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A COMPARATIVE STUDY OF ANTI INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF FENUGREEK (*TRIGONELLA FOENUM GRAECUM*) AND CARAWAY (*CARUM CARVI*) AQUEOUS AND OIL SEED EXTRACTS IN EXPERIMENTAL MODEL OF ULCERATIVE COLITIS

Mai Elsayed[1]* and Rasha H. Hussein[2]

[1]Department of Biochemistry and Nutrition, Women College, Ain Shams University, Cairo, Egypt.

[2]Department of Biochemistry and Nutrition, Women College, Ain Shams University, Cairo, Egypt. Department of Applied Biochemistry, Faculty of Science, Jeddah University, Jeddah, Saudi Arabia.

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***Corresponding Author**
Mai Elsayed
Department of Biochemistry and Nutrition, Women College, Ain Shams University, Cairo, Egypt.

ABSTRACT

Ulcerative colitis is a chronic inflammatory bowel disorder that increase the risk of colon cancer. The usage of natural antioxidants exhibit great interest as a means to avoid oxidative damage. This study evaluated the effect of fenugreek and caraway either in form of aqueous or oil seeds extracts on experimental model of ulcerative colitis in rats. Colitis was induced using of 4% acetic acid solution. Groups were: G1: healthy control group, G2: ulcerative colitis group, G (3, 4) : ulcerative colitis supplemented with fenugreek aqueous and oil extracts, respectively, G (5, 6) : ulcerative colitis supplemented with caraway aqueous and oil extracts, respectively. Colon samples were assayed for oxidative stress and inflammatory biomarkers along with colon histology and DNA fragmentation. Results showed that treatment with fenugreek and caraway either in form of aqueous extract or seed oil extracts protect against ulcerative colitis effects as shown by improvement in antioxidant enzymes level and inflammatory biomarkers, histological and genetical evaluation. These findings indicated that fenugreek and caraway aqueous and oils extracts have defensive effects against acetic acid-induced ulcerative colitis which might due to their anti-inflammatory, antiulcerative and antioxidant properties. Therefore, they can possibly used for successful treatment of ulcerative colitis.

KEYWORDS: Fenugreek - Caraway - Ulcerative colitis- DNA fragmentation -Antioxidant-Inflammation.

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disorder that may develop into colon cancer. Among these factors are : long extent, reduced activity, occurrence of complicating primary sclerosing cholangitis, and insufficient pharmacological remedy. It also may due to environmental, immunological and genetic factors.[1]

Ulcerative colitis occurs primerly in immature blood vessels that are unable to provide sufficient ulcer healing. This pathologic angiogenesis causes inflammation by permitting an increased flooding of immune cells into the affected colonic tissue. The new created vessels can secrete chemokines and proinflammatory cytokines which attract additional immune cells, this would increase the gastrointestinal tract inflammation that leads to oxidative damage in the colonic mucosa.[2] Ulcerative colitis symptoms includes diarrhea and abdominal pain acccompained by rectal bleeding**.** No efficient therapies depends on lowering the colonic lining and reducing the related symptoms.[3]

Medicial herbs are rich sources of biologically active compounds, and they still a main supply of therapy for all population world wide.[4] There is a belief that the usage of artificial drugs is not safe as medicinal plants this increase the need to phyto-pharmaceutical treatment.[5] A lot of plant extracts acquire anti-inflammatory characteristics and screened for their effectiveness to decrease the injury of the mucosal cells induced by proinflammatory cytokines, leading to cytotoxic edema.[6]

Fenugreek (*Trigonella foenum graecum*), a leguminous plant widely grown in Africa, India, and the Mediterranean, has been usually used as a therapeutic herb. Seeds are used as a spice in food preparations in different countries this may due to their flavor and odor. Fenugreek seeds are characterized by, antiandrogenic, antifertilitic, hypolipidaemic, hypoglycemic and antinociceptive properties Fenugreek seeds and leaves extracts have been reported to acquire anti-inflammatory, hypoglycemic, antitumour properties and also are found to be nontoxic. It has been used to treat a number of gastro-intestinal disorders.[7] Fenugreek is abundant sources of quercetin, gallic acid, kaempferol, luteolin and gallic acid. The anti-oxidant ability of the fenugreek extract was found attributed to the incidence of its flavonoids.[8]

Caraway (*Carum carvi)* is a medicinal plant belonging to family Apiaceae and it is located to Asia, Africa and Europe. In addition, it has great benefits in the treatment of gastrointestinal disorders. Caraway could be used as diuretic without any renal side effects. Also, it acts as a protective medicinal plant against many disorders, including ulcers, neoplastic disease, proliferative disorders and hyperglycemic conditions.[9]

Carvone (40–60%), carveol, limonene, dihydrocarveol, thymol in addition to glucosides and flavanoids are considered the major constituents of caraway (*Carum carvi)*. They own powerful chemopreventive properties and have shown its antitumor, antihyperglycemic, antiproliferative, and antimicrobial activities.[10]

This study examined the comparative achieve of administrating fenugreek or caraway aquous and oil seed extracts on ulcerative colitis induced by acetic acid.

