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ORIGINAL ARTICLE

Deguelin Treatment Attenuated the Lipopolysaccharides-Provoked Inflammation in Murine Macrophage and Dextran Sulfate Sodium-Inflamed Acute Colitis in Mice through Suppression of Inflammatory Markers

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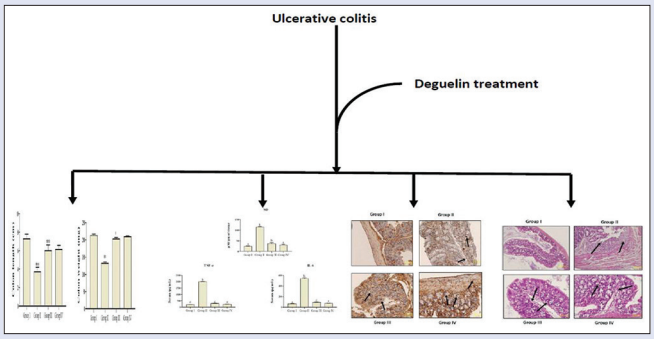
ABSTRACT

Background: Inflammatory bowel disease was a chronic inflammatory disease associated with the gastrointestinal region along with its two main types, i.e., ulcerative colitis and Crohn's disease. The occurrences of colitis were quickly expanding in recent decades worldwide. **Objectives:** In this investigation, we anticipated to examine the curative usefulness of deguelin, a natural herbal rotenoid against the lipopolysaccharide (LPS)-provoked inflammatory responses in murine macrophages and dextran sulfate sodium (DSS)-inflamed acute colitis in replica of mice. **Materials and Methods:** The inflammation response in RAW-264.7 cells was triggered with the LPS administration. The acute colitis was stimulated in BALB/c mice through administering the DSS. The colon length and disease activity index score was evaluated. The statuses of inflammatory arbitrators such as interleukin (IL)-6, tumor necrosis factor-α (TNF-α), and nitric oxide (NO) and enzymatic function of myeloperoxidase were inspected via commercial kits. The matrix metalloproteinase-2 expression in the colon tissues and colon histological examination were done to assess the deleterious effects of DSS in mice. **Results:** The deguelin treatment markedly alleviated the proinflammatory markers augmentation such as IL-6, TNF-α, and NO in the murine macrophages, as well as in DSS-provoked colitic mice. Deguelin markedly reversed the colon shortening and also improved the colon weight. The histopathological investigation of colon tissues exposed the protective effect of deguelin. A marked alleviation in DSS-incited colon inflammation was noted in the deguelin-supplemented mice. **Conclusion:** The findings of this research were established the remedial values of deguelin against the DSS-inflamed colitis in mice. It was clinched that the deguelin can be a promising therapeutic agent to treat the colitis.

Key words: BALB/c mice, deguelin, inflammation, sulfasalazine, ulcerative colitis

SUMMARY

- Dextran sulfate sodium (DSS)-exposed murine displays the declined bodyweight, rectal blood loss, diarrheal condition, and escalated myeloperoxidase actions as an indicator of leukocytes penetration and histological alterations such as mucosal destruction
- Deguelin alleviated the DSS and lipopolysaccharides provoked an escalation of proinflammatory arbitrators in mice replica and murine macrophages, respectively.



Abbreviations used: IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease; LPS: Lipopolysaccharides; DAI: Disease activity index.

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INTRODUCTION

Ulcerative colitis (UC), one of the main subgroups of the inflammatory bowel diseases (IBDs) that mainly mark the colon, rectum, and distinguished via destruction and severe inflammation at colon mucosa.[1] The major signs of UC are bloody stool, diarrhea, and severe abdominal pain, and it could be identified through the colonoscopic analysis. The frequent occurrence of UC possibly directs toward the pathogenesis of colon cancer.[2] The incidence of UC was rapidly

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expanding in recent era worldwide, particularly in developing countries.[3-5] The pathological processes of UC were multifactorial that comprises environmental causes, food habits, living style, genetic propensity, a disparity of intestinal microbial flora, mucosal deficiency, dysfunctions of epithelial barriers, and lessened regulation of immunological responses.[6]

The molecular events of UC were extremely multifarious, and the recent scientific reports emphasized that the inflammation processes were contributed to the pathogenesis of colitis.[7,8] At the progressive stage of UC, the inflammatory modulators, i.e., tumor necrosis factor-α (TNF-α), interleukin-1 β (IL-1β), and interleukin-6 (IL-6), were raised and those inflammatory markers able to cause the inflammatory reactions of the mucosal immune system.[8,9] It has been largely recognized that the macrophages take a vital function in the innate immune inflammatory reactions.[10] As well, the macrophages were viewed to be the main kind of innate immune cells, which is normally stimulated on dextran sulfate sodium (DSS)-provoked acute colitis. The elevated statuses of proinflammatory regulators, IL-1β and TNF-α noted in the DSS-stimulated acute colitis, were proportionate to the harshness of the inflammation. Besides, the enhanced status of IL-1β was identified in the colonic mucosa as well as peritoneal macrophages of DSS-provoked colitis, which showed that the innate immune reactions of macrophages take a crucial function in the UC.[11,12] The DSS is a synthetic agent, which widely utilized to stimulate acute colitis in the animal replicas to scrutinize the curative potentials of sample drugs, extracts, and novel agents. The DSS was efficiently stimulating the destruction of epithelial linings, penetration of immune cells, as well as inflammatory reactions in the colon of test animals. DSS-provoked acute colitis in animal replicas shows the bloody feces, severe diarrhea, losing of body weight, and histological alterations in the colon tissues, which is very similar to the colitis conditions in humans,[13] and these signs are often stared as an essential tool to examine the molecular as well as cellular processes of UC that utilized for examining the therapeutic potential of novel anti-inflammatory agents.[14,15] The triggering of inflammation in ailment condition is illustrious via the innate and adaptive immune reactions, which include elevated proinflammatory modulators discharge in the intestinal area.[16] Those uplifted proinflammatory markers such as IL-1β, TNF-α, and IL-6 able to deteriorate permeability of intestinal linings and mucosal protection system as well as it associates with the sternness of intestinal inflammation in UC.[17] At present, the remedial approach for UC was largely depended on conventional drugs, such as the administration of corticosteroids and immunosuppressive drugs. hence, the urge for exploring the conventional agents with null side effects for the treatment of UC was improved. In modern decades, the exploration of natural herbal-based medicines was amplified and it can be an auspicious approach to develop an effectual agent to the corresponding treatment strategy for UC.[18]

