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THE EFFECT OF SALVIA FRUTICOSA WATER EXTRACT IN ACETIC-ACID-INDUCED COLITIS IN MICE

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THE EFFECT OF SALVIA FRUTICOSA WATER EXTRACT IN ACETIC-ACID-INDUCED COLITIS IN MICE

Abstract

Inflammatory bowel disease (IBD) including Ulcerative colitis (UC) and Crohn's disease, is a chronic condition of the intestine characterized by an uncontrolled inflammatory response. Current biologic treatments developed to treat IBD are expensive and associated with significant side effects; thus, alternative approaches are required. Recently, herbal concoctions have emerged as promising intervention. This study investigates the protective effects of *Salvia fruticosa* (SF) against acetic acid-induced colitis in mice, a model of human inflammatory bowel disease (IBD). Mice were divided into four groups (n=3). IBD was induced with acetic acid (6%). SF was administered by gavage at 1.5ug/kg mimicking folk medicine. SF treatment was given for 2, 4 & 6 days. After treatment, colons were collected for colonic biochemical markers, and histopathological evaluation. SF treatment induced enhancement in the colon's structure and decreased ulcerations and inflammation. It significantly decreased pro-inflammatory cytokines TNF- α , IL-17 by 2.1-fold and 0.9-fold respectively at day2 and 6 and IL-6 by 2.8-fold at day 2 & 1.7-fold at day 6. Also, pro-inflammatory cytokine INF- γ showed a significant 22-fold decrease at day2 in SF-treated colitic group. Moreover, Myeloperoxidase (MPO) and Malondialdehyde (MDA) levels decreased showing the anti-oxidant property of the extract. Anti-oxidant Glutathione (GSH) level and anti-inflammatory cytokine IL-10, remained at normal levels while an increase by 0.9-fold at day 2 and 0.5-fold at day 6 in anti-inflammatory cytokine TGF- β in healthy SF-treated groups was significant. Hence, *Salvia fruticosa* exerts an anti-inflammatory and anti-oxidant effects against IBD as well as it help healing colon ulcers and maintain its architecture.

Keywords

Inflammatory Bowel Disease, Crohn's Disease, Ulcerative Colitis, Cytokines, Myeloperoxidase, Glutathione, Malondialdehyde.

1. INTRODUCTION

The digestive tract also called the Gastrointestinal tract (GI tract) is a continuous canal that starts from the mouth down to the anus and comprises the following organs: oral cavity, pharynx, esophagus, stomach, small intestine and large intestine. Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), hemorrhoids, diverticulitis and anal fissures are all diseases that might affect the GI tract. IBS is a disorder described by altered bowel habits and abdominal pain (Ceuleers, et al, 2016). IBD is a chronic inflammation of the intestine that majorly comprises Crohn's Disease (CD) and Ulcerative Colitis (UC) and is described by the infiltration of inflammatory cells to the intestinal mucosa (Kim & Cheon, 2017). UC occurs when inflammation affects part or portion of the large intestine. CD, on the other hand, is not restricted to the large intestine, but can cause inflammation to any part of the digestive tract (Gohil & Carramusa, 2014). The symptoms of IBD vary according to the condition severity and inflammation location. Most common symptoms include diarrhea that may be associated with blood/mucus or constipation as seen in UC, abdominal pain, weight loss, nausea, and vomiting (McDowell, et al, 2020). IBD prevalence is higher in North America and Europe than is Asia and Africa (McDowell, et al, 2020). IBD pathogenesis is multifactorial and involves inappropriate immune response with genetic susceptibility. Among the genes associated with IBD is the nucleotide-binding oligomerization domain 2 gene (NOD2) that binds the muramyl dipeptide (MDP) present on most types of bacterial peptidoglycan inducing immune responses (Zhang & Li, 2014). NOD2 acts as a bacterial sensor and plays a role in activating the Nuclear Factor Kappa B (NF-KB) signaling pathway. Mutation in NOD2 results in abnormal immune function (Hanauer, 2006). Oxidative stress, where an imbalance between free radicals and antioxidant levels occurs, is known to lead to many diseases including IBD. Enzymes such as the myeloperoxidase (MPO), xanthine oxidase (XO), and cyclooxygenase (COXs) have been described as major players that induce IBD (Tian et al., 2017). The intestinal barrier that is made up of intestinal epithelial cells (IECs) and innate immune cells provides a balance between luminal content and mucosa, any disturbance may lead to UC and CD (Ramos & Papadakis, 2019). Alterations of the apical junctional complex by means of TNF- α and IFN- γ leads to the damage of the barrier's functions upon inflammation, consequent ulcers of the epithelium may occur. Therefore, it is important to accelerated resealing the epithelial barrier to minimize IBD risk. Several treatments are used to cure IBD patients including immune modulators, anti-inflammatory agents, and biologics such as anti-TNF- α therapy that has been associated with side effects and are highly expensive.

Herbal medicine is a major part of complementary and alternative medicine (CAM) (Li, et al, 2011) and has attracted attention for research worldwide due to its minimal side effects and being cheaper than medicinal drugs. *Salvia fruticosa* (SF), known as Sage, is one of the largest genus in the family Lamiaceae (Labiatae) with approximately 1000 species distributed worldwide (Anwar, et al, 2017). Numerous species of *Salvia* have been used in pharmaceutical industries for therapeutic research. *Salvia fruticosa* Miller, also named *S. triloba* or *S. libanotica* is a sage species commonly found in Mediterranean countries. It is also known as East Mediterranean sage or Lebanese sage (Boukhary, et al, 2016). This herb has been used traditionally as a tea for the remedy of several pains, menstrual disorders, colds, and bronchitis (Torun, et al, 2015; Boukhary, et al, 2016). *S. fruticosa* was shown to have a high radical scavenging activity as well as an anti-inflammatory activity against carrageenan-induced mouse paw edema (Boukhary, et al, 2016). SF contains a considerable amount of bioactive components such as polyphenols (hydroxycinnamic acid derivatives), flavonoids and phenolic diterpenes that contribute to its biological benefits that include anti-inflammatory, anti-oxidant and anti-microbial activities (Torun, et al, 2015; Anwar, et al, 2017).

This study aims at investigating the effect of SF water extract on acetic acid induced colitis in Balb/c female mice. Cytokines were measured, *Salvia fruticosa* antioxidant activity against IBD was assessed, and the histological and macroscopical changes were examined.

2. MATERIALS AND METHOD

2.1. Plant Materials

Samples of *Salvia fruticosa* plant (herbarium number: ps-14-12) were collected from Taanayel, Lebanon. The plant was identified by Dr. Georges Tohme, Professor of Taxonomy. The leaves and the stems were air-dried in the shadow before use.

2.2. Plant Extract Preparation (7%)

The dried leaves were ground into fine powder; 7g were soaked in 100ml boiling distilled water for 5 minutes. They were left to simmer for 30 minutes then filtered. The extract was aliquoted and stored at -80°C.

2.3. Experimental Animals

Healthy female Balb/c albino mice (6-8 weeks old) with average weight of 22g were obtained from the animal house at Beirut Arab University. The mice were grouped into cages (10 mice per cage) in air conditioned room with temperature between 22°C to 25°C and a photoperiod of 12L: 12D. Standard laboratory pelleted formula and tap water were provided. The mice were left to acclimate for one week before the beginning of the experiments. Animals were fasted for 24 hours before colitis induction using acetic-acid with free access to tap water. The experimental protocol was approved by the Institutional Review Board at Beirut Arab University, code number 2020-A-0047-S-M-0407.

2.4. Induction of Colitis

To induce colitis, overnight-fasting mice were intra-rectally injected once with 0.1 ml of 6% acetic acid (AA) using a 2mm soft polyethylene tubing according to Nakhai, et al. Mice were held upside down for about 1 min to prevent AA leakage (Nakhai, et al, 2007).

