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Corn Silk (Stigma maydis) Ameliorates Indomethacin–Induced Colitis in Rats via Modulation of Keap1/Nrf2 Signaling Pathway

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Abstract

AS A PUBLIC health problem, ulcerative colitis (UC) is a common inflammatory bowel disease associated with inflammatory perturbation and oxidative stress. Our work investigated the anti-colitic effects of corn silk (*Stigma maydis*) on indomethacin-induced ulcerative colitis and further explored its potential mechanism. Moreover, phytochemical characterization of the corn silk aqueous methanol extract was analyzed by UPLC-ESI-MS/MS analysis to rationalize the results of the biological investigation. Adult male Wistar rats (60) were divided randomly into six groups for 21 days. 1st group (control negative), 2nd group indomethacin (control positive), 3rd group sulfasalazine (positive drug control) at a dose of 100 mg/kg b.w. They were given corn silk orally in a dose of 150 or 300 mg/kg bw /day for 14 days before and after induction of UC on the 15th and 16th day of treatment representing the 4th and 5th groups respectively. The 6th group received (corn silk at a dose 300 mg/kg b.w + sulfasalazine. Our results revealed that corn silk could markedly lighten the adverse impacts of indomethacin by restoring the leukogram to normal, reducing DAI, colon weight, macroscopic damage, colonic MDA and NO levels, Caspase-3 and expressions levels of keap1 and significantly reduced histopathological deterioration. Additionally, it significantly enhanced colon length, Catalase activity and GSH level with elevation of Nrf2 gene expression level compared to control positive group. Overall, the effect of corn silk for alleviating colitis was largely mediated by modulating the signaling pathway of keap1-Nrf2 genes that is attributed to the phytochemical characterization of the phenolic (mainly flavonoids) and hydroxylated fatty acids components in corn silk extract that restored antioxidants and prevented inflammatory and apoptotic cell damages in rats. For that, corn silk holds a promising potential approach for lowering the risk of developing colitis. To the best of our understanding, this study marks the first instance of documenting the protective effect of corn silk against indomethacin-induced colitis.

Keywords: colitis, indomethacin, corn silk, UPLC-ESI-MS/MS, sulfasalazine, antioxidant, Gene expression, Rats.

Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are prevalent gastrointestinal disorders that impact around 0.3 to 0.5 percent of the global populace [1]. IBD is a long-term, sporadic illness that can cause

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abnormal discomfort, decreased appetite, and rectal bleeding. It is characterized by persistent, recurring gastrointestinal system inflammation. IBD has several different causes, the most common being immunological, environmental, nutritional, and genetic [2,3]

There are few effective medications available and our understanding of the pathophysiology of IBD is incomplete [4]. Since there are currently no medications to treat this refractory illness, new treatment options are desperately needed.

One of the substances thought to be utilized in animal models to cause ulcerative colitis is indomethacin. It's a non-steroidal anti-inflammatory medicine (NSAID), commonly used without a prescription and a primary cause of inflammatory bowel disease (IBD) [5]. Intra-intestinal injury and inflammation, alterations in the ileum's serosa and mucosa, mucosal erosion, hyperemia and/or petechial bleeding, and adhesive, hyperemic, or hemorrhagic lesions in the serosa and mesentery are all caused by the administration of indomethacin, which increases mucosal permeability [6].

Even though the exact mechanism causing ulcerative colitis (UC) is still unknown, growing clinical and experimental research proposes that oxidative is a prominent factor in the development of colitis [7,8]. Consequently, several studies have suggested ROS-targeted therapy and antioxidant avoidance as ways to reduce colitis [8,9]. Most people agree that the pathophysiology and course of inflammatory bowel disease (IBD) are significantly affected by oxidative stress, that is characterized by a buildup of hydrogen peroxide (H_2O_2) with reactive oxygen species (ROS) in the intestine [10]. Thus, stimulating the Nrf2-dependent antioxidative response presents a hopeful approach for therapy in managing inflammatory bowel disease (IBD).

An essential leucine zipper redox-sensitive transcriptional factor known as nuclear factor-erythroid 2-related factor 2 (Nrf2) react with antioxidant response elements (ARE) upstream of its target genes to counteract oxidative stress brought on by environmental toxins and illnesses [11,12]. Under normal physiological circumstances, Nrf2 is attached to its suppressor, kelch-like ECH-associated protein 1 (keap1), within the cell cytoplasm. Meanwhile, when subjected to xenobiotics or oxidative stress, the keap1 protein's Nrf2-binding domain is altered, releasing Nrf2, the intern can move into the nucleus and initiate cytoprotection by activating its target genes with small MAF proteins [13].

It has been demonstrated that the immunosuppressive medications and biological therapies used to treat IBD, which target certain inflammatory molecules, are either ineffective or have undesirable side effects. Thus, there is a pressing demand for safer and more effective alternative approaches to treat IBD. Dietary therapies for the management of IBD have gained more and more attention in recent years. These may serve as a preventative and non- pharmacological treatments for IBD since they are less intrusive than traditional medical methods [14,15].

Natural goods are thought to be a key source of novel medications, especially medicinal plants. Following evidence that medications produced from medical plants have significantly improved human health and well-being, medicinal plants offer a promising reservoir of innovative therapeutic molecules [16,17].

