ORIGINAL ARTICLE

Benefit of *Aloe vera* and *Matricaria recutita* Mixture in Rat Irritable Bowel Syndrome: Combination of Antioxidant and Spasmolytic Effects*

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ABSTRACT **Objective**: To evaluate the beneficial effects of a mixture of *Aloe vera* (AV) and *Matricaria recutita* (German chamomile, GC) in an experimental model of irritable bowel syndrome (IBS). **Methods**: IBS was induced by a 5-day restraint stress in rats including the groups of control (water), GC (300 mg/kg), loperamide (10 mg/kg), mixed AV and GC (50: 50 at doses of 150, 300 or 450 mg/kg assigned as Mix-150, Mix-300 and Mix-450, respectively) and the sham group which did not receive any restraint stress and was fed with saline. All medications were administered intragastrically by gavage for 7 days, 2 days as pre-treatment followed by 5 days during induction of IBS every day before restraining. **Results**: The increased tumor necrosis factor alpha (TNF-α), myeloperoxidase (MPO) activity, and lipid peroxidation (LPO) in colonic cells in the control group were significantly decreased in the treatment groups. GC inhibited only small bowel transit while the AV/GC mixture delayed gastric emptying at the doses of 150 and 300 mg/kg. The AV/GC mixture also reduced colonic transit and small bowel transit at the dose of 150 mg/kg. **Conclusions**: The severity of stress-induced IBS was diminished by the AV/GC mixture at all doses used but not dose-dependently, via inhibiting colonic MPO activity and improving oxidative stress status. The effect of the mixture was more effective than GC alone. The present results support effectiveness of the AV and GC combination in IBS.

KEYWORDS *Aloe vera*, *Matricaria recutita*, irritable bowel syndrome, oxidative stress, colitis

The exact etiology of irritable bowel syndrome (IBS) is not known yet and thus effective management of IBS is still a challenge.[1] Visceral hypersensitivity in the development of pain or discomfort, gut dysmotility, 5-hydroxy tryptamine (5-HT) dysregulation, stress and psychological disturbances, previous infection, small intestinal bacterial overgrowth, food intolerance,[1] inflammation, immune activation, and oxidative stress[2] seem involved in the pathophysiology of IBS. Different therapeutic approaches for management of IBS symptoms include antidiarrheals, laxatives, bulking agents,[1] antispasmodics,[3] tricyclic antidepressants/selective 5-HT reuptake inhibitors (SSRIs),[4,5] 5-HT3 receptor antagonists, 5-HT4 receptor agonists,[6,7] and antibiotics/probiotics.[8,9]

In the recent years, some medicinal herbs have been examined experimentally and some were found effective in the management of IBS.[10] New investigations have shown the role of inflammatory mediators in pathogenesis of IBS.[2,11] Elevated tumor necrosis factor alpha (TNF-α), myeloperoxidase (MPO) activity, interleukin (IL)-1β, IL-6, activated neutrophil and eosinophil in IBS patients have been reported.[11] Stress-induced colonic inflammation and increased permeability lead to an increase in cell membrane lipid peroxidation (LPO) and expression of IL-1α mRNA in mucosal biopsies.[11,12] Also, stress facilitates the entry of luminal contents that activate previously-sensitized CD4 T cells in the colon, initiating an inflammatory response.[12] Therefore, it is reasonable to examine the possible effects of antioxidants and inflammatory modulating agents from natural sources.[13]

Aloe vera (AV) grows in hot and dry climates in Asia, Africa, and other tropical parts of the world. There are many studies that have proven its medicinal effects; these effects are thought to be due to the presence of compounds such as polysaccharides, mannans, anthraquinones, and lectinsas.[14] Many studies

