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Potential Anti-inflammatory Effect of Statin on Inflammatory Colitis Induced in Experimental Animals

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ABSTRACT

Ulcerative colitis is a type of inflammatory bowel disease that is chronic, recurring, and remitting. Although the cause of colitis is uncertain, many studies have associated it with changes in immunity, genetics, and environmental factors. The increased proportion of unwanted effects, together with the current treatment's poor therapeutic effectiveness, suggests that new medications with fewer side effects and high effectiveness are needed. Efforts have accordingly been increased towards developing treatments by applying the strategy of " Drug Repurposing " through using existing drugs such as Atorvastatin for treatment of this disease outside the scope of original medical indication.

1. INTRODUCTION

Inflammatory bowel disease (IBD) refers to a group of disorders defined by a tendency for chronic relapsing-remitting inflammatory illness of the large intestine [1].

Inflammatory bowel disease (IBD) is a chronic inflammatory bowel illness characterized by colon tissue ulceration, increased colon epithelial permeability, and significant leukocyte infiltration [2].

Crohn's disease (CD) and ulcerative colitis (UC), the two most common kinds of IBD, are histologically and clinically distinct [3].

Inflammation in the colon is the most common symptom of ulcerative colitis, whereas, with Crohn's disease, inflammation can affect any portion of the gastrointestinal tract (GIT), from the mouth to the anus. UC is a chronic inflammatory bowel disease (IBD) characterized by rectum ulcers and inflammation that can extend to the cecum. UC is a chronic inflammatory bowel disease (IBD) distinguished by inflammation and ulceration of the rectum, which can extend to the cecum. Ulcerative pro colitis is a disease that affects only the most distal region of the colon and the rectum; limited or distal colitis is a disease that affects the entire colon, and pancolitis is a disease that affects the entire colon [4].

The goal of treatment for UC patients is to induce and sustain long-term remission because the disease is usually relapsing and remitting (5). This clinical phase can occur spontaneously or in response to treatment (6); As a result, in the evaluation of new UC treatments, symptom assessments and more objective markers of the underlying disease process, such as endoscopy, are used. Clinical trials in UC must include both disease activity (total Mayo score) and endoscopic and healing procedures [4], [7]. Many pathogenesis factors cause ulcerative colitis: Genetic Predisposition, Environmental factor, Microbial factor, Immunological Factors, Cytokines. Adhesion Molecules and Oxidative Stress. Treatment of ulcerative colitis includes several medication classes. These are aminosalicylic acid agents, Corticosteroids, Immunomodulators, Calcineurin Inhibitors, Tacrolimus Biologicals Antibiotics Therapy and Prebiotics and Probiotics. One of the major concerns of current colitis treatment are side effects and toxicity. statins are a further example of treatment, which has a wide ray of uses, e.g. ulcerative colitis. Additionally, it has anti-inflammatory and antioxidant effects. The most widely prescribed medications worldwide are 3-hydroxyl-3-methyl-glutaryl—coenzyme A reductase (statins). Primary and secondary prevention of cardiovascular diseases, as well as therapy of hypercholesterolemia, are some of the indications for their usage [8]. Statins may have more complex activities than only reducing cholesterol because of their immune-modulatory properties [8]. According to prior studies, statins are linked to a lower use of oral steroids during the acute phase, as well as a lower disease activity index and inflammatory markers in colitis animals [9]. According to another study, statins may be linked to a lower incidence of new-attack Inflammatory bowel disease [10], increasing the probability that these medications could also the anti-inflammatory properties which suggest that they can regulate molecules important for immunomodulation. statin was found to inhibit the production of TNF- and inducible nitric oxide synthetase by microglia and astrocytes. Statins also prevented IFN- -inducible major histocompatibility.

2. Materials and methods

2.1 Preparation of animals

Mice were given free access to tap water but were malnourished for at least 24 hours before being infected with colitis. To avoid coprophagy, the mice were randomly distributed in cages with a large wire–mesh floor during starvation. Water was held two hours before the procedure on the day of the experiment.