The yellowish, thread-like strands known as corn silk are extracted from the female maize blossom and have a variety of medicinal uses is a maize by-product that is high in flavonoids, terpenes, proteins, and carbs and is mostly used to treat diabetes and diuresis [18,19]. Crude fiber, polysaccharides, flavonoids, organic acid, saponins, alkaloids, etc. are the principal chemical components of maize silk [20]. The primary active component of corn silk, according to current pharmacological research, is polysaccharides, which also have multiple biological properties as antidiabetic, hypolipidemic, anti-obesity, antioxidant, hepatoprotective, anti-fatigue, and anticancer effects [21-25]. Guo et al. [21] highlighted the significant antioxidant properties of maize silk in vitro. Pan et al. [26] have shown that maize silk might have anti-diabetic benefits in diabetic mice on high-fat diet with streptozotocin [26]. A subchronic toxicity research conducted on rats showed that eating maize silk had no negative effects and confirmed that it is safe for people to consume [27]. Nevertheless, no research has looked at how corn silk (Stigma maydis) protects albino rats against ulcerative colitis caused by indomethacin. Consequently, the main purpose of our study was to investigate the possible mechanism behind the ameliorated effects of corn silk on the Indomethacin-induced colitis model by assessing alteration in the disease activity index (DAI), histological injury, antioxidants, lipoperoxidation markers, and caspase 3 immunoreactivity. Additionally, the study aimed to elucidate the principle molecular mechanism, particularly focusing on its effect on the keap 1/Nrf2 pathway.

Material and Methods

Chemicals and assay kits

- Indomethacin (Indo): Nile Company for pharmaceuticals, Cairo, Egypt. It is used for induction of ulcerative colitis. Dosage: 7.5 mg/kg b.w by (s.c.) injections 24 h apart for two consecutive days [28,29].
- SALAZO-SULPH PYRINE (Sulphasalazine tablet): It was manufactured by: USP KAHIRA pharma, CAIRO- Egypt. Each enteric coated tablet contains 500 mg sulphasalazine, dissolved in

normal saline 0.9%.Therapeutic indications: for treatment of ulcerative colitis.

Dosage: 100 mg/kg dissolved in 1 ml of saline 0.9% [30].

- Diethyl ether: It was obtained from (Spinreact), it was used to Anesthetize rats [31].
- Malondialdehyde (MDA), Catalase (CAT), Nitric oxide (NO) and reduced Glutathione (GSH) were supplied by BIO-DIAGNOSTIC.

Plant Material

Corn silks (fresh cut stigmata of Zeamays L. poaceae flowers) were purchased from farmers in kfs Herbal farm (Egypt, kfs). The plant was morphologically identified at the department of Botany, Kafrelsheikh University.

Preparation of the extracts of corn silk

Methanolic extracts of the plant was prepared by air-drying and powder preparation. 2 kg of corn silk powder was totally soaked in 10 L of 70% methanol (Merck Company, Germany) at room temperature for 72 h. The resulted extract was then filtered, evaporated using a rotary evaporator under reduced pressure until complete dryness. The solvent of methanolic extract was evaporated and removed that resulted in a semi-solid mass producing 12% w/w. The extract was kept in a clean, dry bottle then placed in a desiccator. The extract was maintained in stock solutions prepared by adding 15 g of extract to 30 ml distilled water to obtain a 300 mg/mL concentration and dissolving 7.5 g of extract in 15 ml distilled water to obtain a 150 mg/mL concentration. Dosage: 150-300 mg/kg [32].

Phytochemical characterization of the corn silk extract using UPLC-ESI-MS/MS analysis

The UPLC-ESI-MS/MS analysis was performed following a published procedure [Assar et al., 2023]. The sample, dissolved in HPLC methanol, was injected (10 µL) after degassing and filtration. A XEVO TQD triple quadrupole mass spectrometer (Waters Corporation, Milford, MA01757 U.S.A.) connected an ACQUITY UPLC- BEH C18 1.7 µm-2.1 × 50 mm column was used. Gradient elution with HPLC grade solvents composed of 0.1% formic acid in water (A) and methanol (B) with a flow rate of 0.2 mL min-1: 0 min, 10% B, 5 min, 30% B, 15 min, 70% B, 22 min, 90% B, 25 min, 90% B, 26 min, 100% B, 29 min, 100% B, 32 min, 10% B, then re-equilibration by adjusting the starting conditions for 3 min. Data processing was accomplished using Maslynx 4.1 software and compounds were provisionally recognized using information from open-access databases and documented research.

Ethical approval

Following the normal operating procedures approved by the Institutional Animal Care and Animal Ethics Committee (IAACUC-KSU), Faculty Veterinary Medicine, Kafrelsheikh University, Egypt.

Animals and feed management

For these experiments, sixty male albino rats were kept in conventional settings with a temperature of 23 ± 1 °C, a relative humidity of 55 ± 1 percent, light and dark cycles of 12 and 12 hours, and unlimited access to standard pellet and water. Male albino rats (150–170 g B.wt.) were utilized in these studies. The Institute animal ethics committee rules for animal care and use at Kafrelsheikh University supported the study procedure. Every safety precaution was done to reduce the stress on the animals.

Experimental design scheme

Following a two-week period of acclimation, sixty male wistar rats were split into six groups at random (n = 10 rats per group) (21 Days).

The 1st group (C–ve): Rats served as the trial's control group and received just normal saline through an orogastric tube for the whole 21-day duration. On the 15th and 16th days of the experiment, two subcutaneous (s.c.) injections of normal saline were given.

The 2^{nd} group (C+ve): (indomethacin group)(INDO) where the rats were treated orally with distilled water continuously for 21 day then administered with two injections of indomethacin (7.5 mg/kg b.w) at the 15^{th}, 16^{th} day of the experiment by subcutaneous (s.c.) route and served as positive control (ulcerated, non-treated).

The 3rd group: where the rats were treated orally with distilled water for two weeks then administered with two injections of indomethacin (7.5 mg/kg b.w) at the 15^{th}, 16^{th} day of the experiment by subcutaneous (s.c.) route with treatment by sulfasalazine (100mg/kg b.w orally) starting from day 15 of the experiment and continue for one week.

The 4^{th} group: Rats were orally administrated corn silk (150 mg/kg b.w orally at the first day of the experiment) continuously for 21 day the whole period of the experiment before and after administration of indomethacin (7.5 mg/kg b.w) at the 15^{th}, 16^{th} day of the experiment.

The 5th group: Rats were orally administrated corn silk (300 mg/kg b.w orally at the first day of the experiment) continuously for 21 day the whole period of the experiment before and after administration of indomethacin (7.5 mg/kg b.w) at the 15th, 16th day of the experiment.