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have demonstrated immunomodulatory and anti-inflammatory activity of the extracts of AV.[15] Several studies suggest protective effects of AV in many diseases via its anti-inflammatory activity. AV heals gastric lesions by reducing leukocyte adherence and TNF-α, and elevating IL-10 level. Also its protective effect on polymicrobial sepsis through attenuating TNF-α, IL-1β, and IL-6 has been reported.[14] It is believed that AV has analgesic effect associated with its anti-inflammatory activity.[15] The other herbal medicine *Matricaria recutita* or *Chamomilla recutita* known as German chamomile (GC) grows over Europe, Western Asia and the USA. The main active constituents of GC are chamazulene, apigenin, bisabolol, terpenoids, α-bisabolol, flavonoids, luteolin, quercetin, tannin, and polysaccharides.[16,17] It has been primarily used as sedative, anxiolytic, and antispasmodic. Its aqueous and ethanolic extracts are mainly used for antiinflammatory, antiseptic, and spasmolytic purposes.[18-20] Regarding above information about pathophysiology of IBS and previous findings about AV and GC, we were interested to examine the effects of AV and GC alone and in combination in an experimental stress-induced IBS.

METHODS

Materials

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol, hexadecyl trimethyl ammonium bromide (HETAB), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), phenol red, malondialdehyde (MDA), ethylene diamine tetra acetic acid (EDTA), O-dianisidine hydrochloride from Merck Chemical Co. (Germany), bovin serum albumin (BSA) from Sigma-Aldrich (Germany), rat specific TNF-α enzyme-linked immunoassay (ELISA) kit from Bender MedSystems GmbH (Austria) were used in this study. Loperamide was obtained from Abidi Pharmaceutical Company, Tehran, Iran.

Extract Preparation

The whole leaves of AV and the dried flower heads of GC were used. The extract powders were obtained from Institute of Medicinal Plants, Academic Center for Education, Culture and Research (ACECR), Karaj, Iran. Aqueous extracts were obtained by infusing in phosphate-buffered water and were prepared just before use and administered by gavage.

Animals

Male Wistar *albino* rats, weighing 200–230 g, from animal house of Faculty of Pharmacy at Tehran University of Medical Sciences were used for the experiment. Animals were maintained under standard conditions of temperature (23±1 ℃), relative humidity (55%±10%), and 12/12 h light/dark cycle, and had free access to standard pellet diet and tap water. Animals were cared under guidelines set by the Tehran University of Medical Sciences Review Board.

Experimental Design

The rats were assigned to six groups with 18 rats in each group, including the sham, control, loperamide and three AV/GC groups. IBS was induced by restraint stress model in all groups except the sham group. For treatment, both the sham (Normal) and model control (Control) groups received saline, the GC group received 300 mg/(kg·d) of GC,[15] the loperamide group received 10 mg/(kg·d) loperamide,[21] and the three AV/GC groups, Mix-150, Mix-300 and Mix-450, were treated with 50:50 mixture extract of AV and GC at doses of 150, 300 and 450 mg/(kg·d), respectively. All medications were administered intragastrically by gavage for 7 days, 2 days as pre-treatment followed by 5 days during induction of IBS before restraining.

Induction of IBS

Restraint stress method for induction of IBS was performed. Animals were lightly anesthetized with inhalation of ether and the restraint was performed using plastic tube restrainers that allowed for a close fit to rats. Diameters of tubes were stocked to fit closely round the whole animal body. The both ends of the tube were closed to avoid any movements in a way that allowed oxygen in and exhaust expel. Stress consisted of 6 h of immobilization starting at 9 am for 5 consecutive days.[12] Loperamide group was used as a standard to compare the capability of plants extracts in gastric emptying, small bowel transit, and fecal pellet excretion within 4 h after the day 5 restraint stress. Biochemical parameters were measured in all groups except for the loperamide group.

