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Research Article

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The protective effect of methylsulfonylmethane on an experimental model of ulcerative colitis in rats

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Abstract Methylsulfonylmethane (MSM) is an organic sulfur-containing natural compound without any toxicity. MSM may have anti-inflammatory and free radical scavenging activity. The present work was done to investigate the possible protective effects of methylsulfonylmethane on acetic acid-induced colitis in rats. Colitis was induced by intracolonic injection of 5% acetic acid. Several parameters including macroscopic score, histopathological and biochemical parameters such as myeloperoxidase activity and glutathione levels were measured following standard assay procedures. Results showed that MSM decreased macroscopic and microscopic colonic damage scores caused by administration of acetic acid. MSM also significantly reduced colonic levels of MPO and increased the levels of GSH compared to acetic acid-induced colitis group. It seems that MSM as a natural product was able to give a nearly complete protection against acetic acid-induced colitis**.**

Keywords methylsulfonylmethane, ulcerative colitis, anti-inflammatory, antioxidant

1. Introduction

Ulcerative colitis is an inflammatory disease of unknown cause, affecting principally the mucosa of the rectum and left colon that exhibits an unpredictable clinical course with remissions and exacerbation [1], characterized by rectal bleeding, diarrhea abdominal pain and cramps, weight loss, cachexia, disrupted digestion and a substantial personal burden [2].

The exact etiology of ulcerative colitis has not been clarified but the current concept of pathogenesis involves the interactions between environmental agents, genetic influences and immunologic abnormalities [3]. Although it is suggested that ulcerative colitis might be an autoimmune disease, potential enteric antigens for the exacerbation of inflammatory bowel disease are luminal bacteria, parasitic nematodes or food allergens [3].

Prolonged or inadequate activation of the intestinal immune system plays an important role in the pathophysiology of chronic mucosal inflammation. Furthermore, infiltration of neutrophils, macrophages, lymphocytes and mast cells, ultimately giving rise to mucosal disruption and ulceration. The infiltrated and activated neutrophils represent an important source of cytokines [4], proteases and lipid mediators that can contribute to intestinal injury, in addition to reactive oxygen (ROS) and nitrogen species (RNS) which are cytotoxic agents, inducing cellular oxidative stress by cross-linking proteins, lipids, and nucleic acids, causing cellular dysfunction and damage [5].

Normally, most tissues possess sufficient amounts of protective enzymatic [SOD (superoxide dismutase), catalase, GSH peroxidase] and nonenzymatic (thiols, ascorbate, α -tocopherol) antioxidants that will decompose most of the injurious oxidizing agents that escape into the surrounding environment thereby limiting "bystander" tissue damage.

However, the uncontrolled overproduction of ROS, as would occur during active episodes of UC, could easily overwhelm these protective mechanisms resulting in oxidative damage to cells and tissue [6].

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 [7].

In the present study, we have focused on MSM (methylsulfonylmethane) as a prophylactic agent of ulcerative colitis induced experimentally in rats by acetic acid 5%.

Methylsulfonylmethane (MSM) is an organic sulfur-containing natural compound without any toxicity. MSM can be found in small quantities in a variety of foods, such as milk, fruits and vegetables [8].

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. In murine models, MSM was shown to effect inflammatory conditions such as rheumatoid arthritis and lupus [9].

2. Materials and Methods

2.1. Animals

Adult male Wistar rats weighing 220–250 g, were placed in cages with wire–net floors in a controlled room (temperature 24–25°, humidity 70–75%, lighting regimen of 12-h light: 12-h dark) and were fed a normal laboratory diet. Rats were deprived of food for 24 h prior to the induction of colitis, but were allowed free access to tap water throughout.

The experimental animals were divided into three groups each consisting of seven animals. Group (1) served as normal control and received 1 ml saline intrarectally following the administration of saline orally.; group(2) served as colitis control and received 1 ml acetic acid5% intrarectally following the administration of saline orally ; group (3) received 1 ml acetic acid intrarectally following the administration of MSM (400 mg/kg/day, orally).