The 6th group: Rats were orally administrated corn silk (300 mg/kg b.w orally at the first day of the experiment) continuously for 21 day the whole period of the experiment before and after administration of indomethacin (7.5 mg/kg b.w) at the 15th, 16th day of the experiment, with treatment by sulfasalazine at dose 100mg/kg b.w orally for one week after colitis induction.

Every day, the rats in each group were evaluated for clinical indications, death rates, body weights, and amounts of food and water they consumed. At day seven of colitis induction, the rats were killed while sedated with diethyl ether. Various sections of the colon were dissected, the adipose tissue removed, and then rinsed with normal saline for both macroscopic and microscopic examination. Samples of the colon were stored at -80°C for subsequent molecular and oxidative stress analysis.

Assessment of disease activity index

The disease activity index (DAI), which uses a score system for diarrhea, weight loss, and the presence of rectal bleeding, was computed to determine the severity of colitis (Table 1). Colitis induced by indomethacin is evaluated by the DAI [He et al., 2019]. DAI was monitored daily in all experimental groups after induction of colitis via an indomethacin injection [33].

Assessment of severity of colitis score and macroscopic damage score

The colon samples were visually inspected either for presence of adhesions or any significant morphological changes immediately following death (Table 2). Colon weight and colon length were determined after separation of the colon from adipose tissue and remind intestine as colon weight (g), colon length (cm) and its weight to length ratio [34].

Blood and colon sampling

Using clean capillary tubes and a moderate ether anesthetic, blood samples were taken from each rat's retro-orbital puncture of the medial canthus of the eyes. The samples were then promptly placed in tubes containing an anticoagulant for total leukocyte and differential counts. After that, rats were sacrificed, dissected and colon were removed, cleaned, dried, weighed, measured for their length and divided into three parts: The first part was shocked in liquid nitrogen, stored at -80 °C for RNA extraction. The second portion was homogenized in buffered saline (10% w/v), stored at -80°C for selected oxidative stress and antioxidant biomarkers analysis. The last part was immediately placed in buffered solution of 10% formalin for pathological and immune histochemistry alterations.

Hematological examination

Blood sample with EDTA anticoagulant (1mg/ml blood) was used to measure white blood cells (WBCs) and differential count by Rayto RT.7600 Auto Hematology Analyzer [35,36].

Estimation of lipoperoxidation and antioxidants biomarkers

Colon homogenates were prepared and used to determine MDA in accordance to Ohkawa et al. [37], nitric oxide (NO) based on the methods of Ingram [38], catalase activity in tissues homogenate by Aebi [39] and Reduced glutathione(GSH) was measured following Owens and Belcher [40]

Histopathological analysis

Freshly excised distal part of colon from each treatment group were rinsed with normal saline and initially kept in a 10% neutral buffered formalin solution (pH 7.4). The samples were fixed, then underwent dehydration using alcohol, followed by clearing in xylene, and subsequent embedding in paraffin for light microscopic analysis. Tissue sections embedded in paraffin, cut to a thickness of 5 µm, were prepared and stained with H&E stain (hematoxylin and eosin) after deparaffinization [41].

Histopathological assessment involved scoring the observed findings based on a four-point scale. This assessment considered factors such as mucosal necrosis, ulceration, submucosal edema, hemorrhage, and inflammation. This scoring was performed across eight high-power fields (HPFs), following a method detailed elsewhere.

Immunohistochemistry analysis

For immunohistochemical examination, paraffin sections were affixed onto specially coated glass slides. These sections were subjected to a series of steps, including clearing in xylene, rehydration, and antigen retrieval using an EDTA solution at pH 8. To minimize nonspecific staining, the slides were treated with 0.3% hydrogen peroxide (H_2O_2) and subsequently blocked with a solution containing 5% bovine serum albumin in Tris-buffered saline (TBS) for duration of 2 hours. Subsequent steps involved the preparation of the slides and their staining with an anti-Caspase-3 antibody obtained from Thermo Fisher Scientific (Waltham, MA, USA). Following staining, the slides subjected to multiple washes with PBS and were then treated with a secondary antibody (EnVision + System HRP, Dako, Santa Clara, CA, USA) for a duration of 30 minutes at room temperature. This was followed by additional washing steps and a 2-minute incubation with diaminobenzidine (DAB, Dako, Santa Clara, CA, USA). Finally, the slides were counterstained with Mayer's hematoxylin stain and covered with glass cover slides. Scanned IHC images (four sections per group) were analyzed using ImageJ software (NIH/Bethesda, Maryland) to quantify the percentage of the area of Caspase-3 positive regions (stained dark brown) compared to non-stained regions. This occurred by applying thresholding techniques, allowing us to determine the mean percentage of caspase-3 positive stained areas, indicative of apoptosis and necrosis [42].

Quantitative determination of mRNA expression of Nrf2 and keap1 using real-time (qRT-PCR)

Total RNA was extracted from colon using Trizol reagent (total RNA isolation kits, INTRON Biotechnology, Inc). Synthesis of complementary DNA (cDNA) was performed using an oligo (dt)

cDNA synthesis kits (INTRON) following the manufacturers direction. The SYPR green (RT-PCR) was performed using BIoRad IQ2 (japan). Rat β-actin was used as internal reference gene. Expression of mRNA levels in each sample was normalized to β-actin gene. The PCR primer sequence was illustrated in Table (3). Both the CT values and amplification curves were analyzed by the stratagene MX3005P software. The CT value of each sample was analyzed against the control group using the ΔΔCt method to assess variations in gene expression of the different samples. The quality of PCR products was measured using 1.5% agarose gel electrophoresis.

Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was used to determine the difference between groups followed by Tukey's-compare all pairs of colums. GraphPad Prism Software version 8.0 was used for statistical analysis of the resulted data and the statistical significance was taken as $p < 0.05$ [45].