Sample Preparation

On the 5th day after restraining, each group was divided into three subgroups with each containing 6 rats. Phenol red was administered by gavage to overnight-fasted rats in two of the three subgroups. The rats in one of the subgroups that received phenol red were anesthetized using pentobarbital sodium (50 mg/kg) after 60 min and those in the

other subgroup were anesthetized after 120 min. The stomach, small intestine and colon were removed by laparotomy and rinsed in cold saline bath. All rats were sacrificed by overdose of ether inhalation following the procedure. Colons were used for biochemical and immunological evaluations and cut open in an ice bath, cleansed gently with cold saline, then weighed and homogenized in 10 volume ice cold potassium phosphate buffer (50 mmol/L, pH 7.4), then 100 μL of the homogenates were taken for ferric-reducing antioxidant power (FRAP) assay and kept in –80 ℃ until analysis. The rest of samples were sonicated and centrifuged for 30 min at 3,500 *g*, and then the aliquoted supernatants were transferred in into micro-tubes and kept in –80 ℃ until analysis. The small intestine was divided horizontally into three equal segments, and the stomach and three intestinal segments were used to determine gastric emptying and small bowel transit. In the second part of the experiment, the third non-fasted subgroups were used to evaluate colonic transit.

Gastric Emptying and Small Bowel Transit Measurement

For determination of gastric emptying and small bowel transit, phenol red recovery method was used as described previously.[22] Animals received 1 mL of 1.5% methylcellulose solution containing 0.5 mg phenol red by gavage. The stomach and three equal segments of the small intestine were homogenized in 100 mL of 0.1 mol/L NaOH for 30 s. The suspension was stored at room temperature for 60 min. Then 5 mL of supernatant was added to 0.5 mL of 20% TCA (W/V) and centrifuged at 3,000 *g* for 20 min. The supernatant was added to 4 mL of 0.5 mol/L NaOH. Finally, the absorbance of the samples was read by a ultraviolet-visible spectrophotometer at 560 nm.[22] A calibration curve was used to measure the concentration of phenol red and the percentage of gastric emptying was derived according to the following formula: 1– amount of phenol red recovered from test stomach x 100/average amount of phenol red recovered from normal stomachs. Small bowel transit was calculated by differences between the amount of phenol red in the first and third segments expressed as a percent of total amount recovered from small intestines.

Colonic Transit Evaluation

To assess colonic transit, fed animals were used to evaluate the fecal excretion. Fecal pellets output in rats were counted for 4 h after the 5th day of 6-h restraint stress and the number of pellets was used to quantify colonic transit.

Biochemical Assays

TNF-α Assay

A rat specific ELISA kit, Bender Med Systems (Vienna, Austria) was used to quantify TNF-α in colon tissues. Amount of cytokine was assessed at the final step by measuring the absorbance of the sample in 450 nm as the primary wave length and 620 nm as the reference wave length by ELISA reader as described by kit brochure. Data were expressed as pg/mg protein of the tissue.

MPO Activity Assay

MPO activity was measured by observing the rate of change in the absorbance of UV spectrophotometer for 3 min in 460 nm as set up and described previously.[23] MPO activity was reported as U/mg protein of the tissue.

LPO Assay

Thiobarbituric acid-reactive substance (TBARS) was used to measure LPO. Specifically, lipid peroxides in the samples reacted with TBA and produced a measurable pink color with maximum absorption at 532 nm in a UV spectrophotometer. The complete procedure has been described previously.[24] Data were reported as mmol/g protein of the tissue.

Total Antioxidant Capacity Assay

The antioxidant capacity of the colon homogenate was determined by measuring the extent of reduction of Fe^{+3} to Fe^{+2} that in complex with TPTZ produced a blue color and was measured at 593 nm in a UV spectrophotometer as described in details in our previous work.[24] Data were expressed as mmol ferric ions reduced to ferrous per gram of colon tissue.

Total Protein of Colon Homogenate Measurement

Total protein of colon homogenates was measured by Bradford method using BSA as the standard. Results were reported as mg/mL of homogenized tissue.

Statistical Analysis

One-way ANOVA followed by Newman Keul's posthoc test for multiple comparisons were used. *P* values less than 0.05 were considered significant. Results were expressed as mean ± standard error of the mean (SEM).

