Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2016; 8(6): 604-609

ISSN- 0975 1556

Research Article

Ameliorative Effect of Olmesartan Medoxomil on Acetic Acid-Induced Colitis in Rats

Darwish Fatima[1*], Al laham Shaza[1], Al-Manadili Ahmad[2]

[1]*Pharmacology & Toxicology Department - Faculty of pharmacy- Damascus University- Damascus-Syria.*
[2]*Oral Histopathology Department - Faculty of Dentistry - Damascus University- Damascus-Syria.*

Available Online: 8th June, 2016

ABSTRACT

Background and aim: The renin–angiotensin system (RAS) is a homeostatic pathway widely known to regulate cardiovascular and renal physiology; however, little is known about its influence in gastrointestinal tissues. There are several reports showing influences by RAS and its key mediator angiotensin II (Ang II) on intestinal epithelial fluid and electrolyte transport. Data are accumulating, suggesting involvement in GI mucosal inflammation and carcinogenesis. Our target was to evaluate the protective alleviating effects of RAS blockade by using Olmesartan Medoxomil (OLM-M) with marked anti-inflammatory and antioxidant actions against acetic acid-induced ulcerative colitis in rats. Materials and methods: Pretreatment with OLM-M at a dose of (5mg/kg, p.o.), 7days before induction of colitis, continued till the 4th day. The severity of colitis in rats was assessed by macroscopic and histopathological examinations. Results: The results suggested that OLM-M attenuated the macroscopic and microscopicolonic damage induced by acetic acid when administrated before induction of colitis (prophylactic effects). Conclusion: OLM-M may be effective in prophylaxis of ulcerative colitis through targeting RAS.

Keywords: ARBs, Olmesartan , Ulcerative colitis , Acetic acid , RAT, Histopathology.

INTRODUCTION

The renin–angiotensin system (RAS) plays a central role in regulating cardiovascular and renal physiology. The RAS was, for many years, thought of as an endocrine system with enzymes and peptides released into the systemic circulation to act on target organs. More recently, it has been recognized that most organs including the brain, kidney, heart, liver, pancreas, reproductive organs, skin and the gastrointestinal tract constitutively express all the components required to allow autonomous function of a local intra-organ RAS, where it performs both paracrine and autocrine functions[1]. The RAS is a very old system with pro-inflammatory effects on different tissues. RAS activation can activate the NF-κB pathway in monocytes and cultured vascular smooth muscle cells[2]. As well as the subsequent production of inflammatory mediator such as tumor necrosis factor α (TNF-α), transforming growth factor β1, interleukin 1β(IL-1β) and monocyte chemotactic protein-1 (MCP-1). Therefore, ACE inhibitors and AT1R antagonists (AT1R-A), commonly used as therapeutic agents for treating hypertension, have additional protective and therapeutic actions[3]. Angiotensin Ⅱ receptors are also found in the intestinal mucosa, and increased colonic mucosal angiotensin Ⅰ & Ⅱ concentrations have been found in IBD patients[4,5]. It has been suggested that the RAS is a potential mediator of colonic inflammation by activating the NF-κB signaling pathway, and the inhibition of RAS could be a beneficial protective target in IBD, downregulating the NF-κB pathway[2]. Inflammatory bowel disease (IBD), including ulcerative colitis(UC) and Crohn's disease (CD) has been a worldwide health-care problem with a continually increasing incidence[6]. As there is no curative treatment, most IBD patients need life-long treatment, and consequently, IBD is associated with a high economic burden on society[7]. Among several candidates of ARBs, Olmesartan medoxomil (OLM-M) has unique anti-inflammatory and antioxidant features owing to the blockade of Ang Ⅱ AT1 receptors[8]. OLM-M also exhibits favorable actions in myocarditis[9,10], atherosclerosis[11], renal injury[12] esophageal strictures[13] and hepatic fibrosis[14]. Therefore, in the present study, (OLM-M) a non-peptide angiotensin Ⅱ receptor antagonist was used as a possible anti-inflammatory and antioxidant drug to investigate protective potential in experimental model of colitis induced by acetic acid (AA) in rats.