Results

Phytochemical characterization of the corn silk extract using UPLC-ESI-MS/MS analysis

The UPLC-ESI-MS/MS analysis detected 54 compounds of which flavonoids and hydroxylated fatty acids were the major constituents (Fig. 1, Table 4). The compounds were identified through their masses and MS/MS fragmentation compared to the published literature [46-56] and free databases as The Human Metabolome Database (HMDB) (https://hmdb.ca/) and Pubchem database (https://pubchem.ncbi.nlm.nih.gov/).

Mortality rate

Mortality rate as seen in (Fig. 2A). Throughout the whole investigation, no aberrant clinical symptoms nor mortality were observed in the control negative group, that was fed a typical diet. Moreover, the control positive group ascertained obvious elevation in mortality rate (4/10) (40%). Meanwhile, sulfasalazine group which was used for treatment of ulcerative colitis showed decreased mortality rate (2/10) (20%). Administration of corn silk (150 mg/kg b.w) decreased mortality rate (3/10) (30%). Supplementation of Corn silk (300mg/kg b.w) also decrease mortality rate (2/10) (20%). Additionally, there was a noticeable drop in the death rate (0/10) when sulfasalazine and corn silk (300 mg/kg b.w.) were administered together (0 percent).

Body weight change

Indomethacin group served as a positive control (ulcerated, non-treated), where the rats were treated orally with distilled water continuously for two weeks then administered with indomethacin (7.5 mg/kg b.w) by two subcutaneous (s.c.) injections at the 15^{th} , 16^{th} day of the experiment and continue five more days. Data showed a significant ($p \leq 0.05$) elevation in body weight from the 1st day till the 16^{th} day after Indomethacin sc injection. The control negative group continue to increase in body weight till the 21^{th} day of experiment. The lowest body weight change was detected in the control positive group given Indomethacin while rat group supplied with corn silk either (300 mg/kg BW) or combination with sulfasalazine exhibited a marked elevated body weight when compared with the control positive group (Fig .2C).

Disease activity index

The effects of Indomethacin, corn silk and sulfasalazine on DAI were summarized in (Fig .2B). Indomethacin treated group demonstrated a significant elevation ($p \leq 0.05$) in DAI in as compared with the control negative one which showed the lowest DAI. Sulfasalazine group revealed significant decrease in DAI in comparison with the control positive group. Moreover, corn silk group (150 mg /kg BW) caused pronounced decrease in DAI but this reduction was still insignificant as compared with the control positive group. While, corn silk (300mg /kg BW) either alone or in combination with sulfasalazine demonstrated a significant ($p \leq 0.05$) improvement in DAI compared to the control positive group.

Scoring severity of colitis

The macroscopic damage score is shown in Fig.3. Indomethacin-treated group showed a significant ($p \leq 0.05$) increase in damage score as compared with the control negative one. While, all other treated groups showed significant ($p \leq 0.05$) decrease in colon damage score when compared with the positive control group.

Colon weight and length

Concerning the colon weight and length as showed in Fig.4.The obtained data revealed a significant ($p \leq 0.05$) increase in colon weight with decrease in colon length of the control positive group as compared with the control negative one (Fig.4A&B). Meanwhile, sulfasalazine group showed a significant decrease in colon weight with a significant ($p \leq 0.05$) increase in colon length as compared with the control positive group. At the same time, simultaneous supplementation of corn silk of both (150 and 300 mg/kg BW) and combination with sulfasalazine treated groups revealed a significant ($p \leq 0.05$) improvement in colon weight with a significant ($p \leq 0.05$) increase in colon length as compared with the control positive group.

Concerning the Colon weight / length ratio as recorded in Fig.4C. The weight / length ratio data reflected a significant ($p \leq 0.05$) increase in the control positive group as compared with the control negative group. On the other hand, Mesalazine-treated group revealed a significant ($p \leq 0.05$)

decrease in weight / length ratio of colon as compared with the control positive group. Similarly, supplementation of corn silk alone or in combination with sulfasalazine showed a significant ($p \leq 0.05$) decrease in weight / length ratio of colon in comparison with the control positive group.

Hematological finding

The impacts of indomethacin and the ameliorative effects of corn silk on the leukogram are seen in Fig.5. A significant increase in WBCs (Fig.5A), lymphocytes (Fig.5B), Monocytes (Fig.5C) and granulocytes count (Fig.5D) were noticed in the indomethacin treated group as compared to the control group. Sulfasalazine and other corn silk-treated group showed a marked ($P \leq 0.05$) decrease in WBCs, lymphocytes, Monocytes and granulocytes count as compared to the indomethacin-treated group. The level of improvement varied in a dose-dependent manner.

Lipid peroxidation and antioxidant biomarkers

The combined effects of indomethacin and corn silk on lipid peroxidation and antioxidant biomarkers were outlined in Fig.6. Indomethacin treated group showed a significant ($p \leq 0.05$) elevation in MDA (Fig. 6A) and nitric oxide (Fig. 6B) with marked inhibition of CAT activity (Fig. 6C) and GSH content (Fig. 6D) when compared to the control negative group. Meanwhile, sulfasalazine group revealed a significant ($p \leq 0.05$) decrease in MDA and NO content and significant increase in CAT and GSH as compared with the control positive group. Corn silk supplementation with both doses (150 or 300mg /kg bw) or in combination with sulfasalazine showed a significant ($p \leq 0.05$) decrease of MDA and NO as compared with the control positive group with a marked elevation in CAT and GSH compared with the control positive one.