2.5. Animal Treatment

Animals were randomly divided into 4 experimental groups of 9 mice in each group.

- Group N: Normal Group: Animals were left to obtain freely the provided tap water throughout the study without any treatment.
- Group AA: Animals received 6% acetic acid rectally only once a day 0 with no additional treatment.
- Group SF: Animals received the aqueous *Salvia fruticosa* extract by gavage at a dose of 1.5 µL/g for 2, 4, or 6 consecutive days.
- Group AA+SF: Animals received 6% acetic acid rectally once at day 0 and then the animals were administered the *Salvia fruticosa* extract 2 hours post induction of colitis. The extract was given over a period of 2, 4 or 6 days.

Mice were sacrificed the following day after the final dosage given, i.e. after 2, 4 and 6 days which is a convenient duration to be able to assess the effect of the extract against IBD (Richard, et al, 2011). After dissection, the intestinal tissues were removed, cleaned and washed using PBS buffer (pH 7.4). Colon length was measured and were sectioned for both histological and biochemical analysis. The ones used for biochemical analysis were directly stored at -80°C while the ones used for histological analysis were preserved in 4% formalin at room temperature.

2.6 Evaluation of Colitis

During the treatment period, the severity of colitis was assessed daily by measuring the body weight change of each mouse and the disease activity index (DAI). DAI was calculated by summing up the scores assessed for body weight change, stool consistency and rectal bleeding according to parameters that are well-established and validated compared to clinical presentation of human IBD (Cooper et al 1993).

2.6.1 Body Weight Scoring

- 0 point= no significant weight loss
- 1 point= 1-5% weight loss
- 2 points= 5-10% weight loss
- 3 points= 10-15% weight loss
- 4 points= more than 15% weight loss

2.6.2 Stool Consistency and Rectal Bleeding scoring

- 0 point= normal, no bleeding
 - 2 points= loose stool, slightly bleeding
 - 4 points= watery diarrhea, gross bleeding
- DAI score = Body weight score + Stool consistency and rectal bleeding score

2.7 Histological Assessment

After dissection of mice, colon tissues were collected and preserved in 4% Formalin. Samples were sent to the Histology Center at BAU Faculty of Medicine for processing. H&E stained slides were examined under light microscope.

2.8 Preparation of Tissue Homogenate

After collecting and cleaning colon samples, the tissues were homogenized in lysis buffer (pH 7.4) which consists of 5ml PBS, 0.5ml PMSF (1mM) and 0.25ml Triton X-100 at a ratio of 1g of tissue per 10ml lysis buffer. Colon pieces were homogenized manually on ice and centrifuged two times at 15,000 rpm for 15 mins at 4°C. The collected supernatants then stored at -80°C.

2.9 Protein Quantification

After tissue homogenization, protein concentration of each homogenate was determined using Bradford Protein Assay. Bovine Serum Albumin (BSA) was used as a standard. The absorbance of the developed color was read using a spectrophotometer at 595nm. Protein quantification of the samples was deduced from the BSA standard curve.

2.10 Oxidative Stress Assays

2.10.1 Reduced Glutathione (GSH) Assay

GSH activity (mg GSH/g protein) was measured by a colorimetric assay using reduced GSH kit according to manufacturer's instructions (Elabscience, cat # E-BC-K051). Dithio-dinitrobenzoic acid can react with sulfhydryl compound of GSH tripeptide to generate a yellow compound.

The optical density was measured at 420nm wavelength.

GSH concentration was calculated using the following formula:

$$\text{GSH activity (mg GSH/g protein)} = \frac{OD \text{ Sample} - OD \text{ Blank}}{OD \text{ Standard} - OD \text{ Blank}} \times \text{Concentration of standard (20} \times 10^{-3} \text{ mmol/L)} \times \text{GSH molecular weight (307)} \times \text{Dilution factor (2)} \times \text{Dilution factor of sample before tested} / \text{concentration of protein (gprot/L)}.$$

2.10.2 Myeloperoxidase (MPO) Colorimetric Assay

MPO concentration in colonic tissues was quantified using Myeloperoxidase (MPO) Colorimetric Assay Kit according to manufacturer's instructions (Elabscience, cat # E-BC-K074). MPO reduces hydrogen peroxide to a complex, where the latter react with o-dianisidine to produce a yellow product. The supernatant was collected and the absorbance was read spectrophotometrically at 460nm.

MPO concentration was calculated using the following formula:

The amount of MPO in 1ml of sample that catalyzes the decomposition of 1μmol H₂O₂ at 37°C for 30 min is defined as 1 unit.

$$\text{MPO activity (U/L)} = \frac{\Delta A}{11.3 \times V \times \frac{m}{V_3}} \times f$$

($\Delta A = OD \text{ sample} - OD \text{ control}$, 11.3 = constant, $V =$ volume of sample added to the reaction (ml), $V_3 =$ volume of reagent 2 application solution added in sample preparation step (ml), $m =$ weight of sample (g), $f =$ the dilution factor of sample before testing).

2.10.3. Malondialdehyde (MDA) Assay

The concentration of MDA of the supernatant of the colonic homogenates was measured according to the method of Buege & Aust (1978). In brief, 50 μ l of the tissue homogenates was added to 100 μ l TBA reagent (prepared by dissolving 0.025g TBA in 5ml of 20% TCA). The samples were placed in a water bath at 88 $^{\circ}$ C for 20 min, left to cool at room temperature and then centrifuged at 1000 rpm for 15 min. 100 μ l of the supernatants was added in an ELISA plate to be measured at 540nm. The MDA content was calculated as TBARS expressed in terms of μ m/mg of protein using a molar extinction coefficient 155 $\text{mM}^{-1} \text{cm}^{-1}$ (Chatterjee & Sil, 2006).

$\text{MDA (uM)} = (A_{\text{sample}} * DF) / (1 * \epsilon \text{ molar absorptivity})$.

2.11 Cytokine Assay

All cytokines were quantified similarly using Mouse ELISA kits according to manufacturer's instructions (Elabscience). TNF- α , IL-6, IL-10, IFN- γ , TGF- β and IL-17 concentrations were measured in the colonic homogenates. Briefly, standard working solutions with different concentrations were prepared and 100 μ L of diluted sample were added to the anti-body coated 96-well plate. The plate was properly sealed and incubated at 37 $^{\circ}$ C for 90min. After incubation, the liquid was removed from the well-plate, without washing, 100 μ L of 1x Biotinylated Detection Ab working solution was added to each well, gently mixed, sealed and incubated at 37 $^{\circ}$ C for 60min. Next, 100 μ L of 1x HRP Conjugate working solution of the corresponding cytokine was added and incubated at 37 $^{\circ}$ C for 30min. After each incubation, the liquid was removed from the well-plate and washed 3-5 times using washing buffer. 90 μ L Substrate reagent of the corresponding was added to each well, liquid coloration was detected and the plate was incubated at 37 $^{\circ}$ C for 15min in shading light. The reaction was terminated by adding 50 μ L Stop solution to the wells. Finally, the plate is read using an ELISA plate reader at 450nm wavelength.

2.12 Statistical Analysis

All statistical analyses were performed using the standard statistical program Graph Pad Prism Ver. 7. The data are expressed as mean \pm standard deviation. Statistical analysis of the obtained results was analyzed using (ANOVA). Statistical significance is reported as **** at P-value ≤ 0.0001 , *** at P-value ≤ 0.001 , ** at P-value ≤ 0.01 and * at P-value ≤ 0.05 .

