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Possible Beneficial Effects of Chia *(Salvia hispanica L.)* and Anise *(Pimpinella anisum L.)* Seeds Oils on Ulcerative Colitis Rat Model

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ABSTRACT

Ulcerative colitis (UC) is one of the inflammatory bowel diseases that have lethal symptoms affecting human health. In this study; the amount of some bioactive constituents of cold pressed chia and anise seeds oils were determined. Furthermore their efficacy alone or in combination to compete with induced UC (30 mg of 2,4-dinitrobenzenesulfonic acid; given intrarectally at the 15th day of the trial) was examined. Fifty five healthy adult male (Sprague -Dawely) rats were divided into five groups; 10 rats each, except UC control group that composed of 15 rats. Group (1): Healthy control, group (2): UC control, while groups (3-5): UC rats supplemented with 1 ml /kg body weight of chia seeds oil, anise seeds oil and 0.5ml of each oil respectively intragastrically for 30 days. Oils chemical analysis revealed that they contain significant amounts of polyphenols, flavonoids and have a strong antioxidant activity. But chia seeds oil has higher contents. UC markedly ($p\leq0.01$) decreased body weight, colon length, red blood cells and platelets counts as well as hemoglobin content with significant ($p\leq0.01$) increase in white blood cells count and colon weight. Increased ($p\leq0.01$) colonic oxidative stress parameters initiated inflammatory cytokines and nuclear factor kappa B **(**NF-κB) production. Also immune response was detected by increased myeloperoxidase (MPO) activity. All these biochemical alterations caused significant reduction ($p\leq0.01$) in colonic antioxidant parameters. Also decreased deoxyribonucleic acid (DNA) fragmentation, apoptosis ($p\leq0.01$) and increased oxidative DNA damage ($p\leq0.01$) initiating tumor formation was indicated. Microscopic examination and records confirmed colonic tissue inflammation and ulceration. On the flip side treatment of rat's colitis by tested oils ameliorated all these investigations and alleviated colitis. The most significant improvements were recorded in the fifth group that supplemented with both oils; followed by treated chia seeds oil group and finally treated anise seeds oil group.

Key Words: Chia- Anise-Oils- Ulcerative Colitis- NF-κB -DNA Fragmentation – Apoptosis.

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are two digestive system disorders .They are known as inflammatory bowel diseases (IBDs). UC is considered as one of the modern

common inflammatory diseases. UC is a multi-factorial disease and its pathogenesis is not clearly understood. UC main symptoms include abdominal pain, diarrhea and bloody mucopurulent stool. If UC is not treated in a timely manner, it may be associated with a high risk of colon cancer ***(Hazel and O'Connor, 2020).***

UC drugs treatment mostly interfere with metabolism and immune responses, resulting in some serious adverse reactions. Alternative treatments, including nutritional supplements, have been given more attention due to fewer side effects ***(Wang et al., 2019).***

Chia *(Salvia hispanica L.)* belongs to *Lamiaceae* family. Chia is a small seed that comes from an annual herbaceous plant, *Salvia hispanica L*. Chia seeds use has tremendously grown as a result of their high nutritional and medicinal values. Chia seeds contain healthy omega-3 fatty acids, polyunsaturated fatty acids, proteins, dietary fiber, vitamins, and some minerals. The seeds are an excellent source of polyphenols and antioxidants, such as caffeic acid, rosmarinic acid, quercetin, myricetin, and others ***(Hrnčič et al., 2020).***

Chia oil is one of the most valuable oils. Different extraction methods have been used to produce chia oil. Varying quality parameters of the oil like percentage yield, purity, fatty acids content, preservation of antioxidant content, and functionality of the oil depend on different extraction methods. These extraction methods include cold pressing, vacuum steam distillation, solvent extraction and supercritical fluids extraction. Cold pressed chia seed oils are purer with better aroma and nutritive value ***(Akinfenwa et al., 2020).***

Anise *(Pimpinella anisum L.)*, is an annual important spice and medicinal plant related to the family *Apiaceae (Umbelliferae)* .Anise seeds are an important natural raw material which is used for pharmaceutics, food, perfumery, as well as cosmetic industries . Anise has antioxidative, anti-inflammatory, anticancer, anti-hemolytic, anti-hyperglycemic and hypolipidemic activities ***(El-Rokiek et al., 2020).***

Anise seeds contain a variety of nutrients and active components including fat, protein, minerals, vitamins and antioxidants. Anise seeds contain essential oils and fatty acids. The main component of essential oils is anethol that stimulates the secretion of gastrointestinal enzyme and appetite as well as biologically inhibits bacteria. The taste and smell of anise are mainly due to the essential oil, which is 80-90% trans-anethole, with other components consisting of cis-anethole, safrole, p-anisaldehyde, estragole, linalool, anisketone, and b-farnesene ***(Helal et al., 2019).***

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This research aimed to investigate the possible beneficial effects of chia and anise seeds oils alone or in combination on ulcerative colitis induced by 2,4-dinitrobenzenesulfonic acid in male rats.

MATERIALS AND METHODS

Materials

Plants

Chia seeds *(Salvia hispanica L.)* and anise seeds *(Pimpinella anisum L.)* were purchased from Ministry of Agriculture, Cairo, Egypt.

Chemicals

DNBS (2,4-dinitrobenzenesulfonic acid) was purchased from Sigma Aldrich Chemical Co. (St. Louis, Missouri, USA). Ethanol (50%) and all other chemicals were of high analytical grade and purchased from El-Gomhoria Company for Chemicals, Cairo, Egypt.

Animals

Fifty five healthy adult male white albino rats (Sprague -Dawely- strain) weighing (200±5) g were used. Animals supplied from Breading Unit of Animal Reproduction Research Institute, Giza, Egypt. All animal experimentations were carried out in conformity with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines and were approved by the Institutional Animal Ethics Committee.

Diet

Balanced diet were prepared according to American Institute of Nutrition AIN-93*M* and adjusted by (***Reeves et al., 1993*).**

Methods

Preparation of oil seeds extracts

Chia and anise seeds were cleaned manually and their oils were extracted by pressing methods on cold in Oil Extraction Unit, National Research Center, Dokki, Giza, Egypt. Oils were given orally at dose level of (1ml/kg body weight).

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Determination of total poly phenols, total flavonoids and total anti-oxidant activity in seeds oils

The total phenolic and flavonoids contents were determined according to the Folin-Ciocalteu procedure ***(Zilic et al., 2012).*** Total anti-oxidant activity in seeds oils were determined using the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) (***Hwang and Do Thi, 2014).*** The experiments were repeated in triplicate.

Induction of colitis

For colitis induction ***Fornai et al. (2006)*** method with minor changes was used. Rats were fasted overnight with free access to water. Rats were anesthetized using sodium barbiturate and given a single dose of (30 mg DNBS; dissolved in 0.25 mL of 50% ethanol) intrarectally administered via a polyethylene PE-60 catheter inserted 7 cm proximal to the anus. Control rats received 0.25 mL of saline solution.