RESULTS

Gastric Emptying

A significant elevation in gastric emptying in the control group (154.33 ± 11.45) was observed when compared to the normal group (100.88 ± 14.37, *P*<0.01). The loperamide group (73.43 ± 5.80) showed a significant reduction in gastric emptying in comparison to the control group (*P*<0.01). The GC group (146.75 ± 9.32) had gastric emptying similar to the control group, demonstrating no inhibitory effect. In contrast, the Mix-150 (90.46 ± 10.07) and Mix-300 (101.53 ± 12.48) significantly reduced gastric emptying in comparison to the control and GC groups (*P*<0.01 and *P*<0.05, respectively), without significant difference compared with the loperamide group. There was no significant difference between Mix-450 (133.25 ± 12.95) and the control group (Figure 1).

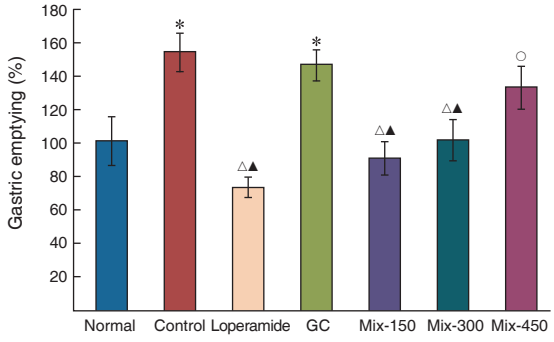

Figure 1. Gastric Emptying Percentages after 60 min

Notes: *P<0.01, compared with the normal group; △P<0.01, compared with the control group; ▲P<0.05, compared with the GC group; ○P<0.01, compared with the loperamide group

Small Bowel Transit

Small bowel transit in the control group (152.47 ± 10.92) was significantly higher when compared to the normal group (85.38 ± 14.75, *P*<0.05). The loperamide group (67.23 ± 10.23) showed a significant reduction in small bowel transit in comparison to the control group (*P*<0.01). Treatment with either GC (90.74 ± 10.83) or Mix-150 (99.95 ± 11.61) significantly inhibited small bowel transit when compared to the control group (both *P*<0.05). No significant difference was observed for Mix-300 (136.56 ± 13.72) and Mix-450 (140.51 ± 19.99) in comparison to the control group (Figure 2).

Colonic Transit as Fecal Pellet Output

Restraint stress caused a significant elevation in colonic transit in the control group (number of pellets excreted: 21.84 ± 0.99) in comparison to the normal group (15.10 ± 0.64, *P*<0.01). Pellet output significantly decreased in the loperamide group (9.13 ± 0.62) when compared to the control group (*P*<0.01) and the normal group (*P*<0.05). GC (20.67 ± 0.84) did not reduce pellet output in comparison to the control group. The reduction was significant in Mix-150 group (15.14 ± 1.49) when weighted against the control group (*P*<0.01). Mix-150 fecal pellets output was also lower in comparison to GC (*P*<0.05). No difference was observed between either the Mix-300 (19.5 ± 1.57) or Mix-450 (18.33 ± 0.86) and the control group (Figure 3).

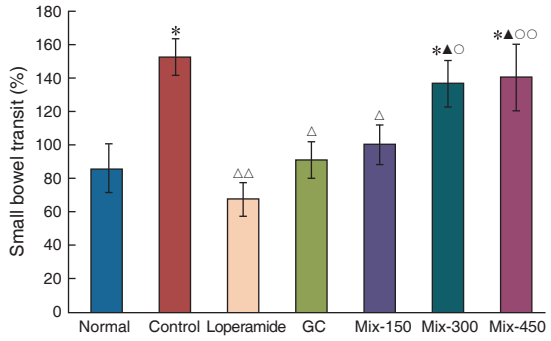

Figure 2. Small Bowel Transit Percentages after 120 min

Notes: *P<0.05, compared with the normal group; △P<0.05, △△P<0.01, compared with the control group; ▲P<0.05, compared with the GC group; ○P<0.05, ○○P<0.01, compared with the loperamide group