MATERIAL AND METHOD

Preparation of the aqueous extracts of the seeds

The fenugreek and carawy seeds were obtained from Ministry of Agriculutre. Seeds were washed with distilled water to exclude extraneous matter, then dried by air and ground into powder in a mixer. One gram of seed powder was mixed woth distelled water (100 ml) in a vortex cyclomixer for l0 minutes and then centrifuged at 10000 rpm. The collected supernatant was used as aqueous extract and was freshly prepared. In this study, each animal was orally given 1 ml of the final aqueous extract containing 0.4g/kg body weight.[11] Caraway and fenugreek seeds oil were purchased from Ministry of Agriculutre tobe administered orally at a dose 0.1ml oil/ 100 g body weight.[12]

Induction of ulcerative colitis

The experimental induction of UC was performed according to Mousavizadeh et al[13] method. Using a 2.7 mm soft pediatric catheter, animals were transrectally administered 2 mL of 4% acetic acid solution (v/v) under light ether anesthesia. To avoid leakage of acetic acid, rats were then holed horizontally for 2 minutes. Similar procedure was performed to control animals using equal volume of normal saline.

Animals

The experimental animals used in the present study were normal adult male albino rats weighing 200 ± 10 g and supplied by the Breading Unit of Animal Reproduction Research

Institute (Gizah, Egypt). Animals were maintained on a natural light/dark cycle and given food and tap water ad libitum. After acclimation period, the animals were left for randomly assigned into six experimental groups (10 rats /group) which were organized as follows**:**

Group 1 (C): Rats were gavaged orally with saline soln

Group 2 (UC): Rats were gavaged orally with saline soln

Group 3 (UC+Fen-ex): Rats were gavaged orally with Fenugreek aqueous extract daily

Group 4 (UC+Fen-oil): Rats were gavaged orally with Fenugreek oil extract daily

Group 5 (UC+Car-ex) : Rats were gavaged orally with Caraway aqueous extract daily

Group 6 (UC+Car-oil): Rats were gavaged orally with Caraway oil extract daily

The administration of seeds extracts lasts for 30 days. At the 31th day, UC was induced in all groups except healthy control group. Then, 24 hr later, animals were sacrificed under deep anesthesia, about 5–6 cm of the colon specimens were dissected and washed with saline solution then weighted.

Analysis of inflammatory Biomarkers and inflammation involved proteins

The following inflammatory biomarkers are assessed in colon tissue : Colonic Myeloperoxidase (MPO) activity was measured, colon samples were weighed the cut into small pieces, and prepared for determination of MPO.[14] The levels of Prostaglandine E2 (PGE2) and Interleukin 1ß (IL-1 ß) in colon tissue were determined using enzyme linked immunosorbent assays (ELISA) according to the manufacturer's instructions (lifespan Biosciences, USA). Inflammation involved proteins : Cyclooxygenase-2 **(**COX-2) was determined using Enzyme-linked Immunosorbent Assay Kit (Cusabio, USA). Readings made directly at 450 nm. Caspase-3 was examined using Enzyme-linked Immunosorbent Assay Kit according to the manufacturer's instructions (Cloud-Clone Corp, USA).

Oxidative stress evaluation

A small portion of colon tissue was homogenized in 3 ml 0.5% hexadecyltrimethyl ammonium bromide in 50mM phosphate buffer (pH 6) and kept in ice bath during homogenization to maintain maximum enzyme stability. Determination of the thiobarbituric acid reaction was performed according to Bose et al.[15] The results were expressed as nmols of malondialdehyde (MDA) per mg of tissue. Superoxide dismutase (SOD) levels was performed according to Misra and Fridovich.[16] Protein Carbonyl (PC) was determined calorimetry using commercial kits (Biovision incorporated, USA).

