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An experimental study on ulcerative colitis as a potential target for probiotic therapy by *Lactobacillus acidophilus* with or without "olsalazine"

Amany A. Abdin [a,*], **Eman M. Saied** [b]

[a] *Department of Pharmacology, Faculty of Medicine, Tanta University, Egypt*
[b] *Department of Pathology, Faculty Of Medicine, Tanta University, Egypt*

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Abstract

Traditional medical treatments for ulcerative colitis (UC) are still compromised by its adverse effects and not potent enough to keep in remission for long-term periods. So, new therapies that are targeted at specific disease mechanisms have the potential to provide more effective and safe treatments for ulcerative colitis. Probiotics is recently introduced as a therapy for ulcerative colitis. In the present study, *Lactobacillus acidophilus* was selected as a probiotic therapy to investigate its effects in oxazolone-induced colitis model in rats that mimics the picture in human. The rats were grouped (8 rats each) as normal control group (Group I), Group II served as untreated oxazolone-induced colitis, Group III oxazolone-induced colitis treated with probiotic *L. acidophilus* (1×10^7 colony-forming units (CFU)/mL/day oral for 14 days), Group IV oxazolone-induced colitis treated with olsalazine (60 mg/kg/day oral for 14 days), Group V oxazolone-induced colitis treated with probiotic *L. acidophilus* and olsalazine in the same doses and duration. Disease activity index (DAI) was recorded, serum levels of C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and intrleukin-6 (IL-6) was assessed as inflammatory markers and the histopathological picture of the colon of each rat was studied. Disease activity index (DAI) showed significant positive correlation with the elevated serum levels of CRP ($r=0.741$, $p<0.05$), TNF-α ($r=0.802$, $p<0.05$) and IL-6 ($r=0.801$, $p<0.05$). Treatment with either *L. acidophilus* (group III) or olsalazine (group IV) resulted in significant reduction in serum levels of CRP, TNF-α and IL-6, as well as disease activity index (DAI). Treatment with combination of *L. acidophilus* and olsalazine (group V) offered more significant reduction in serum levels of CRP, TNF-α, IL-6 and disease activity index (DAI) when compared to either group II (untreated group), group III (treated with *L. acidophilus*) or group IV (treated with olsalazine). So, it was concluded that *L. acidophilus* probiotic could be recommended as adjuvant therapy in combination with olsalazine to achieve more effective treatment for ulcerative colitis. For application in human, this needs to be verified in further clinical studies.

* Corresponding author.
E-mail address: amanynhr@hotmail.com (A.A. Abdin).

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1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that is known easy to relapse.[1] Despite more than a century of existence as a clinical entity, the true origin of ulcerative colitis still remains elusive. Several factors probably contribute to the development of this condition. Genetic susceptibility together with abnormal innate immunoreactivity probably comprises the essential prerequisites for the initiation and perpetuation of ulcerative colitis. The high frequency of ulcerative colitis in the industrialized countries supports the link between environmental factors and its aetiopathogenesis. The link arises from factors modifying the intestinal bacterial profile including western type of diet, use of antibiotics and chemotherapeutics, modern infant nutrition, public health measures and finally the high hygienic standards and sanitation.[2] In brief, the currently accepted model of the pathogenesis of ulcerative colitis is an inappropriate immune response to host microorganisms in genetically susceptible people.[1] The current state of therapy for IBD is not satisfactory. About 33% to 50% of cases with fulminant ulcerative colitis have their colon removed because of failure of conventional medical therapy. Also, patients with IBD frequently experience relapse and traditional medical treatments are not potent enough to keep in remission for long-term periods.[3] The most common protocols include 5-aminosalicylates (5-ASA) as standard therapy for maintaining remission and corticosteroids during acute episodes.[4,5] Olsalazine is a newer 5-ASA agent, well established in the treatment of ulcerative colitis, despite its efficacy and relative tolerability, but it is compromised by its frequent dose-dependent diarrhea.[6,7] Although, glucocorticoids suppress active inflammation very effectively, but its long-term use is associated with high rates of relapse and unacceptable adverse effects which are dose- and duration dependent. So, new therapies that are targeted at specific disease mechanisms have the potential to provide more effective and safe treatments for IBD diseases. One of the most interesting areas of IBD research is the use of probiotics therapy.[1,3] Recently, probiotic therapy has been suggested to ameliorate the milieu of intestine and prolong the time of relapse.[8] So, manipulation of intestinal microbial flora, by use of probiotics, may be a new and promising therapeutic modality in the near future.[2] Their use comes from suggestion that the imbalance between protective and harmful bacteria "dysbiosis", has been strongly postulated as a pro-inflammatory mechanism in ulcerative colitis.[9] Despite inability to isolate a specific pathogen, there is considerable evidence that bacteria play a role in colitis because bowel lesions apparently occur more frequently in areas of highest bacterial concentration; and finally therapeutic manipulation of colonic flora with probiotics can result in symptom improvement.[10] Over the past few years, there is considerable evidence that an abnormal mucosal immune reactivity against enteric bacteria represents the key event leading to intestinal injury in patients with IBD. Molecular biology techniques have shown that the intestinal space of an adult may contain >500 different bacterial species; some of them exert a protective role, whereas others are aggressive.[2]