MATERIALS AND METHODS

Animals

Male albino rats of the Wistar strain weighing (200-350 g) were used throughout this work (supplied from Scientific Research Center, Damascus, Syria). The animals were maintained in a room under standard conditions of light, feeding and temperature, had access to a standard diet and clean drinking water.

Acute colitis model induction and treatment protocols

colonic inflammation under light ether anesthesia was simulated. Administering 2mL of 3% (v/v) AA in 0.9% NaCl intrarectally with a soft and flexible catheter where

**Author for Correspondence*

inserted in to the anus up to a length of 8 cm. The rat was maintained in a head-down position for 30 s to limit expulsion of the solution. Before removing the catheter, 2 mL of air was injected to spread the AA completely in the colon[15] .

Grouping

After one week of acclimatization, the animals were randomly divided into 3 groups (n = 6 per group):

Group (A) (Normal control group): isotonic saline 0.9% (2ml *i.r.*) + vehicle (10ml /kg/day, p.o. CMC 0.5%).

Group (B) (Colitis group): Acetic Acid (2ml of 3% v/v, *i.r.*) + vehicle (10ml /kg/day, p.o. CMC) [AA: Merck, Germany].

Group (C) (AA+ OLM-M): received AA rectally + oral OLM-M [Nutre Specialities private limited]

OLM-M was suspended in 0.5% carboxymethyl cellulose vehicle, administered (5 mg/kg/day) by oral gavage starting 1 week before the induction of AA colitis, and continued till the 4th day post AA instillation. At the end of the experimental period, on the 5 day after induction, all rats were euthanized under deep ether anesthesia. Colonic biopsies were immediately taken for macroscopic scoring and histopathological studies.

Assessment of colitis

Inflammatory Changes in Colonic Mucosa:

The main parameters, used to assesse the degree of colonic inflammation were macroscopic and histological scoring:

macroscopic scoring

The entire colon was excised and opened longitudinally, rinsed with cold saline. The colonic samples were examined visually immediately and by magnifying lens, then scored macroscopically according to the following grading system[16].

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

Histological analysis

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), evaluated microscopically by a pathologist in blinded fashion. The colon microscopic damage was scored as follow[17]:

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

Statistical study

Macroscopic 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

Morphological examination

The macroscopic score observed in group A was 0.0 ± 0.0 (table 2, figure 2). This value indicated that 100% of animals showed normal morphology. There was visible erosions, oedema, ulcer, haemorrage on day 5 following administration of acetic acid in group B**.** Visual evidence of colitis was scored at 3.167± 0.40 (table 2, Figure 2), this value was significantly different as compared to corresponding controls ($p<0.01$) (fig1). Monotherapy with OLM-M (5mg/kg) caused a reduction in macroscopic injury (2.667± 0.33) (table 2, figure 2) but these values did not achieve significance compared to AA alone group(B) (fig1).

Histopatholgical examination

Group A: histopathologic assessment and microscopic images revealed regular colonic mucosa with intact epithelium, crypts and submucosa in the control group. Only one of them showed a mild degree of congestion of blood vessels and mild mixed infiltrates in the lamina propria (fig4).

Group B: histological observation of AA induced ulcerative colitis showed comparatively extensive histological damage with the highest histological score (2.333±0.21) (table 4, fig 5,6) which was significantly different as compared to corresponding controls ($p<0.001$) (fig3). The histopathological features of untreated rats included oedema and diffuse inflammatory cell infiltration in the mucosa. There was deep ulceration of the colonic mucosa extending through the muscularis mucosa. An infiltrate consisting of mixed inflammatory cells, crypt abscesses, proliferation of fibroblast, dilatation in the blood vessels were also noted (fig4).

Goup C: Rats that received pretreatment with OLM-M showed retreating in microscopic injury as seen by reduction in histological score (1.833±0.16) (table 7,8), but this value was not significantly different from the acetic acid control group ($p>0.05$) (fig 3). Most rats in this group experienced the mild mixed infiltrates in the lamina propria and focal superficial ulceration of mucosa with the score 1 and 2 (fig7,8).

