

Original Article

## THE CURATIVE EFFECT OF METHYLSULFONYLMETHANE ON AN EXPERIMENTAL MODEL OF ULCERATIVE COLITIS IN RATS

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### ABSTRACT

**Objective:** Ulcerative colitis is a chronic inflammatory bowel disease of unknown etiology that affects the mucosa and submucosa of the colon and rectum. It is associated with oxidative stress, neutrophil infiltration and production of inflammatory mediators. In the present study, the anti-inflammatory and antioxidant effects of Methylsulfonylmethane (MSM) in treatment the acetic acid (A) induced model for UC in rats were examined.

**Methods:** Ulcerative colitis was induced by intra colonic administration of 2 ml of 4% acetic acid in adult male Wistar rats which were divided into 3 groups: normal control (N), acetic acid (A) control, and MSM (1000 mg/kg). Rats received treatment for six consecutive days. On day 7, the rats were sacrificed, the colon was removed, the body weight, colon weight/length ratio, tissue glutathione (GSH) concentration, and macroscopic evaluations, were performed.

**Results:** The colon weight/length ratio was decreased significantly ( $P<0.05$ ). The glutathione (GSH) concentration was increased significantly ( $P<0.05$ ). The macroscopic parameters were decreased, but it didn't reduce significantly in the MSM treated groups compared to group A. Contrariwise the parameters of group A. The levels of body weight were increased, and colon weight/length ratio was decreased significantly ( $P<0.05$ ). The glutathione (GSH) concentration was increased significantly ( $P<0.05$ ). The macroscopic parameters were decreased significantly in the MSM treated group, compared to the acetic acid-treated group.

**Conclusions:** Methylsulfonylmethane is an effective, safety natural product with little side effects, has a good curative effect in a dose (1000 mg/kg) on experimental Ulcerative colitis induced by acetic acid.

**Keywords:** MSM, Ulcerative colitis, Glutathione, Macroscopic

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### INTRODUCTION

Ulcerative colitis (UC) is chronic and relapsing inflammatory disorder of the gastrointestinal tract, defined by clinical characteristics such as diarrhea, abdominal pains, weight loss, and nausea and by pathological features such as a loss of mucosal integrity and inflammatory cell infiltration [1]. The immune response associated with lymphocytes and macrophages followed by a release of soluble cytokines and other inflammatory mediators [2]. As well as reactive oxygen species (ROS), which cause diminishing of cellular membrane stability and cell death by leading lipid peroxidation [3]. Excessive production of ROS in mucosal cells could cause damage to intestinal epithelial cells, subsequently influences the mucosal integrity or initiate an inflammatory signaling cascade and lead to severe damage in experimental colitis [4].

A number of medical strategies are available, but fewer side effects are better. The present work was conducted to assess the possible anti-inflammatory and antioxidative effects of Methylsulfonylmethane in treating an animal model of colitis induced by acetic acid in rats.

Methylsulfonylmethane (Dimethylsulfone or, MSM) is a naturally occurring organosulfur molecule that can be synthesized commercially from dimethylsulfoxide (DMSO). MSM is naturally present in the human body as it is metabolized from ingested DMSO [5]. Many properties have been attributed to MSM, such as chemopreventive properties, anti-inflammatory activities, anti-atherosclerotic action, prostacyclin (PGI<sub>2</sub>) synthesis inhibition, and free radical scavenging activity [6].

### MATERIALS AND METHODS

#### Animals

Wistar rats weighing (200-250g) were acclimatized for one week before any experimental procedures and were fed with standard commercial rat pellets and allowed water ad libitum. They were kept

at controlled environmental conditions (temperature  $23\pm 2$  °C, humidity  $55\pm 15\%$ , lighting regimen of 12-h light: 12-h dark). All methods performed in this study were in accordance with regulatory guidance on the care and use of experimental animals. Three groups, 6 rats per group, were used, the groups were divided into normal control (N), acetic acid (A) control, and MSM (M). Group N (Normal control group) was received physiological saline intrarectally, following the administration of oral gavage syringe, once daily for 6 consecutive days, starting 24 h after the induction of colitis. Group A (colitis control) received 2 ml acetic acid 4% intrarectally following the administration of saline orally; the group (M) received 2 ml acetic acid intrarectally, following the administration of MSM (1000 mg/kg/day, orally) suspended in normal saline, for 6 d [7].