Probiotics have been defined as "a live microbial food supplement which beneficially affects the host by improving the intestinal microbial balance",[11] and more broadly, World Health Organization,[12] defined a probiotic strain as "a live microorganism which, when administered in adequate amounts, confers a health benefit on the host beyond inherent basic nutrition". Although several probiotic bacteria of human origin are now being exploited commercially e.g. *Lactobacillus acidophilus*, many consumers, consumer organizations, and members of the scientific community are skeptical of such products and their publicized probiotic claims.[13] Thus, concomitant use of probiotics needs rigorously designed controlled trials, to investigate the unresolved issues related to efficacy, dose, duration of use, and single or multistrain formulation.[14]

So, this study was aimed to evaluate the therapeutic effects of the probiotic therapy (*L. acidophilus*) either alone or in combination with olsalazine on experimental ulcerative colitis induced by oxazolone.

2. Materials and methods

2.1. Chemicals and drugs

- *4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one* (oxazolone sensitizing agent (OXA), Sigma-Aldrich Chemical Co.).
- The probiotic *L. acidophilus* (Lactospore, >500 million colony-forming units (CFU) live *L. acidophilus* encapsulated—Repharma Pharmaceuticals, Cairo, Egypt)
- *Olasalazine* (Dipentum, 250 mg capsule—Phamacia&Upjohn Co., Middle East & North Africa, Egypt)

Induction of ulcerative colitis was performed as previously described by Boirivant et al.[15] Briefly, oxazolone solution was prepared by dissolving in 40% (v/v) aqueous ethanol to a final concentration of 7.5 mg/mL and administered once in a dose of 1.1 mL/rat into the colon through a rubber catheter inserted 4 cm inside anal verge under light ether anesthesia. Then, the catheter was removed, and the rat was held vertically for 30 seconds to ensure distribution of the oxazolone within the entire colon and cecum.

This work was conducted on 40 albino rats weighing 150–175 g, allowed for food and water ad libitum through the whole period of the work. They were divided into 5 groups (each of 8 rats) as the following:

Group I: served as normal control group, received 40% aqueous ethanol once in a dose of 1.1 mL/rat into the colon through a rubber catheter under light ether anesthesia.

Group II: served as untreated group with oxazolone-induced colitis.

Group III: oxazolone-induced colitis with concomitant administration of the probiotic *L. acidophilus* orally in a dose of 1×10^7 colony-forming units (CFU)/mL/day for 14 days.[16]

Group IV: oxazolone-induced colitis with concomitant administration of olsalazine orally in a dose of 60 mg/kg for 14 days.[17]

Group V: oxazolone-induced colitis with concomitant administration of both the probiotic *L. acidophilus* and olsalazine in the same mentioned doses and duration.

