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Methanolic Leaf Extract of *Dissotis Rotundifolia* Alleviates Acetic Acid-Induced Ulcerative Colitis in Rats

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ABSTRACT

Dissotis rotundifolia (Sm.) Triana has been used locally in the treatment of inflammatory conditions such as painful swellings and conjunctivitis. This study aimed at investigating the effect of *Dissotis rotundifolia* in acetic acid-induced ulcerative colitis. Sprague Dawley rats were administered with *Dissotis rotundifolia* extract at doses 30, 100 and 300 mg kg^{-1} or sulfasalazine 500 mg kg^{-1} for 8 days. On the 4th day of treatment, colitis was induced by intrarectal administration of 1 ml, 4 %v/v acetic acid. On day 8, animals were sacrificed and parameters such as microscopic and macroscopic colon damage assessed. The extract exhibited significant ($P < 0.0001$) reduction in microscopic and macroscopic colon damage. 30 and 300 mg kg^{-1} of the extract significantly ($P < 0.0001$) inhibited weight loss and colon oedema. The leaf extract of *Dissotis rotundifolia* showed significant amelioration of acetic acid-induced ulcerative colitis which may be attributable to its anti-inflammatory effect.

Keywords: Chronic inflammation, colon damage, *Dissotis rotundifolia*, oedema, ulcerative colitis.

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INTRODUCTION

Ulcerative colitis is a chronic inflammatory disease of the gastrointestinal tract that usually affects the rectum and sometimes extends proximally within the colon[1]. The disease shares a number of similarities with Crohn's disease enough to warrant their description under the collective term Inflammatory Bowel Diseases[1]. However, notable differences exist between the two. Except in the case of backwash ileitis, ulcerative colitis is devoid of small bowel involvement, as opposed to Crohn's disease where patients present with small bowel involvement. Moreover, ulcerative colitis does not affect the upper gastrointestinal tract, and the presentation of hematochezia is common. However, upper gastrointestinal tract is affected in Crohn's disease and hematochezia is rarely seen on presentation[2]. In addition, patchy, segmental and typically transmural inflammation in the gut, characterized by macrophage aggregation that often form noncaseating granulomas are seen in Crohn's disease. Ulcerative colitis, on the other hand, is characterized by significant leukocytic infiltration of the lamina propria and crypts, where they form microabscesses as well as depletion of mucin by goblet cells[3]. Although the exact pathoetiology of ulcerative colitis is not completely understood, it is widely accepted that dysregulated interaction between the commensal enteric flora and gut-associated immune system plays a crucial role in this disease[3,4]. The dysfunction of the mucosal immune system triggers intestinal inflammation *via* the activation of both the innate and acquired immune systems in the gut[5]. Recent mortality data reveals a 10% increase in intermediate and long-term mortality among ulcerative colitis patients with an even higher percentage in patients diagnosed in childhood or adolescence[6], clearly highlighting the need for novel therapies[7].

Medicinal plants play a beneficial role in healthcare. According to Amani et al. (2013) treatment with products of natural origin produces promising results and fewer side effects. One such plant known for its beneficial effects is *Dissotis roundifolia* (Sm.) Triana[8]. The West African native, *Dissotis rotundifolia*, commonly called Pink Lady[9] belongs to the family Melastomataceae[10,11] and is a versatile perennial creeping herb that roots at the nodes[12]. The leaves are ovate to ovate-lanceolate or suborbicular and the leaves are modestly crenuate with an acute apex, and a truncate to short-attenuate base[12,13]. The herb is used traditionally in the treatment of inflammatory conditions such as conjunctivitis and painful swellings[14], asthma[15], sinusitis and bronchitis[16]. The leaf decoction is also used in the treatment of stomach ache and diarrhea[13]. Medicinal plants are excellent sources of lead compounds that may provide new and cost-effective treatment options with tolerable side effect profile which may as well induce

and maintain total remission and also improve upon the quality of life of patients with ulcerative colitis. The aim of this study is to determine the effect of *Dissotis roundifolia* leaf extract (DRE) on acetic acid-induced ulcerative colitis.

METHODOLOGY

Animals

A total of 30 male Sprague Dawley rats (180 – 210 g) were obtained from and maintained in the Animal Housing facility of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The rats were housed in stainless steel cages (34 cm × 47 cm × 18 cm) with soft wood shavings as bedding, and fed with commercially available pellet diet (GAFCO, Tema, Ghana) and given water *ad libitum*.

