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Original Article

Investigation of Effects of *Cynodon Dactylon* Aqueous Extract on the Mice Model Ulcerative Colitis

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Abstract

Background and Aim: *Cynodon dactylon (C. dactylon)* has long been examined for its anti-inflammatory properties. Ulcerative Colitis (UC) is a type of Inflammatory Bowel Disease (IBD). This study investigated the impacts of *C. dactylon* on the inflammatory mediators of UC induced by acetic acid in Wistar rats.

Materials and Methods: The experiment was conducted on 50 male Wistar rats equally divided into five groups. Excluding Group I (negative control), acetic acid was used to induce UC in the rats. The treatment groups received different doses of *C. dactylon* extract as follows: Group II (1 mL of 200 mg/kg orally per day), Group III (1 mL of 200 mg/kg as intra-colonic), Group IV (1 mL of intra-colonic 10 mg/kg Mesalazine), and Group V (1 mL of normal saline) as positive control. The whole treatment period lasted for 10 successive days. Upon the completion of the study, intestinal tissue and blood samples were taken from sacrificed rats. Measured parameters included inflammatory mediators, myeloperoxidase (MPO), superoxide dismutase (SOD), glutathione peroxidase (GPx), and activities of cytokines TNF-α and IL-6. Moreover, animals were evaluated for colon tissue histopathology.

Results: A more significant remedial advantage of *C. dactylon* extract in two forms of trans-rectal and oral administration was observed in the colonic tissue damaged by acetic acid with reduced activities of MPO (2.01±0.152), SOD (0.35±0.62) and GPx (0.47±0.041). Amounts of TNF-α (145±8.544) and IL-6 (125±3.26) were lowered by all treatments. Aqueous extract *C. dactylon* had a more remarkable healing effect on damaged colonic tissue in both forms of trans-rectal and oral administration. Mesalazine and *C. dactylon* extracts were not significantly different in terms of gross damages.

Conclusion: Our observations indicate that *C. dactylon* extract with anti-colitis property can be regarded as an appropriate candidate and a natural source of current medications.

Keywords: *C. dactylon*, Inflammation, Ulcerative Colitis, Rat

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Introduction

Plants have been the sources of isolates and extracts for huge numbers of medications. Therapeutically important metabolites and essential oils have originated from medicinal plants. Various characteristics. Apart from safety, being economical, effective and easily available are the significant therapeutical advantages of medicinal plants in various diseases (1-3). As a perennial grass, *C. dactylon* belongs to the family Poaceae with a wide range of medicinal properties. The plant is grown all over tropical and subtropical regions. The medicinal application involves the entire herb and its root stalk (4, 5). This species naturally grows at northern and eastern Africa, Asia and Australia, and south of Europe. A number of pharmacological activities including antioxidant, antidiabetic, antidiarrheal, hepatoprotective, antiulcer, immunomodulator, CNS depressant, antimicrobial, and germicidal activities have been reported for *C. dactylon* (6-9). Aqueous extract of *C. dactylon* revealed a high antidiabetic potential together with significant hypoglycemic and hypolipidemic advantages. *C. dactylon* possesses crude proteins, carbohydrates, and mineral constituents, oxides of magnesium, phosphorous, calcium, potassium, and sodium (10-12). The entire plant contains β-sitosterol, flavonoids, alkaloids, glycosides and triterpenoids. There are also many chemical constituents viz. hexadecanoic acid, linolenic acid, ethyl ester, hydroquinone, and d-mannose in *C. dactylon* (12, 13). Potential scavenging of 2, 2-diphenyl-1-picrylhydrazyl free radical and nitric oxide are other properties of *C. dactylon*. Such important phytoconstituents as orientin, luteolin, apigenin, and vitexin flavonoids were extracted from this species (14). Because flavonoids inhibit ulcers (UC) in the digestive system, they may play an important role as they have been recognized as anti-inflammatory agents (15). As an inflammatory bowel ailment, UC mainly influences the colonic mucosa. Most forms of UC may be limited to the distal part of the rectum, but most extended forms of UC involve the whole colon. Both genders may develop UC at any age, though, the disease frequently emerges in people aged 15-30 years (16, 17). Although a variety of factors are reportedly the likely etiologic agents, the exact UC pathogenesis is not yet clear. The enzymes involved in the production of inflammatory mediators are affected by genetic factors, infective agents, immunological basis, smoking, medications, and pathological causes (18). Colitis induced by acetic acid is generally employed as an easily inducible model. This kind of colitis is considered to be a model of IBD that is highly similar to human IBD with regard to pathogenesis, histopathological characteristics and inflammatory mediator profile (9).

