WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 4, 204-215. Research Article ISSN 2277– 7105

COLON ULCER INDUCED BY ALENDRONATE AND CURE IT WITH METHYL SULFONYL METHANE

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Article Received on
22 Jan. 2019,

Revised on 11 Feb. 2019,
Accepted on 03 March 2019

DOI: 10.20959/wjpr20194-14474

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ABSTRACT

Background: Bisphosphonates define a class of drugs which are widely indicated to treat osteoporosis both in men and women. Side effects of the oral tablets may include nausea, difficulty swallowing, heartburn, irritation of the esophagus and gastric ulcer. Methylsulfonylmethane (MSM), which has become a popular dietary supplement, was used to treat Bisphosphonates ulceration. Our objective was studying the effect of MSM on treat colon ulcer induced by Alendronate (ALN) in rats using different doses of MSM. The experiments had been done on 8 white wistar rats for each group. The colon ulcer had been induced by administration of Alendronate (20mg/kg/day) by gavage for 4 days. MSM (400mg/kg/day) has been given, for the first therapeutic group, for 4 days after administration of Alendronate, while the other therapeutic group has been given MSM (800 mg/kg/day). The ulcers in rats' colon were examined histologically and microscopically. Results indicated the inability of MSM to treat the colonic ulceration induced by Alendronate in both doses 400 and 800 mg/kg/day for four days. **Conclusion**: MSM both doses (400 mg/kg/day) and (800 mg/kg/day) have no therapeutic effect of colon ulcer induced by alendronate.

KEYWORDS: Gastrointestinal. Colon ulcer. therapeutic. Alendronate. Methyl sulfonyl methane.

1. INTRODUCTION

Isolated ulcers of the large intestine are infrequently encountered by the gastroenterologist but can result in diagnostic uncertainty. The absence of associated colitis is necessary to call the ulceration "isolated." Ulcers may occur singly, as in solitary rectal ulcer syndrome (SRUS), or multiply, as in NSAID-induced colonic ulcers. The etiology is variable and includes infectious causes, medications, ischemia, and anorectal dysmotility.[1]

Bisphosphonates are stable analogues of naturally occurring pyrophosphates. The mechanism of action of these drugs is to inhibit bone resorption through their effects on osteoclast function. Bisphosphonates are poorly absorbed and avidly taken up by bone on active sites of resorption. Alendronate is a second generation nitrogen containing bisphosphonate which is administered daily or once weekly (depending on formulation) and does not impair bone mineralization at doses that maximally inhibit bone resorption.[2] However, recent studies have shown that chronic alendronate use may be associated with side effects primarily involving the gastrointestinal tract, including gastric ulcer and erosive esophagitis.[3]

MSM is a naturally occurring organosulfur molecule and a putative methyl donor. It has anti-inflammatory activities, chemopreventive properties, prostacyclin (PGI2) synthesis inhibition, anti- atherosclerotic action, salutary effect on eicosanoid metabolism, and free radical scavenging activity. Health claims associated with MSM include relief of pain, inflammation, arthritis, allergies, certain parasitic infections and asthma. It is also used to nourish skin, hair and fingernails, due to its sulfur concentration, which contributes to cystine, a sulfur amino acid that is required for the production of keratin.[4]

2. MATERIALS AND METHODS

2.1. Animals

Thirty-two male albino rats of the Wistar strain weighing between 180-250 gram were used for this study. The animals were separated randomly into six cages of four rats each where they were kept for four weeks before the start of the experiment. The animals were housed under standard conditions of temperature (23 ± 2°C), humidity (55 ± 15%) and 12-hour light (7.00 am - 7.00 pm). The cages were constantly cleaned in order to prevent the animals from

contracting disease. They were fed with standard commercial rat pellets and allowed water ad libitum.

2.2. Experimental Design

2.2.1. Grouping: The animals were divided into four groups of eight rats each.

Group One (group A) (NORMAL): Animals were treated with normal saline+ NaOH for four days, then they were treated with normal saline for four days. They were called the control group.

