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Original article

Lactoferrin and/or Psyllium Seed Husk as Potential Therapeutics for Induced Ulcerative Colitis in Rats

Aya Fathy Elmasry [1], Maha G. Soliman [1], Hanaa A. Mansour[2], Wedad A.Hassan[2], Shimaa Attia Atta [3] Sara Mohamed Saber [4].

[1] *Zoology and Entomology Department, Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt*
[2] *Pharmacology department, Egyptian Drug Authority (EDA); National Organization for Drug Control and Research (NODCAR), Cairo, Egypt*
[3] *Immunology department, Theodor Bilharz Research Institute, Giza, Egypt*
[4] *Histopathology Department, Egyptian Drug Authority (EDA). Cairo, Egypt*

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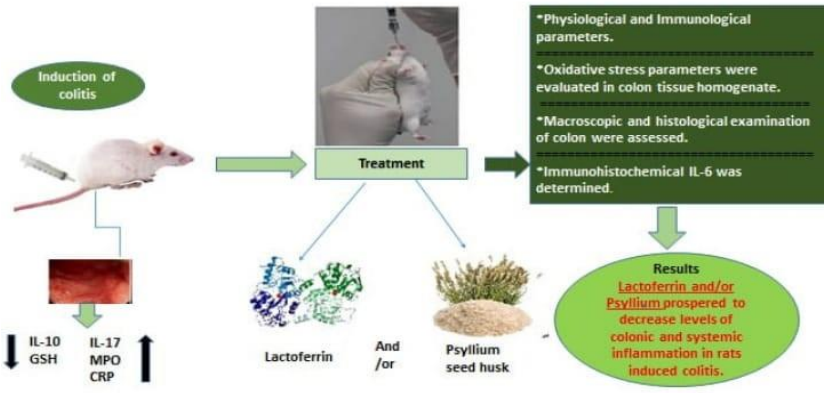
ABSTRACT

Ulcerative colitis is a pathological condition characterized by recurrent colon inflammation, causing symptoms such as diarrhea, nausea and abdominal discomfort. In vitro, studies have demonstrated that Lactoferrin directly impacts intestinal immunity and reduces inflammation by altering the production of inflammatory cytokines in immune cells. *Plantago ovata's* seed husk, known as Psyllium, are frequently taken as a dietary supplement to help with digestive problems. The study investigated the induction of the colitis model in rats using a 1 ml of acetic acid (4%) enema inserted into the rectum. Oral Lactoferrin with a dose of (30 mg/animal/day) and Psyllium seed husk colloid with a dose of (15 mg/animal/day) for 7 days were used as treatments. Animals were divided into seven groups with different treatments. Rats' stool was examined and scored. Serum C- reactive protein (CRP), interleukin-10, and Interleukin-17 were estimated with some oxidative stress parameters evaluated in colon tissue homogenate. Immunohistochemical Interleukin-6 and histological examinations of the colon were assessed. The study found that oral administration of lactoferrin and/or Psyllium significantly improved the severity of colon inflammation by reduction in colon wet-to-dry ratio, colon inflammatory index, serum Interleukin-17, protein expression of interleukin-6 and CRP, colon homogenate malondialdehyde (MDA) and myeloperoxidase (MPO) activity. Also, increased serum Interleukin-10 and glutathione (GSH) levels were determined. The inflamed colons treated with lactoferrin and/or Psyllium showed a mostly normal histopathological examination with minimal erosion. The present study revealed that Lactoferrin and/or Psyllium prospered to decrease levels of colonic and systemic inflammation in rats-induced colitis, hopefully postponing disease progression.

Graphical abstract



∗ *Corresponding author*
E-mail address: elmasry.aya97@gmail.com

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1. Introduction

Inflammatory bowel disease (IBD), a chronic digestive disease involving Crohn's, ulcerative colitis, and unclassified IBD-U, is a complex interplay of immunological, microbial, environmental, and genetic factors causing systemic and local inflammation [1]. Ulcerative colitis is defined as recurrent colon inflammation [2], characterized by continuous mucosal inflammation that extends from the rectum into the colon [3 & 4]. Diarrhea, weight loss, nausea, and abdominal discomfort are the clinical symptoms of this condition that frequently manifest and have an impact on quality of life [2]. Ulcerative colitis (UC) has affected patients' physical, psychological, familial, and social lives badly [5]. The disease involves the invasion of inflammatory cells, colonic barrier rupture, release of cytokines, arachidonic acid metabolites, and production of reactive oxygen species, causing oxidative damage [6].

Together with genetic and environmental variables, the etiopathology is most likely associated with dysregulation of the mucosal immune response toward the local bacterial flora. To lower symptoms or manage inflammation, a variety of drugs are employed. Many other techniques and treatments that go outside the purview of traditional Western medicine are included in herbal medicine. Nonetheless, a restricted number of controlled studies suggest that traditional Chinese medications, including aloe vera gel, wheat grass juice, Boswellia serrata, and bovine colostrum enemas, are effective in treating ulcerative colitis. Herbal remedies may be less dangerous than manufactured medications even if they still carry some risk. Its high patient acceptance rate, effectiveness, relative safety, and affordability may be the reasons for herbal medicine's future advantages. The effectiveness of herbal medication has been examined in hundreds of clinical trials for the treatment of UC, and patients everywhere appear to have embraced it in significant ways. Although there are hazards and advantages linked with herbal treatment, the research is contradictory, intricate, and difficult to understand. In addition to improved regulations to ensure the highest standards of quality and safety, more controlled clinical trials investigating the possible effectiveness of herbal medicine techniques in the treatment of UC are required [7].

The glycoprotein lactoferrin (LF) is made up of single-stranded amino acids and is found naturally in bodily fluids and secretions such as milk, mucus, tears, and saliva. Leucocytes, inflammatory or reproductive tissues, and other tissues can also contain it [8, 9]. Due to its ability to bind iron, it plays a vital function in providing iron to breastfed newborns. Moreover, LF has a wide range of significant bioactive properties, including antibacterial, immunomodulatory, anticancer, antimicrobial, antifungal, and osteogenic properties [10]. Previous in vitro investigations have shown that lactoferrin directly affects intestinal immunity and reduces inflammation by altering the production of immune cell cytokines [11, 12].