Deguelin, a natural herbal rotenoid separated from the *Derris trifoliate* and *Mundulea sericea* (*Leguminosae*), was exhibited numerous pharmacological activities. Deguelin was exposed the potent antitumorigenesis,[19] antiangiogenesis,[20] and antimetastasis[21] functions on different kinds human malignancies. Deguelin also influenced the potent anticancer activity against different types of cancers, i.e., skin papilloma, lung cancer, colon cancer, mammary gland adenocarcinoma, and head squamous cell cancer.[19,22] However, there is no scientific indication to claim the anti-inflammatory efficiency of deguelin against acute colitis in animal models. Current research work was intended to examine the curative benefits of deguelin against lipopolysaccharides (LPSs)-stimulated inflammatory responses in murine macrophages and DSS-provoked acute colitis in mice.

MATERIALS AND METHODS

Chemicals

Deguelin, DSS, sulfasalazine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and all other chemicals and reagents were purchased from Sigma-Aldrich, USA. The ELISA kits to estimate the inflammatory markers were procured from MyBioSource, USA. Antibodies of matrix metalloproteinase-2 (MMP-2) were purchased from SantaCruz Biotech, USA. The additional chemicals were bought in diagnostic grade.

In vitro assays of anti-inflammatory actions of deguelin

Cell line

The murine macrophage cells (RAW-264.7) were purchased from American Type Culture Collection, USA, and preserved in Dulbecco's modified Eagle's medium growth medium supplemented with fetal bovine serum (10%) and antibiotic–antimycotic mix (1%) in a moistened incubator (Borg, Austria) along with 5% CO_2 at 37°C. The cells were reloaded every 2–3 days.

Cell viability assay

The toxicity of deguelin on the RAW-264.7 cells was assessed via the MTT test.[23] The RAW-264.7 cells were loaded on a 96-well plate at 1×10^4 cells/well density. After sustaining of 24 h, the cells were provoked with LPS (100 ng/mL) along with various dosages (1, 2.5, 5, 10, and 25 μg/mL) of deguelin. Later than 24 h of incubation, 10 μL of MTT reagent was mixed to every well and again incubated for 4 h at 37°C. Afterward, the medium was removed and 100 μL of dimethyl sulfoxide was added to liquefy the formazan crystals. Finally, the absorbance was taken at 570 nm with the aid of the microplate reader (Biorad, USA).

Assay of inflammatory cytokines and nitric oxide levels in macrophages

RAW-264.7 cells were loaded in the 24-well plate at 1×10^4 cells/well density and presupplemented with deguelin at two different doses (10 and 15 μg/mL) and maintained for 1 h. Afterward, the cells were provoked along with 100 ng/mL of LPS for the next 18 h. Followed by incubation regimen, the medium was removed and the cells suspension was used to detect the amount of proinflammatory cytokines, TNF-α and IL-6, with the aid of relevant ELISA test kits (MyBioSource, USA). The nitric oxide (NO) status was examined via the Griess reagent (Sigma-Aldrich, USA) with sodium nitrite as standard.[24]

Experimental animals

BALB/c mice (male breed) aged 7 weeks weighing from 18 to 22 g were bought in institutional animal house and continued in organized laboratory situation (26°C ± 1°C, air moisture 60%–70%, light and dark sequence being 12 h) with at most care. All animals were fed with commercial pellet food with water *ad libitum*. The animals were getting used to laboratory situations for 7 days previous to the beginning of the work. The heed and treatment of the rats were completed based on the institutional animal handling guidelines (02-2019).

Experiment design and induction of colitis in mice

All animals have alienated randomly into four groups with six animals. Group-I was provided as control and given only drinking water excluding DSS for 7 days; Group-II was UC-provoked group via providing the 2% of

DSS along with drinking water for 7 days.[25] Group-III was supplemented with 30 mg/kg of deguelin via gavage route alongside to DSS for 7 days. Group-IV animals were given with sulfasalazine (50 mg/kg) via gavage route concurrently to DSS-challenge and provided as a positive control. After the experimental time, all animals were anesthetized by using suggested institutional guidelines through chloroform and fortified via cervical dislocation; then, colon tissues were gathered and used for further examinations.

