



## The Effects of *Hyphaena thebaica* and *Lactobacillus* in Dextran Sodium Sulphate-Induced Ulcerative Colitis in Rats

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### Abstract

Ulcerative colitis (UC) can cause a variety of complications, ranging from colon damage to colorectal cancer. The cause of UC, as well as successful treatment options, remain a mystery. The mechanisms behind the coloprotective benefits of *Hyphaena thebaica* (*H. thebaica*) and *Lactobacillus*, as well as their combined therapy on dextran sodium sulphate (DSS)-induced colitis in rats, are the subject of this study. The experimental panel included seven groups of rats; normal control, negative plus *H. thebaica*, negative plus *Lactobacillus*, UC diseased rats, *H. thebaica* treated, *Lactobacillus* treated, and combination of *H. thebaica* and *Lactobacillus* treated. Clinical evaluation of UC and both macroscopic and microscopic examination scoring was also done. Colonic oxidants/antioxidant stress biomarkers; malondialdehyde (MDA), glutathione (GSH), catalase, myeloperoxidase activity, and superoxide dismutase (SOD) activity were assessed. Colon Nrf2, HO-1 contents, TNF- $\alpha$  as well as caspase and Bax proteins was evaluated. *H. thebaica* in conjunction with *Lactobacillus*, greatly reduced dextran sodium sulphate-induced colon injury (DSS). In addition, the tested drugs dramatically decreased DSS-induced oxidative, inflammatory, and apoptotic activities.

**Keywords:** Ulcerative colitis ; *Hyphaena thebaica*; *Lactobacillus*; Heme Oxygenase-1 (HO-1); Tumor necrosis factor(TNF- $\alpha$ )

## 1. Introduction

Inflammatory bowel disease (IBD) has become a global health concern as the number of sufferers grows. IBDs include Crohn's disease (CD) and ulcerative colitis (UC). UC is a chronic inflammatory disease that affects men and women in their third decade of life and is characterized by colon mucosal inflammation. One theory for the pathophysiology of IBDs, particularly UC, is that a persistent inflammatory response is caused by an imbalance in the gut microbiota (El-Baz et al., 2020; Hao et al., 2014).

Various pro-inflammatory cytokines like interleukin-1 (IL-1) and IL-18 are formed in the mucosal immune response. In an uninfected human gut, there is a bulk of bacteria found like Bacteroidetes, Actinobacteria, Firmicutes, and Proteobacteria (Saber et al., 2021). Physiological changes in the colon mucosa, which signal gut bacteria metabolic activity, have an impact on gut health and immunity. The gut microbiota is a homeostatic organ that decreases harmful bacteria while also preserving the intestinal mucosa's integrity (Fooladi et al., 2015).

Probiotic species that can alter the gut microbiota are included in a variety of pharmacological regimens for the treatment of human and animal diseases. Probiotics are living commensal bacteria with anticarcinogenic and immune-modulating capabilities that boost human immunity (Fooladi et al., 2015; Khalaf & Raizada, 2020). Other lactic acid bacteria (LAB) can impact immune responses if fed in sufficient proportions (El-Baz et al., 2021; Fooladi et al., 2015).

Doum is an Egyptian traditional beverage strong in polyphenolic chemicals. According to various studies, Doum fruit extracts have high levels of flavonoid and phenolic components, which function as antioxidants and antibacterials, lowering negative oxidative stress and preventing illness caused by pathogenic microorganisms. The ability of plant phenolics to scavenge reactive radicals is well established (Seleem & Sciences, 2015).

Sulfasalazine and glucocorticoids are two medications often used to treat IBD. Antibiotics, monoclonal antibodies, and immunosuppressants are often used in cases of severe illness. These medications have negative side effects and are ineffective in treating IBD patients (Volk & Siegel, 2018) (Privitera et al., 2021). Antioxidants have been proven in multiple studies to be useful in the treatment of acute colitis. Some herbal treatments have been proven to be beneficial in a number of animal models, with antioxidant activity being the primary cause. Anti-inflammatory and antioxidant capabilities have been linked to *H. thebaica* (Allam, 2007).

Doum flour extract has been shown to lower the weight and volume of tumour components in previous studies (Bello et al., 2017).

This could be due to the presence of antiproliferative polyphenols, coumarins, and saponins in doum extract. Another aspect of protection by *H. thebaica* extends to renal tissue. In rats, doum flour treatment reduces reactive oxygen species and renal interstitial fibrosis (Sobhy Fahmy Abd Elfatah, 2021).

Adding doum flour to the diet can improve the symptoms of renal failure by acting as an anti-inflammatory. Mice were administered cyclosporine and supplemented with doum flour, their anti-inflammatory state improved significantly, compared to animals given only cyclosporine (Aboshora et al., 2016).

Doum's anti-inflammatory qualities are assumed to be due to the saponin component, which protects against reactive oxygen species and lowers serum transforming growth factor- $\beta$ 1 synthesis.

Other studies have linked the considerable increase in white blood cells produced by *H. thebaica* to the stimulation of bone marrow stem cells. Flavanols increase leucocytic production by preventing oxygen reactive species from damaging both committed and produced hematopoietic bone marrow. Polyphenol conjugates, oxygenated triglycerides, and sphingolipids contained in flour may help *H. thebaica*'s anti-inflammatory effect. (Al-Khalaifah et al., 2020).

The goal of this study is to assess the role of *Lactobacillus* and *H. thebaica* as promising supplements in an ulcerative colitis model induced by dextran sodium sulphate, as well as to understand the underlying mechanisms of protection.