3. RESULTS

3.1 Effect of *Extract* on Body Weight

The body weight of all mice was measured daily throughout the whole period of the study. As shown in Figure 1, the normal control group (N Group) showed increase in body weight of about 9% by day 6. Untreated colitic-mice (Group AA) as well as SF treated colitic-mice (Group AA+SF) showed a significant decrease in their body weight of 12.92 % (P-value 0.0002) and 10.76 % (P-value < 0.0001) at day 2 compared to the normal control group respectively. The first 2 days after colitis induction with acetic acid, the AA group lost 2-3g daily while AA+SF group lost approximately 1-2g. However, it is worth noting that both groups started to regain their weights after day 4.

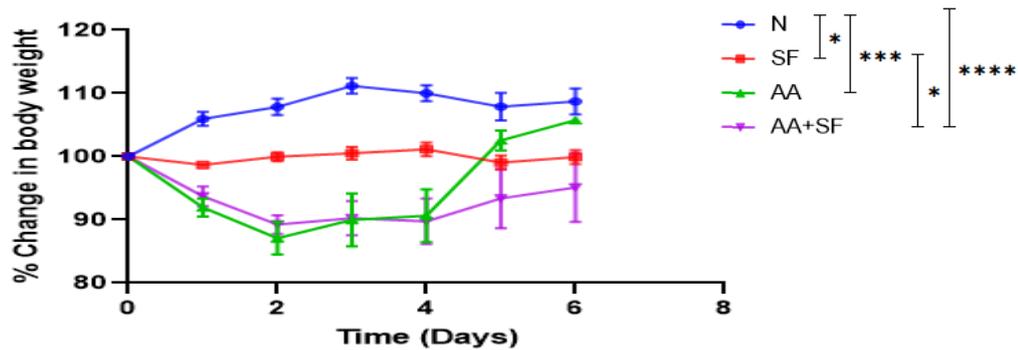


Fig.1: Effect of extract on body weight. Bar graph showing the percentage change in body weight of mice in each experimental group for 6 consecutive days. Data represented is mean \pm SEM (n=4). Asterisks shown in the legend represent significance relative to the normal control, (****), (***) and (*) corresponds to $P < 0.0001$, $P < 0.001$ and $P < 0.05$ respectively.

3.2 Effect of Extract on Colon Length

Macroscopic observation of the colons showed changes in their lengths and thicknesses. The length of the colons was measured after dissection from the cecum to the anus (Figure 2). The healthy group that received SF had colonic length like the normal control unlike the length of the untreated colitic mice that showed a significant 0.3-fold decrease in length (P -value < 0.0001). Upon treating colitic mice with SF extract, the length of the colon increased significantly by 0.1-fold (P -value 0.0016) compared to untreated mice (group SF+AA versus AA group).

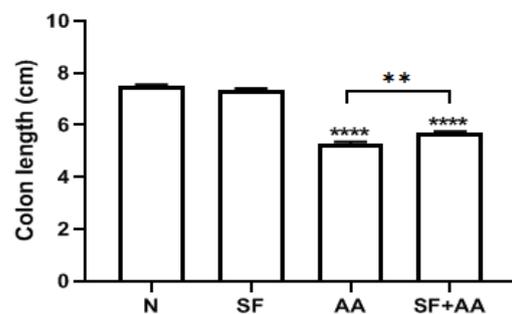


Fig.2: Effect of Extract on colon length. The graph illustrates the colon length in centimeters of each group. Data represented is mean \pm SEM (n= 3). Asterisks shown on bars represent significance relative to the normal control, (****) and (**) corresponds to P -value < 0.0001 and P -value 0.0016 respectively.

3.3 Effect of SF Extract on Stool Consistency and Rectal Bleeding

To assess the severity of colitis, Disease Activity Index (DAI) score was monitored daily by observing stool consistency, rectal bleeding and change in body weight for each mouse. At day 2, untreated colitic-mice showed a significantly higher DAI score compared to normal mice (10.5 vs 0, P -value 0.0079). However, treatment with SF extract induced a significant decrease in DAI score by 71% (3 vs. 10.5, P -value 0.0465). It is important to note that DAI score started showing gradual decrease in colitic groups after day 2 (Figure 3).

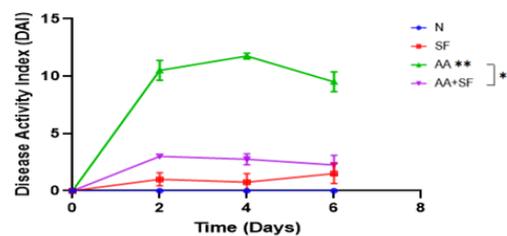


Fig.3: Effect of SF extract on stool consistency and rectal bleeding. The disease activity index score of mice over the 6 days of the experimental study. Data represented is mean \pm SEM (n=4). Asterisks shown on bars represent significance relative to the normal control, (*) corresponds to P -value < 0.05 .

3.4 Histopathological analysis

The macroscopic changes of the colons at different time points are shown in Figure 4. At day 2, colons of the normal group and the SF-treated mice were clear and with no abnormalities as seen in Figure 4-IA and 4-IB respectively. However, colons of colitic mice showed evident signs of inflammation including redness throughout the whole colon, swelling and shrinkage in colon length with loose stool (Figure 4-IC). Treating colitic mice with the aqueous *Salvia fruticose* extract induced a decrease in the inflammatory signs showing less redness and regular size colon and normal stool consistency (Figure 4-ID).

At day 4, similar results were obtained as shown in figure 4-II. Clear colonic tissues were seen in normal and SF mice (Figure 4-II A and B respectively). The colons of untreated colitic mice showed increase in redness and ulcerations (Figure 4-II C) that decreased upon SF treatment (Figure 4-II D).

At day 6, the anti-inflammatory effect of SF was more evident. The colon of SF-treated colitic mice (Figure 4-IIID) was clear and comparable to that of the normal group (Figure 4-IIIA), unlike that of the untreated colitic mice that was still showed signs of inflammation and shrinkage of the colon's length (Figure 4-IIIC). It is worth noting as well that colon tissues of healthy SF-treated mice (Figure 4-IIIB) were thicker than colons of normal mice.

The microscopic analysis verified the macroscopic analysis of the colons. Figure 5-I, 5-II and 5-III shows representative sections of the colons at day 2, 4 and 6 from all experimental groups.

Colons section from Normal and SF-treated mice showed normal colonic mucosa with no distortion in crypt architecture and numerous goblet cells lining them throughout the period of study as shown in Figure 5-I, II, and III A and B, respectively.

At day 2, the colons of the untreated colitic group (Figure 5-IC) showed vasodilation, disruption architecture of crypt (DAC) and infiltration of polymorphonuclear leukocytes (PMNs) (indicated on figures). Treatment of colitic group with SF extract minimized the extent of inflammation as shown in Figure 5-ID.