2.2 Preparation of drugs

On the day of the trial, all medications were freshly prepared before being administered. The medication atorvastatin and the prednisolone were suspended in 25mL distilled water. The dose of atorvastatin was 50 mg/kg [11]. In a dose of 1 mg/kg, Prednisolone was administered as standard therapy [12].
All drugs will be given orally for 10days. (3days before induction and 7days after induction).
Acetic acid Induced colitis is a more used mice model of colitis [13].

2.3 Induction of colitis

Mice that had been starved for 24 hours were mildly anaesthetized with chloroform. The tip of a 6 Eng. catheter was 4 cm proximal to the anus and then cautiously placed into the colon. To induce colitis, a solution of 2 ml acetic acid (4 percent, v/v) in 0.9 percent saline was instilled for 30 seconds into the colon lumen to prevent the intracolonic infusion from leaking out. Using the same manner, mice were given 0.9 percent saline alone in normal control tests [14].

2.4 Experimental protocol

The animals were split into four groups, each one with ten animals as follows:
Group I: was used as a control group and was given normal saline (5ml/kg) rectally.
Group II: was used as an induction group for colitis., was received only 4% v/v acetic acid (5ml/kg) rectally.

Group III: was used as atorvastatin group, were received acetic acid rectally + atorvastatin (50mg /kg) orally. Group IV: was used as the positive control group, were received acetic acid rectally + prednisolone as standard therapy (1mg /kg) orally.

All these drugs were given orally one hour following the induction of colitis for ten days(3day before induction and7 days after induction) by using oral gavage.

Animals were sacrificed by an overdose of chloroform inhalation after 24 hours of starvation, The colon was then removed after the abdomen was quickly dissected and opened.

2.5 Assessment colitis severity

Variable	Acetic acid-induced colitis group	Healthy control group	*p-value*

2.6 Tumor Necrosis Factor-alpha (TNF-α)

TNF is a pleiotropic pro-inflammatory cytokine that has been associated with a variety of biological processes such as cell proliferation, survival, and death. TNF- was quantified in the tissues using an enzyme-linked immunosorbent assay (ELISA) kit, as directed by the manufacturer [15].

2.7 Myeloperoxidase as a pro-inflammatory marker (MPO)

MPO is an enzyme that is common in neutrophils. MPO could be a good measure of neutrophils. For this project, MPO levels are measured and used as an approximation of leukocyte count and therefore degree of inflammation [16].

2.8 Statistical analysis

The statistical package for the social science version (SPSS)23 software program was used to summarize, analyze, and show the data. The mean and standard deviation were used to express quantitative (numerical) variables. A one-way analysis of variance (ANOVA) was used to evaluate the difference in mean of quantitative variables between groups, followed by a post hoc least significant difference (LSD) test to analyze mean differences within groups. Significant was defined as a P value of less than 0.05 (Daniel,2009).

3. Results

Table 1. Comparison of biochemical and oxidative stress parameters in healthy control and acetic acid-induced colitis groups

TNF-α	790 ± 73	299 ± 20	<0.01**
MPO	100 ± 14	30 ± 8	<0.01**

Table 2. Comparison of biochemical and oxidative stress parameters in Acetic acid-induced colitis group and atorvastatin group

Variable	Acetic acid-induced colitis group	High dose atorvastatin group	*P-value*

TNF-α	790 ± 73	420 ± 47	<0.01**
MPO	100 ± 14	45 ± 5	<0.01**

Table 3. Comparison of biochemical and oxidative stress parameters in Acetic acid-induced colitis group and prednisolone group

Variable	Acetic acid-induced colitis group	Prednisolone group	*P-value*
TNF-α	790 ± 73	365 ± 63	<0.01**
MPO	100 ± 14	36 ± 4	<0.01**