Experimental design

All rats were individually housed with constant controlled environments in stainless steel cages and fed on the balanced diet with drinking water *ad libitum* for 7 days to be acclimatized. Animals were classified randomly into 5 groups of ten animals each except ulcerative control group that composed of 15 rats as follow:

Group (1) HCG: Healthy control group, rats were given distilled water daily orally and injected with a single saline dose intrarectally on the 15th day of the experiment.
Group (2) UCCG: Ulcerative colitis control group, rats were given distilled water daily orally and injected with a single DNBS dose (30mg) intrarectally on the 15th day of the experiment**.**
Group (3) UC+CO: Ulcerative colitis rats supplemented with chia seeds oil, rats were given chia seeds oil(1ml/kg) daily orally and injected with a single DNBS dose (30mg) intrarectally on the 15th day of the experiment**.**
Group (4) UC+AO: Ulcerative colitis rats supplemented with anise seeds oil, rats were given anise seeds oil (1ml/kg) daily orally and injected with a single DNBS dose (30mg) intrarectally on the 15th day of the experiment.
Group (5) UC+CO+AO: Ulcerative colitis rats supplemented with chia and anise seeds oil, rats were given chia(0.5ml/kg) and anise(0.5ml/kg) seeds oil daily orally and injected with a single DNBS dose (30mg) intrarectally on the 15th day of the experiment.

All rats were given oil supplements during all the experiment period (30 days), then rats were sacrificed under deep anesthesia. Whole blood samples were collected and colon was separated from all rats.

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Determination of body weight, colon weight and length

Rats were weighed weekly to monitor body weight changes. Colon tissues were removed starting from rectum, longitudinally opened and cleared of fecal remains if present, using 0.9% saline, dried then weighted using electrical balance. The colon length was measured using graduated tap.

Handling of colon samples

Random colon samples were fixed in 10% neutral buffered formalin for microscopic examination and the remaining colon samples were prepared to form tissue homogenate for other analysis. Colon tissues were rinsed in ice-cold phosphate buffered saline PBS (0.02mol/L, pH 7.0-7.2) to remove excess blood thoroughly. Tissues minced to small pieces and homogenized in 500µl of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultra-sonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifuged for 15 minutes at 1500×g. Supernatant was removed and assayed immediately for all biochemical analysis. While colon tissue slices washed in 0.01MPBS; added to tissue protein extraction reagent according to proportion of 1g: 5-10ml and mixed in ice water. After being blended, mixture centrifuged for 10min at 5000-10000rpm. Supernatant tested immediately for nuclear factor kappa B **(**NF-κB).

Biochemical analysis

Complete blood picture (CBC) in whole blood was determined according to ***Dacie and Lewis (1948).*** The colon content of advanced glycation end products (AGEs) and protein carbonyl group (PCG) were determined according to ***Koschinsky et al. (1997)*** and ***Hopps et al. (2014)*** using enzyme linked immunosorbent assay (ELISA) kits of Mybiosource, USA. Nitric oxide (NO) , malondialdehyde (MDA) levels, total antioxidant capacity (TAC) and reduced glutathione (GSH) content in colon tissue homogenate were determined according to ***Montgomery and Dymock.(1961), ohkawa et al.(1979), koracevic et al. (2001) and Beutler et al. (1963)*** respectively using Bio diagnostic kits, Giza, Egypt. Colonic DNA oxidative marker 8-hydroxydeoxyguanosine (8-OHdG), tumor necrosis factor -α (TNF-α), interlukine-1β (IL-1β), interlukine-10 (IL-10), nuclear factor kappa B **(**NF-κB) levels and myeloperoxidase (MPO) activity were determined in colon tissue homogenate using ELISA kits from koma biotech, Korea, Mybiosource, USA and Ray Biotech, USA respectively. DAN fragmentation and caspase-9 gene were determine in colon tissues ***(Tribukait et al., 1975)***.

Microscopic examination of colon tissues

Colon specimens were collected from all rats/ group and then fixed in 10% neutral buffered formalin. Paraffin sections of 50 and 200µm thickness were prepared and stained with hematoxylin and eosin (H&E) and then examined by a light microscope (Olympus BX50, Japan) ***(Suvarna et***

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al., 2018). Histopathological damage in the colonic tissues were graded from (0-4) as follow: (0) indicated no changes; (1) indicated percentage area affected (<10%); (2) indicated percentage area affected (20-30%); (3) indicated percentage area affected (40-60%) and (4) indicated percentage area affected (>60%) ***(Thoresen et al., 1996).***

Statistical analysis

Data were statistically analyzed by Statistical Package for Social Science (Version 20). The significance of differences between more than two groups was evaluated by one way analysis. Values were presented as mean ± standard deviation (S.D.). Statistical Differences between groups were performed using one way ANOVA, the mean difference was significant at ($P \leq 0.01$) level according to ***(Levesque, 2007).***

RESULTS

Total polyphenols, total flavonoids contents and total antioxidant activity of chia and anise seeds oils

The result in table (1) illustrated that each 1 g of the tested chia seeds oil contains 28.84 mg as gallic acid equivalent of total polyphenols, 1.75 mg as catechin equivalent of total flavonoids and 7.37 mg as trolox equivalent of total anti-oxidant capacity while each 1 g of the tested anise seeds oil contains 5.63 mg as gallic acid equivalent of total poly phenols, 0.908 mg as catechin equivalent of total flavonoids and 3.06 mg as trolox equivalent of total anti-oxidant capacity, from these results it is found that chia seeds oil contains higher phenolic , flavonoids and antioxidant activity than anise seeds oil .

Table (1): Total poly phenols, total flavonoids contents and antioxidant activity of chia and anise seeds oils

Oil	Total Polyphenols Content (mg GAE/g)	Total Flavonoids Content (mg CE/g)	Antioxidant Activity (mg TE/g)
Chia seeds	**28.84**	**1.75**	**7.37**
Anise seeds	**5.63**	**0.908**	**3.06**

Effect of chia and/or anise seeds oils supplementation on body weight, colon weight and length in healthy control and ulcerative colitic rats

Data for body weight, colon weight and colon length were illustrated in Fig. 1(A-C). In regard to body weight, the rat body weight increased normally and regularly up to the 15^{th} day of the experiment in all groups while on the 30^{th} day (i.e; after colitis induction by 15 days), the body weight decreased significantly ($p \leq 0.01$) in all diseased groups in comparison with healthy control group that continued in increasing body weight normally . Supplementation with oils improved

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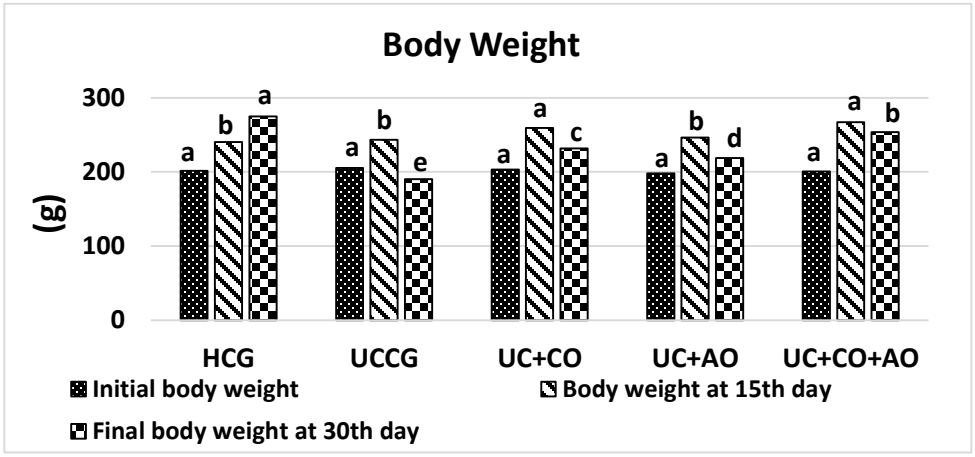
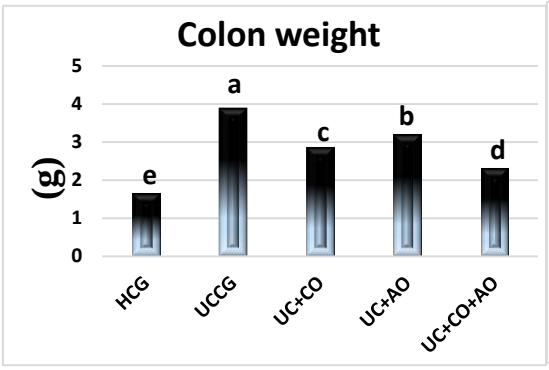
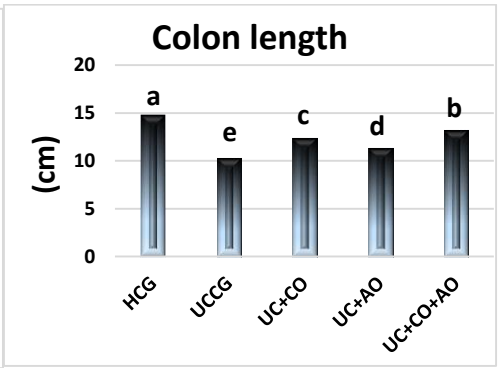
and controlled body weight decrement significantly ($p \leq 0.01$) in comparison with ulcerative colitis control group. Colon weights of ulcerative colitis control group were significantly ($p \leq 0.01$) increased in comparison with healthy control group while supplementation with each oil alone or in combination improved these results significantly ($p \leq 0.01$). Ulcerative colitis significantly ($p \leq 0.01$) reduced colon length in ulcerative colitis control group in comparison with healthy control group. On contrary, oil active constituents preserved and improved the colon length. The most significant improvements were found in the group that supplemented with both oils.