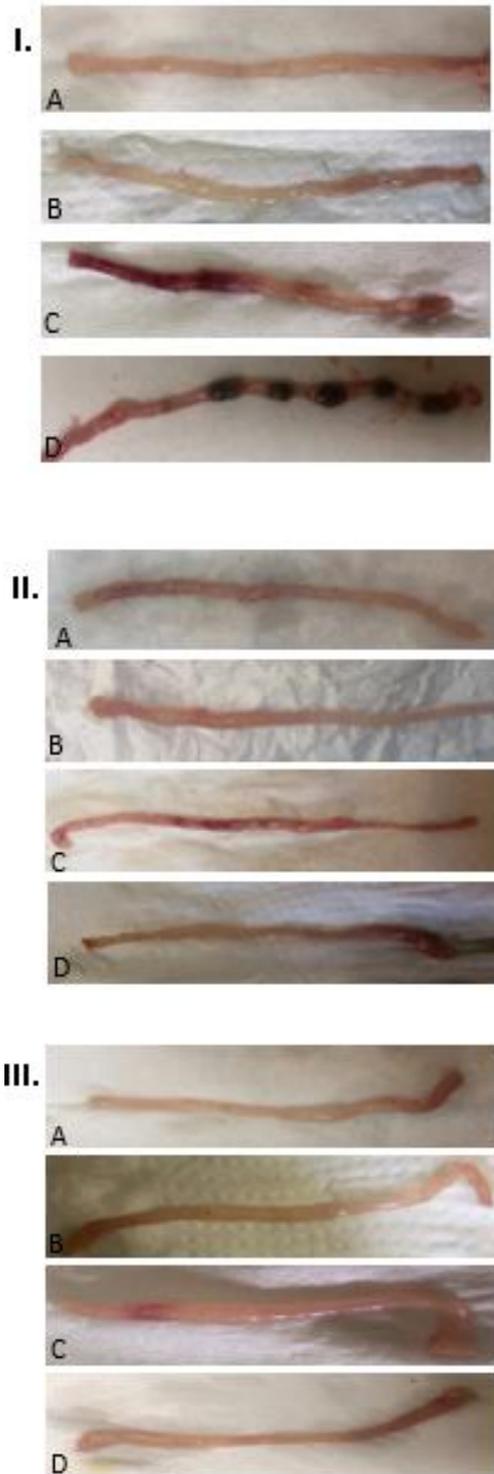


Fig.4: Macroscopic inspection of colon tissues at days 2 (I), 4 (II) and 6 (III) in all experimental groups. A shows colon tissue of normal mice, B shows colon from SF-treated healthy mice, C shows colon of colitic untreated mice, and D shows colon from SF-treated colitic mice.

At day 4, the colons of untreated colitic mice (Figure 5-II C) showed increase in the inflammation signs with more crypt distortion, higher PMNs infiltration and goblet cell loss. Treatment of colitic group with SF extract attenuated these modifications (Figure 5-II D).

At day 6, SF treatment of colitic mice markedly reduce the degree of infiltration and the architecture of the colon is restored (Figure 5-III D), the tissue of the colon seemed regenerated and appeared to be like the normal and SF treated groups (Figure 5-III A and B). Colitic group showed self-repairing with some inflammation mark ((Figure 5-III C) on day 6 but to a lower extent than previous days.

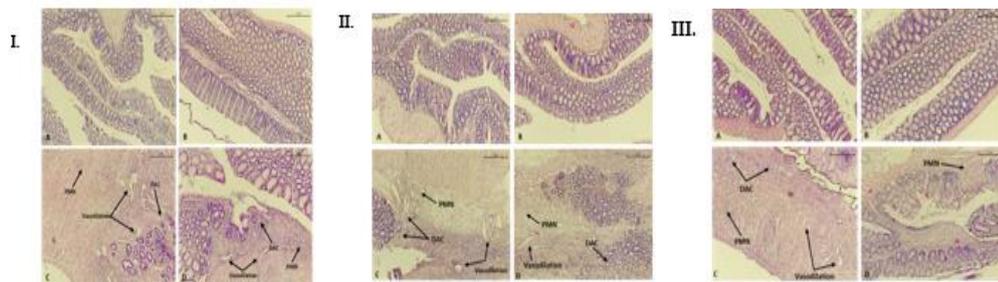


Fig.5: Histology of the colon tissues at day 2 (I), 4 (II) and 6 (III) of all experimental groups. Panel A shows colon tissue for normal groups, Panel B shows colon tissue for SF-treated healthy group, Panel C shows colon tissue for colitic untreated group, Panel D shows colon for SF-treated colitic group. Disrupted architecture of crypt (DAC) and Infiltration of polymorphonuclear leukocytes (PMN) are seen. (H&E 100X).

3.5 Effect of SF extract on oxidative stress levels

3.5.1 Effect of SF Extract on Reduced Glutathione (GSH) Level

The levels of reduced glutathione (GSH) were measured in the colonic homogenate of all mice groups at days 2, 4 and 6. At day 2 (Fig. 6-I), a significant increase in GSH level was observed in the healthy group that received SF extract by 2.83 folds (P-value 0.005) and the untreated colitic group by 3 folds (P-value 0.003) when compared to normal mice. However, the levels of GSH in colitic mice that received the SF extract were significantly lower than that of AA Group (P-value 0.005) and comparable to normal levels.

At day 4 (Figure 6-II), healthy mice that received SF as well as colitic mice showed a decrease in GSH level compared to day 2, while SF-treated colitic mice still shows GSH levels within the normal range (20 mg/mgProt).

At day 6 (Figure 6-III), the GSH level in healthy SF-treated group was still significantly higher than normal group by 2 folds (P-value 0.014). Colitic untreated mice showed similar GSH levels as that of day 4, while SF-treated colitic mice showed a slight increase by 1.3 folds in GSH level when compared to that of day 4.

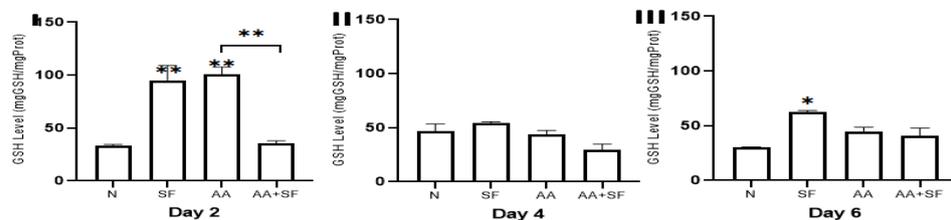


Fig.6: Effect of SF Extract on Reduced Glutathione (GSH) level. I, II, and III correspond to GSH level at day 2, 4 and 6 respectively. Data represented is mean \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (**) and (*) correspond to P-value < 0.01 and P-value < 0.05, respectively.

3.5.2 Effect of SF Extract on Myeloperoxidase (MPO) level

No significant change in MPO levels were observed at day 2 among all experimental groups (Fig. 7-I). The highest level of MPO was observed in the untreated colitic group with an increase of 1.6 folds (P-value 0.41) compared to normal. SF treatment was able to lower it by 1.22 folds (P-value 0.729).

Similarly, no significant changes were observed at day 4 (Fig. 7-II). The level of MPO in untreated colitic mice was still higher by 1.3 folds albeit non-significantly compared to normal control. SF treatment was also able to lower it by 1.9 folds. Furthermore, SF treatment decreased MPO further by 1.2 folds compared to levels at day 2.

At day 6 (Fig 7-III), significant decrease in MPO levels were observed in all groups compared to the normal control. A decrease by 2.5 folds (P-value 0.029), 3.3-fold (P-value 0.009), and 3.3 folds (P-value 0.017) were observed in SF, AA and AA+SF groups respectively.

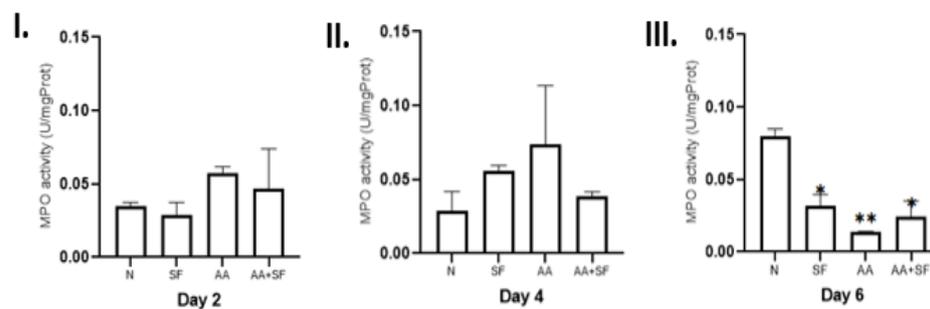


Fig.7: Effect of SF Extract on Myeloperoxidase (MPO) level. I, II and III correspond to MPO level at day 2, 4 and 6 respectively. Data represented is mean determinations \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (***) and (*) corresponds to P-value < 0.01 and P-value < 0.05, respectively.

