 50 mM phosphate buffer, pH6.0, containing 0.167 mg/mL 0-dianisidine hydrochloride and 0.0005% hydrogen peroxide. The change in absorbance was observed spectrophotometerically at 460 nm. One unit of MPO activity is defined as that degrading 1 pmol of peroxide per minute at 25°C[26].

Histopathological examination

The colon tissue was fixed in 10% v/v formalin. The specimens were processed for standard procedure and were then processed in paraffin to expose the lessional and normal area of colon tissue. The blocks were then sectioned according to hematoxylin and eosin method. Five-micrometer thick histological sections were examined under the light microscope and photographs were taken under 40X[27].

Statistical analysis

The results were expressed as Mean ± SEM. N=6, Statistical analysis was done by using one-way ANOVA, followed by Dunnett's test and $P < 0.05$ was considered statistically significant.

Results

Disease activity index (DAI)

The acetic acid treated group significantly increased disease activity index as compared to vehicle control group (P <0.05) at day 21st. Disease activity index significantly decreased in Gr. III (treated with prednisolone as standard control) as compared to Gr. II (treated with acetic acid) (P <0.05) on day 21st and animals treated in Gr. V and VI with varying dose SKB-Gutbiotic significantly (P <0.05) decreased the disease activity index compared to Gr. II on day 21th.(Fig. 1).

Weight, length and macroscopical colon damage

The weight of colon in Gr. II (colitis control) was significantly (P <0.05) increased as compared to Gr. I (vehicle control). Gr. III, V and VI showed a significant (P <0.05) decrease in colon weight as compared to Gr. II. The Gr. II (acetic acid treated group) showed a significant (P <0.05) increase in macroscopical colon damage as compared to Gr. I (vehicle control). Macroscopical damage decreased significantly (P <0.05) in Gr. III, V and VI as compared to Gr. II (Fig. 2).

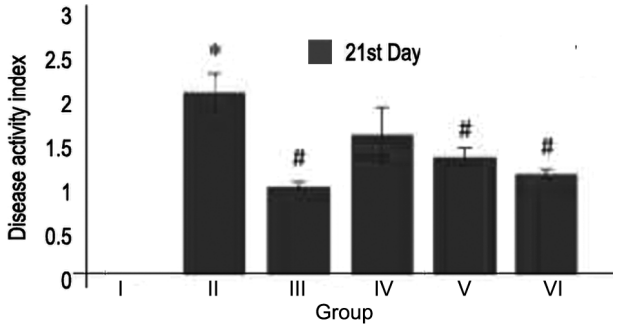

Fig. 1 — Effect of SKB-Gutbiotic (2×10^9 Cfu/kg, 20×10^9 Cfu/kg and 50×10^9 Cfu/kg) on Disease activity index in acetic acid induced ulcerative colitis in rats. [Groups: I, Vehicle conrol; II, Acetic acid (colitis control); III, Prednisolone @2 mg/kg; IV-VI, SKB-Glutbiotic @ 2, 20 and 50 billion Cfu/kg]

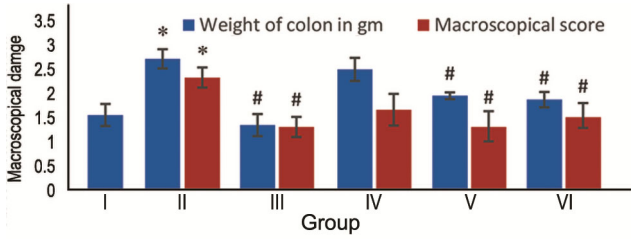

Fig. 2 — Effect of SKB_Gutbiotic (2×10^9 Cfu/kg, 20×10^9 Cfu/kg and 50×10^9 Cfu/kg) on weight of colon and macroscopical damage in acetic acid induced ulcerative colitis in rats. [Groups: I, Vehicle conrol; II, Acetic acid (colitis control); III, Prednisolone @2 mg/kg; IV-VI, SKB-Glutbiotic @ 2, 20 and 50 billion Cfu/kg]

SOD, GSH and MPO levels

Colonic injury induced by acetic acid administration was accompanied by increased SOD content as compared with vehicle control. The SOD content was significantly (P <0.05) decreased in Gr. III, V, and VI as compared with Gr. II (Fig. 3A). Colitis control group showed significant (P <0.05) decrease in GSH level as compared to vehicle control group. The Gr. III treated with standard control prednisolone and Gr. V, and VI significantly (P <0.05) increased GSH level in colon as compared to colitis control (Fig. 3B). The MPO activity as an indicator of neutrophils infiltration, increased significantly after intra-rectal administration of acetic acid. MPO activity was higher in Gr. II (Colitis control) as compared to Gr. I (Vehicle control) (P <0.05). The MPO level was decreased in Gr. III treated with standard control prednisolone compared with Gr. II. The level of MPO was significantly (P <0.05) decreased in Gr. V and VI as compared to Gr. II (Fig. 3C).

