*Egypt. J. Chem.* **Vol. 67**, No. 7 pp. 535 - 545 (2024)

**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Colo-Protective Effect of Grape Seed and Flaxseed Oils Via Caspase 3 and NF-Kb Signaling Inhibition in Experimental Model of Ulcerative Colitis

**Safaa Mostafa Awad[1], Ranwa Ahmed Elrayess[2*], Aziza Tarik Fathy[1]**

[1]*Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt*

[2]*Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt*

**Abstract**

Apoptotic signals, inflammatory mediators, and oxidative stress are up-regulated in ulcerative colitis (UC), which increases the risk of colon cancer. In UC, oils are being investigated as protective agents. Therefore, the current study is concerned with comparing the potential effectiveness of grape seed oil (GSO) and flaxseed oil (FSO) in modulating the risk factors associated with UC condition induced by acetic acid (AA). Twenty-four adult male rats were divided into four groups/6 rats each; control group received distilled water, UC group intrarectal treated with a single dose of 4% AA; GSO and FSO groups administrated 5 mL/kg body weight of grape (Vitis vinifera L.) seed oil or flaxseed (Linum usitatissimum L.) oil, respectively for three weeks before the induction of the UC. Protective effects were determined by measuring tissue Hemoxegenase 1, Monocyte chemoattractant protein 1, Myeloperoxidase, Tumor necrosis factor alpha and Interleukin-10 as well as serum oxidative markers, such as malondialdehyde, and total antioxidant capacity. In addition, macroscopic, histopathological and immunohistochemical examinations of colon tissues. The findings showed that inducing UC in rats activated the pro-inflammatory, apoptotic, and lipid peroxidation pathways. However, the exacerbated effects of ulceration on the colonic mucosa could be effectively reversed by GSO and FSO. Surprisingly, FSO was more efficient to exert its colo-protective effect, as confirmed by histopathological/immunohistochemical changes. Therefore, FSO may be a promising therapy for treating UC.

***Keywords:*** Ulcerative colitis; Acetic acid; Grape seed oil; Flaxseed oil; Colo-protective; Inflammation; Caspase 3; NF-κB

## 1. Introduction

Ulcerative colitis (UC) is a chronic inflammation of the colonic mucosa that causes abdominal pain, weight loss, nausea, and rectal bleeding, all of which diminish the quality of life [1]. If left untreated, UC can raise the chance of developing colorectal cancer and result in death [2]. Due to modern lifestyle, the prevalence of idiopathic inflammatory bowel disease (IBD), including UC, is remarkably high, with an estimated 25 individuals per 10,000 people in the west, as well as, in other regions of the world [3].

The etiology of IBD tends to be the result of an intricate relationship between genetic, environmental, microbial and immunogenic factors that activate in mucosal T-cell immune response, stimulating the release of inflammatory mediators such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), decline of antioxidant enzymes of the colonic mucosa, loss of mucosal barrier integrity, anti-inflammatory markers and cytokines, cell infiltrations, and NF-κB activation, which lead to apoptotic injuries in the colon [4]. Apoptosis has been involved in the pathophysiology of IBD in many studies [5]. Inflammatory response could alter the mucosal barrier function and intestinal integrity resulting in apoptosis [6].

A plant-based diet provides more butyrate and other short chain fatty acids (SCFAs), as well as

*Corresponding author e-mail: ranwa_elrayess@science.suez.edu.eg.; (Ranwa Elrayess).
EJCHEM use only: Received date 29 October 2023; revised date 12 January 2024; accepted date 15 January 2024
DOI: 10.21608/EJCHEM.2024.245275.8797

phytochemicals with anti-inflammatory and antioxidant properties that can alleviate UC with fewer and no adverse effects [7]. Dietary lipids, which are major modulators of the IBD risk, are among the dietary factors that may affect UC disease. Plant-based oils have significant pharmaceutical and food manufacturing interests because they are used as food flavoring agents in a variety of food items [8].

Grape (*Vitis vinifera* L.) seeds oil serves several functions, including cooking (as a food ingredient), cosmetics, disease prevention, and wound healing [9]. Grape seed contains between 8 and 20% oil, as well as varied amounts of phenolic compounds, flavonoids, and unsaturated fatty acids depending on the grape type. Grape seed oil active ingredients have been demonstrated to have a variety of pharmacological activities. The therapeutic effect of grape seed oil on IBD is attributed to its powerful antioxidant action [10].

Flaxseeds (*Linum usitatissimum* L.) oil is frequently used for baked goods like bread, cookies, cakes, and other items [11]. Flaxseed oil is a strong source of α-linolenic acid, which has been shown to reduce inflammatory cytokines produced by macrophages and monocytes as well as improve diarrhea and constipation [12].

The purpose of this study is to determine the bioactive components in grape seed and flaxseed oils and investigate their potential protective role against colonic inflammatory insult induced by acetic acid in rats, which mimic the symptoms of the serious inflammatory bowel disease "ulcerative colitis" and introduce a new functional anti-inflammatory food supplement.

## 2. Material and methods

### *2.1 Chemicals*

All the research chemicals used in the present study were obtained from Sigma Chemical Company in Cairo, Egypt including acetic acid (4%), potassium phosphate buffer (pH 7.5), and formalin (10%).

### *2.2 Plant Oils*

The oils of Grape seed (*Vitis vinifera* L.) and Flaxseed (*Linum Usitatissimum* L.) were obtained from the Agricultural Research Center (Giza, Egypt).

### *2.3. High performance liquid chromatography analysis for plants oils*

An Agilent 1260 series was used for the HPLC analysis. Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 μm) was used for the separation. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were the components of the mobile phase, and the flow rate was 0.9 ml/min. The linear gradient was sequentially programmed into the mobile phase as follows: 0 min (82% A); 0–1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). At 280 nm, the multi-wavelength detector was observed. The injection volume was 5 μl for each sample solution and the column temperature was maintained at 40°C.

### *2.4. Animals*

The Research Ethics Committee, Ain Shams University, Egypt [sci1432304002, approved the experimental plan. All experiments were carried out in accordance with the ethical standards for the use of experimental animals. Twenty-four adult male Sprague-Dawley rats, each weighing 200±20g, were procured from the Cairo University's oncology unit in Egypt. To maintain the essential recommended dietary allowances, rats received a standard commercial pellets diet and free access to water. Animals were housed individually in stainless steel cages under controlled conditions [13]. Animals were prevented from food for sixteen hours with free access to water prior to the induction of the ulcer.