1(A)





1(B) 1(C)

Fig. 1: Effect of chia and/or anise seeds oils supplementation on (A): body weight, (B): colon weight and (C): colon length in healthy control and ulcerative colitic rats. Values are expressed as means ±standard deviation, n=10. Column of histogram with different letter is significantly different at ($p \leq 0.01$) .HCG: Healthy control group, UCCG: Ulcerative colitis control group, CO: Chia seed oil, AO: Anise seed oil.

Impact of chia and/or anise seeds oils supplementation on complete blood picture in healthy control and ulcerative colitic rats

Results tabulated in table (2) illustrated that ulcerative colitis induced bleeding and inflammation in diseased rats that caused significant ($p \leq 0.01$) decrease in red blood cells (RBC's)and platelets (PLT)counts as well as hemoglobin (Hb) content with significant ($p \leq 0.01$)

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increase in white blood cells (WBC's) count in comparison with healthy control group . Supplementation with chia and anise seeds oils significantly ($p \leq 0.01$) improved the CBC results. The most significant amelioration was found in the fifth group that supplemented with both oils.

Table (2): Impact of chia and/or anise seeds oils supplementation on complete blood picture in healthy control and ulcerative colitic rats

Parameter / Group	RBC's (10^6/µl)	PLT (10^3/µl)	Hb (g/dl)	WBC's (10^3/µl)
HCG	11.18±0.83[a]	894.97±2.87[a]	13.96±0.69[a]	10.17±0.45[e]
UCCG	6.53±0.36[e]	567.25±1.41[e]	4.16±0.23[e]	21.83±0.76[a]
UC+CO	8.41±0.26[c]	683.71±1.90[c]	8.79±0.48[c]	17.72±0.61[c]
UC+AO	7.83±0.52[d]	613.89±1.08[d]	6.80±0.35[d]	19.65±0.29[b]
UC+CO+AO	10.07±0.39[b]	781.12±2.09[b]	11.52±0.58[b]	14.53±0.31[d]

There is no significant difference between means have the same letters in the same column, n= 10 rats, ($P \leq 0.01$).
HCG: Healthy control group, UCCG: Ulcerative colitis control group, CO: Chia seed oil, AO: Anise seed oil.

Effect of chia and/or anise seeds oils supplementation on advanced glycation end products (AGEs), protein carbonyl group (PCG), nitric oxide (NO) and malondialdehyde (MDA) levels in healthy control and ulcerative colitic rat's colon.

Data showed in **table (3)** magnified that induction of colitis in rats caused a state of oxidative stress, leading to a massive increase ($P \leq 0.01$) in the levels of oxidative biomarkers like AGEs, PCG, NO and MDA in ulcerative colitis control group colon when compared to healthy control group. On the other hand, supplementation with chia and anise seeds oils caused noticeable decrement ($P \leq 0.01$) in the levels of oxidative biomarkers in ulcerative colitic rats' colon when compared with ulcerative control group. It was noticed that the most significant improvements ($P \leq 0.01$) were recorded in the fifth group that supplemented with both oils.

Table (3): Effect of chia and/or anise seeds oils supplementation on AGEs, PCG, NO and MDA Levels in healthy control and ulcerative colitic rats

Parameter / Group	AGEs (ng/mg)	PCG (ng/mg)	NO (µmol/g)	MDA (µmol/g)
HCG	1.46±0.05[e]	2.16±0.16[e]	5.43±0.79[e]	3.88±0.16[e]
UCCG	27.91±1.86[a]	10.43±0.75[a]	32.15±1.61[a]	19.80±1.13[a]
UC+CO	15.65±0.37[c]	5.22±0.27[c]	20.64±0.36[c]	7.48±0.44[c]
UC+AO	21.09±0.60[b]	7.81±0.68[b]	29.59±1.23[b]	13.51±0.85[b]
UC+CO+AO	11.32±0.72[d]	4.19±0.32[d]	17.60±0.72[d]	5.89±0.39[d]

There is no significant difference between means have the same letters in the same column, n= 10 rats, ($P \leq 0.01$).
HCG: Healthy control group, UCCG: Ulcerative colitis control group, CO: Chia seed oil, AO: Anise seed oil.

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Impact of chia and/or anise seeds oils supplementation on colonic reduced glutathione (GSH) level and total antioxidant capacity (TAC) in healthy control and ulcerative colitic rats

Table (4) clarifies that colonic GSH level and TAC were significantly ($P \leq 0.01$) decreased in ulcerative colitis control group when compared with healthy control group. It was understandable from the results that chia and anise seeds oils contain antioxidants that caused a detectable enhancement ($P \leq 0.01$) in colonic antioxidant parameters of ulcerative colitic supplemented groups when compared with ulcerative colitis control group. The synergistic effect of both oils caused the most improving action on colonic antioxidant status.

Table (4): Impact of chia and/or anise seeds oils supplementation on colonic GSH Level and TAC in healthy control and ulcerative colitic rats

Parameter / Group	GSH (mg/g)	TAC (mmol/g)
HCG	8.65±1.07[a]	28.54±1.35[a]
UCCG	2.04±0.36[e]	11.73±0.48[e]
UC+CO	6.78±0.49[c]	17.98±0.72[c]
UC+AO	3.47±0.21[d]	14.14±0.51[d]
UC+CO+AO	7.12±0.68[b]	22.61±1.02[b]

There is no significant difference between means have the same letters in the same column, n= 10 rats, ($P \leq 0.01$).
HCG: Healthy control group, UCCG: Ulcerative colitis control group, CO: Chia seed oil, AO: Anise seed oil.

Effect of chia and/or anise seeds oils supplementation on colonic tumor necrosis factor -α (TNF-α), interlukine-1β (IL-1β), interlukine-10 (IL-10), nuclear factor kappa B (NF-κB) levels and myeloperoxidase (MPO) activity in healthy control and ulcerative colitic rats

Results in Fig. 2(A-C) illustrated that ulcerative colitis induced an inflammatory status in colonic tissue resulted in significant ($P \leq 0.01$) increase in TNF-α, IL-1β, IL-10, NF-κB levels and MPO activity in ulcerative colitis control group in comparison with healthy control group. On contrary chia and anise seeds oils prohibited inflammatory cascades and pathways causing significant ($P \leq 0.01$) decrease in TNF-α, IL-1β, IL-10, NF-κB levels and MPO activity in supplemented groups in comparison with ulcerative colitis control group. The most significant improvements were recorded in the fifth group that supplemented with both oils.