## 2. Material and methods

### 2.1. Experimental design

All experimental techniques were authorized by our local Animal Care Committee at Delta University in Egypt. The experiments were conducted using the Laboratory Animals Guide, which was published by the National Institutes of Health in the United States. The Vaccine and Immunization Authority (Helwan, Cairo, Egypt) supplied 60 male Sprague–Dawley rats weighing 170–200 g, which were kept in a controlled environment (22°C, 12 h light/12 h dark cycle). All animal procedures were approved by the Ethical Committee of the Faculty of Pharmacy Delta University for Science and Technology (FPDU14/2022), which is in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals had unlimited access to food and water. After a week of acclimation, rats were randomly separated into seven groups, each with eight rats:

1. Negative group (negative)
2. Negative plus *H. thebaica* (negative + HT)
3. Negative plus Lactobacillus (negative + Lacto)
4. Dextran sodium sulphate group (ulcerative colitis), 4% DSS in drinking water from 7<sup>th</sup> day to 13<sup>th</sup> day
5. Ulcerative colitis plus *H. thebaica* 7 days before induction and continue for 2 weeks (ulcerative + HT)
6. Ulcerative colitis plus lactobacillus 7 days before induction and continue for 2 weeks (ulcerative colitis+ Lacto)
7. Ulcerative colitis plus combination (Ulcerative colitis + Combinations)

## 2.2. Induction of colitis

Acute colitis was produced in rats given ad libitum access to 4 % w/v DSS (MW 30–40 kDa) in pathogen-free water. Seven days later, DSS administration was halted. The rats were euthanized on the 15th day following induction (Martin et al., 2016).

## 2.3. Preparation of HT

The root was donated by Mansoura University's plant department, faculty of agriculture. The root of the plant was sun dried for 6 hours before being crushed into a fine powder and sieved (Hossam et al., 2018). Every day, 5 gram of powder was dissolved in 100 ml of distilled water and stored at room temperature.

## 2.4. Microorganism and feeding procedure

*Lactobacillus delbrueckii* and *Lactobacillus fermentum* were donated by Rameda Pharma Co. in Giza, Egypt, as 10 billion microbe cells per sachet. The suspension was prepared by dissolving sachets in drinking water and concentration was adjusted to  $2.7 \times 10^8$  CFU/mL where each rat in probiotic group received 0.5ml administered through stomach tube. A similar volume of PBS was administered to rats in the control group (Garcia-Castillo et al., 2019)

## 2.5. Disease activity index and Macroscopic damage index

The disease activity index (DAI), which assessed a percent body weight loss calculated as the difference between starting weight at disease induction point and weight at the end of study relative to the starting weight, stool consistency, and extensive bleeding, was used to determine disease intensity. Each parameter was graded as follows: diarrhea (0, normal; 1 and 2, loose stools; 3 and 4, watery diarrhea); percentage body weight loss (0, none; 1, 1–5 %; 2, 6–10 %; 3, 11–20 %; and 4, >20 %); and bloody stool (0, none; 1, 1–5 %; 2, 6–10 %; 3, 11–20 %; and 4, >20 %). The Macroscopic Damage Index (MDI) was determined. In a nutshell, MDI is the sum of each animal's scores. This approach was developed using a single-blinded visual assessment of intestinal injury. The MDI scoring criteria for colonic macroscopic damage were based on a random scale ranging from 0 to 4 points. The following were the scoring criteria: No macroscopic signs, 0; mucosal erythema alone, 1; mild mucosal edema with minor mucosal bleeding or erosions, 2; moderate mucosal edema with substantial mucosal bleeding or erosions, 3; severe edema and tissue necrosis, 4. (Chen et al., 2007)

## 2.6. Preparation of colonic homogenate

According to Buege and Aust (1978) a variable speed homogenizer was used to homogenize 10 % w/v colon homogenate in ice-cold KCl (1.15 %, pH 7.4). (According to OMNI International).

After that, the homogenate was centrifuged at 4 °C for 30 min at 4000 rpm. The supernatants were separated and employed in biochemical calculations.

### **2.7. Assessment of superoxide dismutase (SOD), reduced glutathione (GSH) concentration, catalase activity, activity and total antioxidant capacity (TAC)**

Nishikimi et al. (1972) approach was used to assess SOD activity, while the Beutler et al. (1963) method was used to measure GSH concentration. Aebi (1984) demonstrated how to measure catalase activity and TAC in the colon.

### **Assessment of serum myeloperoxidase (MPO) and C-reactive protein (CRP)**

According to the manufacturer's instructions, CRP-Latex was used to measure C-reactive protein levels (Chemelex, Spain). Krawisz et al. (1984) explained how to check for colonic myeloperoxidase. All tests were carried out using a commercially available kit, following the manufacturer's instructions (Biodiagnostic, Giza, Egypt).

### **2.8. Estimation of colon Nuclear Factor, Erythroid Derived 2 Like Protein 2 (Nrf2) Heme Oxygenase-1 (HO-1) contents**

According to the manufacturer's instructions, ELISA assay kits (Cloud-Clone Co., Houston, USA) were used to detect Nrf2 content and HO-1 activity.

### **2.9. Assessment of TNF- $\alpha$ and IL-1 $\beta$**

TNF- $\alpha$  and IL-1 ELISA test kits were used to assess the inflammatory cytokine content of the colon (eBioscience Inc., San Diego, CA, USA).

### **2.10. Assessment of Caspase and BAX**

Caspase-3 and Bax proteins expression, the pro-apoptotic markers were evaluated in colon tissue homogenates according to the manufacturers' instruction, using ELISA kits purchased from (BioVision Inc., Catalog #E4592 Milpitas, USA), (Cusabio, Cat. # CSB-EL002573RA, Houston, USA), respectively.