### *2.5. Animal Design and Grouping*

Animals were randomly divided into four categories (6 rats/group); Group 1: Control, Group 2: Ulcerative colitis (UC), Group 3: Grape seed oil (GSO) treated group and Group 4: Flaxseed oil (FSO) treated group. The control and UC groups received distilled water, while GSO and FSO groups received grape seed oil and flaxseed oil, respectively at 5 mL/ kg b.wt. [14, 15] by oral gavage daily for 3 weeks before induction of UC. After that animals were fasted for 16 h except providing them with water ad libitum, and anesthetized intraperitoneally with valium (10 mg/kg) and ketamine (50 mg/kg) mix for induction of UC.

### *2.6. Induction of Ulcerative Colitis*

A single dose of 2 ml acetic acid (4%) was administered intrarectally to induce ulcerative colitis [16] with the aid of flexible plastic catheter (diameter = 2 mm) that was inserted 8 cm into the colon through the anus, then the animals were kept with their heads down for 5 minutes to prevent from the solution leakage. In the control group, distilled water was instilled. Seventy-two hours after the induction of colitis, oil treatment was discontinued, rats were sacrificed, and the colon tissues were collected.

### *2.7. Blood and Tissue Sampling*

Rats were sacrificed at the end of the trial and serum was obtained from blood samples by allowing them to stand at a temperature of 37°C for 15 minutes, centrifugation for 20 minutes at 4000 rpm, and then removing the serum and storing it in plastic vials at -20°C until it was used to measure the antioxidant and oxidative stress indices. Colon was removed from the anus to the ileocecal junction and washed with saline solution for measurement of its weight and length and for macroscopic examination. After examination it was homogenized in a potassium phosphate buffer solution (pH 7.5) and the homogenate centrifuged at 6000 rpm for 15 minutes at 4°C and the supernatants were collected for additional biochemical analyses. For histological and immunohistochemical analysis, colonic tissues were kept in 10% formalin.

### *2.8. Assessments of Colitis*

#### *2.8.1. Evaluation of the Disease Activity index (DAI)*

During the experimental trial, the disease activity index (DAI) was assessed to determine the severity of ulcerative colitis by an investigator who recorded the stool consistency, body weight, and rectal bleeding of each animal according to previously described protocol [17]. The DAI score was calculated as the sum scores of all the parameters mentioned.

#### *2.8.2. Assessment of Colon Weight-to-Length Ratio (W/L)*

The anus to caecum of rats' colons were removed and emptied before being weighed. Each rat's colon weight and length were measured, and the relative colon weight to length ratio—which serves as a marker of disease-related oedema and wall thickening-was computed by dividing the wet weight of colon by its length [18].

#### *2.8.3. Macroscopic examination of colonic damage*

Colons from the animals were examined macroscopically to score the visible injuries in each group using a scale extending from zero to four, as 0 for normal coloration with no damage, + for slight injury, + + for haemorrhagic streaks, + + + for moderate ulceration, and + + + + for severe ulcerated colon [19].

### *2.9. Determination of the pro- and anti-inflammatory markers levels in colon tissue*

Inflammatory markers levels: Hemoxegenase 1 (HO-1), Monocyte chemoattractant protein 1 (MCP-1), Myeloperoxidase (MPO), Tumor necrosis factor alpha (TNF-α) and Interleukin-10 (IL-10) were quantified in colonic tissues using ELISA kits (Catalog No. MBS764989, CSB E07429r, RDR-MPO-Ra, CSB E11987r, and MBS764911) according to the manufacturer's protocol and absorbance was measured at 450 nm.

### *2.10. Determination of Oxidative Stress and Antioxidant biomarkers in serum*

Lipid Peroxidation biomarker; malondialdehyde (MDA) and the antioxidant biomarker; total antioxidant capacity (TAC) levels were estimated in serum using a colorimetric assay kit (Sigma Aldrich Chemical Co., St. Louis, USA) following the manufacturer's instructions.

### *2.11. Histological Examination*

The colon samples were fixed with 10% buffered formalin and embedded in paraffin. From each block, sections of 3µm thickness were submitted, mounted, stained by hematoxylin and eosin (H&E), scanned, and photographed. Colon sections were examined blindly for Histopathological findings and scored semi-quantitatively as follows [20]: inflammation (none = 0, slight = 1, moderate = 2, and severe = 3), hemorrhage (none = 0, slight = 1, moderate = 2, and severe = 3), inflamed area/extent (mucosa = 1, mucosa and submucosa = 2, and transmural = 3), crypt damage (none = 0, basal 1/3 damaged = 1, basal 2/3 damaged = 2, only the surface epithelium is intact = 3, and entire crypt and epithelium are lost = 4), and percent involvement (1–25% = 1, 26–50% = 2, 51–75% = 3, and 76–100% = 4). The histopathological changes were evaluated from 20 random microscopic fields per treatment group using light microscopy (magnification 40×).

### *2.12. Immunohistochemical preparation*

The immunohistochemical staining for Nuclear Factor Kappa B (NF-κB) and Caspase 3 were performed using 10% goat serum to block the unspecific binding, followed by using either the specific anti- NF-κB or anti-caspase 3 antibodies to detect their cellular content in colon tissue [21]. Brown color indicated Positive reaction. Reactivity area percentage was estimated using Image J software, Japan from 20 random fields/treatment.

### *2.13. Statistical Analysis*

The Statistical Package for Social Science (SPSS) version 16.0 was used to statistically analyze the results. The software used was SPSS Inc. and Microsoft Windows. Data were expressed as (mean ± SD) using mean and standard deviation. The statistical differences between groups were determined by using the ANOVA test followed by Duncan's test [22], and the significance was determined at $P<0.05$.

## 3. Results

### *3.1 HPLC analysis of bioactive components in GSO and FSO*

In case of grape seed oil, the phenolic compounds identified were 48% gallic acid, 11% chlorogenic acid, 9% syringic acid, 6% methyl gallate, 5% catechin, 5% ferulic acid, 5% vanillin, 5% naringenin, 3% daidzein, 2% coumaric and 1% cinnamic acid as shown in Fig 1. The phenolic compounds identified in flaxseed oil, were 60% gallic acid, 8% vanillin, 6% caffeic acid, 5% rosmarinic acid, 4% rutin, 4% quercetin, 3% syringic acid, 3% naringenin, 2% coumaric acid, 2% ferulic acid, 2% daidzein and 1% cinnamic acid as shown in Fig 2.