This study aimed to assess the potent anti-inflammatory activity of *C. dactylon* in comparison with mesalazine, as a standard medication, on UC induced by acetic acid in male Wistar rats.

Materials and Methods

Herbal extraction

Samples of *C. dactylon* were acquired from a local market and identified by a botanist affiliated to the Faculty of Agriculture, Urmia University (Herbarium code: 514). The specimens were finely grounded by a mixer, and the aqueous extraction was carried out as reported previously. We observed the animal care and the general protocols for animal management in compliance with the rules of the Ministry of Health and Medical Education of the I.R. of Iran, endorsed by the Medical Ethics Committee of the University (19).

Animals

A total of 50 male Wistar rats (200 ± 20 g) were obtained from Pasteur Institute, Iran. The animals were kept under conventional conditions *viz*. 22 ± 1 °C, 55 ± 5% relative moisture, and a light/dark cycle of 12/12 h. Animal welfare was maintained based on the National Institute of Health Guide for the Care and Use of Laboratory Animals. After the rats fasted for 24 h, 4% acetic acid (1 mL) was instilled rectally to induce UC (20). As shown in Table 1, the animals were randomly assigned to five equal isolated groups. Different treatments were applied after 24 h. All rats were subjected to euthanasia by cervical dislocation and sampling on the 10^{th} day.

Evaluation of Myeloperoxidase (MPO) Levels

MPO activity was assessed according to a previous procedure and in accordance with the manufacturer's

instructions (ab119605) (21). In brief, after homogenizing the colon tissue the supernatant was used to determine the MPO concentration. Using a UV–visible spectrophotometer, the absorbance of the samples was measured at 460 nm followed by the calculation of MPO activity with a standard curve.

MPO is an influential marker of neutrophil influx into colonic tissues. The proteins that were extracted from the colonic tissues were used to evaluate the MPO levels in the excised colon that was weighed, homogenized in 0.1 M phosphate buffer (pH 7.4) and centrifuged. The supernatant was used to determine the MPO concentration. Absorbance was measured at 460 nm. MPO activity was represented as U/g protein and defined as the quantity of enzyme degrading 1 µmol peroxide per minute at 37˚C.

Antioxidant Enzyme Activities

Superoxide dismutase (SOD) (ab80946) and glutathione peroxidase (GPx) (ab104448) enzymes were quantified by commercial kits (22).

Cytokine Assay

The contents of IL-6 and TNF-α in the colon tissue were measured by a commercial enzyme-linked immunosorbent assay (ELISA) kit (Peprotech, UK) as instructed by the manufacturer. In Brief, Cytokine ELISA Kit rests on the standard tenet of a sandwich enzyme-linked immunosorbent assay. A mouse monoclonal antibody peculiar to each cytokine is coated on a 96-well plate. Standards and test samples that are added to the wells and cytokines present in a sample are bound by the immobilized antibody. Subsequently, a cytokine-specific biotinylated polyclonal antibody from the goat is added. Following the washing away, the unbound biotinylated antibody with PBS or TBS buffer, the avidin-biotin-peroxidase complex is added to the wells. The wells were washed again with PBS or TBS buffer to remove the unbound conjugates. HRP substrate TMB used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to make a blue product that turns yellow following the adding of an acidic stop solution. The density of yellow color is related to the cytokine captured onto the plate. The OD (optical density) values were measured by an ELISA reader at 450 nm wavelength. The cytokine amount of each sample was calculated using standard curves derived from standard samples (23).

Histological Investigation

Samples were prepared with H & E staining for examination of the histopathologic pattern under a light microscope. The results of staining scored from 0 to 3 based on the levels of inflammatory cells infiltration, thickening of the muscle layer, and mucus increase. Finally, numbers obtained from each of the parameters collected showed the severity of the lesion (22).

Statistical Analysis

Data were analyzed by SPSS (Ver. 21) and presented as means ± SD. Significant differences were to be found out by one-way analysis of variance (ANOVA) using Tukey as the post-hoc test ($p < 0.05$).

Results and Discussion

After scored damage of tissues (Fig.1B), the negative

Table 1: Experimental Setup and Treatments Used in This Study.

Group no.	Abbreviation	Treatment
1	Negative control (NC)	1mL normal saline, enema
2	Extract Oral (EO)	1 ml of a solution of 200 mg/kg Extract (Orally)
3	Extract Enema (EE)	1 ml of solution of 200 mg/kg Extract (intracolonic)
4	Mesalazine Enema (AE)	1 ml of the solution of 10 mg/kg Mesalazine (intracolonic)
5	Positive Control (PC)	1mL normal saline, enema.