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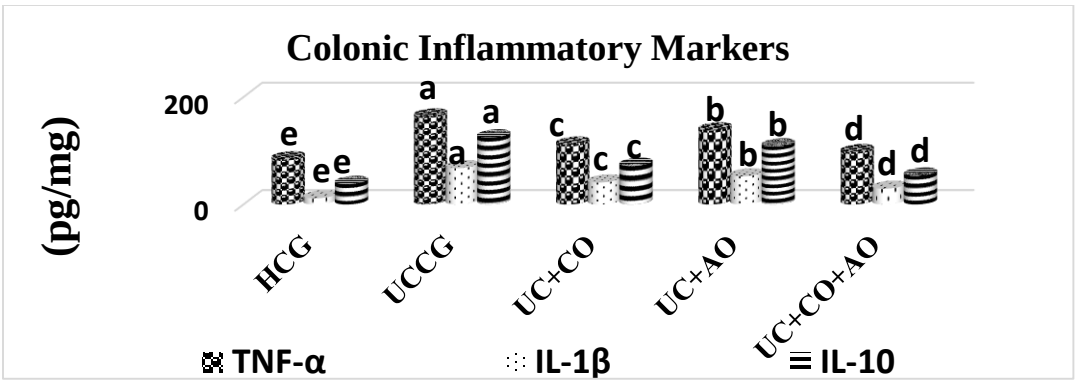

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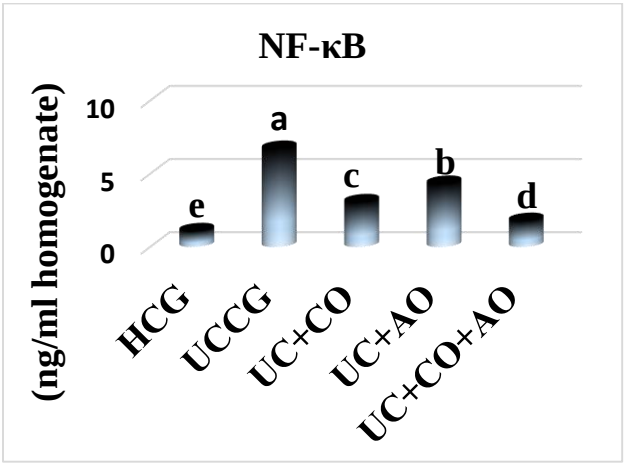

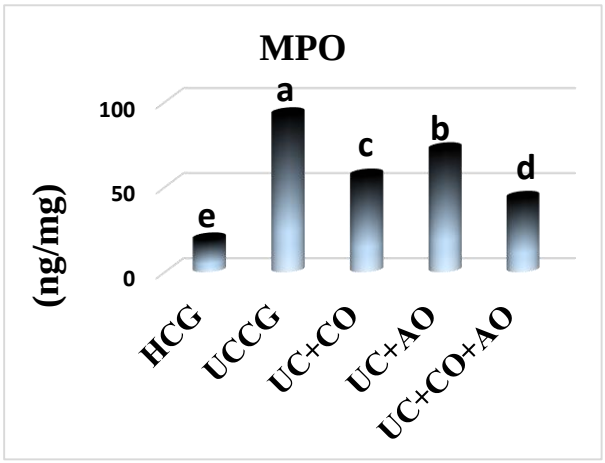

2(B) 2(C)

Fig. 2: Effect of chia and/or anise seeds oils supplementation on (A): colonic tumor necrosis factor -α (TNF-α), interlukine-1β (IL-1β) and interlukine-10 (IL-10) levels, (B): colonic nuclear factor kappa B (NF-κB) level and (C): colonic myeloperoxidase (MPO) activity in healthy control and ulcerative colitic rats. Values are expressed as means ±standard deviation, n=10. Column of histogram with different letter is significantly different at (p≤0.01). HCG: Healthy control group, UCCG: Ulcerative colitis control group, CO: Chia seed oil, AO: Anise seed oil.

Impact of chia and/or anise seeds oils supplementation on colonic DAN fragmentation, caspase-9 gene percent and 8-hydroxydeoxyguanosine (8-OHdG) level in healthy control and ulcerative colitic rats

Results tabulated in table (5) showed that induction of colitis by DNBS in rats caused significant ($P \leq 0.01$) decrease in DAN fragmentation and caspase-9 gene percent with obvious ($P \leq 0.01$) increase in 8-OHdG level in colonic tissues of ulcerative colitis control rats on comparison with healthy rats. Supplementation with oils improved significantly ($P \leq 0.01$) these results in all supplemented groups especially the group that supplemented with oils mixture.

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Table (5): Impact of chia and/or anise seeds oils supplementation on colonic DAN fragmentation, caspase-9 gene percent and 8-OHdG level in healthy control and ulcerative colitic rats

Parameter / Group	DAN fragmentation (%)	Caspase-9 gene (%)	8-OHdG (ng/mg)
HCG	91.68±1.61[a]	14.83±1.13[a]	0.83±0.08[e]
UCCG	38.19±0.23[e]	8.62±0.32[e]	10.64±0.93[a]
UC+CO	69.98±0.74[c]	10.94±0.57[c]	5.24±0.46[c]
UC+AO	51.37±0.29[d]	9.14±0.76[d]	7.36±0.54[b]
UC+CO+AO	86.50±0.51[b]	12.35±0.84[b]	2.18±0.21[d]

There is no significant difference between means have the same letters in the same column, n= 10 rats, ($P \leq 0.01$).
HCG: Healthy control group, UCCG: Ulcerative colitis control group, CO: Chia seed oil, AO: Anise seed oil.

Effect of chia and/or anise seeds oils supplementation on microscopic changes in colon of healthy control and ulcerative colitic rats

Microscopic findings are exhibited in Fig. 3(A-B) of healthy control group colon showed normal histological layers including (mucosa, submucosal, muscularis propria and serosa) without inflammation and necrosis. In contrast microscopic findings in Fig. 4(A-B), revealed that administration of DNBS to ulcerative colitis control group led to sever hemorrhagic enteritis with large diffuse necrotic, hemorrhagic and ulcerated intestinal mucosa with significant loss of glandular elements. Sever submucosal edema and congested blood vessels were shown accompanied with sever inflammatory cells infiltrates from different populations. On the other hand, the administration of tested oils led to the reduction of DNBS -induced colonic damage. The administration of chia seeds oil to ulcerative colitis rats in Fig. 5(A-B) showed moderate mucosal protective efficacy with moderate restoration of some glandular elements alternated with focal necrotic mucosal lesions records, persistence records of submucosal edema as well as inflammatory cells infiltrates .On the flip side ulcerative colitis rats treated with anise seeds oil in Fig. 6(A-B) showed the lesser results records than that of DNBS control group. While, ulcerative colitis rats treated with mixed oils in Fig. 7(A-B) showed significant higher protective efficacy of glandular mucosa with minor records of mucosal erosions or ulceration. Mild inflammatory cells infiltrates were observed accompanied with moderate submucosal edema.