At the end of the work (14 days), all rats were sacrificed, blood samples were obtained, centrifuged at 3000 ×g and serum was stored at −20 °C for further spectrophotometric

Table 1 Disease activity index (DAI) and serum levels of CRP, TNF-α and IL-6 in the different studied groups

Parameter	Group I ($n=8$)	Group II ($n=8$)	Group III ($n=8$)	Group IV ($n=8$)	Group V ($n=8$)	*F* value	*p* value
Serum CRP (mg/dl)	7.9 ± 0.37	16.89 ± 0.74 $p_1<0.001$	14.3 ± 0.42 $p_2<0.05$	14.1 ± 0.37 $p_2<0.05$	11.7 ± 0.57 $p_2<0.001$ $p_3<0.05$ $p_4<0.05$	43.589	<0.001
Serum TNF-α (pg/mL)	11.21 ± 0.87	36.80 ± 2.57 $p_1<0.001$	27.81 ± 1.68 $p_2<0.05$	25.46 ± 1.90 $p_2<0.01$	15.90 ± 0.64 $p_2<0.001$ $p_3<0.01$ $p_4<0.01$	35.958	<0.001
Serum IL-6 (pg/mL)	39.8 ± 1.01	69.4 ± 1.25 $p_1<0.001$	54.2 ± 1.47 $p_2<0.001$	53.7 ± 1.38 $p_2<0.001$	44.7 ± 1.09 $p_2<0.001$ $p_3<0.001$ $p_4<0.01$	81.480	<0.001
Disease activity index (DAI)	–	5.14 ± 0.49	3.88 ± 0.17 $p_2<0.05$	3.41 ± 0.16 $p_2<0.01$	1.95 ± 0.18 $p_2<0.001$ $p_3<0.01$ $p_4<0.05$	21.436	<0.001

n = number.
Scheffe test:
p_1 = oxazolone-induced colitis (group II) vs control (group I).
p_2 = oxazolone-induced colitis treated by *Lactobacillus acidophilus* (group III), olsalazine (group IV) and combination of *L. acidophilus* with olsalazine (group V) vs untreated group (group II).
p_3 = oxazolone-induced colitis treated by combination of *L. acidophilus* with olsalazine (group V) vs *L. acidophilus* treated group (group III).
p_4 = oxazolone-induced colitis treated by combination of *L. acidophilus* with olsalazine (group V) vs olsalazine treated group (group IV).

assay of CRP (mg/dl),[18] TNF-α and IL-6 (pg/mL),[19] using the commercial available ELISA kits for rats. Disease activity index (DAI) was derived by scoring three major signs (weight loss, diarrhea, and rectal bleeding) divided by 3,[20] DAI = [% body weight loss + diarrhea score + rectal bleeding score] / 3. The appearance of diarrhea was defined as mucus/fecal material adherent to anal fur. The appearance of rectal bleeding was defined as diarrhea containing visible blood with or without mucus. The absence/presence of either diarrhea or rectal bleeding was given a score of 0 or 1, respectively.

2.2. Histopathological analysis

Colon of rats were excised, washed with saline and then fixed in 10% natural buffered formalin solution, embedded in paraffin, cut into tissue sections, and stained with hematoxylin and eosin (H&E). The stained sections were examined by light microscope for evidence of colitis using the following criteria: presence of inflammatory cell infiltration, cytoplasmic mucin depletion, presence of crypt abscesses, crypt distortion, and regenerative changes in the form of nuclear enlargement and increased mitotic activity,[21] cases treated with drugs were examined for histological signs of resolution.

3. Statistics

Values of the measured parameters were expressed as mean ± SEM. One way-ANOVA test (*F* value) was used to detect significance of the difference among more than two arithmetic means, followed by "Scheffe test" to test the difference between each two means. Pearson's correlation coefficient was applied to correlate between the parameters. The significance was considered at *p* values <0.05. All the statistical analyses were processed using Statistical Program of Social Sciences (SPSS) for windows, version 10.0.

4. Results

As shown in Table 1; oxazolone-induced colitis (group II) caused significant elevation in serum CRP levels, TNF-α and IL-6 when compared to the control group (group I).

In comparison to rats with untreated oxazolone-induced colitis (group II), treatment of oxazolone-induced colitis by either *L. acidophilus* (group III) or olsalazine (group IV) resulted in significant reduction in serum CRP and IL-6 levels.