Group Two (group B) (Alendronate ALN): Animals were treated with Alendronate (20 mg/kg/day) for four days, then they were treated with normal saline for four days.

Group Three (group C) (MSM) (400 mg/kg/day): Animals were administrated with Alendronate (20 mg/kg) for four days, then they were treated with 400 mg/kg of Methylsulfonylmethane for four days.

Group four (group D) (MSM) (800 mg/kg/day): Animals were administrated with Alendronate (20 mg/kg) for four days, then they were treated with 800 mg/kg of Methylsulfonylmethane for four days.

2.2.2. Ulcer Induction

Animals were singly housed and fasted for 18h in wide mesh bottom cages, allowed free access to water except for the last hour before the last dose of the medication. Alendronate was administrated by gavage (20 mg/kg/day) for four days.[5] Then they were euthanized and killed under deep ether anesthesia.

2.2.3. Operative procedure

Immediately after the animals were killed, the entire colon starting from caecum was excised, longitudinally split. Washed with ice-cold saline to remove fecal residues.

3. Macroscopic damage score

The colonic samples were scored macroscopically according to the following grading system: 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.[6]

4. Histopathological study

After formalin fixation (10% during 24 hours), each excised sample block was processed for histological evaluation. The sample block was first dehydrated by immersion in progressively increasing concentrations of ethanol and then xylene. Following this, the dehydrated tissue was immersed in melted paraffin at 60 0C for 3 h before being embedded in a paraffin block. Sections 5 microns thick were cut by using an 82-spence microtome. The sections were then deparaffinized by treatment with xylene, ethanol and water. Tissues were stained with haematoxylin and eosin (H&E) and then left in the fume cupboard overnight.[4]

All groups were histopathologically assessed by using following score:[7]
0=normal; 1=mild mixed infiltration in the lamina propria; 2= focal superficial ulceration of the mucosa only; 3= deep ulceration penetrating colonic wall through mucosa till muscularis mucosa and severe inflammation; 4=necrosis through large bowel wall.

5. Statistical analysis

All obtained values were expressed as mean ± standard deviation (SD). Data analyses were achieved using prism (Version 5) statistical package. Lesion score and histological score (non-parametric values) analyzed using the Kruskal–Wallis nonparametric analysis of variance with mann-whitney comparison test. P values less than 0.05 were considered Statistically significant.

6. RESULTS

6.1. Macroscopic study

The macroscopic findings of opened colon showed healthy mucosa in most samples with no swelling or redness, and some tiny pinpoint hemorrhages in Control group (group A). But we find two samples with hemorrhage lines and large erosions. in Alendronate group (group B) After administration of Alendronate the colon mucosa showed large erosions and many hemorrhage places. There is significant difference when comparing group B with control group ($p<0.05$).

While treating with MSM 400 mg/kg/day one of the rats had dead, and the other rats showed erosions in colon mucosa, and the macroscopic finding showed swelling and redness in most samples. There was significant difference when comparing curative group, which took MSM 400 mg/kg/day (group C) with Alendronate group (group B); $p<0.05$.

After treating with MSM 800 mg/kg/day, all rats' colon showed healthy mucosa, with no blood spots or erosions, Thus there was significant difference when comparing curative group which took MSM 800 mg/kg/day (group D) with Alendronate group (group B); $p<0.05$.

Table 1: Effect of MSM on alendronate-induced colon injury macroscopically.

Group	Control	Alendronate	MSM 400 mg/kg/day	MSM 800 mg/kg/day
Grade	1±0.5669	4.5±0.3273	2.714±0.3595	2.125±0.3981

Values are expressed as mean±SD (Standard Deviation), one-way ANOVA followed by mann-whitney's test as compared to control.