### **2.11. Histopathological examination**

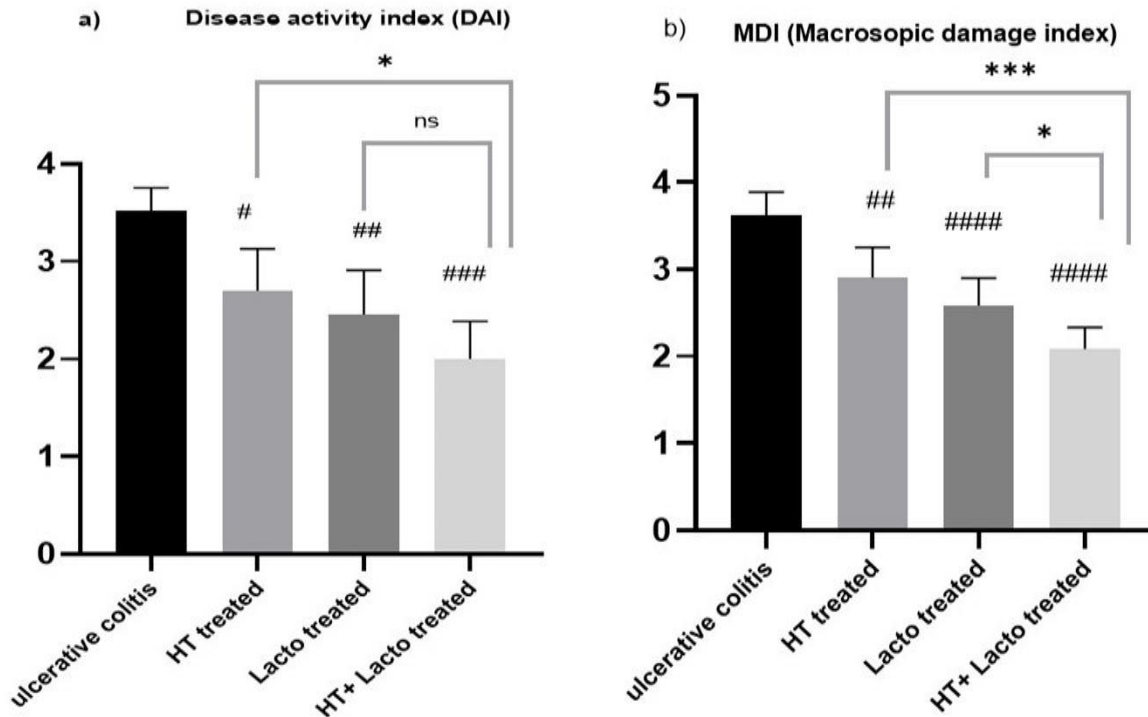
An appropriate part of the lower colon was excised and treated by soaking it in a 10% neutral buffered formalin solution (pH 7.4). The slice was then transversely sliced, paraffin embedded, and stained with hematoxylin and eosin (Hx&E) for microscopic evaluation of DSS-induced colon damage, particularly to the mucosal and submucosal layers, as well as responsiveness to Lactobacillus and THtreatment. A histopathologist who was uninformed of the experimental groups examined the tissues under the microscope in a random order.

### **2.12. Statistics**

The results are stated as Mean  $\pm$  SE. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer's test for parametric data, and Kruskal-Wallis test followed by a Dunn's tests for non-parametric scoring.  $p < 0.05$  was recognized significant. Data analysis was conducted using Graph prism version 8.

