Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 128 (2019) 52786-52790

ISSN: 2229-712X

The Protective Effect of Methyl Sulfonyl Methane on Peptic Ulcer Induced by Alendronate

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ARTICLE INFO

Article history:

Received: 17 January 2019;

Received in revised form:

20 February 2019;

Accepted: 1 March 2019;

Keywords

Ulcer,

Protective,

Alendronate,

Methyl Sulfonyl Methane.

ABSTRACT

Gastric ulceration is a common adverse effect of many medications. Bisphosphonates (BPs) that is used for osteoporosis, is associated with peptic ulcer manifested -in its severe state- in hemorrhage and perforation. Methyl sulfonyl methane (MSM) which is used for osteoarthritis was used to prevent BPs ulceration. Our objective was to study the effect of MSM on preventing peptic ulcer induced by Alendronate (ALN) in rats. The experiments had been done on eight white Wister rats for each group. The gastric ulcer has been induced by administration of Alendronate (20mg/kg/day) by gavage for 4 days. MSM (400mg/kg/day) has been given for the protective group for 4 days before administration of Alendronate. The gastric ulcers in rats' stomachs were examined histologically and microscopically. The ulcer index and protective index were measured, and then the statistical analyses were carried out. The results showed that the Administration of MSM before Alendronate inducing ulcer led to a reduction in ulcer index and showed significant difference comparing with morbidity group. The conclusion was that MSM (400 mg/kg/day) has a protective effect of peptic ulcer induced by alendronate.

Introduction

Ulceration of the gastrointestinal mucosa is caused by disruption of the normal balance of the corrosive effect of gastric juice and the protective effect of mucus on the gastric epithelial cells. The most common sites for ulcers are the stomach and the first few centimetres of the duodenum. More rarely they occur in the oesophagus, following reflux of gastric juice, and round the anastomosis of the stomach and small intestine, following gastrectomy[1]. Mucosal erosions or ulceration arise when the caustic effects of aggressive factors (acid, pepsin, bile) overwhelm the defensive factors of the gastrointestinal mucosa (mucus and bicarbonate secretion, prostaglandins, blood flow, and the processes of restitution and regeneration after cellular injury). Over 90% of peptic ulcers are caused by infection with the bacterium Helicobacter pylori or by use of nonsteroidal anti-inflammatory drugs (NSAIDs)[2]. Other medications can cause peptic ulcer such as steroids, bisphosphonates (BPs), potassium chloride, chemotherapeutic agents (e.g., intravenous fluorouracil)[3].

Bisphosphonate drugs include alendronate, ibandronate, risedronate and zoledronic acid They stick to the surfaces of the bones and slow the breakdown of old bone[4]. All bisphosphonates cause gastro-intestinal side effects. Patients should not take these tablets at bed-time and should be advised to stay upright for at least 30 min after taking them[5]. Some amino bisphosphonate anti- osteoporosis drugs, such as alendronate, have been reported to cause irritation of the upper gastrointestinal tract, and in some cases severe esophagitis[6].

Methylsulfonylmethane MSM also known as dimethyl sulfone $(CH_3)_2SO_2$ [7]. MSM is the oxidised form of dimethyl sulfoxide. It is found in very low amounts in fruits, corn, tomatoes, tea, coffee, and milk[8]. It has been found to occur naturally in asparagus[9]. In the presence of ozone and high-energy ultraviolet light, MSM (along with dimethyl sulfoxide [DMSO]) is formed from dimethyl sulfide, taken up into atmosphere, returned to the earth in rainfall, and taken into the root systems of plants[10]. 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[11]. Because of MSM's sulfur content, it is used by the body to maintain normal connective tissues. MSM may have anti-inflammatory activities, chemopreventive properties, prostacyclin (PGI2) synthesis inhibition, anti-atherosclerotic action, salutary effect on eicosanoid metabolism, and free radical scavenging activity[12].

Amirshahrokhi et al. and Al-bitar et al. have studied the protective effect of methylsulfonylmethane in ulcerative colitis induced by acetic acid[13,14]. Nevertheless no one has studied the efficacy of MSM in protection of gastric ulcer.