Drugs and Chemicals

Sulfasalazine was purchased from Pfizer Inc, New York, USA; disodium monohydrogen phosphate, sodium chloride and formaldehyde were purchased from Sigma-Aldrich Chemical Co, St Louis, USA; hematoxylin and eosin stain was purchased from Abbey Color, Philadelphia, USA; glacial acetic acid was purchased from Eastman Chemical Company, Kingsport, Tennessee, USA.

Collection of Plant material

The fresh leaves of *Dissotis rotundifolia* was collected from the area around Kakum National Park, Cape Coast, Ghana in January 2012 and was authenticated by the Curator at the Herbarium of the Department of Environmental Science, School of Biological Sciences, University of Cape Coast. A voucher specimen (No. 107346) has been deposited at the herbarium. The leaves were thoroughly washed and dried in the shade for 3 weeks. Subsequently, the partially dried plant material was oven-dried at 40°C for 3 h and then pulverized into powder.

Preparation of Extract

The extraction of the crude extract was carried out as described by Rath et al. (1995) and Kweku et al. (2018) [17,18]. Briefly, 60 g of the powdered leaves was transferred into a 1 L Erlenmeyer flask followed by the addition of 210 ml of 70% methanol. The neck of the flask was plugged tightly with cotton wool, and the contents of the flask mixed by placement on an orbital shaker (IKA HS/KS260 basic orbital shaker-Werke-GmbH & Co. KG Germany) at a speed of 200 rpm for 72 h. The powdered leaves were allowed to be drenched in the methanol for 72 hours, after which the resulting mixture was then filtered using a Whatman No.1 filter paper into a 500 ml flat bottom flask, and the filtrate discarded appropriately. The obtained filtrate was subsequently concentrated us-

ing a rotary evaporator (BÜCHI rotavapor R-200, Germany). The concentrated crude preparation obtained was then dried in an oven at a temperature of 50°C to obtain 6.9% ($^{w}/_{w}$) of dried powdered extract, henceforth referred to as *Dissotis rotundifolia extract* (DRE). DRE was stored at −8°C until required for use.

$$\% \ Yield = \frac{A_1}{A_o} \times 100 \ \&$$

Where A_o was the mass of the leaf sample and A_1 was the mass of the crude extract.

Induction of colonic injury and body weight determination

Acetic acid-induced ulcerative colitis was induced in rats as described by Fabia et al. (1992) and Osafo et al. (2019)[19,20]. Male Sprague Dawley rats (180 – 210 g) were randomly divided into six groups (n = 5) and treated as follows:

Group I: normal saline (0.9% $^{w}/_{v}$) *p.o* for 8 days.

Group II: normal saline (0.9% $^{w}/_{v}$) *p.o* for 8 days and 1 ml, 4.0% acetic acid ($^{v}/_{v}$) intrarectally on day 4.

Group III: sulfasalazine (500 mg kg^{-1} *p.o.*) for 8 days and 1 ml 4.0% acetic acid intrarectally on day 4.

Groups IV-VI: *Dissotis rotundifolia* leaf extract (DRE) 30, 100 and 300 mg kg^{-1} *p.o.* respectively for 8 days and 1 ml 4% acetic acid intrarectally on day 4.

Intrarectal administration of 4.0% acetic acid was made under anaesthesia. Body weight changes were monitored every morning, before feeding, over the 8-day period and the effect of DRE on the overall percentage change in body weight in relation to body weight on day 1, was expressed as area under the time course curve (AUC).