Table 2 Incidence (%) of diarrhea and rectal bleeding in different groups of oxazolone-induced colitis

Group	Diarrhea *n*, (%)	Rectal bleeding *n*, (%)
Group II (untreated)	8, (100%)	5, (62.5%)
Group III (treated by *Lactobacillus acidophilus)*	4, (50%)	4, (50%)
Group IV (treated by olsalazine)	3, (37.5%)	2, (25%)
Group V (treated by combination of *L. acidophilus* with olsalazine)	2, (25%)	–

n = number, % = percent incidence.

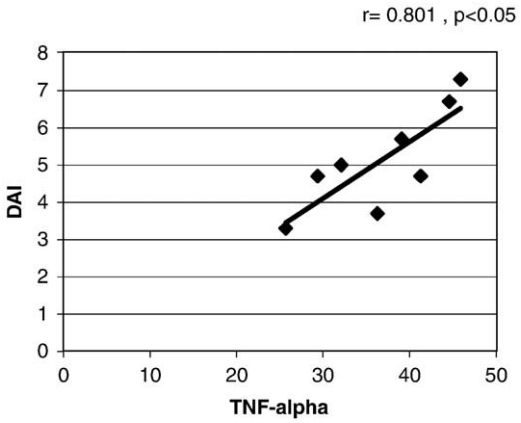

Figure 1 Correlation between DAI and TNF-alpha in oxazolone-induced colitis (Group II).

Also, treatment with combination of *L. acidophilus* and olsalazine (group V) resulted in significant reduction in serum CRP levels, serum TNF-α levels, serum IL-6 levels and disease activity index (DAI) when compared to the untreated group (group II).

When group V (treated with combination of *L. acidophilus* and olsalazine) was compared to either group III (treated with *L. acidophilus*) or group IV (treated with olsalazine), it showed significant reduction in serum levels of CRP, TNF-α and IL-6 as well as decrease in the disease activity index (DAI).

On recording disease activity index (DAI), the highest incidence of diarrhea and rectal bleeding was observed in rats with untreated oxazolone-induced colitis (group II) [$n=8$, (100%) and $n=5$, (62.5%); respectively]. The rats treated with either *L. acidophilus* (group III) or olsalazine (group IV) showed moderate incidence of diarrhea and rectal bleeding [$n=4$, (50%) and $n=4$, (50%); respectively for group III] and [$n=3$, (37.5%) and $n=2$, (25%); respectively for group IV]. The lowest incidence of diarrhea with no rectal bleeding was observed in the rats treated with both *L. acidophilus* and olsalazine (group V) [$n=2$, (25%)] (Table 2).

In oxazolone-induced colitis (group II), the disease activity index (DAI) showed significant positive correlation with

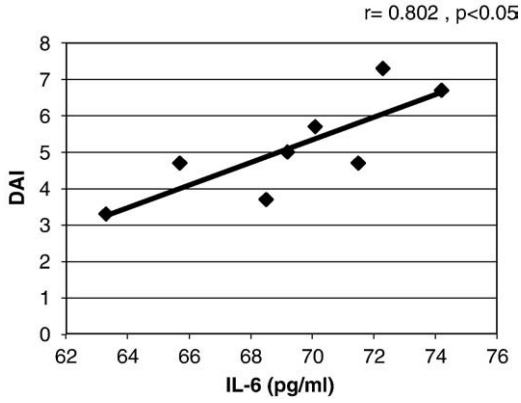

Figure 2 Correlation between DAI and IL-6 in oxazolone-induced colitis (Group II).

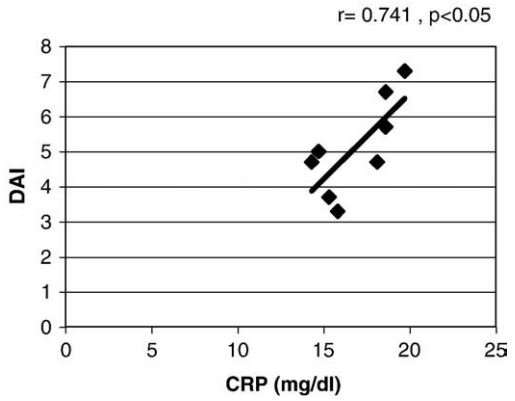

Figure 3 Correlation between DAI and CRP in oxazolone-induced colitis (Group II).