Materials and methods

Animals

Twenty four male albino rats of the Wistar strain weighing between 180-250 g 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 theexperiment. 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.

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Experimental design

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

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

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

Group Three (group C) (MSM): Animals were treated with 400 mg/kg of Methylsulfonylmethane for four days before Alendronate administration (20 mg/kg) for four days.

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 [15]. Then they were euthanized and killed under deep ether anesthesia.

Operative procedure[16]**:**

Immediately after the animals were killed, stomachs were dissected out, cut along the greater curvature. The mucosae were rinsed with cold normal saline to remove blood contaminant, if any.

Assessment of mucosal damage[17]**:**

The stomachs were examined and rated for pathology according to the following arbitrary scale: 0 = no damage; 1 = blood in lumen; 2= pin point erosions; 3 = 1-5 small erosions (<2 mm); 4 = >5 small erosions; 5 = 1-3 large erosions (>2 mm); 6 = >3 large erosions.

Ulcer index (UI) and preventive ratio of each of the groups pretreated with extract were calculated using the methods Nwafor et al. (2000).[18].

UI= degree of ulceration × percentage of group ulcerated/ 100

Preventive ratio= UI(Ulcerated group-protected group)/UI (Ulcerated group) × 100

Degree of ulceration= Total ulcer score/ No. of animals ulcerated

Tissue preparation[19]:

The tissues were fixed with 4% formaldehyde in tris-buffered saline (TBS) and dehydrated by a graded ethyl alcohol (EtOH) series increasing in concentration (30%, 50%, 70%, 80%, 90%) diluted in TBS, and finally in 100% EtOH. The EtOH was then replaced by Xylene before they were embedded in paraffin wax and mounted. The paraffin block was cut into thin sections (4-8 µm thickness) using a sledge microtome. Serial sections were prepared on glass slides. Afterwards, the sections were deparaffinized and rehydrated by a graded EtOH series decreasing in concentration (98%, 90%, 70%, 50%, 30%) ending in water. After drying the samples were stored dust-free until further use.

The severity and the degree of mucosal damage were assessed according to modified Sedny scale, so that the following grades were obtained: Grade (0): no mucosal lesions; Grade (1): mucosal edema, congestion, and neutrophils infiltration; Grade (2): surface mucosal erosion. Grade (3):≤2 Gastric ulcers. Grade (4): > 2 Gastric ulcers[20].

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.

Results

Macroscopic Study

The macroscopic findings of opened stomach showed healthy mucosa in most samples with some tiny pinpoint hemorrhages in Control group (group A). After administration of Alendronate the stomach mucosa appeared macroscopically ulcerated and hemorrhagic in Alendronate group (group B) compared to control group ($p<0.05$). Administration of MSM inhibited Alendronate-induced ulcer formation, so that the macroscopic findings showed less ulcerated regions and pinpoint hemorrhages. Thus there is significant difference when comparing protective group which took MSM (group C) with Alendronate group (group B); $p<0.05$.

Table 1. Effect of MSM on ulcer severity, ulcer index, and preventive index in Alendronate- (ALN; 20 mg/kg, p.o.) induced gastric injury.

Group	Number of animals	Ulcer severity	Ulcer Index	Preventive index
Control	8	0.625±0.9161	1.6	-
Alendronate	8	5±0.9258	5	-
MSM	8	2.625± 1.061	2.625	0.475

Values are expressed as mean±SD (Standard Deviation), one-way ANOVA followed by mann-whitney's test as compared to control. AlN.: alendronate; p.o.: per oral

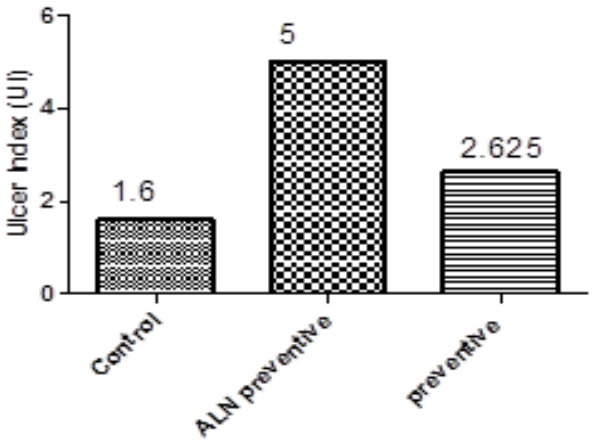

Figure 1.A. comparing ulcer index between groups.