2.2. Induction of colitis

To investigate the etiology of UC, animal models of experimental colitis have been developed and used to evaluate anti-inflammatory and antioxidant effects of MSM.

Rats were slightly anesthetized with ether and a rubber catheter was inserted into the rectum such that the tip was 8 cm inside the anus. 1 ml of 5%acetic acid in 0.9nacl was applicated intracolonic.

On the fourth day after the induction of colitis, rats were euthanized and the distal 8 cm of the colon was resected for macroscopic scoring, histopathological examination and biochemical studies. After macroscopic examination, the colonic sample from each rat cut into two parts, one for the histological examination and the other for the biochemical analysis.

3. Assessment of colitis

3.1. Macroscopic scoring

After resection of the distal colon, the specimen was flushed out with cold saline solution and opened by a longitudinal incision and fixed in 10% buffered formalin for further histological examination. 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 [10].

3.2. Histopathological study

After formalin fixation (10% during 24 hours), then 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 5microns 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.

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.

3.3. Biochemical assays

Colonic tissue samples were frozen in liquid nitrogen and stored at −80 °C until time of assay [11].

3.3.1. Measurement of glutathione levels:

Glutathione was determined as described by Akerboom and Nair [12,13].

The measurement of GSH uses a kinetic assay in which catalytic amounts (nmoles) of GSH cause a continuous reduction of 5-5' -dithiobis(2-nitrobenzoic acid) (DTNB) to TNB and the GSSG formed is recycled by glutathione reductase and NADPH. The GSSG present will also react to give a positive value in this reaction. The reaction rate is proportional to the concentration of glutathione up to 2 mM. The yellow product, 5-thio- 2-nitrobenzoic acid (TNB) is measured spectrophotometrically at 412 nm. The assay uses a standard curve of reduced glutathione to determine the amount of glutathione in the biological sample.

3.3.2. Determination of myeloperoxidase activity:

MPO activity had been used as index of leukocyte adhesion and accumulation in several tissues including the intestine. Myeloperoxidase (MPO) activity was measured in the colonic mucosa, according to method as described by Roelofs [14]:

MPO measured by using rat MPO ELISA kit. The rat MPO ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a wrorking time of 3½ hours.

4. Statistical Analysis

The results were expressed as the means+ standard error of mean (S.E.M.). Statistical significances were assessed by Turkeys' test following one-way analysis of variance (ANOVA). Lesion score and histological score were expressed as medians and compared using Krustal–Wallis nonparametric ANOVA followed by Dunn's multiple comparison tests. Differences with a P value less than 0.001 ($P<0.001$) were considered significant.

5. Results

5.1. Macroscopic Results

Table (1) shows that 6 /7 of colitis group (85.7%) belong to grade 5 and 14.3% to grade 6.We noticed that 4 rats which received MSM (57.14%) belong to grade 1 and 42.85% to grade 2.

Table 1

Macroscopic score	Number of cases		
	Normal group	Colitis group	Colitis+ MSM group
0	7	0	0
1	0	0	4
2	0	0	3
3	0	0	0
4	0	0	0
5	0	6	0
6	0	1	0

Administration of 1 ml 5% acetic acid caused macroscopic evidence of extensive colonic mucosal injury. The mucosa appeared macroscopically ulcerated, hemorrhagic, oedematous and necrotic compared to normal control group ($p<0.001$).

Administration of MSM prevented the development of acetic acid-induced ulcerative colitis.