Macroscopic and Microscopic colon damage assessment

Colonic injury with acetic acid was induced in Sprague Dawley rats as described above. At the end of the 8-day period, animals were euthanized by cervical dislocation before large bowels were excised. Colons were extirpated and macroscopic damage was assessed as described by Kimball et al. (2004) using a 5-point scale based on the weight, consistency of the stool found within as well as length, measured from 1 cm above the anus to the top of the cecum[21]. Disease activity index (DAI) for each group was calculated as a sum of the individual macroscopic damage score. To evaluate microscopic colon damage by light mi-

croscopy, samples of the distal colon were fixed immediately in 10% neutral buffered formalin solution, embedded in paraffin, cut into transversal sections and mounted on glass slides. Sections were deparaffinized and stained with hematoxylin and eosin stain (H&E). In each specimen, six random fields of view were analyzed for microscopic colonic damage and scored by two double-blinded trained observers as described by Dieleman et al. (1998)[22]. Three independent parameters were measured: inflammatory cell infiltration (0-3: none, slight, moderate, severe), extent of injury (0-3: none, mucosal, submucosal, transmural), and crypt damage (0-4: none, basal $^{1}/_{3}$ damage, basal $^{2}/_{3}$ damage, only surface epithelium lost, entire crypt and epithelium lost). DAI was computed as the sum of individual damage scores with 10 as the maximum possible score.

Hematological analysis

Colitis was induced as described in Section 2.2.3. At the end of the 8-day period, the rats were euthanized by cervical dislocation and blood samples were collected from the jugular vein. A full blood count was performed on the blood samples using hematology analyzer (BC-2800, Mindray, Shenzhen, China).

Colon oedema assessment

Acetic acid-induced colonic injury was induced in rats as described in Section 2.2.3. At the end of the 8-day period, colons were excised and cut open by longitudinal incisions. The resected colons were washed thoroughly with normal saline to remove all residual fecal matter. As described by Morteau et al. (2000), degree of oedema was assessed by calculating the colon weight to length ratio[23].

Statistical analysis

Data was presented as Mean ± SEM. One-way ANOVA followed by Dunnett's multiple comparison test was employed in analyzing obtained experimental data. Graphs were plotted using GraphPad Prism for Windows Version 5.01 (GraphPad, San Diego, CA, USA).

RESULTS AND DISCUSSION

The control rats with colitis showed a progressive decrease in body weight after the induction of colon damage (Figure 1A) with a significant ($P < 0.0021$) loss in total body weight when compared with the control rats without colitis (Figure 1B). Treatment with the DRE at doses 30 and 300 mg kg^{-1} resulted in no significant ($P > 0.05$) changes in weight compared to the control group without colitis (Figure 1A) with no significant ($P > 0.05$) differences between the total loss of body weight over the course of the study when compared with the con-

trol group without colitis (Figure 1B). At dose 100 mg kg^{-1} of DRE, a total body weight loss (Figure 1B) was observed. The total loss of body weight at 100 mg kg^{-1} when compared with the rats without colitis was significant ($P < 0.0021$) (Figure 1B). In sulfasalazine-treated rats, there was decrease in body weight after colonic injury (Figure 1A) with a significant ($P < 0.021$) loss in total body weight after the 8th day when compared to the control rats without colitis (Figure 1B).

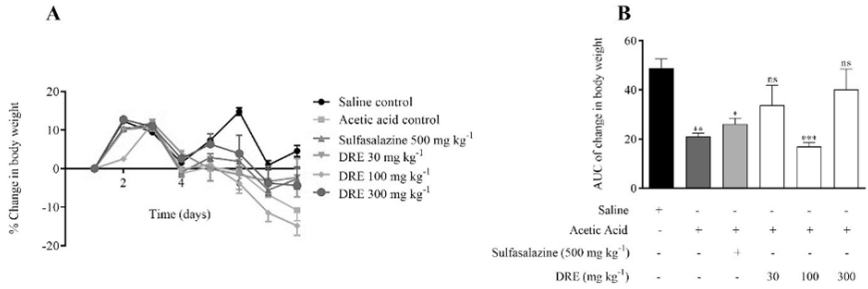

Figure 1: Effect of *Dissotis rotundifolia* on rat body weight in acetic acid-induced colitis.

The body weight was monitored as the percentage change in baseline body weight (A). Total body weight measured during the study period was calculated as area under the time course curves, AUC (B). ***$P = 0.009$, **$P = 0.0034$, *$P = 0.0185$, $^{ns}P > 0.05$ when compared with saline control group. AUC, area under the curve; DRE, *Dissotis rotundifolia* extract; Sulf, Sulfasalazine; AA, Acetic acid.