Assay of disease severity

The preclinical study of DSS-provoked acute colitis in mice was examined via the method of Zhang *et al.*[26] Disease activity index (DAI) was measured based on the scores allocated to each disease sign that contains loss of body weight, change in stool consistency, and the occurrence of bloody stool. Simply, DAI score was measured as the summation of bodyweight declining (score 0 denotes none, 1 as 1%–5%, 2 as 5%–10%, 3 as 10%–20%, and 4 as over 20%, in that order), occurrence or lack of bloody feces (score 0 designates negative hemoccult, 2 as positive hemoccult, 4 as gross bleeding, respectively), and stool consistency (score 0 denotes well-formed pellets, 2 means loose stools, and 4 means diarrhea).

Assay of myeloperoxidase function

Myeloperoxidase (MPO) enzyme function was assessed via the procedure described by Khan *et al.*[25] Detached colon tissues form control and deguelin-treated colitis mice were homogenized along with 0.1 M phosphate buffer (pH 6.5); then, it was spun at 11,000 rpm for 25 min at 4°C. Resulting supernatant (0.1 mL) was added to 2.9 mL of reaction mixture that contains 0.16 mg/mL of o-dianisidine hydrochloride and the 0.0005% of hydrogen peroxide, and it was incubated for 5 min at 37°C, and finally, the absorbance was taken at 460 nm with the help of spectrophotometer.

Assay of proinflammatory mediators and nitric oxide levels in colon tissue

The separated colon tissues from control and deguelin-treated colitis animals were homogenized with the 50 mM of buffered saline (pH 7.4) along with 1% of protease enzyme-inhibiting complexes. The 10% of homogenate was spun at 7000 rpm for 25 min, and the upper aqueous phase was used to detect the proinflammatory cytokines such as IL-6 and TNF-α with the help of relevant ELISA test kits (MyBioSource, USA) in accordance with the protocols stated by the manufacturer. The total amount of NO was detected via the reaction with Griess reagent (Sigma-Aldrich, USA) and sodium nitrite was employed as standard.[27]

Immunohistochemical study of colon tissue

The collected colon tissues of control and deguelin-treated colitis animals were fixed on paraffin and cut at 5 μm size. Then, the sections were handled with 0.5% of Triton × −100 at 37°C for 35 min. The sections were washed in phosphate-buffered saline (PBS) and incubated with rhodamine-phalloidin at 1:60 dilution in PBS for 40 min at 37°C. Sections were then blocked with 2% of bovine serum albumin (BSA) for 1 h at 37°C, and it was incubated with primary antibodies, anti-MMP-2 (SantaCruz Biotech, USA) 15 g/mL in 2% of BSA in buffered saline for 1 h at 37°C. After cleaning thrice with saline sections were incubated with rhodamine-conjugated mice-specific secondary antibodies (SantaCruz Biotech, USA) 1/100 in 2% of BSA in saline for 45 min at 37°C, and finally, it was scrutinized beneath the optical microscope (Olympus, Tokyo, Japan).

Histological study of colon tissue

The removed colon tissues from control and deguelin-treated colitis animals were treated with 10% of formalin and entrenched on paraffin. Then, the tissues were sliced into 5 μm size and stained with hematoxylin and eosin (H and E). Finally, the sections were observed beneath the optical microscope at ×40, and histological changes stimulated by DSS and protective efficiency of deguelin were restrained.

Statistical assessment

The statistical examination was done with SPSS (Version 17) (SPSS Inc., Chicago, IL) statistical tool. Data were represented as mean ± standard deviation. One-way ANOVA after by Duncan's Multiple Range Test (DMRT) quantity analysis was utilized to assess the statistical relevance among the variable groups. Data are viewed as statistically significant if the $P < 0.05$.

RESULTS

Cytotoxic effects of deguelin in murine macrophage cells

The cytotoxic level of deguelin in murine macrophage cells (RAW-264.7) was assessed via the MTT test. No cell death was found in the control group. Figure 1 shows that 5 and 15 μg/mL of deguelin did not show any cytotoxicity to RAW-264.7 cells, while 20–50 μg/mL of deguelin influenced mild cytotoxicity to macrophage cells, and a slight reduction in the cells was noted. 5 and 15 μg/mL of deguelin have exhibited an analogous result with the untreated control [Figure 1], which reveals that those dosages were not cytotoxic to murine macrophage cells.

Deguelin treatment alleviates the proinflammatory regulators and nitric oxide accretion in lipopolysaccharide-provoked RAW-264.7 cells

The LPS challenge to the RAW-264.7 cells results in the magnificent elevation in proinflammatory arbitrators (IL-6 and TNF-α), as well as NO, which is quite conflicting to untreated control cells. However, the deguelin (10 and 15 μg/mL)-pretreated RAW-264.7 cells along

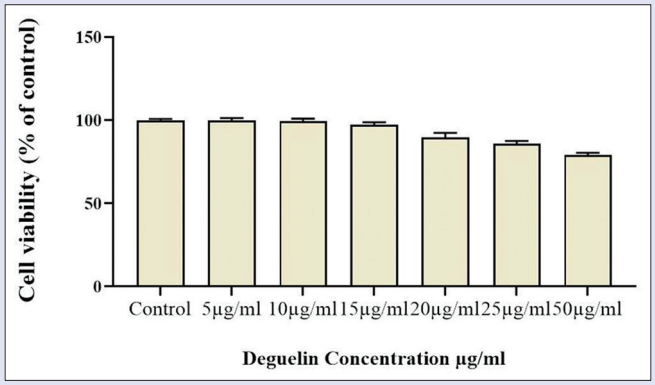

Figure 1: Effect of deguelin on lipopolysaccharide-treated murine macrophage cells viability. All values are depicted as mean ± standard deviation ($n = 6$). The level of statistical significance was calculated by one-way ANOVA followed by the DMRT test; note: $^{*}P < 0.05$ when compared with the control group and $^{\#}P < 0.05$ when compared with the lipopolysaccharides treated group. Figure 1 evidencing that the 5 and 15 μg/mL of deguelin did not display any cytotoxicity to RAW-264.7 cells. However the 20–50 μg/mL of deguelin possessed a mild cytotoxicity to the macrophage cells

