Protective Effects of *Hyssopus Officinalis* and *Medicago Sativa* Extracts in Salmonella-Induced Colitis by Regulating Antioxidant and Inflammatory Mediators

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Received: December 27, 2021

Published: January 31, 2022

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Abstract

Background: *Salmonella Typhimurium* causes gastroenteritis which is characterized by diarrhea. Nowadays, instead of using anti-bacterial therapies to treat colitis, there is a growing interest in alternative natural products with antibacterial or anti-inflammatory properties. The aim of this study was to investigate the effects of *Hyssopus officinalis* (Hyssop) and *Medicago sativa* (Alfalfa), alone and in combination, on salmonella-induced colitis in mice.

Methods and results: Graded doses of 50% ethanolic extracts (25, 50, and 75 mg/kg) for 8 days, showed 50 mg/kg for Hyssop and 75 mg/kg for Alfalfa as an optimal effective dose against Salmonella induced colonic damage score which this dosage was further studies in salmonella-induced colitis for other parameters. Our data showed administration of extracts, not only reduced Lipid Peroxidation, myeloperoxidase activity, and nitric oxide levels but also inhibited decreasing of total antioxidant capacity in the colitis group. Also, following oral administration, the extracts had a significant reduction in pro-inflammatory mediators including interleukin-1 β and tumor necrosis factor- α , which had better results in the Mix group. Moreover, the Hyssop and Mix group decreased the histological score and Cox-2 mRNA expression, while Nrf-2 gene expression in the treatment groups did not show a significant change compared to the colitis group.

Conclusions: In conclusion, Oral Hyssop and Alfalfa extracts, alone and in combination, alleviate the symptoms, oxidative stress, and inflammation of salmonella-induced colitis, and combination therapy with *Hyssopus officinalis* and *Medicago sativa* may provide a promising dietary approach for the management of acute bacterial colitis.

Keywords: *Hyssopus Officinalis*; *Medicago Sativa*; Salmonella; Colitis; Inflammation; Antioxidant

Introduction

Salmonella enterica serotype Typhimurium is associated with gastroenteritis in humans, which has been one of the most common foodborne poisonings. commonly, the complaint does not

ultimate in addition than a few days and is self-limiting, however, now and again the infection may be more dangerous, with fluid and electrolyte loss in patients, babies, and the elderly and might have an effect on death [1]. The most, not unusual place method of

contaminating salmonella is thru food and water, which upon entry, the bacterium attaches to the surface of the intestinal mucosal epithelium after which enters the vacuoles in the intestinal cells or circulates via cellular junctions and circulates all through the body [2].

Hyssopus officinalis (Hyssop), the mint family is a perennial culinary and medicinal plant. It has a totally robust highly spiced taste and generally utilized in folk medicine [3]. As a medicinal plant, Hyssop is utilized in viral infections along with the common cold, cough, sore throat, bronchitis, and asthma. Tea crafted from herb leaves is powerful in neurological problems and toothache. In addition to its specific aroma, many essential oils, and their remoted additives additionally exhibit muscle relaxant, antibacterial, and antifungal activity [4,5].

Alfalfa, additionally called Lucerne or *Medicago sativa*, is a plant that has been grown as feed for farm animals for masses of years. *Medicago* in the granules is mentioned to have antibacterial, anti-inflammatory properties [6] even as the root has anti-antioxidant action [7], flowers stated for anti-inflammatory [8], a plant has also been reported to exhibit anti-tumor [9] and anti-fungal actions [10].

This research paper aims to investigate the therapeutic effects of ingesting the combined extract of *Hyssopus officinalis* and *Medicago Sativa*; due to their known background, prevention and the healing of inflammation and oxidative stress caused by colitis specifically, salmonella induced gastroenteritis. This research was conducted by studying anti-oxidative and cytokine markers in colon and observing histopathological differences to measure the results of the extracts.

Materials and Methods

Plant collection and extract preparation

Plants were purchased from Pakanbazar Co. (Isfahan, Iran), and ten grams of them were extracted with 100 milliliters of ethanol: water (70%), being macerated at room temperature ($25 \pm 3^\circ\text{C}$) for 3 days with occasional shaking. The solvent was evaporated, and the extracts were concentrated to the desired level and stored at -20°C .

Microbiological analysis

In a separate study, *in vitro* antibacterial susceptibility tests of extracts were done in the department of biology (Faculty of sci-

ence, Gazi University, Turkey) using a serial concentration of 50, 100, 150 and 200 mg/ml following the approved standards of the international committee for clinical laboratory's standard against *S. Typhimurium*. The disk-diffusion assay used was adapted from Taylor, *et al.* methods [11].