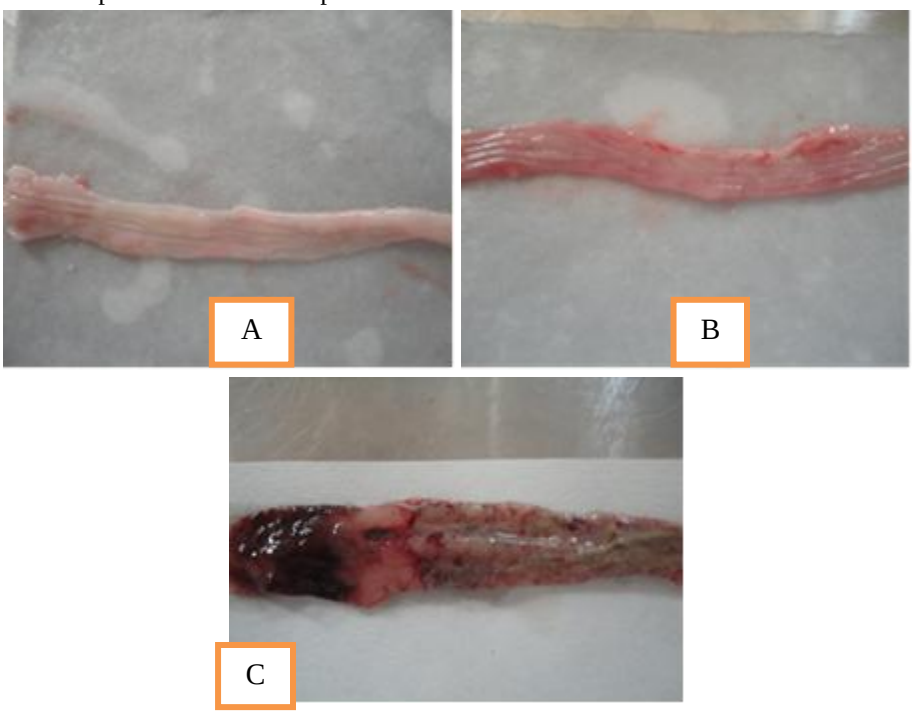

Figure 1: A: normal group (grade 0); B: MSM group (grade 1); C: colitis group (grade 6)

There was a significant protection from ulceration and necrosis in the group which had received MSM compared to acetic acid group. Administration of MSM significantly reduced the severity of inflammation compared to acetic acid group.

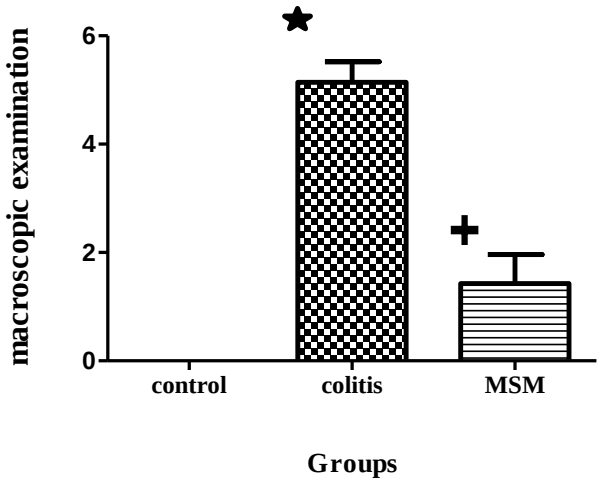

Figure 2: The macroscopic grading for the three groups tested. Statistically significant difference between control group and colitis group was observed. Also a statistically significant difference between colitis group and MSM group was noticed at the level of P < 0.001.

★ P<0.001 as compared to saline control, ✚ P<0.001 as compared to colitis control.

5.2. Histological Results

Table (2) shows that 6 /7 (85.7%) of colitis group belong to grade 3 and 14.3% to grade 4.We noticed that 7/7 (100%) of rats which received MSM belong to grade1.

Table 2

Histological grade	Number of cases		
	Normal group	Colitis group	Colitis+ MSM group
0	7	0	0
1	0	0	7
2	0	0	0
3	0	6	0
4	0	1	0

In our study, 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. Infiltration of inflammatory cells was slightly observed in the colonic mucosa of MSM group, but there was no necrosis or ulceration of mucosa.

