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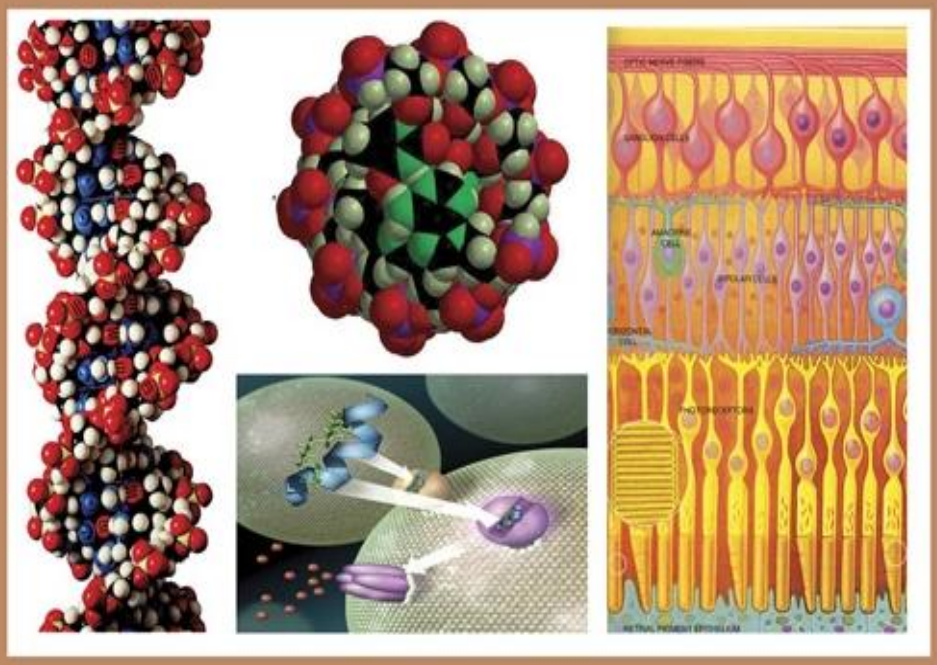

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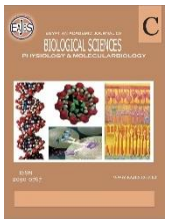

The Impact of Flaxseed *(Linum usitatissimum L.)* and Psyllium Seed *(Plantago Ovata P.)* Oils on Hemogram, Oxidative Stress and Inflammation in Ulcerative Colitis Rat Model

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ABSTRACT

Ulcerative colitis (UC) is one common type of inflammatory bowel disease characterized by prolonged inflammatory conditions. Plant oils are thought to offer an alternative to pharmaceutical medications for diseases such as UC. This study aimed to investigate the curative effects of flaxseed and psyllium seed oils on the UC model induced by 70% ethanol intrarectal injection (0.5 mL/100g body weight). Animals were separated into 4 groups; the control group received saline and the experimental groups received (500 mg kg^{-1}b.wt.) of flaxseed and psyllium seed oils. Therapeutic effects were determined by measuring hematological parameters; (Red blood cells (RBCs), white blood cells (WBCs) (total and differential) and platelets counts, hemoglobin (Hb) concentration, hematocrit (packed cells volume) PCV, erythrocytes indices), colonic oxidative stress parameters; malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT) and myeloperoxidase (MPO), as well as the colonic inflammatory parameters; interleukin-1β (IL-1β), interleukin-10 (IL-10), prostaglandin I2 **(**PGI2) and leukotriene B4 (LTB4) in addition to, histological examinations of colon tissues. The results of the current investigation demonstrated the antiulcer properties of tested oils; FSO and PSO. Tested oils improved oxidative stress and inflammatory indicators in colon tissues, hematological parameters, and modified colon structural and histological changes. Oils from psyllium and flaxseed may be utilized to treat acute UC induced by alcohol.

INTRODUCTION

Ulcerative colitis (UC) is a type of chronic inflammatory bowel disease (IBD) characterized by abdominal discomfort, difficulty defecation and bloody looseness, which can enormously reduce the quality of life (Segal *et al.*, 2021). UC if neglected can also increase the danger of colorectal cancer and results in the morbidity and death associated with this disease (Rose and Strombom, 2020).

Consuming alcohol and alcoholic beverages increase susceptibility for colonic hemorrhage and have a positive correlation with gastrointestinal ulceration such as peptic ulcer and ulcerative colitis (Boligon *et al.*, 2014). This is explained by the proposition that alcohol modifies the microflora profile and induced intestinal inflammation (Ramos and Kane, 2021).

Many of the serious complications of UC include massive bleeding that may cause anemia due to blood loss (Ramos and Papadakis, 2019). In addition to decreased red blood cell count, all blood picture strictures have been affected such as hemoglobin concentration, packed cell volume or hematocrit value, platelets count and white blood cell count either total or differential count (Adedara *et al.*,2017).

Adrenal cortical steroids, salicylates, immunosuppressants, and anti-TNF-α agents are common ingredients in conventional ulcerative colitis medications, which frequently have serious adverse effects like a nuisance, nausea, vomiting, malaise, abdominal discomfort, joints pain, and infection when taken frequently and in large doses. (Lopez *et al.,* 2018). Therefore, active dietary complements with no or fewer adverse effects are upright contestants as lines for treatment and even prevention of UC. Plant diets that depend mainly on plant-origin foods can significantly reduce the danger or deterioration in UC patients (Moayyedi *et al.,* 2015).