Experimental protocol and design

Healthy adult male mice ($25 \pm 5\text{g}$) were procured from the laboratory animal center of Kermanshah University of Medical Science, Iran. They were allowed to adapt to the laboratory environment for one week and had free access to filtered water, and mice chow pellets were housed in solid bottom polypropylene cages under controlled temperature ($20 - 22^\circ\text{C}$) and humidity and light/dark (12/12h) cycles. Before inducing colitis, mice were inoculated intragastrically with 20 mg of streptomycin by oral gavage 24h prior to infection [12]. Animals were infected orally with 108 *Salmonella* in 0.1 ml sterile LB broth. Control mice were given 0.1 ml sterile LB broth. An initial dose response study was undertaken with *Hyssopus officinalis* (Hyssop) and *Medicago Sativa* (Alfalfa) (25, 50 and 75 mg/kg) against *Salmonella*-induced against colonic damage score (Fig. 1B). An optimal effective healing dose of extracts were then selective for future work on inflammation, oxidative stress and histopathology changes. At the 8th day, after blood sampling, animals were killed under deep anesthesia. The colon was excised and rinsed in saline to remove fecal residue, then measured the length. A small section from each colon was placed in 10% formalin.

Assessment of colitis

The animals were weighed and monitored for the appearance of the gross rectal bleeding and stool consistency daily throughout the experimental period. The overall disease severity of each animal in this study was assessed through the disease activity index score which was used for colitis evaluation as described earlier by Cooper, *et al.* [13].

Determination of total antioxidant capacity (TAC) levels in colon homogenate

Spectrophotometer analysis with the aid of a colorimetric assay kit (Naxifer™, Navand Salamat Co., Iran) was used to estimate the concentrations of colon levels of ferric reducing antioxidant power (FRAP). This procedure is based on the ability of colon homogenates to reduce iron III (Fe^{3+}) to iron II (Fe^{2+}) in the presence of 2,4,6-Tripyridyl-S-triazine (TPTZ). A complex with blue color and

maximum absorbance appeared in 593 nm with a reaction of Fe²⁺ and TPTZ.

Determination of lipid peroxidation (LPO) levels in colon homogenate

To evaluate the lipid peroxidation products in tissue samples of the colon, malondialdehyde (MDA) was measured by a Nalondi™ assay kit (Navand Salamat Co., Iran). MDA levels can be detected by the absorbance at a wavelength of 532 nm using a standard curve of MDA.

Determination of nitric oxide (NO) levels in Colon homogenate

The total NO content of the homogenized colon was measured according to the Griess reaction, which is a procedure provided by the manufacturer (Natrix™ assay Kit; Navand Salamat Co., Iran). In the Griess reaction, NO rapidly converts into nitrite, which is an acidic environment, and then converts into HNO². After adding Sulfanilamide, HNO² forms a diazonium salt that reacts with N-(1-Naphthyl) ethylene diamine dihydrochloride to form an azo dye, which could be measured at 570 nm.

Determination of myeloperoxidase activity (MPO) in colon homogenate

The Myeloperoxidase activity was determined using the excised colons were homogenized in 0.1 M phosphate buffer (pH 7.4) and then centrifuged at 12,000 g for 15 minutes in 4°C, the supernatant fraction was used for the measurements of MPO content in colons using the MPO detection kits (Nampox™, Navand Salamat Co., Iran).

Measurement of serum and colonic cytokine levels

Colon homogenates from each animal were prepared using cold Tris-HCl buffer (pH 7), centrifuged at 12000rpm for 10min at 4 °C. The amounts of inflammatory cytokines (TNF-α and IL-1b) in serum and lyse colon were using standard sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instruction.

RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted with the RNX-Plus Solution Kit (Sinaclone, Iran). The extracted RNA was applied for cDNA synthesis kit (Sinaclone, Iran) immediately. The PCR reaction was performed by a thermal cycler Rotor-Gene Q (Qiagen, Germany) and the Master

Mix (Ampliqon, Denmark). The PCR re-actions for mRNAs expression consisted of 95 °C for 7 min (denaturing cycle) followed by amplification cycles (40 cycles) at 90 °C for 15 s (annealing cycle) and 72 °C for 20 s (extension cycle). The sequences of all the primers are listed in table 1, and β-actin was used as a housekeeping gene.