Clinically, toxins including acids, cause anorexia and induces vomiting by stimulating vagal afferent serotonergic nerves that connect with the chemoreceptor trigger zone in the floor of the fourth ventricle[24]. Owing to this, anorexia with pain can be regarded as a protective reflex that prevents absorption of toxins into the body by reducing the passage of chyme through diseased parts of the gastrointestinal tract[25]. This results in the avoidance of food and malabsorption with subsequent weight loss. The inhibition of weight loss by the extract indicates its ability to reduce anorexia, pain and malabsorption associated with mucosal and submucosal layer necrosis possibly by inhibiting afferent vagal nerve stimulation by ulcer-induced acid related pain.

The control rats with colitis macroscopically exhibited extensive colonic damage (Figure 2A) and significantly ($P < 0.0001$) high disease activity index compared to the control rats without colitis (Figure 2B). Macroscopic colonic damage (Figure 2A) and disease activity index (Figure 2B) significantly improved with sulfasalazine treatment when compared to the control rats with colitis. Treatment with DRE at doses of 30 and 300 mg kg^{-1} resulted in a significant improvement in mac-

roscopic scores (Figure 2A) and DAI (Figure 2B) compared to the control rats with colitis. However, a significant ($P < 0.01$) improvement in macroscopic scores (Figure 2A) and disease activity index (Figure 2B) was achieved at 100 mg kg^{-1} of DRE.

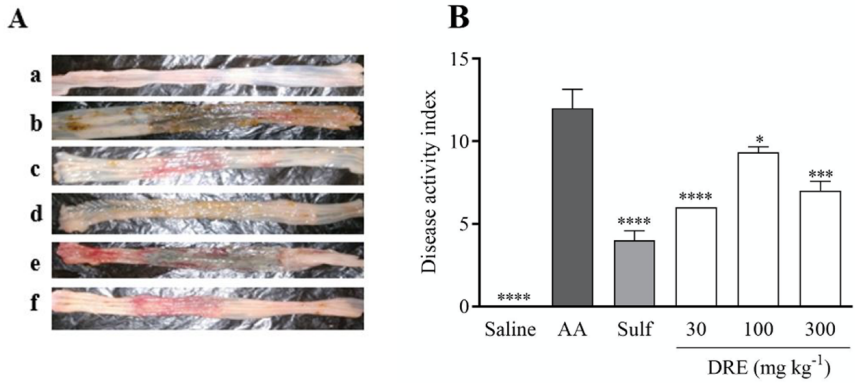

Figure 2: Effect of *Dissotis rotundifolia* on macroscopic acetic acid-induced colonic damage in rats.

Colons were extirpated and examined for: weight, the consistency of the stool found within as well as gross macroscopic appearance and length. (A) Representative slides of colon (a, untreated control; b, AA treatment only; c, AA + 500 mg kg^{-1} sulfasalazine; d, AA + 30 mg kg^{-1} DRE; e, AA + 100 mg kg^{-1} DRE; f, AA + 300 mg kg^{-1} DRE. (B) Disease Activity Index. ****P < 0.0001, ***P < 0.001 and *P < 0.01 when compared with the control rats with colitis. AA, acetic acid; DRE, *Dissotis rotundifolia* extract; Sulf, Sulfasalazine.

Intrarectal administration of acetic acid in the control rats with colitis caused massive loss of mucosal architecture characterized by massive mucosal ulceration (red arrow), loss of cellular detail, heavy inflammatory cell infiltration (green circle), slight thickening of the muscularis mucosa (red rectangle), crypt abscess formation (green arrow) and goblet cell depletion (Figure 3B). Treatment with the extract at 30 mg kg^{-1} resulted in a decrease in mucosal ulceration and the distortion of crypt architecture with no observable crypt abscess formation. At 30 mg kg^{-1} reduced infiltration of the mucosa, submucosa and lamina propria by inflammatory cells, chiefly neutrophils and lymphocytes, compared to the control rats with colitis was observed (Figure 3D). At 100 and 300 mg kg^{-1}, the extract reduced mucosal and submucosal inflammation with a slight loss of cellular detail, crypt distortion (green rectangle) and crypt abscess formation. Neutrophil and lymphocyte infiltration were reduced with less pronounce mucosal and submucosal ulceration compared to the control rats with colitis (Figure 3E and F). However, DRE at all concentrations significantly (P

< 0.0001) reduced the gross microscopic damage scores (Figure 4). Treatment with sulfasalazine 500 mg kg^{-1} resulted in a significant (P < 0.0001) reduction in microscopic damage score (Figure 4) by decreasing mucosal ulceration and the infiltration of inflammatory cells into the lamina propria, muscularis mucosa and submucosa and also by improving cellular detail (Figure 3C).