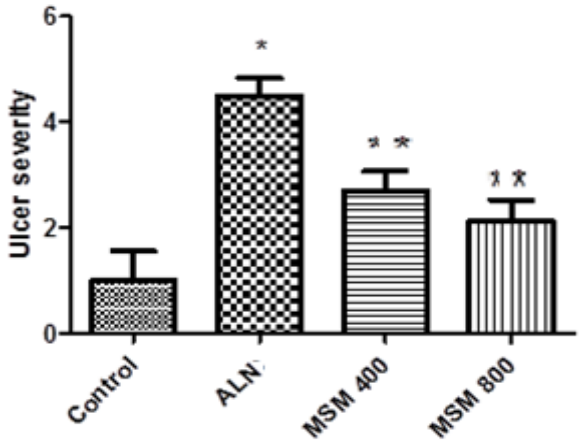

Figure 1: Comparing ulcer severity macroscopically between groups.

** significant difference between group B and group A.*

*** significant difference between group C and group B. and between group D and group B.*

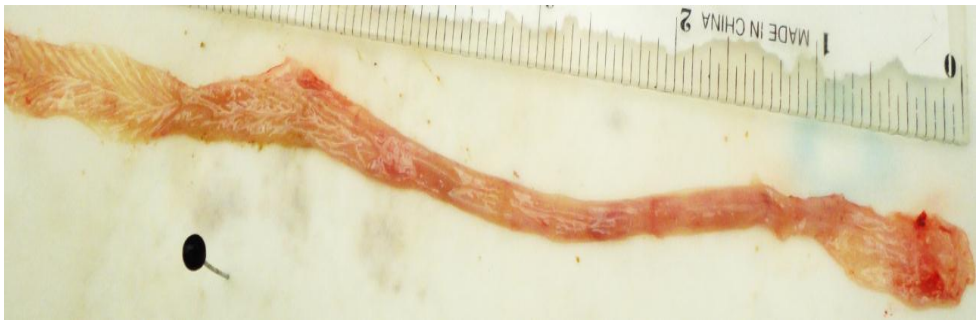

Figure 2: Normal colon with some redness- Control group- grade 1.

Figure 3: Swelling and redness - MSM group 400mg/kg/day- grade 4.

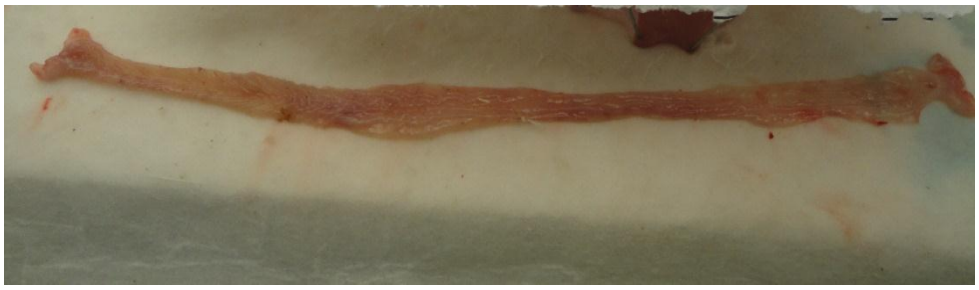

Figure 4: Normal colon mucosa with blood spots- MSM group 800mg/kg/day- grade 2.

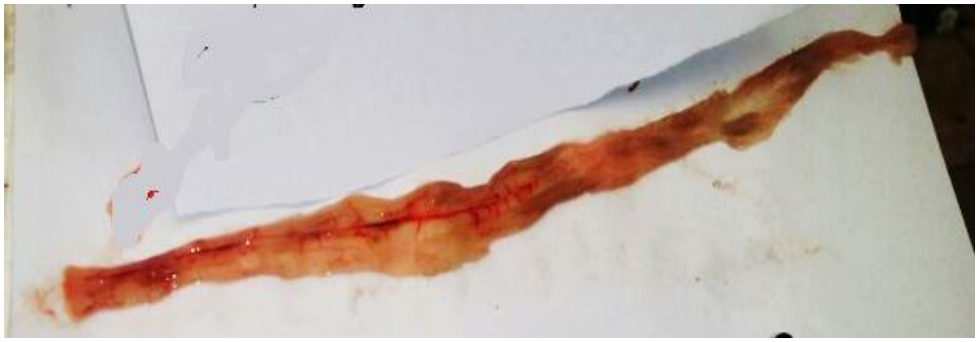

Figure 5: Large erosions with necrosis- ALN group- grade 5.

