AMELIORATIVE EFFECT OF AVENA SATIVA (OAT) IN DINITROBENZENE SULPHONIC ACID INDUCED INFLAMMATORY BOWEL DISEASE IN RATS

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ABSTRACT

Inflammatory bowel disease (IBD) is a chronic relapsing gastrointestinal tract disease. There is an upsurge of IBD cases worldwide and there is no gold standard therapy and the drugs used to treat IBD are having many major side effects. Thus, there is a need for a better treatment option. This study aimed to evaluate the prophylactic role of Avena sativa (oat) in 2, 4-dinitro benzene sulphonic acid (DNBS) (120 mg kg⁻¹) induced IBD in rats. Animals were randomly allocated to five groups- negative control, model control receiving only DNBS, group receiving A. sativa extract (500 mg kg⁻¹ and 1 g kg⁻¹ p.o.) and the last group receiving sulphasalazine (100 mg kg⁻¹, p.o.). Colitis-induced rats treated with A. sativa and sulphasalazine restored their body weight, stool consistency, and bleeding in stool and significantly improved several biochemical parameters such as colonic glutathione content, lactate dehydrogenase, myeloperoxidase and lipid peroxides levels as compared to the model control group. Findings suggest that A. sativa possesses antioxidant and anti-inflammatory activity and can be useful in treating IBD.

Keywords: Avena sativa (oat), inflammatory bowel disease, sulfasalazine, DNBS

INTRODUCTION

Inflammatory bowel disease (IBD) is a complex, chronic relapsing disease characterized by ulcerative colitis (UC) and Crohn’s disease (CD). The most common symptoms of IBD include severe diarrhea, anemia, weight loss, severe abdominal pain and several other events that may lead to complications. No single factor is responsible for relapsing conditions and the four major factors include genetic susceptibility, imbalance in immune responses, gut microbiome and environmental factors. A study on epidemiology states that the burden of IBD is increasing in developing countries, including Asian countries and the prevalence and incidence of IBD in India are increasing making it a burden on the Indian population. It is assumed that changes in lifestyle, environmental factors and genetic predisposition in developing countries have some major impact on the increasing IBD cases.

There is no gold standard treatment for treating IBD. Some conventional therapies include aminosalicylates, glucocorticoids, sulfasalazine, antibiotics and immunomodulators, which provide temporary relief. Various adverse effects associated with these drug treatments include nausea, vomiting, headache and severe fatigue. Some major side effects of conventional drugs include bone marrow suppression, hepatitis, pulmonitis and reduction in sperm counts in males. Some patients receiving sulphonamides show severe hypersensitivity reactions. Systemic corticosteroids produce severe complications in around 5 % of patients. There is no specific treatment approach for IBD. The combination of various classes of drugs increases the risk of serious infection, malignancies, increased risk of cardiovascular events and thromboembolic events. Some newer class of drugs like anti-TNF-α agents, IL-23 inhibitors, and sphingosine 1-phosphate receptor modulators are also used to treat IBD, but these drugs are contraindicated in diabetic patients and they also increase cholesterol level. Thus, there is a need for a newer therapeutic approach to treat IBD. Plants in ayurveda contain many bioactive compounds, which have numerous health benefits. Those bioactives exhibit various activities including antimicrobial, antioxidant activities and anti-inflammatory activities. Many herbal...
medicines can be used as an alternative source and are more compatible due to lesser side effects as compared to conventional drug therapies. The current study was designed to find a cost-effective treatment with fewer side effects and was conducted to explore the therapeutic effect of *Avena sativa* (oat) on DNBS-induced inflammatory bowel disease in rats.

*A. sativa* Linn., commonly known as oat, belongs to the Poaceae family. Oat has numerous health benefits. It is used to control blood pressure and it also possesses hypercholesterolemic activity. *A. sativa* contains avenathramides, soluble fibers like β-glucan and prolamines (avenins), glutamic acid, C-glycosyl flavones, avenacosides (spirostanol glycosides), Vit. E, enzymes (alpha amylase, phosphatase, tyrosine, maltase, lipase), and a lipid (avenothionin). Oat exhibits anti-oxidant activity and anti-inflammatory activity, and it may be due to avenathramides and β-glucan. Oat is used in treating diarrhea and dysentery and is traditionally used in treating colitis. The study aimed to investigate the effect of *A. sativa* in the mitigation of DNBS-induced IBD in rats.