## **Results**

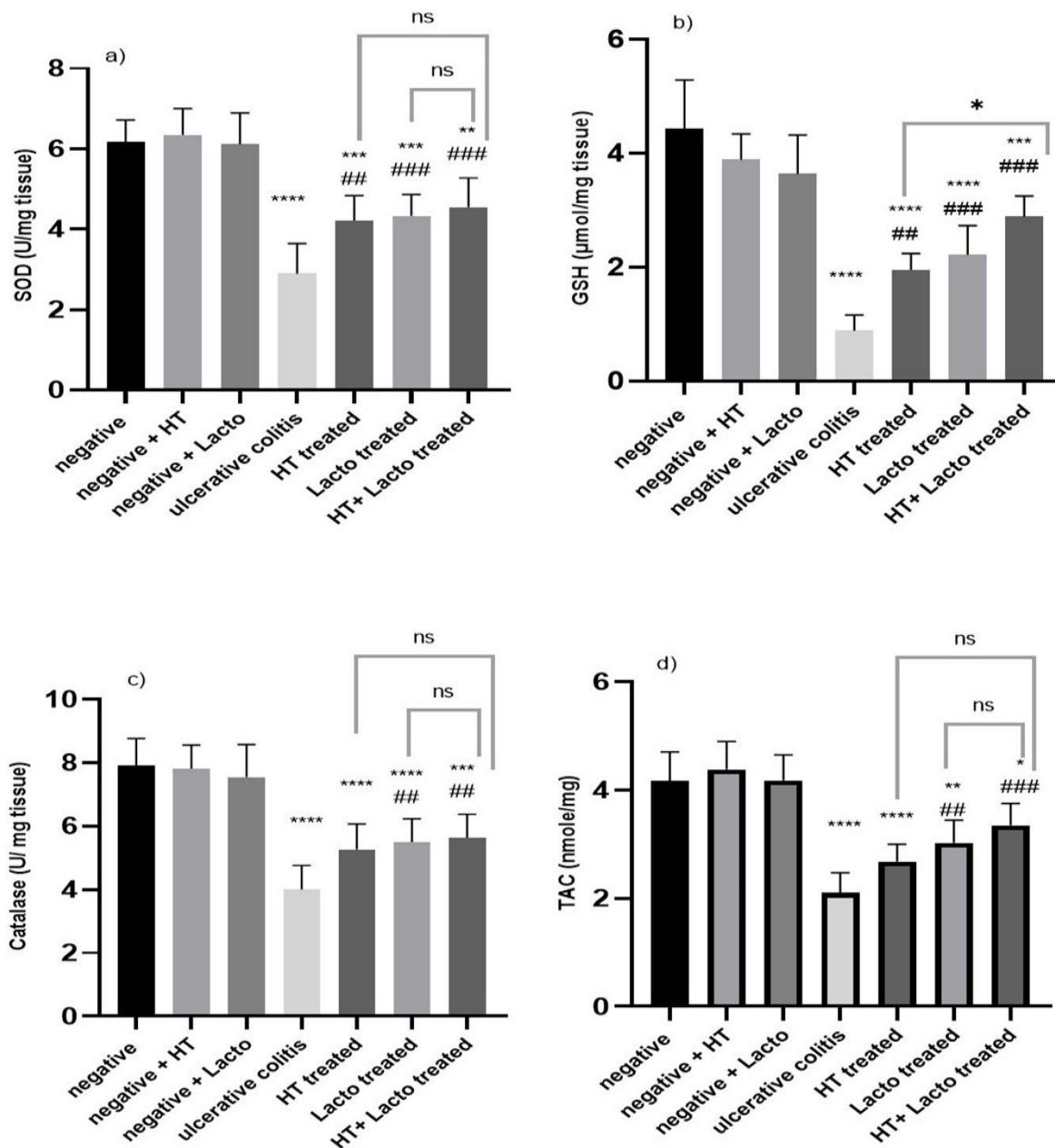
As shown in Fig. 1-a, DAI improved dramatically in the HT treated group, Lacto treated group, and combination therapy group. There is also a significant improvement when comparing combination therapy to HT-treated gp. In addition, as seen in Fig.1-b, all treated groups improved significantly in MDI. When compared to solo therapy, combo therapy improves significantly.



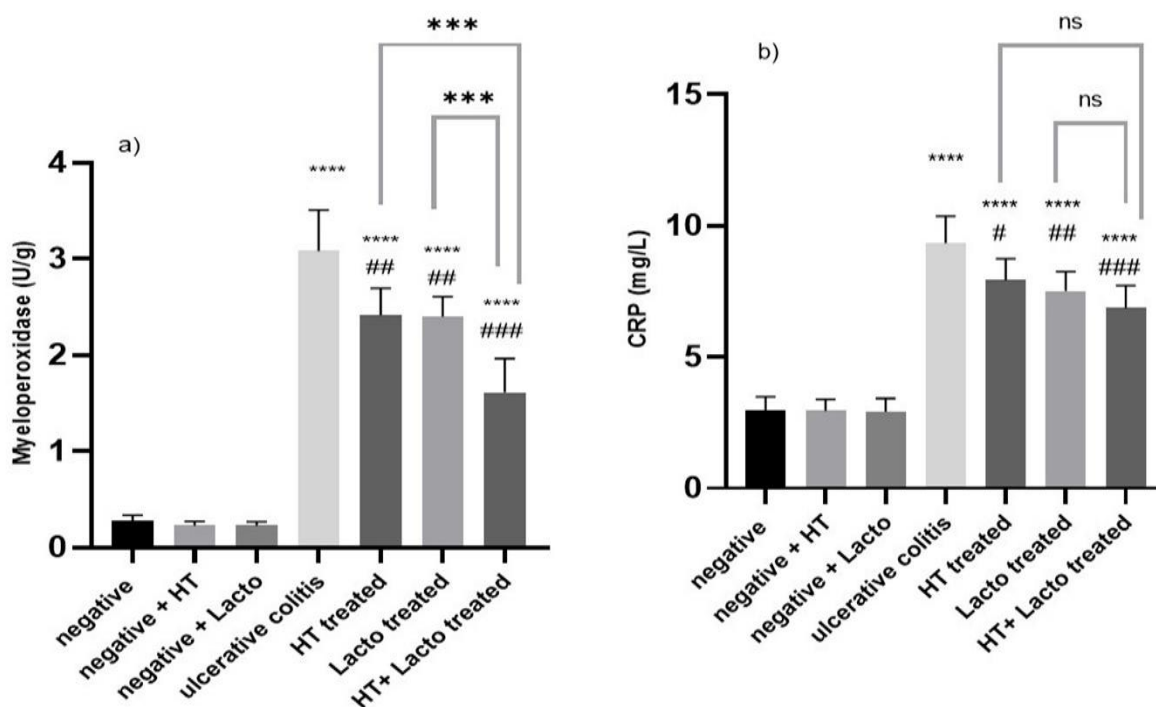
**Fig. 1 Effect of *Lactobacillus* and *Hyphaena thebaica* on: (a) DAI, (b) MDI. Ulcerative colitis:** positive diseased group; **HT treated:** Ulcerative colitis treated with HT extract; **Lacto treated:** Ulcerative colitis treated with *Lactobacillus*; and **HT+Lacto treated:** Ulcerative colitis treated with HT extract + *Lactobacillus*. # ( $p < 0.05$ ) vs positive group, ## ( $p < 0.01$ ) vs positive group, ### ( $p < 0.001$ ) vs positive group, #### ( $p < 0.0001$ ) vs positive group. \* ( $p < 0.05$ ) vs negative group, \*\* ( $p < 0.01$ ) vs negative group, \*\*\* ( $p < 0.001$ ) vs negative group, \*\*\*\* ( $p < 0.0001$ ) vs negative group.