Histopathological findings

The histopathological analysis of the normal control group manifested an intact colon structure characterized by well-arranged mucosal crypts and submucosal components, devoid of any inflammatory manifestation (Table 5 and Fig.7A). Conversely, rats exposed to indomethacin in the positive control group exhibited severe ulcerative hemorrhagic lesions accompanied by extensive confluent necrosis along the mucosal lining. Also, fibrin deposition within the submucosal layers, along with sever infiltration of inflammatory cells particularly throughout the mucosa, and sometimes lamina propria, and muscular layers, underscored the severity of induced colitis (Fig.7B). Treatment with sulfasalazine in the therapeutic group (G3) generated moderate improvements, evidenced by slight enhancement in crypt arrangement and epithelial coverage. However, signs of inflammatory cell infiltration and superficial epithelial necrosis persisted, but with reduced edema and diminished fibrin deposition in the lamina propria (Fig.7C). Interestingly, the administration of corn silk low-dose (150 mg/kg), prior to colitis induction, revealed protective effects against mucosal necrosis, accompanied by residual infiltration of inflammatory cells and beside an increased level of goblet cells (Fig.7D).On the same pattern, corn silk with higher-dose (300 mg/kg) yielded more robust mucosal preservation, with markedly reduced inflammatory cell infiltration and evident expansion of goblet cell proliferation. Additionally, crypt elongation was notable in this group, suggesting a potential for tissue regeneration (Fig.7E). Co-treatment involving the combination of corn silk (300 mg/kg) and sulfasalazine resembled the histological features observed in the non-treated control group, implying a significant ameliorative effect (Fig.7F). Collectively, these histopathological findings underscore the potency of corn silk and sulfasalazine in mitigating the deleterious effects of indomethacin-induced ulcerative colitis, evident in the restoration of colon tissue architecture and modulation of inflammatory responses Table (4).

Immunohistochemical Evaluation of Caspase-3 expression

The percentage of apoptotic cells exhibiting immune reactivity was most pronounced in group 2 (Fig.8B). This finding was notably distinct from the control group (Fig.8A), which displayed the lowest level of caspase 3 immune-reactivity. Moreover, rats received a large dose of corn silk, (300 mg/kg), showed only marginal improvement in the apoptosis percentage (Fig.8E) when compared to both the control positive group and rat group pre-treated with a low dose of corn silk, (150 mg/kg) (Fig.8C & 8D, respectively). Corresponding to the H&E results, rats received co-treatment of corn silk at 300 mg/kg and sulfasalazine displayed a low apoptotic percentage (Fig.8F), which closely resembled that of the control negative group. There was no noticeable difference noted between the two groups.

Molecular investigation

The expression of *keap1 and Nrf2* genes was determined by qRT-PCR which showed the transcriptional alterations in their expression levels in colon of the examined rats. Indomethacin injection caused a significant increase in *keap1* expression levels while markedly decreased *NrF2* ($P \leq 0.05$) expression in comparison with the control negative group. Sulphasalazine group showed a significant ($p \leq 0.05$) elevation in *NrF2* with a significant decrease in *keap1* expression level as compared with the control positive group while simultaneous supplement with *corn silk* at both doses either (150 or 300 mg/kg b.w) decreased the expression of *keap1* gene while enhanced the *NrF2* gene expression when compared with the control positive group. In the same time the co-administration of corn silk at

300mg /kg BW with sulfasalazine exhibited a significant ($p \leq 0.05$) decline in *keap1* gene and raised *NrF2* gene expression level as portrayed in Fig.9A&B.

Discussion

UC is a common digestive system inflammatory condition that is persistent and recurring. It negatively affects patients' physical and emotional well-being and may raise their risk of colon cancer [57,58]. Currently, corticosteroids, aminosalicylates, immunosuppressors, together with other biological therapies aim to treat the inflammation and clinical signs of ulcerative colitis (UC). However, there may be unfavorable side effects from these drugs. Consequently, research aimed for creating safer, more efficient, and more focused UC treatments is required [**59**]. In this study we intended to explore the ameliorative effect of Corn silk on indomethacin induced colitis in rats.

In comparison to the other groups, the current study showed a substantial increase in DAI and other macroscopic damage in control positive rats. In comparison to the other groups, the current study showed a substantial increase in DAI and macroscopic damage in control positive rats. Numerous indications and symptoms of ulcerative colitis (UC) are similar to those of humans, such as diarrhoea, emaciation, melena, mucosal ulcerations, colon lengthening, and infiltration of inflammatory cells [60]. This rise in DAI and macroscopic damage might be linked to decreased appetite, less food consumed, which results in weight loss, decreased faecal output, and increased water consumption [61]. Histopathological lesions, a high macroscopic damage score, an elevated death rate percent (40%) and sporadic ulceration that resulted in bloody diarrhoea were the reasons given by others for the rise in DAI. A comparable outcome was attained by Allam et al. [62].

In addition, the control positive rats exhibited a marked decrease in colon length and gain in colon weight when compared to the normal negative rats. This could be explained by cellular swelling brought on by the extracellular water shifting into the cells linked to neutrophil and macrophage infiltration. Additionally, the results of goblet cell hyperplasia, vascular dilatation, cellular turgor, and submucosal edoema are consistent with Jagtap et al. [63].

The hematopoietic system is an extremely sensitive mechanism for determining the potential risks that medications and poisons pose to human health [64]. Our study indicated that s.c injection of indomethacin caused marked increase in the WBCs, lymphocytic, granulocytic and monocytic count when matched with the control negative one. However, WBCs count act as inflammatory indices in routine clinical practice for determine UC. The higher number of WBCs in Indomethacin group may owe to infiltration of inflammatory cells as lymphocytes and neutrophils consequence to increase of inflammatory response during colitis in experimental rats [65]. Neutrophils establish a protective barrier separating the gut lumen from the tissue beneath and are essential for maintaining intestinal homeostasis. Moreover, it promoted colon mucosa repair by releasing mediators that are essential for the resolution of inflammation. Despite the foregoing, in order to alleviate the symptoms of ulcerative colitis, inflammatory cell infiltration was required [66]. Increases in T-lymphocyte populations and mucosal macrophage activation in intestinal inflammation have been linked to immunological activation, according to certain studies. This inflammation is thought to be a significant aspect of the disease's aetiology [67]. Corn silk was found to have beneficial effects against Indomethacin by decreasing total leukocytic count about to normal levels. The advantageous benefits of corn silk on oxidative stress and inflammation were further supported by our research. Through their ability to increase the count of macrophages and neutrophils that initiate a self-sustaining phlogogenic loop, oxidants directly contribute to the chronic inflammatory process [68].