Recent years have seen a rise in the use of herbal therapy due to the availability of cultivated and wild plants, offering new therapeutic agents with less hazardous side effects [13]. *Plantago* spp. is an annual plant that grows widely over most of the planet and is used to treat several illnesses [14]. *Plantago ovata* plant native regions are in Asia, the Mediterranean, and North Africa. Crushed seeds and herbal are used to make Psyllium husk with high soluble fiber content. *Plantago ovata* seed is described in Iranian traditional medicine as a useful treatment for gastrointestinal conditions such as diarrhea, ulcerative colitis, hemorrhoids, and constipation. Nevertheless, studies indicate that Psyllium consumption is advantageous for numerous bodily organs, such as the pancreas and the heart. Psyllium is also sometimes used as a food thickener [15]. Psyllium is an anionic polysaccharide consisting of L-arabinose, D-xylose, and D-galactonic acid. The aqueous extract of *P. ovata* seed has a high percentage of hemicellulose [16]. Polysaccharides can regulate gastrointestinal function by controlling viscosity, satiety, large bowel fermentation, and anti-inflammation actions, making them a promising strategy for improving overall health [17].

This study aims to evaluate lactoferrin and/or Psyllium seed husk as potential therapeutics for induced ulcerative colitis in albino rats.

2. Materials and methods

Adult male albino rats weighing 150-200 g were used in the present study. The animals were housed in clean cages and had free access to food and water *ad libitum*. They were maintained at 21–24˚C and 40–60% relative humidity with a 12-h light-dark cycle. Animals were accommodated for one week before the experiment. Every animal procedure was carried out in compliance with the guidelines set forth by the National Organization for Drug and Central Ethics Committee (NODCA Ethics Committee Acceptance No. NODCAR/31/1/2022).

Rats were fasted overnight with free access to water and then anesthetized using thiopental (20 mg/kg i.p.) [18]. Colitis was induced by a single enema of 1 ml acetic acid (4%) according to ***Bademci et al.*** [19], and ***Yamada et al.*** [20], It was instilled using a medical-grade polyethylene catheter (external diameter 2 mm) inserted into the rectum of rats at a depth of 4.5 cm proximal to the anus verge according to ***Matuszyk et al.*** [21]. Different treatments started after 24 hours, according to ***Low et al.*** [22] for seven days [23]. The control groups were instilled with physiological saline instead of an acetic acid solution.

2.1. Experimental design

A pure strain of 56 adult male albino rats were divided into 7 groups at random.

Group1: Normal control group (n: 8 rats)

Group2: Acute colitis group (n: 8 rats) colitis was induced by a rectal enema with 1 ml of 4% acetic acid according to ***Yamada et al.*** [20]

Group3: Lactoferrin treated group (n: 8); Rats were given lactoferrin (30mg/animal/day) orally for 7 days, (Pravotin, 30 sachets, 2 gm Wight for one sheet lactoferrin concentration 100mg/sheet, Meivo international for pharmaceutical industries, Hygint pharmaceuticals company, Alexandria -Egypt)

Group4: Psyllium seed husk colloid treated group (n: 8); Rats were orally administrated (by using a gastric tube) with Psyllium seed husk colloid

(15mg/animal/day) for 7 days, (colomucil, 100% natural seed husk), Lipids Egypt for Pharmaceutical and Medical products,6th October City, Giza, Egypt)

Group5: Acute colitis lactoferrin treated group (n: 8 rats). Rats were instilled with 1 ml of 4% acetic acid enema followed by administrating lactoferrin (30mg/animal/day) orally for 7 days similar to ***Togawa, et al.*** [24]

Group6: Acute colitis psyllium seed husk colloid treated group (n: 8 rats); Rats were instilled with 1 ml of 4% acetic acid enema followed by administrated Psyllium seed husk colloid (15mg/animal/day) orally for 7 days (by using a gastric tube) for 7 days according to ***Bagheri et al.*** [15]

Group7: Acute colitis lactoferrin - psyllium seed husk treated group (n: 8); Rats were instilled with 1 ml of 4% acetic acid followed by administration of lactoferrin - Psyllium seed husk orally for 7 days.

2.2. Tissue collection and preparation

Rats were weighed at the beginning and the end of the experiment according to ***Wess et al.*** [25]. Twenty-four hours after the last treatment, on the 8th day post acetic acid installation, the stool was inspected and recorded for each rat, then rats were euthanized by cervical dislocation, and collected blood in dry clean centrifuge tubes. Clear serum was separated and stored at – 80 C° for estimating the C- reactive protein titer (bioMérieux, Egypt), IL-10, and IL-17 (NOVA Rat interleukin ELISA kits, China). A laparotomy was immediately performed. The distal 10 cm portion of the colon was excised, freed of adherent adipose tissue, longitudinally split, washed with saline to remove fecal residues, and weighed.

The colons were photographed and then assessed for macroscopic damage scoring and determination of the area of colonic lesions. One segment of the colon was fixed in 10% buffered formol saline for assessment to measure colon histological scoring and immunohistochemical analysis of IL-6 protein. The remaining colon tissue was divided into 2 parts, one segment about 1 cm in length was weighed and used for wet/dry ratio; the other part was homogenized. Changes in body weight were recorded [26]. Weights and lengths of excised colons were listed, and the colon weight/length ratio (colon index) was calculated [27]. Following the guidelines of good cleanliness, animal cadavers, and tissue samples were handled carefully.

2.3. Preparation of colon tissue homogenate:

The colon segment was homogenized with ice-cold double distilled water 50 Mm phosphate buffer (pH 7.4) [28] using a glass homogenizer fitted with a glass pestle [Ezstir DAIHAN Scientific Co., Ltd., Korea] to prepare 10% w/v homogenates. The colon homogenates were centrifuged at 4000 r.p.m. for 15 min at 4°C using a cooling centrifuge (Hermile Labortechnik, Wehingen, Germany), and the obtained supernatants of the homogenates were divided into several aliquots. These aliquots were stored at -80 °C until assayed later [29].