SOD and GSH levels in the UC group are much lower than in the negative group, as seen in Fig. 2.a, b. Both metrics demonstrate a significant improvement in ALL of the treated groups. Only the lacto and combination treatments improved catalase and TAC significantly, as shown in Fig.2 c, d. Combination therapy exhibited no advantage over solo therapy in all of the aforementioned criteria except GSH.

The UC group had a considerable increase in myeloperoxidase and CRP, which is greatly reduced in all treatment groups (Fig.3-a,b). Furthermore, when compared to single medication, combination therapy reduces myeloperoxidase significantly, while this effect is not shown with CRP.



**Fig. 2** Effect of *Lactobacillus* and *Hyphaena thebaica* on oxidative stress markers: (a) SOD (U/mg tissue), (b) GSH (μmol/mg tissue), (c) Catalase (U/mg tissue), and (d) TAC (nmol/mg tissue). **Negative:** Control group; **Negative+HT:** Control+HT; **Negative+Lacto:** Control+*Lactobacillus*; **Control + Lactobacillus;** **Ulcerative colitis:** positive diseased group; **HT treated:** Ulcerative colitis treated with HT extract; **Lacto treated:** Ulcerative colitis treated with *Lactobacillus*; and **HT+Lacto treated:** Ulcerative colitis treated with HT extract + *Lactobacillus*. # ( $p < 0.05$ ) vs positive group, ## ( $p < 0.01$ ) vs positive group, ### ( $p < 0.001$ ) vs positive group, #### ( $p < 0.0001$ ) vs positive group. \* ( $p < 0.05$ ) vs negative group, \*\* ( $p < 0.01$ ) vs negative group, \*\*\* ( $p < 0.001$ ) vs negative group, \*\*\*\* ( $p < 0.0001$ ) vs negative group.

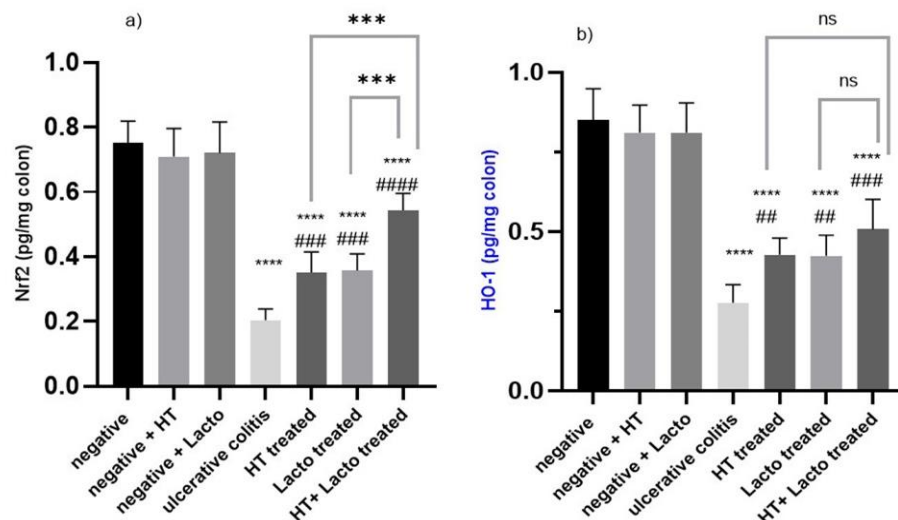


**Fig. 3 Effect of *Lactobacillus* and *Hyphaena thebaica* on: (a) myeloperoxidase (MPO) (U/gm tissue), (b) CRP (mg/L). Negative:** Control group; **Negative+HT:** Control+HT; **Negative+Lacto:** Control+*Lactobacillus*; Control + *Lactobacillus*; **Ulcerative colitis:** positive diseased group; **HT treated:** Ulcerative colitis treated with HT extract; **Lacto treated:** Ulcerative colitis treated with *Lactobacillus*; and **HT+Lacto treated:** Ulcerative colitis treated with HT extract + *Lactobacillus*. # ( $p < 0.05$ ) vs positive group, ##( $p < 0.01$ ) vs positive group, ### ( $p < 0.001$ ) vs positive group, #### ( $p < 0.0001$ ) vs positive group. \* ( $p < 0.05$ ) vs negative group, \*\*( $p < 0.01$ ) vs negative group, \*\*\* ( $p < 0.001$ ) vs negative group, \*\*\*\* ( $p < 0.0001$ ) vs negative group. \* ( $p < 0.05$ ) vs HT+ Lacto group, \*\*\*( $p < 0.001$ ) vs HT+ Lacto group

