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Research Article

Therapeutic Effect of Olmesartan Medoxomile Alone and in Combination with Sulfasalazine in Experimentally Ulcerative Colitis Model in Rats

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ABSTRACT
Background: Ulcerative colitis is a chronically recurrent inflammatory bowel disease of unknown origin. There is, as yet, no cure for this condition (other than colectomy in ulcerative colitis). Accumulating evidence has indicated the implication of angiotensin Ⅱ in the pathogenesis of inflammatory bowel diseases (IBD) via its proinflammatory features. The present study aimed to investigate the potential therapeutic effects of Omesartan Medoxomil (OLM-M) alone and in combination with sulfasalazine (SZ) on acetic acid induced- ulcerative colitis in rats. Methods: A total of 35 male wistar rats were included in the study. Animals were divided into 5 groups (n = 7): group (normal control group), groupⅡ (acetic acid group), groupⅢ (acetic acid+Sulfasalazine 360 mg/kg p.o used as reference), groupⅣ (acetic acid+ Omesartan medoxomil 5 mg/kg p.o), groupⅤ (acetic acid+Sulfasalazine 360 mg/kg+ Olmesartan medoxomil 3 mg/kg p.o). Rats received treatment for seven consecutive days after induction of colitis by intra-rectal acetic acid (2ml 4% v/v) administration. Rats were sacrificed under ether anesthesia for assessment of the colonic mucosal injury using body weight loss, colon weight / length ratio, macroscopic damage, histological study, as well as by biochemical measurement of reduced glutathione (GSH). Results: Our results showed that SZ, OLM-M and their combination decreased body weight loss, weight/length ratio, macroscopic and microscopic colonic damage scores caused by administration of acetic acid. Also significantly increased the levels of glutathione compared to acetic acid-induced colitis group.
Conclusion: The results suggested that intracolonic instillation of acetic acid produced acute colitis. Both SZ and OLM-M exerted anti-inflammatory and antioxidant effects on acetic-acid-induced colitis. In addition, OLM-M potentiated the anti-inflammatory and antioxidant effect of SZ.

Keywords: colitis, Sulfasalazine, Olmesartan, Angiotensin, reduced glutathione.

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic, relapsing, immunologically mediated disorders that are collectively referred to as inflammatory bowel diseases (IBD)[1]. UC primarily affects the mucosal lining of the colon and rectum, whereas CD can involve any segment of the gastrointestinal tract[2]. Patients with ulcerative colitis are at high risk of developing colorectal carcinoma[3]. The main clinical manifestations are abdominal pain, diarrhea, mucous, bloody, and purulent stools, recurrent attacks and relapse[4]. Although the etiology of IBD remains largely unknown, it involves a complex interaction between the genetic, environmental or microbial factors and the immune responses[5]. Activation of intestinal immune system is associated with excessive generation of inflammatory cytokines such as tumor necrosis factor- α (TNF- α) which amplifies the inflammatory cascade by triggering the generation of other proinflammatory cytokines and enhancing the recruitment of macrophages and neutrophils[6]. IL-1β appears to be a primary stimulator of diarrhea the main symptom of intestinal inflammation. Infiltration of neutrophils result in the production of cytotoxic reactive oxygen species (ROS) that are destructive on intestinal cell macromolecules, ultimately leading to mucosal disruption and ulceration[2]. While a number of medical strategies are available, many of these have substantial side-effects including immune suppression; thus, newer approaches are greatly needed[7]. Sulfasalazine is one of the standard and orally used drugs for the conservative management of IBD[8]. The benefits of sulfasalazine generally are dose related. Therefore, high doses of sulfasalazine may be necessary to induce remission. Some patients cannot tolerate high dose because of nausea and stomach upset[9]. It is necessary to search for special target of inflammatory cascades in UC so as to improve the therapeutic effects. The renin-angiotensin system (RAS) is well known to have various physiologic roles[10], including effects on vascular tone, hormone secretion, tissue growth, and neuronal activities[11]. The role of RAS in inflammatory process is gaining wide spread interest in recent times[12] . Angiotensin Ⅱ (AngⅡ), the main effector peptide of the renin angiotensin system, has potent proinflammatory features

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Table 1: The effects of SZ, OLM-M and their combination on the body weight in AA induced ulcerative colitis in rats. Parametric data were expressed as mean ± S.E.M (n =7). AA; Acetic Acid, (NC); normal control, (CC); colitis control, SZ; Sulfasalazine, OLM-M; Olmesartan Medoxomile

Parameter Group	Initial body weight	Final body weight	Body weight change %
Group Ⅰ(NC)	251.9±10.94	267.0±10.03	+6.18±1.32
Group Ⅱ(CC)	262.4±11.93	224.1±8.73	-14.15±3.01
Group Ⅲ(SZ)	267.1±8.34	270.9±9.47	+1.3±1.59
Group Ⅳ(OLM-M)	315.0±15.84	304.3±18.86	-3.51±2.59
Group Ⅴ(SZ+OLM-M)	298.4±11.35	303±13.39	+1.44±1.69