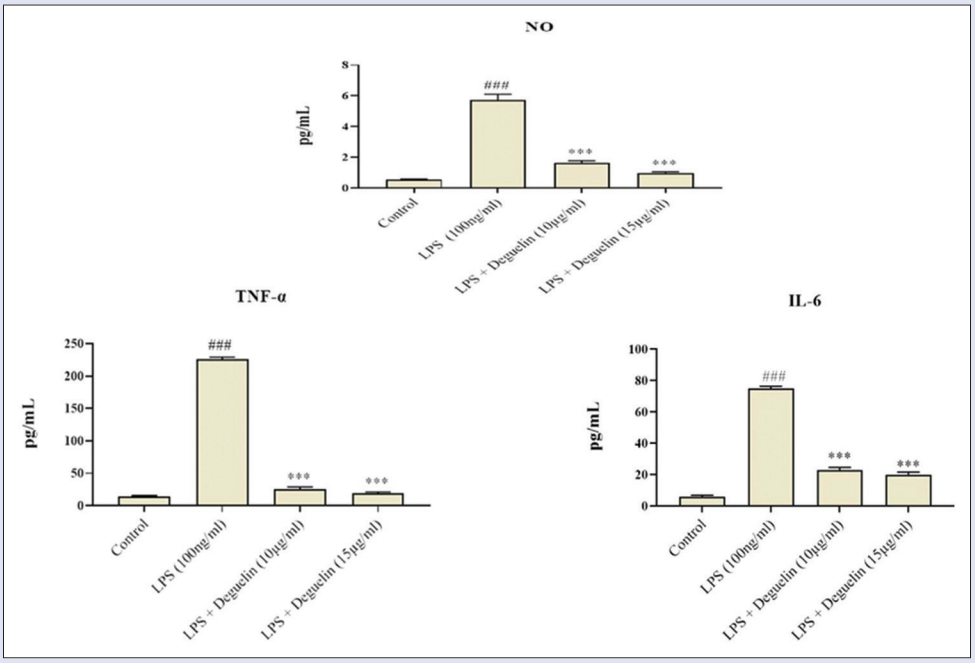

Figure 2: Effect of deguelin on pro-inflammatory cytokines level in lipopolysaccharides -treated murine macrophage cells. All values are depicted as mean ± standard deviation ($n = 6$). The level of statistical significance was calculated by one-way ANOVA followed by the DMRT test; note: $^{*}P < 0.05$ when compared with the control group and $^{\#}P < 0.05$ when compared with the lipopolysaccharides treated group. The treatment with the 10 and 15 µg/mL of deguelin to the AW-264.7 cells along with lipopolysaccharides provoking displayed diminished statuses of interleukin-6, tumor necrosis factor-α, and nitric oxide while comparing it to the lipopolysaccharides alone challenged cells

with LPS provoking exhibited a diminished ($P < 0.05$) statuses of IL-6, TNF-α, and NO [Figure 2], when comparing it to LPS alone challenged cells. 15 µg of deguelin was markedly assuaged the IL-6, TNF-α, and NO statuses and reinstated to near-normal range in LPS provoked RAW-264.7 cells.

Deguelin recovered the bodyweight and disease activity index score in dextran sulfate sodium-provoked colitic mice

The bodyweight was drastically decreased and the DAI score was escalated radically in the DSS-provoked colitis mice that are differentiated to untreated control [Figure 3]. Deguelin (30 mg/kg) treatment to DSS-challenged colitis mice showed appreciable regain ($P < 0.05$) in the bodyweight as well as alleviated the DAI score close to normal range when comparing it to DSS-provoked group. The standard drug sulfasalazine (50 mg/kg) treatment also revert back the DAI score and raised the bodyweight in DSS-challenged colitis mice [Figure 3].

Deguelin suppressed the myeloperoxidase action in colon tissue of dextran sulfate sodium-challenged colitic mice

The enzymatic action of MPO was severely uplifted in the colon tissues of DSS-provoked colitis mice than unprovoked control. The treatment with 30 mg/kg of deguelin was extremely ($P < 0.05$) repressed the enzymatic function of MPO in DSS-provoked colitis mice, which is straight contrast to DSS alone-treated mice [Figure 4]. Deguelin treatment noticeably suppressed the MPO function and showed a similar outcome to the control. The treatment with sulfasalazine (50 mg/kg) was also suppressed an MPO action in the colon tissues of colitis mice.

