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Research Article

Beneficial Effects of MSM Treatment on the Development of Experimental Colitis Induced by Acetic Acid

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ABSTRACT
Ulcerative colitis (UC) is a chronic and relapsing inflammatory disorder of the gastrointestinal tract. A number of medical strategies are available. 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*: The groups were divided into normal control (N), acetic acid (A) control, and MSM (400mg/kg). Rats received treatment for six consecutive days after induction of colitis by intra-rectal acetic acid (2ml 4% v/v) administration. On day 7, the rats were sacrificed, colon was removed, and the macroscopic, biochemical, and histopathological 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 and histopathological parameters were decreased, but it didn't reduce significantly in the MSM treated groups compered to group A. Contrariwise the parameters of group A. *Conclusions*. The anti-inflammatory and antioxidant effects of MSM in treating the UC are due to its potential to reduce the colon weight/length ratio, and increased GSH.

Keyword: MSM, Ulcerative colitis, glutathione, macroscopic, histopathological.

INTRODUCTION

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[1]. Many properties have been attributed to MSM, such as chemopreventive properties, anti-inflammatory activities, anti-atherosclerotic action, prostacyclin (PGI2) synthesis inhibition, and free radical scavenging activity[2]. Ulcerative colitis (UC) is a 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[3]. There is evidence for an intense local immune response associated with recruitment of lymphocytes and macrophages followed by release of soluble cytokines and other inflammatory mediators[4].In addition to reactive oxygen species (ROS), which cause impairment of cellular membrane stability and cell death by leading lipid peroxidation[5]. Several studies found that excessive production of ROS in mucosal cells induced by inflammatory and immune responses could directly or indirectly cause damage of intestinal epithelial cells, subsequently influences the mucosal integrity or initiate an inflammatory signaling cascade and lead to severe impairment in experimental colitis[6]. An additional group of redox active molecules termed RSS are formed in vivo under conditions of oxidative stress[7]. A number of medical strategies are available, but the less side effects is better. The present work was conducted to assess the possible anti -inflammatory and anti- oxidative effects of MSM in treatment an animal model of colitis induced by acetic acid in rats.

MATERIAL & METHOD

Animals

Wister rats weighing (250-300g) 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 ± 2°C, humidity 55 ± 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 2ml acetic acid 4% intrarectally following the administration of saline orally; group (M) received 2 ml acetic acid intrarectally, following the administration of MSM (400 mg/kg/day, orally) suspended in normal saline, for 6 days[8].

Induction of experimental colitis in rats

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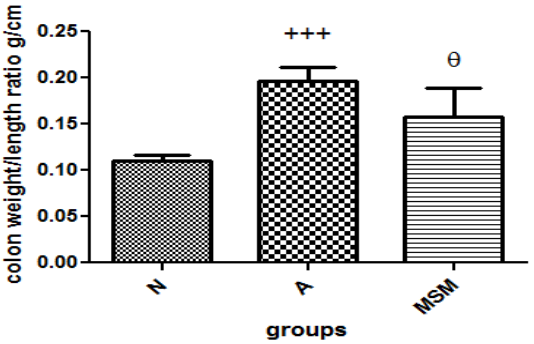


Figure 1: The effects of MSM on colon weight / length in C induced ulcerative colitis in rats. Data are presented as means ± SD (n =6). (N); normal control, (A); colitis control, (M) MSM groups. +++ Significant difference as compared to normal control group at $p<0.05$. Θ Significant difference as compared to A group at $p<0.05$.

Table 1: Macroscopic score of different experimental groups.

Group Macroscopic Score	N	A	M
0	6(100%)	-	-
1		-	1(16.6%)
2	-	-	-
3	-	-	3(50%)
4	-	-	-
5	-	2(33.3%)	-
6	-	4(66.6%)	2(33.3%)

Table 2: Histological score of different experimental groups.

Group histological score	N	C	MSM
0	6(100%)	-	-
1	-	-	1(16.6%)
2	-	-	3(50%)
3	-	2(33.3%)	2(33.3%)
4	-	4(66.6%)	-

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 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 solution spreading out[9].

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, and histological study. In addition the 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.

colon weight / length ratio

The entire colon starting from caecum was excised, longitudinally split. Washed with ice-cold saline to remove fecal residues. The length (cm) and weight (g) was measured. Each colon was gently stretched, 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[10].

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 a pathologist in blinded fashion. Histopathologically were assessed by using following score[6]**:** 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.