Homogenates were used for the determination of malondialdehyde (Biodiagnostic, Giza, Egypt), [30] and glutathione homogenates (Biodiagnostic, Giza, Egypt) according to Beutler, et al., [31] and myeloperoxidase (MPO) activity in colon tissues described by ***Bradley et al.*** [32].

2.4. Statistical Analysis

The study used arithmetic mean and standard error, ANOVA, Tuky-Kramer's post hoc test, Instat software, and GraphPad prism for data analysis, with statistical significance at $P<0.05$.

3. Results

3.1. Immunological parameters

The study found that acetic acid-induced ulcerative colitis significantly decreased serum levels of the anti-inflammatory cytokine Interleukin-10 (IL-10) levels and increased serum inflammatory markers Interleukin-17 (IL-17) and C-reactive protein (CRP) compared to the normal control group. However, lactoferrin or psyllium-treated groups significantly increased serum IL-10 levels and decreased serum IL-17 levels and CRP as compared with the normal group, whereas. in acute colitis rats treated with lactoferrin and/or psyllium significantly increased serum IL-10 levels as compared with acute ulcerative colitis rats and improved the severity of acetic acid-induced colon injury, as evidenced by reduced serum IL-17 levels and serum CRP protein levels as compared with the acute ulcerative colitis group (Fig. 1).

3.2. Physiological Parameters

I. ***Body weight change and body weight percentage change:***

All groups showed an increase in body weight change when compared with normal control at ($p < 0.05$) except the Ulcerative colitis – Psyllium group which revealed a non-significant decrease in body weight change (The UC-Psyllium group has no significance change in body weight because of the high standard deviation). Lactoferrin groups showed a significant increase in body weight change and body weight percentage compared to the normal control group at ($p < 0.05$) (Table 1).

II. ***Colon relative weight and colon wet to dry weight ratio.***

Ulcerative colitis induced by acetic acid rectal instillation resulted in a significant increase in relative colon weight and colon wet-to-dry ratio as compared with the normal control group at $p<0.05$. Ulcerative colitis treated with oral administration of lactoferrin and/or psyllium was accompanied by normalization of relative colon weight and significantly improved severity of colon injury, as evidenced by a reduction in the colon wet-to-dry ratio in treated rat groups as compared with the ulcerative colitis group at $p<0.05$. As soon as the result of lactoferrin or psyllium-treated groups without ulcerative colitis is very close to the normal control group (Table 1).

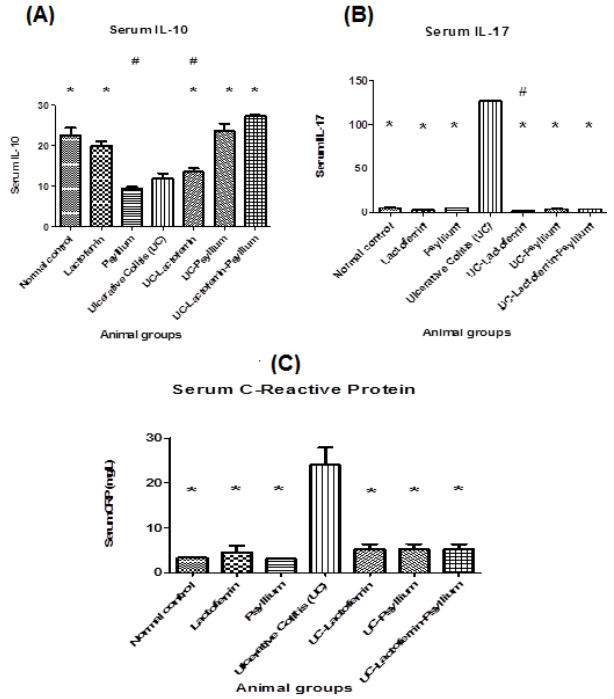

Fig. (1): Effects of Lactoferrin and / or Psyllium oral administration on (A): Serum IL-10, (B): Serum IL-17, and (C): Serum C - reactive protein (CRP) as compared with rats subjected to acetic acid-induced ulcerative colitis. # Significant difference from the control group at $p < 0.05$. * Significant difference from ulcerative colitis group at $p < 0.05$.

III. Colon total macroscopic damage scoring and colon ulcer

The ulcerative colitis group revealed a significantly increased number of colon ulcers and significant increase in colon total macroscopic damage scoring as compared with normal control group colons, while lactoferrin and/or psyllium without ulcerative have normal colon appearance as compared with normal control rat colons at ($p < 0.05$) (Fig..2) and at the same time UC- lactoferrin and /or UC-psyllium groups showed a significant improvement in severity of colon injury as evidenced by reduction in colon total macroscopic damage scoring as compared with Ulcerative colitis group at $p<0.05$, but these therapeutic treated groups were still significantly different when compared with control group at $p<0.05$ (Table 2).

IV. Colon length and Colon Inflammatory Index

The study found that acetic acid-induced ulcerative colitis in rats resulted in a significant reduction in colon length as compared with normal control rat. However, treatment with lactoferrin or psyllium normalized colon length in ulcerate rats and improved the severity of inflammation by reducing the colon inflammatory index compared to the acetic acid-treated group at $p<0.05$ (Table 2). Lactoferrin or psyllium treated groups without ulcerative showed normal colon as compared with normal control group.

Table (1): Effect of Lactoferrin and /or Psyllium administration on change in body weight, body weight percent change, colon relative weight, and colon wet/dry weight ratio in rats with acetic acid–induced ulcerative colitis.