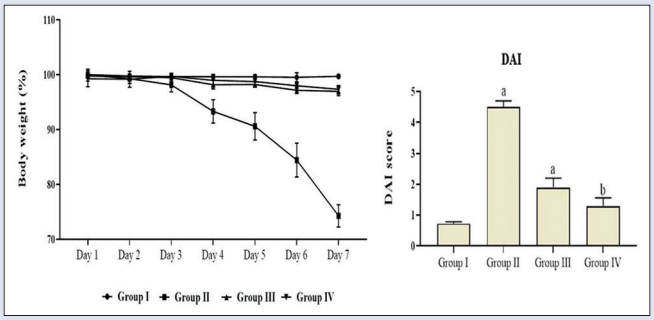

Figure 3: Effect of deguelin on bodyweight and disease activity index score in dextran sulfate sodium-treated colitic mice. All values are depicted as mean ± standard deviation ($n = 6$). The level of statistical significance was calculated by one-way ANOVA followed by the DMRT test; note: $^{*}P < 0.05$ when compared with the control group and $^{\#}P < 0.05$ when compared with dextran sulfate sodium-treated group. The deguelin (30 mg/kg) treatment to the dextran sulfate sodium-challenged colitis mice displayed appreciable regain in the body weight and also diminished the disease activity index score, which is close to the normal level when comparing it to the dextran sulfate sodium-induced animals

Deguelin improved the colon weight and length in dextran sulfate sodium-provoked colitic mice

Figure 5 shows the severe diminution in length and weight of colon in DSS-provoked colitic mice, which shows the sternness of DSS. The treatment with deguelin (30 mg/kg) was astonishingly ($P < 0.05$) escalated the colon weight and length in colitic mice, while comparing it to DSS-only–challenged mice [Figure 5]. The 50 mg/kg of sulfasalazine treatment has also endorsed the length and weight of colon in colitic mice.

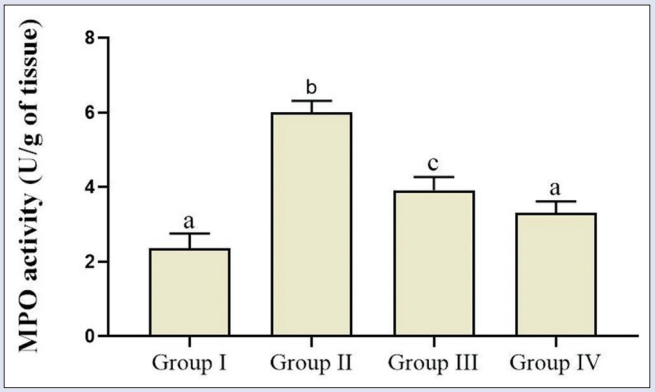

Figure 4: Effect of deguelin on colon weight and length of dextran sulfate sodium-induced colitic mice. All values are depicted as mean ± standard deviation (n = 6). The level of statistical significance was calculated by one-way ANOVA followed by the DMRT test; note: *P < 0.05 when compared with the control group and #P < 0.05 when compared with dextran sulfate sodium-treated group. The treatment with 30 mg/kg of deguelin was appreciably suppressed the enzymatic actions of myeloperoxidase in dextran sulfate sodium-provoked colitis mice, which is quite contrast to the dextran sulfate sodium-alone–treated mice

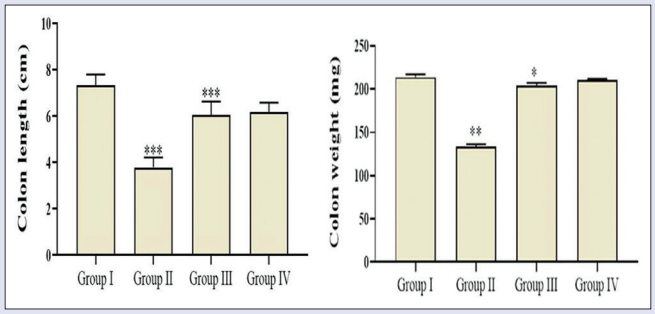

Figure 5: Effect of deguelin on myeloperoxidase activity in the colon tissues of dextran sulfate sodium-induced colitic mice. All values are depicted as mean ± standard deviation (n = 6). The level of statistical significance was calculated by one-way ANOVA followed by the DMRT test; note: *P < 0.05 when compared with the control group and #P < 0.05 when compared with dextran sulfate sodium-treated group. The treatment with the deguelin (30 mg/kg) was astoundingly enhanced the colon weight and length while comparing it to the dextran sulfate sodium-only–challenged experimental mice

Deguelin assuages the proinflammatory markers and nitric oxide accumulation in dextran sulfate sodium-provoked colitic mice

The statuses of proinflammatory indicators (IL-6 and TNF-α) were strikingly escalated in the serum, as well as the status of NO were also increased severely in the colon tissues of DSS-challenged colitic mice, which is straight contrast to untreated control. Deguelin (30 mg/kg) treatment was disclosed the astonishing (P < 0.05) attenuation of IL-6 and TNF-α statuses in the serum and also lessened the NO status in the colon tissues of DSS-provoked colitic mice, while comparing it to DSS-only–provoked mice [Figure 6]. The standard drug sulfasalazine (50 mg/kg) as well displayed the marked reduction in IL-6 and TNF-α. 2.5 μg of deguelin was markedly assuaged the IL-6, TNF-α, and NO statuses and reinstated to near-normal range in DSS-incited colitic mice.

Deguelin repressed the expression of matrix metalloproteinase-2 expression in colon tissues of dextran sulfate sodium-incited colitic mice

Immunohistochemical studies of the colon tissue of untreated normal mice showed the weak expression of MMP-2, while the expression patterns of MMP-2 were harshly uplifted in the colon tissue of DSS-incited colitic mice, which is contrary to control. Figure 7 shows that the 30 mg/kg of deguelin treatment was noticeably repressed the MMP-2 expression in the colon tissue of colitic mice. The treatment along with 50 mg/kg of sulfasalazine to colitic mice was also remarkably suppressed the MMP-2 expression that correlating to the outcomes of deguelin treatment [Figure 7].