Lipid peroxidation and oxidative stress are important factors in ulcerative colitis pathophysiology [69]. In both UC patients and animal models, the use of NSAIDs such as indomethacin resulted in oxidative damage. Free radical concentrations rose in colon tissue samples from individuals affected by this illness. ROS play a vital part in the start of illness. The exaggerated production of reactive oxygen species (ROS) in colitis results from widespread infiltration of inflammatory cells and abscesses in the small intestinal mucosal crypts. These results in damage to colon cells and permeability epithelial barrier due to oxidative stress, which leads to invasion of pathogens and amplified infiltration inflammatory cell and damage [70]. This was created in large quantities due to cellular damage. Comparing the control negative group to the control positive group of rats, it was found that there was an increase in MDA and NO. These findings are in accordance with results obtained by Gomaa et al. [71] and Cervantes-García et al. [72]. Antioxidants that are enzyme-based, such as catalytic antioxidants (CAT) and non-enzymatic antioxidants, such as glutathione (GSH), are essential for preventing the overproduction of free radicals in disease states [73,74].

In the current work, the antioxidant enzymatic activities were markedly decreased in the colon tissue by administration of the indomethacin, that obviously proposed the role of oxidative stress in GIT tissue damage. Additionally, with gradual increase in the dose of corn silk extract alone or in combination with sulfasalazine, antioxidant levels

approach to normal level and it helps manage the disease conditions. Knowing that the beneficial effects of the plant extracts are principally due to the flavonoids content, that has remarkable anti-inflammatory activities [**75**]. An elevation in the antioxidant molecule GSH levels in the blood of mice was consistent with the radical scavenging properties of the maize silk extract. An elevation in the antioxidant molecule GSH levels in the blood of mice was consistent with the radical scavenging properties of the maize silk extract. Free radicals are directly scavenged by GSH [76]. In diabetic animal model, the blood GSH concentration was able to be restored with the administration of natural dietary extracts [77].

Upon using the aqueous acetone corn silk extract in vitro it was found that the polyphenol content as well as antioxidant activity positively correlate with corn silk's antioxidant capacity at first [78]. Test tube investigations demonstrated the corn silk extracts' ability to scavenge radicals and chelate iron [79,80]. Human breast cancer cells have been linked to the cornsilk extract's antioxidant properties [75], Neuroblastoma human cells [81], Rat pancreatic β-cell clones [82], and CSP2, a polysaccharide that was isolated from corn extract [21]. In animal studies, feeding healthy albino mice cornsilk extract on demand for 28 days boosted the amount of reduced glutathione in their kidneys and the activities of antioxidant enzymes [83]. In a different experiment on animals, dietary cornsilk extract prevented glutathione peroxidase from declining due to a high salt diet [84] and reduced the oxidative damage brought on by radiation in mice [32]. Furthermore, taking flavonoids derived from cornsilk orally shown protective benefits against oxidative stress during intense exercise [85] and in diabetes caused by streptozotocin in mice [86].

In our study, the phytochemical analysis highlighted the polyphenolic (mainly flavonoid) compounds and the oxygentated and unsaturated fatty acids in the corn silk extract. Natural polyphenolic compounds are very effective antioxidant and anti-inflammatory agents in addition to other health benefits [87]. Maysin, the major flavonoid in corn silk, showed the ability to trigger the expression of intracellular antioxidant enzymes [81]. Fatty acids have shown antioxidatnt and antiinflammatory potentials [88]. These components identified in the corn silk extract may account for the exerted biological results in our study.

Histopathological findings aree in accordance with the recorded macroscopic damage score, both oxidative and anti-oxidative biomarkers. In the current study, colon from rats in the negative control group exhibited typical histological features of the epithelium, intestinal glands and intestinal lumen. Meanwhile, the control positive rats (severe colitis) revealed wall thickening, severe ulcerative hemorrhagic lesions accompanied by extensive confluent necrosis along the mucosal lining. Also, fibrin deposition within the submucosal layers, along with sever infiltration of inflammatory cells particularly throughout the mucosa, and sometimes lamina propria, and muscular layers, underscored the severity of induced colitis These data were in harmony with those obtained by Baumgart and Carding [89], and Magro et al. [90]. Our findings demonstrated that the application of corn silk, as demonstrated by a marked reduction in inflammatory cell infiltration and a clear elevation in goblet cell proliferation, ameliorated the pathological alterations and symptoms typical of the colitis that rats develop when exposed to methacin. According to earlier research, cornsilk contains luteolin [91] and allantoin [92], which may have anti-inflammatory or anti-oxidative properties [93-95].

Additionally, immunohistochemical investigation in our work revealed elevated expression level of caspase-3 in Indomethacin-treated group when compared to the control negative animals. The mechanism of Indomethacin induced caspase-3 elevation was described by El-Maraghy et al. [96]. On the contrary, corn silk prevented apoptosis and protected rats from increased caspase-3 expression, that is matching previous research results [97]. Corn silk possesses anti-apoptotic effect through attenuating oxidative stress and downregulate of Caspase 3. Through increased mitochondrial membrane integrity, corn silk inhibits the cytochrome c release, a crucial component of the intrinsic apoptotic cascade. This results in decreased apoptosis, as demonstrated by immunohistochemistry, which showed a significant drop in Caspase 3.

The nrf2/are cascade is known to be activated by a number of methods, including directly increasing nrf2 expression levels in post-transcription and/or post-translation sage, or by blocking the keap1- nrf2 connection to cause nrf2 nuclear translocation [11-13, 55,98,99].