Histopathological analysis

Colon tissue samples from each group was set in 10% formalin solution. Then, sectioned, deparaffinized. Colon sections were stained with hematoxylin and eosin (H&E) and evaluated by a pathologist.

DNA fragmentation analysis

Small portion of colon tissues were homogenized and incubated in 100 mM Tris-HCl (pH 8.0), 25 mM EDTA, and 0.1 µg/ml proteinase K at 60 ºC for 3 hr. DNA was extracted and examined by an ultraviolet transilluminator according to Sambrook et al.[17]

Statistical analysis

Data was statistically analyzed using SPSS computer Program. The results were presented as mean ± SE. The differences between mean values were determined by analysis of variance (ANOVA test).

RESULTS

- **Biochemical results**

Results of table (1 and 2) showed acetic acid resulted in a significant increase ($p< 0.01$) in PGE2 significantly when compared to control group. Similar results were obtained for COX-2 and IL-1 ß, MPO and CASP3 when compared to control group. A general improvement was observed in inflammatory biomarkers in all treatment groups after administration of either fenugreek (aqueous or oil extracts) or caraway (aqueous or oil extracts). Administration of Fenugreek as aqueous extract or as oil ameliorates the increased level of PGE2, COX-2, IL-1 ß, MPO and CASP3 significantly ($p< 0.01$) by (60%, 39%), (37%, 35%), (36%, 27.7%), (30%, 48%) and (30.5%, 25.5%) respectively. While administration of Carawy as aqueous extract or as oil ameliorates the increased level of PGE2, COX-2, IL-1 ß, MPO and CASP3 significantly ($p< 0.01$) by (5.3%, 28.9), (39%, 62.4%), (19%, 30.2%), (36%, 20.8) and (22.2%, 13.%) respectively. Comparing results showed that fenugreek aqueous extract has the most potent effect of inflammatory biomarkers.

Table (1): Effect of different treatments on inflammatory markers (PGE2, IL-1 ß, MPO) in control and ulcerative colitic rats.

Groups /Parameters	PGE2 ng/mg	IL-1 ß Pg/mg	MPO ng/mg
G 1 (C)	7.75±0.56	115.4±7.15	6.72±0.46
G 2 (UC)	24.61±15.61*	223.2±9.64*	13.48±0.27*
G3(UC+Fen-ex)	10.01±1.01**	142.8±1.73**	9.37±0.06**
G4 (UC+Fen-oil)	15.11±3.51**	161.5±2.88**	10.05±0.35**
G5 (UC+Car-ex)	23.3±2.69*	180.9±5.11**	10.49±0.09**
G6 (UC+Car-oil)	17.7±2.3**	155.9±6.43**	11.68±0.38**

Note: **means ±SD are significant (P<0.01) compared with normal control group (1), **means ±SD are significant (P<0.01) compared with Ulcerative colitis group (2).*

Abbreviation: PGE2, Prostaglandine E2; IL-1 ß, Interleukin 1ß; MPO, Myeloperoxidase.

Table (2): Effect of different treatments on inflammation involved proteins (COX-2, CASP3) in control and ulcerative colitic rats.

Groups/ Parameters	COX-2 ng/mg	CASP3 ng/mg
G 1 (C)	11.9±1.65	2.36±0.057
G 2 (UC)	38.3±2.96*	5.93±0.07*
G3(UC+Fen-ex)	24.2±1.86**	4.17±0.74**
G4 (UC+Fen-oil)	24.9±2.65**	3.10±0.02**
G5 (UC+Car-ex)	23.4 ±2.77**	3.80±0.2**
G6 (UC+Car-oil)	14.2±2.36**	4.7±0.16**

Note: **means ±SD are significant (P<0.01) compared with normal control group (1), **means ±SD are significant (P<0.01) compared with Ulcerative colitis group (2).*

Abbreviation: COX-2, Cyclooxygenase-2*;* CASP3, caspase -3.

Acetic acid affects levels of oxidative enzymes (Table 3), this resulted in a significant ($p< 0.01$) increment of PC and MDA as compared to control group (Table 3). Whereas, a significant reduction was observed in SOD level of colitic rat group. For SOD levels, comparing treatment groups, best result was obtained in G6 (colitic rats treated with caraway oil) which inceased SOD level significantly ($p< 0.01$), bring it near normal level. Treatment of colitic rats with fenugreek extract (G2) did not reduce the elevated levels of PC significantly, while other treatments (Fen-oil), (Car-ex) and (Car-oil) could reduce it. For MDA, all treatments could reduce elevated MDA level significantly ($p< 0.01$). In general, fenugreek oil has the most potent effect on oxidative stress, followed by caraway extract.