Animal groups	Body weight change (g)	Body weight percentage change %	Colon relative weight (mg/g body weight)	Colon wet-dry weight ratio (W/D Ratio)
Normal control	**5.38 ± 0.78***	**2.54±0.41**	**5.87±0.29***	**3.9± 0.23***
Lactoferrin	**47.00± 4.1#**	**29.6 ±2.57*#**	**7.09 ±0.24**	**4.25± 0.10***
Psyllium	**30.67± 5.4**	**17.7 ±3.11**	**7.25±0.11**	**4.06± 0.13***
Ulcerative Colitis (UC)	**18.25± 10.7**	**11.2± 7.48**	**9.21±1.09**	**5.34± 0.21#**
UC-Lactoferrin	**38.25± 8.8#**	**28.3± 2.4*#**	**6.64±0.42**	**3.98 ±0.17*#**
UC-Psyllium	**- 4.0± 3.8**	**4.6 ±1.27**	**7.6±0.78**	**3.98 ±0.16***
UC-Lactoferrin-Psyllium	**18.29 ± 5.8**	**12.1 ±2.67**	**7.65±0.41**	**4.35± 0.11***

Values of body weight change, body weight percent change, colon relative weight, and colon wet/dry weight ratio are expressed as mean ± SEM. UC; ulcerative colitis. # Significant difference from the control group at $p < 0.05$.
* Significant difference from ulcerative colitis group at $p < 0.05$.

Table (2): Effect of Lactoferrin and / or Psyllium administration on colon total macroscopic damage scoring, colon length, colon Inflammatory Index, and number of colon ulcers.

Animal groups	Colon total macroscopic damage scoring	Colon length (cm)	Colon Inflammatory Index [Colon weight (mg)/Colon length (cm)]	Number of colon ulcers
Normal control	**0.5±0.04***	**18.95± 0.41***	**78.66± 2.11***	**0.0 ±0.0***
Lactoferrin	**2.0± 0.57***	**18.37± 0.64***	**79.75± 3.88***	**0.0 ±0.0***
Psyllium	**1.66± 0.42***	**17.87±0.21**	**80.65± 4.53***	**0.0 ±0.0***
Ulcerative Colitis (UC)	**8.12± 0.44#**	**15.06± 0.47**	**103.5± 3.96**	**4.12±1.2**
UC-Lactoferrin	**3.25± 0.25*#**	**16.99± 0.53**	**98.59± 4.61**	**0.0 ±0.0***
UC-Psyllium	**4.62± 0.82*#**	**15.73±1.18#**	**84.79± 7.70**	**0.37± 0.26***
UC-Lactoferrin-Psyllium	**3.14± 0.79*#**	**18.10± 0.73***	**81.93± 4.64***	**0.12± 0.12***

Values of colon total macroscopic damage scoring, colon length, colon inflammatory index, and number of colon ulcers in rats with acetic acid–induced ulcerative colitis are expressed as mean ± SEM. UC; ulcerative colitis.
Significant difference from the control group at $p < 0.05$. * Significant difference from ulcerative colitis group at $p < 0.05$.

III. Colon gross macroscopic examination

The study examined colons of rats treated with lactoferrin and psyllium, gross macroscopic examination of colons from the naive control group showed intact mucosa and serosa with no signs of tissue damage or haemorrhage Fig (2-A). Lactoferrin-treated colons and psyllium -treated colons showed a normal appearance comparable to normal control rats Fig (2-B & C).

The acetic acid ulcerative colitis group upon examination showed extensive necrosis of tissue over a wide surface area with severe haemorrhage, where the mucosal lining was damaged with visible erosions Fig (2-D, E & F). In lactoferrin treated group, little evidence of bleeding was seen, and lactoferrin shielded the colon against acetic acid-induced mucosal injury and tissue necrosis, - Fig (2-G). Psyllium or psyllium combined with lactoferrin treatment groups did not exhibit significant tissue erosion, nor did they exhibit significant damage to the rat colons, which show improvement with minimal congestion Fig (2-H & I). Macroscopic scores were assigned to numerically quantify tissue damage table (2).

3.3. Oxidative stress parameters

The ulcerative colitis group resulted in a significant decrease in glutathione **(**GSH**)** and an increase in colon homogenate malondialdehyde (MDA) content and myeloperoxidase (MPO) activity as compared with control rats ($p < 0.05$). The combined therapy of lactoferrin and psyllium with ulcerative resulted in a significant increase in colon GSH content with a significant decrease in colon homogenate malondialdehyde (MDA) content and myeloperoxidase (MPO) activity as compared to the acetic acid-treated group ($p < 0.05$). On the other hand, in the non-ulcerative lactoferrin or psyllium-treated group, the results were comparable to that of the normal control group. (Fig. 3)

4. Histopathological and Immunohistochemical examination

4.1. Colon microscopic histopathological examination

The colon's mucosa was found to be composed of crypts and tubular glands, with lamina propria and submucosa and muscular layers. Both lactoferrin and psyllium control sections showed apparently normal colons as compared with normal control group Fig. (4- B&C). Acetic acid administration caused significant pathological changes, including crypt distortion, hyperplasia of epithelial cells, loss of goblet cells, and ulcerated surface epithelium as compare with normal control group (fig. 4-D, E, F, G, H, I). However, colons with ulcerative colitis that treated with lactoferrin and/or psyllium showed normal colon structure, with minimal erosion changes as compared with acute colitis group. Remarkable improvement was observed after treatment with psyllium alone or lactoferrin and psyllium where colon tissue looked comparable to control (Fig. 5- A, B, C).

4.2. Colon histological damage scoring

Ulcerative colitis induced by acetic acid revealed a highly significant increase in colon histological damage scoring as compared with the normal control group at ($p < 0.05$). All test groups were significantly lower than the Ulcerative colitis group at ($p < 0.05$).