Gene	Primer Sequence (5'-3')
Nuclear factor erythroid 2-related factor 2 (Nrf-2)	F: CTGAACCTCTGGACGGGACTA
	R: CGGTGGGTCTCCGTAAATGG
Cyclooxygenase-2 (Cox-2)	F: CCACTTCAAGGGAGTCTGGA
	R: AGTCATCTGCTACGGGAGGA
B-actin	F: GTGCTATGTTGCTCTAGACTTCG
	R: ATGCCACAGGATTCCATACC

Table 1: List of primer sequences for mice PCR.

F: Forward, and **R:** Reverse.

Assessment of colon histological damage

Fixed colons were embedded in paraffin and cut into 5 mm sections. Tissues were stained with hematoxylin and eosin (HandE) and Periodic Acid Shift (PAS) using standard protocols. Tissue pathology in the infected colon was scored using H and E-stained sections as previously described [14].

Statistical analysis

All experimental results were expressed as the mean ± standard deviation. Statistical analysis was performed using Student's t-test or one-way analysis of variance with Tukey's post-hoc test with GraphPad Prism 9 (GraphPad Software, Inc., La Jolla, CA, USA). P < 0.05 was considered to indicate a statistically significant difference.

Results

Antimicrobial susceptibility and MIC

In vitro antimicrobial tests showed susceptibility tests against *S. Typhimurium* with extracts showing antibacterial activity with an inhibition zone ranging from 4 to 9 mm for Alfalfa and 7 to 11 mm for Hyssop (Figure 1A). Hyssop extract exhibited a stronger antibacterial activity against *S. Typhimurium* tested than Alfalfa. The activity profile for MICs of extracts (Figure 1A) showed a much lower MIC (0.13 mg/mL) which indicating a strong antibacterial activity on *S. Typhimurium*, while this amount for Alfalfa was 0.26 mg/mL (Figure 1A).

Experimental colitis and study of physical parameters

Control group which was given LB broth, did not show any colonic mucosal damage and inflammation or at the end of experiment while, colitis group led to significant increase in colonic mucosal damage score (6.19 ± 0.43). Extracts when given in graded concentration of 25, 50 and 75 for 8 days after induction of colitis showed decrease in damage score in comparison with the colitis group. Therefore, the concentrations of 75 mg/kg for Alfalfa and 50 mg/kg Hyssop showing percentage decrease in damage score > 50% was selected for future studies such as biochemical and histopathology analysis (Figure 1B).

Salmonella-induced colitis led to gradual decrease in body weight (BW) as observed from day 3 onwards till 8th day of study. Significant decrease in BW was observed from the 5th day in comparison with the control group. Treatment with Alfalfa (75 mg/kg), Hyssop (50 mg/kg) and the combination of these two plants (Mix group: 75 mg/kg Alfalfa and 50 mg/kg Hyssop) for 8 days after colitis induction showed reversed the decrease trend in BW (Figure 1C). The DAI of the colitis group was significantly ($p < 0.0001$) elevated on day 8 compared to the control group. Treatment with 50 mg/kg Hyssop ($p < 0.0113$) and mix of extracts ($p < 0.0006$) markedly reduced the DAI score in comparison with colitis group (Figure 1D).

Figure 1: A: Antibacterial activity and minimum inhibitory concentration (MIC) of Hyssop and Alfalfa extracts on Salmonella Typhimurium. MIC for Hyssop was significantly lower than Alfalfa, also, zone of inhibition range for Alfalfa was 4 to 9 mm and it was 7 to 11 mm for Hyssop extract. B: Effects of graded dosage of 70 % ethanolic extracts on Salmonella-induced colitis, which revealed that 50 mg of Hyssop and 75 mg/kg of Alfalfa could reduce percentage decrease in damage score (PDDS). C: changes in body weight (BW) in 8 day of treating with extracts. Colitis group show significant decreasing in BW percent of 0-day value in compare with control group. D: Assessment of disease activity index (DAI) in salmonella-induced colitis model. The DAI of the Mix group presented a significant ($p < 0.001$) decrease when compared to the colitis group. Values are mean ± SE of 5 mice in each group.

Effects on total antioxidant capacity (TAC)

Salmonella enhanced TAC in colonic homogenate in comparison with control group ($p < 0.0001$). In extracts groups, the reduction of TAC was prevented and the comparison of the extract groups with the colitis group (0.26 ± 0.06) showed that have higher levels of TAC, but the interesting point is that the antioxidant power of Alfalfa (0.43 ± 0.06) according to this parameter compared to the other two treatment groups, i.e. mix (0.80 ± 0.14) and Hyssop (0.74 ± 0.04) is less and there was no significant ($p > 0.05$) (Figure 2A).