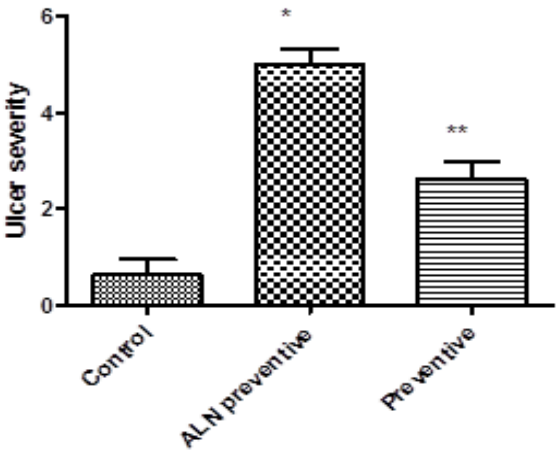

Figure1.B. Comparing ulcer severity macroscopically between groups.

* significant difference between group B and group A.

** significant difference between group C and group B.

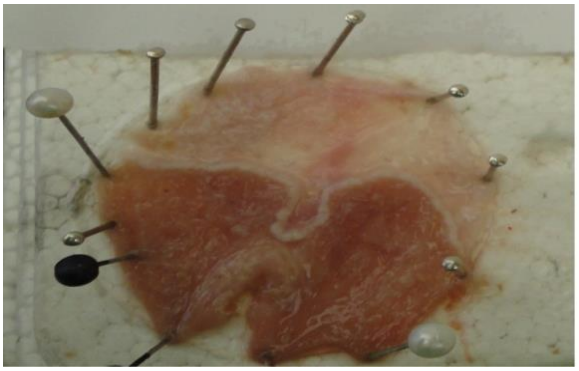

Figure 2.A. Normal stomach- Control group- grade 0.

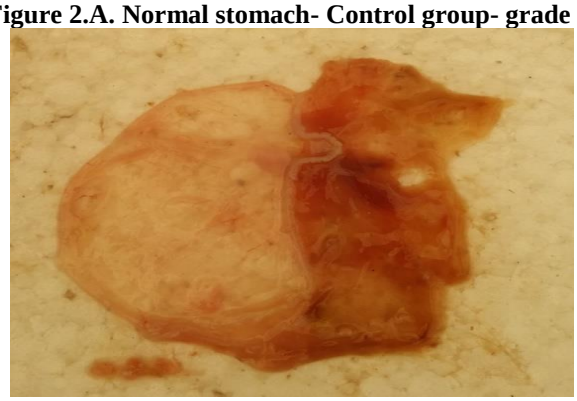

Figure 2.B. Large erosion- ALN group- grade 5.

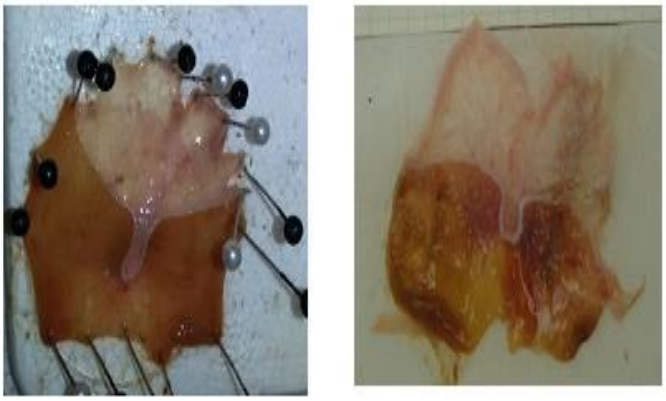

Figure 2.C. Large erosions with hemorrhage- ALN group- grade 6.