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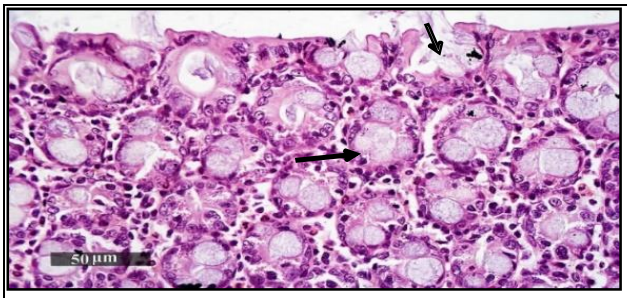

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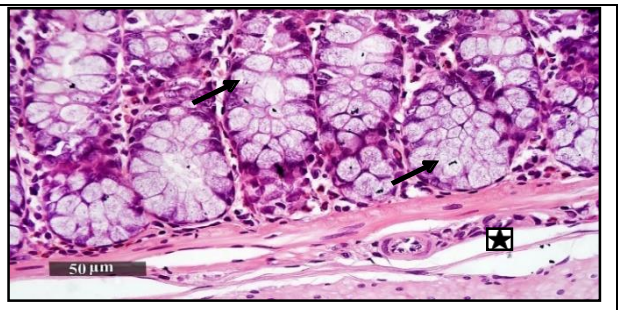
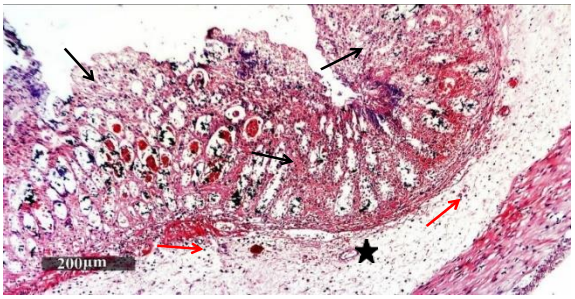
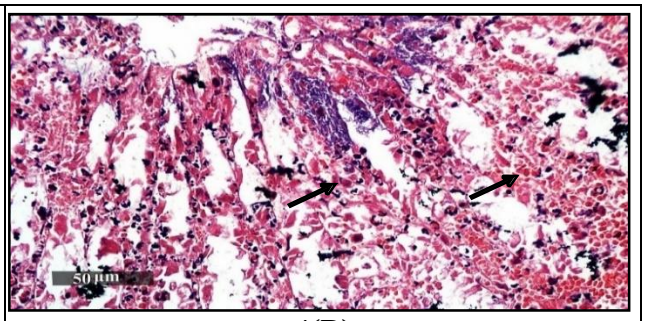


3(B)

Fig. 3 (A-B): Healthy control samples demonstrated normal histological structures of intestinal wall with intact lining mucosa including intestinal crypts with abundant goblet cells and intact enterocytes ***(arrow)***. Intact submucosal layers with minimal inflammatory cells infiltrates ***(star)*** with intact vasculatures as well as outer muscular coat (H & E X 400).

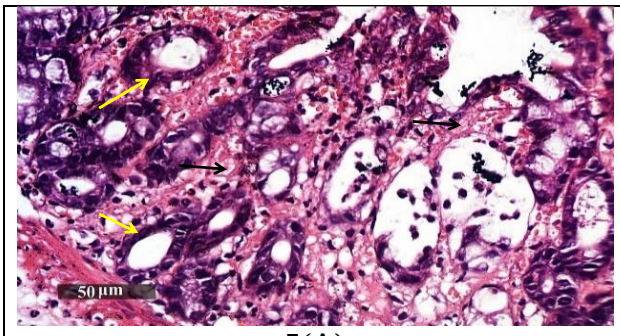


4(A)

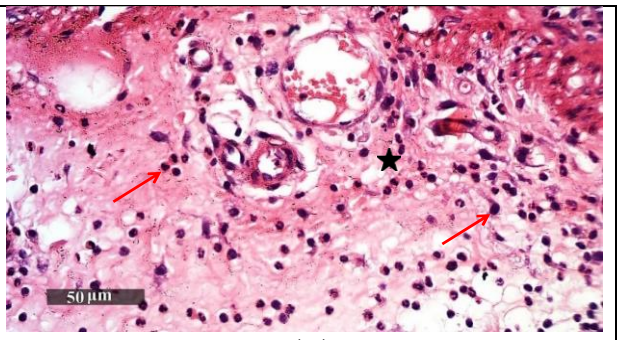


4(B)

Fig. 4 (A-B): Ulcerative colitis control samples showed sever hemorrhagic enteritis with large diffuse necrotic, hemorrhagic and ulcerated intestinal mucosa with significant loss of glandular elements ***(black arrow).*** Sever submucosal edema and congested blood vessels ***(star)*** were shown accompanied with sever inflammatory cells infiltrates from different populations ***(red arrow)*** (H & E X 400)**.**



5(A)



5(B)

Fig. 5 (A-B): Ulcerative colitis rats treated with chia seeds oil colon samples showed moderate mucosal protective efficacy with moderate restoration of some glandular elements ***(yellow arrow)*** alternated with focal necrotic mucosal lesions records ***(black arrow)*** persistence records of submucosal edema ***(star)*** as well as inflammatory cells infiltrates ***(red arrow)*** (H & E X 400)**.**

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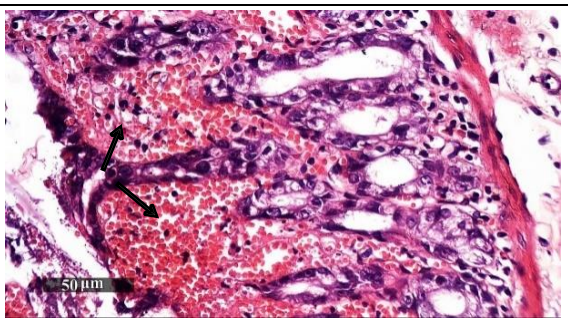
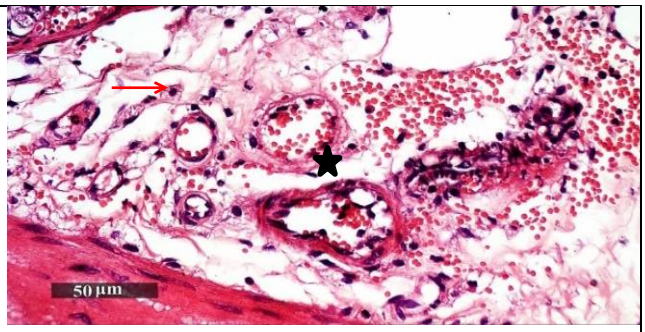
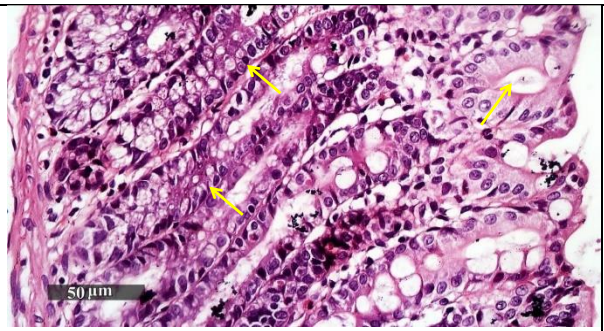


6(A)

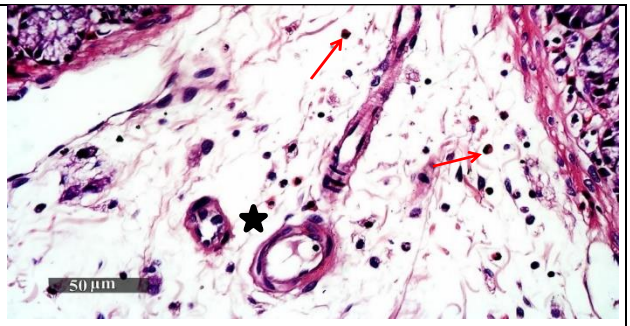


6(B)