6.2. Microscopic Study

The colon epithelium of control group contained swelling vessels. Besides mild mixed infiltration in the lamina propria in most samples, and the mucosa showed some light erosions in the control group. The Alendronate group was very ulcerated. Most samples showed deep ulceration penetrating colonic wall through mucosa till muscularis mucosa and severe inflammation. There was significant difference when comparing group B with control group ($p<0.05$). The curative group with MSM 400 mg/kg/day did not show respond to the treatment, thus three samples showed deep ulceration reached the muscalaris mucosa, three other samples showed surface ulceration in mucosa, while one rat had dead during treatment.

There was no significant difference when comparing with group B ($p>0.05$). Seven samples of the group D (MSM 800 mg/kg/day) take the evaluation score of 2, because it showed

surface erosions and swelling blood vessels. The last sample have a large erosion reached muscalaris mucosa. There was no significant difference comparing with group B ($p>0.05$).

Table 2: Effect of MSM on alendronate-induced (ALN; 20 mg/kg, p.o.) colon injury histologically.

Group	Control	Alendronate	MSM (400 mg/kg/day)	MSM (800 mg/kg/day)
Grade	1±0.2673	2.375±0.183	2.286±0.2857	2.125±0.125

Values are expressed as mean±SD (Standard deviation), one-way ANOVA followed by mann-whitney's test as compared to control.

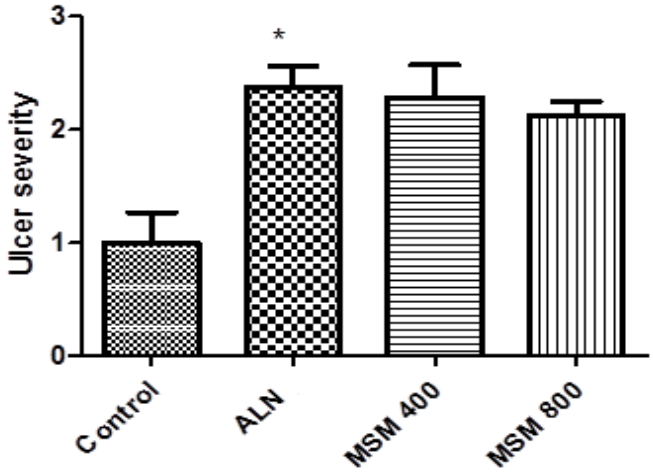

Figure 6: Comparing ulcer severity histologically between groups.

** significant difference between group B and group A.*

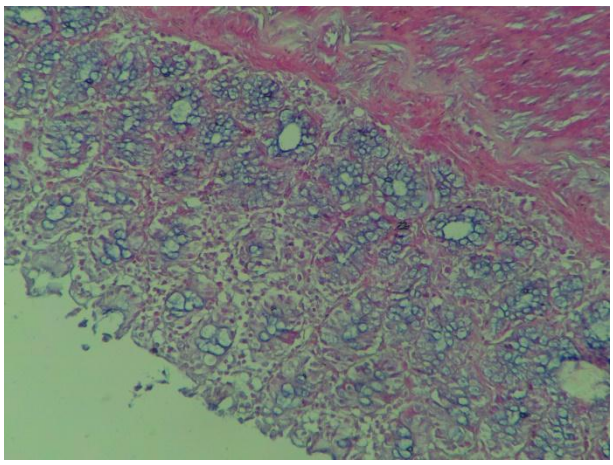

Figure 7: Microscopic sketch for colon (x20 magnification)- control group- grade1.