Table (3): Effect of different treatments on oxidative biomarkers (SOD,PC,MDA) and DNA fragmentation in control and ulcerative colitic rats.

Groups/ Parameters	SOD U/mg	PC Nmol/mg	MDA Nmol/mg
G 1 (C)	4.73±0.45	3.48±0.04	2.20±0.46
G 2 (UC)	2.34±0.2*	8.07±0.45*	6.10±0.23*
G3(UC+Fen-ex)	3.16±0.85**	8.16±0.45*	4.55±0.40**
G4(UC+Fen-oil)	3.06±0.12*	6.02±0.11**	4.10±0.23**
G5 (UC+Car-ex)	3.20±0.24*	6.24±0.38**	5.05±0.58**
G6 (UC+Car-oil)	4.01±0.11**	6.70±0.21**	4.75±0.59**

Note: **means ±SD are significant (P<0.01) compared with normal control group (1), **means ±SD are significant (P<0.01) compared with Ulcerative colitis group (2).*

Abbreviation: *MDA,* malondialdehyde*; PC, protein carbonyl; SOD, supeoxide dismutase.*

The present study was supported by determination of DNA fragmentation level as shown in Fig. (1). It was shown that UC causes the maximum DNA fragmentation rate as compared to experimental groups. On the other hand, supplementation with fenugreek and carawy aqueous and oil extracts protect DNA from damage.

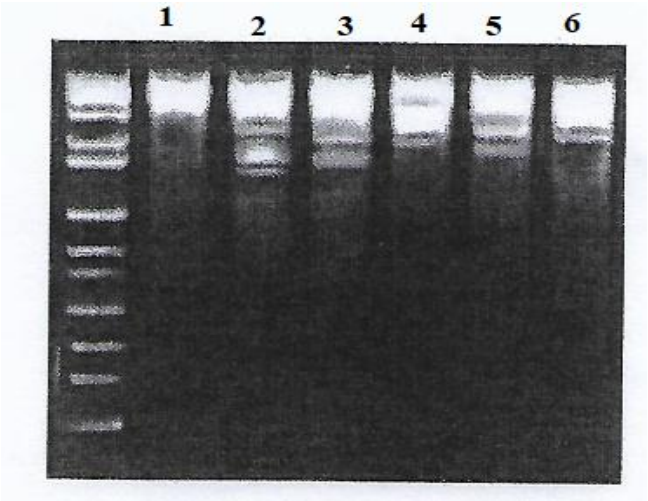

Figure 1: DNA fragmentation analysis by agarose gel electrophoresis. Lanes show results from 1000 bp marker, lane 1: Control group 99% fraction, lane 2: Ulcerative colitis group 45% fraction, lane 3: (UC+fen-ex) group 62% fraction, lane 4: (UC+fen-oil) group 89% fraction, lane 5: (UC+Car-ex) group 54% fraction, lane 6: (UC+Car-oil) group 65% fraction.

Microscopic examination Results

Microscopically, colon of control, untreated rat showed the normal histological layers (mucosa, submucosa, musculosa and serosa) (Figures. 2, A & B). In contrary, colon of rats

from group 2 (UC) rats, revealed ulcerative colitis with mucosal necrosis associated and submucosal oedema and cells infiltration (Figures. 3, C & D). Meanwhile, colon of rats from group 3 (UC+ Fen-ex) showed focal necrosis of the mucosa and submucosal inflammatory cells infiltration (Figs. 4, E&F) and mucosal inflammatory cells infiltration. Examined sections from group 4 (UC+Fen-oil) revealed improved picture as examined sections showed submucosal oedema and inflammatory cells infiltration (Figures.5 J&H) as well as mucosal and submucosal inflammatory cells infiltration. Moreover, sections from group 5 (UC+ Car-ex) revealed only mucosal and submucosal inflammatory cells infiltration (Figures. 6, I & J). On the other hand, colon of rats from group 6 (UC+ Car-oil) showed mucosal necrosis accompanied with with mucosal and submucosal cellular infiltration (Figures. 7, K & L).