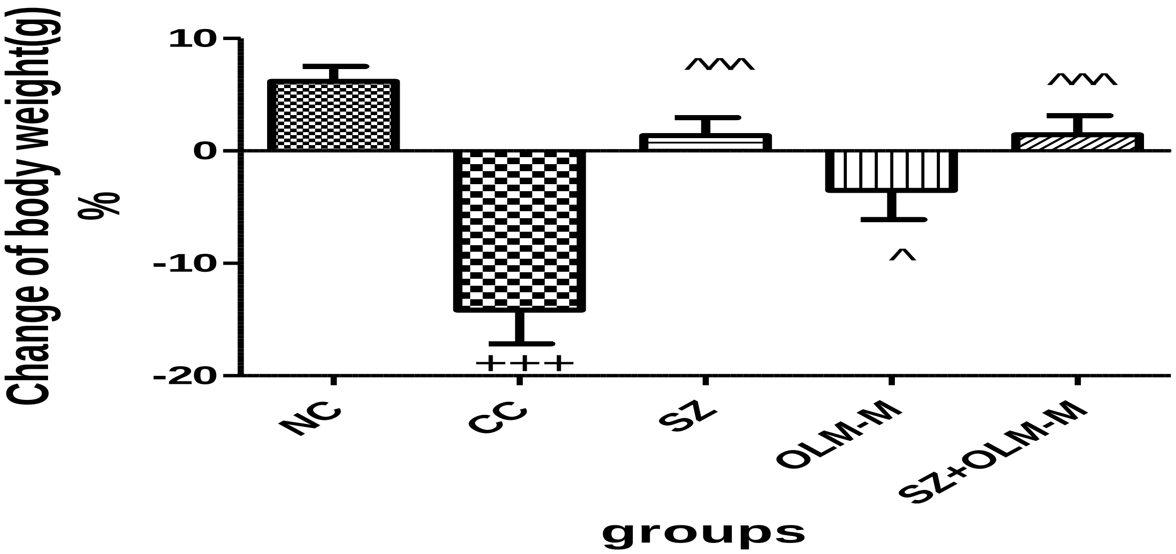

Figure 1: The effects of SZ, OLM-M and their combination on the body weight in AA induced ulcerative colitis in rats. Data are presented as means ± SEM (n =7).
+++ Significant difference as compared to normal control group at p<0.001. ^^^ Significant difference as compared to colitis group at p <0.001.
^ Significant difference as compared to colitis control group at p <0.05.

linked with the pathogenesis of several chronic inflammatory disorders including IBD[13]. Indeed, (Hirasawa et al., 2002) reported colonic localization of RAS[14] .via its actions on angiotensin Ⅱ type1(AT1) receptors Angiotensin Ⅱ promotes tissue inflammation through upregulation of adhesion molecules, increasing vascular permeability, and thus, enhancing neutrophil infiltration, which contributes to gut ulceration[15]. It also increases the release of proinflammatory cytokines such as TNF-a probably, through activation of NF-κB. Additionally, Ang Ⅱ triggers oxidative stress via activation of NADH/NADPH oxidase with consequent generation of superoxide anions[13]. Moreover, Ang Ⅱ is known to regulate motility in the intestine, as well as ions and water absorption via receptors in the mucosa and muscle[16]. The expression of angiotensin converting enzyme (ACE) and its associated receptors have been identified within the gastrointestinal mucosa, and their abundance has been shown to increase in states of intestinal injury and colitis in both animal models and humans[7]. Accumulating evidence has indicated the efficacy of the drugs that affect the angiotensin Ⅱ such as angiotensin converting enzyme inhibitors (ACE-I)[17,18] , Angiotensin Ⅱ receptor antagonists (ARBs)[19,20] and renin inhibitor (Aliskiren)[12] in the attenuation of colon injury in experimental colitis. Because combination treatment is the preferred strategy for most patients of ulcerative colitis[21]. These findings encouraged us to investigate the potential therapeutic effects of OLM-M alone and in combination with sulfasalazine on acetic acid induced- ulcerative colitis in rats, an experimental model of human UC.

Acetic acid (AA) induced UC model is an easily inducible model of UC in rats bears similarities to acute human UC in terms of histopathological appearances[22]

MATERIAL & METHOD

Animals

Adult male Wistar rats weighing (250-315 g) were procured from the Scientific Research Center, Damascus, Syria. The animals were kept at controlled environmental conditions (temperature 23 ± 2°C, humidity 55 ± 15%, lighting regimen of 12-h light: 12-h dark). They were acclimatized for one week before any experimental

Table 2: The effects of SZ, OLM-M and their combination on colon weight / length in AA induced ulcerative colitis in rats. Parametric data were expressed as mean ±S.E.M (n =7). AA; Acetic Acid, (NC); normal control, (CC); colitis control, SZ; sulfasalazine, OLM-M; Olmesartan Medoxomile.