DISCUSSION

UC is an inflammatory disease in which patients experience cyclic bouts of clinical symptoms including diarrhea, rectal bleeding, and anemia resulting from intestinal inflammation, edema, and ulceration of the colonic mucosa[18]. Treatment of IBD is associated with substantial morbidity in patients with both Crohn's disease and ulcerative colitis[19]. The role of the RAS in health and disease is well defined in a number of organs, but is poorly characterised in the gastrointestinal tract. Initial interest in the RAS in the gastrointestinal tract is based on the fact that several components of the RAS are highly expressed in the small and large intestine of rodents and humans[3].

Table 1: Macroscopic score of different experimental groups.

Group Macroscopic Score	Group A	Group B	Group C
0	6(100%)	-	-
1		-	-
2	-	2(33.33%)	3(50%)
3	-	1(16.66%)	2(33.33%)
4	-	3(50%)	1(16.66%)
5	-	-	-
6	-	-	-

Table 2: The effects of OLM-M on gross lesion score (mean ± S.E.M.) in AA induced ulcerative colitis in rats (n =6). AA ; Acetic Acid , OLM-M; Olmesartan Medoxomile.

Group	Gross lesion score
Group A	0.0 ± 0.0
Group B	3.167± 0.40
Group C	2.667± 0.33

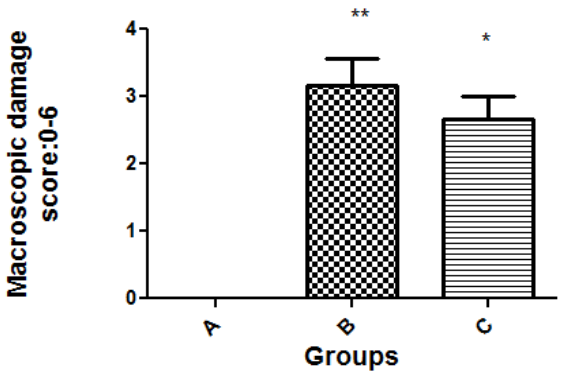

Figure 1: The effects of OLM-M on gross lesion score. Data are presented as means ± SEM (n =6). **Significant difference as compared to normal control group at p<0.01.

Table 3: Histological assessment of inflammation in different experimental groups.

Group histological score	A	B	C
0	5 (83.33%)	-	
1	1(16.66%)	-	1(16.66%)
2		4 (66.66%)	5(83.33%)
3		2 (33.33%)	
4			

The potential role of the RAS in mucosal inflammation in IBD provides an exciting opportunity for therapeutic intervention. Experimental studies have contributed significantly to advancing the understanding of the