Deguelin protected the colon tissue architecture in dextran sulfate sodium-inflamed colitic mice

The protective effects of deguelin in acute colitis-provoked mice were assessed via the H and E staining method. Figure 8 shows that the deleterious effects of DSS in colon tissues of colitic mice exhibited broken mucosal deposits and epithelial cell linings. DSS-only–incited mice displayed the mucosal inflammation, crypt loss, severe fibrosis, and penetration of leucocytes with ulcerations. The normal epithelial and goblet cells along with slight hemorrhage were noted in deguelin (30 mg/kg)-treated colitic mice [Figure 8]. Deguelin treatment also alleviated leucocyte permeation, crypt damages, and mucosal ulcerations. The usual intestinal tissue integrity and epithelial linings were detected in deguelin-treated colitic mice. The 50 mg/kg of sulfasalazine treatment as well exposed the appreciable protection to t colon cells against DSS-inflamed colitis in mice [Figure 8].

DISCUSSION

IBD was a recurrent inflammatory disease associated with the gastrointestinal region along with its two foremost types, i.e., UC and Crohn's disease (CD), and the distinct reasons behind the progression of IBD are still unclear.[28] IBD was known as an immunological-regulated intestinal disease distinguished by persistent mucosal destruction through inflammation in the intestinal region.[29] UC is a persistent disease renowned through unrestrained inflammation in the colon mucosal region; over the years, the incidence of UC was constantly escalated in throughout the world, particularly in developing countries.[30] The detailed causes of UC were not clearly defined, yet but the preceding reports highlighted that it was multifarious; the malfunction of the intestinal epithelial barrier was regarded as one of the imperative factors that eventually escorts to the pathogenesis of UC.[31]

The exact causes and pathological processes of IBD were not visibly studied yet, nevertheless, the corticosteroids and immune-regulatory drugs are often used as a mainstay treatment for IBD; conversely, a lot of problems are associated with those drugs such as deleterious effects, deprived responses, and lessen effectual.[32,33] It is hugely essential to inspect the remedial agents with enhanced effectual and null side effects to the IBD patients.[10,34] Due to the steadily escalating occurrences of IBD worldwide and reduced effectual of currently employed drugs with side effects, it was essential to develop the novel herbal-derived compounds or drugs with the enhanced therapeutic potency and null adverse effects. Moreover, the reports from the primary healthcare system were evidenced by the growing attention in the adopting of plant-derived products and herbal-based supplements as a substitute therapy for inflammatory ailments such as IBD.[35,36] In this exploration, the deguelin, a natural herbal rotenoid found in many plants such as *D. trifoliate* (*Leguminosae*), was subjected to inspect the LPS-provoked inflammatory responses in murine macrophages and DSS-provoked acute colitis in mice replica.

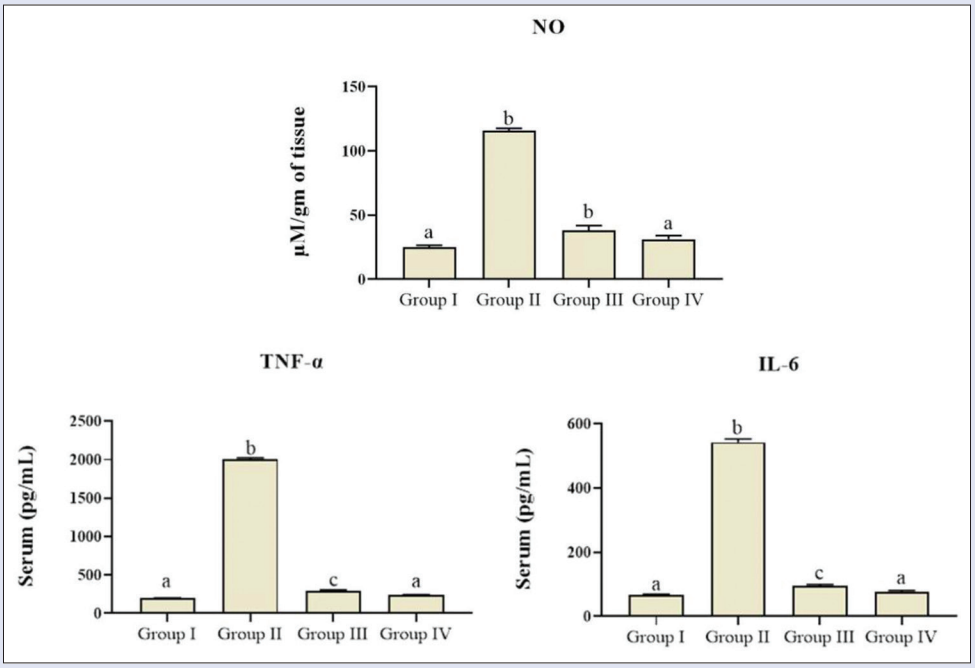

Figure 6: Effect of deguelin on nitric oxide and proinflammatory cytokines level in the serum of dextran sulfate sodium-induced colitic mice. All values are depicted as mean ± standard deviation ($n = 6$). The level of statistical significance was calculated by one-way ANOVA followed by the DMRT test; note: $^{*}P < 0.05$ when compared with the control group and $^{\#}P < 0.05$ when compared with dextran sulfate sodium-treated group. Deguelin (30 mg/kg) treatment was disclosed the astonishing attenuation in the interleukin-6 and tumor necrosis factor-α statuses in the serum and also diminished the nitric oxide status in the colon tissues of dextran sulfate sodium-provoked colitic mice while comparing it to dextran sulfate sodium only provoked mice