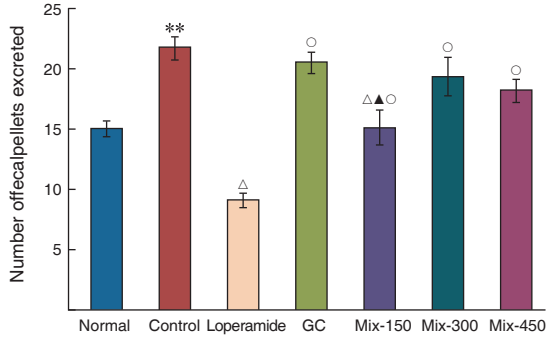

Figure 3. Fecal Pellet Excretion within 4 h after the Day 5 Restraint Stress

Notes: *P<0.05, **P<0.01, compared with the normal group; △P<0.01, compared with the control group; ▲P<0.05, compared with the GC group; ○P<0.01, compared with the loperamide group

Colonic TNF-α

Restraint stress caused a significant elevation in colonic TNF-α protein production (pg/mg protein) in the control group (169.74 ± 10.11) when compared to the normal group (71.88 ± 8.70, *P*<0.01). GC treatment was able to diminish the level of TNF-α (113.26 ± 8.67) comparing to the control group (*P*<0.05). The animals in all 3 Mix groups, Mix-150 (115.60 ± 7.51), Mix-300 (107.50 ± 12.88) and Mix-450 (109.15 ± 11.91), showed

a significant decrease in TNF-α in comparison to the control group (P<0.05). There was no significant difference in TNF-α protein production between three Mix groups and the GC group (Figure 4).

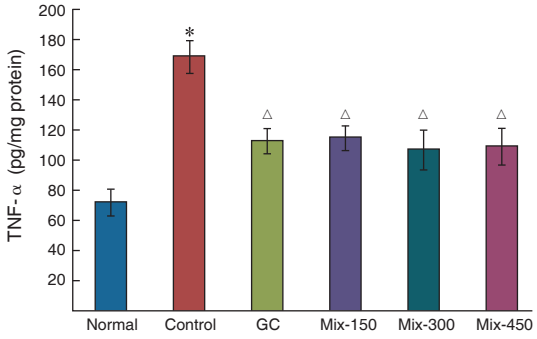

Figure 4. TNF-α Level in Colon

Notes: *P<0.01, compared with the normal group; △P<0.05, compared with the control group

Colonic MPO Activity

There was a significant increase in colonic MPO activity (mmol/g colon) in the control group (27.65 ± 1.189) comparing to the normal group (4.98 ± 0.77, P<0.01). MPO activity in the GC treated group (20.80 ± 0.14) was significantly lower than that of control (P<0.01). All doses of Mix extract, Mix-150 (21.08 ± 2.37), Mix-300 (13.55 ± 0.51), Mix-450 (21.97 ± 1.52) significantly decreased MPO activity in comparison to the control group (P<0.05). Moreover, Mix-300 lowered MPO activity significantly more than GC group (P<0.01, Figure 5).

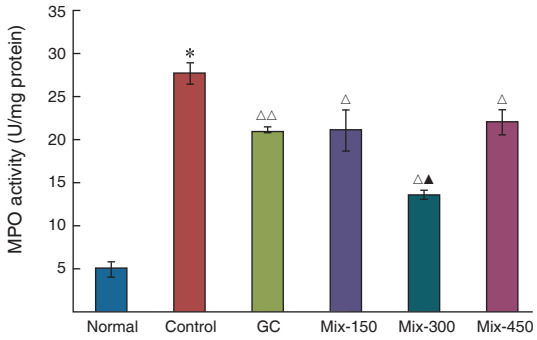

Figure 5. MPO Activity in Colon

Notes: *P<0.01, compared with the normal group; △P<0.05 △△P<0.01, compared with the control group; ▲P<0.01, compared with the GC group

Colonic LPO as TBARS

TBARS (μg/g colon) was significantly higher in the control group (39.84 ± 0.30) when compared to normal group (10.84 ± 0.46, P<0.01). TBARS decreased in the GC group (30.37 ± 0.99) in comparison to the control group (P<0.01). There was a significant decrease in TBARS in all Mix groups, Mix-150 (22.11 ± 1.12), Mix-300 (20.10 ± 1.04) and Mix-450 (21.89 ± 2.23), in comparison to the control group (P<0.01). In addition, all Mix groups showed lower TBARS when compared with the GC group (P<0.01). There was no significant difference among the three Mix groups (Figure 6).