Parameter Group	Colon weight (g)	colon length(cm)	colon weight / length (g/cm)
Group Ⅰ(NC)	1.82±0.052	16.50±0.393	0.110±0.0021
Group Ⅱ(CC)	2.75±0.099	14.00±0.293	0.196±0.0051
Group Ⅲ(SZ)	2.24±0.106	15.59±0.556	0.145±0.0098
Group Ⅳ(OLM-M)	2.34±0.108	15.43±0.581	0.154± 0.0123
Group Ⅴ(SZ+OLM-M)	2.24±0.086	16.21±0.653	0.138±.0054

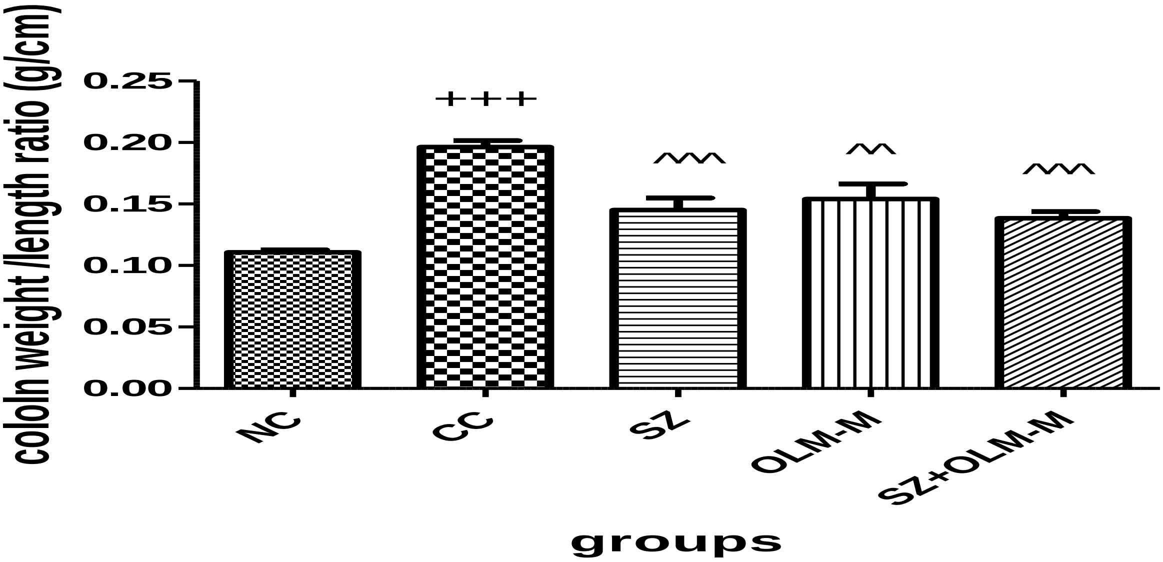

Figure 2: The effects of SZ, OLM-M and their combination on colon weight / length in AA induced ulcerative colitis in rats. Data are presented as means ± SEM (n =7).
+++ Significant difference as compared to normal control group at p<0.001.
^^^ Significant difference as compared to colitis group at p <0.001.
^^ Significant difference as compared to colitis control group at p <0.01.

procedures and were fed with standard commercial rat pellets and allowed water ad libitum.

Drugs and chemicals

Olmesartan medoxomil was obtained from Nutre Specialities private limited.

Sulfasalazine was obtained from Kanawati Medical Products.

Acetic acid (AA) (99-100%) was purchased from Merck company. All other chemicals and solvents were of highest grade commercially available.

Glutathione Assay Kit for direct assay of reduced glutathione in colonic tissue was provided by Abnova Chemical Company (Taiwan).

Experimental design and treatment protocol

animals were randomly divided into five groups (7 rats per group):

- Group Ⅰ (Normal control group): received physiological saline intra rectally instead of 4% acetic acid + oral vehicle (10ml/kg/day sodium CMC, 0.5% w/v).
- Group Ⅱ(AA control group) : received single dose of acetic acid (AA) intrarectally (2ml 4% v/v in saline, i.r.) + oral vehicle(10ml/kg/day sodium CMC, 0.5%) .
- GroupⅢ (reference group): received (AA) intrarectally + SZ (360 mg/kg/day, p.o.) [8, 23]
- GroupⅣ (OLM-M group) : received (AA) intrarectally + OLM-M(5 mg/kg/day, p.o.)[24,25]
- Group Ⅴ(combination group): received (AA) intrarectally+ SZ(360 mg/kg/day, p.o.) + OLM-M (3 mg/kg/day, p.o.).

All drugs were given by oral gavage syringe once daily for seven consecutive days, starting 24 h after the induction of colitis, and were suspended in sodium carboxy methyl cellulose solution (vehicle) (sodium CMC 0.5%w/v), which does not affect the severity of acetic acid-induced ulcerative colitis[26].