Due to the most analogous characteristics with the human IBD as well as the expediency of animal replica utilization, the DSS-incited colitis replica was widely used as an investigational mice replica to study the curative potency of sample drugs. DSS-exposed murine displays the declined bodyweight, rectal blood loss, diarrheal condition, and escalated MPO actions as an indicator of leukocytes penetration and histological alterations such as mucosal destruction.[37,38] The deguelin supplementation noticeably alleviated the DSS-inflamed colitic signs and pathological alterations in the colitis mice replica. Deguelin strikingly assuaged the DAI score in the DSS-inflamed colitic mice replica.

The preceding reports emphasized that the escalated enzymatic actions of MPO was noted in the DSS-incited UC animal replica. The permeation of neutrophils to the colon tissues eventually deteriorates the MPO functions, and it was regarded as an indicator of neutrophil penetration and trepidation of the inflammatory mechanism that equally can activate the pathological processes and signs of UC.[8,39] Thereby, it is crucial to inspect the status of enzymatic actions of MPO in the colon tissues to examine the extent of colonic injury. Preceding reports were also proved that the neutrophil penetration to the damaged colon tissue can speed up the destruction of colon tissues through enzyme MPO.[40,41] In our work, the supplementation with the deguelin was repressed the MPO enzyme activity in the colon tissues of the DSS-provoked colitic mice replica. It may simplify alleviating the leukocyte permeation to the colon tissues, and it was established through the histological examinations that exhibited not as much of severe ulcerations and inflammation in the colon tissues of DSS-incited mice.

Earlier reports proved that the macroscopic inspection of the colon in IBD patients showed the dispersed mucosa, erythema, and edema along with ulcerations, whereas in the investigational animal replica displayed the lessening and shortening of the colon.[42,43] Precisely, the outcomes of our study exposed that the DSS-inflamed murine exhibited a drastic shortening of the colon. Conversely, the treatment with deguelin was astonishingly regained the colon contraction and shortening in the DSS-inflamed colitic mice.

The escalated statuses of inflammatory modulators, i.e., IL-1β, IL-6, IL-12, and TNF-α, are the divergent characteristics of the inflammatory diseases, particularly IBD. Predominantly, the augmented status of IL-1β was distinguished in the origin of inflammation on the colon tissues that eventually escort to the IBD.[44] The macrophages take enormous functions in regulating the intestinal homeostasis of the colon mucosa in IBD patients. They can accrete enormous amounts of inflammatory modulators, i.e., IL-6, TNF-α and IL-1β, which can activate the strong inflammation and further deteriorate the harshness colitic condition. The stimulated macrophages can accrete the vast levels of IL-1β directly linked with the UC progression. It was also mentioned that the decreasing of those modulators alleviates the incidence and progression of IBD.[45-47] Our findings showed that the deguelin supplementation was remarkably alleviated the proinflammatory markers escalation in the LPS-provoked murine macrophages as well as DSS-inflamed colitic mice.

MMPs were viewed as vital arbitrators of inflammation in retort to the gastric tissue damage at the stage of ulceration. The deprivation of the extracellular matrix takes a crucial function in the expression of ulcerative sores during gastric ulceration coordinated through the MMPs. Moreover, the instigation and differential modulation of MMPs such as MMP-2 and MMP-9 in the extracellular matrix deprivation of gastric tissues were regarded as a prominent feature, which is found during the ulceration.[48] The immunohistochemical investigation of