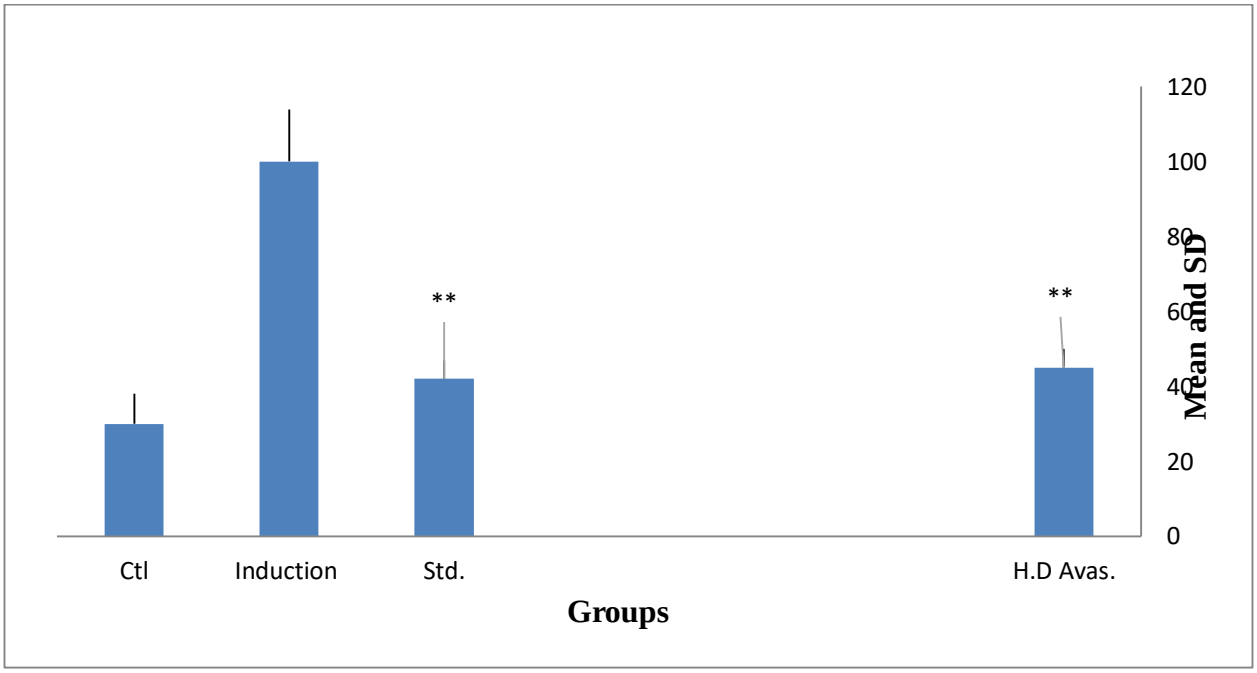

Figure 1: show myeloperoxidase (MPO) concentration in the control and all study Groups. Comparison between means of MPO in all groups. (N=10), **=p<0.01.

Ctl:control, Std:stander, H.D:high dose, Avas:Atorvastatin, SD:stander deviation

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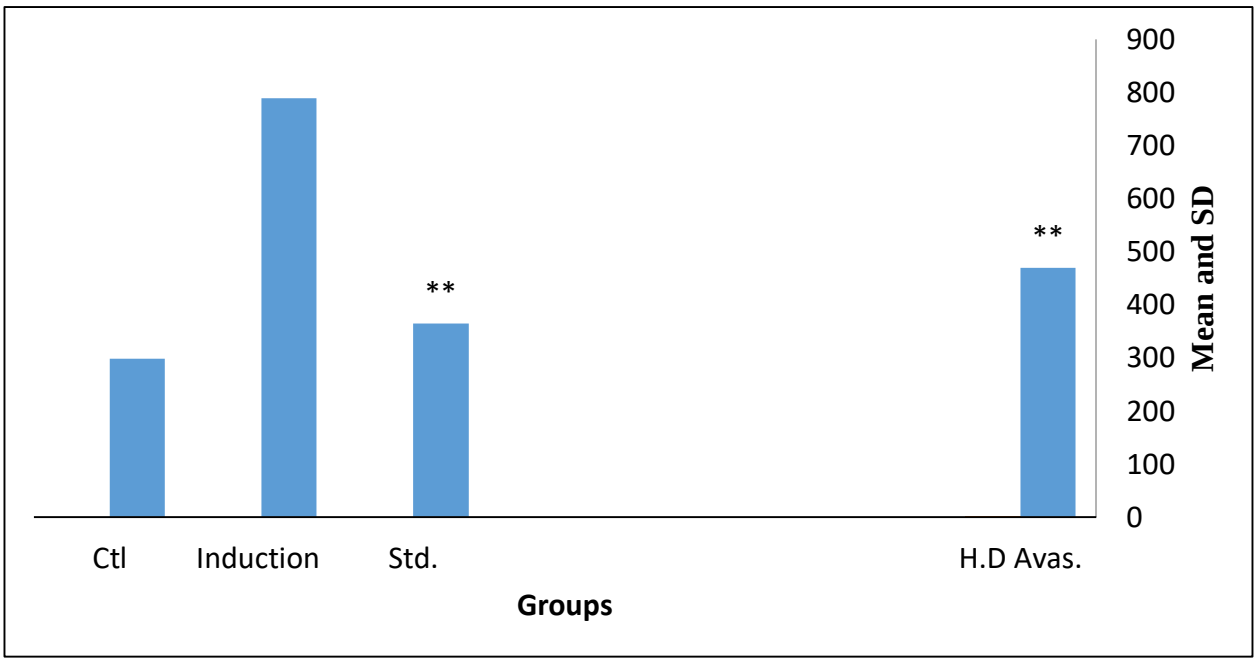