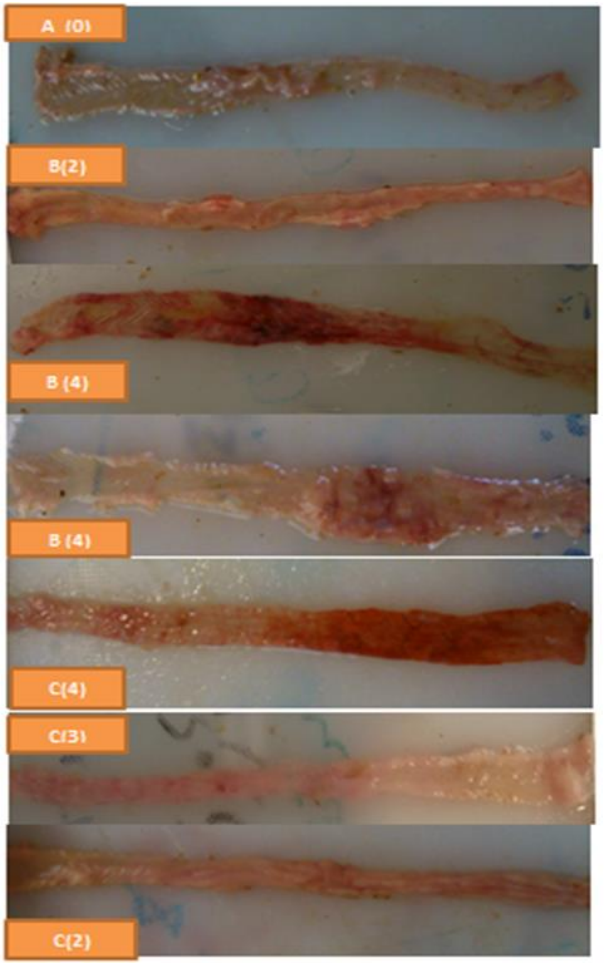

Figure 2: Photographic representation of colon from each group A, B, C.

Table 4: The effects of OLM-M on histological score (mean ± S.E.M.) in AA induced ulcerative colitis in rats. (n =6). AA; Acetic Acid, OLM-M; Olmesartan Medoxomile.

Group	histological score
Group A	0.166±0.16
Group B	2.333±0.21
Group C	1.833±0.16

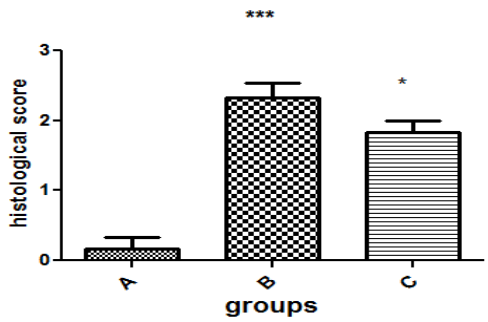

Figure 3: The effects OLM-M on histological score. Data are presented as means ± SEM (n =6). ***Significant difference as compared to normal control group at p<0.001.

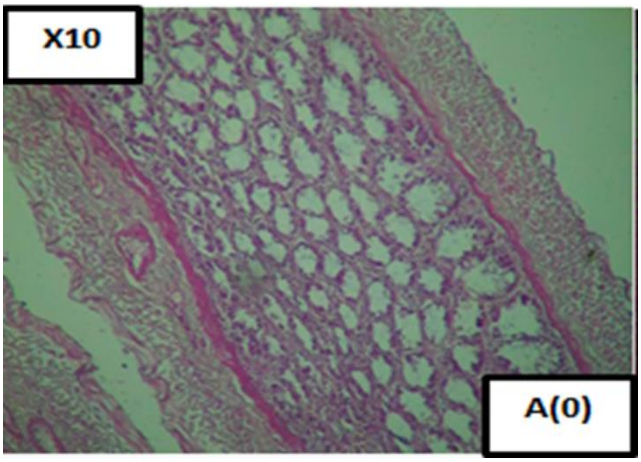

Figure 4: Histological section of colonic wall in a rat from Group 1 (Normal).
Microscopic grade: 0. Note: normal
(H&E stain, 10x magnification)

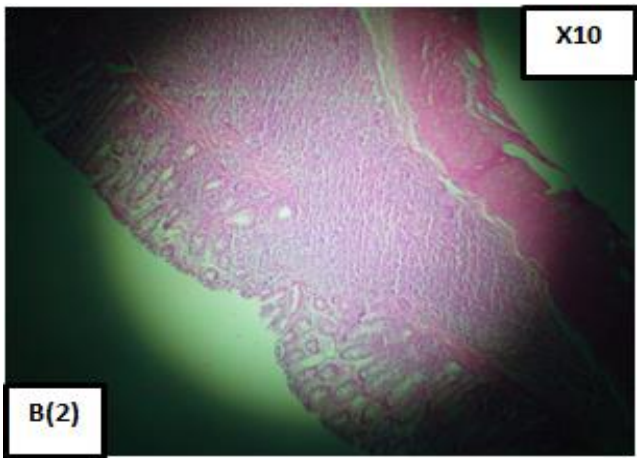

Figure 5: Histological section of colonic wall in a rat from Group 2 (Colitis control).
Microscopic grade: 2. Note: focal superficial ulceration of mucosa only, moderate cryptitis and crypt abscess
(H&E stain, 10x magnification)