*Induction of experimental colitis in rats*rats were fasted for 24 h with free access to water before induction of colitis. Colitis was induced in rats using 2 ml acetic acid 4% or saline alone (normal control group) via intra-colonic

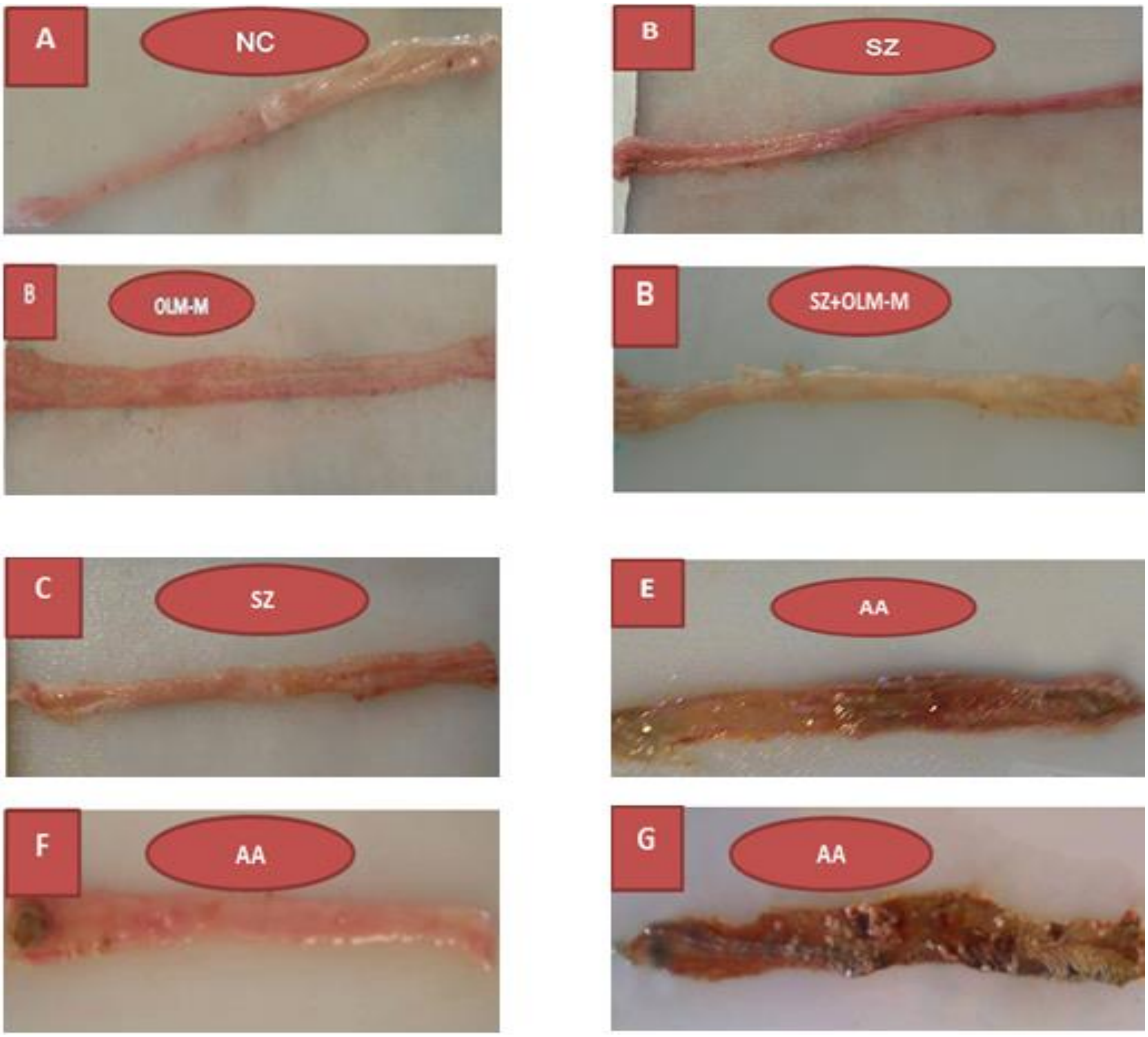

Figure 3: Macroscopic appearances of colons from acetic acid colitis in rats A: normal group (grade0) showing normal mucosa with intact epithelial surface , B: Sulfasalazine-treated group, Olmesartan- treated group and combination treated group (grade1) showing swelling or redness , C: Sulfasalazine-treated group (grade 2) showing swelling and redness , E: acetic acid group (grade 4) showing more than two ulcers or one large ulcer , F: acetic acid group (grade 5) showing mild necrosis and G: acetic acid group (grade 6) showing severe necrosis .

Table 3: Macroscopic score of different experimental groups.