In the current work, Indomethacin administration resulted in downregulation of nrf2 levels and upregulation of keap1 levels, meanwhile, treatment with corn silk extracts altered the results in both low and high dose cornsilk-treated groups, the expression levels of nrf2 was markedly increased compared with the Indomethacin group. Therefore, corn silk extracts may retrieve oxidative balance and mitigate colitis through activating the keap1/nrf2 signaling pathway, that may be attributed to the rich polyphenols content in the extracts [**100**]. Previous research have demonstrated that polyphenols content have the ability to inhibit ROS, as well as various roles including degrading keap1, inhibiting the keap1 and nrf2 combination, and in the same time stimulating the keap1-nrf2 signaling pathway. These polyphenols from corn silk extract can subsequently

bind to Keap1 protein, in the same time activate nrf2 in order to control the expression level of downstream antioxidant proteins, thus altering vascular inflammation. As a result, activating the keap1-nrf2 signaling pathway via dietary polyphenols supplementation may be an efficient strategy to either prevent or treat UC [101].

The nrf2/kaep pathway is a possible therapeutic method for the suppression of inflammation because it may activate an endogenous defense system to withstand cellular oxidative stress and ameliorate oxidative damages [102]. Furthermore, nrf2 activation lessens inflammatory reactions that are harmful and damages cells. The fact that nrf2 activation may regulate the cytochemical microenvironment and activate a number of signaling pathways that prevent inflammation is demonstrated by all these protective effects [103]. Our study confirmed that the corn silk mitigated both inflammation and oxidative stress in experimental colitis via activating nrf2 bringing about an overall recovery in both macroscopic and histological parameters.

Conclusion

For the first time, our study demonstrated that maize silk extract might alleviate colitis caused by indomethacin via modifying the Keap1-Nrf2 signalling pathway. Additionally, the presence of phytoconstituents can alter apoptotic and inflammatory effects, pointing to the anticolitic impact of maize silk extract and perhaps enhancing the antioxidative defence system. These findings imply that maize silk cans be used as a natural, functional food or medication to treat colitis.

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Conflicts of interest

There are no conflicts to declare. The authors declared no competing interests.

TABLE 1. Scoring of disease activity index (DAI).

Score	Weight loss %	Stool consistency	Occult/gross bleeding
0	0	Normal	Normal
1	1-5%	-	-
2	5-10%	Loose stools	Occult blood
3	10-15%	-	-
4	> 20%	Diarrhea	Gross bleeding+ mucous

TABLE 2. Criteria for scoring of gross morphologic damage of colon

Score	Appearance
0	Normal
1	Localized hyperemia, no ulcer
2	Ulceration without hyperemia
3	Ulceration with inflammation at one site
4	Two or more sites of discrete ulceration and inflammation

TABLE 3. Primer sequences of the genes investigated by real-time RT-PCR Forward (F) and reverse primer (R) sequence of β-actin, Nrf2 and Keap1 and Gene bank accession numbers.

Gene	Primer sequence (5'-3')	Accession No./ References
Rat ß-actin	For: AGTGTGACGTTGACATCCGTA Rev: GCCAGAGCAGTAATCTCCTTCT	**NM_031144.3** **(43)**
Nuclear factor (erythroid-derived2) -like 2 (Nrf2)	For: GCTGCCATTAGTCAGTCGCTCTC Rev: ACCGTGCCTTCAGTGTGCTTC	**XM_006234398.3** **(43)**
Kelch-like ECH-associated protein 1 (Keap1)	For: GTGGCGGATGATTACACCAAT Rev: GAAAAGTGTGGCCATCGTAGC	**NM 057152** **(44)**

TABLE 4. Phytochemical profile of corn silk extract by UPLC-ESI-MS/MS in negative and positive ion modes.

NO.	Rt min	$[M-H]^-$ *m/z*	$[M+H]^+$ *m/z*	MS^2 Ions *m/z*	Identification
1	0.74		381*	381	Homovanilic acid glucuronide
2	0.75	179		225. 89	Glucose
3	0.95	179		225. 89	Fructose
4	0.96	173		173	Unknown
5	1.11	187		169, 125	Benzoic acid derivative
6	1.77	353		179, 283	Chlorogenic acid
7	5.94	607		461, 446, 430	2''-*O*-L-Rhamnosyl-6-C-fucosyl-3'-methoxy luteolin
8	5.94	577		269	Apigenin-7-*O*-rutinoside (isorhoifolin)
9	5.94	577		559,473,415,353	Apigenin-6-C-rutinoside
10	5.94	447		429,378,327,285	Luteolin-8-C-glucoside (Orientin)
11	6.16	547		337,367,415,457,465	Puerarin xyloside
12	6.16	563		503, 473, 443, 383, 381, 353	Schaftoside
13	6.52		593	449, 285 (retusin)	Retusin-7-*O*-neohesperidoside
14	6.61	575		429, 413, 411, 337, 327, 298, 285	Maysin
15	6.61	593		285	Luteolin-7-*O*-neohesperidoside
16	6.61		449	287	Cyanidin glucoside
17	6.70	461		299	Chrysoeriol-*O*-hexoside
18	6.70	593		533, 503, 473, 353	Vicenin 2
19	6.87	473		341, 310, 293, 283, 175, 117	Vitexin 6''-acetyl hexoside
20	7.07		284	147,121,93	Paprazine
21	7.07	431		341, 310, 293, 283, 175, 117	Vitexin
22	7.07	559		441, 413, 397, 195, 185,159	Apimaysin
23	7.18	589		571,560,466,463	Manniflavanone
24	7.18	589		454,443,425,381	3'-*O*-Methyl maysin
25	8.52	299		284,271,227,201,165	Hispidulin
26	8.52	299		271, 207, 163	Chrysoeriol
27	8.64	425**		329, 313, 298, 282	Dimethoxy isorhamnetin
28	8.77	325		307, 289, 271, 83, 59	Trihydroxylinolenic acid
29	8.77	327		209, 171, 185	Trihydroxy octadecadienoic acid
30	9.40	329		261,167	Vanilloyl hexose
31	10.17	329		211, 199	Trihydroxy octadecenoic acid
32	10.99	331		213, 201	Trihydroxy octadecanoic acid
33	11.09	311		293,275,253,235,223	Dihydroxy octadecadienoic acid
34	11.79	311		293,275,253,235,223	Dihydroxy octadecadienoic acid isomer
35	11.91	311		293,275,253,235,223	Dihydroxy octadecadienoic acid isomer
36	11.92	309		209, 185, 171, 137, 99	Hydroperoxy-octadecatrienoic acid
37	12.05	311		293,275,253,235,223	Dihydroxy octadecadienoic acid isomer
38	12.80	313		295,277,194,183	(+/-)12(13)-DiHOME
39	13.10	313		295,277,251,201,171	(+/-)9(10)-Dihydroxy-12Z-octadecenoic acid
40	13.50	315		297, 141	Dihydroxy stearic acid
41	13.80	285		257,240,216,198,174	Luteolin
42	14.13				
43	15.65	293		185, 125, 113	Oxo-octadecadienoic acid isomers
44	15.97				
45	17.13				
46	14.25	315		297, 141	Dihydroxy stearic acid isomer
47	15.06	295		277, 177	Hydroxy octadecadienoic acid
48	15.29	295		277, 177	Hydroxy octadecadienoic isomer
49	18.97	277		83, 59	α-Linolenic acid
50	20.49	279		261,205, 59	Linoleic acid
51	21.96	255		237,209,187	Palmitic acid
52	21.96	355		337, 311, 309, 135, 59, 44, 41	Tetracosahexaenoic acid (Nisinic acid)
53	25.51	271		253,225,205	16-Hydroxy hexadecenoic acid
54	26.91	355		337, 311, 309, 135, 59, 44, 41	Tetracosahexaenoic acid (Nisinic acid) isomer