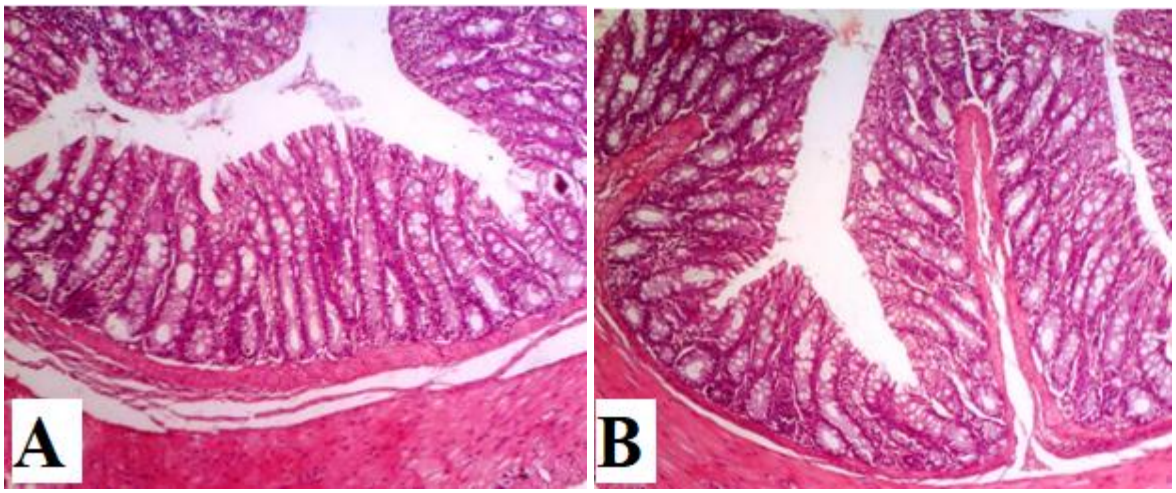

Figure 2 (A & B): Colonic Histopathological examination of (Control) group showing normal histological layers (mucosa, submucosa, musculosa and serosa) (H & E X 100).

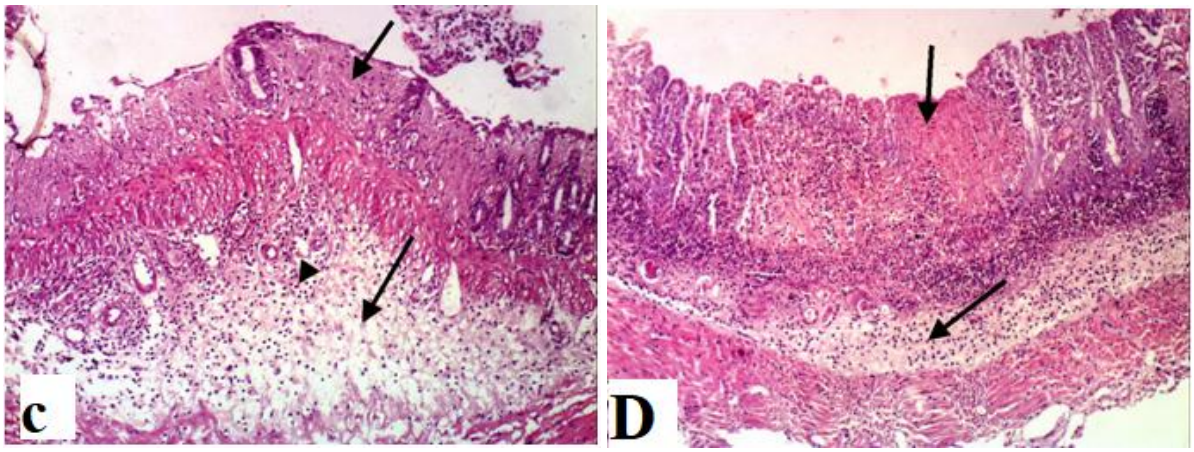

Figure 3 (C & D): Colonic Histopathological examination of (Ulcerative collitic) group showing ulcerative colitis. Notice marked necrosis of the mucosa associated with inflammatory cells infiltration in the mucosa and submucosa (H & E X 100).

