

**METHYLSULFONYLMETHANE AND GREEN TEA EXTRACT REDUCED OXIDATIVE STRESS AND INFLAMMATION IN AN ULCERATIVE COLITIS.**VALANTINA AL BITAR<sup>1\*</sup>, DR SHAZA LAHAM<sup>2</sup>

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*Received: 17 February 2013, Revised and Accepted: 15 March 2013***ABSTRACT**

Ulcerative colitis is characterized by oxidative stress, leukocyte infiltration and upregulation of proinflammatory cytokines. The purpose of the study was to examine the protective effects of methylsulfonylmethane and green tea extract on acetic acid induced colitis in rats, a model for inflammatory bowel disease. Methylsulfonylmethane is an organosulfur compound, naturally occurring in green plants and it has anti-inflammatory and antioxidant effects. Green tea extract contains polyphenolic compounds especially catechins which have anti-inflammatory and antioxidant effects by removing free radicals and increasing intracellular antioxidant defense. Colitis was induced by intracolonic instillation of 1 ml of 5% of acetic acid. On the fourth day after the induction of colitis, the distal colon was resected for macroscopic score, histopathological and biochemical such as myeloperoxidase and glutathione levels. Our results showed that methylsulfonylmethane, green tea extract and their combination decreased macroscopic and microscopic colonic damage scores caused by administration of acetic acid, also significantly reduced colonic levels of myeloperoxidase while increased the levels of glutathione compared to acetic acid-induced colitis group. Conclusion -It seems that methylsulfonylmethane as a natural product has a better protective effect on experimental ulcerative colitis compared with green tea extract, or the combination between them.

**Keywords:** Ulcerative colitis, methylsulfonylmethane, green tea extract, anti-inflammatory, antioxidant.**INTRODUCTION**

UC is a disorder involving the large intestine characterized by contiguous inflammation of the colonic lamina propria with subsequent injury and disruption of the mucosal barrier<sup>1</sup>. The cause of UC is still unknown, but several factors have been implicated. These include environmental factors, genetic factors, microbial pathogens, altered levels of inflammatory mediator and defects in immunoregulation.<sup>2</sup>

Activated neutrophils and macrophages are major components of active lesions in UC. Large numbers of neutrophils and macrophages pass out of the circulation and enter the inflamed mucosa and submucosa of the large intestine during acute inflammation, leading to overproduction of ROS<sup>3</sup> via activation of the phagocyte-associated, ROS-producing NADPH oxidase. Normally, most tissues possess sufficient amounts of protective enzymatic (SOD, catalase, GSH peroxidase) and nonenzymatic (thiols, ascorbate,  $\alpha$ -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.<sup>4</sup>

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.<sup>5</sup>

In addition to ROS, RNS also play important role in the pathophysiology associated with this model of inflammation. The two most extensively studied ROS and RNS, O<sub>2</sub><sup>-</sup> and NO, are known to exert profound, and often opposing, effects in inflamed tissues. These actions of O<sub>2</sub><sup>-</sup> and NO can be manifested as impaired endothelium-dependent vasodilatation, activation of nuclear transcription factors, and the subsequent production of inflammatory cytokines, enhanced recruitment and activation of leukocytes, accelerated apoptosis, and parenchymal cell necrosis.<sup>6</sup> Neutrophils can also release proteases and lipid mediators that can contribute to intestinal injury.<sup>7</sup> Macrophages produce certain cytokines, such as TNF and IL-1, the levels of which are often increased in both animal models and patients with UC.<sup>8</sup>

The present work was conducted to assess the possible anti-inflammatory and anti-oxidative effects of green tea extract, MSM and the combination between them in an animal model of colitis induced by 5% acetic acid in rats.

Green tea (*Camellia sinensis*)<sup>9</sup> contains several antioxidants, including polyphenols of the catechin.<sup>10</sup>

The main catechins in green tea are EGCG, epicatechin-3 gallate, epigallocatechin, and epicatechin. All green tea catechins appear to have antioxidant capacity and EGCG seems to be the principle.<sup>11</sup>

Catechins have been reported to have many pharmacological properties such as effects of antioxidative, antimutagenic, antitumor, hypotensive and antiulcer, virus inactivation, reduction of blood glucose levels and induction of apoptosis.<sup>12</sup>

MSM is a naturally occurring organosulfur molecule and a putative methyl donor. It has anti-inflammatory activities, chemopreventive properties, prostacyclin (PGI<sub>2</sub>) synthesis inhibition, anti-atherosclerotic action, salutary effect on eicosanoid metabolism, and free radical scavenging activity.<sup>13</sup>

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<sup>14</sup>

**MATERIALS AND METHODS****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.

**Induction of colitis**

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 applied intracolonic.<sup>15</sup>

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. The experimental animals were divided into six 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 acid 5% 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) for 4 days; group (4) received 1 ml acetic acid 5% intrarectally following the administration of green tea extract (100 mg/kg/day, orally) for 10 days; group( 5) received 1 ml saline intrarectally following the administration of MS (400 mg/kg/day, orally) & green tea extract (100 mg/kg/day, orally); group( 6) received 1 ml acetic acid 5% intrarectally following the administration of MSM(400 mg/kg/day, orally) & green tea extract (100 mg/kg/day, orally).