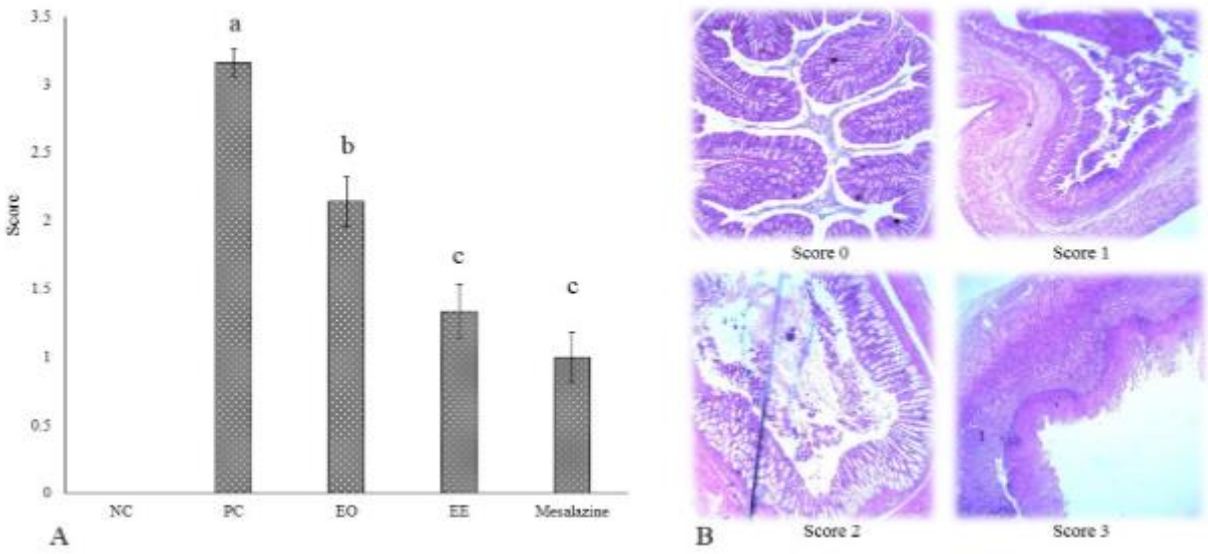

Figure 1. Results of blind analysis of histopathological sections of colon tissue (A). Remarkable statistical distinctions between groups in each index are exhibited by the distinct superscript letter ($p < 0.05$). Score 0: normal structure of the epithelial cells and basal area observed, Score 1: high recovery of microvilli in some epithelial cells and in the basal area the inflammatory cell infiltration was roughly absent observed, Score 2: slight recovery of microvilli in certain epithelial cells and in the basal area the inflammatory cell infiltration were observed, Score 3: the loss of microvilli, swollen mitochondria with the loss of cristae and nuclear alteration including the dilation of the nuclear envelope was also observed (B).

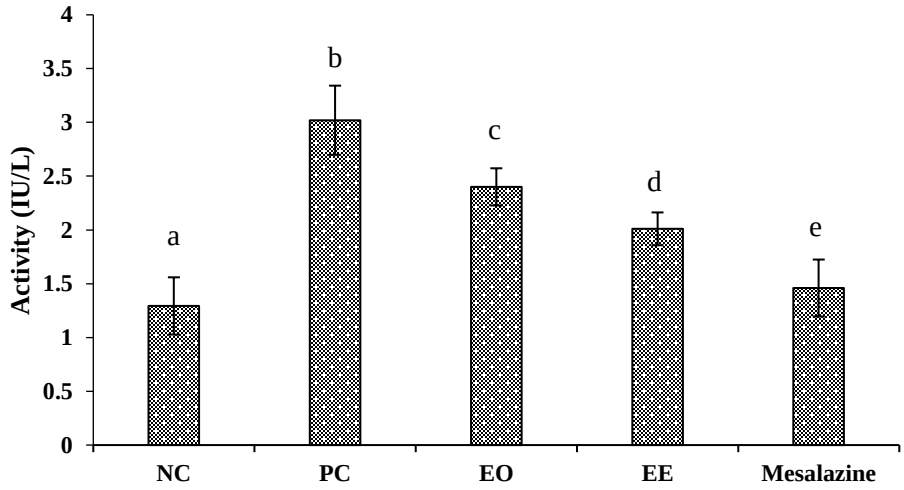

Figure 2. Changes of myeloperoxidase activity in colon tissues of rats in different groups. Remarkable statistical distinctions between groups in each index are exhibited by the distinct superscript letter ($p < 0.05$).