Plant-based diet ensures a greater amount of butyrate and other SCFAs, as well as provides phytochemicals that have anti-inflammatory and antioxidant properties (Li *et al.,* 2015). Additionally, a plant-based diet provides a more favorable microbiome profile which contributes to UC treatment (Li *et al.,* 2019). From the dietary aspects, that may affect UC disease dietary fats which are important modulators of the IBD risk. Plant natural oils have important pharmacologic, biotechnological and food manufacturing requests; they are used as food flavoring and stabilizers in many food products (Kuna *et al.,* 2019).

The flaxseed is also known as *(Linum usitatissimum L.)* which is belonging to *Linaceae*. Flaxseed oil is widely utilized for baked foods such as bread, cookies, cakes and others (Morshedzadeh *et al., 2019*). Flaxseed oil is a rich source of α-linolenic acid which was reported to have a lowering effect on macrophages and monocytes-induced inflammatory cytokines in addition to, its improving effect on blood lipid profile by inhibition of cholesterol synthesis and activating hepatic cholesterol excretion (Reifen *et al.,* 2015). Also, flaxseed oil improves both constipation and diarrhea (Palla *et al., 2016*). Administration of flaxseed oil revealed colon mucosal and submucosal protecting effects with improving colon structural and histological changes in ethanol induced-colitis mice (Palla and Gilani, 2015).

Psyllium seed (*Plantago ovata*) is belonging to the family *Plantaginaceae.* Psyllium seed oil was reported to improve UC symptoms such as diarrhea, constipation, incomplete evacuation, and bloating (Amer *et al.,* 2019). Psyllium also has hypocholesterolemia effects, by doubling the activity of two enzymes that contributed to plasma cholesterol clearing; cholesterol 7α-hydroxylase and HMG-CoA reductase (Bagheri *et al.,* 2018). Psyllium oil has been used for various medicinal effects and continued treatment of IBD, UC, colon cancer and diabetes mellitus (El-Feky *et al.,* 2018). The high pasty contents of psyllium seeds oil have also procreated an interest in their use as a medicinal excipient (Serafim *et al.,* 2020).

This study aimed to investigate the antiulcer effects of flaxseed and psyllium seed oils and their improving effects on hematological, oxidative and inflammation parameters in an ulcerative colitis rat model.

MATERIALS AND METHODS

1.Chemicals:

All chemicals that were used in this study were purchased from Sigma for Chemicals Company, Cairo, Egypt. Ethanol (Purity: 96%), NaOH (Normality: 0.01 N), potassium phosphate (pH: 7.5) buffer, and formalin (10%).

2.Plant Oils:

The tested plant oils of Flaxseed *(Linum Usitatissimum L.)* and Psyllium seed *(Plantago Ovata)* were purchased from PHATRADE Company, Cairo, Egypt.

3.Animals: Forty male albino rats from (Sprague-Dawley) strain weighing (200±20g)

were obtained from the oncology unit-(NCI), at Cairo University, Egypt. All experiments were performed following the guidelines for the ethical use of experimental animals. Rats were offered a standard commercial pellets diet (maintaining essential recommended dietary allowances) and tap water *ad libitum* and kept individually in stainless cages in constant conditions (NRC, 1995). The animals were prevented from food for 16 hrs. before ulcer induction with free access to water.

4.Animal Design and Grouping:

For the induction of UC model, rats were intrarectal (ir.) injected with ethanol (70%) (0.5 ml. $100g^{-1}$ b.wt.) (Shosha *et al.*, 2022). The normal control group received an ir. saline injection. The UC + flaxseed oil (FSO) and UC + psyllium seed oil (PSO) groups were given flaxseed and psyllium seed oils (500 mg. kg^{-1} b. wt.) respectively for 7 days later after ethanol ir. injection (Zhoua *et al.*, 2020).

4.Blood and Tissue Sampling:

At the end of the experimental period (7 days), rats were sacrificed. Blood samples were collected from all groups in a heparinized test tube for hematological examination. Colon was excised from the ileocecal junction to the anus. Colon tissue was homogenized in a buffer solution of potassium phosphate (pH 7.5) and then the homogenate was centrifuged at 6×10^3 rpm for 15 minutes at 4°C the supernatants were sucked out for further biochemical analysis. Colonic specimens were preserved in 10% formalin and paraffin-embedded for histopathological examination.

5.Determination of Hematological Markers:

According to Coles, (1986), the hematological parameters were determined including; Red blood cells (RBCs), white blood cells (WBCs) (total and differential) counts, hemoglobin (Hb) concentration, hematocrit (packed cells volume) PCV, erythrocytes indices (mean corpuscular volume; MCV, mean corpuscular hemoglobin; MCH and mean corpuscular hemoglobin concentration MCHC) and platelets count.

6.Determination of Oxidative Stress Markers In Colon Tissue:

Lipid peroxidation products were quantified by measuring malondialdehyde (MDA) using an MDA lipid peroxidation test kit (Sigma Aldrich Chemical Co., St. Louis, USA) according to the manufacturer's instructions. MDA level was calculated as (nmol/mg). Superoxide dismutase (SOD) activity was measured using the SOD test kit (Sigma Aldrich Chemical Co., St. Louis, USA) according to the manufacturer's instructions. SOD activity was calculated as activity (%/mg) relative to the corresponding protein content. Reduced glutathione (GSH) level was determined using the GSH assay kit (Sigma Aldrich Chemical Co., St. Louis, USA) according to the manufacturer's instructions. GSH value was calculated as (nmol/mg). Myeloperoxidase (MPO) activity was measured using the MPO assay kit (Sigma Aldrich Chemical Co., St. Louis, USA) according to the manufacturer's instructions. MPO activity was calculated in (ng/mg).