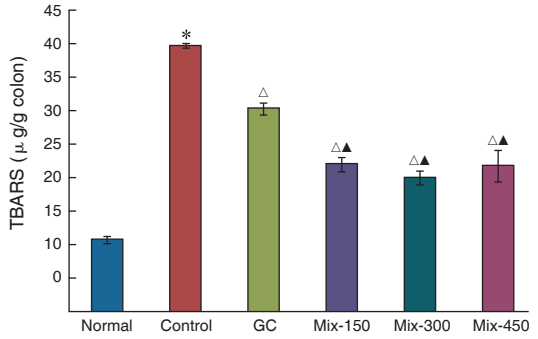

Figure 6. LPO as TBARS in Colon

Notes: *P<0.01, compared with the normal group; △P<0.01, compared with the control group; ▲P<0.01, compared with the GC group

Colonic Total Antioxidant Capacity as FRAP

FRAP (mmol/g colon) in the control group (311.65 ± 10.75) was significantly lower than that of the normal group (603.06 ± 10.44, P<0.01). FRAP was significantly increased in the GC group (498.92 ± 14.67) in comparison to the control group (P<0.01). The Mix groups, Mix-150 (700.68 ± 23.06), Mix-300 (725.94 ± 15.77) and Mix-450 (739.01 ± 21.39) showed a significant increase in FRAP in comparison to the control group (P<0.01) to a level that was higher than that of the normal group. Moreover, a significant increase was observed in all Mix groups when compared to the GC group (P<0.01). There was no significant difference among the Mix treated groups (Figure 7).

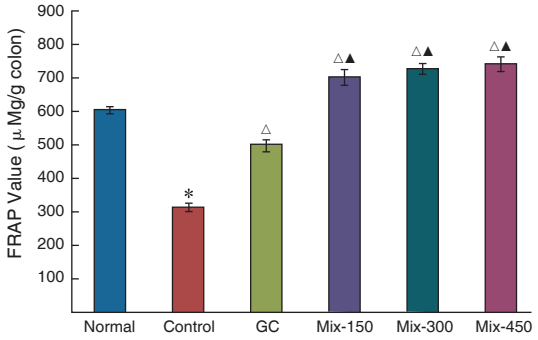

Figure 7. Ferric-reducing Anti-oxidant Power of Colon

Notes: *P<0.01, compared with the normal group; △P<0.01, compared with the control group; ▲P<0.01, compared with the GC group

DISCUSSION

On investigating the beneficial anti-inflammatory and antioxidant effect of AV and GC, two known

herbal extracts on rat stress-induced IBS model, we observed that GC and AV/GC mixed extracts are able to ameliorate the increased TNF-α, MPO, TBARS and also improved total antioxidant defense of colonic tissues. Moreover, the modulator effect of the mixed extract on increased gastrointestinal (GI) transit was seen with the lowest tested dose (150 mg/kg) and such effect may be attributable to GC's spasmolytic activity as such effect was also observed with the GC treatment.

Studies have shown the association between sensitization of GI nervous system and immune activation in IBS[11] while there was also evidence supporting the existence of oxidative stress in IBS.[13] In this study, we performed a reliable IBS model by use of restraint stress on the basis of the belief that stress and psychological factors are involved in IBS. Stress induces secretion of corticotropin-releasing factor from hypothalamus that leads to initiation of inflammatory processes via increasing intestinal permeability and mast cell degranulation, and decreasing the colonic transit time. It acts on CRF receptor-1 (CRFR1) in the nervous central system and GI tract, and stimulates parasympathetic nervous system, resulting in acceleration of lower GI tract motility.[25] The process includes extra uptake of macromolecular protein antigens that initiate or exacerbate inflammation and immune reaction.[26] It is believed that stress-induced colonic inflammation increases cell membrane LPO and expression of IL-1α mRNA in mucosal biopsies.[27] Also stress increases the colonic permeability, facilities the entry of luminal contents that activate previously sensitized CD4 T cells in the colon initiating the inflammatory reaction.