3.5.3 Effect of SF Extract on Malondialdehyde (MDA) Level

MDA levels were measured in colonic homogenates of all mice at day 2, 4 and 6 (Figure 8). The highest level of MDA was observed at day 2 in the untreated colitic group with a significant 1.3-fold increase (P-value 0.0159) compared to normal. Upon SF treatment, MDA level significantly decreased by 0.7-fold (P-value 0.0308) compared to untreated colitic group, while at day 4 and 6 no significant changes were observed in MDA levels.

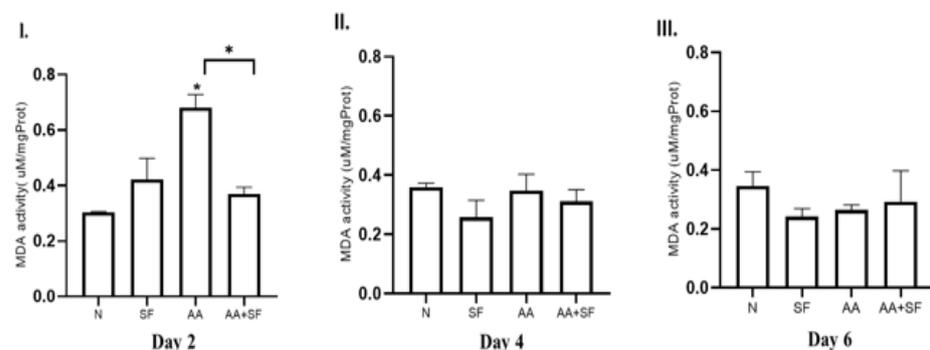


Fig.8: Effect of SF on Malondialdehyde (MDA) level. I, II, and III correspond to MDA level at day 2, 4 and 6 respectively. Data represented is mean \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (*) correspond to P-value < 0.05.

3.6 Effect of SF extract on Levels of Inflammation Markers

3.6.1 Effect of SF extract on TNF- α level

At day 2 (Fig 9-I), SF treatment in colitic mice induced a significant 1.6-fold decrease in TNF- α levels compared to the untreated colitic group (P-value 0.047).

At day 4 (Fig. 9-II), no significant changes in the levels TNF- α of all groups were observed compared to the normal control. However, the levels were overall slightly lower compared to the levels of day 2 and where the SF-treated colitic mice exhibited the least levels.

At day 6 (Fig. 9-III), similar results were obtained where no significant changes in TNF- α levels were observed in SF and AA mice compared to control. The only significant decrease was observed in SF-treated colitic mice (2.1-fold, P-value 0.043) compared to normal group.

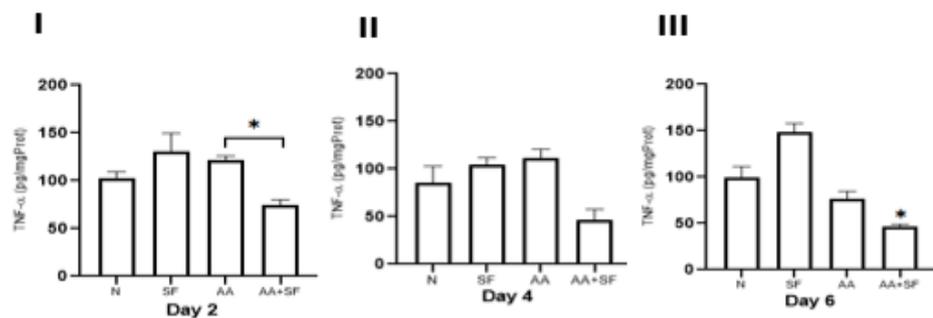


Fig. 9: Effect of SF extract on TNF- α level. I, II and III correspond to TNF- α level at day 2, 4 and 6 respectively. Data represented is mean \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (***) and (*) corresponds to P-value < 0.01 and P-value < 0.05, respectively.

3.6.2. Effect of SF extract on IL-6 level

At day 2 (Fig. 10-I), SF treatment of colitic mice induced a significant 2.8-fold decrease in IL-6 levels compared to untreated colitic mice (P-value 0.023).

At day 4 (Fig. 10-II), all experimental groups showed lower concentration of IL-6 compared to day 2. However, no significant changes were observed among the groups.

At day 6 (Fig. 10-III), a significant 1.7-fold decrease in IL-6 levels was observed in SF-treated colitic mice compared to untreated ones (P-value 0.0006).

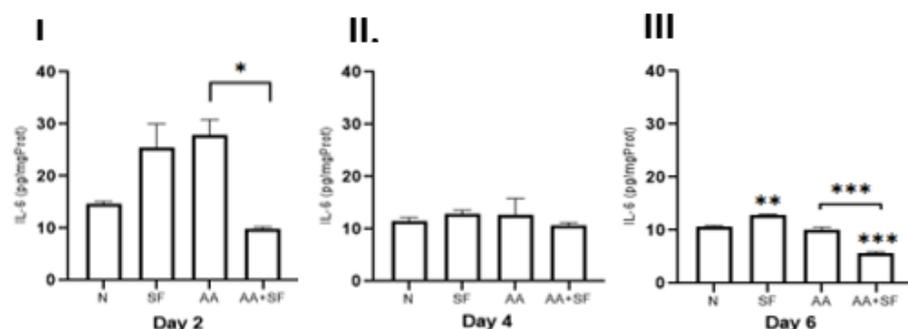


Fig. 10: Effect of SF extract on IL-6 level. I, II and III correspond to IL-6 level at day 2, 4, and 6 respectively. Data represented is the mean \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (***), (***) and (*) corresponds to P-value < 0.001, P-value < 0.01 and P-value < 0.05, respectively.

3.6.3. Effect of SF extract on IFN- γ level

At day 2 (Fig. 11-I), a significant 2.2-fold decrease was observed in SF-treated colitic mice compared to the untreated colitic mice (P-value 0.019).

Similar results were seen at day 4 (Fig. 11-II). The untreated colitic mice showed significant higher levels of IFN- γ compared to normal mice (2 folds, P-value 0.041). SF treatment of colitic mice caused a significant 2.7-fold decrease in IFN- γ levels compared to untreated ones (P-value 0.015).

At day 6 (Fig. 11-III), the levels of IFN- γ was significantly lower in all experimental groups compared to normal control (P-value 0.012), with SF treated colitic mice exhibiting the lowest levels. The levels of IFN- γ decreased to lower values compared to day 4.

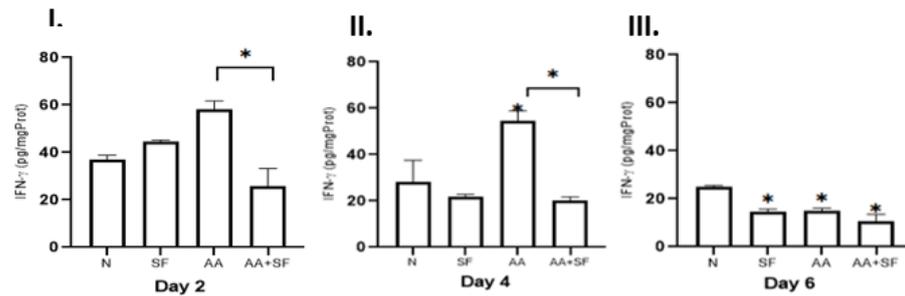


Fig.11: Effect of SF extract on IFN- γ level. I, II and III correspond to IFN- γ level at day 2, 4 and 6 respectively. Data represented is mean \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (*) corresponds to P-value < 0.05.