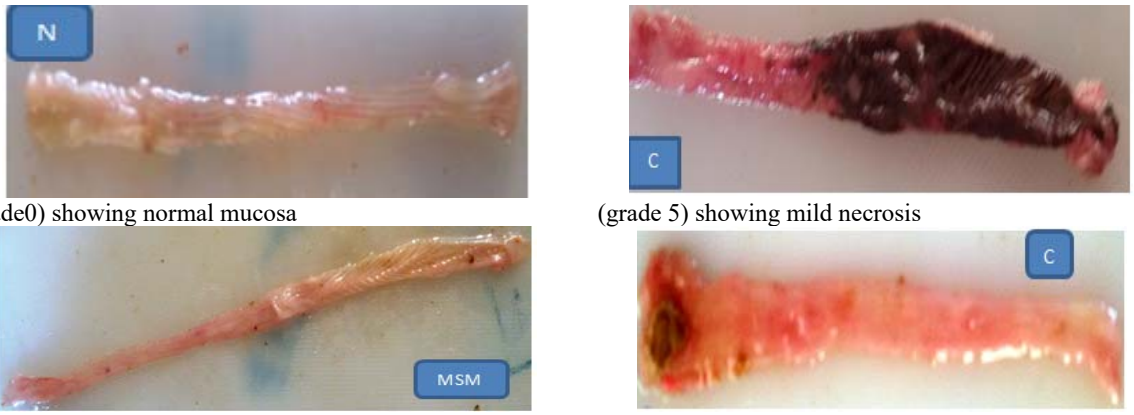
Reduced glutathione

Glutathione Assay Kit (Abnova) for direct assay of reduced glutathione in colonic tissue was used. Colonic tissue samples were frozen in liquid nitrogen, stored at −80 °C until time of assay. Colon GSH levels were determined as previously described by Blenn[11], based on the reaction of 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB) with the glutathione present to form yellow product. The optical density (OD) measured at 412 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.

Statistical analysis

Data analyses were achieved using prism (Version 5) statistical package. Data were presented as means ± standard diviation(SD)**.** 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



(grade0) showing normal mucosa (grade 5) showing mild necrosis

(grade 2) showing swelling and redness (grade 3) showing one or two ulcers

Figure 2: Macroscopic appearances of colons, N; normal group, MSM group, C; acetic acid group.

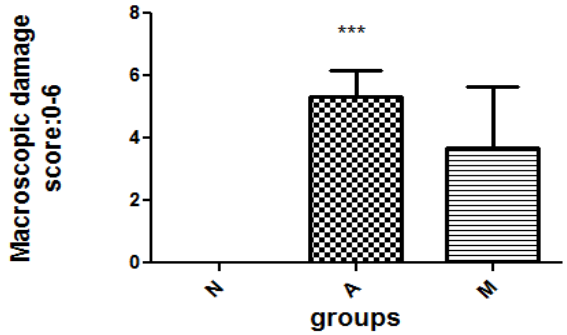


Figure 3: The effects of MSM on the macroscopic changes in Acetic Acid induced ulcerative colitis in rats. Parametric data were expressed as mean ± S.D (n =6). (N); normal control, (A); colitis control, (M) MSM groups. *** Significant difference as compared to normal control group at $p<0.05$.

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 observed in colitis animals relative to normal control. The rats in the A group showed a significant increase in the colonic weight/length ratio ($p<0.05$), compared with that of normal control group. MSM treatment exerted an intestinal anti-inflammatory effect, for 6 days, by a significant decrease in the weight/length ratio compared with that of group A ($p<0.05$).

Macroscopic scoring

After intracolonic administration of 2ml 4% acetic acid, there was a macroscopic evidence of extensive colonic mucosal injury. The mucosa appeared macroscopically ulcerated, hemorrhagic, oedematous and necrotic compared to normal control group ($p<0.05$). Thus the morphological score in the (A) group was significantly increased as compared to normal control group ($p<0.05$). Administration of MSM therapeutically alleviate the severity of the gross lesion, but the morphological score was not statistically significant as compared to (A) group ($p<0.05$).

Histological results

Infiltration of small round cells and polymorphonuclear leukocytes to lamina properia, deep ulceration of muscularis mucosa, severe inflammation and necrosis through large bowel wall were observed in acetic acid induced colitis animals.

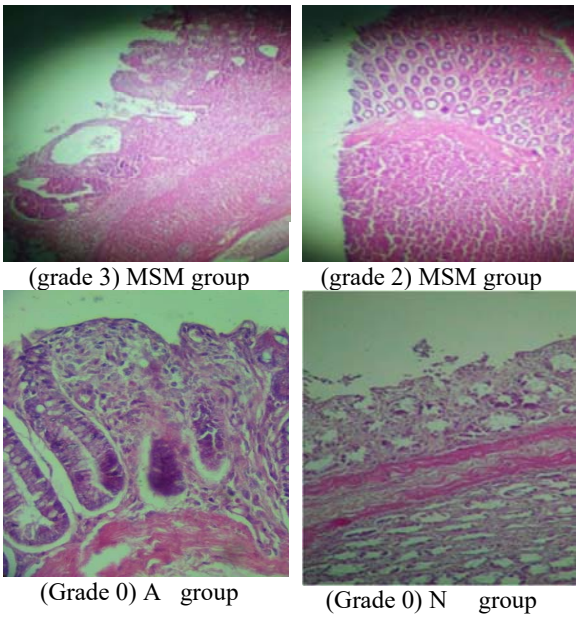
Colon sections from control group revealed normal morphology with an intact architecture of colon tissues in all animals of the normal control group (Figure 4). On the other hand, colons of A group revealed significant tissue injury with high scores of microscopic damage indicating erosion of the lining 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, the 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. A medium necrosis or ulceration of mucosa were, in administration of MSM as therapy, resulted in reduce the severity of the injury, less necrosis and overall less visible changes compared to acetic acid control group. However, the values obtained with this dose of MSM (400mg/kg) were not significantly different from the acetic acid control group.