control group received score 0, that is the least score possible. Groups that receive *C. dactylon* extract (oral, enema), and mesalazine (enema) represented that epithelium was not totally destroyed. Consequently, they receive 2 for the level of epithelium damage. Given the observed alterations created in the tissue, the score pertains to the group that receive extract (oral, enema) along with mesalazine was 1 (Fig. 1A). Statistical analysis exhibited that compared to other groups, the group that receive the synergistic treatment of mesalazine and extract intracolonically (enema) represented a greater reduction of acetic acid-induced ulcers ($p < 0.05$). Furthermore, other treatment groups represented

remarkably lower damage to the colon compared with the positive control group ($p < 0.05$).

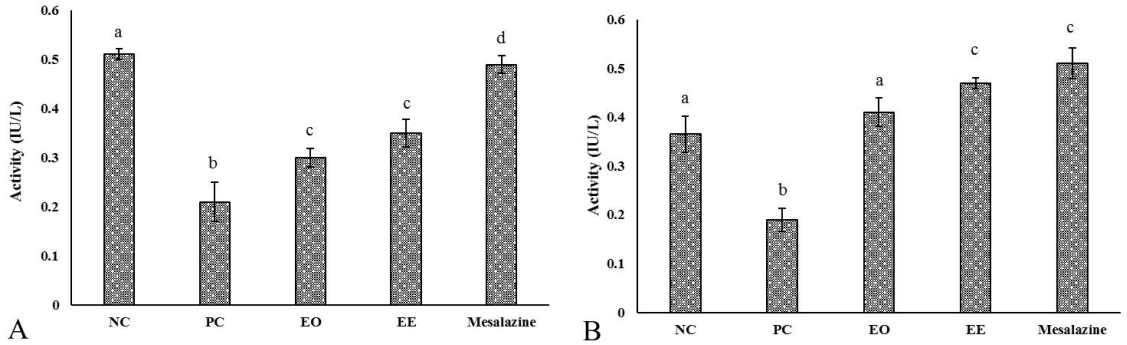

Figure 3. Evaluation the levels of superoxide dismutase activity (A) and glutathione peroxidase activity (B) in colon tissues of rats in different groups. Remarkable statistical distinctions between groups in each index are exhibited by the distinct superscript letter ($p < 0.05$).

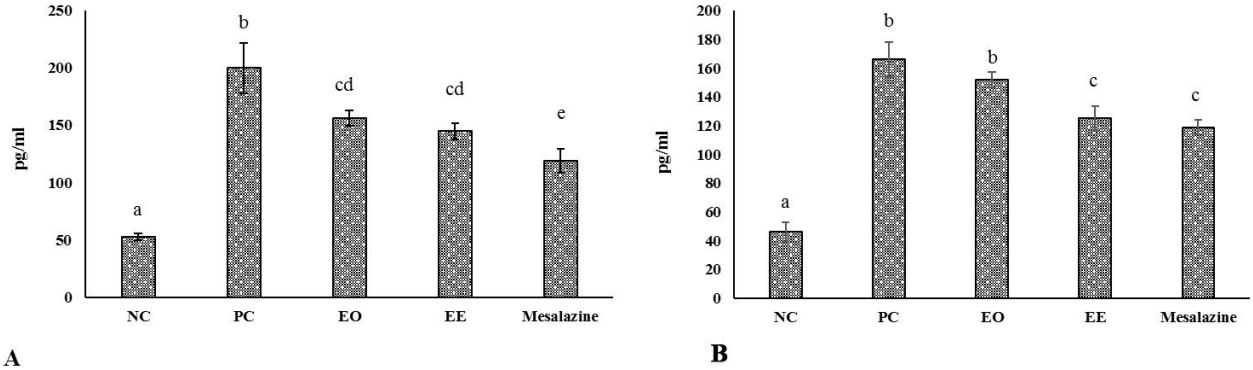

Figure 4. Assessment of TNF-α (A) and IL-6 (B) levels in colonic tissues rats with colitis different groups. Remarkable statistical distinctions between groups in each index are exhibited by the distinct superscript letter ($p < 0.05$).