TABLE 5. Inflammatory and degenerative changes count for H&E in control and different treated groups.

	Mucosal ulceration	Mucosal necrosis	Hemorrhage	Edema	Inflammation
Control negative	-	-	-	-	-
Control Positive (INDO)	+++	+++	++	++	+++
INDO + Sulphasalazine	-	+	-	-	++
INDO + Corn silk (150mg /kg BW)	-	+	-	-	++
INDO + Corn silk (300mg /kg BW)	-	+	-	-	+
INDO+ Sulphasalazine+ Corn silk (300mg /kg BW)	-	-	-	-	+

Score of lesions, (− negative, + = mild, ++ = moderate, +++ = severe).

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يعمل حرير الذرة (Stigma maydis) على تحسين التهاب القولون الناجم عن الإندوميتاسين في الفئران عبر تعديل مسار إشاراتKeap1 / Nrf2

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الملخص

كمشكلة صحية عامة، يعد التهاب القولون التقرحي (UC) أحد أمراض الأمعاء الالتهابية الشائعة المرتبطة بالاضطراب الالتهابي والإجهاد التأكسدي. بحث عملنا في التأثيرات المضادة للمغص لحرير الذرة (Stigma maydis) على التهاب القولون التقرحي الناجم عن الإندوميتاسين واستكشف آليته المحتملة. علاوة على ذلك، تم تحليل التوصيف الكيميائي النباتي لمستخلص الميثانول المائي لحرير الذرة من خلال تحليل UPLC-ESI-MS/MS لترشيد نتائج التحقيق البيولوجي. تم تقسيم ذكور فئران ويستار البالغة (60 عامًا) بشكل عشوائي إلى ست مجموعات لمدة 21 يومًا. المجموعة الأولى (السيطرة سلبية)، المجموعة الثانية الإندوميتاسين (السيطرة إيجابية)، المجموعة الثالثة سلفاسالازين (إيجابية السيطرة على المخدرات) بجرعة 100 ملغم/كغم من وزن الجسم. تم إعطاؤهم حرير الذرة عن طريق الفم بجرعة 150 أو 300 ملجم/كجم من وزن الجسم/اليوم لمدة 14 يومًا قبل وبعد تحريض التهاب الكبد في اليومين الخامس عشر والسادس عشر من العلاج يمثلان المجموعتين الرابعة والخامسة على التوالي. تلقت المجموعة السادسة (حرير الذرة بجرعة 300 ملغم / كغم من وزن الجسم + سلفاسالازين. كشفت نتائجنا أن حرير الذرة يمكن أن يخفف بشكل ملحوظ من التأثيرات الضارة للإندوميتاسين عن طريق إعادة مخطط الكريات البيض إلى طبيعته، وتقليل DAI، ووزن القولون، والتلف العياني، وMDA القولون ومستويات NO وCaspase-3 ومستويات تعبيرات keap1 وقللت بشكل كبير من التدهور النسيجي المرضي، بالإضافة إلى ذلك، عززت بشكل كبير طول القولون ونشاط الكاتلاز ومستوى GSH مع ارتفاع مستوى التعبير الجيني Nrf2 مقارنة بمجموعة السيطرة الإيجابية بشكل عام تم التوسط إلى حد كبير في استخدام الحرير للتخفيف من التهاب القولون عن طريق تعديل مسار الإشارة لجينات keap1-Nrf2 التي تعزى إلى التوصيف الكيميائي النباتي للمكونات الفينولية (الفلافونويدات بشكل رئيسي) ومكونات الأحماض الدهنية الهيدروكسيلية في مستخلص حرير الذرة الذي أعاد مضادات الأكسدة ومنع تلف الخلايا الالتهابية وموت الخلايا المبرمج. ولهذا السبب، يحمل حرير الذرة طريقة واعدة لتقليل خطر الإصابة بالتهاب القولون. وفقًا لفهمنا، تمثل هذه الدراسة أول مثال لتوثيق التأثير الوقائي لحرير الذرة ضد التهاب القولون الناجم عن الإندوميتاسين.

الكلمات الدالة: التهاب القولون. الإندوميتاسين. حرير الذرة؛ سلفاسالازين. مضادات الأكسدة، التعبير الجيني، الفئران