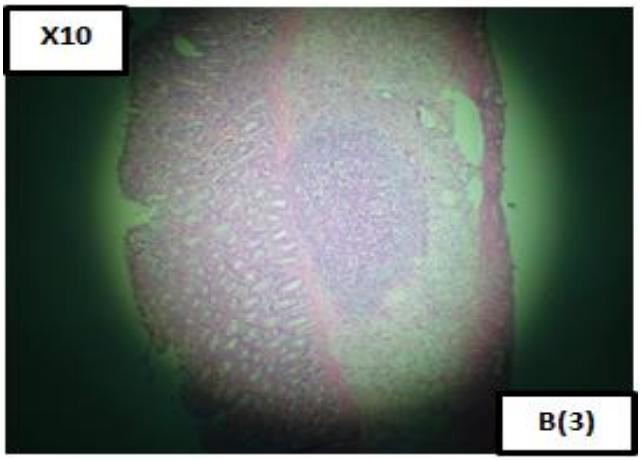

Figure 6: Histological section of colonic wall in a rat from Group 2 (Colitis control).
Microscopic grade: 3. Note: deep ulceration penetrating colonic wall through mucosa till muscularis mucosa and severe inflammation**.**
(H&E stain, 10x magnification)

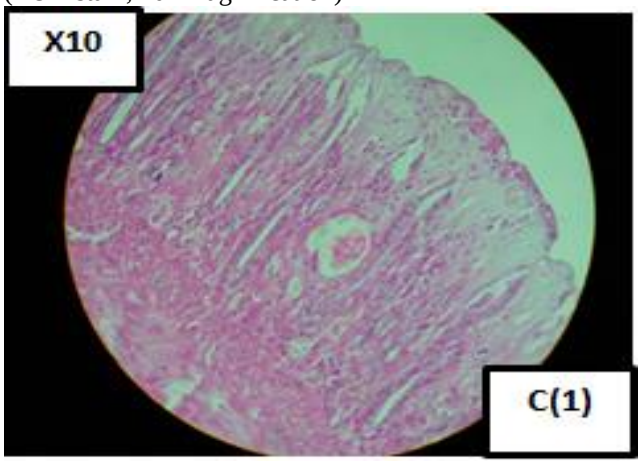

Figure 7: Histological section of colonic wall in a rat from Group 3 (OLM-M).
Microscopic grade: 1. Note: mild mixed infiltrates in the lamina propria**.**
(H&E stain, 10x magnification)

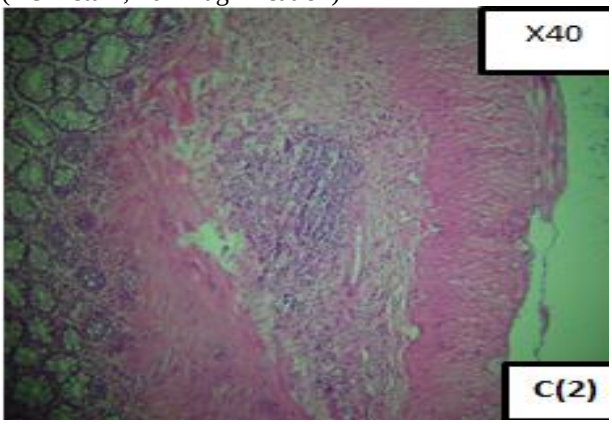

Figure 8: Histological section of colonic wall in a rat from Group 3 (OLM-M).
Microscopic grade: 2. Note: focal superficial ulceration of mucosa only, moderate cryptitis and crypt abscess**.**
(H&E stain, 40x magnification)