Group Macroscopic Score	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	Group Ⅴ
0	-	-	-	-	7(100%)
1	7(100%)	5(71.42%)	5(71.42%)	-	
2	-	2(28.57%)	2(28.57%)	1(14.28%)	-
3	-	-	-	-	-
4	-	-	-	1(14.28%)	-
5	-	-	-	2(28.57%)	-
6	-	-	-	3(42.85%)	-

administration. On day 0 under light ether anesthesia, a soft and flexible catheter lubricated with glycerine (2 mm inner diameter) was inserted to the anus for 8 cm then acetic acid was carefully injected. The rats were maintained in a head-down position for 30 seconds in order to prevent solution spreading out. After a 30-second period of exposure, excess fluid was withdrawn and the colon was then flushed with saline[27]. Before removing the catheter, 2 mL of air was injected to spread the AA completely in the colon[28].

Table 4: Histological assessment of inflammation in different experimental groups:

Group histological score	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	Group Ⅴ
0	7(100%)	-	-	-	-
1	-	-	5 (71.42%)	7(100%)	7(100%)
2	-	1(14.28%)	2(28.57%)	-	-
3	-	1(14.28%)	-	-	-
4	-	5(71.42%)	-	-	-

Tissue collection and preparation

24 hours following completion of the experiment, On the 8th day rats were sacrificed under deep ether anesthesia and their distal colon was removed for the evaluation of body weight, colon weight / length ratio, macroscopic damage, and histological study. As well as by biochemical measurement of reduced glutathione (GSH)**.**

Measured parameters for assessment of colonic damage

Clinical finding

During the study, rats were checked daily for body weight, behavioral changes, food intake, rectal bleeding and stool consistency. The body weight of animals was measured at regular time intervals from day 0 to 7. and change of body weight (%) was calculated**.**

colon weight / length ratio

The abdomen was immediately opened. The entire colon starting from caecum was excised, freed of adherent adipose tissue, longitudinally split and washed with ice-cold saline to remove fecal residues. The length(cm) and weight (g) was measured. The colon was dried between two filter papers then weigh. Each colon was gently stretched and the distance from the colocecal junction to anus was measured, from which weight/length (g/cm) ratio, as indirect marker of inflammation was calculated.

Macroscopic scoring

The colonic samples were examined immediately by naked eye and magnifying lens for gross inflammatory changes according to the criteria as follows:

0=no inflammation; 1=swelling or redness; 2=swelling and redness; 3=one or two ulcers; 4=more than two ulcers or one large ulcer; 5=mild necrosis; 6=severe necrosis[29]**.**

Histopathological examination

Segments of colon were fixed in 15% formalin for 24 h. The specimens were first dehydrated by immersion in progressively increasing concentrations of ethanol, then were cleared in xylene. Following this, the dehydrated tissue was immersed in melted paraffin at 55-60 ^{0}C for 3 h. Sections 5 microns thick were cut by using microtome (Leica RM2155). The sections were then deparaffinized by treatment with xylene, ethanol and water. Tissues were stained with haematoxylin and eosin (H&E) and were evaluated microscopically by apathologist in blinded fashion. All groups were histopathologically assessed by using following score[3]**.**

0= normal

1=mild mixed infiltrates in the lamina propria.

2=focal superficial ulceration of mucosa only, moderate cryptitis and crypt abscess

3=deep ulceration penetrating colonic wall through mucosa till muscularis mucosa and severe inflammation

4=necrosis through large bowel wall

Reduced glutathione

Colonic tissue samples were frozen in liquid nitrogen and stored at −80 °C until time of assay. Colon GSH levels were determined as previously described by Lindenmaier, Blenn, and Wang[30,31] based on the reaction of 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB) with the glutathione present to form yellow product. The optical density measured at 405 nm is directly proportional to glutathione concentration in the sample by using microplate reader (Elisys Uno Human Germany). The glutathione content was expressed as μM/g tissue. OD:optical density**.**

Statistical analysis

Data analyses were achieved using a software program Graph Pad Prism version 5**.** Data were presented as means ± standard error (S.E.M)**.** For parametric data One way analysis of variance (ANOVA) was used followed by Tukey-Krammer multiple comparison test. Lesion score and histological score (non-parametric values) analyzed using the Kruskal–Wallis nonparametric analysis of variance with Dunn's multiple comparison test. P values less than 0.05 were considered Statistically significant.

RESULTS

Clinical findings, general observation and body weight change.