Effects on lipid peroxidation (LPO)

Colitis group significantly increased the mean MDA level to 22.85 ± 3.41 nmol/g colon weight ($p < 0.0001$) compared to the control (2.99 ± 0.72). The groups receiving the extracts individually and in combination showed a decrease ($p < 0.0001$) in the level of MDA compared to the colitis group (Figure 2B).

Figure 2: Effects of extracts, alone and in combination on salmonella-induced changes in free radicals A: Total antioxidant Capacity (TAC), B: Lipid peroxidation marker (MDA), C: Nitric Oxide (NO) and D: Myeloperoxidase activity in colonic mucosa.

Values are mean $\pm$ SE of 5 mice in each group.

Effects on nitric oxide (NO)

To investigate whether the extracts have potential against nitrosative stress in salmonella-induced gastroenteritis. As shown in figure 2C, NO production was strongly induced in salmonella-stimulated colitis, while treatment with extracts notably inhibit the salmonella-induced NO production. Interestingly, Hyssop (17.23 ± 2.25 nmol/g colon weight) had more inhibition of NO production than Alfalfa (25.25 ± 2.04 nmol/g colon weight).

Effects on myeloperoxidase (MPO) activity

The MPO activity is a marker of neutrophil infiltration, which plays an important role in polymorph-nuclear infiltration. As shown in figure 2D, colitis group showed significant increase in MPO level (4.13 ± 1.04) in the colonic mucosal when express as U/mg colon weight compared to control (0.79 ± 0.16). Our data revealed that, Hyssop and Alfalfa groups couldn't decrease MPO activity compared to colitis group but in combination therapy for these extracts, significantly ($p < 0.05$) inhibited salmonella induced MPO activity.

Effect on levels of TNF- α in the serum and colon homogenate

The TNF- α concentration in the serum and colon of control mice was 83.38 ± 11.02 pg/ml and 431.50 ± 55.17 pg/g tissue, respectively. Colitis group significantly ($p < 0.0001$) increased serum and colon TNF- α concentration (191.53 ± 20.84 pg/ml and 1217.69 ± 22.04 pg/g colon weight). Meanwhile, extracts whether using as single or combined, had prevented TNF- α in colon homogenate and serum from increasing (Figure 3A and B).

Effect on levels of IL-1 β in the serum and colon homogenate

ELISA assays were performed to evaluate the level of IL-1 β . Compared to normal controls, IL-1 β protein level were significantly ($p < 0.0001$) up-regulated in serum (from 149.89 ± 16.71 to 2263.20 ± 49.41 pg/ml) and colon of colitis group (from 14.44 ± 1.12 to 32.09 ± 5.90 pg/g Colon). Treatments with Hyssop or simultaneously administration of Hyssop and Alfalfa significantly down-regulated IL-1 β in comparison to the colitis group. Alfalfa at 75 mg/kg did not have a significant effect on IL-1 β . Analysis of the results in colonic tissue showed that, like the serum, it increased after colitis in the tissue, and in the groups treated with the extracts were able to prevent the increase of this marker in the tissue (Figure 3C and D).

Figure 3: Violin plots showing comparison of serum and colon homogenates concentrations of selective proinflammatory cytokines in salmonella-induced colitis. A: TNF-α in serum, B: TNF-α in colon tissue, C: IL-1β in serum, D: IL-1β in colon tissue. Values are mean ± SE of 5 mice in each group.

Effect on Nrf-2 expression in colon tissue

Nrf2 is a main regulator of detoxification, which has been shown to activate the transcription of genes encoding for antioxidant enzymes. Thus, the role of Nrf-2 in mediating the protective effects of extracts on salmonella-induced colitis was examined. Compared with the healthy control mice, colitis group had markedly lower expression levels of Nrf-2 (Figure 4A). However, mRNA levels of this gene in treated groups which was detected using RT-PCR, did not show a significant change compared to the colitis group (Figure 4B).

Effect on Cox-2 expression in colon tissue

The mRNA levels of Cox-2 as an important precursor of prostacyclin, which is expressed in inflammation, were measured by RT-PCR of colon of mice. As shown in Figure 4A-C, Cox-2 was significantly expressed in colonic samples in the colitis group after acute colitis induced by salmonella. Interestingly, preventive treatment in each of treat's groups markedly reduced the up-regulation of Cox-2 in colonic tissue.