etiology of IBD. One of the well-established models of induced colitis the acetic acid model was chosen in the present study to detect the morphological and pathological changes associated with colitis[20]. In the present study, the experimental induction of colitis with instillation of 2 ml of 3% acetic acid into the colon was successfully achieved, and confirmed by macroscopic damage and histopathological findings such as congestion, haemorrhages, oedema and leukocytic infiltration. This result is in agreement with previous reports in animal models of colitis, which demonstrated similar macroscopic and microscopic pictures and tissue damage after induction of colitis using acetic acid[15]. In this study, the influence of pretreatment with OLM-M on the course of acetic acid-induced colitis have been investigated. The results demonstrate that daily OLM-M treatment of rats markedly improves acetic acid-induced colonic lesions as confirmed by macroscopic and microscopic examination. That

macroscopic and microscopic lesion scores of the colitis group were reduced by treatment with OLM-M, but the drug did not cause a significant inhibition of colonic lesions as compared to acetic-acid controls. Our findings are in support of other investigators who have recently identified potential benefit in blocking the RAS pathway in inflammatory bowel disease. El-Medany et al (2011) reported the beneficial effect of valsartan and captopril in AA induced ulcerative colitis in rats[21]. Moreover these data are in concert with Nagib et al (2013) which demonstrate that OLM-M decreased significantly the production of TNF-α and PGE2 in a dose-related manner. These effects were clearly reflected on microscopic findings showing attenuation of tissue damage, reduction in cell infiltration and mucosal ulceration, especially with the higher dose of OLM-M in dextran sodium sulphate (DSS) induced ulcerative colitis[22]. Duration of treatment was a determinant in the effectiveness of ARB on Protection against chemically induced colitis. Nevertheless, the safety record for ARBs usage is strong and may thus have a strong appeal for clinical applications. Previously modulation of the RAS with inexpensive, safe pharmaceuticals used by millions worldwide is an attractive therapeutic strategy for application to human autoimmune diseases.

CONCLUSION

Olmesartan medoxomil, a new AT1 antagonist, manifested a protective effects against AA-induced ulcerative colitis, when used in a dose 5mg/kg through its anti-inflammatory and antioxidant effects, as proven by histopatholgical study. This suggests that the clinical application of ARBs may have a potential therapeutic role.