7.Determination of Inflammation Markers In Colon Tissue:

Interleukin-1β (IL-1β) level was determined in colon tissue homogenate using ELIZA kit provided by (Sigma Aldrich Chemical Co., St. Louis, USA) following the manufacturer's instructions. The IL-1β level was calculated as (pg/mg). Interleukin-10 (IL-10) was determined in colon tissue homogenate using ELIZA kit provided by (Sigma Aldrich Chemical Co., St. Louis, USA) following the manufacturer's instructions. The IL-10 level was calculated as (pg/mg). Prostaglandin E2 (PGI2) level in colon tissue was determined using ELISA kit provided by (Sigma Aldrich Chemical Co., St. Louis, USA). PGI2 level was calculated as (pg/mg). Leukotriene-B4 (LTB4) level in colon tissue was determined using

ELISA kit provided by (Sigma Aldrich Chemical Co., St. Louis, USA). LTB4 level was calculated as (pg/mg).

8.Histological Examination Of Colon Tissue:

Colonic samples of rats from tested groups in 10% formalin were purified in xylene and paraffin-embedded at 56°C for 24 hours. Paraffin beeswax tissue blocks were prepared for 4 µm sectioning using a microtome slide. The resulting tissue sections were collected on glass slides, deparaffinized, stained with hematoxylin and eosin then alizarin red, and examined using a light microscope (Bancroft *et al.*, 1996).

9.Statistical Analysis:

Statistical analysis of results was done by using version 16.0 of the Statistical Package for Social Science (SPSS). Microsoft Windows and SPSS Inc. were utilized. The data were expressed as (mean ± SD) using the mean and standard deviation. Using ANOVA test (one-way analysis of variance) to detect statistical differences between groups; the mean difference was significant at the ($P< 0.05$) level (Levesque, 2007).

RESULTS AND DISCUSSION

1. The Effect of Flaxseed and Psyllium Seed Oils on Hematological Markers in Tested Groups:

From the results in Table (1) it was clear that in the ulcerative colitis UC group there were significant ($p<0.05$) decreases in RBCs, Hb level and PCV values by about 24.72%, 30.05% and 27.87% respectively when compared with the healthy control group. While administration of tested oils FSO and PSO resulted in significant ($p<0.05$) increases in these parameters as compared with UC group. With respect to platelets count, there was a significant ($p<0.05$) increase in UC group by about 24.50% as compared with the healthy control group. While administration of tested oils FSO and PSO resulted in a significant ($p<0.05$) decrease in platelets count by about 13.80% in FSO group and 17.76% in PSO group as compared with the untreated UC group. Concerning the RBCs indices showed non-significant changes.

The results in Table (2) showed a significant ($p<0.05$) increase in WBCs total count in UC group by about 23.03% as compared with the control group. While administration of tested oils FSO and PSO resulted in a significant ($p<0.05$) decrease in total WBCs count by about 20.42% in FSO group and 16.64% in PSO group as compared with UC group. Regarding previous reports, there was a clear association between gastrointestinal ulceration and increased neutrophil count with decreased lymphocytes count. The results in Table (2) showed a significant ($p<0.05$) increase in neutrophile count by about 32.46% and a significant ($p<0.05$) decrease in lymphocytes count by about 36.40% in UC group as compared with their normal values in the control group. While administration of tested oils FSO and PSO resulted in significant ($p<0.05$) decreases in neutrophil count accompanied by significant ($p<0.05$) decreases in lymphocytes count as compared with UC group.

Additionally, the monocyte count confirmed the inflammation progression due to ulceration in UC group as it recorded a significant increase by about 100%in UC group as compared with control group. While administration of tested oils FSO and PSO resulted in significant ($p<0.05$) decreases in monocyte count by about 62.5% in FSO group and 50% in PSO group as compared with UC group. Eosinophil and basophil counts indicated significant increases in UC group and this confirmed their role in inflammatory diseases. While administration of tested oils FSO and PSO resulted in significant ($p<0.05$) decreases in eosinophil and basophil counts that confirmed the anti-inflammatory effects of tested oils.

In the present study, we established that treatment with flaxseed and psyllium seed oils improved the development of ethanol-induced UC through regulation of oxidation and inflammation responses, reducing bleeding-induced anemia and ameliorating ulceration condition. Our findings in hemogram parameters went in

accordance with the previous study of Farombi *et al.,* (2016) who found that the use of psyllium oil showed significant antithrombotic activity through decreased platelets count in ethanol-induced ulcerative colitis. Also, Ge et al., (2015) found that pre-treated mice with psyllium oil orally for 7 consecutive days induced a significant elevation in the count of RBC and PCV %. In the same line Fletcher and Swift, (2017) said that eosinophils and basophils assessed 14 days after treatment rats with psyllium oil at a dose of (2,000 mg. kg^{-1} b. wt.) recorded a non-significant difference compared with those of control. Moreover, Ghozlan *et al.,* (2017) reported that psyllium oil reduced the leukocyte activation during inflammation and so help in the prevention of increased neutrophil and monocyte counts during inflammation which reflected in decreased production of inflammatory mediators such as LTB-4. Furthermore, Gupta *et al.,* (2021) found that the PCV, Hb concentration and lymphocytic % from total WBCs count were significantly increased due to psyllium oil consumption. Meanwhile, the monocyte % was a significant decrease with no significant differences in basophil and eosinophil in U C rats treated with psyllium oil at a dose (100 and 200 mg/kg/daily for 21 days). Kalili *et al.*, (2018) reported that administration of psyllium oil in UC rats showed marked raise in RBCs count, Hb, PCV% and lymphocyte count and a marked decrease in platelets, WBC, neutrophil, and monocyte with non-significant changes in eosinophil and basophil counts when compared with positive control (acetic acid ulcerative rats) group.