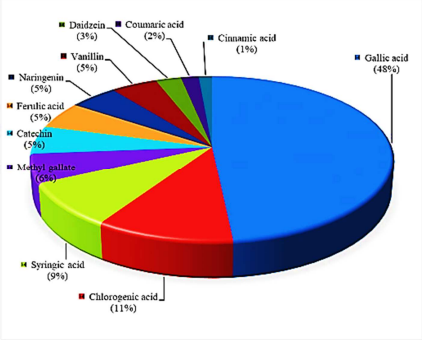


**Fig. 1** Bioactive components of GSO

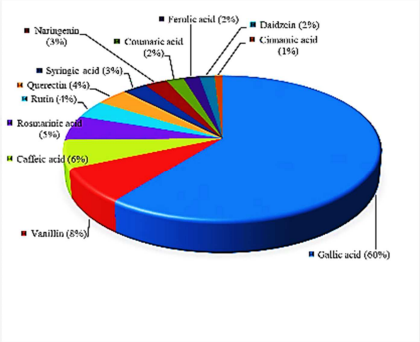


**Fig. 2** Bioactive components of FSO

### *3.2 Effect of GSO and FSO on disease activity index (DAI)*

Rectal bleeding, stool consistency, and percentage of weight loss were used to assess the severity of the disease and how effectively the response to the treatment. Based on the findings in Fig 3, the mean DAI was substantially higher in the UC group as compared to the control group ($p<0.05$). In contrast to UC group, pretreatment with GSO and FSO significantly reduced the UC' DAI ($p<0.05$).

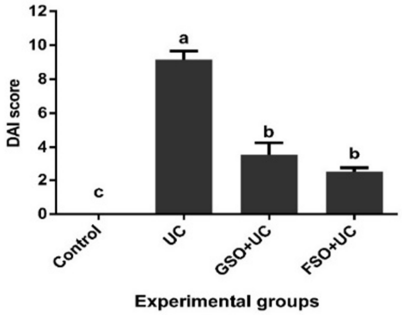


**Fig. 3** Mean values of DAI in the different experimental groups. Different superscript letters indicate significant difference at $P < 0.05$ using ANOVA followed by Duncan's

### *3.3 Effect of GSO and FSO on colon weight to length ratio (W/L)*

The ratio of rat colon weight to length (g/cm) was calculated for each group and it was found that the W/L ratio of the control group was 0.34±0.03 which increased considerably to 0.44±0.03 in the UC group (Fig. 4) however, this was reduced to 0.37±0.09 and 0.33±0.04 in GSO and FSO groups, respectively when compared to the UC group ($p<0.05$).

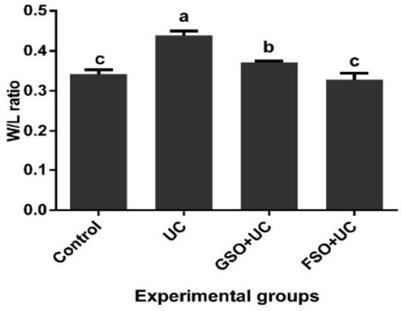


**Fig. 4** Mean values of W/L ratio in the different experimental groups. Different superscript letters indicate significant difference at P <0.05 using ANOVA followed by Duncan's.

### *3.4 Effect of GSO and FSO on colon macroscopic examination*

The intestinal mucosa in the control group exhibited no lesions or redness (score 0), whereas the intestinal mucosa in the UC group had a severe ulcerated colon with haemorrhage (score + + + +). The GSO group displayed haemorrhagic streaks (score + +), while the FSO-treated group displayed slight colon injury (score +). Based on the findings of the macroscopic evaluation of colons, FSO was more successful in restoring the intestinal mucosal integrity to a level that was like that of the normal intestinal mucosa (Fig. 5).

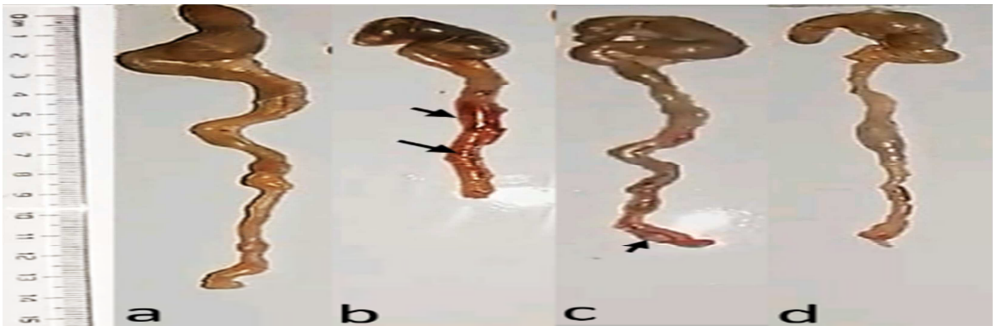


**Fig. 5** Macroscopic examination of rat's colons in the experimental groups: (a) control group showed normal colored colon without any damage (score 0); (b) UC group showed severe ulcerated colon (score + + + +); (c) GSO group showed haemorrhagic streaks (score + +); (d) FSO group showed slight injury (score +).

*3.5 Amelioration of the pro- and anti-inflammatory markers levels in colon tissue*

The obtained results in Table 1 demonstrated that acetic acid instillation significantly increased ($p< 0.05$) the inflammatory mediators; MPO activity, TNF-α and MCP-1 levels in colonic tissue of UC group as compared to the control group. However, the anti-inflammatory cytokine IL-10 and the antioxidant biomarker HO-1 are significantly decreased ($p< 0.05$) in colonic tissue of UC group when compared to the control group. It was obvious that oral administration of GSO and FSO had an anti-inflammatory effect reflected by a significant ($p< 0.05$) reduction in MPO activity, TNF-α and MCP-1 levels, by 45.1%, 61.7% and 37.3%, respectively with GSO and 51%, 61.7% and 43.1% respectively with FSO as compared to UC group. On the other hand, GSO significantly elevated IL-10 by 108.8% as well as HO-1 by 189.7%. Whereas FSO raised IL-10 by 106.7% and HO-1 by 175.9%, respectively when compared to UC group.