Figure 2: show Tumor Necrosis Factor(TNF) concentration in the control and all Study Groups. Comparison between means of TNF in all groups. (N=10), **=p<0.01.

Ctl:control, Std:stander, H.D:high dose, Avas:Atorvastatin, SD:stander deviation.

4. Discussion

4.1 Effect of acetic acid on colonic mucosa in mice

Our research also corroborates the work by [18] who described the increased level of expression of pro-inflammatory cytokine (TNF-α) and these findings corroborate the model's efficacy in producing results on ulcerative colitis in humans, where an increase in pro-inflammatory cytokines is related to disease severity [19]. And their appearance in UC had long been associated with colonic inflammation. TNF- is a critical pro-inflammatory cytokine released from lymphocytes and macrophages in the early stages of inflammation, inducing many inflammatory genes and promoting the expression of pro-inflammatory cytokines, it can produce different mediators which responsible for severe inflammation that contributes to the development of UC. The present study demonstrated that the colitis group was associated with"a significant increase in the level of MPO and levels in the colonic tissue. This finding is comparable with an observation of [20- 23] has indicated that oxidative stress results from the shift of equilibrium between the pro-oxidant and anti-oxidant systems in favour of the pro-oxidant system which result in excessive production of free oxygen radicals and neutrophil infiltration". Myeloperoxidase is a hemoprotein enzyme that catalyzes the production of various reactive species and is abundantly released from neutrophil granules by inflammatory stimuli [24].

4.2 Effect atorvastatin in acetic acid-induce colitis in mice

In Acetic acid colitis mice, atorvastatin further lowered pro-inflammatory cytokine (TNF-α). By inhibiting the Acetic Acid-induced increase in (TNF-α) expression in colonic tissue, which is commonly overexpressed in this model of colitis, it significantly reduced the severity of intestinal inflammation in experimental colitis and caused a marked reduction in inflammation in the colon of animals with ulcerative colitis [25].

Atorvastatin treatment significantly reduced IL-17 levels as we also observed on TNF-α (IL-17 is a pro-inflammatory cytokine that promotes T-cell priming and stimulates the production of numerous pro-

inflammatory cytokines by fibroblasts, endothelial cells, macrophages, and epithelial cells) [26], [11].

Atorvastatin in this dose significantly decreases MPO in Acetic acid colitis mice [25]. These results refer to the inhibition of neutrophil aggregation by statins may be responsible for protecting Acetic acid-induced colonic mucosal injury because MPO activity, an index of tissue-related neutrophil aggregation, is significantly elevated in the colonic mucosa after Acetic acid administration [27]. At last, Statins may have a dose-dependent effect, as two trials demonstrated only a decrease in histological activity with these dosing regimens [28], [29].

4.3 Effect prednisolone in acetic acid-induce colitis in mice

The prednisolone therapy was successful in reducing pro-inflammatory mediators such as TNF-α. The levels of the mediator were significantly restored at TNF-α as compared to the UC [31]. Prednisolone was a highly reduced MPO activity than the acetic acid colitis group [32].

5. Conclusion

In summary, HMG COA (Atorvastatin) is a potent anti-inflammatory and anti-oxidants that have been shown to be effective in the treatment of acetic acid-induced colitis in mice. biochemical parameters cytokine (TNF-α) has positive relationships with oxidative stress marker (MPO).

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