Additionally, the present findings are similar to the previous results of Kim *et al.*, (2018) which demonstrated that treatment with flaxseed oil significantly improved hematological parameters in relation to RBCs count PCV % and the authors recommended the addition of flaxseed oil to the daily diet to enhance hematopoiesis. Along with the known benefits of flaxseed oil for constipation and loose stools, flaxseed had also been described as beneficial for the treatment of hemorrhoids (Keshteli *et al.*, 2019). It was found to have significant improvement in reducing bleeding and dramatically reducing clogged hemorrhoidal cushions, thereby reducing symptoms of anemia associated with bleeding in UC (Kakodker & Multu, 2017).

Table 1. The effect of flaxseed and psyllium seed oils on erythrogram and platelets counts in tested groups**.**

Groups /Parameters	RBCs 10^6/µL	Hb g/dl	PCV %	MCV fL	MCH pg	MCHC %	Platelets 10^3/µL
G1 Control	8.05±0.98	13.08±0.81	41.84±1.95	48.90±1.00	15.80±0.75	32.32±0.51	588.00±6.11
G2 UC	6.06±0.93*	9.15±0.41*	30.18±0.98*	48.35±1.08	14.68±1.30	30.37±1.28	732.00±8.13*
G3 UC+FSO	9.16±1.07**	13.81±0.85**	43.82±0.88**	49.48±1.11	15.60±1.51	32.00±0.39	631.00±8.50**
G4 UC+PSO	8.91±0.75**	13.02±068**	40.55±0.90**	49.55±1.12	15.99±1.31	32.21±1.49	602.00±6.33**

Data were expressed as mean ± SD (n=10). * Indicated a significant difference at $p<0.05$ compared to G1 control. **Indicated a significant difference at $p<0.05$ compared to G2 UC.

Table 2. The effect of flaxseed and psyllium seed oils on leukogram in tested groups.

Groups /Parameters	WBCs 10^3/µL	Neutrophil 10^3/µL	Lymphocytes 10^3/µL	Monocytes 10^3/µL	Eosinophil 10^3/µL	Basophil 10^3/µL
G1 Control	10.55±1.54	7.27±1.69	2.83±0.89	0.40±0.11	0.05±0.00	0.00±0.00
G2 UC	12.98±1.51*	10.08±1.68*	1.80±0.60*	0.80±0.13*	0.21±0.01*	0.09±0.00*
G3 UC+FSO	10.33±0.83**	7.17±2.11**	2.78±0.24**	0.30±0.07**	0.06±0.01**	0.00±0.00
G4 UC+PSO	10.82±1.27**	7.46±2.17**	2.88±0.50**	0.40±0.13**	0.08±0.00**	0.00±0.00

Data were expressed as mean ± SD (n=10). * Indicated a significant difference at $p<0.05$ compared to G1 control. **Indicated a significant difference at $p<0.05$ compared to G2 UC.

2. The Effect of Flaxseed and Psyllium Seed Oils on Oxidative Stress Markers in Tested Groups:

The oxidative stress parameters that were estimated in colon tissue were illustrated in Figure (1a & b). There were significant ($p<0.05$) decreases in antioxidant non-enzymatic molecule GSH level and CAT enzyme activity in UC group by about 66.55% and 57.94% respectively as compared with their corresponding control group. In addition to lipid peroxidation marker MDA and neutrophil-derived peroxidase enzyme MPO; which contributes to tissue damage during inflammation due to (ROS) production. There were significant ($p<0.05$) increases in their levels by about 63.92% and 280% respectively as compared with their normal levels in the control group.

While treatment with tested oils FSO and PSO resulted in significant ($p<0.05$) increases in antioxidant markers and significant ($p<0.05$) decreases in oxidation markers and these results confirmed the antioxidant effects of tested oils FSO and PSO. In FSO group the percentages of increment in antioxidant markers GSH and CAT were 142.11% and 97.75% respectively. while the percentages of decrement in oxidation markers MDA and MPO were 53.28% and 36.64% respectively as compared with their corresponding UC group levels. Additionally, in PSO group the percentages of increment in antioxidant markers GSH and CAT were 160% and 102.47% respectively. while the percentages of decrement in oxidation markers MDA and MPO were 45.56% and 33.72% respectively as compared with their corresponding UC group levels.