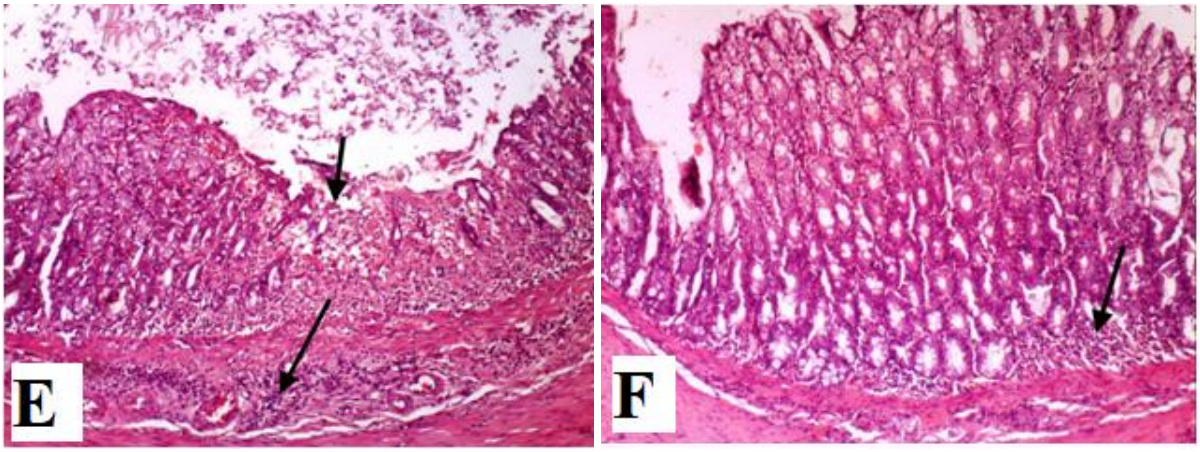

Figure 4 (E & F): Colonic Histopathological examination of (UC+Fen-ex) group showing moderate necrosis, mild mucosal inflammatory cells infiltration (H & E X 100).

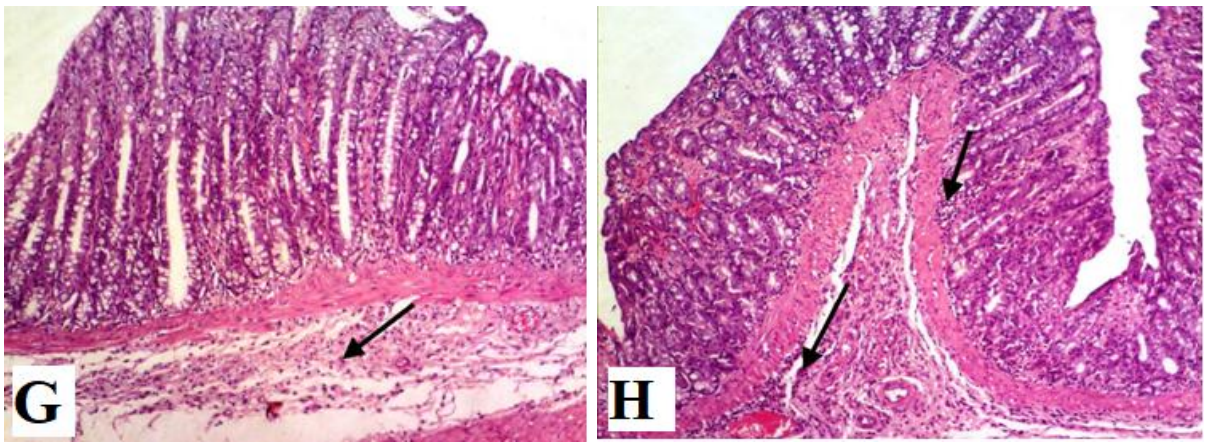

Figure 5 (G & H): Colonic Histopathological examination of (UC+Fen-oil) group, slight improvement, no necrosis appear, showing mild mucosal and moderate submucosal inflammatory cells infiltration (H & E X 100).