#### Assessment of colitis

##### 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.<sup>15</sup>

##### 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 °C 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.

**All groups were histopathologically assessed by using following score.<sup>5</sup>**

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.

##### Biochemical assays

Colonic tissue samples were frozen in liquid nitrogen and stored at -80 °C until time of assay.<sup>7</sup>

##### Measurement of glutathione levels

Glutathione was determined as described by Akerboom and Nair.<sup>16-17</sup> This measurement of GSH uses a kinetic assay in which catalytic amounts (nmoles) of GSH cause a continuous reduction of 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, 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.

##### Determination of MPO activity

MPO activity was measured in the colonic mucosa, according to method as described by Roelofs.<sup>18</sup>

MPO was 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 working time of 3½ hours.

##### Statistical analysis

The results were expressed as the means+ standard error of mean (S.E.M.). Statistical significances were assessed by Turkey's 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 test. Differences with a P value less than 0.001 (P<0.001) were considered significant.

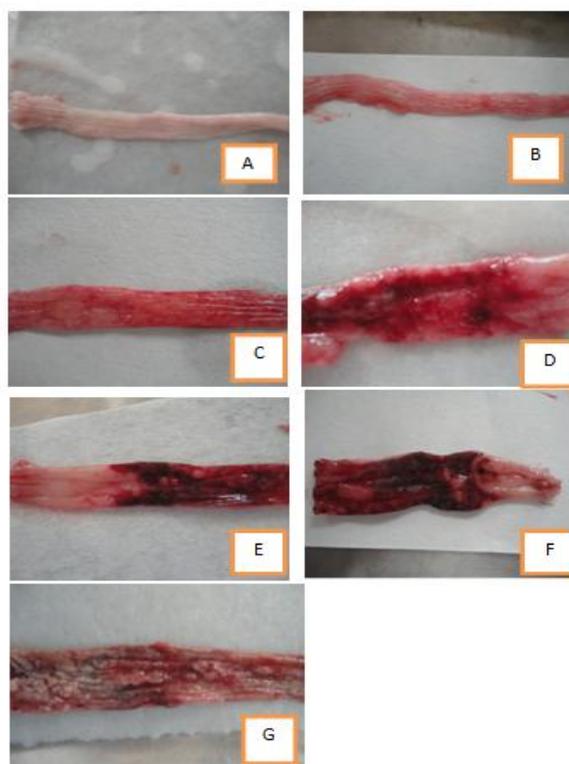
## RESULTS

### Macroscopic results

On the fourth day after intracolonic administration of 1 ml 5% 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<0.001).

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.

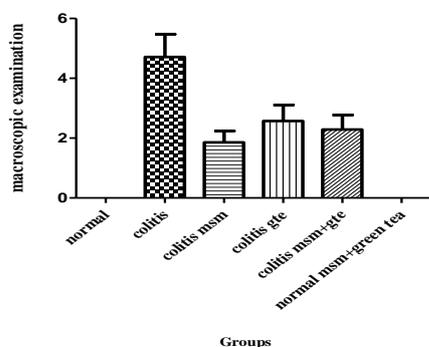
Macroscopic evaluation of the distal colon after green tea extract pretreatment revealed the presence of swelling and redness, but significantly reduced the severe ulceration and necrosis. Although the combination of MSM with green tea extract revealed significantly protection from ulceration and necrosis, but in comparison to MSM group and green tea extract group, we noticed that MSM has superior effect in protection of inflammation and intestinal injury than MSM & green tea group and green tea group.



**Fig.1. A, normal group (grade 0); B, colitis MSM group (grade 1); C, colitis & combination group (grade 2); D, colitis green tea extract group (grade 3); E, acetic acid group (grade 4); F, acetic acid group (grade 5) and G, acetic acid group (grade 6).**

Acetic acid administration resulted in ulceration and necrosis in colonic mucosa.

MSM, green tea extract and their combination significantly reduced the intestine injury.