Oxidative stress can occur due to an increase in the oxidant level and/or a decrease in the antioxidant system, which is thought to be involved in the development of chronic diseases such as UC (Jin *et al.,* 2017). Both CAT and GSH play important roles in the antioxidant system. As an antioxidant enzyme, CAT can convert peroxide radicals into H_2O and O_2, and GSH is an important intracellular free radical scavenger (Kobayashi *et al.,* 2020). In addition to lipid peroxidation marker MDA; the product of lipid peroxidation by free radicals and neutrophil-derived peroxidase enzyme MPO; that contributes to tissue damage during inflammation due to (ROS) production and it was reported by Omer, (2018) to increase during inflammation conditions with an increased neutrophil count. In the present study, the activity of CAT and content of GSH were significantly decreased while MDA content and MPO activity were significantly increased in colon tissue of the UC group, whereas after treatment with flaxseed and psyllium seed oils oxidative condition was reversed by increasing antioxidant GSH molecule and CAT enzyme activity in addition to, decreasing oxidant MDA molecule and MPO enzyme activity.

Ethanol oxidation is an integral part of alcohol metabolism, leading to the excessive formation of ROS that leads to cell damage in the colon upon ethanol consumption (Naouar *et al.,* 2016). Ethanol administration significantly decreases the concentrations of total thiol and GSH, resulting in the destruction of cellular lipid components and consequently inducing oxidative damage in the colon, as evidenced by increased MDA levels (Naqshbandi *et al.,* 2013).

Additionally, there was a significant increase in MPO activity in the colon of rats given ethanol, indicating induction of inflammation with markedly increased leukocyte infiltration into the colon tissue. MPO possesses cytokine-like properties and is known to activate neutrophils for the further production of inflammatory mediators (Ogata *et al.,* 2017). Additionally, MPO uses H_2O_2 to generate hypochlorite through a process related to ROS production and subsequent tissue damage. Interestingly, treatment with the tested oils FSO and PSO was observed in the present study to modulate the deleterious effect induced by ethanol in UC models. This was indicated by an improvement in oxidative and inflammatory

status due to the antioxidant and anti-inflammatory components in flaxseed and psyllium seed (Omar, 2018).

Consistent with the previous observations of Olamilosoye *et al.,* (2018), flaxseed oil showed essential antioxidant properties that could improve the antioxidant status of the gut. α-linolenic acid can be converted in vivo to eicsopentaenoic acid and docosahexaenoic acid, which can enhance GSH synthesis. On the other hand, under the state of oxidative stress, lipid peroxidation is inevitably generated in cell and subcellular membranes and causes irreversible damage to cell structure and function (Palla *et al.,* 2020). It is confirmed by the previous reports of Nakayama *et al.,* (2015) the level of lipid peroxidation product MDA was significantly increased in colitis induced by intrarectal injection of ethanol, while the administration of flaxseed oil reduced the levels of MDA in colon tissue of the CU group lowered. Furthermore, as an indicator of inflammation, MPO could accelerate oxidative tissue damage, whereas the increase in MPO activity in the colon in rats treated with intrarectal ethanol injection was significantly inhibited by flaxseed oil treatment (Tyagi *et al.,* 2017).

The present findings went in hand with Wei *et al.,* (2016) who found that treatment with psyllium oil once daily at a dose (1000 mg. kg^{-1} b. Wt.) for five consecutive days in rats showed a significant elevation in the values of hepatic and blood GSH. Moreover, Zeng *et al.,* (2017) recorded a significant increase in serum GSH level and CAT in hyperglycemic rats after administration of (200 mg. kg^{-1} b. Wt.) psyllium oil for 21 days. Similarly, this result is in accordance with Young *et al.,* (2019) who discovered that psyllium oil has antioxidant and anti-inflammatory activities through the inhibition of NO production and reduction of IL-1β levels. Furthermore, Rose and Strombom, (2019) mentioned that psyllium oil administration in a broiler-fed basal diet significantly increased the anti-inflammatory cytokine IL-10 levels due to phenolic compounds.

It is important to note that the antioxidant effect of *Plantago psyllium* seeds might be due to their antioxidant potential which is represented by their rich content of flavonoids, alkaloids, polyunsaturated fatty acids, sulfur-containing amino acids, and metabolites (Stantos *et al.,* 2018). Furthermore, high levels of caffeic acid in *Plantago psyllium* seeds that possess scavenging free radicals' properties and increasing expression of antioxidant enzymes give an explanation of the antioxidant activities of *Plantago psyllium* seeds. Asaad *et al.,* (2020) revealed that GSH levels significantly ameliorated in the tissues of ketoprofen-induced hepatorenal toxicity rats after oral treatment with psyllium oil. Also, this result was accepted by Bancil and Pollius, (2015) who indicated that NO level was significantly reduced in the inflamed rat colon diet supplemented with 5% psyllium oil for 2 weeks before trinitrobenzene sulfonic acid colitis induction. In the same manner, Bekhit *et al.,* (2018) found the diet supplemented with 5% psyllium oil for 13 weeks before evaluation of the colonic inflammatory status significantly reduced the NO level in genetic mutation rats.