#### Induction of experimental colitis in rats

Rats 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 administration. On day 0 under light ether anesthesia, a soft and flexible catheter (2 mm inner diameter) was inserted to the anus for 8 cm, to inject the acetic acid. The rats were maintained in a head-down position for 30 seconds in order to prevent the solution from spreading out [8].

#### Tissue collection and preparation

On the 7th day, rats were sacrificed under deep ether anesthesia, their distal colons were removed to evaluate the colon weight/length ratio, macroscopic damage. In addition, the biochemical measurement of reduced glutathione (GSH).

#### Measured parameters for assessment of colonic damage

##### Clinical finding

Change the body weight (%) was measured at regular time intervals from the first day. The rats were observed for rectal bleeding, and stool consistency.

### Colon weight/length ratio

The entire colon starting from the caecum was excised, longitudinally split. Washed with ice-cold saline to remove fecal residues. The length (cm) and weight (g) were measured. Each colon was gently stretched, the distance from the colorectal junction to anus was measured, from which weight/length (g/cm) ratio, as an indirect marker of inflammation was calculated.

### Macroscopic scoring

The colonic samples were examined immediately by the 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 [9].

### Reduced glutathione

Glutathione Assay Kit (Abnova) for the direct assay of reduced glutathione in colonic tissue was used. Colonic tissue samples were frozen in liquid nitrogen, stored at  $-80^{\circ}\text{C}$  until time of the assay. Colon GSH levels were determined as previously described by Blenn [10], based on the reaction of 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB) with the glutathione present to form a yellow product. The optical density (OD) measured at 412 nm is directly proportional to glutathione concentration in the sample by using a microplate reader (Elisys Uno Human Germany). The glutathione content was expressed as  $\mu\text{M/g}$  tissue.

### Statistical analysis

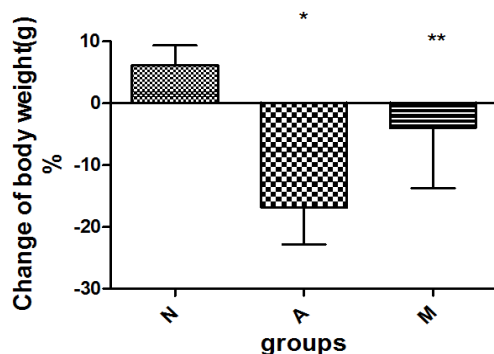
Data analyses were achieved using Prism (Version 5) statistical package. Data were presented as means $\pm$ standard deviation (SD). For parametric data, one-way analysis of variance (ANOVA) was used followed by Tukey's tests multiple comparisons. Lesion score (non-parametric values) analyzed using the nonparametric Mann-Whitney U test. P values of less than 0.05 were considered statistically significant.

## RESULTS

### Body weight change

Progressively body weight loss with weakness and decreased food intake were observed after 24 h of administration of acetic acid.

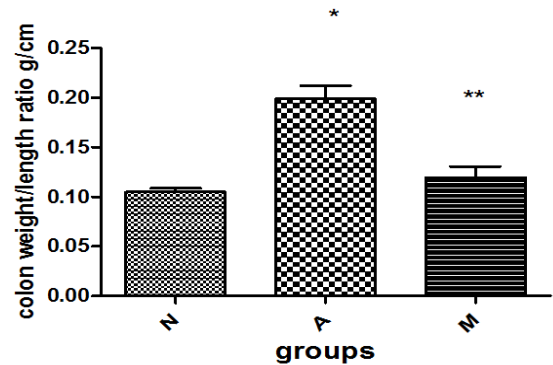
Compared with the normal group which revealed an increase in body weight, the body weight of group A at the end of the experiment was significantly reduced ( $p<0.0001$ ). While the body weights of the treated group with MSM were significantly increased compared with the colitis group at ( $p<0.0001$ ) fig. 1.