Figure 4: A: Expression of Nrf-2 and COX-2 in colon of mice detected by RT-PCR analysis. B: mRNA from colonic extracts was analyzed by semiquantitative RT-PCR for Nrf-2. Expression is presented as a ratio of gene/β-actin and C: mRNA from colonic extracts for COX-2, respectively.

Histopathological studies of the colon

Colon length is a useful indication of colitis and was therefore measured as a marker of inflammation. So, after killing, the length of the colon from the colocaecal junction to the anal verge was measured. There was a significant shortening of the colon in groups in which colitis is induced compared with control group. The oral administration of Alfalfa improves this inflammatory marker compared with Colitis treatment alone. Unlike previous markers, Hyssop could not have beneficial effects on this marker ($p > 0.05$) (Figure 5B). Mix treatment reversed a Salmonella-induced decrease of crypt depth in mouse colon (Figure 5D). Histologic analyses of colonic sections which stained with hematoxylin and eosin (H and E) revealed complete disruption of the colonic architecture in colitic mice, whereas Hyssop and Mix-treated colitic mice display a better preservation of tissue architecture and reduced epithelial denudation, crypts distortion, and leukocyte infiltration of the lamina

propria (Figure 5A). Consistent with the histological findings, the crypt damage of Mix-treated colitic mice was significantly lower than in Salmonella-induced enteritis mice (Figure 5C). Furthermore, the reduction in neutrophil infiltration observed by histological inspection in Hyssop and Mix groups were confirmed by the

reduced MPO activity detection in colon tissues from Mix-treated group (Figure 2D). Altogether, HandE analysis strongly support the view that Simultaneous use of two combinations could ameliorate salmonella-induced experimental colitis.

Figure 5: A: Images of hematoxylin and eosin (H and E) and Periodic acid shift (PAS)-stained colon tissue samples.

Magnification, $\times 40$. B: Colon Length (cm). C: Crypt damage

scoring. D: Crypt length (%). Values are mean $\pm$ SE of 5 mice in each group.

Discussion

Changes in intestinal microbiota are critical players in a large number of intestinal and extra-intestinal diseases. When the homeostasis of intestinal flora is destroyed, a big variety of dangerous microorganisms can result in oxidative stress-related pathways within the host cell, main too complicated interactions, including the atypical differentiation and apoptosis of intestinal cells,

destruction of the epithelial barrier, and inflammation response. For example, *Helicobacter* and *Salmonella typhimurium* are major pathogenic bacteria inflicting intestinal oxidative stress and disorder of intestinal homeostasis. Most salmonella-caused infection is severe [15], and if the treatment is not successful leads to severe inflammation and has the capacity to provoke gastrointestinal cancer. The outcomes of the study showcase that the animals with salmonella-induced colitis advanced pathological damage and simi-

larly pathological differentiation, mainly in inputting inflammatory cells within the specimen. The presence of inflammatory cells and gastrointestinal tissues has been reported in previous studies [16]. Additionally, notable weight reduction has been distinguished in the salmonella group, while in the treatment groups, weight reduction was prevented. One of the most important causes of weight reduction observed in the colitis group has been due to diarrhea, moreover, damage to the gastrointestinal system caused a decrease in the absorption and digestion of nutrients [17] which these results aligned with disease activity index (DAI) data. According to our findings, Hyssop and Mix treatments improve DAI compared to colitis mice, modulating critical proteins involved in inflammation and oxidative stress regulation. Most likely the extracts used were able to be improved DAI in parallel with the increased barrier integrity, suggesting that modulating tight junctions [18]. The length of the colon has been found to decrease in colitis groups; this factor is also used to predict inflammation [19]. In treatment groups, the Hyssop group has been shown to prevent a decrease in colon length, which aligns with research done by Micovic, *et al.* in which they used this extract, and it highlighted anti-inflammatory qualities in colon cells due to its possession of flavonoids and phenolic acids, tannins [20].