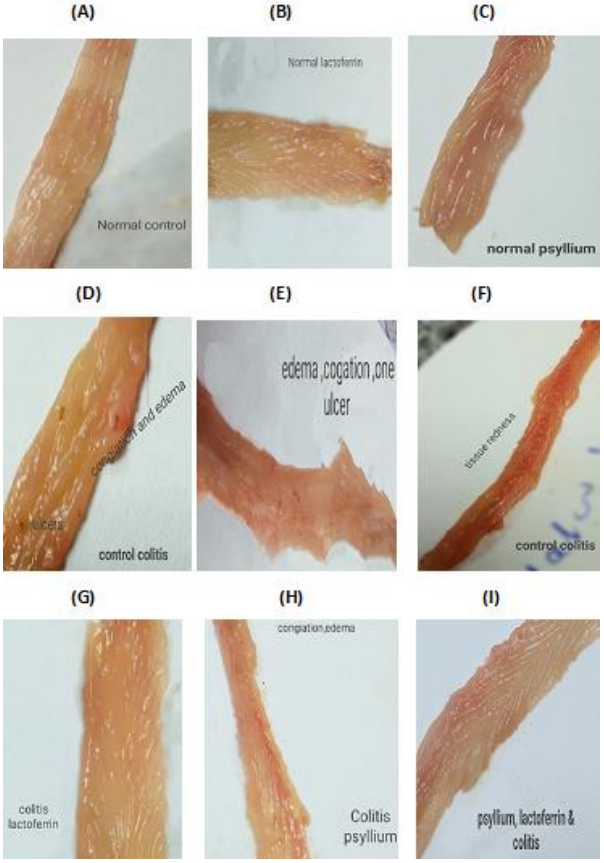

Fig. (2): Effects of Lactoferrin and / or Psyllium administration on acetic acid-induced macroscopic damage on rat colon. (A): naive control, (B): Lactoferrin, (C): Psyllium, (D, E & F): ulcerative colitis tissue necrosis, wide surface area with severe hemorrhage, mucosal lining damage with visible erosions and ulcers, (G): ulcerative colitis treated with lactoferrin showing normal appearance, (H): ulcerative colitis treated with psyllium showing slight hyperemic rat colon (I): ulcerative colitis treated with lactoferrin combined with psyllium revealing slightly hyperemic mucosa, with no ulcer or erosion.

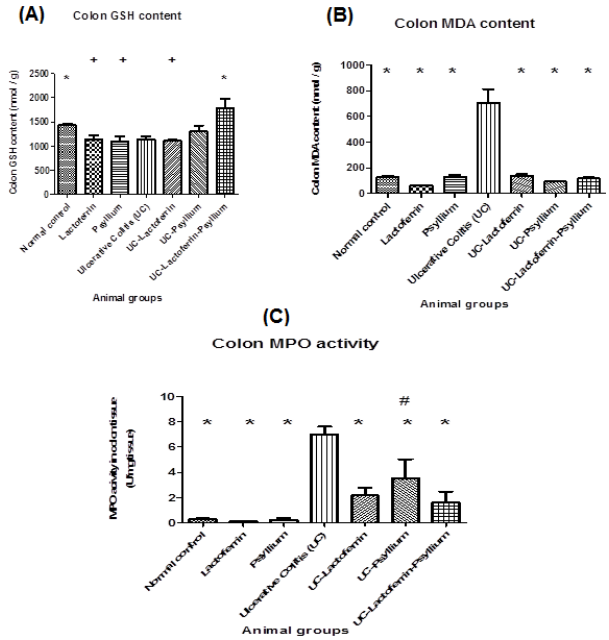

Fig. (3): Effects of Lactoferrin or /and Psyllium oral administration on (A): Colon GSH content, (B): Colon MDA content, and (C): Colon MPO activity as compared with rats subjected to acetic acid-induced ulcerative colitis. # Significant

difference from the control group at $p < 0.05$. * Significant difference from ulcerative colitis group at $p < 0.05$.

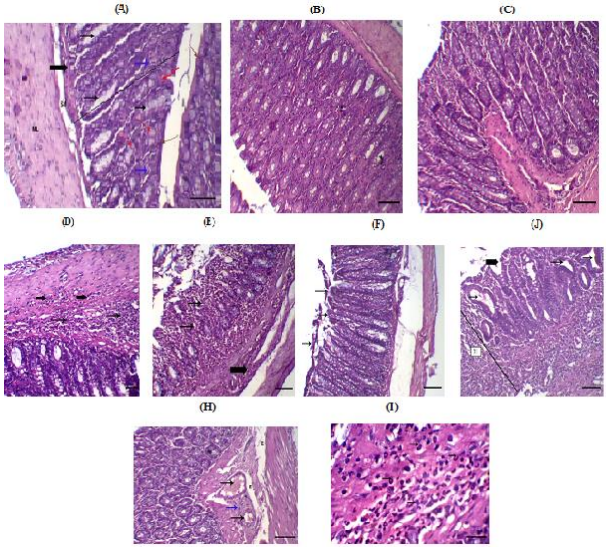

Fig. (4): Photomicrograph of colon tissues of Control group (A): showing: mucosal layer (line), crypt (black arrow), lamina propia (blue arrow), surface epithelium (brown arrow), goblet cell (red arrow), muscularis mucosa (thick arrow), submucosal layer (SM), muscular layer (ML). (B): colonic tissues from Lactoferrin treated group showing the normal architecture of the colon. (C): colonic tissues from Psyllium treated group showing the normal architecture of the colon (H& E X100). Colitis group [D – I]: (D): inflammation in submucosa layer (thin arrow), inflammation in muscular submucosal layer (thick arrow); (E): crypitis (thin arrow), dense inflammatory cell aggregates in submucosal layer (thick arrow). note: marked loss of goblet cells; (F): damage and erosion of epithelium (arrow); (J): distortion of crypt architecture with loss of goblet cells (thin arrow), ulceration of epithelium (thick arrow), transmural inflammation (TI); (H): congested dilated blood vessel (black arrow), edema (E), perivascular inflammation (blue arrow) (H&E. X:100); (I): mixed leukocytes infiltration in mucosal layer (thin arrow) (H & E. X400).

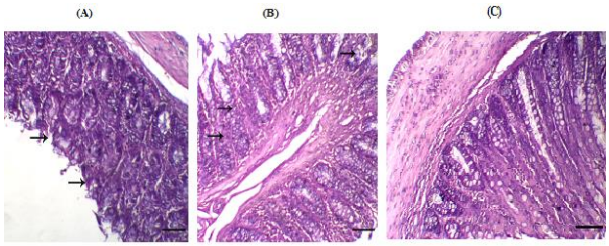

Fig. (5): Photomicrograph of colon tissues from colitis-treated groups (A): Lactoferrin-treated colitis group showing minimal erosion changes in the epithelium (arrow) and normal colon tissue; (B) Psyllium-treated colitis group showing destructed crypt (arrow) and normal colon tissue; (C): combined Lactoferrin and Psyllium treated colitis group showing normal architecture of colon (H&E. X100).