**Table 1:** Effects of GSO and FSO on pro- and anti-inflammatory markers of UC induced by AA

| Group | HO-1 (ng/mg) | MCP-1 (pg/mg) | MPO (pg/mg) | TNF-α (pg/mg) | IL-10 (pg/mg) |
|---|---|---|---|---|---|
| **Control** | 11.1±0.65 [a] | 116.9±1.00 [d] | 38.7±4.40 [d] | 22.2±1.89 [c] | 190.1±2.90 [a] |
| **UC** | 2.9±0.27 [c] | 261.4±5.70 [a] | 116.3±2.70 [a] | 88.1±5.88 [a] | 79.6±4.86 [c] |
| **GSO + UC** | 8.4±0.46 [b] | 163.8±6.59 [b] | 63.8±1.03 [b] | 33.7±3.13 [b] | 166.2±0.69 [b] |
| **FSO + UC** | 8.0±0.58 [b] | 148.8±6.34 [c] | 57.0±3.39 [c] | 33.7±3.51 [b] | 164.5±0.96 [b] |

The results are presented as mean ± SD (n = 6) per group. Mean values with different superscript letters within the same column are significantly different at $P<0.05$ using ANOVA followed by the Duncan's.

*3.6 Modulation of oxidative stress and antioxidant parameters in serum*

Table 2 illustrates the oxidative stress and the antioxidant biomarkers estimated in serum. There was significant ($p<0.05$) reduction in TAC level in UC group by about 54.8% comparing to the control group. Regarding lipid peroxidation marker MDA, there was significant elevation in its level of about 206.9 % as compared with UC group. While treatment with GSO and FSO resulted in significant ($p<0.05$) raise in antioxidant biomarker with significant reduction in lipid peroxidation marker. In GSO and FSO groups the percentage of increment in TAC level were 80.4 % and 88.2%, while the percentages of decrement in MDA level were 57.4% and 58.5%, respectively as compared with their corresponding UC group levels.

**Table 2:** Effects of GSO and FSO oxidative stress and antioxidant biomarkers of UC induced by AA

| Group | MDA (nmol/ml) | TAC (mM/L) |
|---|---|---|
| **Control** | 60.9±4.01 [c] | 107.1±1.60 [a] |
| **UC** | 186.9±0.28 [a] | 48.4±4.68 [d] |
| **GSO + UC** | 79.7±3.82 [b] | 87.3±2.17 [c] |
| **FSO + UC** | 77.5±1.07 [b] | 91.1±1.86 [b] |

The results are presented as mean ± SD (n = 6) per group. Mean values with different superscript letters within the same column are significantly different at *P<0.05* using ANOVA followed by Duncan's.

*3.7 Histological examination*

Sections of control colon tissue showed normal structure with few inflammatory cell's infiltration (Fig. 6a), while colon of the UC group showed severe histological alternation including focal surface ulceration, formation of lymphoid follicles, with areas of drop out glands. Distortion of glands and crypts, areas of edema with chronic inflammatory cells infiltrate within lamina propria were also recorded (Fig. 6b-c). The GOS group revealed significant changes when compared to UC group, surface epithelium was restored was no crypt distortion but still exhibit focal area of drop out glands and edema with chronic inflammatory cells infiltrate within submucosa (Fig. 6d). However, FOS group showed restoration of the colon tissue to its normal structure (Fig. 6e). Pretreatment with natural oils under investigation remarkedly protects against UC stimulate colon tissue damage. It was noticed that the protective effect of FSO was noticeably higher than that of GSO as evidenced by the histopathological scoring (Table 3).

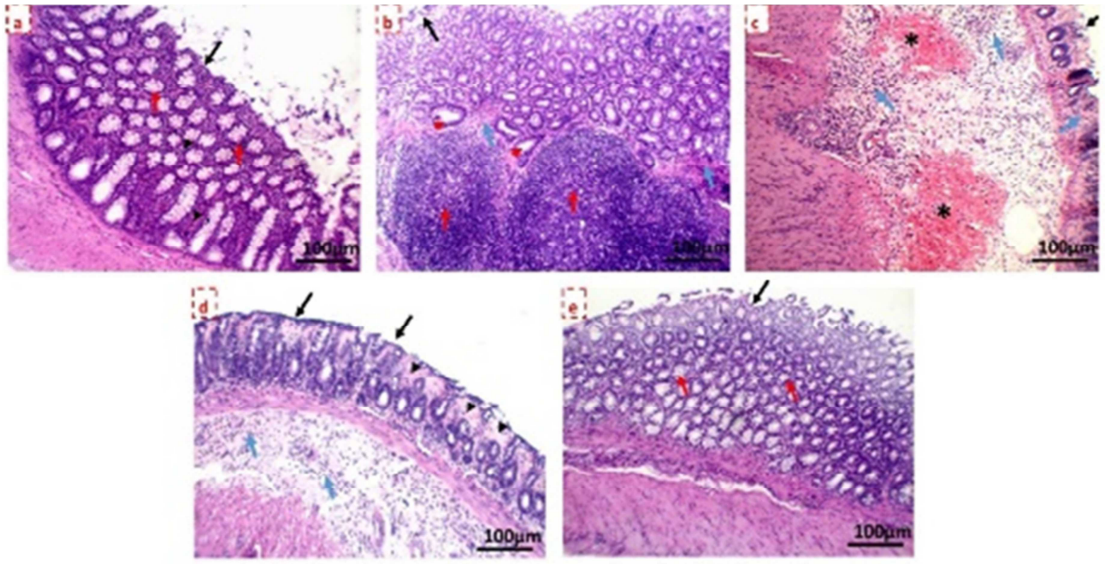


**Fig. 6** Histopathological examination of colon tissue from all experimental groups stained with H&E. (a) control group showing uniform colonic tissue with regular epithelial cell covering (black arrow), overlying lamina propria showing few chronic inflammatory cell infiltrates (arrowheads) and uniform mucous secreting colonic glands (red arrows). (b&c) UC group exhibited focal surface ulceration with focal area of drop out glands (black arrow), with underlying dense chronic lympho-palsmacytic infiltrate involving submucosa and lamina propria, forming lymphoid follicles (red arrows), distortion of the glands and crypts (red arrowheads), edema with chronic inflammatory cells infiltrate within lamina propria (blue arrows) and hemorrhage (star). (D) GOS group displayed restored surface epithelium (black arrow) with focal area of drop out glands (black arrowheads). There is edema and chronic inflammatory cells infiltrate within submucosa (blue arrows). (e) FSO group showing complete restoration of colon tissue with regular epithelial cell covering (black arrow) and colonic glands (red arrows)

**Table 3** Histological observation for all experimental groups

| Treatment | Inflammation | Inflamed area/extent | Crypt damage | Percent involvement |
|---|---|---|---|---|
| **Control** | 0 | 0 | 0 | 0 |
| **UC** | +++ | +++ | +++ | +++ |
| **GSO + UC** | ++ | ++ | 0 | ++ |
| **FSO + UC** | + | + | 0 | + |

*3.8 Immunohistochemical expression of Caspase 3 and NF-κB*

Immunohistochemical evaluation of Caspase 3 and NF-κB showed lowest expressions in the control group, while its expression was significantly ($p<0.05$) increased in the UC group as evidenced by the strong immunopositivity reaction indicated by the area of brown color staining. However, pretreatment with GSO and FSO significantly ($p<0.05$) reduced the immunohistochemical expression of Caspase-3 and NF-κB in the tissue. It was minimal in the tissue pretreated with FSO (Fig.7-8).