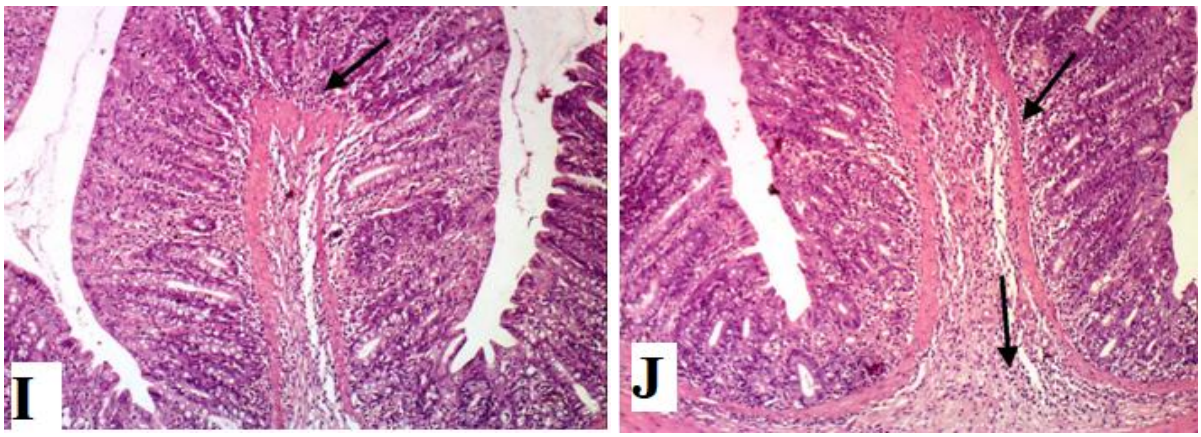

Figure 6 (I & J): Colonic Histopathological examination of (UC+Car-ex) group, slight improvement, no necrosis appear showing mild mucosal and moderate submucosal inflammatory cells infiltration, (H & E X 100).

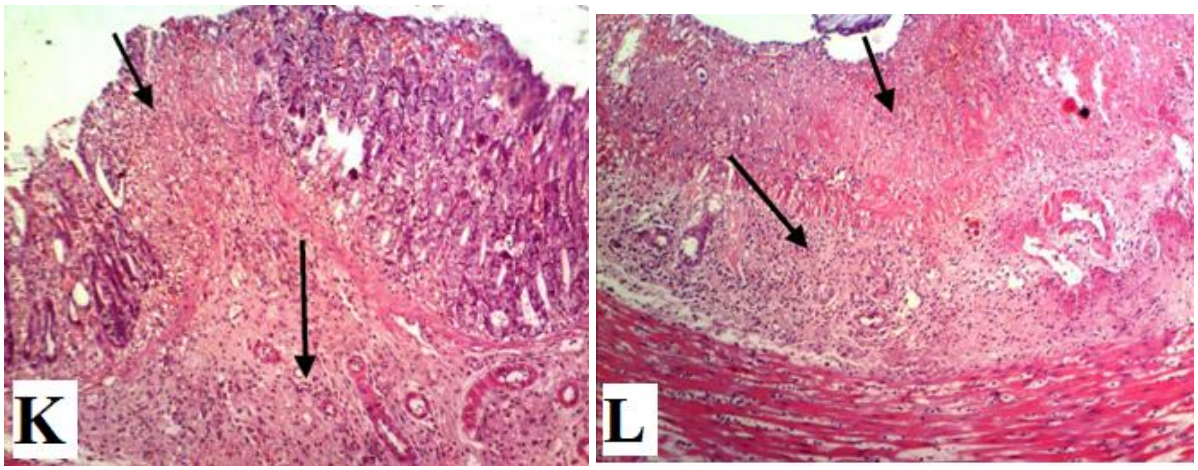

Figure 7 (K & L): Colonic Histopathological examination of (UC+Car-oil) group, showing mild necrosis of the mucosa associated with inflammatory cells infiltration in the mucosa and submucosa (H & E X 100).

DISCUSSION

This study compares the intestinal anti-inflammatory and antioxidant activity of fenugreek and caraway aqueous and oil extracts in the acetic acid model of rat colitis. Noticeable protective effect was shown as aresult of fenugreek and caraway administration either in form of aqueous or oil extract that was manifested by reduction of colon inflammation, ulcers, and colitis score.

Different studies indicated medicinal plant usage considered a new advance for the management of experimentally induced ulcerative colitis.[18,19,20] They revealed that the acetic acid increased colonic inflammatory markers and oxidative enzymes levels, with a reduction in colonic SOD levels as compared to the healthy rats. In addition, the acetic acid disturbs the functions of the immune cells that leads to improper immune reactions**.**

Proinflammatory cytokines are concerned with the incedince of ulcerative colitis. Furthermore, treatments could concentrate on reducing proinflammatory cytokines this might be could be helpful for the avoidance of ulcerative colitis.[21] In the present study, supplementing Fenugreek or Caraway either in form of seed oil or aquous extract mitigated the harmful effect of acetic acid. Comparing results obtained from different treatments revealed that, Fenugreek extract posses the most potent reducing action for PGE2, IL-1 ß, CASP3 and MPO. While for COX-2 the most potent reducing action was seen in caraway oil group.[22]

Caraway is rich in terpenoid, flavonoids, fatty acids, triacylglycerols, polysaccharides, lignin and polyacetylenic compounds that can reduce inflammatory cytokines and chemokines.[23] It seems that caraway is anticolitis with reducing the production of PGE2 in human polymorphonuclear leucocytes.[24] The most present component of *C. carvi* which is known as an anti-inflammatory agent is carvone. Carvone inhibits 5-lipoxigenase and cyclooxygenase activity so it can decrease biosynthesis of leucotriens and prostaglandins. This is due to carvone and limonene which considered as mucoprotective and have antiulcerogenic effects.