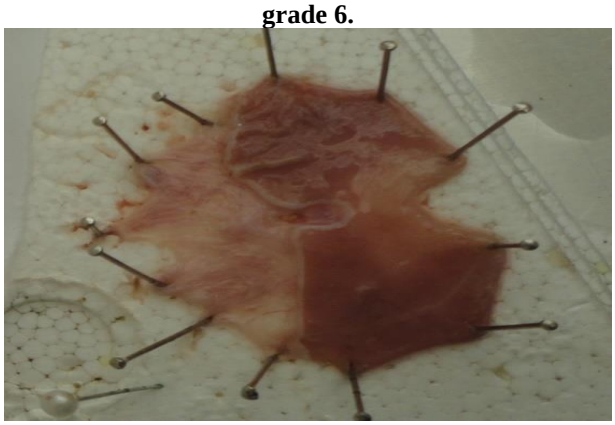

Figure 2.D. pinpoint blood- MSM group- grade 1.

Microscopic Study

The control group stomach mucosa showed normal mucosa with some vessels congestions. The Alendronate group was totally ulcerated. Most samples showed mucosal erosion, mucosal edema, congestion, and neutrophils infiltration. There was significant difference when comparing group b with control group ($p<0.05$). there was no ulceration in MSM group, except for some tiny erosion in some samples, and there was significant difference when comparing with group B ($p<0.05$).

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

Group	Control	Alendronate	MSM
Grade	1.125±0.3536	2.875±0.3536	1.750±0.4629

Values are expressed as mean±SD (Standard deviation), one-way ANOVA followed by mann-whitney's test as compared to control. AlN.: alendronate; p.o.: per oral

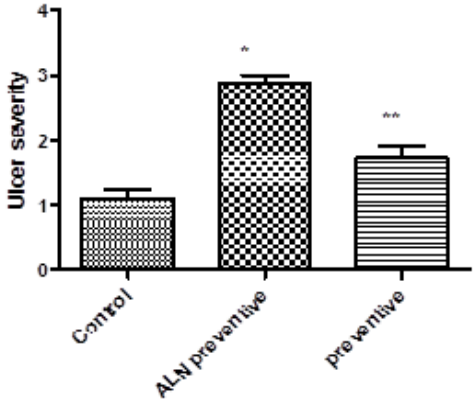

Figure 3. Comparing ulcer severity histologically between groups.

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

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

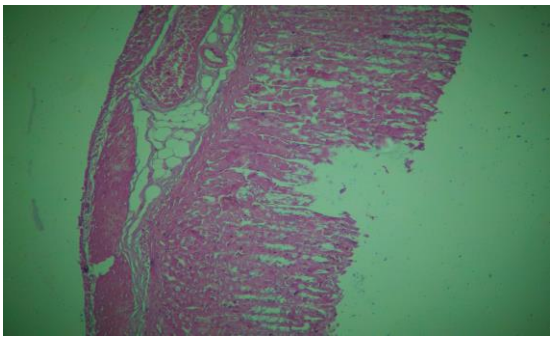

Figure 4.A. Microscopic sketch for stomach (x10 magnification)- control group- grade 1.