(mild mixed infiltration in the lamina propria.**)**

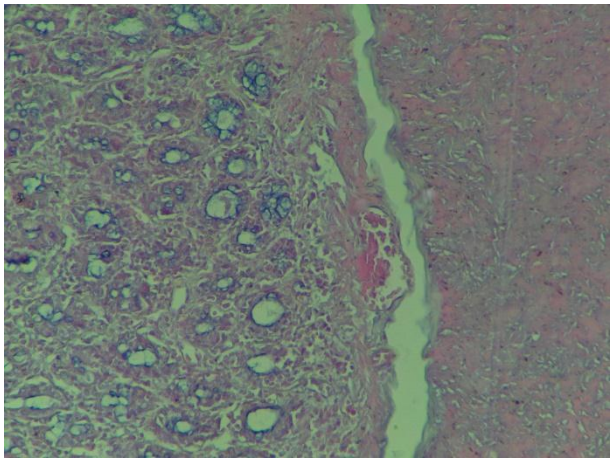

Figure 8: Microscopic sketch for colon (x20 magnification)- MSM 400mg/kg/day group- grade1.

(mild mixed infiltration in the lamina propria, and swelling blood vessels)

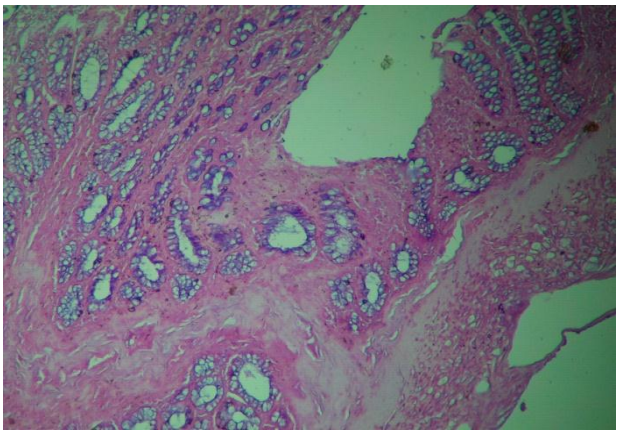

Figure 9: Microscopic sketch for colon (x20 magnification)- ALN group- grade2.

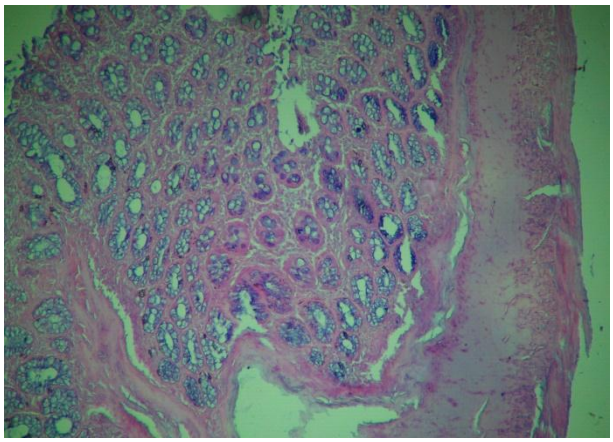

Figure 10: Microscopic sketch for colon (x20 magnification)- MSM 800mg/kg/day group - grade2.

7. DISCUSSION

Esophageal injury caused by alendronate is well characterized, but there are no reports of injury to the colon except one clinical study. Biopsy specimens from the lesion revealed granulation tissue with reactive-appearing overlying epithelium.[8] The study was on two patients who had been initiated treatment with alendronate within 6 months of detection of the colonic ulcer. Other known causes of colon ulcers were excluded by histopathologic examination and culture of biopsy specimens. After discontinuation of alendronate, there was complete ulcer healing in both patients.[8]

In our study, the ulcer had been induced by administration of Alendronate 20mg/kg/day for four days after dissolve it in saline. This dose caused colon injury presented in large erosions and many hemorrhage places, and it caused in some samples mild necrosis. The histological study showed deep ulceration penetrating colonic wall through mucosa till muscularis mucosa and severe inflammation. It also showed infiltration of neutrophils and macrophages to the mucosa. This represent an early characteristic of inflammation. The neutrophils, once in contact with the gut mucosa and the lipopolysaccharides, actively produce pro-inflammatory cytokines, interleukin-1 (IL-1), tumour necrosis factor alpha (TNF-α). It was observed that cytokines stimulate monocytes, macrophages and lymphocytes, responsible for maintaining inflammation.[9]

The mechanism of alendronate-induced mucosal toxicity has not been completely elucidated. Experimental evidence suggests that bisphosphonates directly irritate the GI mucosa and may compromise the hydrophobic mucosal phospholipid layer.[8]