Glutathione levels

Rectal administration of acetic acid significantly reduced the concentration of endogenous antioxidant glutathione as compared to control group. Treatment of animals with MSM significantly increased the glutathione concentration compared to acetic acid group ($p<0.05$).

DISCUSSION

Induction of colitis by acetic acid in rats is one of standardized methods to produce an experimental model of inflammatory bowel disease. Several major causative factors in the initiation of human colitis such as enhanced vasopermeability, prolonged neutrophils infiltration and increased production of inflammatory mediators are involved in the induction of this animal model[4]. Neutrophils play a crucial role in the development and full



(grade 3) MSM group (grade 2) MSM group

(Grade 0) A group (Grade 0) N group

Figure 4: Histological appearance of colonic tissue sections, original magnification×10

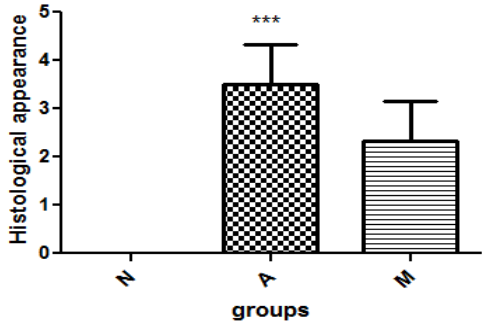


Figure 5: The effects of MSM on the histological changes in Acetic Acid induced ulcerative colitis in rats. Parametric data were expressed as mean ± S.D (n =6). (N); normal control, (A); colitis control, (M); MSM group. *** Significant difference as compared to normal control group at p<0.05.

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 remodelling of injured tissue[12]. 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[13]. 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[14]. IL-1β appears to be a primary stimulator of diarrhea the main symptom of intestinal inflammation. 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

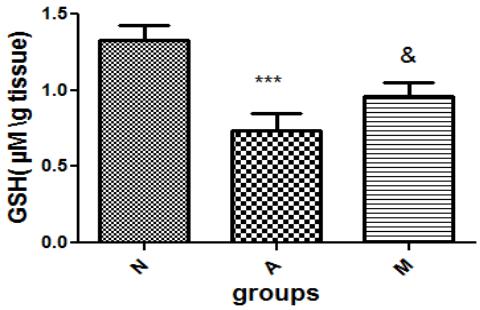


Figure 6: The effect of MSM on GSH levels in acetic acid induced ulcerative colitis in rats. Data are presented as means ± SD (n =6). (N); normal control, (A); colitis control, (M) MSM groups. *** Significant difference as compared to normal control group at p<0.05. & Significant difference as compared to colitis control group at p <0.05.

inflammatory response as evidenced by body weight loss, reduction in food intake, increase colonic weight / length ratio. 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[15]. Consequently the increased colonic weight / length ratio confirms 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 increase in diarrhea with mucous and blood. Similar results were observed by Amirshahrokhi, that showed redness, oedema, ulcer and necrosis in acetic acid group[10]. 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[15]. This could be due to direct damaging effects of acetic acid as well as alterations in epithelial function produced either directly or indirectly by products released from activated mast cells, as Madhavan et al. reports[16]. Transient local ischemia might contribute to the acute injury, Mucosa and submucosal inflammation followed initial injury was associated with activation of arachidonic acid pathways[17]. Acetic acid metabolism by colonic enzymes provides superoxide anions and H2O2 which contribute to its colonic toxic effects[18]. These results are in agreement with Ghatule, who showed that intra-colonic administration of acetic acid indicated significant increase in colonic mucosal damage, necrosis and ulcerations[19]. 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 plants kingdom, that without side effects. So treatment of rats with the MSM (400mg/kg) for 6 days, for the first study, 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 present study revealed the development of acetic acid-induced colitis, in relation to macroscopic and histological features of inflammed tissue which are used to quantify the severity of inflammation. 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. Beside the anti-inflammatory effect to the MSM, there is the antioxidant effect. Treatment with MSM in this study reversed 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 free radical scavenger, which would further add to the efficiency of MSM as an antioxidant[20]. Also, MSM has been reported to increase antioxidant defense (glutathione), as well as decrease the actual production of ROS. As with pro-inflammatory biomarkers, MSM resulted in a lowering of multiple oxidative stress biomarkers[21]. Other studies showed that sulfur, which is the main component of MSM, is an important constituent of amino acid(s)[22], 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[23]. Sulfur amino acids are also involved in the synthesis of intracellular antioxidants (glutathione, taurine etc.) and in the methionine sulfoxide reductase antioxidant system[24].

CONCLUSION

Although the present study is the first report on MSM (400mg/kg) tretment for 6 days, it had some limitations, which need to be addressed in ongoing investigations. MSM was effective to ameliorate experimental colitis, and increased GSH, that suggest 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. More studies are strongly recommended to determine the immune mechanism, and study more doses with longer duration.

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