REFERENCES

1. Garg M, Angus PW, Burrell LM, Herath C, Gibson PR, Lubel JS. Review article : the pathophysiological roles of the renin – sangiotensin system in the gastrointestinal tract. Aliment Pharmacol Ther. 2012;35:414–28.
2. Gonzalez-Villalobos RA, Shen XZ, Bernstein EA, Anjulia T, Giani JF, Blackwell W-LB, et al. Rediscovering ACE: Novel insights into the many roles of the angiotensin-converting enzyme. J Mol Med. 2013;91(10):1143–54.
3. Okawada M, Koga H, Larsen SD, Showalter HD, Turbiak AJ, Jin X, et al. Use of Enterally Delivered Angiotensin II Type Ia Receptor Antagonists to Reduce the Severity of Colitis. Dig Dis Sci. 2011;56(9):2553–65.
4. Jaszewski R, Tolia V, Ehrinpreis M., Bodzin J., Peleman R., Korlipara R, et al. Increased colonic mucosal angiotensin I and II concentrations in Crohn's colitis. Gastroenterology. 1990;98(6):1543–8.
5. Hirasawa K, Sato Y, Hosoda Y, Yamamoto T, Hanai H. Immunohistochemical localization of angiotensin II receptor and local renin-angiotensin system in human colonic mucosa. J Histochem Cytochem. 2002;50(2):275–82.
6. Zhang Y, Li Y. Inflammatory bowel disease : Pathogenesis. World J Gastroenterol. 2014;20(1):91–9.
7. Lee C, Chun J, Wook S, Joo S, Pil J, Sung J. Enalapril inhibits nuclear factor- κ B signaling in intestinal epithelial cells and peritoneal macrophages and attenuates experimental colitis in mice. Life Sci. 2014;95(1):29–39.
8. Fliser D, Buchholz K, Haller H. Antiinflammatory Effects of Angiotensin II Subtype 1 Receptor Blockade in Hypertensive Patients With Microinflammation. Circulation. 2004;110:1103–7.
9. Seko Y. Effect of the angiotensin II receptor blocker olmesartan on the development of murine acute myocarditis caused by coxsackievirus B3. Clin Sci. 2006;110:379–86.
10. Sukumaran V, Watanabe K, Veeraveedu PT, Gurusamy N, Ma M, Thandavarayan RA, et al. Olmesartan , an AT 1 Antagonist , Attenuates Oxidative Stress , Endoplasmic Reticulum Stress and Cardiac Inflammatory Mediators in Rats with Heart Failure Induced by Experimental Autoimmune Myocarditis. Intteerrnattiionall Jourrnall off Biiollogiiccall Scciieenccees. 2011;7(2):154–67.
11. Hirohata A, Yamamoto K, Miyoshi T, Hatanaka K, Hirohata S, Yamawaki H, et al. Impact of Olmesartan on Progression of Coronary Atherosclerosis. J Am Coll Cardiol. Elsevier Inc.; 2010;55(10):976–82.
12. Vinuesa SG de, Goicoechea M, Kanter J, Puerta M, Cachofeiro V, Lahera V, et al. Insulin Resistance, Inflammatory Biomarkers, and Adipokines in Patients with Chronic Kidney Disease: Effects of Angiotensin II Blockade. J Am Soc Nephrol. 2006;17:206–12.
13. Dereli M, Krazinski BE, Ayvaz S, Aksu B, Kanter M, Uzun H, et al. A novel approach for preventing esophageal stricture formation : olmesartan prevented apoptosis. Folia Histochem Cytobiol. 2014;52(1):29–35.
14. Sipal S, Halici Z, Kiki I, Polat B, Albayrak A, Albayrak F, et al. Comparative study of three angiotensin II type 1 receptor antagonists in preventing liver fibrosis in diabetic rats : stereology , histopathology , and electron microscopy. J Mol Hist. 2012;43:723–35.
15. Astha S, Ramica S. Research on anti-inflammatory and anti-oxidant activity of Ocimum sanctum L . ethanolic extract in ulcerative colitis. Wudpecker J Med Plants. 2013;2(6):066–73.
16. Amirshahrokhi K, Bohlooli S, Chinifroush MM. The effect of methylsulfonylmethane on the experimental colitis in the rat. Toxicol Appl Pharmacol. 2011;253(3):197–202.
17. Medhi B, Prakash A, Avti PK, Saikia UN, Pandhi P, Khanduja KL. Effect of Manuka honey and sulfasalazine in combination to promote antioxidant defense system in experimentally induced ulcerative colitis model in rats. Indian J Exp Biol. 2008;46:583–90.
18. Mahmoud BL, Shady AM, Kifafy MA, El-seify GH, Eldamhogy RA, Omara RS. The effect of royal jelly versus sulfasalazine on acetic acid-induced colitis in adult albino rats. Menoufia Med J. 2015;28:748–56.
19. Sueyoshi R, Ignatoski KMW, Daignault S, Okawada

M, Teitelbaum DH. Angiotensin converting enzyme-inhibitor reduces colitis severity in an IL-10 knockout model. Dig Dis Sci [Internet]. 2013;58(11):3165–77. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3859685&tool=pmcentrez&rendertype=abstract

20. Shivanandappa B, Mahendran S, Biradar MI, Raj P, Srivastava K, Badami S, et al. Protective effect of embelin against acetic acid induced ulcerative colitis in rats. Eur J Pharmacol. Elsevier B.V.; 2011;654(1):100–5.
21. El-medany AH, Guemei AA, Hagar HH, El-medany JH, Baraka AM. Comparative study between effect of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on acetic acid- induced ulcerative colitis in rats. Int Res J Pharm Pharmacol. 2011;1(6):100–8.
22. Nagib MM, Tadros MG, Elsayed MI, Khalifa AE. Anti-inflammatory and anti-oxidant activities of olmesartan medoxomil ameliorate experimental colitis in rats. Toxicol Appl Pharmacol. 2013;271(1):106–13.