**Fig. 1: The effects of MSM on body weight in acetic acid-induced ulcerative colitis in rats. Data are presented as means $\pm$ SD (n =6). (N); normal control, (A); colitis control, (M) MSM groups, \*Significant difference as compared to normal control group at  $p<0.0001$ , \*\*Significant difference as compared to A group at  $p<0.0001$**

### Colon weight/length ratio

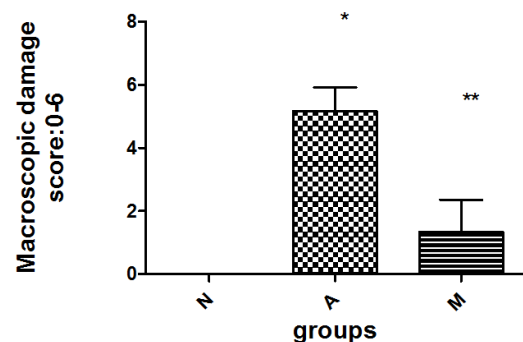
A corresponding increase in the colon weight to length ratio is a reliable marker of colon inflammation that was observed in colitis animals relative to normal control. MSM treatment group exerted significant decrease in the weight/length ratio compared with that of group A ( $p<0.0001$ ), which has shown a significant increase in the colonic weight/length ratio compared with that of the normal control group.



**Fig. 2: The effects of MSM on colon weight/length in in acetic acid-induced ulcerative colitis in rats. Data are presented as means $\pm$ SD (n =6). (N); normal control, (A); colitis control, (M) MSM groups, \*Significant difference as compared to normal control group at  $p<0.0001$ , \*\*Significant difference as compared to A group at  $p<0.0001$**

### Macroscopic scoring

Administration of MSM therapeutically after intra colonic administration of 2 ml 4% acetic acid, has treated the severity of the gross lesion, therefore the morphological score was significantly decreased as compared to (A) group ( $p<0.0044$ ). While in group A, the mucosa appeared macroscopically ulcerated, hemorrhagic, oedematous and necrotic. Thus, the morphological score in the (A) group was significantly increased as compared to the normal control group ( $p<0.0025$ ).



**Fig. 3: The effects of MSM on the macroscopic changes in Acetic Acid-induced ulcerative colitis in rats. Parametric data were expressed as mean $\pm$ S. D (n =6). (N); normal control, (A); colitis control, (M) MSM groups, \*Significant difference as compared to normal control group at  $p<0.0025$ , \*\*Significant difference as compared to A group at  $p<0.0044$**

### Glutathione levels

Treatment of animals with MSM significantly increased the glutathione concentration compared with that of group A ( $p<0.0001$ ). While rectal administration of acetic acid significantly reduced the concentration of endogenous antioxidant glutathione as compared to the control group ( $p<0.0001$ ).

Table 1: Macroscopic score of different experimental groups

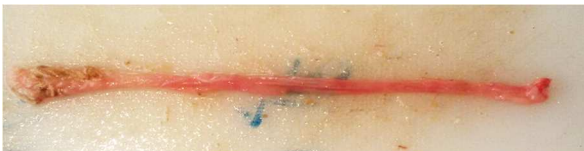
Group macroscopic score	N	A	M
0	6(100%)	-	1(16.6%)
1	-	-	3(50%)
2	-	-	1(16.6%)
3	-	-	-
4	-	1(16.6%)	-
5	-	3(50%)	-
6	-	2(33.3%)	-



A. Showing normal mucosa



B. Showing mild necrosis



C. Showing one or two ulcers

Fig. 4: Macroscopic appearances of colons, N; normal group, MSM group, C; acetic acid group

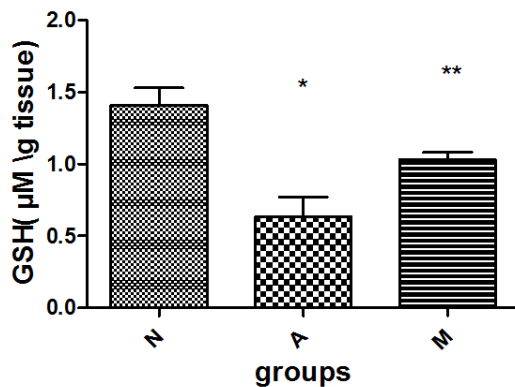


Fig. 5: The effect of MSM on GSH levels in acetic acid-induced ulcerative colitis in rats. Data are presented as means±SD (n =6). (N); normal control, (A); colitis control, (M) MSM groups, \*Significant difference as compared to normal control group at p<0.0001, \*\*Significant difference as compared to A group at p<0.0001