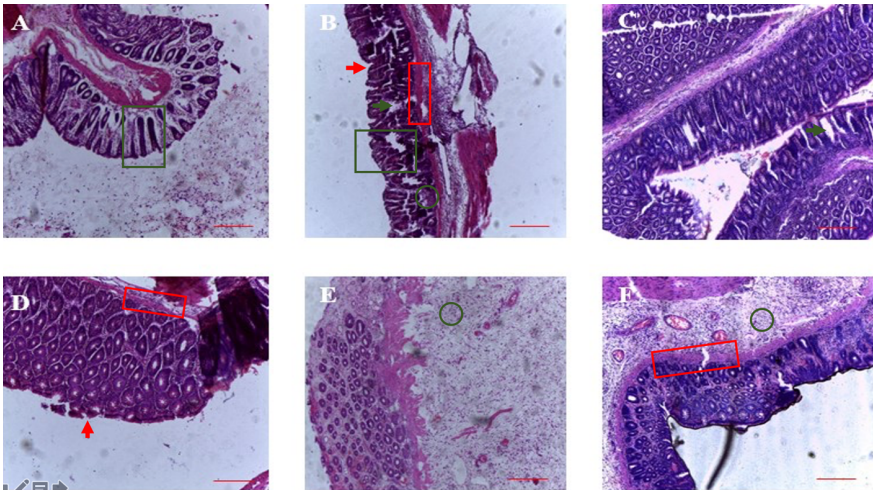

Figure 3: Histopathology of effect of *Dissotis rotundifolia* extract on acetic acid-induced ulcerative colitis in rats.

Control rats without colitis (A), control rats with colitis (B), sulfasalazine 500 mg kg^{-1} (C), DRE 30 mg kg^{-1} (D), DRE 100 mg kg^{-1} (E) and DRE 300 mg kg^{-1} (F). Scale bar represents 100 μm of tissue. DRE, *Dissotis rotundifolia* extract.

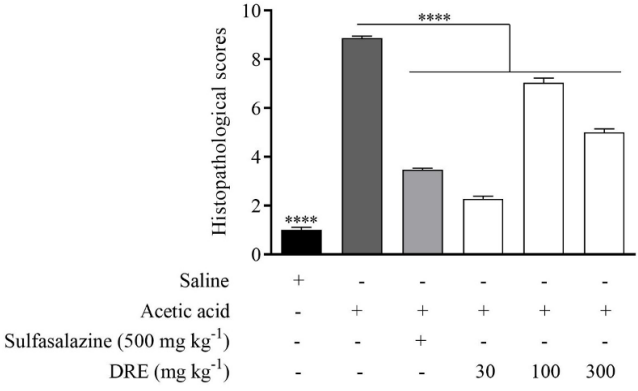

Figure 4: Histopathological score of effect of *Dissotis rotundifolia* extract on the colon.

****P < 0.0001 when compared with control rats with colitis. DRE, *Dissotis rotundifolia* extract.

Analysis of blood samples collected from the jugular vein of the animals showed significant hematological imbalances in the control rats with colitis (Figure 5). Treatment with sulfasalazine and DRE (30, 100 and 300 mg kg^{-1}) resulted in significant decrease in the number of WBCs compared to the control rats with colitis (Figure 5A). However, decrease in the number of WBCs upon treatment with DRE 30 and 300 mg kg^{-1} was significant compared to treatment with sulfasalazine ($P < 0.0001$) (Figure 5A). Significant increase in neutrophil number ($P < 0.0001$) as compared to the rats without colitis was observed in the acetic acid challenged group (Figure 5B). Neutrophil number was significantly reduced upon treatment with DRE (30, 100 and 300 mg kg^{-1}), however, treatment with sulfasalazine resulted in no significant decrease in neutrophil number when compared with the control rats with colitis (Figure 5B). Upon treatment with sulfasalazine and DRE (30, 100 and 300 mg kg^{-1}), significant ($P < 0.0001$) decrease in the lymphocyte number (Figure 5C) and percentage (Figure 5E) compared to the control rats with colitis was observed. Treatment with DRE 30 and 300 mg kg^{-1} resulted in a significant decrease in the number of platelets ($P < 0.0001$) while with sulfasalazine and DRE 100 mg kg^{-1} significant increase in platelets ($P < 0.0001$) was observed when compared to the control rats with colitis (Figure 5D). Treatment with sulfasalazine and DRE (30 and 100 mg kg^{-1}) resulted in a significant increase in mean cell hemoglobin ($P < 0.0001$) (Figure 5F). However, at a dose of 300 mg kg^{-1}, significant decrease in mean cell hemoglobin was observed ($P < 0.0001$) (Figure 5F). The effects observed at 300 mg kg^{-1} were indicative of myelosuppression.