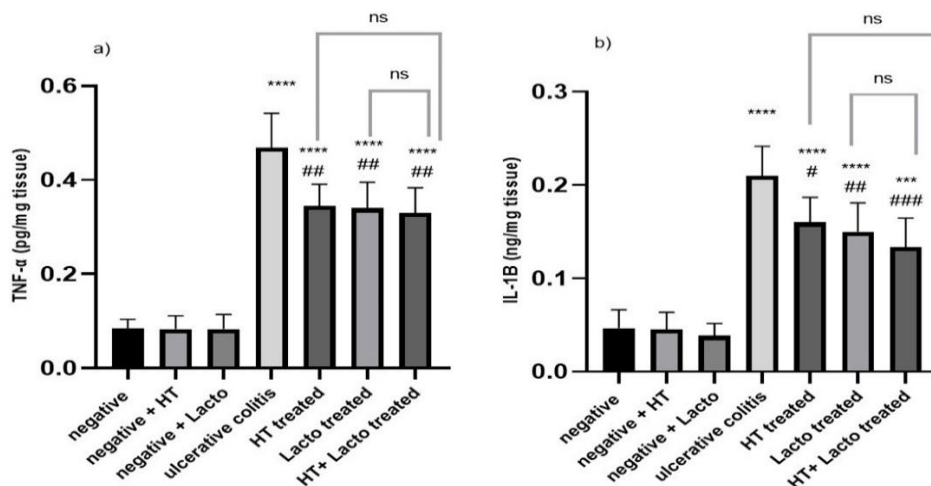
All treated groups exhibit significant improvements in Nrf2 and HO-1 as compared to the UC group (Fig.4-a,b). Furthermore, as demonstrated in Fig. 4-a, as compared to solo therapy, combination therapy had a significant effect on Nrf2.

TNF- and IL-1 $\beta$  levels in the UC group are significantly higher, as seen in Fig.5a,b. Both values were significantly lower in all treatment groups, however combination therapy had no effect as compared to solo therapy.

As seen in Fig. 6-a,b, the UC group had significantly higher levels of caspase-3 and BAX. The Lacto group and combined treatment dramatically reduced both levels, whereas the HT group had no effect. When compared to solo therapy, combined therapy has a noticeable effect on caspase-3 and Bax in the same scenario.

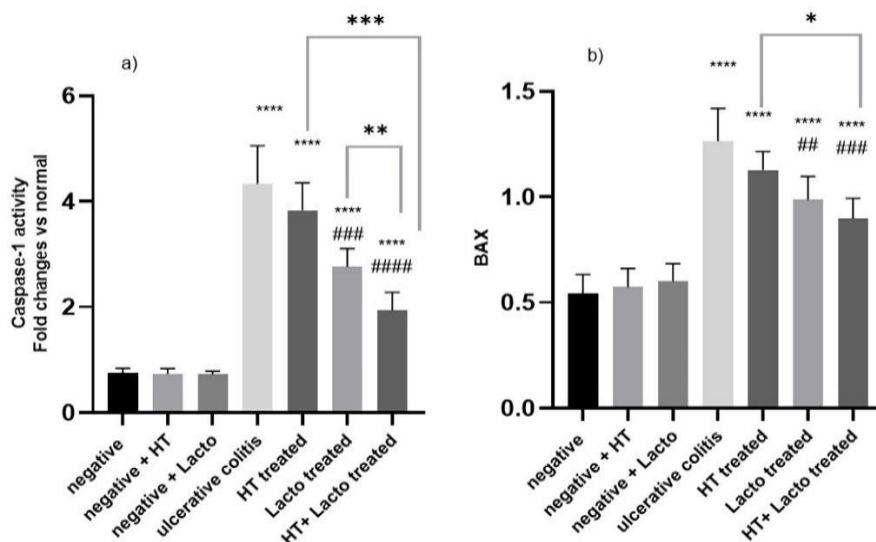


**Fig. 4 Effect of *Lactobacillus* and *Hyphaena thebaica* on: (a) Nrf2 (pg/mg colon), (b) HO-1 (pg/mg colon).** **Negative:** Control group; **Negative+HT:** Control+HT; **Negative+Lacto:** Control+Lactobacillus; **Control + Lactobacillus:** positive diseased group; **HT treated:** Ulcerative colitis treated with HT extract; **Lacto treated:** Ulcerative colitis treated with Lactobacillus; and **HT+Lacto treated:** Ulcerative colitis treated with HT extract + Lactobacillus. # (p<0.05) vs positive group, ##(p<0.01) vs positive group, ### (p<0.001) vs positive group, #### (p<0.0001) vs positive group. \* (p<0.05) vs negative group, \*\* (p<0.01) vs negative group, \*\*\* (p<0.001) vs negative group, \*\*\*\* (p<0.0001) vs negative group.



**Fig. 5 Effect of *Lactobacillus* and *Hyphaena thebaica* on: (a) TNF-α (pg/mg tissue), (b) IL-1β (ng/mg tissue).** **Negative:** Control group; **Negative+HT:** Control+HT; **Negative+Lacto:** Control+Lactobacillus; **Control + Lactobacillus:** positive diseased group; **HT treated:** Ulcerative colitis treated with HT extract; **Lacto treated:** Ulcerative colitis treated with Lactobacillus; and **HT+Lacto treated:** Ulcerative colitis treated with HT extract + Lactobacillus. # (p<0.05) vs positive group, ##(p<0.01) vs positive group, ### (p<0.001) vs positive group, #### (p<0.0001) vs positive group. \* (p<0.05) vs negative group, \*\* (p<0.01) vs negative group, \*\*\* (p<0.001) vs negative group, \*\*\*\* (p<0.0001) vs negative group.





**Fig. 6 Effect of *Lactobacillus* and *Hyphaena thebaica* on: (a) Caspase-1, (b) Bax. Negative:** Control group; **Negative+HT:** Control+HT; **Negative+Lacto:** Control+*Lactobacillus*; **Control + *Lactobacillus*:** Control + *Lactobacillus*; **Ulcerative colitis:** positive diseased group; **HT treated:** Ulcerative colitis treated with HT extract; **Lacto treated:** Ulcerative colitis treated with *Lactobacillus*; and **HT+Lacto treated:** Ulcerative colitis treated with HT extract + *Lactobacillus*. # ( $p < 0.05$ ) vs positive group, ##( $p < 0.01$ ) vs positive group, ### ( $p < 0.001$ ) vs positive group, #### ( $p < 0.0001$ ) vs positive group. \* ( $p < 0.05$ ) vs negative group, \*\*( $p < 0.01$ ) vs negative group, \*\*\* ( $p < 0.001$ ) vs negative group, \*\*\*\* ( $p < 0.0001$ ) vs negative group.