DISCUSSION

Induction of colitis by acetic acid in rats is one of the standardized methods to produce an experimental model of inflammatory bowel disease. Several major causative factors in the initiation of human colitis such as increased production of inflammatory mediators, enhanced vasopermeability, and prolonged neutrophils infiltration are concerned with the induction of this animal model [9-10].

Infiltration of neutrophils results in the production of cytotoxic reactive oxygen species (ROS) that are destructive on intestinal cell macromolecules, ultimately leading to mucosal disruption and ulceration [11].

In this study, the results showed that administration of acetic acid resulted in a significant infiltration of inflammatory cells in the injured colon. Acetic acid produced a large inflammatory response as evidenced by body weight loss, and increase colonic weight/length ratio that can be considered a reliable and sensitive indicator of the severity of UC. The 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. Consequently, the increased colonic weight/length ratio confirms the intensification of intestinal infiltrations and consequent intestinal oedema.

Acetic acid caused a substantial degree of tissue injury associated with deep ulceration penetrating colonic wall through mucosa till muscularis mucosa, severe inflammation and necrosis. Rats showed an increase in diarrhea with mucous and blood. Similar results were observed by Amirshahrokhi that showed redness, oedema ulcer, and necrosis in the acetic acid group. The mechanism by which acetic acid 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 [12]. Transient local ischemia might contribute to the acute injury, Mucosa and submucosal inflammation followed initial injury were associated with activation of arachidonic acid pathways [13]. Acetic acid metabolism by colonic enzymes provides superoxide anions and H<sub>2</sub>O<sub>2</sub> which contribute to its colonic toxic effects [14]. These results are in agreement with Ghatule, who showed that intra-colonic administration of acetic acid indicated a significant increase in colonic mucosal damage, necrosis and ulcerations [15].

A number of medical strategies are available, many of these have substantial side-effects including immune suppression; thus, newer approaches are greatly needed, especially from the plant's kingdom, that without side effects. So, treatment of rats with the MSM (1000 mg/kg) for 6 d, for the first study, cured the tissue damage in rat model of colitis induced by acetic acid as confirmed from its effects, as evidenced by lowered the incidence of diarrhea, improved food intake by increasing the body weight, and decrease the colonic weight/length ratio contraries the ulcerative colitis induced by acetic acid. Also, the macroscopic features in MSM group exerted upgrading the extent and severity of inflammation by treating ulceration and necrosis that has been very obviously significantly different from the acetic acid control group.

Besides the anti-inflammatory effect to the MSM, there is the antioxidant effect. Treatment with MSM in this study inverted colonic GSH depletion and restored the levels toward the normal value suggesting an antioxidant action of MSM. As many studies demonstrated that MSM acts as a free radical scavenger, which would further add to the efficiency of MSM as an antioxidant [16]. Other studies showed that sulfur, which is the main component of MSM, is an important constituent of amino acids [17], which 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 ROS [18]. Sulfur amino acids are also involved in the synthesis of intracellular antioxidants (glutathione, taurine, etc.) and in the methionine sulfoxide reductase antioxidant system [19].

This dose of MSM (1000 mg/kg) has improved its ability to cure the ulcerative colitis much better than the MSM (400 mg/kg) as has shown in previous study [20].

#### CONCLUSION

MSM (1000 mg/kg) was effective to cure experimental colitis and increased GSH that suggests a useful therapeutic activity for MSM as an anti-inflammatory and antiulcerative medicinal plant for UC. Oral administration of MSM could be considered as an alternative remedy for UC.

#### AUTHORS CONTRIBUTIONS

All the author have contributed equally

#### CONFLICT OF INTERESTS

Declare none

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