Hematology study

The impact on hematological parameters in rats treated with SKB-Gutbiotic and the mean values are presented in Fig. 4 and Table 1. Acetic acid induced colitis significantly increased (P <0.05) white blood cell (WBC) count, neutrophilcount (NEU) as compared with vehicle control. However, administration of prednisolone significantly lowered (P <0.05) white blood cell (WBC) count and neutrophil count (NEU) count when compared with colitis control group. Gr. V and VI treated with SKB-Gutbiotic also significantly decreased (P <0.05) white

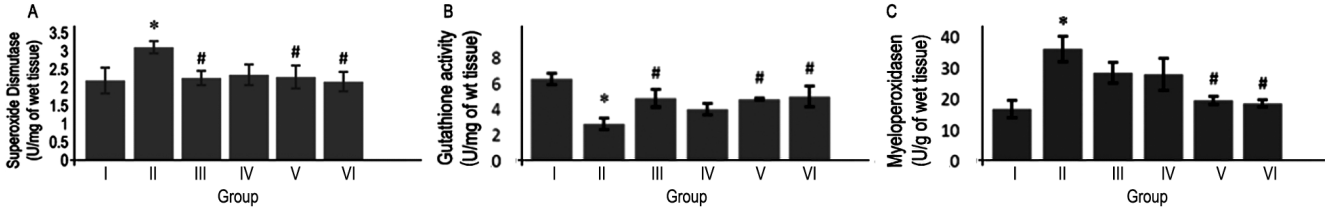

Fig. 3 — Effect of SKB_Gutbiotic (2×10^9 Cfu/kg, 20×10^9 Cfu/kg and 50×10^9 Cfu/kg) on (A) Superoxide dismutase activity; (B) Glutathione activity; and (C) Myeloperoxidase activity in acetic acid induced ulcerative colitis in rats. [Groups: I, Vehicle conrol; II, Acetic acid (colitis control); III, Prednisolone @2 mg/kg; IV-VI, SKB-Glutbiotic @ 2, 20 and 50 billion Cfu/kg]

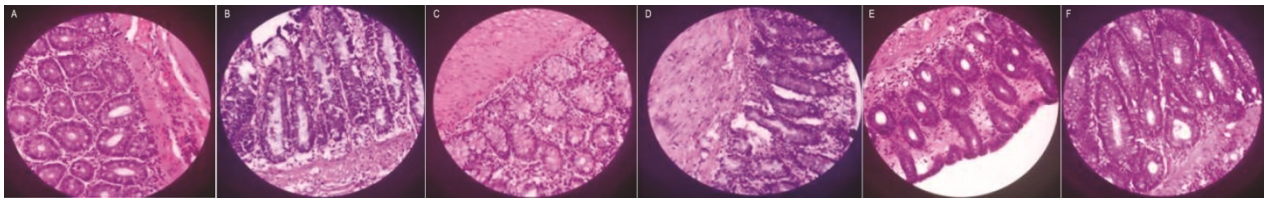

Fig. 4 — Histopathological examination of colon tissue section (A) Control (Gr. I) showed normal structure, normal epithelial architecture; (B) Gr. II treated with acetic acid (4% v/v) showed severe epithelial erosion including edema and necrosis, also showed enlarged crypts cell with large crypt abscess, distraction of goblet cell, accumulation of mucosae and infiltration of leukocytes and lymphocytes in lamina propria and ulceration; (C) Gr. III treated with prednisolone (2 mg/kg, p.o.) showed reduction in epithelial erosionnormalarchitecture of crypts cell and reduce distraction of goblet cell as compared to control group; (D) Gr. IV treated with SKB-Gutbiotic (2×10^9 Cfu/kg, p.o.) showed reduced destruction of epithelial cell, improved architecture of crypts with small crypts abscess and infiltration of lymphocytes of in lamina propria when but effects were less than colitis control; (E) group treated with SKB-Gutbiotic (20×109 Cfu/kg, p.o.) showed improved epithelial cell damage, normal architecture of crypts and goblet cell as compared with colitis control; and (F) group treated with SKB-Gutbiotic (50×10^9 Cfu/kg, p.o.) showed reduction in epithelial erosion, reduced lengthening of crypts cell, reduced mucosa accumulation and improved goblet cell structure damage as compared to colitis control group.