After 24 h of administration of acetic acid, animals developed hematochezia, diarrhea and progressively body weight loss with weakness and decreased food intake. All these symptoms began to be blunted in the Ⅲ,Ⅳ and Ⅴ groups at day 4. At the end of the experiment, 9 rats died: 5 from group Ⅱ, 3 from Ⅳ, and 1 from group Ⅲ, that was due to bleeding or perforation of the colon. Figure(1) shows that the intracolonic administration of acetic acid has caused the body weight to decrease in group Ⅱ . Compared with that of the normal control group which revealed increase in body weight (+6.18%), the body weight of the AA control group at the end of the experiment was significantly reduced by (-14.15%) ($p<0.001$). The body weights of the groups treated with SZ, OLM-M and combination between them were significantly increased compared with colitis control group at ($p<0.001$, $p<0.05$, $p<0.001$ respectively).(Table1, Figure1).

colon weight / length ratio

Reduction in colon length, an increases in colon weight, and a corresponding increase in the colon weight to length ratio a reliable marker of colon inflammation were

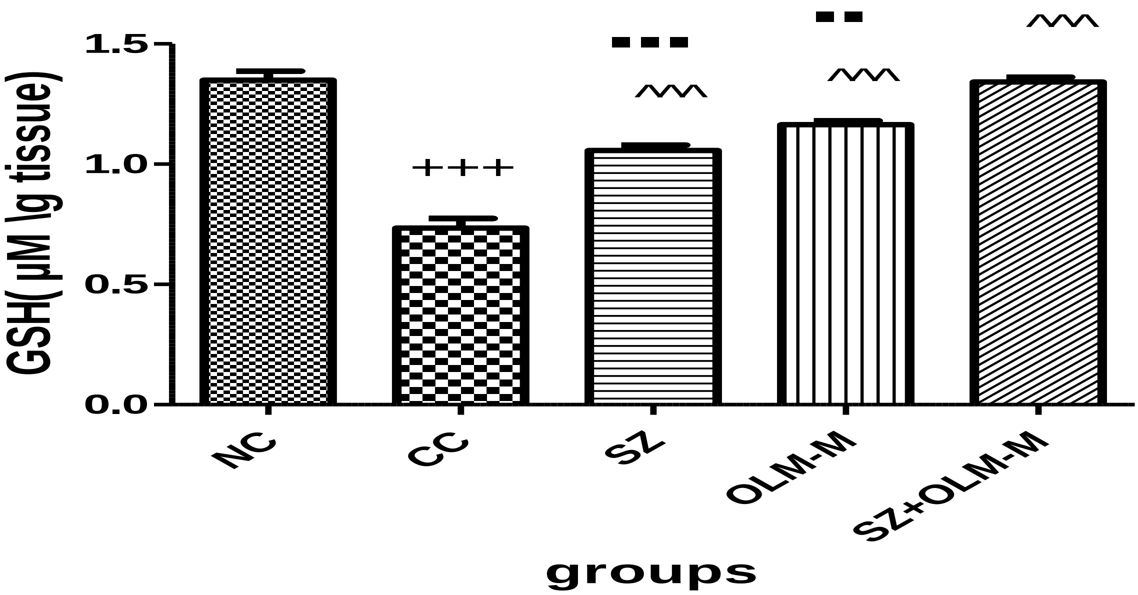

Figure 4: The effects of SZ, OLM-M and their combination on endogenous anti-oxidant (GSH) levels in AA induced ulcerative colitis in rats. Data are presented as means ± SEM (n =7).
+++ Significant difference as compared to normal control group at p<0.001. ^^^ Significant difference as compared to colitis control group at p <0.001.
▪▪▪ Significant difference as compared to combination group at p <0.001.
▪▪ Significant difference as compared to combination group at p<0.01.

Table 5: oxidative stress in rat colon was evaluated by assessing GSH

Group	Glutathione (GSH) Mµ / g tissue
Group Ⅰ(NC)	1.349±0.037
Group Ⅱ(CC)	0.734±0.040
Group Ⅲ(SZ)	1.059±0.060
Group Ⅳ(OLM-M)	1.164±0.016
Group Ⅴ(SZ+OLM-M)	1.341±0.019

observed in colitic animals relative to normal control. The rats in the AA control group showed asignificant increase in the colonic weight/length ratio (p<0.001)(Table 2) compared with that of normal control rats. SZ treatment for 7 days exerted an intestinal anti-inflammatory effect that included a decrease in the weight/length ratio compared with that of AA control group (p<0.001) (Table2). As well as OLM-M treated group rats showed asignificant decrease (p<0.01) in the ratio of colon weight/length compared with that of AA control rats. Combination of SZ and OLM-M significantly improved colon weight / length ratio compared with that of AA control rats (p<0.001). However, the difference between SZand OLM_M group was not statistically significant. also the difference between combination group and SZ alone group and OLM-M alone group was not statistically significant(p>0.05).

Macroscopic scoring

There was no evidence of mucosal injury in any specimen from control groups. In contrast, rectal administration of acetic acid as an inducing agent resulted in the production of severe colonic mucosal lesions. The colonic mucosa appeared macroscopically edematous, hemorrhagic, ulcerated and necrotic compared to the normal control group. Thus the morphological score in the AA group was significantly increased (p<0.001) as compared to normal control group (fig 3). Administration of SZ and OLM-M therapeutically reduced the severity of the gross lesion but this reduction in morphological score was not statistically significant as compared to AA group (p> 0.05). However, combination of SZ and OLM-M effectively decreased the morphological changes as compared to AA group (p<0.05), but this decrease in morphological score in combination group did not achieve significance compared to SZ and OLM-M alone treated groups (P>0.05).