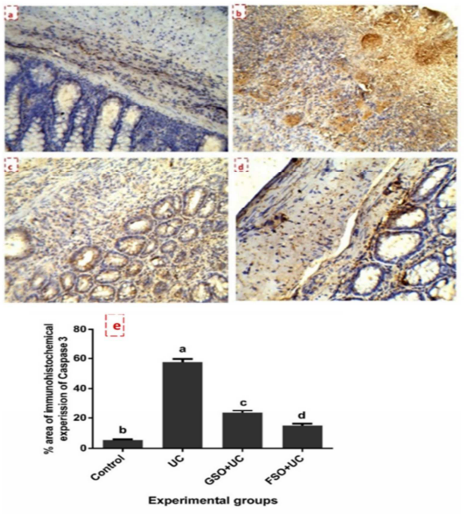


**Fig. 7** Immunohistochemical evaluation of active caspase 3 in the colon of different experimental groups (200x). (a) Control, normal level of caspase 3 expression; (b) UC group showing extensive expression of caspase 3 (brown staining) (c) minimal expression, (d) FSO group showing weak expression; (e) Histogram of the mean percentage areas of caspase 3, in the colon tissue of different experimental groups. Different superscript letters indicate significant differences at $p<0.05$.

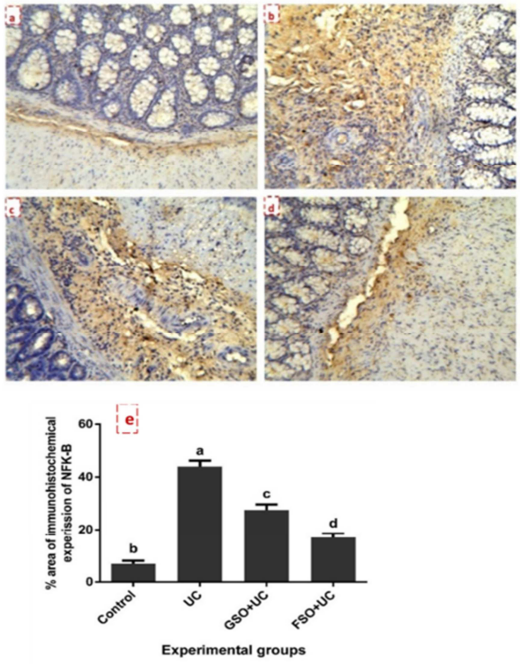


**Fig. 8** Immunohistochemical evaluation of active NF-κB in the colon of different experimental groups (200x). (a) Control, normal level of NF-κB expression; (b) UC group showing extensive expression of NF-κB (brown staining) (c) minimal expression, (d) FSO group showing weak expression; (e) Histogram of the mean percentage areas of NF-κB, in the colon tissue of different experimental groups. Different superscript letters indicate significant differences at $p<0.05$.

## 4. Discussion

UC is one of the most common types of IBD which mainly targets colon and rectum [23]. The main cause for the disease is unknown but it could be related to environmental and genetic factors activating inflammatory and immunological responses in the colon [24]. The use of natural or herbal medicines as therapeutic agents has recently drawn significant interest on a global scale for the prevention and/or treatment of several disorders [25]. The colo-protective properties of GSO and FSO and their key pathways in decreasing damage in the AA-induced UC in rats. The results of the current study emphasized that no abnormalities were observed in rats after grape seed oil or flaxseed oil treatment during the experiment, suggesting that the dose of these oils employed in the current study may be suitable for use in animal experiments.

The acetic acid induced UC rat model is a well-established rodent model that exhibits multiple characteristics of human UC, including weight loss, mucosal ulcerations, bloody stools, severe diarrhea, colonic shortening, and inflammation of the cells lining the mucosa [26]. Therefore, to assess the activity of UC and treatment response, the percentage of stool consistency, body weight loss, and rectal bleeding were taken into consideration. DAI measures the severity of inflammation and contributes to intestinal mucosa damage [27]. Hyperosmolarity in the intestinal lumen and/or enhanced permeability of the intestinal cells may be the causes of the diarrhea encountered in UC rats after AA induction [28]. Additionally, weight assessment is considered a reliable indicator of the severity of intestinal inflammation therefore, the noticed weight reduction has a strong association with the clinical and biochemical alterations associated with UC. According to the assessment of DAI, the presence of acute bleeding promotes the medical condition to worsen [29]. The observed improvement in DAI and the protective activities of GSO and FSO against UC may be attributed to their bioactive components (as shown in Fig. 1 and 2) which have beneficial antioxidant and anti-inflammatory activities, thus functioning in lowering oxidative stress and inflammation leading to protection against bowel injury [30, 31].

The crypt abscess, in which the crypt epithelia are damaged, is the characteristic pathology of UC. Polymorphonuclear leukocytes invade the lamina propria. Colon mucosal architecture is destroyed because of the injured crypts; thus, the colon shortens due to severe scarring. As a result, the proportion of the colon W/L would rise. In this context, it is noted that AA-induced increment in the W/L ratio has been

greatly mitigated by GSO and FSO which may be due to the beneficial anti-inflammation activity. Although inflammation causes edema, GSO and FSO were able to remove the inflammatory cells from the site of the inflammation, and this enhanced removal may also be responsible for the decreased colon weights in the treatment groups [9, 32].