3.6.4. Effect of SF extract on IL-10 level

At day 2 (Fig. 12-I), a significant increase in the concentration of IL-10 was observed in SF-treated healthy mice by 6.8 folds and untreated colitic mice by 6 folds compared to normal mice (P-value < 0.001). SF treatment of colitic mice induced a significant 3.5 folds decrease in IL-10 levels compared to untreated colitic mice (P-value < 0.001).

At day 4 (Fig. 12-II), the level of IL-10 dramatically decreased by 2.3 folds in untreated colitic mice compared to normal mice (P-value 0.029). Similarly, IL-10 level significantly decreased in SF-treated colitic group compared to normal group (P-value 0.017).

At day 6 (Fig. 12-III) no significant change was noticed between groups as well as when compared to the levels at day 4.

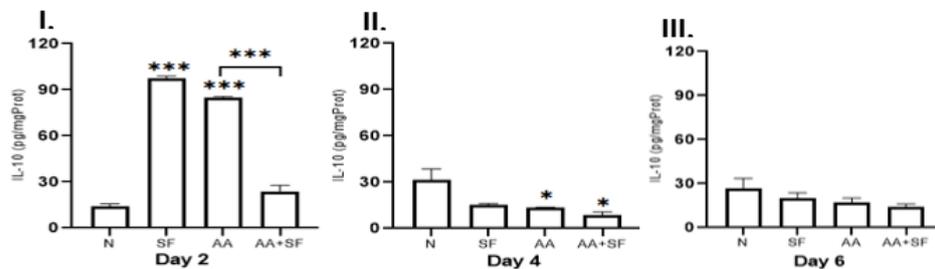


Fig.12: Effect of SF extract on IL-10 level. 12-I corresponds to IL-10 level at day 2, 12-II corresponds to IL-10 level at day 4 and 12-III corresponds to IL-10 level at day 6. Data represented is mean \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (***) and (*) corresponds to P-value < 0.001 and P-value < 0.05, respectively.

3.6.5. Effect of SF extract on IL-17 Level

At day 2 (Fig 13-I), the level of IL-17 significantly increased in the untreated colitic group by 1.6-fold (P-value 0.004) compared to the normal group. Upon treatment of colitic mice with the SF extract, the level of IL-17 decreased significantly by 1.6-fold (P-value 0.004) and was comparable to that of the normal control mice.

At day 4 (Figure 13-II), no significant changes were seen among the groups.

At day 6 (Figure 13-III), a significant 0.5-fold (P-value 0.037) increase in IL-17 level was observed in healthy mice administered with SF extract. Furthermore, a significant decrease by 0.9-fold (P-value 0.049) was obtained compared to the normal group.

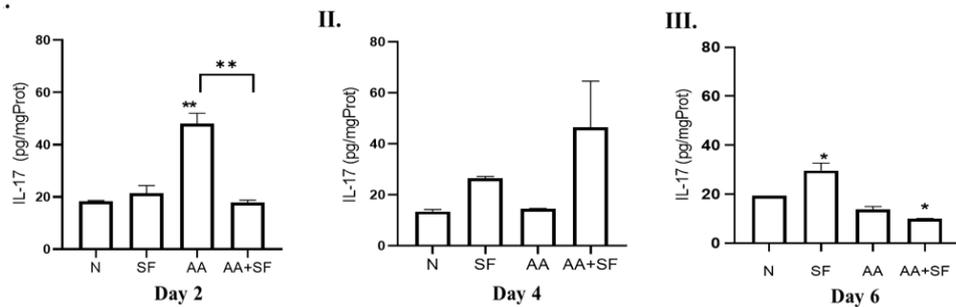


Fig. 13: Effect of SF extract on IL-17 level. Panel I correspond to IL17 level at day 2, Panel II corresponds to IL-17 level at day 4 and Panel III corresponds to IL-17 level at day 6. Data represented is mean \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (**) and (*) corresponds to P-value < 0.01 and P-value < 0.05, respectively.

3.6.6. Effect of SF extract on TGF- β Level

At day 2 (Figure 14-I), a significant increase in the concentration of TGF- β was observed in SF-administered healthy mice by 0.9-fold (P-value 0.006) and untreated colitic mice by 0.6-fold (P-value 0.024) compared to normal mice. SF treatment of colitic mice induced a significant 1.4-fold decrease in TGF- β level compared to untreated colitic mice (P-value 0.005).

At day 4 (Figure 14-II), no significant change was noticed between groups. However, the level of TGF- β among groups on day 4 was lower than the level of TGF- β in day 2.

At day 6 (Figure 14-III), a significant increase in TGF- β level in SF-treated healthy group by 0.5-fold (P-value 0.003) and a decrease in SF-treated colitic group by 0.8-fold (P-value 0.006) in comparison with normal group. Also, TGF- β level significantly decreased in SF-treated colitic group by 0.7-fold compared to untreated colitic group (P-value 0.012).

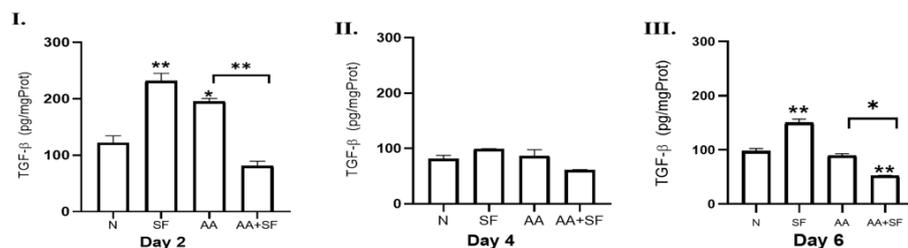


Fig. 14: Effect of SF extract on TGF- β level. Panel I correspond to TGF- β level at day 2, Panel II corresponds to TGF- β level at day 4 and Panel III corresponds to TGF- β level at day 6. Data represented is mean \pm SEM (n=3). Asterisks on bars represent significance relative to the control, (**) and (*) corresponds to P-value < 0.01 and P-value < 0.05, respectively.

4. DISCUSSION

IBD including Ulcerative colitis and Crohn's disease is an inflammatory disease characterized by chronic relapsing intestinal inflammation, which can lead to weight loss, abdominal pain, diarrhea, and blood in stool, accompanied with macroscopic colonic alteration such as ulceration. The disease incidence is raising worldwide and current treatments, like anti-inflammatory agents, immune-modulators, biologics and CAM, have been associated with multiple side effects such as cytotoxicity, susceptibility to infectious diseases, bone marrow suppression and allergic reactions. Herbal medicine had grabbed researchers' attention due to its association with less side effects as well as its effectiveness against multiple diseases. *Salvia fruticosa* is an East Mediterranean plant that has been used in treating diseases due to its antioxidant and anti-inflammatory effects (Boukhary et al., 2015 & Bassil, et al., 2016). Studies have shown that ulcerative colitis can be induced in animals treated with acetic acid mimicking the disease in humans (Low, et al., 2013; Fabia, et al 1992). In the present study, we demonstrate the anti-inflammatory and antioxidant effect of SF water extract in colitic mice induced by 6% acetic acid.

Treatment with SF water extract showed improvement in body weight, colon length, DAI score, and in histopathological assessment. A slightly higher weight loss was observed in colitic SF-treated compared to the untreated colitic mice at day 6. These results build on existing evidence where SF increased body weight in alloxan-induced diabetic mice (Raafat, et al, 2013). The disease severity assessed by DAI score was noticeably improved in the colitic group that received SF. As for the consistency of stool, it was harder and more compacted compared to the untreated group that showed watery & bloody stool. Moreover, colon length is inversely related to the severity of inflammation, the observed findings showed a shortening of colon length in acetic acid-induced colitis; while, in SF-treated colitic groups, intestinal length was effectively restored.