Histopatholgical study

There was a good correlation between macroscopic and histological scores in each study group. Colon sections from control group revealed an intact architecture of colon tissues and hundred percent of the animals in this group showed normal morphology. On the other hand, colons of AA group revealed significant tissue injury with high scores of microscopic damage indicating erosion of the lining mucosal epithelium, degenerative changes in the crypt epithelium deep ulceration of muscularis mucosa, severe inflammation and necrosis through large bowel wall. Diffuse mixed inflammatory cells infiltration (neutrophils, eosinophile and lymphocytes) were detected in the mucosa including the lamina propria, in addition to submucosa and muscularis. As well as submucosal edema, vasculitis, dilatation and congestion in the blood vessels, crypt abscesses, proliferation of fibroblast and hemosiderin precipitation that indicative to haemorrhage were observed. Administration of SZ as therapy resulted in reduce in the severity of the injury of the large intestine but the values obtained with this dose of SZ (360mg/kg) were not significantly different from the acetic acid control group (p>0.05). Significant histologic improvement was observed and reduced the histopathological scores in OLM-M treated group compared to the group treated with only AA (P <0.05). In group Ⅴ, microscopic injury observed with SZ(360mg/kg) plus OLM-M (3mg/kg) was significantly less than AA group (P <0.05) and the anti-inflammatory effect produced by this combination was more distinct but was not statistically significant(p>0.05) than that obtained after treatment with SZ (360 mg/kg,po.) alone and similar to OLM-M (5 mg/kg, p.o.) alone.

Reduced glutathione (GSH)

AA-induced oxidative stress in rat colon was evaluated by assessing GSH as shown in table (5) and fig(4). AA significantly reduced GSH level in the colon by 45.9% as compared to the control group (p< 0.001). Administration of sulfasalazine resulted in an elevation of tissue GSH concentration by about 30.47% as compared to AA-colitis group (p<0.001). GSH content was signifiantly increased in the group treated with OLM-M (5mg/kg) by 37.06% as compared to animals that received AA alone (p< 0.001). Also asignificant increase in the mucosal glutathione concentration was observed in SZ & OLM-M group by 45.5% as compared to acetic acid group (p< 0.001). this combination treatments group was able to reach the control value. The increase was substantially higher with combination treatments group than sulfasalazine and Olmesartan medoxomile used alone (p <0.001 and P<0.01).

Table (5): The effects of SZ, OLM-M and their combination on endogenous anti-oxidant (GSH) levels (µM /g tissue) in AA induced ulcerative colitis in rats. Parametric data were expressed as mean ±S.E.M (n =7). AA; Acetic Acid, (NC); normal control, (CC); colitis control, SZ; sulfasalazine, OLM-M; Olmesartan Medoxomile.

DISCUSSION

Various experimental models of colitis mimicking the active phase of the disease have been developed to test the potential beneficial effects of various drugs. One of the most commonly employed models is acetic acid induced colitis in rats. The model of induced colitis through intra rectal injection of acetic acid presents advantage over other experimental models. Such advantages include easy availability of aggressor reagent, low cost, reproducibility and similarity to UC in humans[32]. The mechanism by which AA induces colitis involves the entry of protonated form of acid into the epithelium where it dissociates to liberate protons causing intracellular acidification that might account for the epithelial injury[33]. Transient local ischemia might contribute to the acute injury, Mucosa and submucosal inflammation followed initial injury was associated with activation of arachidonic acid pathways[34]. AA metabolism by colonic enzymes provides superoxide anions and H2O2 which contribute to its colonic toxic effects[35]. The results of the present study demonstrate that acetic acid induced colitis model in rats is a reproducible technique and produces a large inflammatory response as evidenced by body weight loss, reduction in food intake, colonic shortening, increase colonic weight / length ratio, characteristic acute colonic lesions such as mucosal oedema, haemorrhage and necrosis. Furthermore, the lesions are associated with changes in biochemical parameters which include depletion of GSH. The RAS from an evolutionary point of view is a very old system with pro-inflammatory effects on different tissues. In addition to endocrine effects, it also has paracrine and autocrine actions[36]. It is noteworthy that colitis is likely to be multifactorial, and recent data indicate that ARBs prevented some, but not all of the inflammatory stimuli in this model. Hence the importance of Investigation on the participation of this class of drugs with other treatments used in inflammatory bowel disease.