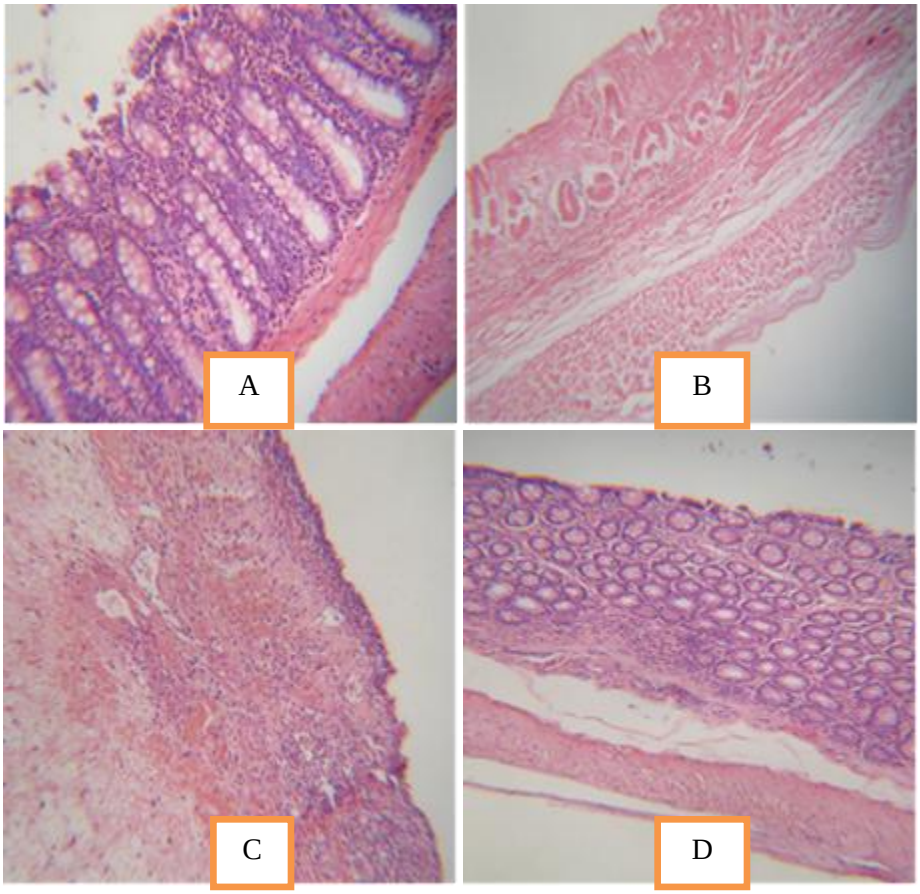

Figure 3: No histological alteration was observed in the colon section from normal group (A). Mucosal injury was produced after acetic acid administration characterized by necrosis through large bowel wall (B), deep ulceration till muscularis mucosa with severe inflammation(C). Pretreatment with MSM (400 mg/kg day PO) corrected the disturbances in morphology associated with acetic acid (D).

So administration of MSM significantly reduced the severe inflammation, ulceration and necrosis compared to acetic acid group.

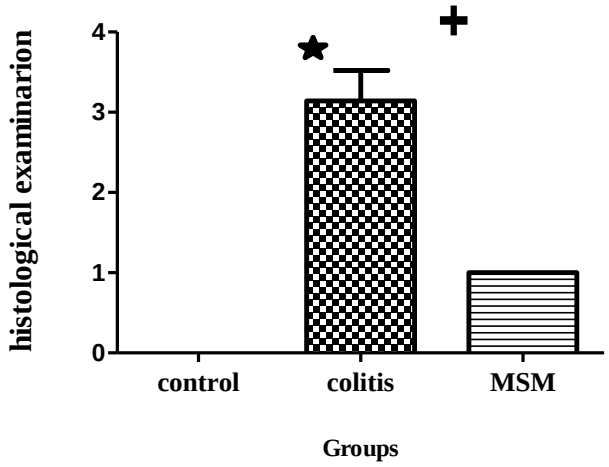

Figure 4: Effect of MSM on histological score of damage at the fourth day after acetic acid administration. Pretreatment with MSM (400mg/kg/day, PO) significantly decreased the histological score. Statistically significant difference among groups at the level of P < 0.001was observe.