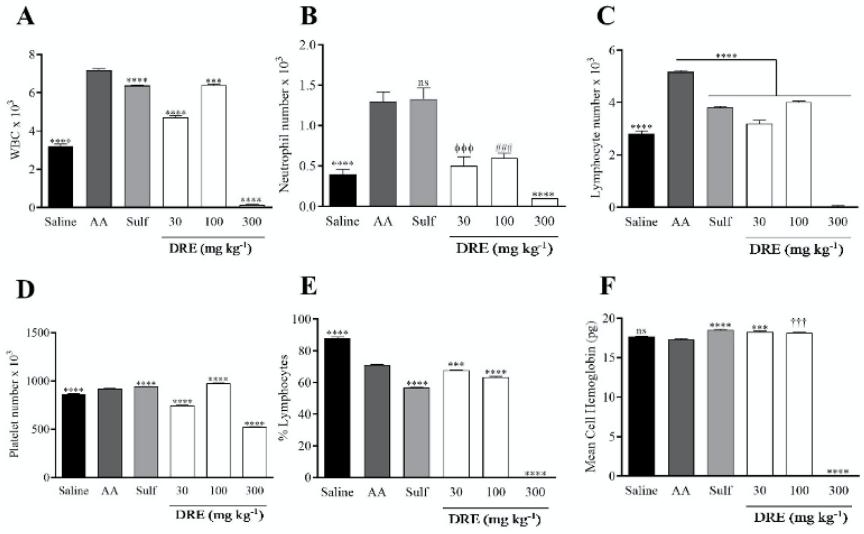

Figure 5: Effect of *Dissotis rotundifolia* extract on hematological parameters in acetic acid-induced colitis in rats.

$^{****}P < 0.0001$, $^{***}P = 0.0001$, $^{\phi\phi\phi}P = 0.0002$, $^{\#\#\#}P = 0.0007$, $^{\dagger\dagger\dagger}P = 0.003$, $^{ns}P > 0.05$ when compared with control rats with colitis. DRE, *Dissotis rotundifolia* extract; Sulf, Sulfasalazine; AA, Acetic acid.

The ability to inhibit necrosis of the colon, by DRE, is also seen in the reduction of intestinal hemorrhage evidenced by the decreased observation of melena and the subsequent increase in mean cell hemoglobin. Colonic damage led to the infiltration of the mucosa, submucosa and lamina propria by inflammatory cells chiefly neutrophils. The infiltrated neutrophils produce large amounts of reactive oxygen species which activates proteolytic enzymes causing endothelial cell damage. The extract was able to attenuate mucosal and submucosal necrosis by preventing oxidative stress-mediated tissue damage following initial tissue insult and also by promoting the recovery of mucosal integrity following mucosal damage.

After colons were extirpated and the colon weight to length ratio determined, the control rats with colitis showed significant ($P < 0.0001$) degree of oedema (Figure 6), compared to the control rats without colitis. Treatment with sulfasalazine resulted in a significant reduction ($P < 0.0001$) in oedema compared to the control rats with colitis. Significant reduction ($P < 0.0001$), in the degree of oedema, compared to the control group with colitis, resulted from the treatment with DRE at doses 30 mg kg^{-1} and 300 mg kg^{-1} respectively (Figure 6). However, treatment with DRE at 100 mg kg^{-1} resulted in no significant decrease ($P > 0.05$) (Figure 6), in total oedema compared to the control rats with colitis.