CRP ($r=0.741$, $p<0.05$), TNF-α ($r=.802$, $p<0.05$) and IL-6 ($r=.801$, $p<0.05$) (Figs. 1–3).

4.1. Histopathological examination

Histopathological examination of the studied cases of oxazolone-induced colitis (group II) revealed the presence of inflammatory infiltrate composed of neutrophils, lymphocytes, plasma cells, eosinophils and histiocytes, present in lamina propria invading base of the crypts (Fig. 4B). In advanced cases, crypt abscesses (collections of neutrophils in the glandular lumen) were present (Fig. 4D); lymphoid follicles with germinal centres were also observed. Other histopathological features typical of ulcerative colitis were also present in the form of crypt distortion (Fig. 4C), cytoplasmic mucin depletion (Fig. 4B and C), and nuclear enlargement and increased mitotic activity as an indication of regenerative changes.

In oxazolone-induced colitis with concomitant administration of the probiotic *L. acidophilus* (group III), there was partial remission in the form of partial resolution of the inflammatory infiltrate as well as partial restoration of cytoplasmic mucin and crypt regularity (Fig. 4E), however, residual histological signs of ulcerative colitis were still present compared to group II.

In oxazolone-induced colitis with concomitant administration of olsalazine (group IV), there was better response compared to group III, manifested by greater degree of remission in the form of more regression of the inflammatory infiltrate in addition to more restoration of cytoplasmic mucin and crypt regularity (Fig. 4F), the residual histological signs of ulcerative colitis were less than group III.

In oxazolone-induced colitis with concomitant administration of both the probiotic *L. acidophilus* and olsalazine (group V), the highest response was obtained and the greatest degree of remission was observed compared to both group III and group IV, as the inflammatory infiltrate was scanty and cytoplasmic mucin as well as crypt regularity were almost totally restored (Fig. 4G).

5. Discussion

Although, several methods have been reported to produce experimental models of colitis,[22–24] the current work was conducted on oxazolone-induced colitis (UC) model. The choice of this model in the present work was based on the