## Discussion

Ulcerative colitis, an inflammatory colon disease, is diagnosed in 10 to 20 people per 100,000 each year. Although a small fraction of ulcerative colitis patients do not respond to first- or second-line treatment, the vast majority of patients can be put into remission. As a result of contemporary medications, a large number of people are experiencing negative side effects. As a result, researchers are constantly looking into new ulcerative colitis therapy alternatives. Humans have been found to benefit from probiotics in terms of gut microbial balance, gut barrier function, and local immunological response.

In the current study, *Lactobacillus* and *H. thebaica* significantly reduced oxidative stress and inflammatory indicators. They considerably lower myeloperoxidase as well. People with more severe IBD exhibit a stronger expression of MPO. MPO levels show the sort of reaction when assessing a treatment's efficacy. It might be a useful diagnostic and predictive tool for those with IBD. (Hansberry et al., 2017). High levels of flavonoids, including luteolin, which prevents lipid oxidation by scavenging free radicals or by other mechanisms like singlet oxygen quenching, metal chelation, and lipoxygenase inhibition, were found to be responsible for these effects. (Saravanan & Leelavinothan, 2006; Wang et al., 2011). Another experiment found that the aqueous extract of *H. thebaica* showed antioxidant properties due to its high water-soluble phenolic content (Hsu et al., 2006).

Five flavone glycosides were isolated and found in *H. thebaica* to shed light on the most likely mechanism underlying the plant's hepatoprotective properties, which can also extend to other organs like the intestinal wall in UC patients. (Aremu & Fadele, 2011; Mohamed et al., 2009).

*H. thebaica* has antioxidant capabilities that may make pretreatment beneficial for the gut wall's ability to fend off DSS's harmful effects. The presence of flavonoids, coumarins, and saponins in *H. thebaica* was confirmed by phytochemical investigation (Eldahshan et al., 2009; Shalaby & Shatta, 2013).

One of the phenolic acids from *H. thebaica*, coumaric acid, may prevent the oxidation of lipids in the liver by scavenging free radicals generated by  $\text{HgCl}_2$ . Flavonoids, which are phenolic compounds found in plants, prevent lipid oxidation (Necib et al., 2013).

The lamina propria is protected from other luminal bacteria by the barrier formed by probiotics, which also strengthens the mucosal immune system. Additionally, probiotics can alter mucus production and consistency, resulting in a thicker mucus layer that defends against invasive microorganisms (Okumura & Takeda, 2016). They trigger the production of defense-enhancing immunoglobulins (Ig), including secretory IgA, as well as a number of defense-enhancing defensins and bacteriocins into the lumen of the intestine (Monteagudo-Mera et al., 2019).

Probiotics can activate dendritic cells, reducing their receptivity and inflammatory response to bacteria in the lumen and increasing the anti-inflammatory and decreasing the pro-inflammatory properties of the mucosal immune system. This latter pathway seems to be particularly important in ulcerative colitis (Zhu et al., 2022). Through a variety of mechanisms, probiotics can lessen the contribution of luminal bacteria to the initiation and maintenance of a gut inflammatory response. Probiotics can induce dendritic cells to become slightly less reactive and receptive to bacteria in the lumen, increasing the mucosal immune system's anti-inflammatory and decreasing its pro-inflammatory properties. (Wang et al., 2021).

This pathway appears to be quite important in ulcerative colitis. Probiotics diminish luminal bacteria's role in the establishment and maintenance of a gut inflammatory response through a number of different techniques. The antioxidant activity of *H. thebaica* is likewise quite high, triggering the system of enzymes that scavenges free radicals (Al-Masri et al., 2012).

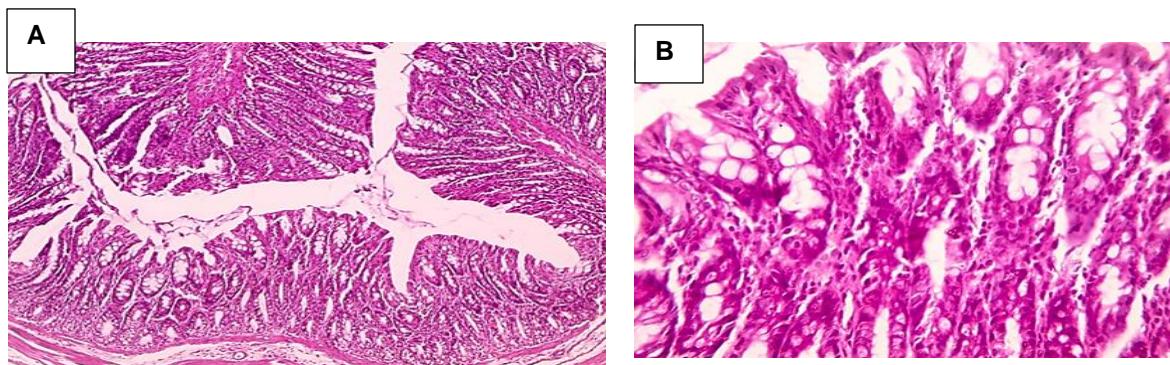
The combination of lacto and *H. thebaica* treatment dramatically improved apoptotic markers in the current investigation. *B. bifidum* raised the expression of TLR-2, COX-2, and PGE2, while decreasing apoptosis in the intestinal epithelium. (Islam et al., 2011). Sphingolipids, oxygenated triglycerides, and polyphenol conjugates are thought to contribute to the anti-inflammatory effects of *H. thebaica*. Also, the abundant GSH present in *H. thebaica* reacts with toxic substances which are associated to progression, the antioxidant potential of *H. thebaica* has been reported in different literatures and it's mainly associated with significant modulation GSH and MDA levels (Abdel Rahman et al., 2022) (Farrag et al., 2020)