Colon visualization was also used to assess the macroscopic damage and it was found that colon ulcerations and erosions were among the features reported. AA is known to cause ulcers, reddening, and bleeding in the colon. Pretreatment with GSO and FSO markedly improved wound healing and repaired colon damage on a macroscopic level. The production of pro-inflammatory cytokines like TNF-α and IL-6, which are responsible for the disease's main signs and symptoms, may be exacerbated by persistent inflammation causing a damage in the tissues surrounding the site of inflammation [33]. GSO and FSO significantly lowered the proinflammatory cytokines which might help explain the overall protective impact observed. Additionally, oxidative stress plays a significant role in the development of UC [16]. In this context, GSO and FSO antioxidative function in lowering ROS may boost their anti-inflammatory function and increase protection against colon injury due to presence of gallic acid which is the main phenolic acid in both oils as well as several bioactive components as shown in Fig. 1 and 2 with good anti-inflammatory and antioxidant effects. These results were confirmed by the histological evaluation in which the AA-treated group exhibited severe colonic injury, evidenced by the formation of lymphoid follicles, with areas of drop out glands, distortion of glands and crypts, edema with chronic inflammatory cells infiltrate. These results are in harmony with previous studies [6, 25]. However, pretreatment with GSO and FSO obviously alleviated AA-induced colonic damage and preserved the whole functional colon structure, however, FSO exhibited more protective effect than GSO. This may indicate the ability of GSO and FSO to protect the colon and prohibit the disease progression.

Our findings clarified that AA causes UC by increasing inflammatory cytokines and mediators while lowering anti-inflammatory cytokines and biomarkers. These findings are consistent with an early study [1]. MPO is a peroxidase enzyme found mostly in neutrophil granulocytes. It is an excellent indicator of tissue inflammation, damage, and neutrophil infiltration. Acetic acid increased colonic MPO levels, indicating neutrophil infiltration. It specifies that neutrophil accumulation contributes to the UC induce oxidative injury [34]. Acetic acid causes UC by causing widespread intracellular acidification, which leads to the erosion of mucosal barriers and eventually destroys the colonic epithelium, which increases the activation of inflammatory cytokine-releasing cells such as monocytes and macrophages. Inflammatory cytokines generate a variety of chemokines and enhance chemotaxis [35].

Pretreatment with GSO and FSO before UC induction exerted anti-inflammatory and anticolitic effects through up regulation of IL-10 and HO-1 levels and downregulation of inflammatory mediators. In addition, amelioration to neutrophil infiltration as evidenced by diminished the colon MPO level and development of histological features [7, 9]. These oils significantly reduced leucocyte infiltration, downregulated neutrophil infiltration, and dramatically attenuated histopathological alterations. So that, these oils exert a protective effect against AA-induced UC [36]. Gallic acid (GA) which is present at high concentration in both oils, suppressed the MPO level and pro-inflammatory cytokines IL-1/6and TNF-α, as well stimulated the release of anti-inflammatory cytokines IL-4/10 by inhibiting the IκB/NF-κB pathway. Through inhibiting the NF-κB signaling pathway, the pro-inflammatory factors COX-2 and iNOS are consequently reduced [37]. Other mechanisms by which GA protects against UC include decreased IL-21 and IL-23 expression, activation of the Nrf2 gene and its downstream, and hence increased HO-1 levels [38]. GA also promotes deoxyribose oxidation and neutralizes free radicals while also providing anti-oxidative properties and suppressing the regulation of proinflammatory cytokines [39].

Furthermore, FSO contains roughly 57% of the Omega-3 fatty acid alpha-linolenic acid (ALA), which is six times more than typical fish oil. Eicosapentaenoic acid (EPA) is the precursor of the PG3a class of anti-inflammatory prostaglandins and resolvins. Docosahexaenoic acid (DHA) is the precursor of docosanoids, also known as 'resolvins' or 'protectins,' which are similar to eicosanoids in their anti-inflammatory and immune-regulatory properties. DHA is thought to have anti-inflammatory effects in the intestine and to lower the risk of colon cancer, which may be mediated through interactions with certain signaling proteins in membranes [40].

MDA, a commonly used biomarker of lipid peroxidation and oxidative stress, was shown to be increased in the UC group, although TAC was significantly reduced [41]. Oxidative stress can occur because of an increase in oxidant levels and/or a decrease in the antioxidant system and is hypothesized to be involved in the development of

chronic diseases such as UC [42]. It has been shown that elevated levels of NF-κB relate to increased levels of oxidative stress and inflammatory cytokines, as well as depletion of antioxidant biomarkers, all of which are the causes of greater colon epithelial damage, which contribute to UC. Furthermore, MPO uses $H_2O_2$ to generate hypochlorite, an action that contributes to ROS generation and consequent tissue damage.

Interestingly, pretreatment with the tested oils GSO and FSO has significantly reduced MDA and elevated TAC levels to modulate the harmful effect induced by AA in UC models. This was demonstrated by a decrease in oxidative and inflammatory status because of the antioxidant and anti-inflammatory bioactive components found in those oils. Chlorogenic acid present in GSO may possibly exert its action through potentiating the antioxidant Nrf-2 pathway that may be the main cause for minimizing the histopathological alternation changes in colon tissue [43]. In harmony with a previous observation [44], FSO demonstrated an important antioxidant property that could increase the antioxidant status of the gut. In vivo, α-linolenic acid can be converted to DHA and EPA, which can boost the production of antioxidant biomarkers.

Furthermore, the immunohistochemical detection in the current work revealed extensive expression of NF-κB and caspase-3 in A.A treated group as compared to the normal control group. The elevated level of NF-κB in our study was connected to increased levels of oxidative stress and inflammatory cytokines, both of which are the causes of increased colon epithelial injuries. Mucosal barrier function and intestinal integrity can be altered by this inflammatory action, leading to apoptosis through upregulation of pro-apoptotic caspase 3, leading to colitis [1, 45].

On the other hand, pretreatment with GSO and FSO greatly reduced apoptotic damage while also maintained the intestinal integrity and mucosal barrier functions by decreasing the expression of NF-κB and caspase 3. Gallic acid, the principal active ingredient in both oils, is a very safe phenolic acid with anti-inflammatory properties. It has been found that GA suppressed inflammation and apoptosis via suppressing phosphorylation of NF-κB and therefore, inhibiting the NF-κB pathway, in addition, down regulating caspase 3 level [46].

## 5. Conclusion

The current study showed that both GSO and FSO markedly protected rats from developing UC caused by AA, but FSO exhibited higher efficiency. The favorable effect may have been due to the control of the inflammatory factors and/or antioxidant activity. Moreover, the use of FSO as a preventative medication may be advantageous, and its brief administration may result in the desired colo-protective effect. However, to clarify GSO and FSO use and dosage for people, more studies on toxicity and safety should be carried out.

## 6. Author contribution

All authors contributed to the study's conception and design. Material preparation, data collection, analysis, and writing the first draft of the manuscript were performed by safaa M. Awad, Aziza T. Fathy, and Ranwa A. Elrayess. All authors approved the final manuscript. All authors have agreed to the published the manuscript.