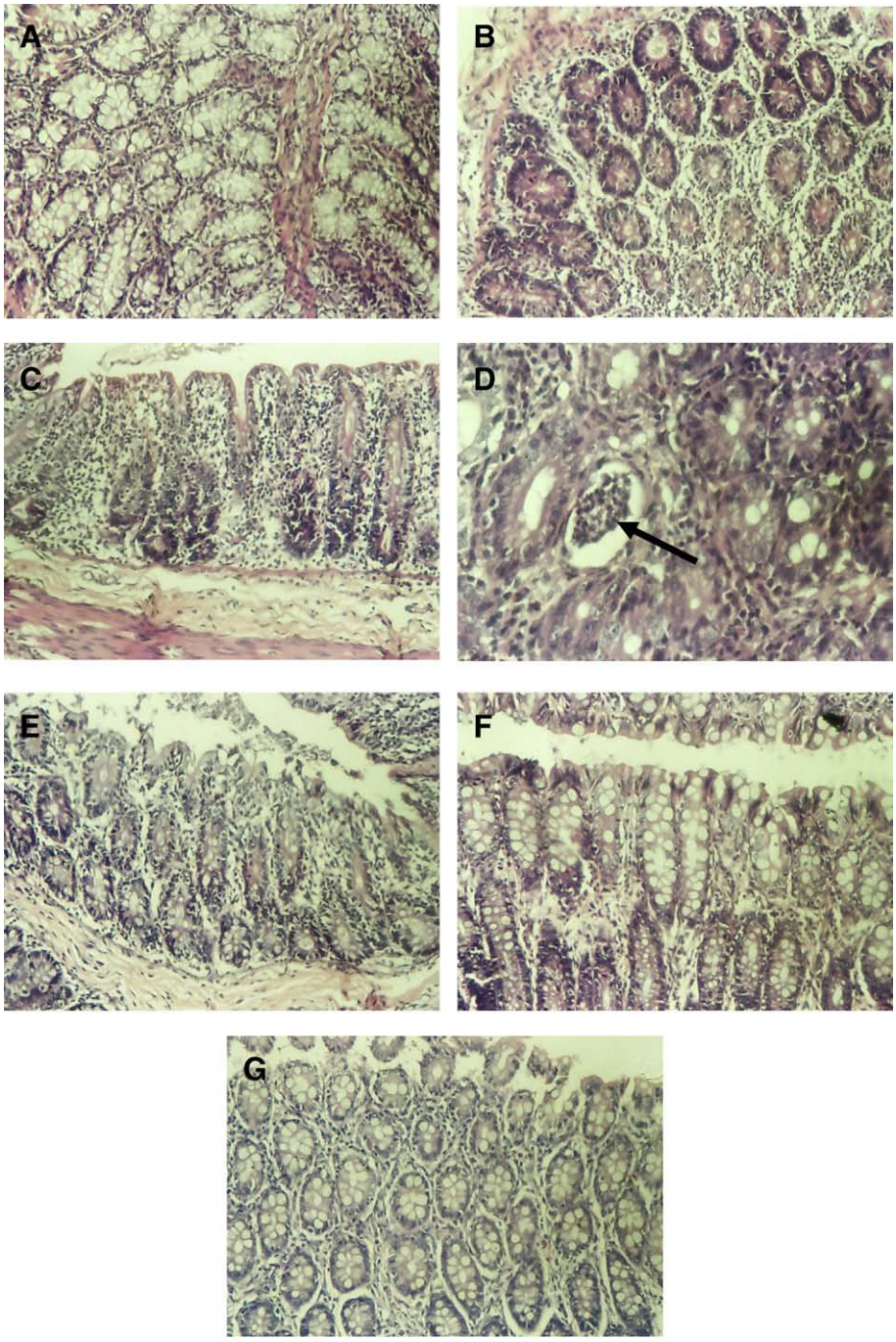

Figure 4 Histologic examination of the colonic sections (H&E) (×200): Group I (control) showing normal colonic glands with abundant mucin-secreting goblet cells, regular uniform glands with no infiltration by inflammatory cells (A). Group II (oxazolone-induced ulcerative colitis) showing increase in number of inflammatory cells in lamina propria, with invasion of the base of crypts and progress towards crypt lumina in an attempt to form crypt abscess. The glands also show mucin depletion (B), marked distortion of the crypts as well as cytoplasmic mucin depletion (C), and crypt abscess (collection of neutrophils in the glandular lumen "arrow" (D) (×400). Group III (ulcerative colitis treated with probiotic *Lactobacillus acidophilus*) showing partial remission in the form of partial resolution of the inflammatory infiltrate as well as partial restoration of cytoplasmic mucin and crypt regularity, however, residual histological signs of ulcerative colitis in the form of cytoplasmic mucin depletion, nuclear enlargement and infiltration by inflammatory cells are still present (E). Group IV (ulcerative colitis treated with olsalazine) showing greater degree of remission with more diminution of the inflammatory infiltrate and more restoration of cytoplasmic mucin and crypt regularity (F). Group V (ulcerative colitis treated with combined therapy probiotic *L. acidophilus* and olsalazine) showing greater degree of remission than each of the drugs alone, there is greater restoration of cytoplasmic mucin and crypt regularity as well as scanty inflammatory infiltrate (G).

previous findings of many authors,[15,25,26] who reported that oxazolone initiates a typical picture of ulcerative colitis mimics that occurs in human. Moreover, histological examination showed morphological similarities between this model and human UC.[26] The histopathological pictures obtained in the present work confirmed such similarity in morphological alterations of the colon. A recent report showed that use of the haptenating agent oxazolone (OXA) to induce colitis promotes production of Th-2 type cytokines, resulting in lesions characterized by Th-2 responses that is known to be involved in the pathogenesis of ulcerative colitis, provides a new way to evaluate the efficacy of new therapeutic agents for ulcerative colitis.[26] It has been established that TNF-α and IL-6 are cytokines related to Th-2 response pattern.[27]