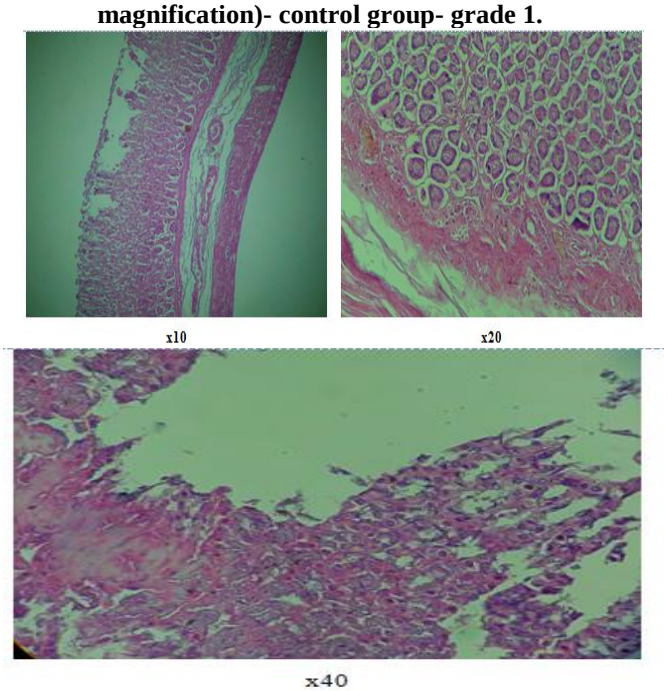

Figure 4.B. Microscopic sketch for stomach- ALN group- grade 2.

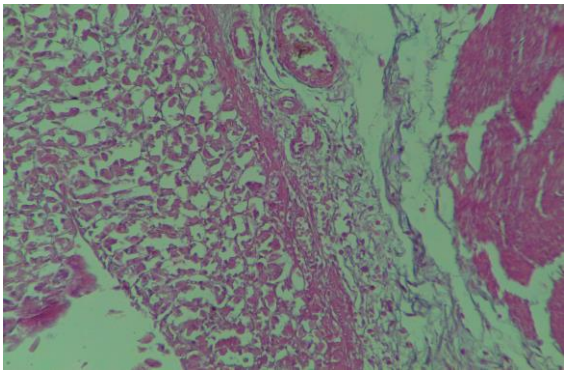

Figure 4.C. Microscopic sketch for stomach (x20 magnification)- ALN group- grade 3.

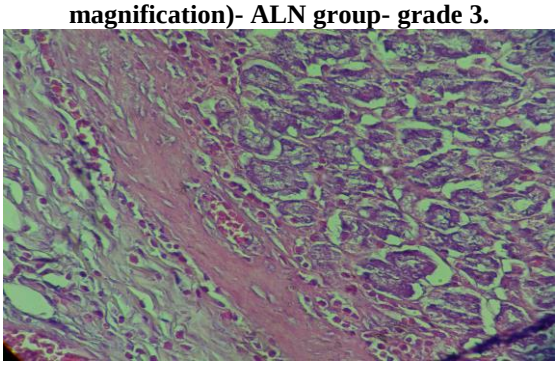

Figure 4.D. Microscopic sketch for stomach (x40 magnification)- MSM group- grade 1.

Discussion

Among the various nitrogen-containing bisphosphonate under development or on the market, data from animal and human studies suggest that agents configured with primary amino side chains, such as alendronate, may have increased potential for causing gastric damage[21]. In our study, the ulcer was induced by administration of Alendronate 20mg/kg/day for four days after dissolve it in saline with sodium hydroxide to reach pH=7, because the studies showed that the irritative effects is dependent on the pH of drug solution and the action is more potent at pH 7 than pH 4 [22]. This dose cause increase in ulcer severity and ulcer index. Similar results were shown by Şener et al. study that demonstrated, an administration of Alendronate 20mg/kg/day for four days by gavage increase gastric acidity significantly, and chronic administration of Alendronate cause significant ulceration and bleeding in all of the animals[15]. Our results also were compatible with Amgase et al. study, which found that alendronate, given to fasted rats, produced ulcers in the antrum after re-feeding for 3 days. These ulcers were accompanied by a marked increase in vascular permeability, and a highly significant relationship was observed between the area of lesions and the increased permeability. Indeed, histological observation revealed that these ulcers were accompanied with severe edema and inflammatory cell infiltration in the submucosa. It was also found that the damaged mucosa was covered with a white cap, mainly composed of inflammatory cells and fibrin-like substances[23].