The increase in IL-1β, IL-6, and TNF-α secreted by peripheral blood mononuclear cells has been reported in IBS patients.[2] Interestingly, the present results indicated that treatment with the AV/GC extract reduced the TNF-α level in IBS rats. In support, the potential of AV in reduction of leukocyte adherence and TNF-α, besides elevating IL-10 levels, and healing of gastric ulcers has been reported.[28] Preliminary studies have suggested that oral AV gel may reduce symptoms and inflammation in patients with ulcerative colitis.[29] It has been demonstrated that AV could inhibit the elevation of both TNF-α and IL-1β when used as a protective treatment in septic animals.[20] Aloe-imodin, one of the AV ingredients, dose-dependently inhibited inducible nitric oxide (iNOS) mRNA expression and nitric oxide (NO) production. Furthermore, suppression of cyclooxygenase 2 (COX-2) mRNA and prostaglandin E_2 (PGE_2) expression were observed. Aloin and aloe-imodin are the major anthraquinones in AV, possessing polyphenolic structure and anti-inflammatory effects.[30]

On the other hand, GC has been used for its anti-inflammatory and analgesic purposes in many studies. Some of constituents of GC including apigenin, luteolin, terpene compounds, chamazulene, α-bisabolol, quercetin, myricetin, and rutin have been shown to have anti-inflammatory activities. Chamazulene, α-bisabolol, and apigenin have been shown to possess the highest anti-inflammatory activity against pro-inflammatory agents by inhibiting COX-2, iNOS and leukotrine expression.[17] Usually, inflammation results in expression and stimulation of the enzyme COX-2, which produces inflammatory mediators. GC affects COX-2 pathway, causing a reduction in lipopolysaccharide (LPS)-induced COX-2 mRNA and protein expression, without influencing COX-1 expression.[31] Apigenin, another component of GC, was shown to be able to interfere with leukocyte adhesion and suppress IL-1α, prostaglandin, TNF-α, IL-6, and IL-8.[32] In another study, on pretreated rats with oral apigenin (50 mg/kg), up-regulation of TNF-α was effectively blocked 5 h after treatment.[33] Also, TNF-α was inhibited in mice pretreated with 50 mg/kg apigenin.[34]

The increase in MPO activity as determinant of leukocyte infiltration was observed in the control group has been already reported.[12] We observed a decrease in MPO activity in all Mix groups as well as the GC treatment group. There is evidence that AV has anti-edema effect correlating with its ability to decrease the neutrophil migration into the peritoneal cavity, anti-inflammatory activity of the extracts of AV gel is via inhibition of COX pathway.[35] In addition, GC has been shown to suppress both inflammation and leukocyte infiltration.[36]

In this study, we used TBARS and FRAP assays to indicate oxidative stress status in colon samples. Oxygen toxicity and related free radical reactions are implicated in numerous pathophysiological conditions, including inflammation. Restraint stress caused an increase in LPO that was reduced by various doses of the AV/GC extract and GC in an association with an increase in the FRAP levels. In support, anti-oxidative effect of AV in liver and kidney of diabetic

rats has been shown that seem mediated through its component called aloeresin derivatives.[37] Other components of AV for its anti-oxidative effect are phenols, flavonoids, ascorbic acid, β-carotene and α-tocopherol.[38] GC has been used therapeutically for conditions in which oxidative stress is supposed to be implicated due to its phenolic content.[16,17]

Disturbances in GI transit in IBS are categorized into two major subtypes, including diarrhea- and constipation-predominant. The present model was a diarrhea-predominant IBS as presented by accelerated gastric emptying and intestinal transit in animals. As mentioned above, stress has some effects on GI motility such as delayed gastric emptying. Cortico trophin releasing hormone is one of the stress-related neuropeptides which plays an important role in mediating delayed gastric emptying induced by stress.[39] It has been demonstrated that restraint stress increases CRH mRNA in paraventricular nucleus (PVN) of the hypothalamus.[40] In continuous stress models, gastric emptying delayed in the first day whereas accelerated on days 3 and 5 via ghrelin secretion, an appetite-regulating factor and increasing GI motility.[41] We observed a significant increase in gastric emptying in animals after 5 days of stress that was controlled in loperamide, Mix-150 and Mix-300 while was not affected by GC.