The implication of proinflammatory cytokines in pathogenesis of UC arises from observation that changes in inflammatory mediators at the molecular level could precede gross clinical changes scored by several weeks.[28] The present work evidenced such relationship by the positive correlation between the highly scored disease activity index (DAI) and the elevated serum levels of TNF-α IL-6 and CRP in the rats with untreated ulcerative colitis. Many studies,[29–31] reported that tumor TNF-α and IL-6 have been elevated in inflammatory bowel conditions and proposed to play an integral role in its pathogenesis. It is known that pathogenic bacteria trigger intestinal inflammation by secreting enterotoxins that increase epithelial cell permeability and impairing epithelial cell metabolism resulting in increased uptake of antigens, bacterial products and endotoxins into lamina propria; followed by activation of immune cells; secretion of proinflammatory cytokines as well as products of reactive oxygen metabolites and proteases; and finally mucosal damage occurs.[32] A theory suggested TNF-α production of reactive oxygen species (ROS); ROS in turn activate nuclear factor-kappa B (NF-κB), which then enhances further TNF- α production, propagating a vicious cycle.[4] In addition, apoptosis of intestinal epithelia was found induced by a lot of proinflammatory cytokines such as TNF-α, IL-1ß, and interferon gamma during the process of intestinal inflammation.[33] Also, in ulcerative colitis, IL-6 participates in a variety of critical functions, including Tcell growth and differentiation, as well as B-cell proliferation.[3] Although, Umehara et al.[34] and Goral et al.,[35] investigated the correlation between these cytokine molecules and clinical activity and found that serum levels of IL-6 were significantly high and in proportionately with disease activity, but on the other hand, serum levels of TNF-α were within the normal range in most of cases, but this argued results was not explained by the authors. Nevertheless, this could be contributed to the fact that TNF-α is useful as a means of monitoring disease activity, although it is not specific to UC.[4]

Probiotics have been used in humans for almost a century and widely recommended for the treatment of a variety of ills assumed to be of colonic origin, including diarrhea, constipation, bloating, and flatulence. More recently, probiotics have been evaluated in the management of specific colonic disorders such as inflammatory bowel disease.[36] However, it was established that different probiotic bacteria might be distinct in their immunological effects due to differences in their cell wall structures.[37] In order to further select the strain with the most promising prophylactic or therapeutic effect on intestinal inflammation, it is important to select strains in a relevant in vivo animal model.[38] To achieve this task, probiotic strain should have the following criteria: human origin, nonpathogenic behavior, resistance to technologic processes (i.e., viability and activity in delivery vehicles), resistance to gastric acidity and bile toxicity, adhesion to gut epithelial tissue, ability to persist within the gastrointestinal tract, production of antimicrobial substances, ability to modulate immune responses.[4,13] Bifidobacteria and lactobacilli are commonly used as probiotics commercially.[39] However, lactobacilli (mainly *L. acidophilus*) were reported to be superior on Bifidobacteria in such criteria.[40] So, the *L. acidophilus* was selected in the present work to be given in a live formula, where it caused an improvement in the course of the ulcerative colitis. The live formula is important because it was demonstrated that nonviable bacteria were cleared more rapidly and probiotic bacteria must remain viable in the gut at least 48 to 72 h to be effective; that is the time required for any particulate antigen to induce gut immunostimulation.[41,42] This fact is a very important finding, indicating the importance of daily administration in a dose established for each probiotic bacterium to have an adjuvant effect without induction of oral tolerance.[43] Borruel et al.,[44] emphasized that some bacterial strains (e.g. *Lactobacillus*) down regulate the release of proinflammatory cytokines and induce the apoptosis of activated lymphocytes. In general, the beneficial effects of probiotics was contributed to competition with microbial pathogens for the limited number receptors present on the surface of epithelia, and inhibition of invasion by enteropathogenic bacteria,[45] fermentation of dietary fiber forming short chain fatty acids and other acids products that can induce specific pH,[46] and counteract the apoptosis of intestinal epithelia through a dose-dependent increase of the anti-apoptotic protein Bcl2.[37,47]