## Declarations

**Conflicts of interest:** The authors have no competing interests to declare.

**Formatting of funding sources:** Not applicable.

**Data availability:** Data supporting findings are presented within the manuscript.

**Ethics approval:** This study was carried out and approved by the Research Ethics Committee, Ain Shams University, Egypt [sci1432304002], and all efforts to minimize rats suffering during the study were applied.

**Consent to participate and publish:** This study does not deal with any human participants.

## 7. References

1. [1] M. Shahid, M. Raish, A. Ahmad, Y.A. Bin Jardan, M.A. Ansari, A. Ahad, K.M. Alkharfy, A.L. Alaofi, F.I. Al-Jenoobi, Sinapic acid ameliorates acetic acid-induced ulcerative colitis in rats by suppressing inflammation, oxidative stress, and apoptosis, Molecules, 27 (2022) 4139.
[2] S. Rose, A. Strombom, Ulcerative Colitis-Prevention and Treatment with a Plant-Based Diet, Advanced Research Gastroenterology & Hepatology, 5 (2020) 101-106.
[3] A.M. Aleisa, S.S. Al-Rejaie, H.M. Abuohashish, M.S. Ola, M.Y. Parmar, M.M. Ahmed, Pretreatment of Gymnema sylvestre revealed the protection against acetic acid-induced ulcerative colitis in rats, BMC Complement Altern Med, 14 (2014) 49.
[4] F.A. Silva, B.L. Rodrigues, M.L. Ayrizono, R.F. Leal, The Immunological Basis of Inflammatory Bowel Disease, Gastroenterol Res Pract, 2016 (2016) 2097274.
[5] M. Raish, M. Shahid, Y.A. Bin Jardan, M.A. Ansari, K.M. Alkharfy, A. Ahad, I.A. Abdelrahman, A. Ahmad, F.I. Al-Jenoobi, Gastroprotective Effect of Sinapic Acid on Ethanol-Induced Gastric Ulcers in Rats:

Involvement of Nrf2/HO-1 and NF-κB Signaling and Antiapoptotic Role, Front Pharmacol, 12 (2021) 622815.
[6] A.A. Ali, E.N. Abd Al Haleem, S.A. Khaleel, A.S. Sallam, Protective effect of cardamonin against acetic acid-induced ulcerative colitis in rats, Pharmacol Rep, 69 (2017) 268-275.
[7] A.A. El-Rhman, 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, Egyptian Academic Journal of Biological Sciences, C Physiology & Molecular Biology, 14 (2022).
[8] L. Kuna, J. Jakab, R. Smolic, N. Raguz-Lucic, A. Vcev, M. Smolic, Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options, J Clin Med, 8 (2019) 179.
[9] F. Hosseinzadeh, M. Salehi, N. Tanideh, D. Mehrabani, A. Sayarifard, A. Sedighi, The Healing Effect of Grape Seed Oil Enema with or without Sesame Oil in Acetic Acid Induced Ulcerative Colitis of Rats, World J Plast Surg, 6 (2017) 176-182.
[10] M.E. Martin, E. Grao-Cruces, M.C. Millan-Linares, S. Montserrat-De la Paz, Grape (Vitis vinifera L.) seed oil: A functional food from the winemaking industry, Foods, 9 (2020) 1360.
[11] N. Morshedzadeh, S. Shahrokh, H.A. Aghdaei, M.A. Pourhoseingholi, V. Chaleshi, A. Hekmatdoost, S. Karimi, M.R. Zali, P. Mirmiran, Effects of flaxseed and flaxseed oil supplement on serum levels of inflammatory markers, metabolic parameters and severity of disease in patients with ulcerative colitis, Complementary therapies in medicine, 46 (2019) 36-43.
[12] A.H. Palla, N.T. Iqbal, K. Minhas, A.H. Gilani, Flaxseed extract exhibits mucosal protective effect in acetic acid induced colitis in mice by modulating cytokines, antioxidant and antiinflammatory mechanisms, Int Immunopharmacol, 38 (2016) 153-166.
[13] N.R. Council, Nutrient requirements of laboratory animals: 1995, (1995).
[14] P.V. Turner, T. Brabb, C. Pekow, M.A. Vasbinder, Administration of substances to laboratory animals: routes of administration and factors to consider, J Am Assoc Lab Anim Sci, 50 (2011) 600-613.
[15] A. Dugani, A. Auzzi, F. Naas, S. Megwez, Effects of the oil and mucilage from flaxseed (linum usitatissimum) on gastric lesions induced by ethanol in rats, Libyan J Med, 3 (2008) 166-169.
[16] S.S. Al-Rejaie, H.M. Abuohashish, M.M. Al-Enazi, A.H. Al-Assaf, M.Y. Parmar, M.M. Ahmed, Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats, World J Gastroenterol, 19 (2013) 5633-5644.
[17] H.S. Cooper, S.N. Murthy, R.S. Shah, D.J. Sedergran, Clinicopathologic study of dextran sulfate sodium experimental murine colitis, Lab Invest, 69 (1993) 238-249.
[18] M. Duijvestein, M.E. Wildenberg, M.M. Welling, S. Hennink, I. Molendijk, V.L. van Zuylen, T. Bosse, A.C.W. Vos, E.S. de Jonge-Muller, H. Roelofs, Pretreatment with interferon-γ enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis, Stem cells, 29 (2011) 1549-1558.
[19] S.K. Kulkarni, Hand book of experimental pharmacology, Vallabh prakashan, 1987.
[20] Y. Zhang, X. Zhao, Y. Zhu, J. Ma, H. Ma, H. Zhang, Probiotic Mixture Protects Dextran Sulfate Sodium-Induced Colitis by Altering Tight Junction Protein Expressions and Increasing Tregs, Mediators Inflamm, 2018 (2018) 9416391.
[21] S.S. Laurentino, S. Correia, J.E. Cavaco, P.F. Oliveira, L. Rato, M. Sousa, A. Barros, S. Socorro, Regucalcin is broadly expressed in male reproductive tissues and is a new androgen-target gene in mammalian testis, Reproduction, 142 (2011) 447-456.
[22] R. Levesque, SPSS programming and data management: A Guide for SPSS and SAS user. 3rd Edition, USA; (2007) 32-45
[23] N.J. Talley, S. O'connor, Clinical examination: a systematic guide to physical diagnosis, Elsevier Health Sciences, 2014.
[24] D.C. Baumgart, S.R. Carding, Inflammatory bowel disease: cause and immunobiology, The Lancet, 369 (2007) 1627-1640.
[25] G. Owusu, D.D. Obiri, G.K. Ainooson, N. Osafo, A.O. Antwi, B.M. Duduyemi, C. Ansah, Acetic Acid-Induced Ulcerative Colitis in Sprague Dawley Rats Is Suppressed by Hydroethanolic Extract of Cordia vignei Leaves through Reduced Serum Levels of TNF-α and IL-6, Int J Chronic Dis, 2020 (2020) 8785497.
[26] G. Wang, B. Xu, F. Shi, M. Du, Y. Li, T. Yu, L. Chen, Protective Effect of Methane-Rich Saline on Acetic Acid-Induced Ulcerative Colitis via Blocking the TLR4/NF-κB/MAPK Pathway and Promoting IL-10/JAK1/STAT3-Mediated Anti-inflammatory Response, Oxid Med Cell Longev, 2019 (2019) 7850324.
[27] H.M. Ismail Abo El-Fadl, M.F.A. Mohamed, Targeting endoplasmic reticulum stress, Nrf-2/HO-1, and NF-κB by myristicin and its role in attenuation of ulcerative colitis in rats, Life Sci, 311 (2022) 121187.
[28] Y. Yan, V. Kolachala, G. Dalmasso, H. Nguyen, H. Laroui, S.V. Sitaraman, D. Merlin, Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis, PloS one, 4 (2009) e6073.
[29] L. Solomon, S. Mansor, P. Mallon, E. Donnelly, M. Hoper, M. Loughrey, S. Kirk, K. Gardiner, The dextran sulphate sodium (DSS) model of colitis: an overview, Comparative clinical pathology, 19 (2010) 235-239.
[30] R. Lazcano-Silveira, X. Jia, K. Liu, H. Liu, X. Li, M. Hui, Carbon 60 Dissolved in Grapeseed Oil Inhibits Dextran Sodium Sulfate-Induced Experimental Colitis, J Inflamm Res, 15 (2022) 4185-4198.
[31] Q. Zhou, L. Ma, W. Zhao, W. Zhao, X. Han, J. Niu, R. Li, C. Zhao, Flaxseed oil alleviates dextran sulphate sodium-induced ulcerative colitis in rats, Journal of Functional Foods, 64 (2020) 103602.