The macroscopically observations showed different results from Amirshahrokhi et al. and Al-bitar et al.[4,6] who induced ulcerative colitis in rats by using acetic acid. These showed ulcerated mucosa, hemorrhagic, oedematous and necrotic compared to normal control group, while this study didn't show the same necrotic. Otherwise, the microscopically observations showed inconsiderable difference between those studies and the recent study. They all showed deep ulceration of muscularis mucosa, severe inflammation and necrosis through large bowel wall. In the other hand, Amirshahrokhi et al. and Al-bitar et al. results showed infiltration of small round cells and polymorphonuclear leukocytes to lamina properia,[4,6] that is not shown in this study. Thus, this colon ulceration is not totally the same model of ulcerative colitis induced by the acetic acid.

Nagar described Isolated ulcers of the large intestine as ulcers that are not associated with an underlying colitis and may be an incidental finding on screening colonoscopy or present with abdominal pain, hematochezia, chronic gastrointestinal bleeding, and rarely, perforation. He suggested that the common cause of isolated colonic ulcers is the use of nonsteroidal anti-inflammatory drugs (NSAIDs).[1] This kind of ulceration is also not the same of the ulceration in this study. Besides, there was no gastrointestinal and hemorrhagic symptoms in the experimental rats.

Neutrophils play a crucial role in the development and full manifestation of gastrointestinal inflammation, also neutrophil infiltration into inflamed tissue plays a crucial role in the destruction of foreign antigens and in the breakdown and remodeling of injured tissue.[10] 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.[11] 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 pro-inflammatory cytokines and enhancing the recruitment of macrophages and neutrophils.[12]

Methylsulfonylmethane (MSM), or dimethylsulfone, is a popular dietary supplement. It is found in small amounts in many foods including unpasteurized milk, grains, meat, eggs and fish. It is also found in raw vegetables including broccoli, peppers, Brussels sprouts, onions, asparagus and cabbage.[13] Our study demonstrated, according to microscopic findings, the inability of MSM (400 mg/kg/day) of treating the colonic ulcer induced by alendronate in rats. Otherwise, the macroscopic findings have showed a mild recovering in rats' activity and general health. Similar results were observed by Laham et al who used other model of ulcerative colitis to test the anti –inflammatory and antioxidant potential of MSM. Their study showed that treatment of rats with the MSM (400mg/kg) for 6 days, cured the tissue damage in rat model of colitis induced by acetic acid as verified from its effects, as evidenced by lowered the incidence of diarrhea, improved food intake, and colonic weight/length ratio decrease, reverse the acetic acid induces colitis group. The macroscopic and histological changes in MSM exerted a mild amelioration of the extent and severity of inflammation by treating ulceration and necrosis. However, the values obtained with this dose of MSM (400mg/kg) for 6 days, were not significantly different from the acetic acid control group.[14]

The Microscopic statically scores were not significantly different also when using 800 mg/kg/day of MSM. This is the first study of the effect of MSM in a dose of 800 mg/kg/day in treating colonic ulcer induced by Alendronate.

MSM has an anti- inflammatory and antioxidant activity at colorectal sites that is due to its effect on promoting antioxidant status (GSH), decreasing free radicals and myeloperoxidase responsible for tissue damage and delayed healing. But with all these activities it wasn't effective in treat the damage of colon induced by alendronate. That may indicate that the mechanism of alendronate in causing the colon ulceration may be immune mechanism in case of protecting the body from the ulcerating Alendronate.

This kind of the inflammation may be the same inflammation induced by the hapten 2,4,6-trinitrobenzenesulfonic acid in previous study. That chronic inflammation of the intestine may occur as a consequence of increased permeability of the mucosa to a luminal antigen which cannot be readily cleared by the immune system.[15]

8. CONCLUSION

Administration of Methylsulfonylmethane MSM (400 mg/kg/day) for four days has no ameliorative effect of colon ulcer induced by alendronate. Also, the dose of 800 mg/kg/day of MSM, couldn't treat the colon ulcer induced by alendronate in rats.

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