Macroscopic and microscopic observations confirmed the therapeutic effect of *Salvia fruticosa* extract on acetic acid induced colitis. The histopathological observations showed that SF treatment led to reduction of the inflammatory response. The cells regenerated and the architecture of the colon was restored and re-organized as opposed to the untreated group that showed noticeable PMN infiltrations and crypts disruption.

There are several anti-oxidative mechanisms within the GI tract including the enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), along with non-enzymatic scavengers, such as glutathione. Recently, it has been suggested that peripheral immune cells in patients with active CD produce higher levels of SOD activity and H₂O₂ (Beltran et al. 2010). *Salvia fruticosa*, in addition to its role as a tissue regenerative agent, is known to possess an antioxidant role. The level of reduced GSH, MPO and MDA were measured in all experimental groups. The MDA level in our study showed a significant increase in colitic mice at day 2 compared to the control group. In contrast, SF treatment induced a decreased MDA level in colitic mice during that period. GSH content was significantly increased in healthy mice that administered SF at day 2, then it decreased afterwards to normal level, while SF treatment in colitic mice showed approximately normal levels of GSH throughout the whole experimental duration implying that SF treatment during colitis helps to prevent the changes in GSH levels. Similar observations were obtained by Hamza et al., where SF was found to normalize GSH content and prevent MDA elevation in liver, heart and kidney of diabetic rats. As for myeloperoxidase (MPO), its level increased in colitic mice compared to control group, however SF treatment appeared to decrease its activity. These results are comparable with a previous study that has proven the anti-oxidant activity of SF in streptozotocin-induced diabetic rats (Hamza, et al, 2016). It was suggested that the SF anti-oxidant activity is mainly attributed to its excessive polyphenols and terpenes content (Ravipati, et al, 2012; Fu, et al, 2013).

The anti-inflammatory potential of SF is surely related to its strong antioxidant. To achieve this conclusion, the levels of inflammatory markers TNF- α , IL-6, IL-10, IFN- γ , TGF- β and IL-17 that change according to inflammation severity were measured. As expected, induction of colitis by acetic acid significantly increases the level of pro-inflammatory cytokines (TNF- α , IL-6, IFN- γ and IL-17) compared to the normal, ultimately, due to the activation of monocytes and macrophages in intestinal inflammation (Fournier, & Parkos, 2012). SF treatment decreased pro-inflammatory cytokines (TNF- α , IFN- γ , IL-6 and IL-17) in SF-treated colitic group throughout the study period. SF was shown to decrease and suppress pro-inflammatory cytokine TNF- α and IL-6 up to 85% and 56% respectively in LPS-challenged Balb/C mice (Bzour, et. al., 2011). It was

shown that carnosol extracted from SF significantly reduced TNF- α cytokine in inflamed skin (Yeo, et al., 2018). Other SF components such as α -thujone and β -thujone reduce the production of the proinflammatory cytokines TNF- α , IL-1 β , and IL-6 (Siveen & Kuttan, 2011). As for anti-inflammatory cytokines, SF induce a significant increase in TGF- β in healthy SF-treated groups as compared to the normal, also, a significant decrease in TGF- β production was shown in colitic group treated with SF compared to untreated colitic group at day 2 and day 6. Colitic mice that were given SF treatment showed a significant decrease in IL-10 production as compared to the untreated colitic mice ($P < 0.001$). The results confirm that inflammation in the treated group has decreased upon SF treatment. The anti-inflammatory activity of SF may be partly attributed to the overall effects of the phenolics and other plant constituents having potent anti-inflammatory actions similar to diclofenac. SF has been tested for its anti-inflammatory effect and both the aerial parts and the roots exhibited significant protection against carrageenan-induced mouse paw edema (Boukhary, et al 2016).

Salvia officinalis is a plant that belongs to the same family as *Salvia fruticosa* (Labiatae/Lamiaceae). This herb was proven to possess similar properties as SF. Pharmacological findings for *S. officinalis* that are reported include anticancer, anti-inflammatory, anti-oxidant and antimicrobial effects (Ghorbani, A., & Esmailizadeh, M., 2017). Noteworthy, *S. fruticosa* has a similar chemical profile as *S. officinalis* implying that it might possess similar activities that should be investigated (Couladis, M. & Koutsaviti, A., 2017). Previous studies have shown the presence of rosmarinic acid, carnosol, and/or carnosic acid in *Salvia officinalis*, major components of SF (Vieira, et al 2020).

After exploring the anti-inflammatory, anti-oxidant and regenerative effect of *Salvia fruticosa*, our findings suggest that SF is one of the herbal medicines that have a potential in treating IBD.

5. CONCLUSIONS

Upon treating acetic-acid induced colitic mice with 1.5 μ L/g of *Salvia fruticosa* aqueous extract for 2, 4 and 6 days. *Salvia fruticosa* aqueous extract induced enhancement in the colon's structure and decreased ulcerations and inflammation. It significantly decreased inflammation as assessed through the measurement of the pro-inflammatory cytokines. TNF- α , IFN- γ , IL-6 and IL-17 were decreased in the colitic group treated with SF compared to the untreated one at day 2, 4 and 6. Moreover, Myeloperoxidase (MPO) and Malondialdehyde (MDA) levels decreased upon treatment showing the anti-oxidant property of the extract. Anti-oxidant Glutathione (GSH) level and anti-inflammatory cytokine IL-10, remained at normal levels during the treatment process. SF induced a significant increase in anti-inflammatory cytokine TGF- β in healthy SF-treated groups. Hence, *Salvia fruticosa* exerts an anti-inflammatory and anti-oxidant effects against IBD as well as it help healing colon ulcers and maintain its architecture.

REFERENCES

- Anwar, M.A., Samaha, A.A., Ballan, S. *et al.* *Salvia fruticosa* Induces Vasorelaxation In Rat Isolated Thoracic Aorta: Role of the PI3K/Akt/eNOS/NO/cGMP Signaling Pathway. *Sci Rep* **7**, 686 (2017). <https://doi.org/10.1038/s41598-017-00790-9>.
- Bassil, M., Daher, C., Mroueh, M., & Zeeni, N. (2015, October 23). *Salvia Libanotica* improves glycemia and Serum lipid profile in rats fed a high fat diet. Retrieved February 03, 2021, from <https://www.ncbi.nlm.nih.gov/pubmed/26497894>.
- Beltrán, B., Nos, P., Dasí, F., Iborra, M., Bastida, G., Martínez, M., O'Connor, J. E., Sáez, G., Moret, I., & Ponce, J. (2010). Mitochondrial dysfunction, persistent oxidative damage, and catalase inhibition in immune cells of naïve and treated Crohn's disease. *Inflammatory bowel diseases*, *16*(1), 76–86. <https://doi.org/10.1002/ibd.21027>
- Boukhary, R., Raafat, K., Ghoneim, A., Aboul-Ela, M., & El-Lakany, A. (2016, January 03). Anti-Inflammatory and Antioxidant activities of *Salvia fruticosa*: An HPLC determination of Phenolic contents. Retrieved February 04, 2021, from <https://doi.org/10.1155/2016/7178105>.
- Bzour, J., Oran, S., Khaleel, M., Mashallah, S., & Bustanji, Y. (2011, October 7). Inhibitory effects of methanolic extract of *Salvia fruticosa* Mill. on pro-inflammatory cytokines production in RAW 264.7 in vitro cellular model and in Balb/c mice in vivo animal model. Retrieved February 06, 2021, from <https://doi.org/10.5897/JMPR11.586>.