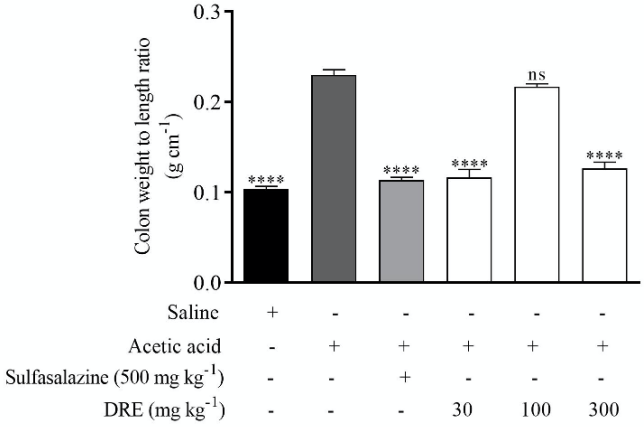

Figure 6: Effect of *Dissotis rotundifolia* extract on colon oedema in acetic acid-induced colonic damage in rats.

$^{****}P < 0.0001$, $^{ns}P > 0.05$ when compared with the control group with colitis. DRE, *Dissotis rotundifolia extract*.

Inflammation causes the activation of the components of the immune system both cellular and systemic, causing the recruitment and activation of immune cells such as leucocytes, neutrophils, etc. Chemical mediators such as chemokines, cytokines and free radicals released by the innate immune cells causes leucocyte recruitment to the injury site leading to elimination of the insult with activation of the adaptive immune response by natural killer and dendritic cells[26]. The inflammatory mediators generated can modulate cell proliferation, death and differentiation and amplify the response to the initial injury[27]. This implies that, inhibition of the proliferation of immune or inflammatory cells such as in myelosuppression, will lead to the attenuation of the inflammatory process and a decrease in disease activity. The decreased plasma cellular components by the extract indicates bone marrow suppression. Though inconclusive, it can however be inferred that DRE suppresses the proliferation, differentiation and maturation of inflammatory cells from the bone marrow reducing their recruitment to the site of injury thereby attenuating the inflammatory process.

Functional activation of mast cells during mucosal inflammation causes *de novo* synthesis and release Arachidonic acid metabolites such as prostaglandins and leukotrienes, PAF, chemokines and cytokines[28,29]. The increased production and release of these mediators alters vascular permeability and enhance vasodilation[30]. The increased vascular permeability as a result of acetic acid-induced colonic damage led to a decrease in intravascular oncotic pressure and an increase in the oncotic pressure of the interstitial fluid following the escape of protein-

rich fluid from plasma into the extravascular space. This, together with increased vascular hydrostatic pressure following vasodilation, resulted in the outflow of intravascular fluid and its subsequent accumulation in interstitial spaces. The net increase in the extravascular fluid volume resulted in oedema of the colon. Thus, the significant inhibition of colon oedema by DRE indicates the role of DRE in inhibiting intestinal vascular permeability and vasodilation by attenuating the activities of various vasoactive mediators such as prostaglandins and histamine on their respective receptors. Claudin-1 is one of the commonest proteins that enhances or maintains colonic epithelial barrier function[30] and reduction in its levels has been observed in the gut mucosa following induction of colon injury with acetic acid[31]. Reduction in claudin-1 levels leads to a decrease in tight junction formations with subsequent development of oedema following the escape of intravascular fluid into the interstitial spaces. The reduction in colon oedema by DRE signifies its ability to enhance claudin-1 formation in addition to its probable inhibitory effect on vasoactive mediators such as prostaglandin and histamine.

The aqueous methanol leaf extract of *Dissotis rotundifolia* is effective and useful in experimental ulcerative colitis. It decreases ulcerative colitis-associated weight loss, macroscopic and microscopic colon damage, and colon oedema due to its anti-inflammatory property.

STATEMENT OF ETHICS

In accordance with internationally accepted principles for laboratory animal use and care (EEC Directive of 1986: 86/609 EEC), the animals were considerately handled throughout the experiment. Additionally, all animal experiments were approved by the Department Ethics Committee [Approval Number DP-COL/2018/007. Valid from 1st June 2018 to 31st May 2019].

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTION

OKY, Performed the experiment and drafted the manuscript; NO, Conceived the idea of the experimental design and analysed the obtained the data; AOA, Data analysis and interpretation; LBE, Drafted the manuscript.

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