Probiotics currently can be administered in dairy yogurts and also in a form of sachets or capsules.[48] It must be noted that dairy fermented products (e.g. yogurt) are declined from use in the present work because the studies evidenced low viability of probiotics in market fermented preparations.[49] Although *L. acidophilus* tolerate acid, a rapid decline in their numbers in yogurt has been observed,[50,51] so it may not be suitable for use as dietary adjuncts in fermented foods.[40] A number of factors have been claimed to affect the viability of probiotic bacteria in yogurt, including acid and hydrogen peroxide produced by yogurt bacteria, oxygen content in the product, and oxygen permeation through the package,[52] concentration of sugars (osmotic pressure), incubation temperature, fermentation time and storage temperature.[53,54] Accumulation of hydrogen peroxide in growth media can occur because lactobacilli do not possess catalase enzyme.[40]

Although administration of olsalazine showed improvement of oxazolone-induced colitis in the present study, but its combination with probiotic *L. acidophilus*, rather caused remarkable amelioration when compared to either probiotic or olsalazine as monotherapy. This improvement was manifested as the lowest incidence of diarrhea and rectal bleeding with marked reduction in DAI and the inflammatory markers. Many other studies emphasized the beneficial effects of addition of probiotic therapy to conventional drugs for ulcerative colitis,[55–58] but none of them conducted on the strain *L. acidophilus* in combination with olsalazine used in the current work. To achieve a satisfactory curative effect

without toxicity, the best therapy for UC is to administer the active 5-aminosalicylic acid (5-ASA) and to reach diseased areas in sufficient concentration.[59] Olsalazine sodium gives a solve to this problem and developed as an alternative to sulphasalazine that is made up of 5-aminosalicylate (5-ASA) and sulfapyridine, to avoid side effects attributable to sulphapyridine moiety.[60] Olsalazine is a prodrug, formed of 2-molecules of 5-ASA jointed by diazo bond. In the current work, olsalazine was administered orally based on the fact that the inflammation in UC extends proximally from the rectum to whole colon in a continuous fashion, involving mucosa and submucosa.[1] So, while rectal rout provides high concentration, but it may be not suitable for treatment of UC because the drug propagates for short distance, at best, up to the splenic flexure only.[61] When it is taken orally, it is poorly absorbed and only a very small amount (absorbing rate < 5%) is absorbed before reaching colon, and so it reaches the colon in a high concentration,[62,63] where it is split by bacterial azoreductase to release two molecules of 5-ASA per unit compared with one 5-ASA per unit for sulphasalazine.[64] In addition, olsalazine was found to be superior on mesalazine which showed systemic absorbing rate 5–6 fold higher than olsalazine, a finding that may have long-term safety implications by reducing the potential risk of nephrotoxicity during long-term maintenance treatment of ulcerative colitis.[65,66] The only frequent side effect of olsalazine is the dose-related diarrhea (affected ~28%), which is contributed to its intact action on small intestine, and seems to be due to an unusual combination of stimulating bicarbonate, chloride, and water secretion and inhibiting absorption.[67] However, the present work did not record such side effect and even showed improvement in incidence of diarrhea and the overall disease activity index (DAI) by olsalazine treatment either when given alone or in combination with *L. acidophilus* probiotic. The present work confirmed the anti-inflammatory action of olsalazine by its significant inhibition of TNF-α, IL-6 and CRP as inflammatory markers. It was reported that 5-ASA is the curative moiety with anti-inflammatory effects locally by blocking production of prostaglandins and leukotrienes, inhibition of chemotaxis, inhibition of NF-κB and scavenging oxygen radicals providing antioxidant activity.[1,4] Despite these advantageous criteria about olsalazine, but there is still considerable rate of relapse (16%) and withdrawal from treatment due to diarrhea (19%) in spite of the relatively good clinical remission recorded in these studies.[67] This could be explained by the fact that microscopic evidence of inflammation often persists despite clinical and sigmoidoscopic remission and may predict relapse.[68] In a recent study, histologic remission was found to be lower than symptomatic remission in ulcerative colitis cases treated with olsalazine.[59] From the results obtained in the present study, it was concluded that *L. acidophilus* probiotic could be recommended as adjuvant therapy in combination with olsalazine to achieve more effective treatment for ulcerative colitis. For application in human, this needs to be verified in further clinical studies.

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