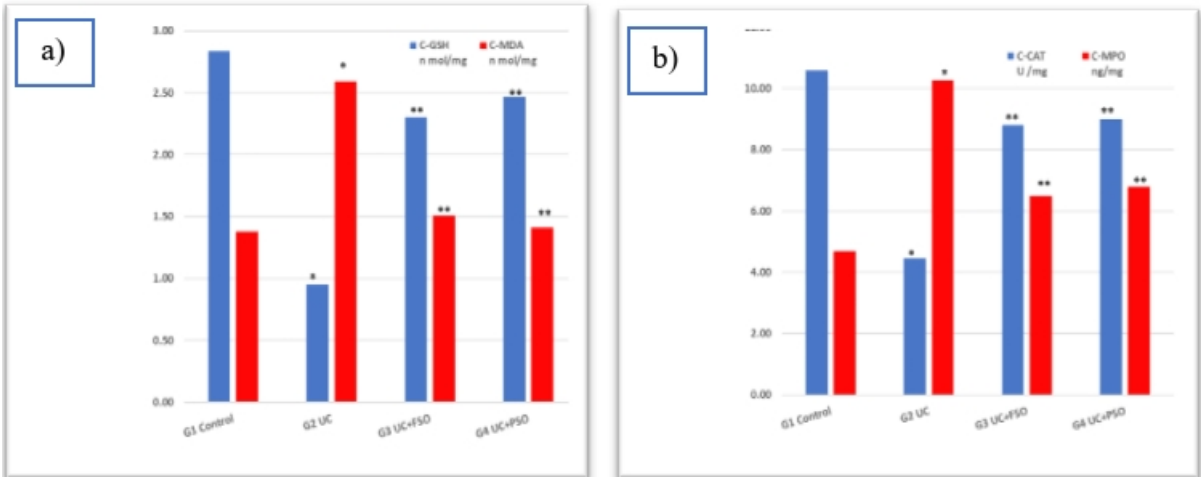

Fig. 1. Effect of flaxseed and psyllium seed oils on oxidative stress markers in tested groups. a) Colon levels of reduced glutathione (C-GSH) and malondialdehyde (C-MDA). b) Colon catalase (C-CAT) and myeloperoxidase (C-MPO) activities. * Indicated a significant difference at $p<0.05$ compared to G1 control. **Indicated a significant difference at $p<0.05$ compared to G2 UC.

3. The Effect Of Flaxseed And Psyllium Seed Oils On Inflammation Markers In Tested Groups

The results of inflammation markers that were illustrated in Figure (2a & b**)** showed significant ($p<0.05$) increases in inflammatory mediators IL-1β and LTB4 by about 87.70% and 74.29% respectively as compared with the control group. In addition to significant ($p<0.05$) decreases in anti-inflammatory mediators IL-10 and PGI2 by about 66.06% and 59.29% respectively as compared with the control group.

Considering the administration of tested oils FSO and PSO they exhibited significantly ($p<0.05$) decrements in inflammatory mediators and significant ($p<0.05$) increments in anti-inflammatory mediators. These results confirmed the anti-inflammatory effects of tested oils. In FSO group the percentages of decrement of inflammatory mediators IL-1β and LTB4 were 39.45% and 19.88% respectively. While the percentages of increment of anti-inflammatory mediators IL-10 and PGI2 were 124.51% and 37.20% respectively as compared with their corresponding UC group levels. With respect to PSO group, the percentages of decrement of inflammatory mediators IL-1β and LTB4 were 36.83% and 38.52% respectively. While the percentages of increment of anti-inflammatory mediators IL-10 and PGI2 were 118.14% and 49.15% respectively as compared with their corresponding UC group levels.

The previous findings of Chiba *et al.*, (2018) indicated the therapeutic and well-being potential of psyllium that was mainly attributed to its antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic and immunoprotect properties. Treatments supplemented with psyllium oil have reflective effects on several inflammatory mediators, such as adiponectin, IL-1β, C-reactive protein, and TNF-α. The anti-inflammatory activity of psyllium has also been reported by De Oliveira *et al.*, (2019) due to the high concentration of flavonoids that are present in psyllium seed. Moreover, psyllium oil may contribute to pain and inflammation reduction by inhibiting inflammatory PGs (such as PGF2α) production through inhibiting COX-2 enzyme activity. While activating the production of physiological PGs through the activation of COX-1 enzyme activity (Elnaggar *et al.*, 2016). Additionally, Bagheri *et al.*, (2018) revealed that psyllium induced an immunomodulatory effect by its antibacterial effect and significantly decreased the elevated

LTB-4; the strong chemotaxis inflammatory cytokines in experimentally dextran sodium sulphate ulcerative colitis mice.

Flaxseed oil was reported by EL-Newary *et al.,* (2017) to have a bactericidal effect against pathogens implicated in ulcerative colitis and inflammatory bowel disease, which may contribute to managing immune dysregulation and cytokine production. Hence, flaxseed oil administration may reduce the aggressive capability of *E. coli* in IBD and downregulate NF-kβ pathway, which results in a reduction of IL-1β, LTB-4, COX-2, and caspase-mediated cell death (Sun *et al.,* 2018a). Henceforward, flaxseed may become an excellent remedy that helps to reduce risks of UC and IBD due to its protective effects on colonic mucosal barriers, anti-neutrophilia and improving microbiome profile by its antibacterial effect. (Sun *et al.,* 2018b). Flaxseed oil was demonstrated by Sukari *et al.,* (2019) as a protective potential for the UC, which was constant with the previous report that flaxseed oil reduced inflammatory indicators and severity of disease (Tao *et al.,* 2017). The α-linolenic acid is the main bioactive compound of the flaxseed oil was reported to have anti-inflammatory and hypolipidemic effects (Tong *et al.,* 2017).