Fig. 6 (A-B): Ulcerative colitis rats supplemented with anise seeds oil colon samples showed moderate hemorrhagic enteritis with large diffuse necrotic, hemorrhagic and ulcerated intestinal mucosa with significant loss of glandular elements ***(black arrow).*** Moderate submucosal edema and congested blood vessels ***(star)*** were shown accompanied with sever inflammatory cells infiltrates from different populations ***(red arrow)*** (H & E X 400)**.**



7(A)



7(B)

Fig. 7 (A-C): Ulcerative colitis rats supplemented with chia and anise seeds oil mix colon samples showed significant higher protective efficacy of glandular mucosa ***(yellow arrow)*** with minor records of mucosal erosions or ulceration**.** Mild inflammatory cells infiltrates were observed ***(red arrow)*** accompanied with moderate submucosal edema ***(star)*** (H & E X 400)**.**

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Table (6): Histopathological lesions score in colon tissue of all experimental group

Histopathological lesions	Colon tissue				
	HCG	UCCG	UC+CO	UC+AO	UC+CO+AO
Hemorrhagic enteritis	0	4	0	2	0
Necrotic, hemorrhagic and ulcerated intestinal mucosa	0	4	1	2	0
Congested blood vessels	0	3	0	1	0
Inflammatory cells infiltrates	0	3	1	2	1

DISCUSSION

Nowadays the turning to alternative medicine became a great challenge in the area of biomedicine. Alternative medicine aims to achieve targeted treatment of different diseases with least possible side effects together with low cost. During the current research possible treating effects of chia and/or anise seeds oils on ulcerative colitis induced by 2,4-dinitrobenzenesulfonic acid in male rats was investigated.

Bioactive components of tested oils were determined; the results showed both cold pressed chia seeds oil and anise seeds oil are rich in phenolic compounds, flavonoids and have antioxidant activities, moreover chia seeds oil contains higher phenolic and flavonoids content also it exhibit higher antioxidant activity than anise seeds oil. These results suggested that both oils should be studied well as they can have beneficial therapeutic effects against various diseases.

Ulcerative colitis (UC) is one of the challenging diseases especially due to the pain and various adverse consequences associated with it. The treatment objectives for UC include prevention and treatment of complications and reestablishment of nutritional deficits to improve patient's quality of life. Normal and current drug treatments of UC are usually accompanied by the progression of different side effects affecting patient compliance and quality of life. Limiting use of these drugs for prolonged periods of time is advised. So the use of alternative and complementary treatments has prompted by UC patients. The use of herbal remedies became interesting and continuously increasing ***(Kayal and Shah, 2020).***

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UC caused a significant reduction ($p \leq 0.01$) in body weight, colon length with significant increase ($p \leq 0.01$) in colon weight in ulcerative colitis control group in comparison with healthy control group this may be due to the major symptoms of UC in both human and animal that include diarrhea, inflammation, rectal bleeding, and appetite loss causing weight reduction. The rats did not show any symptoms before colitis induction days 1–15, but after induction; rats had diarrhea and loose feces on day 16, especially for ulcerative colitis control group. On day 17, feces with occult blood were observed in ulcerative colitis control group and that treated with anise oil. Then bloody feces were found in ulcerative colitis control group rats on day 18 and afterwards. Moreover, the body weight decreased significantly through the experiment associated with erosion and inflammation of separated colon especially in ulcerative colitis control group.

It has been reported that use of plants extracts which have antibacterial and antioxidant activities could prevent the weight loss and also enhance the rat weight in animal model of UC. It seems that the correction of weight changes in response to any agents may be due to treatment of the colon ulcers and also improving the overall health conditions .Supplementation with chia and /or anise seeds oils improved these results significantly due to their nutrients and also active constituents including polyphenols and flavonoids that opposed UC lethal symptoms ***(Dubey and Singh, 2020).***

UC induction affected CBC results causing significant reduction ($p \leq 0.01$) in RBC's, PLT and Hb levels with increased ($p \leq 0.01$) WBC's content significantly .Decreased RBC's and Hb levels due to rectal bleeding associated with bloody feces while decreased PLT content due to direct result of the thrombopoiesis disorder often observed in the early phases of systemic inflammatory progression as well as spontaneous platelet aggregation. On the other hand observed increased WBC's content is associated with inflammatory processes and immune response caused by UC disease ***(Cioffi et al., 2015).*** Chia and anise seeds oils improved the CBC results and corrected blood constituent levels due to their nutritional content of protein, minerals and vitamins that support erythropoiesis as well as their antioxidant and anti-inflammatory properties which protect blood cells components ***(Akinfenwa et al., 2020 and El-Rokiek et al., 2020).***

The pathogeneses of UC is associated with elevated levels of reactive oxygen species (ROS) and this could be attributed to the massive infiltration of poly-morpho-nuclear and mononuclear leukocytes. In fact, free radical production is a key mechanism for the development of colonic inflammation in experimental colitis models. To regulate overall ROS levels, the intestinal mucosa possesses a complex of antioxidant defense system; the efficacy of the antioxidant system is impaired during inflammation status, partially as a result of auto-oxidation. Uncontrolled and persistent oxidative stress with overproduction of ROS and inadequate antioxidant systems will cause tissue degeneration and injury. Oxidative stress not only induces direct damage to intestinal cells, but also causes unregulated redox signaling, leading to NFκB activation and subsequent overexpression of pro-inflammatory cytokines and adhesion molecules ***(Motawea et al., 2020).***

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UC is characterized by an abnormal activation of the gut immune system, which results in local chronic inflammation. To trigger immune-mediated inflammation, it is possible to use haptenating agents, chemical compounds typically dissolved in ethanol. The ethanol allows these compounds to pass through the mucosal barrier. They then act upon either microbial or autologous proteins in the colon, rendering them immunogenic and thus provoking the abnormal activation of the immune system. DNBS is considered as one of the most common haptenating agents; it consistently induces chronic inflammation. DNBS was selected as the model inducer agent because of the symptomatic and morphologic similarities between human UC and DNBS -induced UC ***(Martin et al., 2017).***

In the current research, It is noticed that UC caused a strong oxidative stress status in colonic tissues particularly in ulcerative colitis control group by increasing levels of colonic AGEs, PCG, NO and MDA with decreasing colonic antioxidant content and capacity by decreasing GSH level and TAC in affected colon tissues. This status of oxidative stress initiated and were associated with inflammatory cascades resulting in increased various inflammatory markers including TNF-α, IL-1β, IL-10, NF-κB levels and immune response indicated by increased MPO activity in diseased colonic tissues. Chia and anise seeds oils bioactive components opposed oxidative stress, inflammation and immune reactivity correcting these parameters values. Synergistic effect of supplemented oils constituents achieved the most significant improvements.