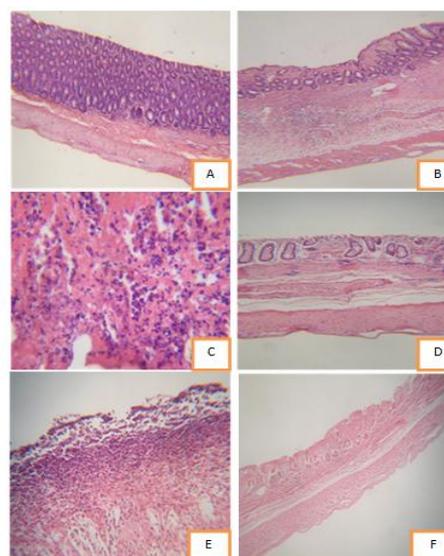


**Figure 2:** Effects of MSM, (gte) green tea extract and combination of MSM with green tea extract on macroscopic score of damage in each study group at the fourth day after acetic acid administration.

★ P<0.001 as compared to normal group, ● P<0.001 as compared to colitis group

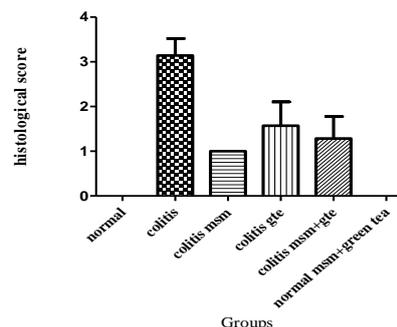
**Histological results**

In our study, infiltration of small round cells and polymorphonuclear leukocytes to lamina propria, 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. So administration of MSM significantly reduced the severe inflammation, ulceration and necrosis compared to acetic acid group. Green tea extract pretreatment resulted in a significant reduce in the severity of the injury of the large intestine compared to acetic acid group. Green tea extract group appeared infiltration in lamina propria and focal superficial ulceration of the mucose only, but it significantly reduced the severe inflammation, deep ulceration and necrosis compared to acetic acid group. (Green tea & MSM) group appeared significantly reduction of ulceration and severe inflammation compared to acetic acid group, but in comparison to MSM group and green tea extract group, we noticed that MSM has a higher suppressive effect of inflammation and ulceration than green tea extract and combination between them.



**Figure 3:** Histological appearance of colonic tissue sections in A, normal group (grade 0); B, colitis MSM group (grade 1); C, colitis & combination group (grade 2); D, colitis green tea extract group (grade 2); E, colitis group (grade 3) and F, colitis group (grade 4).

Pretreatment with MSM, green tea extract and their combination reduced the histological alterations caused by acetic acid administration, however MSM was the best.

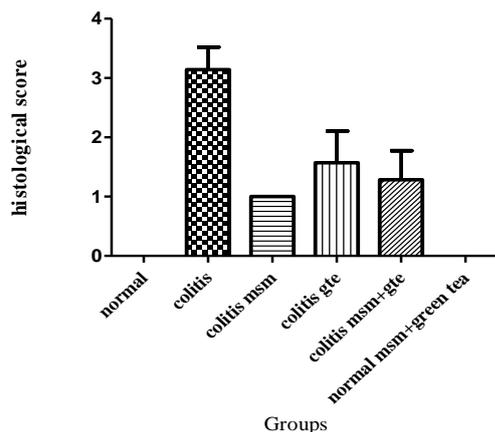


**Figure 4:** Effects of MSM, green tea extract and combination of MSM with green tea extract on histological score of damage in each study group at the fourth day after acetic acid administration.

★ P<0.001 as compared to normal group, ● P<0.001 as compared to colitis group

**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. A significant increase in the glutathione concentration was observed in green tea extract group compared to acetic acid group. Also the mucosal GSH concentration was significantly increased in MSM & green tea extract group as compared to acetic acid group. The increase was substantially higher with MSM alone than green tea extract alone and the combination of green tea extract with MSM.



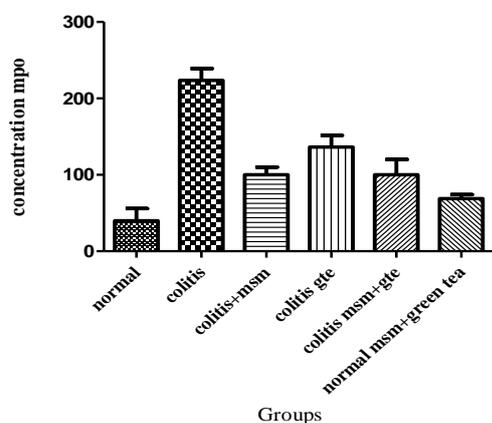
**Figure 5: Effects of MSM, green tea extract and the combination of green tea extract with MSM on colonic GSH content in acetic acid induced ulcerative colitis in rats. Values are means ± SD.**

★  $P < 0.001$  as compared to normal group, ■  $P < 0.001$  as compared to colitis group

#### MPO activity

To study the anti-inflammatory and antioxidant activity of MSM and green tea extract 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 of rats with MSM caused a significant reduction in MPO level as compared to acetic acid group. Green tea extract inhibited acetic acid-induced increase in MPO levels. MPO activity was also significantly inhibited by the combination of green tea extract with MSM. The decrease was substantially lower with MSM alone than green tea extract alone and the combination of green tea extract with MSM.