IL-1β as a pro-inflammatory cytokine is usually associated with colitis and is also used as a biomarker for colitis progressing to cancer, it was observed that inhibition of IL-1β signaling pathway avoids the progression of colitis (Ungaro *et al.,* 2017). In our study, FSO markedly reduced the over-expression levels of colonic IL-1β by the lessening of activated monocytes and macrophages infiltration in colon tissue, whereas the activated monocytes and macrophages were primary sources of the inflammatory mediators (Wang *et al.,* 2015). Additionally, ethanol intrarectal injection reduced PGI2 production, a well-known anti-inflammation prostaglandin and its deficiency can progress or exaggerate colitis in rodent animals (Wang *et al.,* 2016). We demonstrated that flaxseed oil somewhat increased the expression of IL-10 and PGI2. Flaxseed oil improved various circumstances in the rats UC, which displayed an abnormal pattern of inflammation.

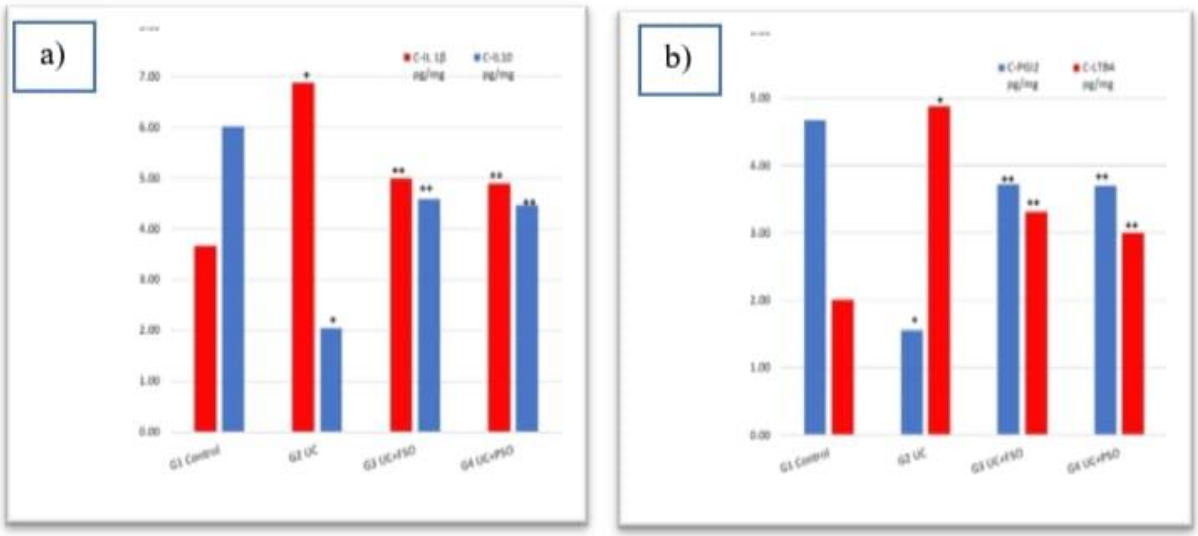

Fig. 2. Effect of flaxseed and psyllium seed oils on inflammation markers in tested groups. a) Colon levels of interleukin-1β (C-IL1β) and interleukin-10 (C-IL10). b) Colon levels of prostaglandin I2 (C-PGI2) and leukotriene B4 (C-LTB4). * Indicated a significant difference at $p<0.05$ compared to G1 control. **Indicated a significant difference at $p<0.05$ compared to G2 UC.

4. The Effect of Flaxseed and Psyllium Seed Oils on Colon Tissue Histological Examination in Tested Groups:

The results of the histological examination of colon sections in all tested groups were presented in Figure (3 a-d). The colon section of the healthy control group showed no histopathological changes, and normal histological structure of the colonic wall; the mucosal, submucosal and muscularis serosa as shown in Figure (3a). Induction of UC by intrarectal injection of ethanol caused focal ashing of the mucosal epithelial cells with fluid and inflammatory cells trapping in the underlying thin layer of connective tissue that lines mucosa. The submucosal layer showed fluid and few inflammatory cells trapping, as well as increased number and size of the lymphoid follicle with goblet cells depletion as shown in Figure (3b).

Flaxseed oil (FSO) treatment ameliorated the histopathological damage of the colon induced by ethanol as showed few fluids and inflammatory cells trapping in the thin layer of connective tissue that lining mucosa as well as in the submucosa associated with fluid trapping in the submucosa with increased goblet cells number as showed in Figure (3c). PSO treatment affected the mucosal layer by decreasing lymphoid follicle size, decreasing ashing in mucosal epithelial associated with decreased fluid trapping in the submucosa, few inflammatory cells trapping in the submucosal layer and increased goblet cells number as shown in Figure (3d).

Ethanol increased the permeability of blood vessels, enhanced the progression of inflammatory mediators and increased the erosion process all of these bad effects resulted in the degradation of colon structure and desquamation of mucosa barrier (Guzman-Gomez *et al.,* 2018). In the present study, ir. injection of ethanol caused clinical symptoms of colitis, whereas it significantly increased the oxidation and inflammatory markers (Strate *et al.,* 2015). These observations were previously indicated by Amer *et al.,* (2019) who reported that ethanol ir. injection aggravated the clinical indicators of colitis in the injected rats and caused a reduction in colonic antioxidant GSH level and CAT activity with increased MDA level and MPO activity. In the microscopical examination of the colon tissue, using H&E stain, there was severe mucosal ulceration with goblet cell degradation, and erosion of all colon layers (Palla *et al.,* 2020).