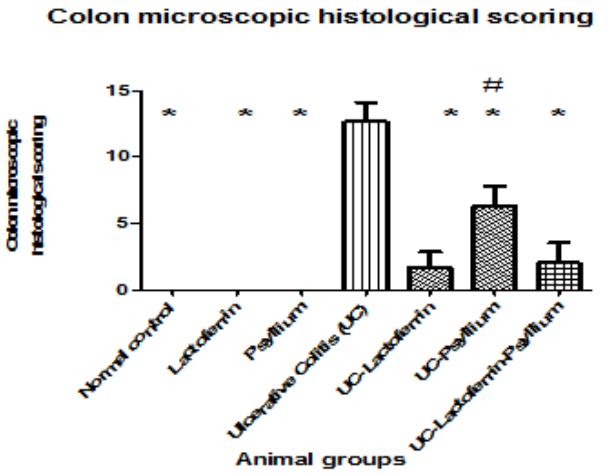

Fig. (6): Effects of lactoferrin and /or psyllium oral administration on colon histological damage scoring as compared with rats subjected to acetic acid-induced ulcerative colitis**.**

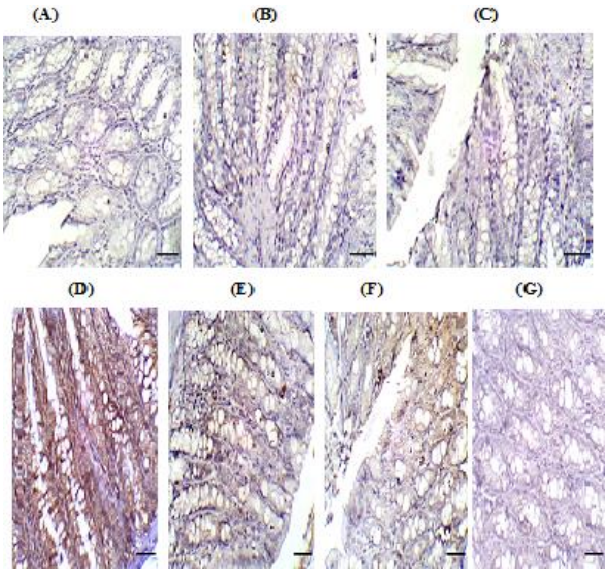

Fig. (7): IL-6 Immunohistochemical photomicrograph of colon tissues from (A) control group showing nearly –ve immunoexpression of IL-6; (B)and (c) Lactoferrin and Psyllium treated groups showing also nearly –ve immunoexpression of IL-6; (D) Immunoreactive IL6 antibody are observed in colonic mucosa and the lamina propria of acetic acid-induced ulcerative colitis; (E) lactoferrin treated colitis group showing mild to moderate immunoexpression of IL-6; (F) psyllium treated colitis group showing mild to moderate immunoexpression of IL-6; (G) combined lactoferrin and psyllium treated colitis group showing mild immunoexpression of IL-6 (x100).

4.3. Colon immunohistochemical examination

The study assessed colon immunohistochemical sections for interleukin-6 (IL-6) protein expressions. Results showed IL-6 was mainly distributed in the mucosa and submucosa layers of colons. Lactoferrin and psyllium control treated groups showing nearly –ve immunoexpression of IL-6 as compared with normal control group. In the acetic acid group, IL-6 expression was significantly increased as compared with normal control group, while in ulcerative colitis rats treated with lactoferrin and/or psyllium, it was significantly reduced as compared with ulcerative colitis group. The results of immunohistochemistry were expressed as optical density (OD). Data of optical density in each group were presented as means ± SEM (Table 10). (fig. 6).

5. Discussion

Human inflammatory bowel disease has a different etiology than acetic acid-induced ulcerative colitis. However, both conditions have similar pathophysiological characteristics and are responsive to medication. Intestinal alterations as a result of acetic acid induction, such as mucosal ulcers, weight loss, hemorrhage, and inflammation, are frequent in humans with inflammatory bowel

disease IBD [33]. Additionally, both diseases are characterized by the invasion of inflammatory cells like neutrophils into the damaged colon, rupturing of the colonic barrier, and the release of inflammatory mediators like cytokines and arachidonic acid metabolites with the production of reactive oxygen species (ROS), which causes oxidative damage [6].

In vitro studies have demonstrated that lactoferrin directly impacts intestinal immunity and reduces inflammation by altering immune cell cytokines production [11, 12].

Plantago ovata's seed and husk are used as fiber supplements for treating constipation and gastrointestinal diseases [34]. The seed contains hemicellulose, fermentable fiber, and butyric acid, which can prevent atherosclerosis, diabetes, obesity, hypercholesterolemia, Crohn's disease, constipation, and diarrhea [35]. Butyric acid also has anti-cancer properties and may aid in ulcerative colitis treatment [36].

It's interesting to note that the ulcerative colitis group in this study demonstrated a considerable gain in body weight that agreed with ***Harper and Zisman's*** study which mentioned that excessive body weight increases concurrently with IBD [37], also, Flores et al., reported that 32.7% of IBD patients were obese [38]. According to ***Jarmakiewicz-Czaja et al.*** [39], the primary factor contributing to excess body weight may be the result of UC, which causes a decrease in physical activity. Additionally, a shift in the distribution of adipose tissue may be the root of weight gain, which could result in the buildup of excessive amounts of body fat [40]. These reported findings explain the recorded increase in body weight that was encountered in this study. On the contrary, other studies defy the findings [41, 42].

According to ***Bretin et al.*** [43] psyllium protects against diet-induced obesity (DIO). Several randomized controlled studies have demonstrated that psyllium also aids in weight loss in persons who are overweight or obese [44]. That may be related to its long-known capacity to sequester luminal BA, lowering blood cholesterol levels, raise serum bile acid levels, and encourage intestinal regularity [45, 46].