Clearly, the free radicals produced in the inflammatory condition like UC caused peroxidation of lipids in the colonic tissues**.** The intensity of the lipid peroxidation may be measured by the level of MDA**.** Also carbonyl compounds produced by lipid peroxidation may function as secondary pathogenic factors, causing further protein and membrane lesions and so increasing advanced glycation end products formation. This may in turn exaggerate oxidative stress, forming a vicious cycle and initiating NO synthesis. Carbonyl compounds could also cause DNA mutations and breaks, driving malignant progression of UC ***(Cordeiro et al., 2020).***

Glutathione is a significant intracellular non-enzymatic antioxidant. In reduced form, GSH, is a more prevalent agent that is highly expressed in the cytoplasm, nucleus, and mitochondria. GSH has been utilized as a biomarker for both inflammation and oxidative stress. Decreased GSH content is associated with decreased TAC in affected colonic tissues. Experimental colitis models normally show decreased GSH levels and TAC content. A lower GSH level has been observed in DNBS induced colitis and can be restored to a normal level by antioxidants ***(Khairy et al., 2018).***

Marineli et al. (2015) reported that dietary chia seeds oil reduced oxidative stress *in vivo*, since it improved antioxidant status by increasing GSH level associated with reducing lipid peroxidation indicated by decreased MDA level. Also ***Rahman et al. (2017)*** reported that phenolic compounds of chia seeds oil are potent antioxidants in foods and are essential for biological

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systems because of their redox properties. Chia phenolic components prevent cell damage and are associated with many other effects. They reduce the risk of development of several diseases because of their antioxidant activity.

Antioxidant activity of anise seeds oil was observed *in vitro* in inhibiting copper catalyzed oxidation of human low-density lipoproteins (LDL); such an activity correlated well with total phenol content of the oil ***(Teissedre and Waterhouse, 2000).***The antioxidant potential of oil from anise seeds was studied. The antioxidant activities were assessed by inhibition of linoleic acid peroxidation, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, Fe^{3+} reducing power, and various lipid peroxidation assays. This findings showed that the anise seeds oil showed highest antioxidant activity, on comparison with butylated hydroxyl toluene and beta hydroxyl acids ***(Singh et al., 2008).***

The TNF-α is secreted by T helper type 1(Th1) cells mediating the cellular immune responses detected in this study. Increased TNF-α revealed the dysfunction of immune regulation in Th1 cells due to exposure of intestinal mucosa to DNBS. MPO is a functional sign and activation marker of neutrophils. MPO activity change represents an interruption in the functional state of neutrophils. The intestinal mucosa of the colon was stimulated by DNBS, resulting in neutrophil infiltration and MPO release. Hence, the inhibition of MPO activity and the regulation of TNF-α, IL-10, and IL-1B levels can affect the progression of UC.NF-κB is a powerful pro-inflammatory transcription factor that can promote inflammatory processes. Moreover, excessive NO production as well as many cytokines can lead to promotion of the NF-κB pathway ***(Kamalian et al., 2020).***Chia and anise seeds oils ameliorated inflammatory and immune response to DNBS colitis model.

Catrysse and Van Loo (2017) showed that chia consumption inhibit the activation of NF-κB and TNF-α, leading to improve in anti-inflammatory body capacity. Anti-inflammatory effects was observed in other study by ***Poudyal et al. (2013)*** that examined anti-inflammatory effects of chia seeds omega-3 unsaturated fatty acids resulting from chia consumption in rats. Anti-inflammatory characteristics could be due the phenolic compounds, and other bioactive components present in chia seeds, such as vitamins, minerals, and antioxidant substances.

The results of ***Da Silvaa et al. (2019)*** showed that IL-10 mRNA expression diminished in the chia supplemented groups because the pro-inflammatory factors (NF-κB and TNF-α) decreased in these groups.

DNBS is a potent DNA damaging agent and carcinogenic agent that induces intestinal and colonic tumors in rodents. It is noticed from results that DNBS caused significant reduction in

DNA fragmentation and caspase-9 expression with increased oxidative DNA damage indicated by increased formation of 8-OHdG in colon of ulcerative colitis control group. Analysis of biopsies from ulcerative colitis patients demonstrated that disease-associated occludin down-regulation was accompanied by and correlated with reduced caspase-3 expression and with decreased caspase-9 expression. *In vitro*, cytokine-induced occludin down-regulation resulted in reduced caspases expression and resistance to intrinsic and extrinsic pathways of apoptosis, demonstrating an overall protective effect of inflammation induced occludin loss. Defects in the cascade of apoptosis-related events during neoplastic development could well affect the execution of apoptotic death and disrupt homeostasis regulation of the colonic tissue ***(Kuo et al., 2019).***

In this study, DNA strand damage was prevented by biological activity of chia and anise seeds oils. Damage of DNA is a free radical-mediated process that includes base modification, production of strand breaks, DNA–protein cross linking and abnormal chromosomal arrangements , these results showed that tested oils may serve as good sources of food ingredients that minimize DNA damage caused though free radical-mediated process. Oils also regulated apoptosis process through activation of its cascades and signaling molecules including caspase-9 preventing progression to cancerous cells.

Boota et al. (2018) reported that anise seeds oil exhibited remedial potential, including cell-defensive, anti-inflammatory and DNA protective properties .Also anise seeds oil has positive effect on DNA damage.

Microscopic examination showed that colon section in DNBS induced colitic group, had sever hemorrhagic enteritis with large diffuse necrotic, hemorrhagic and ulcerated intestinal mucosa with significant loss of glandular elements. Sever submucosal edema and congested blood vessels were shown accompanied with sever inflammatory cells infiltrates. The use of DNBS, altered the colon architectures, whereas daily administration of tested oils is a protective factor of colon tissue in mitigating its harmful effects in experimental colitis. Colon histological examination of supplemented groups showed that chia and anise seeds oils treatment enhanced the tissue damage and lessened surface ulceration, this may due to their major active constituent of proteins, minerals, vitamins, fatty acids and also polyphenols and flavonoids.

Plant-based remedies are becoming a promising approach for treating various inflammatory disorders because of their strong anti-oxidative and anti-inflammatory effects. According to a review of animal models of acute and chronic colitis, when treated with different polyphenols, clinical symptoms of the animal colitis were largely alleviated ***(Zhang et al., 2020).*** In addition, some studies have focused on the improvement of UC by using nutraceutical supplements (prebiotics, probiotics, synbiotics and fish oil), and other natural compounds. For instance, gallic acid, an active component in tested oils, exhibits a potential protective effect on induced UC in

mice ***(Tian et al., 2017).***Tested oils regulated the production of cytokines and preserved the epithelial barrier function, indicating their importance in opposition of the pathogenesis of DNBS-UC model.

CONCLUSION

Study results proved that chia and anise seeds oils active components were able to correct ulcerative colitis degenerative and lethal effects. Using oils mixture was more efficient in ameliorating colitis than using each oil alone evidenced by biochemical analysis and microscopic examination of colon tissues. It is advised to add chia and anise seeds to human foods recipes and to encourage preparation of their cold pressed oils to be available for human beings due to their pharmacological properties and health beneficial effects.

AUTHORS' CONTRIBUTIONS

Dr. Alyae M. S. Gabal and Dr. Huda E. Megahd designed the study, made the protocol, managed the experimental, biochemical and statistical work done, performed the literature searches, completed and revised the manuscript writing.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

AUTHORS' FUNDING

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REFRENCES

Akinfenwa AO, Cheikhyoussef A, Cheikhyoussef N, Hussein AA. Cold pressed chia *(Salvia hispanica L.)* seed oil. Green Technology, Bioactive Compounds, Functionality, and Applications. Chapter 15 .Academic Press. Science Direct. 2020.181-190.

Beutler E, Duron O, Kelly MBJ. Improved methods for the determination of blood glutathione. The J. of Lab. and Clin. Med. 1963; 61: 882-888.

Boota T, Rehman R, Mushtaq A, Kazeroon EGI. Anise: A review on benefits, biological activities and potential uses. IJCBS. 2018; 14:110-114.

Hopps E, Canino B, Montana M, Lo Presti R, Averna MR, Caimi G. Behavior of the total antioxidant status in a group of subjects with metabolic syndrome. . Diabetes Met. Syndr.2014; 8(3):166-169.