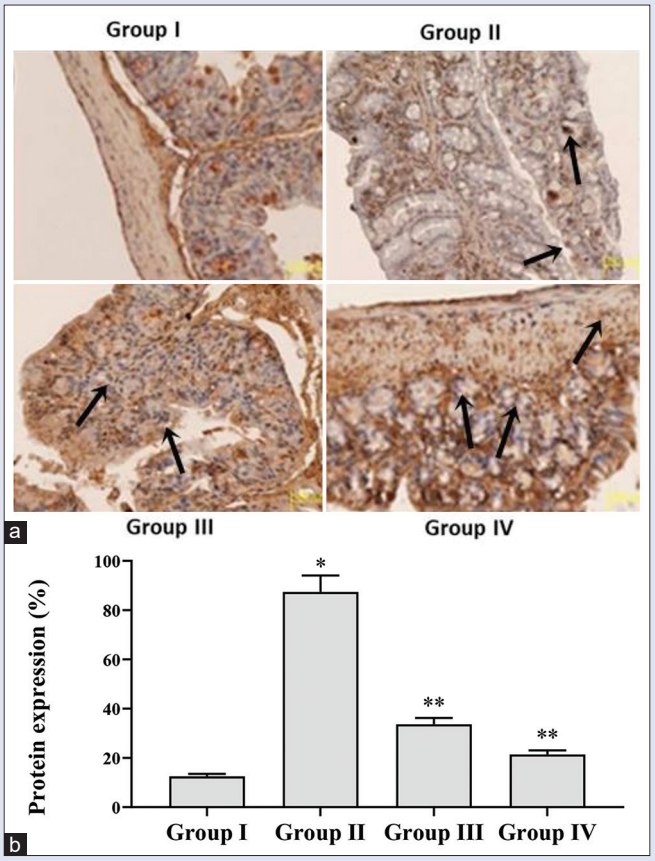

Figure 7: Effect of deguelin on matrix metalloproteinase-2 expression by immunohistochemical analysis of colon tissues of dextran sulfate sodium-induced colitic mice. (a) Control animals displayed slight matrix metalloproteinase-2 expression (Group-I). A drastically elevated expression pattern of matrix metalloproteinase-2 was noted in the dextran sulfate sodium-provoked colitic mice (Group-II). Deguelin treated colitic mice revealed nearly very least matrix metalloproteinase-2 expression (Group-III). Sulfasalazine-treated mice also displayed the near-normal expression of matrix metalloproteinase-2 (Group-IV). (b) The graphical representation of matrix metalloproteinase-2 expression in the colon tissues of experimental animals. All values are depicted as mean ± standard deviation (n = 6). The level of statistical significance was calculated by one-way ANOVA followed by the DMRT test; note: *P < 0.05 when compared with the control group and **P < 0.05 when compared with dextran sulfate sodium-treated group

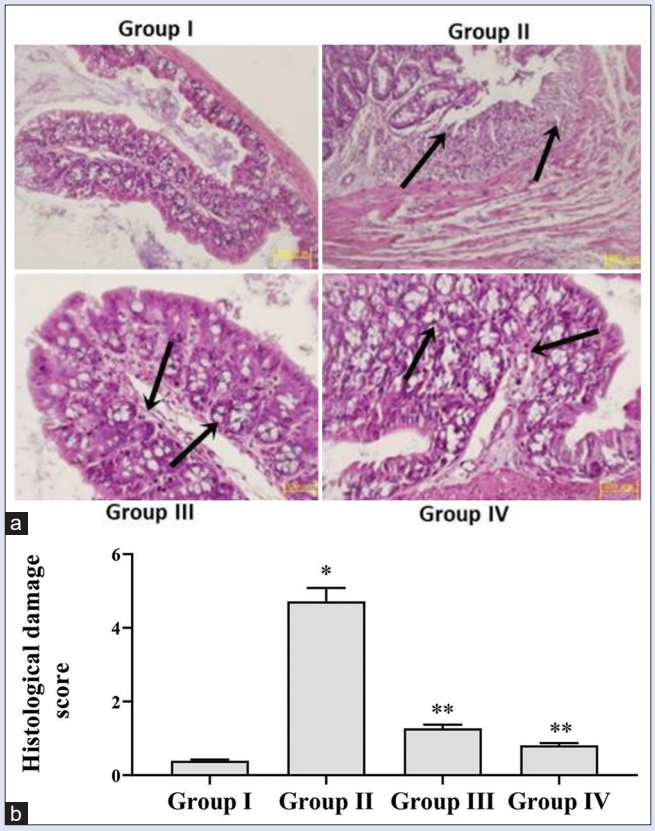

Figure 8: (a and b) Histopathological analysis of colon tissues of control and experimental animals. (a) Control animals displayed the undamaged normal tissue architecture (Group-I). dextran sulfate sodium-provoked animals revealed the escalated neutrophil infiltration, severe inflammation and ulceration in the colon tissues (Group-II). Deguelin-supplemented colitic mice displayed the almost normal tissue architecture with a very minimal sore (Group-III). Sulfasalazine-treated mice showed near-normal tissue structures (Group-IV). (b) Showing the graphical representation of histological changes in the colon tissues of experimental animals. All values are depicted as mean ± standard deviation (n = 6). The level of statistical significance was calculated by one-way ANOVA followed by the DMRT test; note: *P < 0.05 when compared with the control group and **P < 0.05 when compared with dextran sulfate sodium-treated group

colon tissues of DSS-inflamed colitic mice has shown the augmented expression of MMP-2; on the contrary, the deguelin treatment was astonishingly lessened the overexpression of MMP-2 in the colon tissues of DSS-incited colitic mice. The severe ulcerations, inflammation at colon tissues, and crypt deformities may inspect through the microscopic histological investigation.[49] The gathering of neutrophils among the epithelial crypts and intestinal mucosa was straightly connected with the epithelial destructions in the UC.[50] It was already mentioned that the permeation of immune cells was able to activate the inflammation along with the secondary inflammatory products at the stage of the pathological development of UC.[51] The outcomes of histological examining of our work proved that the deguelin treatment markedly alleviated the leukocytes penetration, cryptic damages, and mucosal ulcerations and improved the intestinal tissue integrity and epithelial linings in the DSS-inflamed colitic mice. Based on these findings, it was clear that the deguelin suppressed the inflammation in RAW-264.7 cells and demonstrated a potent anticolitis effectual against the DSS-inflamed acute UC in mice replica via alleviating the inflammation responses.

CONCLUSION

The outcomes of our study showed that the deguelin supplementation was displayed the considerable therapeutic influence against the DSS-inflamed UC in mice replica. Deguelin allayed that the DSS and LPS provoked an escalation of pro-inflammatory arbitrators in mice replica and murine macrophages, respectively. The DSS-inflamed colon inflammation and histological destructions were markedly alleviated by deguelin treatment. Those findings were disclosing the remedial usefulness of deguelin against UC. It was concluded that the deguelin can be a hopeful curative agent for the healing of UC.

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Conflicts of interest

There are no conflicts of interest.

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