The study found that lactoferrin and psyllium-treated colons exhibited normal appearance in colon gross macroscopic examination, while acetic acid ulcerative colitis group showed extensive tissue necrosis, hemorrhage, and visible erosions of the mucosal lining [22]. Lactoferrin directly affects intestinal immunity and reduces inflammation by altering immune cell's cytokines production [11, 12]. Treated groups that received psyllium or psyllium combined with lactoferrin did not show extensive damage to rat colons, and there were no erosions on the tissues due to the anti-inflammatory, antibacterial, and anti-tumor properties of lactoferrin and psyllium [14, 47].

The current study found that ulcerative colitis caused by acetic acid rectal instillation significantly increased colon relative weight, which may be primarily due to inflammation in the rectum [48]. The ratio of the colon's wet weight to the body weight, as reported by ***Qelliny et al.*** [49], could reveal a higher ratio associated with colitis.

Ulcerative colitis resulted in a significant increase in the colon wet-to-dry ratio as compared with control rat colons. The recorded increase in colon weight in the colitis group is due to the mucosa being velvety and edematous [50]. A similar observation was recorded by ***Greca et al.*** [51], who used the colon wet-dry weight ratio to evaluate *edematous* changes in different tissues. The study found that oral administration of lactoferrin or psyllium significantly reduced the severity of colon injury in rats compared to those treated with acetic acid.

The ulcerative colitis group resulted in a significant increase in colon inflammatory index that was similar to ***Qelliny et al***. who found that a larger ratio was recorded with colitis [49]. Interestingly, the angle of the mucosal folds decreased significantly in the chronic colitis group, which may be due to acute inflammation recovery and mucosal dehydration that leads consequently to shortness of colon length [52]. Lactoferrin and /or psyllium oral administration treatment improved the severity of colon inflammation as evidenced by a reduction in colon inflammatory index, psyllium can be considered to have prebiotic potential. In general, the health-promoting effects of prebiotics include supporting the growth of bacteria beneficial to the host and increasing the production of short-chain fatty acids (SCFA) such as butyrate and propionate previously shown to be positive for colonic health [53]. Oral administration of lactoferrin in TNBS-treated rats attenuated all of the inflammatory responses, such as increased colonic weight-to-body weight ratio, macroscopic signs of inflammation, and increased histological inflammation score, which suggest that the lactoferrin suppressed TNBS-induced colitis [24].

According to ***Ordás et al.*** [54], ulcerative colitis includes erythema, granularity, friability, erosions, ulcerations, and spontaneous bleeding. Lactoferrin and/or psyllium oral administration treatment reduced the number of colon ulcers significantly in treated rat groups. As a reason for the anti-inflammatory, antibacterial, and anti-tumor properties of lactoferrin and psyllium [14, 47].

Ulcerative colitis induced by acetic acid shows a decrease in serum IL-10 as compared with control rats. According to Wan et al., reducing IL-10 production in UC patients [55] is due to numerous situations and illnesses that have demonstrated the function of regulatory B cells (Bregs) in suppressing immune responses [56]. Bregs have been shown to affect chronic metabolic disorders [57] and spontaneous colitis [58]. Early research linked IL-10, which came to be known as the hallmark of Breg suppression, to this immunomodulation [58, 59, and 60].

Lactoferrin and/or psyllium UC groups have a significant increase in the level of serum IL-10 as compared with ulcerative colitis rats in our study. Ulcerative rats receiving combined treatment have revealed significantly elevated serum IL-10; as reported by ***Togawa et al.*** [24], who showed that significant decreases in the proinflammatory cytokine tumor necrosis factor and increases in the anti-inflammatory cytokine interleukin IL-10 were brought about by lactoferrin. Those findings indicated that lactoferrin prevents colitis in rats by balancing out cytokine imbalances and modifying the immune system.

The pre-probiotic, which includes psyllium was shown to have the ability to upregulate IL-10. According to reports by other authors, pre-probiotics have a positive effect on immunomodulation, highlighting the fact that pro-inflammatory cytokines, including chemokines and chemokine receptors, are inhibited by IL-10 and other cytokines, which causes intestinal inflammation [61]. Our study revealed that IL-10 as an anti-inflammatory mediator was increased by psyllium; this finding matches the result of ***Abd El-Rhman.*** [62].

Acetic acid-induced ulcerative colitis in rats led to a significant rise in serum IL-17 levels, as shown by ***Yu, et al.*** [63]. Intestinal bacterial colony-stimulating factor (ILC) stimulates the production of cytokines such as TNF-α, IFN-γ, and IL-17 in large quantities. The immune response is then triggered by these cytokines to eradicate pathogens. Conversely, over-activation of intestinal lining cells (ILCs) is the cause of inflammatory bowel disease (IBD) and intestinal inflammation [64, 65]. Our results in lactoferrin conform to those of Hwang et al., who showed a significant decrease in circulating IL-17. Because of the variety of immune-modulating abilities of lactoferrin (LF), since, LF could suppress harmful inflammatory reactions and stimulate the growth of T cells [66].

The health-promoting effects of prebiotics including psyllium support the growth of bacteria beneficial to the host and increase the production of short-chain fatty acids (SCFA) such as butyrate and propionate previously shown to be positive for colonic health [53]. A sign suggested that intestinal levels of SCFAs and the microbiota were elevated by the bacterial fermentation of fibers. In addition, psyllium supplementation prevented colitis in mice by lowering their inflammatory response [67].

Dupraz et al. [68] demonstrated a dichotomy between γδ T cells that produce IL-17 and IL-22 in the small intestine, the cecum, and the colon and how the gut microbiota regulates each of these three compartments differently. In the lamina propria of the small intestine, the gut microbiota stimulates γδ T cells to produce IL-17 and IL-22, whereas in the colon and cecum, the reverse effect is seen. They discovered that the microbiota in the colon and cecum produces metabolites called short-chain fatty acids (SCFAs), which are important regulators of the γδ T cells' production of IL-17 and IL-22 in the gut lamina propria Histone deacetylase (HDAC) activity is inhibited by these metabolites, especially propionate, which is produced by the gut microbiota in the colon and cecum and can directly alter the functional characteristics of γδ T cells.