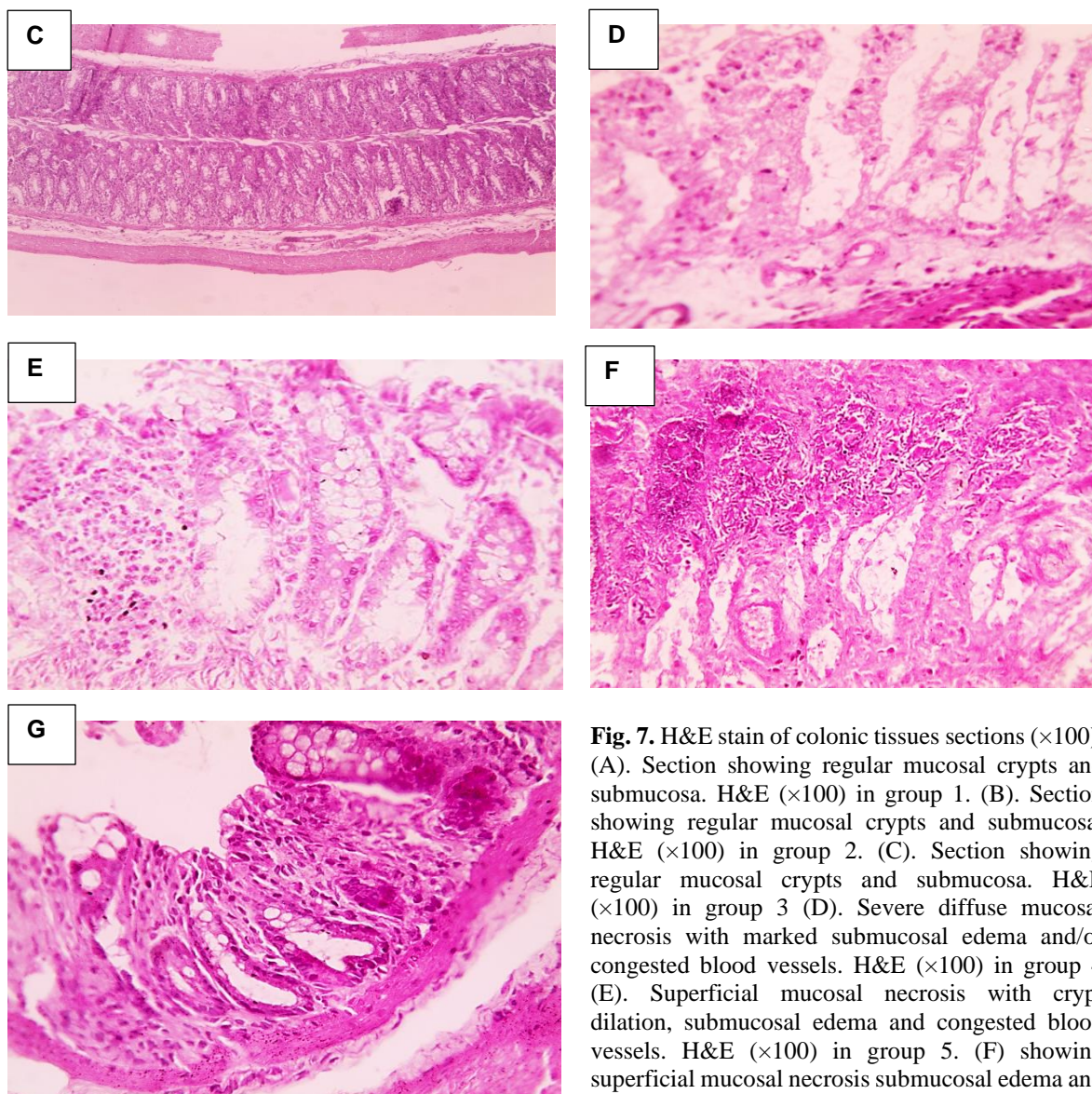
### Conclusion

*H. thebaica* in conjunction with *Lactobacillus*, greatly reduced dextran sodium sulphate-induced colon injury (DSS). In addition, the tested drugs dramatically decreased DSS-induced oxidative, inflammatory, and apoptotic activities.

### Disclosure

The author reports no conflicts of interest in this work.





**Fig. 7.** H&E stain of colonic tissues sections ( $\times 100$ ). (A). Section showing regular mucosal crypts and submucosa. H&E ( $\times 100$ ) in group 1. (B). Section showing regular mucosal crypts and submucosa. H&E ( $\times 100$ ) in group 2. (C). Section showing regular mucosal crypts and submucosa. H&E ( $\times 100$ ) in group 3 (D). Severe diffuse mucosal necrosis with marked submucosal edema and/or congested blood vessels. H&E ( $\times 100$ ) in group 4 (E). Superficial mucosal necrosis with crypt dilation, submucosal edema and congested blood vessels. H&E ( $\times 100$ ) in group 5. (F) showing superficial mucosal necrosis submucosal edema and congested blood vessels. H&E ( $\times 100$ ) in group 6. (G) focal areas of mucosal necrosis, crypt dilation with submucosal edema and congested blood vessels. H&E ( $\times 100$ ) in group 7.

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