The present study highlights the therapeutic effects of OLM-M, an Ang Ⅱ AT-1 receptor antagonist alone and in combination with sulfasalazine in acetic acid induced colitis, an experimental model of human IBD. There was significant reduction in the body weight in the AA group compared to normal control which exhibit marked increase in body weight. Administration of SZ, OLM-M and combination between them as therapy lowered the incidence of diarrhea, improved food intake and attenuated body weight loss as compared AA control group. This effect of Olmesartan medoxomile is attributed to that Ang Ⅱ causes a marked anorexigenic effect and weight loss, and high circulating levels of Ang Ⅱ may contribute to the anorexia, wasting, and cachexia[37]. These observations are in accord with previous studies[12,20].

Colonic weight /length ratio can be considered a reliable and sensitive indicator of the severity of UC. Weight of colon is raised due to the inflammation and also because of the increased activity of the fibroblasts leading to the overgrowth of muscularis mucosa[33]. consequently, the increased colonic weight / length ratio confirms intensification of intestinal infiltrations and consequent intestinal oedema. Treatment of rats with SZ and OLM-M (5mg/kg) for 7days following induction of colitis significantly reduced colonic weight / length ratio. These findings are in agreement with the earlier study which showed that aliskiren at higher dose (10mg/kg,i.p.) significantly improved colon weight/length ratio in dextran sulfate sodium (DSS) induced colitis in mice[12]. Moreover, Wengrower et al.(2004) demonstrated that captopril reduced colonic weight / length ratio in a model of TNBS colitis[38]. furthermore, Nagib et al(2013) documented the beneficial effects demonstrated with pre treatment of rats with10mg /kg OLM-M are similar to or even greater than those obtained with sulfasalazine in DSS colitis model[2]. In the present study combination therapy with SZ plus OLM-M caused asignificant reduction in colon weight/length ratio and this effect was superior to those of either agent alone.

The morphological changes which occurred in this study were similar to the other study that showed redness, oedema, ulcer and necrosis in AA group[29]. In our study, acetic acid administration caused a substantial degree of tissue injury associated with congestion, haemorrhages, oedema, leukocytic infiltration, deep ulceration penetrating colonic wall through mucosa till muscularis mucosa, severe inflammation and degenerative changes such as necrosis .These histological features are consistent with the features described in previous reports[27]. Our macroscopic and histological analysis demonstrated the ability of SZ and OLM-M to ameliorate intestinal damage and inflammation. It was found that the morphological scores were reduced by SZ and OLM-M but neither agent caused a significant inhibition of colonic lesions as compared to acetic-acid controls. The effects of combination SZ (360mg/kg) plus OLM-M(3mg/kg) on macroscopic injury were superior to those of either agent alone and these values achieve significance compared to AA alone. Microscopic findings showing attenuation of tissue damage, reduction in cell infiltration and mucosal ulceration with OLM-M (5mg/kg). These findings are in line with El-Medany et al (2011) that demonstrated that rats received captopril or valsartan, therapeutically showed significant amelioration in these parameters as compared to acetic acid group[36]. Interestingly, Dereli et al (2014) confirm that administration of OLM-M can effectively reduce fibrosis and limit the damage to the esophageal tissue in the animal model of caustic burn[24]. These favorable anti inflammatory actions of OLM-M, were linked with inhibiting the release of TNF- α, was previously reported in various organ maladies such as hypertensive patients with microinflammation[39] , insulin resistance[40], atherosclerosis[41] and bleomycin-induced pulmonary fibrosis[42]. Combination therapy with SZ (360mg/kg) plus OLM-M (3mg/kg) was associated with a significantly reduced in microscopic colonic damage. Data derived from this study indicate that dual therapy has a synergistic effect in reducing inflammation and promoting mucosal repair than SZ (360mg/kg) alone. Our study appears to be the first of its kind as we were not able to find references in the english literature.

The gastrointestinal (GI) tract is a key source of ROS. They are generated from several sources including stimulated PMNs, eosinophils, xanthine oxidase, colonic bacteria and epithelial lipoxygenase[43]. The depletion of glutathione is considered a crucial event of colonic damage occurring both in human UC and in animal.In the present work, acetic acid-induced colonic lesions are associated with significant depletion in GSH levels which is in agreement with the previous findings[36,44]. Data obtained from the treatment of animals with SZ and OLM-M shows significant increase in GSH level as compared to the AA group. It is noticeable that OLM-M showed better improvements but not significant than sulfasalazine. Our results are in agreement with earlier studies that showed the antioxidant effect of OLM-M on experimentally induced liver fibrosis in rats[45].

Co-administration of SZ(360mg/kg, p.o.) with OLM-M(3mg/kg, p.o.)
produced substantial elevation of tissue GSH concentration, and a more documented antioxidant response in comparison with the SZ(360mg/kg, p.o.) and OLM-M(5mg/kg, p.o.) used alone.

CONCLUSION

In conclusion, this study indicates the efficacy of OLM-M in AA-induced UC. These effects, which are comparable or even better than sulfasalazine, are possibly attributed to its anti-inflammatory via modulation of the immune system and antioxidant properties. Combination of OLM-M and SZ has shown greater efficacy than single SZ treatment.

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