Stress-induced acceleration of colonic transit has the same mechanism in which stress stimulates central and peripheral CRH receptors, vagal efferent and peripheral 5-HT3 receptors. CRH that is released in response to restraint stress stimulates secretion of 5-HT that result in increases from the proximal colon to the lumen intestinal and colonic transit.[26] The present results demonstrate that restraint stress significantly increases small bowel and colonic transit. Treatment with GC and loperamide inhibited small bowel transit while GC had no effect on pellet output and did not inhibit colonic transit. In the present study, only Mix-150 influenced intestinal transit and decreased pellet output comparable to that of loperamide group. GC was able to inhibit this acceleration in GI tract and regulate motility by its antispasmodic effect. The antispasmodic effects of GC in isolated guinea-pig ileum was comparable to papaverine, a smooth muscle relaxing drug.[42] The efficacy of GC on spasms induced by acetylcholine and histamine has been also demonstrated.[43] Apigenin at 12.5–50 mg/kg reduced both small and large intestinal transit time in castor oil-induced diarrheic mice.[44] As reported, inhibition of cAMP phosphodiesterase seems to be the mechanism underlying the spasmolytic activity of chamomile.[45] Apigenin affects benzodiazepine (BDZ) receptors and produces anxiolytic and sedative effects.[17] In a study, inhalation of GC oil vapor reduced a stress-induced increase in plasma adrenocorticotropic hormone (ACTH) while flumazenile, a BDZ antagonist blocked this effect of GC on ACTH.[46] Binding to BDZ and gaba aminobutyric acid receptors have been reported for apigenin.[47] To date, no clinical trial has examined the GI effects of chamomile alone. In a trial investigating efficacy of GC in children with colic, administration of chamomile/pectine was more effective in controlling didarrhea than placebo.[48]

Some of constituents of AV are anthranoids which increase both colonic and small intestine transit time.[49] In combination of AV and GC at the dose of Mix-300 and Mix-450, as the dose of AV rose the small intestine transit increased in comparison to GC-300. We hypothesize that at the dose of Mix-300 and higher, the laxative effects of anthraquinones were predominant and led to higher small intestine transit time. On the other hand, at the dose of Mix-150, the synergistic effect of GC and AV led to decrease in small intestine and colonic transit in comparison to the control group. Since the herbal remedies usually contains various components that might have potential synergistic or even antagonistic pharmacological effects, therefore, the situation is very complicated and that is why a dose-dependent effect cannot be seen.

In conclusion, our findings show that in effects of Mix-150, a synergism between AV and GC in reduction of whole GI transit time is evident. Restraint stress, as an IBS model, initiated a low-grade inflammatory and oxidative stress in GI as presented by an increase in TNF-α, MPO activity and LPO, and a decrease in anti-oxidant power of colon tissue and GI tract transit time. The pathological findings are supported by our recent findings on the ability of *Hypericum perforatum* in protecting IBS in rats,[50] melatonin,[51] and benzodiazepines.[52] In conclusion, the inhibitory effects of the AV/GC mixture on GI transit and colonic inflammation and oxidative stress in the rat model of IBS support its effectiveness in the treatment of IBS.

Authors' Contribution

Azar Asadi-Shahmirzadi did majority of the

experiment, analyzed data and helped drafting the manuscript. Shilan Mozaffari helped during animal study, and helped drafting the manuscript. Yara Sanei helped in the animal and biochemical experiments. Maryam Baeeri conducted the biochemical experiments. Reza Hajiaghaee provided herbal preparations. Hamid Reza Monsef-Esfahani advised in herbal information and the idea. Mohammad Abdollahi conceived, designed the experiment, supervised, and reviewed the entire study and edited the manuscript. All authors have read and approved the final manuscript.

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