Cecal γδ T cells are programmable in the thymus and do not require microbiota to produce IL-17 and IL-22. On the other hand, chemicals produced from the microbiome affect ILC3s and γδ T cells differently. Propionate directly inhibits the synthesis of IL-17 and IL-22 by intestinal γδ T cells; however, this SCFA raises the percentage of IL-22+ ILC3s without changing the production of IL-17 by this population [69].

In our study, the level of serum C-reactive protein (CRP) in the ulcerative colitis group initiated a significant increase in serum CRP as compared with control rats. According to ***Fagan et al.*** [70], C-reactive protein levels were raised in ulcerative colitis due to an increase in mucosal inflammation in the ulcerative group. Although lactoferrin and/or psyllium treatments have significantly reduced the level of serum CRP in ulcerative colitis rats, according to ***Bharadwaj. et al.*** [71], CRP was modestly reduced with milk ribonuclease-enriched lactoferrin supplementation, which directly affects intestinal immunity and reduces inflammation by altering the production of cytokines in immune cells [11,12]. Previous research by ***Chiba et al.*** [72], suggested that psyllium had the potential for both therapeutic and well-being, with its anti-inflammatory, hypoglycemic, hypolipidemic, antioxidant, and immunoprotective qualities being its primary sources of benefit**. De Oliveira *et al*.** have also documented psyllium's anti-inflammatory properties. [73]

According to ***Jin et al.*** [74], oxidative stress can be brought on by an increase in oxidants or a reduction in the antioxidant system. It is believed that oxidative stress has a role in the development of chronic disorders like UC. Glutathione (GSH) is essential to the antioxidant system. Given its antioxidant properties, GSH plays a crucial role in scavenging intracellular free radicals [75]. Shaikh Omar demonstrated that the lipid peroxidation marker MDA, as well as the result of lipid peroxidation by free radicals and the neutrophil-derived peroxidase enzyme MPO, contribute to tissue damage during inflammation due to ROS generation [76].

In this study, the colon tissue of the UC group had significantly higher MDA content and MPO activity but significantly lower GSH content. In contrast, the oxidative condition was reversed after treatment with psyllium seed husks by increasing the antioxidant GSH content along with decreasing oxidant MDA molecules and MPO enzyme activity. This is according to ***Abd El-Rhman*** [62].

It is noteworthy to remember that *Plantago* psyllium seeds may have an antioxidant impact because of their substantial metabolites, sulfur-containing amino acids, alkaloids, polyunsaturated fatty acids, and flavonoids, all of which indicate their potential for antioxidant activity [77]. In our study oral administration of lactoferrin treatment improved colon ulcerative colitis oxidative stress as evidenced by significantly decreased MDA and MPO content in treated rat groups, and a significant increase of colon GSH content as compared to acetic acid treated group, in accordance to **Han N, *et al.*,** who proved that LF reduces lipid peroxidation (MDA) in lung tissue homogenate and increases the GSH content [78].

The colon's histologic appearance showed normal colons, with crypts extending to the muscular mucosa as straight tubular glands. The crypts were lined with tall columnar cells and goblet cells, with lamina propria spaces, that were approved by ***Abd El-Rhman*** [62]. Acetic acid administration caused significant pathological changes in colonic mucosa, including distortion of the crypt, hyperplasia of epithelial cells, and ulcerated surface epithelium. This was supported by previous studies by ***Wang et al.*** [79] and ***Abd El-Rhman*** [62]. Histological examination of colonic samples from rats given lactoferrin and psyllium showed that the colon tissue in these groups looked like that of the normal control group

The colon treated with lactoferrin and/or psyllium showed normal colonic structure, suggesting lactoferrin may aid in mucosa healing and inflammation reduction in ulcerative colitis patients [78]. The study found improvement in colitis treatment with psyllium alone, as it reduced lymphoid follicles and mucosal epithelial cells, resulting in less fluid, inflammatory cells, and increased goblet cells [61]. It was discovered in 1973 that T cells secrete a soluble substance called IL-6, which is crucial for B cells to produce antibodies. The IL-6 pathway has been identified as a critical route involved in immune control, health, and dysregulation in numerous disorders since it was discovered more than 40 years ago [80].

In the present study, immunohistochemical examination revealed significantly increased IL-6 expression in the colons of the ulcerative colitis animal group. In the acetic acid group, IL-6 was dramatically raised compared with normally controlled rats. Increased levels of cytokines, including TNF-α and IL-6, have been linked to colitis, according to previous reports of ***Ansari et al.*** [81]. This agreed with **Nakase et al**., who focused on IL-1β, IL-6, tumor necrosis factor-α, T helper (Th) 1-, Th2-, and Th17-associated cytokines which are expressed at relatively higher levels in the intestinal tissues of patients with UC. However, their expression levels depend on the disease stage and patient characteristics. This complex pathology of UC may induce differences in responses to therapy [82]. Lactoferrin and psyllium treatment significantly reduced IL-6 protein expression in ulcerative colitis rats. **Wang *et al.***, found that lactoferrin induced the expression of TGF-β and IL-10 while downregulating the inflammatory factors TNF-α, IL-1β, and IL-6 in the colon tissue of colitis mice. It follows that lactoferrin may be used as a supportive therapy to help heal the colitis colonic barrier and lessen colonic inflammation [79]. Consequently, there was acceptance by ***Hu et al.*** [83], that psyllium seed husks downregulated serum interleukin IL-6 and enhanced the function of the intestinal barrier and gut flora. Findings pointed to the potential benefits of PSH supplementation for improving intestinal microecology. Research on developing cardiovascular risk markers with *Plantago* psyllium demonstrated a considerable reduction in IL-6 levels [84].

6. Conclusion

The present study revealed that; lactoferrin and/or psyllium, a dietary supplement, have been shown to improve intestinal immunity and reduce inflammation by altering inflammatory cytokines in immune cells. A study in rats showed that oral administration of lactoferrin and/or psyllium significantly improved colon inflammation severity, reducing colon wet-to-dry ratio, colon inflammatory index, and increased levels of interleukin-10 and GSH, hopefully postponing disease progression.

I suggest traditional Chinese medications and some herbal treatments like Boswellia serrata, bovine colostrum enemas, aloe vera gel, and wheat grass juice, which have been effective in treating ulcerative colitis in some studies.

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