★ P<0.001 as compared to saline control. ✚ P<0.001 as compared to colitis control.

5.3. Glutathione levels

Rectal administration of acetic acid significantly reduced the concentration of endogenous antioxidant glutathione as compared to control group. Pretreatment of animals with MSM significantly increased the glutathione concentration compared to acetic acid group

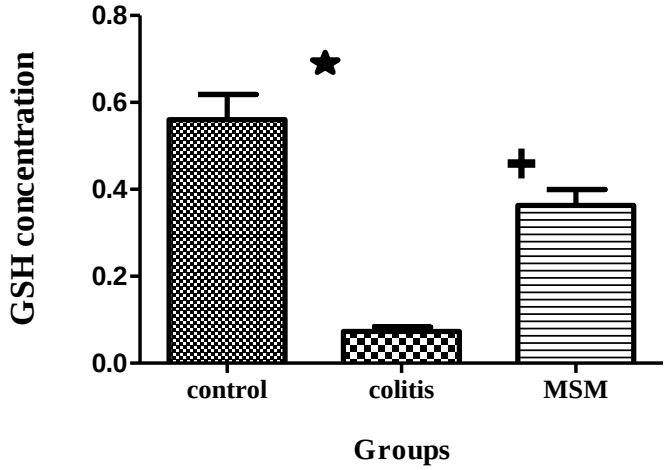

Figure 5: Effects of MSM on colonic glutathione (GSH) content in acetic acid induced ulcerative colitis in rats. MSM significantly increased GSH levels of the colonic tissues as compared to those in the colitis group. Values are means± SD. Statistically significant difference among groups at the level of P < 0.001was observed.

★ P< 0.001 as compared to control group. ✚ P< 0.001 as compared to colitis group.

5.4. Myeloperoxidase activity

To study the anti-inflammatory and antioxidant activity of MSM in acetic acid-induced colitis, the levels of MPO in colonic tissues were measured.

Tissue MPO levels significantly increased (P<0.001) following intrarectal administration of acetic acid. Pretreatment with MSM significantly reduced MPO activity compared to those in the colitis group.

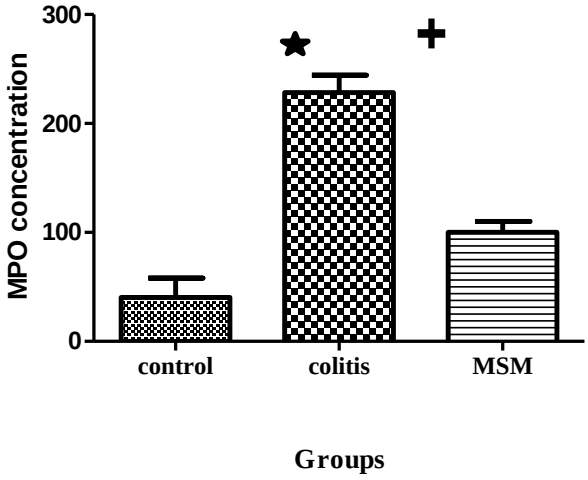

Figure 6: Effects of MSM on the levels of myeloperoxidase (MPO) activity of colonic tissue. MSM 400 mg/kg given orally reduced the levels of MPO in the colon of rats with acetic acid-induced colitis. Values are means ±SD, statistically significant difference among groups at the level of P < 0.001was observed.

★ P< 0.001 compared to normal group. ✚ P<0.001 compared to colitis group.

6. Discussion

Various experimental models of colitis mimicking the active phase of the disease have been developed to test the potential beneficial effects of various drugs. One of the more commonly employed models is acetic acid induced colitis in the rat [15].

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, increased production of inflammatory mediators and reactive oxygen metabolites are involved in the induction of this animal model [11].