The pH of alendronate in the vehicle used in this study was 7, so it is very unlikely that its detrimental effects were due to pH- related irritancy. It is also unlikely that alendronate's detrimental effects were due to an elevation of gastric acid secretion, since the drug was found not to significantly alter basal or pentagastrin-stimulated acid secretion in the rat[6].

Lichtenberger et al. illustrate the mechanism of bisphosphonate induced ulcer in which it is associated with their ability to compromise the surface hydrophobic phospholipid barrier of the tissue. This bisphosphonate effect on the surface barrier may trigger the development of mucosal injury and possible ulceration[24].

Amagase showed that BPPs applied to the gastric mucosa caused a decrease in transmucosal potential difference of the stomach, suggesting a disruption of surface epithelial cells due to direct action[23]. In other study, he suggested that alendronate impaired the healing of gastric ulcers, at least partly through: 1. dysregulation of the expression of basic fibroblast growth factor (bFGF), and vascular endothelium-derived growth factor (VEGF), the important growth factors for vascularization/granulation. 2. as well as suppression of the stimulatory action of epidermal growth factor (EGF) on epithelial proliferation/migration[25].

Şener et al found that the malondialdehyde (MDA) levels that indicate lipid peroxidation of the membranes were significantly increased after ALD, demonstrating tissue damage. However, this increase in lipid peroxidation may partly be due to the free radicals generated by neutrophils. In the same study it has been found that taurine (TAU) $C_2H_7NO_3S$, which is a sulfur-containing, semi-essential amino acid, as an antioxidant might prevent ALD-induced gastric damage through attenuation of the damage, and enhancement of the regenerative response of the damaged gastric cells. Tissue content of Glutathione (GSH) was also increased in rats treated with TAU. This could be attributed to the role of TAU's antioxidant action against lipid peroxidation, thus conserving the GSH antioxidant system[16].

Methylsulfonylmethane (MSM), also known as dimethyl sulfone, is an organic sulfur compound mainly present in foods such as fruits and vegetables, and in beverages as well[(26)]. Our study demonstrated the ability of MSM in protection of Alendronate induced ulceration in rats. Thus administration of MSM led to significantly reduction in deep ulcers macroscopically and histologically. We believe that is the first study, which study the relation between MSM and peptic ulcer induced by ALN. Similar results were observed by Amirshahrokhi who used model of ulcerative colitis to test the anti –inflammatory and antioxidant potential of MSM[13].

This study suggest that MSM $C_2H_6O_2S$ can protect from Alendronate induced ulceration with a similar mechanism of taurine $C_2H_7NO_3S$, because they both have the same structure and the same mechanisms. Thus, they both can donate sulfur and have antioxidant properties.

Beside the anti-inflammatory effect to the MSM, there is the antioxidant effect[27]. Thus, our study have the same results with Takeuchi et al. study which approve that Antioxidative drugs, such as SOD or allopurinol, as well as rebamipide (a mucosal protective drug), are effective to reduce the severity of BPP-induced antral ulceration, while antisecretory drugs, such as omeprazole (a proton pump inhibitor), have no effect[28].

Bohlooli et al. study suggested that MSM pretreatment could alleviate hepatic injury induced by acetaminophen intoxication, this may be through its sulfur donating and antioxidant effects. Thus, it is possible to suggest that MSM as a pretreatment agent has a potential to be investigated as an agent in limiting the drug-induced oxidative damage[29].

MSM can increase the efficacy of antioxidant system by increasing Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity. It also increase levels of reduced glutathione (GSH), and decrease levels of malondialdehyde (MDA). Beside its role as free radical scavenger[30].

Other studies explained the efficacy of MSM in donating Sulfur. Sulfur containing amino acids contribute substantially to the maintenance and integrity of cellular systems by influencing cellular redox state and cellular capacity to detoxify toxic compounds, free radicals and reactive oxygen species[31].

Conclusion

Administration of Methylsulfonylmethane MSM (400 mg/kg/day) give protective effect of peptic ulcer induced by alendronate.

Acknowledgment

Special thanks to Damascus university for fond this project. And to dr. Elias Bshara and ph. Rama Mousleh for their efforts in accomplishment this project.

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