**Figure 6: Effects of MSM, green tea extract and the combination of green tea extract with MSM on the levels of myeloperoxidase (MPO) activity in the colon of rats with acetic acid-induced colitis. Values are means ± SD. ★  $P < 0.001$  compared to normal group, ■  $P < 0.001$  compared to colitis group.**

#### 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<sup>19</sup>

and reactive oxygen metabolites generated by oxidative metabolism are involved in the induction of this animal model.<sup>20</sup>

In our study, acetic acid administration 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.

Our results showed that acetic acid-induced ulcerative colitis was associated with macroscopic, microscopic and biochemical changes compared to control group.

A significant decrease in the glutathione level was observed in acetic acid group compared to control group.

The depletion of glutathione is considered a crucial event of colonic damage occurring both in human UC and in animal. It could be a consequence of enhanced production of free radicals and/or could represent a specific disorder due to an impaired activity of GSH synthesizing enzyme.<sup>21</sup>

Glutathione is a tripeptide - thiol, one of the most prominent low molecular weight thiol found in mammals. GSH is the most powerful intracellular antioxidant and plays a role in the detoxification of reactive oxygen species (ROS) and drug metabolites<sup>22</sup> 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.<sup>23</sup>

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

MPO is a biochemical marker of neutrophil infiltration in the damaged tissue. It is an abundant heme enzyme released from storage granules following activation of neutrophils by inflammatory stimuli that catalyzes the formation of a number of reactive species.<sup>24</sup>

In our experiment, the colonic MPO activity, an index of neutrophil activation and inflammation was decreased in MSM-pretreated animals, therefore, reduction in the activity of this enzyme can be display the anti-inflammatory effects of MSM.

Pretreatment with MSM in this study protected against colonic GSH depletion and restored the levels toward the normal value suggesting an antioxidant action of MSM, because it provides organic sulfur to the synthesis of glutathione.<sup>25</sup>

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)- $\kappa$ B 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 ROS. As with pro-inflammatory biomarkers, MSM resulted in a lowering of multiple oxidative stress biomarkers.<sup>26</sup>

Other studies showed that sulfur is an important constituent of amino acid(s),<sup>27</sup> 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.<sup>28</sup>

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. An additional group of redox active molecules termed 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.<sup>29</sup>

Sulfur amino acids are also involved in the synthesis of intracellular antioxidants (glutathione, taurine etc.) and in the methionine sulfoxide reductase antioxidant system.<sup>30</sup>

This study chose green tea extract as a protective agent to examine its benefits in rat colitis. Our results showed that the macroscopic and histological scores of rats in green tea extract-pretreated group

were significantly improved compared to that in the acetic acid group.

Our study demonstrated that green tea extract prevented tissue damage in rat model of colitis induced by acetic acid as verified from its effects on biochemical changes. MPO activity was decreased significantly in green tea group; these results are in agreement with Miyako Mochizuki who showed that EGCG significantly inhibited MPO activity in TNBS-induced colitis in rats.<sup>31</sup>

A significantly increased in GSH was observed in green tea group compared to acetic acid group.

According to that green tea extract pretreatment of rats with acetic acid-induced colitis can reduce the extent of colonic mucosa injury by its antioxidant and anti-inflammatory effects.

Previous study showed that green tea extract may exert a protective effect on the gastrointestinal mucosa and may prevent atrophy of the intestinal mucosa and promote healing of mucosal damage.

Green tea also contains catechins which act as antioxidants and free radicals scavengers. Besides acting as a scavenger for reactive oxygen and nitrogen species, tea also enhances expression of intracellular endogenous antioxidants such as glutathione, glutathione reductase, glutathione peroxidase, glutathione-S-reductase, catalase, and quinone reductase.<sup>32</sup>

Several studies demonstrated that Catechins chelate metal ions such as copper and iron to form inactive complexes and prevent the generation of potentially damaging free radicals.<sup>33</sup>

In our study, the activity of MPO in the colonic mucosa was decreased significantly in green tea & MSM group, as well as a GSH activity increased significantly in this group compared to acetic acid group. The combination of MSM with green tea extract also attenuated both macroscopic and microscopic changes compared to acetic acid group.

The combination of MSM and green tea extract showed less beneficial effect than MSM group and more beneficial than green tea extract group.

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.

The combination between MSM and green tea extract attenuated acetic acid induced colitis in rats as evidenced by their ability to prevent GSH depletion and reduce MPO activity.

## CONCLUSION

Both MSM and green tea extract act by reducing the oxidant load at inflammatory site and improved UC in both alone and combination between them, however MSM alone showed more beneficial.

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