Our results demonstrated that acetic acid caused a substantial degree of tissue injury associated with an infiltration of polymorphonuclear cells, deep ulceration penetrating colonic wall through mucosa till muscularis mucosa, severe inflammation and necrosis.

Mahgoub showed that intracolonic administration of acetic acid resulted in submucosal oedema, hemorrhage and inflammatory cell infiltration [15]. Also Mustafa found that acetic acid caused mucosal ulceration and heavy neutrophil infiltration of the mucosa and submucosa [5].

In our study, a significant decrease in the glutathione level was observed in acetic acid group compared to control group. Glutathione is a tripeptide - thiol, one of the most prominent low molecular weight thiol found in mammals [16]. GSH is the most powerful intracellular antioxidant and plays a role in the detoxification of reactive oxygen species (ROS) and drug metabolites via catalysis by glutathione-S-transferases (GST) and glutathione peroxidases (GPx). As a consequence, the ratio of reduced and oxidized glutathione (GSH: GSSG) serves as a representative marker of the antioxidative capacity of the cell [17].

Many studies demonstrated the depletion of glutathione is considered a crucial event of colonic damage occurring both in human ulcerative colitis and in animal respectively.[18]

Administration of acetic acid caused a significant elevation of colonic levels of myeloperoxidase (MPO) compared to those in the control group.

MPO is a membrane-bound enzyme found almost exclusively in neutrophils and to a lesser extent in monocytes, its level may provide an estimate of neutrophil infiltration in tissues. Our findings, therefore, indicated a dramatic increase in neutrophil infiltration into the intestinal mucosa after inflammation induction. The neutrophils may contribute to the production of reactive oxygen metabolites via activation of their NADPH oxidase and secretion of myeloperoxidase into extracellular space [19].

We noticed that colonic MPO activity, an index of neutrophil activation and inflammation was decreased in MSM-pretreated animals.

Reduction of MPO activity in the colonic mucosa as well as macroscopic and histological findings of the absence of inflammatory cellular infiltration following the administration of MSM may indicate its antioxidant and anti-inflammatory effects in the prevention of acetic acid induced colitis

Because MSM is a source of sulfur and provides organic sulfur to the synthesis of glutathione which plays a key role in controlling the redox state of the cell through several mechanisms, including scavenging of ROS and keeping the enzyme GSH peroxidase in a reduced state [11], Pretreatment with MSM in this study protected against colonic GSH depletion and restored the levels toward the normal value suggesting an antioxidant action.

Similar results were observed by Amirshahrokhi who used the same model of colitis to test the anti –inflammatory and antioxidant potential of MSM.

Many studies have demonstrated that MSM inhibits the translocation of the p65 subunit of nuclear factor (NF)-kß to the nucleus, thus minimizing downstream events associated with local and systemic inflammation. Indeed, MSM may minimize the expression of pro-inflammatory cytokines. MSM has been reported to increase antioxidant defense (glutathione), as well as decrease the actual production of reactive oxygen species (ROS). As with pro-inflammatory biomarkers, MSM resulted in a lowering of multiple oxidative stress biomarkers [8].

Another studies showed that sulfur is an important constituent of amino acid(s) [20], 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 reactive oxygen species [21].

Reactive species have their origin in enzymatic synthesis, environmental induction, or by the further chemical reaction of an active species with other endogenous molecules to generate a second-generation reactive species. These second-generation species possess a different spectrum of activity to the parent species, with different redox reactions and biological targets. We now propose that an additional group of redox active molecules termed “reactive sulfur species” (RSS) are formed in vivo under conditions of oxidative stress. RSS are likely to include disulfide-S-oxides, sulfenic acids, and thiyl radicals, and are predicted to modulate the redox status of biological thiols and disulfides [22]. Also sulfur amino acids are involved in the synthesis of intracellular antioxidants (glutathione, taurine etc.) and in the methionine sulfoxide reductase antioxidant system [23].

7. Conclusion

Our results suggested that 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.

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