Catrysse L, Van Loo G. Inflammation and the metabolic syndrome: The tissue specific functions of NF-kB. Trends Cell Biol. 2017; 27(6): 417–429.

Cioffi M, De Rosa A, Serao R, Picone I, Vietri MT. Laboratory markers in ulcerative colitis: Current insights and future advances. World J Gastrointest. Pathophysiol. 2015; 6(1): 13–22.

Cordeiro ND, Freitas RHC, Fraga CAM, Fernandes PD. Therapeutic effects of anti-inflammatory N-acylhydrazones in the resolution of experimental colitis. J. Pharmacol. Exp. Ther. 2020; 374(3):420-427.

Da Silvaa BP, Toledoa RCL, Grancieria M, Moreiraa MED, Medinab NR, Silvac RR , Costad NMB, Martino HSD. Effects of chia *(Salvia hispanica L.)* on calcium bioavailability and inflammation in wistar rats. Food Res. Int. 2019; 116: 592–599.

Dacie JV, Lewis SM. Practical Hematology. 6th ed. London, UK: ELBS and Churchill Livingstone, 1984.

Dubey V, Singh S. Alternative herbal medicines for prevention and treatment of ulcerative colitis. EJMCM. 2020; 7(7): 4900-4907.

El-Rokiek KG, Ibrahim ME, Saad El-Din SA, El-Sawi SA. Using anise *(Pimpinella anisum L.)* essential oils as natural herbicide. J. Mater Environ. Sci. 2020; 11(10):1689-1698.

Fornai M, Blandizzi C, Antonioli L, Colucci R, Bernardini N, Segnani C, De Ponti F, Del Tacca M. Differential role of cyclooxygenase 1 and 2 isoforms in the modulation of colonic neuromuscular function in experimental inflammation. J. Pharmacol. Exp. Ther. 2006; 317: 938–945.

Hazel K, O'Connor A. Emerging treatments for inflammatory bowel disease .Ther. Adv. Chronic Dis. 2020; 11: 1–12.

Helal EGE, Abd-El-Aziz MA, Ahmed SS. Effect of anise *(Pimpinella Anisum L.)* as phytoestrogen on some sex hormones and biochemical parameters .The Egyp. J. of Hos. Med. 2019; 75 (1):1918-1922.

Hrnčič MK, Ivanovski M, Cör D, Knez Z. Chia seeds *(Salvia hispanica L.)*: An overview—phytochemical profile, isolation methods, and application. Molecules. 2020**;** 25(11):1-20.

Hwang ES, Do Thi N. Effects of extraction and processing methods on antioxidant compound contents and radical scavenging activities of laver (*Porphyra tenera*). Prev. Nutr. and Food Sci. 2014; 19: 40-48.

Kamalian A, Sohrabi A M, Dolatshahi M, Afshari K, Shamshiri S, Momeni Roudsari N, Momtaz S, Rahimi R, Abdollahi M, Abdolghaffari AH. Interventions of natural and synthetic agents in inflammatory bowel disease, modulation of nitric oxide pathways. World J. Gastroenterol. 2020; 26(24): 3365-3400.

Kayal M, Shah S. Ulcerative colitis: Current and emerging treatment strategies. J. Clin. Med. 2020; 9(1): 94-106.

Khairy H, Saleh H, Badr AM, Marie MAS. Therapeutic efficacy of osthole against dinitrobenzene sulphonic acid induced-colitis in rats. Biomed. Pharmacother. 2018; 100(4): 42-51.

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J. of Clin. Path. 2001; 54 (5): 356–361.

Koschinsky T, He CJ, Mitsuhashi T, Bucala R, Liu C, Buenting C, Heitmann K, Vlassara H. Orally absorbed reactive advanced glycation end products (glycotoxins): An environmental risk factor in diabetic nephropathy. Proc. Natl. Acad. Sci. USA. 1997; 94 (12):6474–6479.

Kuo WT, Le Shen O, Shashikanth N, Drizella M, Wu L, Zha J, Edelblum KL, Wang Y, Steven P, Jerrold R. Inflammation-induced occludin downregulation limits epithelial apoptosis by suppressing caspase-3 expression turner. Gastroenterol. 2019; 157: 1323–1337

Levesque S. SPSS programming and data management: A Guide for SPSS and SAS users, 4th edition. Chicago, Spss Inc. 2007.

Marineli RDS, Lenquiste SA, Moraes EA, Maróstica MR. Antioxidant potential of dietary chia seed and oil (*Salvia hispanica L.*) in diet-induced obese rats. Food Res. Int. 2015; 76:666–674.

Martin R, Chain F, Miquel S, Motta JP, Vergnolle N, Sokol H. Using murine colitis models to analyze probiotics-host interactions. FEMS Microbiol. Rev. 2017; 41 (1):49–70.

Montgomery HAC, Dymock JF. The determination of nitrite in water. Analyst, 1961; 86: 414–416.

Motawea MH, Abd Elmaksoud HA , Elharrif MG, Desoky AA, Ibrahimi A. Evaluation of anti-inflammatory and antioxidant profile of oleuropein in experimentally induced ulcerative colitis. Int. J. Mol. Cell Med. 2020; 9(3):224-233.

Ohkawa H, Ohishi W, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Ann. Clin. Biochem. 1979; 95 (2):351-358.

Poudyal H, Kumar SA, Iyer A, Waanders J, Ward LC , Brown L. Responses to oleic, linoleic and alpha −linolenic acids in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. J. of Nutr. Biochem. 2013; 24(7):1381–1392.

Rahman MJ, De Camargo AC, Shahidi F. Phenolic and polyphenolic profiles of chia seeds and their *in vitro* biological activities. J. Funct. Foods. 2017; 35: 622–634.

Reeves PG, Nielsen FH, Fahey GC. AIN93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad Hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr.1993; 123: 1939-1951.

Singh G, Kapoor I PS, Singh P, De Heluani CS, Catalan CAN. Chemical composition and antioxidant potential of essential oil and oleoresins from anise seeds (*Pimpinella anisum L.*). Int. J. of Ess. Oil Ther. 2008; 2(3):122–130.

Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and Practice of histological techniques. Churchill Livingstone Elsevier, Oxford. 8th Edition. 2018; 1- 672.

Teissedre PL, Waterhouse A. Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils Varieties. J. Agric. Food Chem. 2000; 48(9):3801-3805.

Thoresen M, Bagenholm R, Loberg EM, Apricena F, Kjellmer I. Posthypoxic cooling of neonatal rats provides protection against brain injury. Arch. Dis. Child. 1996; 74(1): 3–9.

Tian T, Wang Z, Zhang J. Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. Oxid. Med. Cell Longev.2017; 2017: 4535194.

Tribukait B, Moberger G, Zetterberg A. Methodological aspects for rapid flow cytofluorometry for DNA analysis of human urinary bladder cells.1975 In: Haenen C, Hillen H, Wessels S, eds. Pulse cytophotometry, part 1. European Press Medicon, Ghent: 55-60.

Wang J, Zhang C, Guo C, Li X. Chitosan ameliorates DSS-induced ulcerative colitis mice by enhancing intestinal barrier function and improving microflora. Int. J. Mol. Sci. 2019; 20(22):5751.

Zhang L, Cao N, Wang Y, Wang Y, Wu C, Cheng X, Wang C. Improvement of oxazolone-induced ulcerative colitis in rats using andrographolide. Molecules. 2020; 25:76.

Zilic SA, Akıllıoglu SG, Jankovic M, Gökmen V. Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and de branned flour. J. of Cereal Sci. 2012; 56:652-658