- Ceuleers, H., Spaendonk, H. V., Hanning, N., Heirbaut, J., Lambeir, A., Joossens, J., Augustyns, K., De Man, J. G., De Meester, I., & De Winter, B. Y. (2016). Visceral hypersensitivity in inflammatory bowel diseases and irritable bowel syndrome: The role of proteases. *World Journal of Gastroenterology*, 22(47), 10275-10286.
- Chatterjee, Mary & Sil, Parames. (2006). Hepatoprotective effect of aqueous extract of *Phyllanthus niruri* on nimesulide-induced oxidative stress in vivo. *Indian journal of biochemistry & biophysics*. 43. 299-305.
- Cooper, H. S., Murthy, S. N., Shah, R. S., & Sedergran, D. J. (1993). Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Laboratory investigation; a journal of technical methods and pathology*, 69(2), 238–249.
- Couladis, M. & Koutsaviti, A. (2017). Chemical composition of the essential oils of *Salvia officinalis*, *S. fruticosa*, *Melissa officinalis*, and their infusions. *Ratarstvo i povrtarstvo*. /54. 36-41. <https://doi.org/10.5937/ratpov54-12365>.
- Fabia, R., Willén, R., Ar'Rajab, A., Andersson, R., Ahrén, B., & Bengmark, S. (1992). Acetic acid-induced colitis in the rat: a reproducible experimental model for acute ulcerative colitis. *European surgical research. Europäische chirurgische Forschung. Recherches chirurgicales europeennes*, 24(4), 211–225. <https://doi.org/10.1159/000129209>.
- Fakhoury, M., Negrulj, R., Mooranian, A., & Al-Salami, H. (2014). Inflammatory bowel disease: clinical aspects and treatments. *Journal of inflammation research*, 7, 113–120. <https://doi.org/10.2147/JIR.S65979>.
- Fournier, B. M., & Parkos, C. A. (2012). The role of neutrophils during intestinal inflammation. *Mucosal immunology*, 5(4), 354–366. <https://doi.org/10.1038/mi.2012.24>.
- Fu, Z., Wang, H., Hu, X., Sun, Z. & Han, C.. (2013). The pharmacological properties of salvia essential oils. *Journal of Applied Pharmaceutical Science*. 3. 122-127. [10.7324/JAPS.2013.3723](https://doi.org/10.7324/JAPS.2013.3723).
- Ghorbani, A., & Esmaeilizadeh, M. (2017). Pharmacological properties of *Salvia officinalis* and its components. *Journal of traditional and complementary medicine*, 7(4), 433–440. <https://doi.org/10.1016/j.jtcme.2016.12.014>.
- Gohil, K., & Carramusa, B. (2014). Ulcerative colitis and Crohn's disease. P & T: a peer-reviewed journal for formulary management, 39(8), 576–577.
- Hamza, A., Ahmed, S., Elwy, H. & Radwan, O. (2016). *Salvia fruticosa*-mediated antioxidant protection against oxidative stress in streptozotocin – induced diabetic rats' role of α -glucosidase activity. *International Journal of Pharmacy and Biological Sciences*. 6. 2321-3272. [10.21276/ijpbs.2016.6.3.16](https://doi.org/10.21276/ijpbs.2016.6.3.16).
- Hanauer, S. B. (2006). Inflammatory bowel disease: Epidemiology, pathogenesis, and therapeutic opportunities. *Inflammatory Bowel Diseases*, 12, S3–S9.
- Kim, D. H., & Cheon, J. H. (2017). Pathogenesis of Inflammatory Bowel Disease and Recent Advances in Biologic Therapies. *Immune network*, 17(1), 25–40. <https://doi.org/10.4110/in.2017.17.1.25>.
- Li, R., Alex, P., Ye, M., Zhang, T., Liu, L., & Li, X. (2011). An old herbal medicine with a potentially new therapeutic application in inflammatory bowel disease. *International journal of clinical and experimental medicine*, 4(4), 309–319.
- Low, D., Nguyen, D. D., & Mizoguchi, E. (2013). Animal models of ulcerative colitis and their application in drug research. *Drug design, development and therapy*, 7, 1341–1357. <https://doi.org/10.2147/DDDT.S40107>
- McDowell, C., Farooq, U., & Haseeb, M. (2020). Inflammatory Bowel Disease. In StatPearls. StatPearls Publishing
- Nakhai, L. A., Mohammadirad, A., Yasa, N., Minaie, B., Nikfar, S., Ghazanfari, G., Zamani, M. J., Dehghan, G., Jamshidi, H., Boushehri, V. S., Khorasani, R., & Abdollahi, M. (2007). Benefits of *Zataria multiflora* Boiss in Experimental Model of Mouse Inflammatory Bowel Disease. *Evidence-based complementary and alternative medicine : eCAM*, 4(1), 43–50. <https://doi.org/10.1093/ecam/nel051>
- Raafat, K., Boukhary, R., Aboul-Ela, M., El-Lakany, A. (2013). Endogenous Lebanese Plants Treating Diabetes and Related Complications. *Nat Prod Chem Res* 1:112. <http://dx.doi.org/10.4172/2329-6836.1000112>.
- Ravipati, A. S., Zhang, L., Koyyalamudi, S. R., Jeong, S. C., Reddy, N., Bartlett, J., Smith, P. T., Shanmugam, K., Münch, G., Wu, M. J., Satyanarayanan, M., & Vysetti, B. (2012). Antioxidant

- and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. *BMC complementary and alternative medicine*, 12, 173.
<https://doi.org/10.1186/1472-6882-12-173x>
- Ramos, G. P., & Papadakis, K. A. (2019). Mechanisms of Disease: Inflammatory Bowel Diseases. *Mayo Clinic proceedings*, 94(1),155–165.
<https://doi.org/10.1016/j.mayocp.2018.09.013>
 - Siveen, K. S., & Kuttan, G. (2011). Thujone inhibits lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice. *Canadian journal of physiology and pharmacology*, 89(10), 691–703. <https://doi.org/10.1139/y11-067>.
 - Tian, T., Wang, Z., & Zhang, J. (2017). Pathomechanisms of Oxidative Stress in Inflammatory Bowel Disease and Potential Antioxidant Therapies. *Oxidative medicine and cellular longevity*, 2017, 4535194.
 - Torun, M., Dincer, C., Topuz, A., Sahin-Nadeem, H., & Ozdemir, F. (2015). Aqueous extraction kinetics of soluble solids, phenolics and flavonoids from sage (*Salvia fruticosa* Miller) leaves. *Journal of food science and technology*, 52(5), 2797–2805.
<https://doi.org/10.1007/s13197-014-1308-8>
 - Vieira, S. F., Ferreira, H., & Neves, N. M. (2020). Antioxidant and Anti-Inflammatory Activities of Cytocompatible *Salvia officinalis* Extracts: A Comparison between Traditional and Soxhlet Extraction. *Antioxidants (Basel, Switzerland)*, 9(11), 1157. <https://doi.org/10.3390/antiox9111157>
 - Yeo, I. J., Park, J. H., Jang, J. S., Lee, D. Y., Park, J. E., Choi, Y. E., Joo, J. H., Song, J. K., Jeon, H. O., & Hong, J. T. (2018). Inhibitory effect of Carnosol on UVB-induced inflammation via inhibition of STAT3. *Archives of Pharmacal Research*, 42(3), 274–283.
<https://doi.org/10.1007/s12272-018-1088-1>.
 - Zhang, Y. Z., & Li, Y. Y. (2014). Inflammatory bowel disease: pathogenesis. *World journal of gastroenterology*, 20(1), 91–99.