Administration of flaxseed oil significantly reversed the elevated levels of peroxidation of lipids and significantly reduced MDA content and significantly increased GSH content in a hepatotoxicity rat model by ethanol intraperitoneal injection (Subermann *et al.,* 2017). The degradation of the colonic mucosal layer was significantly refilled with the treatment of flaxseed oil (500 mg. kg^{-1}b. wt.) as compared to ethanol (80%) induced colitis (Tao *et al.,* 2017). Psyllium oil also protected the different histological alterations (bleeding, inflammation, corrosions, and ulcer) caused in the colonic mucosa of ethanol-injected rats (Bagheri *et al.,* 2018). Psyllium oil was reported to produce an antiulcerogenic effect against ethanolic-induced colon ulcers, in a dose-dependent manner accompanied by a reduction in fluid trapping and leukotrienes with increased mucus production and PGI2 expression (El-Feky *et al.,* 2018).

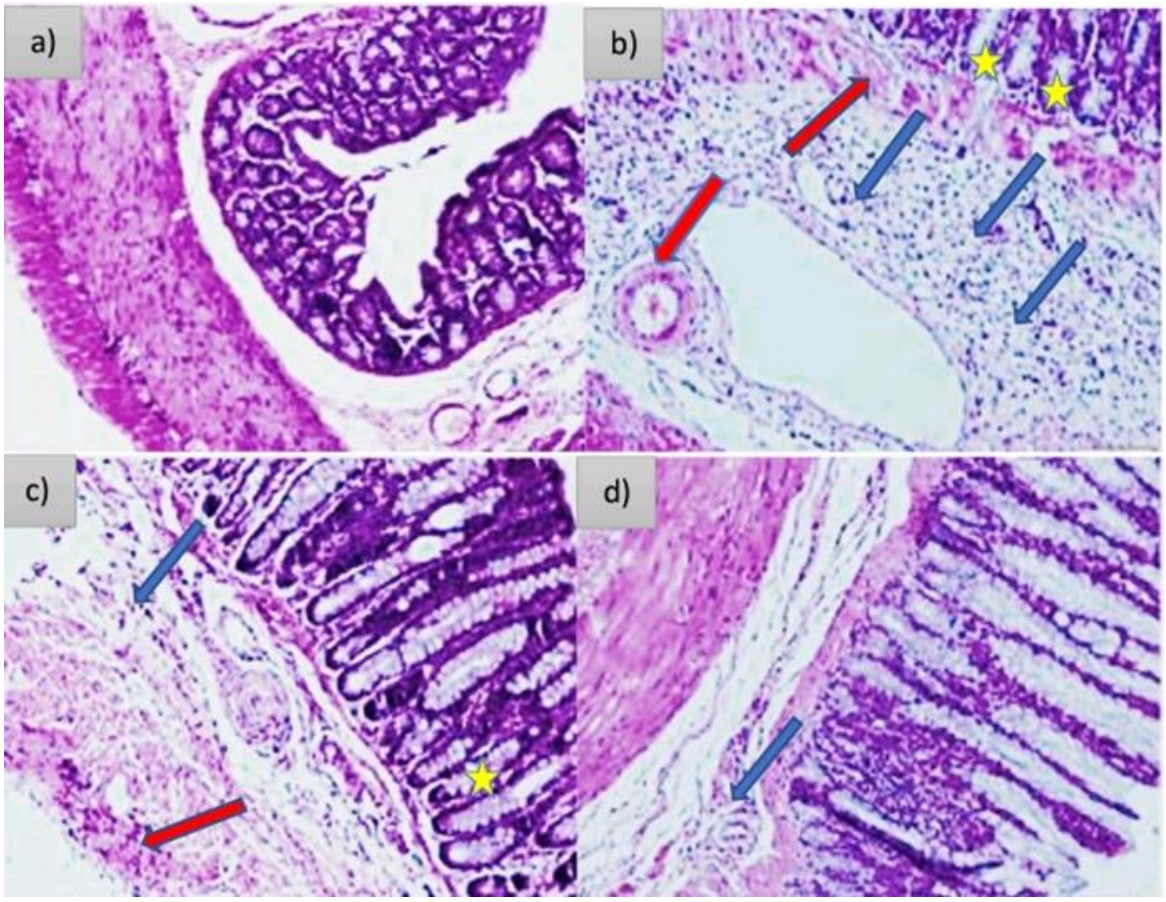

Fig. 3. Histological examination of the colon in tested groups. a) Section of control group showing; no histopathological changes. b) Section of UC group showing inflammatory cells (blue arrow), ulcerative erosions (red arrow) and goblet cell depletion (yellow stars) with lymphoid follicular hyperplasia. c) Section of FSO group showing some inflammatory cells associated with edema in mucosa. d) Section of PSO group showing decreasing lymphoid follicle size with decreasing ashing in mucosal epithelial and few inflammatory cells.

CONCLUSION

The present study indicated that flaxseed and psyllium seed oils against acute UC in rats significantly enhanced hematological parameters with a significant decrease in inflammatory cells neutrophils, monocytes and increased lymphocytes. Both two tested oils, exhibited downregulation of oxidative stress indices in colon tissue by reduction of MDA level, MPO activity and increasing GSH level and CAT activity in treated groups compared to the ulcer control group. In addition to, their anti-inflammatory effects by increasing colonic PGI2 synthesis and decreased inflammatory mediators' secretion. Collectively, we can say anti-inflammatory PGs can decrease inflammation cascades and improve antioxidant status. The constructive effects of tested oils FSO and PSO on anti-inflammatory PGs contributed to controlling the progression of ulcerative damage induced by ethanol.

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