Previous clinical and research studies indicated that in inflamed colon, the antioxidant balance is disturbed by multinucleated WBC-derived oxidant and increased MPO activity, which can subsequently cause various colonic diseases [21]. Our data revealed that extracts can prevent a decrease in antioxidant capacity or increase Lipid peroxidation, due to their antioxidant effects by possessing chemicals such as phenol [22,23]. Reflecting this study's results, the combined group of extracts seems to have been able to prevent the reduction of TAC, which can be concluded that the combination of herbs improves oxidative stress [24]. Malondialdehyde (MDA) is the product of free radical oxidation and lipid peroxidation which indicates the degree of peroxidation and damage [25]. Hyssop prevents the increase of MDA better than even combining two extracts. Amount of After Salmonella induced colitis, MPO levels (a marker of neutrophil activity) increased several folds; this finding is consistent with earlier findings that suggested neutrophils play a key role in inflammation, causing excessive production of pro-inflammatory cytokines and ROS, which have been implicated in inflammation development [20]. Intestinal inflammation or Damage to the colonic mucosa has been found to be associated with an overproduction

of NO by the inducible isoform of NO synthase [26]. Therefore, it has been postulated that antioxidants could accelerate the healing process by destroying the free radicals, so eliminating ROS is likely to be important strategy in colitis treatment.

Many studies have suggested the role of microbial content of intestinal in pathogenesis of colitis and shown increases in concentration of bacteria progressively with disease severity [27]. Hyssop exhibited considerable level of inhibition against Salmonella compared to Alfalfa. Studies have shown that both extract have antimicrobial and anti-inflammatory properties for a variety of bacteria [28], which our data corroborates with the finding of recent study, where they have revealed that *Hyssopus officinalis* be able to inhibit the growth of Salmonella with a lower concentration due to the substances contained in it [20].

Bacterial infection and invasion of the intestine induce inflammatory responses, including upregulation of proinflammatory cytokines [29]. Greater expression of tumor necrosis factor alpha (TNF- α) in colon tissue were demonstrate in salmonella induced colitis [30] which causes histopathological damage, organ dysfunction, and is able to mediate cell survival by activating NF- κ B signaling [31]. TNF- α mediates the depletion of goblet cells during *S. Typhimurium* infection in mice, as pre-treatment with anti-TNF- α antibody restores the goblet cell numbers and mucin profiles [30]. We, however, saw an increase in goblet cell numbers on infection in colon of colitis group (Fig. 3A). This study's results showed that IL-1b decrease in serum and colon tissue after treating with extracts. The cytokine interleukin 1 β is an important mediator of inflammatory processes capable of inducing eicosanoid production, T-cell activation, and development subsequent mucosal inflammation [32]. Induction of interleukin 1 β messenger RNA in enterocytes is causally related to the subsequent inflammatory changes seen in acute experimental colitis which recent studies have shown that some herbal therapies improve colitis in this way [33].

Oxidative stress in inflamed colonic tissue can induce Nrf2 gene expression that might play a role in arresting both inflammatory response and oxidative damages of Colitis [34]. Consistent with the results of antioxidant markers in this study, expression of Nrf-2 was reduced in the colitis group, but the comparison of the expression of it in the extract groups with the colitis group did not show a significant difference. Nuclear factor erythroid-related factor 2 (Nrf2)

is a redox-sensitive transcription factor which plays an essential role in protection against oxidative stress [35], so one of hypothesis could be that following the use of extract with high antioxidant properties, no changes in the Nrf2 pathway occur due to the balance of the body's antioxidant status. Cyclooxygenase-2 (COX-2) is overexpressed in ulcerative colitis due to *S. typhimurium* [36]. Our data revealed that unlike previous parameters on this article, Hyssop could not reduce the mRNA level of COX-2 compared to other groups which seems to be have another way to reduce the effects of Salmonella's side effects. In an article compiled by Bernal-Bayard and Ramos-Morales, Salmonella evade or manipulate physical barriers, innate immunity and adaptive immunity of digestive system. In other hand, Salmonella manipulates the autophagy process and evades the adaptive immune system by interacting with dendritic cells, and T and B lymphocytes [37] which the results of interleukin and MPO activity in our study also indicate a modulation in the immune system.

This research study confirms that extracts of *Hyssopus officinalis* and *Medicago sativa* have significant anti-inflammatory impacts in salmonella-induced colitis mouse models. Suppression of the inflammatory process by *Hyssopus officinalis* and *Medicago sativa* is linked with reduced oxidative stress and reduced measures of pro-inflammatory molecules in colonic tissues. The most important point found in this study was that Alfalfa alone has less anti-inflammatory effects than Hyssop, but in combination with this plant could enhance the beneficial effects of Hyssop. This research attests that combination therapy with *Hyssopus officinalis* and *Medicago sativa* could be used as therapeutic agents for the treatment of salmonella induced colitis.

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