Table 1 — Effect of SKB_Gutbiotic (2×10^9 Cfu/kg, 20×10^9 Cfu/kg and 50×10^9 Cfu/kg) on hematological parameters of rats in acetic acid induced ulcerative colitis

Treatment group	WBC (per cu. mm.)	NEU (%)	LYM (%)	RBC (m/cu. mm)	HB (g/dL)
Gr. I: Vehicle Control (10 mL/kg, p.o.)	13425.0±1438.96	52.75±4.04	40.5±4.29	7.83±0.03	14.45±0.18
Gr. II: 4% Acetic acid (2 mL, p.r.)	17197.3±486.69*	71.25±3.77*	26.5±3.62*	6.17±0.35*	11.7±0.62*
Gr. III: Prednisolone (2 mg/kg, p.o.)	7225.0±657.48#	54.5±4.66#	39.75±5.36	7.80±0.21#	14.55±0.46#
Gr. IV: SKB-Gutbiotic (2×10^9 Cfu/kg, p.o.)	14100.0±1073.0#	60.5±4.92	38.0±4.16	6.88±0.61	13.27±0.58
Gr. V: SKB-Gutbiotic (20×10^9 Cfu/kg, p.o.)	11466.5±520.68#	50.0±4.16#	45.33±2.66#	7.98±0.36#	14.53±0.33#
Gr. VI: SKB-Gutbiotic (50×10^9Cfu/kg, p.o.)	10500.0±2176.77#	50.75±4.96#	44.0±4.69#	7.81±0.36#	14.05±0.77#

[n=6, All values are expressed as Mean ± SEM, *p< 0.05 compared with vehicle control and #P <0.05 compared with acetic acid control group. All data are analyzed by one way ANOVA followed by Dunnett's test]

blood cell (WBC) count and neutrophil count (NEU) count when compared with colitis control group. Acetic acidtreatment significantly lower (P <0.05) lymphocyte count (LYM),red blood cell (RBC) count and hemoglobin (Hb) concentration when compared with vehicle control groups. The Gr. V & VI treated with SKB-Gutbiotic significantly (P <0.05) increased lymphocyte count (LYM), red blood cell (RBC) count and hemoglobin (Hb) concentration when compared with colitis control group.

Discussion

SKB-Gutbiotic contains three different nonpathogenic bacteria *Bacillus subtilis* SKB 2074, *Bacillus lechiniformis* SKB 1809 and *Bacillus vallismortis* SKB 1701, and posses antimicrobial, antidiarrheal and antioxidant activity. SKB-Gutbiotic showed better tolerance to pH (3.5-9.5), bile salts and acids, stable at high temperatures. SKB-Gutbiotic reduces high concentrations of ammonium nitrate, hydrogen sulphide and other harmful gases[21]. With the above desired characteristics, SKB-Gutbiotic is considered as a potential probiotic in human and animal healthcare.

The study highlights the protective effect of SKB-Gutbiotic against ulcerative colitis in rats. Induction of colitis in rats using acetic acid is a classical and well-established method to produce an experimental model of colitis similar to human colitis[28]. Probiotics, natural products and synthetic drugs have been shown to exhibit therapeutic and prophylactic efficacy in a number of animal models of experimental colitis[29-31]. Disease activity index (DAI) is one of the most important parameter in study of colitis. DAI is an average score of weight loss, stool consistency and bleeding. In the present study, animals treated with acetic acid showed significant increase in disease activity index as compared to the vehicle control group. Earlier probiotics studies have also shown protective effect by reducing DAI and reducing score of macroscopical damage[32,33]. SKB-Gutbiotic (20×10^9 and 50×10^9 Cfu/kg) showed significant decrease in DAI and was able to prevent macroscopical damage and reduced the weight of colon significantly as compared with colitis control group.

There is a strong correlation between IBD and oxidative stress. Increased production of free radicals, such as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) associated with the dysregulated immune response, is associated with the tissue injury[34]. SOD is an enzyme existing in

mitochondria as well as cytosol which is responsible for the maintenance of redox balance in the tissue[35,36]. The increased level of reactive oxygen species causes deregulation of cellular redox balance[37]. SOD reduces superoxide to water (O^{2-} to H_2O) thus playing a pivotal role in scavenging of oxidative free radicals. Impaired action of this enzyme or its associated enzymes is linked to the oxidative stress[38]. Inflammation-associated ROS are primarily sourced from the 'oxidative burst', elicited by the phagocytic activity of neutrophils and macrophages. This activity induces oxidative damage via activation of membrane-bound NADPH oxidase (NOX) to rapidly generate large amounts of locally formed $O^{2\bullet-}$ and H_2O_2 following dismutation of $O^{2\bullet-}$ by SOD[39]. Amaretti *et al*.[40] have demonstrated the protective effect of probiotics *in vitro* and *in vivo* study, using *Lactobacillus* and *Bifidobacterium*, and related to the intestinal activities, i.e., secretion of enzymes and promotion of the production of antioxidant biomolecules such as exopolysaccharides that has shown *in vitro* antioxidant and free-radical scavenging activities. In our study, SKB-Gutbiotic (20×10^9 and 50×10^9 Cfu/kg) significantly reduced SOD probably by secretion of enzymes and promotion of the production of antioxidant biomolecules such as exopolysaccharides.