[32] A.H. Palla, A.-u.-H. Gilani, S. Bashir, N. Ur Rehman, Multiple mechanisms of flaxseed: Effectiveness in inflammatory bowel disease, Evidence-Based Complementary and Alternative Medicine, 2020 (2020).
[33] H. Ogata, T. Hibi, Cytokine and anti-cytokine therapies for inflammatory bowel disease, Curr Pharm Des, 9 (2003) 1107-1113.
[34] B. Zaware, R. Gilhotra, S. Chaudhari, The potential of caesalpinia crista leaves in the treatment of ulcerative colitis in laboratory animals, Journal of Drug Delivery and Therapeutics, 8 (2018) 374-381.
[35] E. Saldanha, A. Saxena, K. Kaur, F. Kalekhan, P. Venkatesh, R. Fayad, S. Rao, T. George, M.S. Baliga, Polyphenols in the prevention of ulcerative colitis: a revisit, Dietary Interventions in Gastrointestinal Diseases, (2019) 277-287.
[36] Y. Zhou, D. Wang, H. Duan, S. Zhou, J. Guo, W. Yan, The Potential of Natural Oils to Improve Inflammatory Bowel Disease, Nutrients, 15 (2023).
[37] L. Zhu, P. Gu, H. Shen, Gallic acid improved inflammation via NF-κB pathway in TNBS-induced ulcerative colitis, Int Immunopharmacol, 67 (2019) 129-137.
[38] B. Khodayar, M.H. Farzaei, A.H. Abdolghaffari, R. Bahramsoltani, M. Baeeri, F. Sabbagh Ziarani, M. Mohammadi, R. Rahimi, M. Abdollahi, The protective effect of the gallic acid against TNBS-induced ulcerative colitis in rats: role of inflammatory parameters, Journal of Iranian Medical Council, 1 (2018) 34-42.
[39] J. Bai, Y. Zhang, C. Tang, Y. Hou, X. Ai, X. Chen, Y. Zhang, X. Wang, X. Meng, Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases, Biomed Pharmacother, 133 (2021) 110985.
[40] A.M. Dugani, Comparative effect of flaxseed oil and fish oil in acetic acidinduced colitis in rats, (2012).
[41] G. El-Akabawy, N.M. El-Sherif, Zeaxanthin exerts protective effects on acetic acid-induced colitis in rats via modulation of pro-inflammatory cytokines and oxidative stress, Biomed Pharmacother, 111 (2019) 841-851.
[42] B.R. Jin, K.S. Chung, S.Y. Cheon, M. Lee, S. Hwang, S. Noh Hwang, K.J. Rhee, H.J. An, Rosmarinic acid suppresses colonic inflammation in dextran sulphate sodium (DSS)-induced mice via dual inhibition of NF-κB and STAT3 activation, Sci Rep, 7 (2017) 46252.
[43] L. Cao, W. Han, S.G. Lee, J.H. Shin, Anti-Inflammatory Activity of Chlorogenic Acid on Macrophages: A Simplified Simulation of Pharmacokinetics Following Ingestion Using a Windup Syringe Pump, Applied Sciences, 13 (2023) 627.
[44] K.P. Olamilosoye, R.O. Akomolafe, O.S. Akinsomisoye, M.A. Adefisayo, Q.K. Alabi, The aqueous extract of Ocimum gratissimum leaves ameliorates acetic acid-induced colitis via improving antioxidant status and hematological parameters in male Wistar rats, Egyptian Journal of Basic and Applied Sciences, 5 (2018) 220-227.
[45] H.A. Fawzy, M.F. El-Yamany, H.M. Fawzy, E.M. Fikry, Mesenchymal stem cells attenuate acetic acid-induced colitis in rats via immunomodulatory effects, Azhar International Journal of Pharmaceutical and Medical Sciences, 2 (2022) 96-107.
[46] T.Y. Yu, Y.M. Feng, W.S. Kong, S.N. Li, X.J. Sun, G. Zhou, R.F. Xie, X. Zhou, Gallic acid ameliorates dextran sulfate sodium-induced ulcerative colitis in mice via inhibiting NLRP3 inflammasome, Front Pharmacol, 14 (2023) 1095721