According to the obtained data, MPO concentrations of the colon tissue were significantly higher and lower in PC and NC groups respectively, than all other treatments ($p < 0.05$). The PC group contained significantly lower tissue MPO concentration than those in EO, EE, and Mesalazine groups ($p < 0.05$). The Mesalazine group, however, indicated a more obvious reduction (Fig. 2). Fig. 3 represents the comparison of SOD (Fig. 3A) and GPx (Fig. 3B) as two antioxidant enzymes in colon tissue. Approximately, a similar pattern was established in the evaluation of both SOD and GPx levels ($p < 0.05$). Compared to the NC group, PC animals showed significantly elevated levels of TNF-α (Fig. 4A) and IL-6 (Fig. 4B) in the colon. Contrarily, the rats treated with EE, EO, and mesalazine exhibited significantly higher values of TNF-α and IL-6. Figure 4 displays significant differences between treated animals and PC group regarding the concentrations of TNF-α and IL-6. In this research, the effect of aqueous extract from *C. dactylon* was studied on acetic acid induced colitis. According to the results, the extract of *C. dactylon* could obviously reverse the inflammatory mediators of the trial colitis. A significant reversal in the severity of colitis was observed with oral and clyster administration of *C. dactylon* together with intracolonic instilment of 4% acetic-acid, which was comparable to mesalazine. Apparently, the extract is capable of resolving inflammatory mediators as demonstrated by the decreased biochemical markers MDA, nitrite, GSH, and contents of TNF-α and IL-6 in the colon. Earlier studies have investigated various plants and derivatives as potential treatments for UC. The hydroalcoholic extract of *Teucrium polium* has been reported to increase colon tissue healthy cells, mitigate the inflammation severity, and alleviate colonic tissue inflammation (24). Moreover, 7-day administration of licorice hydroalcoholic extract led to decreased intestinal epithelium damages, and activities of TNF-,

IL-6 and NO and SOD (25).

C. dactylon clearly plays a crucial role in ethnomedicinal and traditional systems. This is supported by a variety of pharmacognostic and pharmacological properties of its extract reported previously (26). Existing evidence indicates the crucial contribution of *C. dactylon* in the reversal of inflammation, candidal and bacterial infections, lipidemic and glycemic disorders, reproductive system and fertility, type II diabetes mellitus, and breast cancer (27). The inflammatory reaction and injurious nature are promoted and proliferated through the production of inflammatory cytokines (TNF-α and IL-6), which are known as the targets of curative interventions (28, 29). Our data suggest significantly decreased TNF-α and IL-6 levels and pathological damage in the treated animals and PC group as a result of *C. dactylon* extract administration. *C. dactylon* extract has been shown to possess an anti-inflammatory effect via the down-regulation of pro-inflammatory cytokines (IL-6, TNF-α, IL-1) and to increase serum levels of IL-10 as seen in the treatment groups, hence, the compound can be regarded as a regulator of the immune system (30). *C. dactylon* extract was also found to exert an anti-inflammatory effect via the down-regulation of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1, and to elevate serum levels of IL-10 in NMRI-mice following challenge with REV1 vaccine (30).

Oxidative stress essentially contributes to the pathophysiology of UC. In UC, the migration of neutrophils to the colon tissue was induced by an initial elevation of free radicals, secondary hypoxic conditions, and inflammatory chemokines resulting in the spread of inflammation and oxidative stress in the colon by means of arachidonic acid metabolites, cytokines and another chemokine (31, 32). The reversal of the antioxidant capacity of IBD patients is obvious, even in the asymptomatic phase of the disease. Intestinal cells contain various enzymatic and non-enzymatic antioxidants, including superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT), for eliminating reactive oxygen and nitrogen substances. However, a surplus of free radical formation improves lipid peroxidation and may diminish antioxidant barriers (33). The ethanolic extract of *C. dactylon* contains saponins, tannins, flavonoids, and phenols, which exhibit substantial antioxidant properties through their ability to hinder reactive oxygen or nitrogen compounds (34). The anti-inflammatory and antioxidative activities of the above compounds are also advantageous as they prevent the arachidonic acid cascade and hinder phospholipase-1, lipoxygenase and cyclooxygenase (35-37). Accordingly, significant increases in SOD and GPx levels were found herein in the rats treated with *C. dactylon* extract.

During the neutrophil respiratory burst and formation of reactive oxygen, myeloperoxidase often emerges in neutrophils and generates hypohalous acids (38). The activity of myeloperoxidase, hence, can be considered as a neutrophil infiltration biomarker (39). The present data further indicate that the administration of *C. dactylon* extract in two trans-rectal and oral forms can reverse MPO activity in the colon.

Conclusion

The aqueous extract of *C. dactylon* is recommended as an encouraging approach for UC treatment. This research, however, is a preliminary animal study that calls for further investigations in the future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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