Glutathione (GSH) plays an important role in coordinating the body's antioxidant defense process, it is a non-protein thiol in living organisms[41]. GSH is involved in the synthesis and repair of DNA, assists the recycling of vitamins C and E, blocks free radical damage, enhances the antioxidant activity of vitamin C, facilitates the transport of amino acids and play a critical role in detoxification[41]. The elevated level of GSH protects cellular proteins against oxidation and also directly detoxifies ROS. Glutathione plays important role in protection of gastric mucosa and depletion of glutathione is associated with experimental colitis[42]. During inflammation, GSH level decreases resulting in severe colon mucosal injury. In some animal model of glutathione deficiency, rats treated with an inhibitor of glutathione synthesis develop severe intestinal epithelial degeneration and diarrhea, suggesting that glutathione is essential in the protection of the intestine against luminal oxidants[43]. In our study, acetic acid treatment showed significant reduction of colonic tissue GSH level as compared with vehicle control group. We observed that SKB-Gutbiotic (20×10^9 and 50×10^9 Cfu/kg) showed significant antioxidant action in GSH by increasing the content of glutathione.

MPO is an enzyme present in neutrophils and at a much lower concentration in monocytes and macrophages. The level of MPO activity is directly proportional to the neutrophil concentration in the inflammed tissue. Therefore, measurement of MPO activity has been considered as a quantitative and sensitive assay for acute intestinal inflammation. In addition, increased MPO activity has been reported to be an index of neutrophil infiltration and inflammation[44]. Pretreatment with SKB-Gutbiotic (20×10^9 and 50×10^9 Cfu/kg) showed decreased activity of MPO enzyme reducing the infiltration of neutrophils.

Alteration in hematological parameters due to tissue damage is an important clinical manifestation of inflammatory bowel diseases. Thus, an assessment of hematological parameters can be used to determine the degree of disease state of colitis[45]. Alabi Q. et al.(2017) reported that in acetic acid induced colitis in rats showed reduction in red blood cell counts with subsequent decline in hemoglobin concentration. The observed decrease in the number of RBCs, accompanied by a decreased HB, seems to confirm that colitis probably caused excessive blood loss as a result of serious gastrointestinal tract bleeding, hemolysis of red blood cell and poor absorption of iron in the intestine. Pretreatment with SKB-Gutbiotic (20×10^9 and 50×10^9 Cfu/kg) may reduce excessive blood loss by improving red blood cell and HB count.

White blood cell count is the most commonly used inflammatory index in routine clinical practice for determining UC activity[46]. In the present study, increase in WBC count in colitis control group may confirm that colitis has been confirmed. The SKB-Gutbiotic (20×10^9 and 50×10^9 Cfu/kg) has reduced inflammation as indicated by reduced WBC levels in animals. In UC, the neutrophil granulocyte is one of the most important infiltrating leukocytes and has been proposed to contribute significantly to the development of tissue injury and inflammation seen in this disease[47]. Neutrophil accumulation in the intestinal mucosa may have been a consequence of increased recruitment of neutrophils and from defective apoptosis[48]. Several chemotactic molecules play a significant role in the recruitment of neutrophils from the blood into inflamed tissue. The

neutrophil is the key cell responsible for the active nonspecific inflammatory response and is closely tied to the destructive tissue cascades by secretion of interleukin-1, interleukin-6, myeloperoxidase, and elastase[49]. In the present study, increase in neutrophil count in colitis may indicate accumulation of neutrophils and tissue damage. The SKB-Gutbiotic in dose 20×10^9 and 50×10^9 Cfu/kg has significantly reduced the neutrophil count. The mucus layer that covers the colonic epithelium is also an important component of its defense mechanisms. Increased mucin secretion by goblet cells helps protect the mucosa[50]. In our study, SKB-Gutbiotic has improved epithelial cell architecture by increasing goblet cell with increased mucin secretion as indicated in histopathology study.

Conclusion

In the present study, oral administration of SKB-Gutbiotic (20×10^9 Cfu/kg, 50×10^9 Cfu/kg) showed protective effect against acetic acid induced induced ulcerative colitis and significantly improved disease activity index and SOD, Glutathione and Myeloperoxidase activity. Thus, SKB-Gutbiotic has demonstrated the potential for its use in the treatment of ulcerative colitis. More safety and efficacy studies of formulations containing SKB-Gutbiotic in clinical set up are required to be undertaken in future.

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Conflicts of interest

Authors declare no competing interests.

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