Meanwhile, The oil fraction of caraway seeds contains considerable amounts of oxygenated monoterpenes. Caraway aldehyde was found to be the main component of seed oil (53.6%) as well as of herb oil (40.5%).[25] Khayyal and coworkers demonstrated that different Caraway extract produced anti-ulcerogenic effect against indomethacin-induced gastric ulcer accompanied by reduction in gastric acid output and leukotriens synthesis while mucus secretion and prostaglandin E2 release were enhanced. This was attributed to the radical scavenging,and spasmolytic effects.[26]

With regard to its protective effects, animal studies in colitis have shown that saponin diosgenin, a compound in fenugreek, suppresses inflammation. Fenugreek rich in Diosgenin that found to suppress gut inflammation. It also down-regulated different inflammatory biomarkers.[27]

Ulcerative colitis caused oxidative stress accompanied by elevated amounts of free radicals that leads to mucosal injuries and harming of cell membrane, and apoptosis leading to lipid peroxidation and increasing MDA levels.[28] Results of this study revealed that all treatments could affect antioxidant status in different manners, while caraway oil affects superoxide scavenging power while caraway extract came in the second position, on the other hand, fenugreek oil had a powerful free radical scavenging power, as compared to other treatments. Caraway seeds oil proved to have potent therapeutic effects.[29] This due to its antioxidative and radical scavenging properties and considered effective in prevention and treatment of oxidative stress associated diseases.[30] The beneficial activity of *C. carvi* could also be attributed to its antioxidant properties of the caraway phenolic compounds.[31] It has known that the major compounds occurring in caraway are α-pinene carvacrol, γ-terpinene, carvone, limonene, linalool, fenchen carvenone, *p*-cymene, carveol and camphene.[32]

The study showed that fenugreek oil had a powerful free radical scavenging power, as compared to other treatments. Also, supplemetation with fenugreek extracts reduces the incidence of colon cancer and restrain antioxidant enzyeme levels.[33] Fenugreek seed oil has been shown to counter the increased lipid peroxidation and alterations in the content of circulating antioxidant molecules.[34] The protective effects of extracts may due to their high lipid solubility similar to volatile oil constituents of seed oil that could be absorbed readily from gastrointestinal tract and considered effective on experimentally induced colitis.[21]

Kaviarasan et al[35] also found the polyphenolic compounds of fenugreek seeds to have cytoprotective effect during ethanol-induced liver damage in Chang liver cells. Astudy by Kaviarasan et al[36] reported signficantly reduced levels of lipid peroxidation products and protein carbonyl content, increased activities of antioxidant enzymes, and restoring levels of thiol groups by administration of polyphenol extract of fenugreek seed to ethanol-fed rats.

Histopathological studies confirmed that necrosis and severity of inflammation were due to the harmful effects of acetic acid on the colonic mucosa, this alter the functions of the epithelium. Such alterations in the intestinal barrier role by mucosal cells and their products during experimental colitis were reported.[37] Corroborating these results, the histological analysis of the colon tissues revealed that animals treated with fenugreek and caraway either in form of aqueous extract or oil represents less alterations as compared to acetic acid treated group.

Results in figure (1) showed that acetic acid induced DNA oxidadive damage, which was modified by fenugreek supplementation either in form of aqueous extract or oil, a study by Xue et al[38] revealed that aqueous extract of fenugreek provided protection against functional and morphologic injuries in the kidneys of diabetic rats by increasing the activities of antioxidants and inhibiting accumulation of oxidized DNA in the kidney. Fenugreek seed oil is rich in active compounds known as terpenenes, these nutrients, along with antioxidants, increase the ability to fight cancer tumors.[39] On the other hand, the clear effect of caraway on DNA fragmentation occurs due to its properties.

IN CONCLUSION

Results demonstrated that fenugreek and caraway either in form of aqueous extract or seed oil exerted a defensive and protective effects against acetic acid induced colitis in rats probably throughout their antiinflammatory and antioxidant properties, as evidenced by efficient

modulation of the severity and degree of colon injury beside the histopathological and genetic lessening of the alterations caused by acetic acid.

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