**MATERIALS AND METHODS**

**Preparation of *A. sativa* extract**

*A. sativa* was collected from the field during the flowering period. Whole grains with the aerial part were rinsed with water and dried. The powder was extracted with 70% methanol in the Soxhlet apparatus and the extract was further evaporated in a rotary evaporator. 500 mg kg\(^{-1}\) and 1 g kg\(^{-1}\) hydro-methanolic extract of *A. sativa* were selected as the doses for the study and were coded as AS I and AS II.

**Preliminary phytochemical screening**

Preliminary phytochemical screening was carried out on *A. sativa* extract for the detection of phytoconstituents. Tests for the presence of major phytoconstituents like proteins, saponins, carbohydrates, alkaloids, flavonoids, phenols and lipids were carried out as per the standard method.

**Animal selection**

Male albino Wistar rats weighing 250-300 g were chosen for the study and placed in hygienic cages. Animals were fed with a standard pellet diet and potable water *ad libitum* for seven days before the commencement of the experiment. The study protocol (SPCP/IAEC/RP-016/13) was approved by Institutional Animal Ethics Committee at Sardar Patel College of Pharmacy.

**Experimental study design**

Animals were randomly allocated into five different groups with six animals in each group. Group I (NC) negative control group received the normal rat chow diet throughout the study. Group II (MC) model control group received only DNBS. Group III and Group IV (AS-I and AS-II) received *A. sativa* (500 mg kg\(^{-1}\) and 1g kg\(^{-1}\) p.o.), respectively. Rats of group V (STD) received standard sulfasalazine 100 mg kg\(^{-1}\), p.o. throughout the study. After 10 days of the study, rats of Groups-II to V were kept on fasting and on the next day IBD was induced by intracolonic administration of DNBS. 120 mg kg\(^{-1}\) DNBS in 50% ethanol was prepared and administered via rectal route using a polypropylene tube inserted 8 cm deep into the colon. Treatment with *A. sativa* and sulfasalazine was started from day 0 and continued till day 18 (Fig. 1). On the 11\(^{th}\) day after inducing IBD body weight of all rats was measured. Stool consistency and rectal bleeding were scored daily. At the end of the study, rats were euthanized and the colon was isolated and washed with saline. The colon was cut open for macroscopic assessment, histopathological assessment and estimation of biochemical parameters.
Table I: Weight loss scoring

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No weight loss</td>
<td>0</td>
</tr>
<tr>
<td>More than 0 to 5% weight loss</td>
<td>1</td>
</tr>
<tr>
<td>More than 5 to 10% weight loss</td>
<td>2</td>
</tr>
<tr>
<td>More than 10 to 15% weight loss</td>
<td>3</td>
</tr>
<tr>
<td>More than 15 to 20% weight loss</td>
<td>4</td>
</tr>
<tr>
<td>Weight loss &gt; 20%</td>
<td>5</td>
</tr>
</tbody>
</table>

Table II: Stool consistency scoring

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal stool</td>
<td>0</td>
</tr>
<tr>
<td>Pasty and semisolid stool</td>
<td>2</td>
</tr>
<tr>
<td>Liquid stools that stick to the anus</td>
<td>4</td>
</tr>
</tbody>
</table>

Table III: Rectal bleeding scoring

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No bleeding</td>
<td>0</td>
</tr>
<tr>
<td>Hemooccult</td>
<td>2</td>
</tr>
<tr>
<td>Gross bleeding</td>
<td>4</td>
</tr>
</tbody>
</table>

Table IV: Colonic mucosal damage index scoring (Macroscopic scoring)

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ulceration, no inflammation</td>
<td>0</td>
</tr>
<tr>
<td>No ulceration with local hyperemia</td>
<td>1</td>
</tr>
<tr>
<td>Ulceration seen without hyperemia</td>
<td>2</td>
</tr>
<tr>
<td>Ulceration and inflammation at only one site</td>
<td>3</td>
</tr>
<tr>
<td>Ulceration and inflammation seen at two or more sites</td>
<td>4</td>
</tr>
<tr>
<td>Ulceration area expanded more than 1 cm</td>
<td>5</td>
</tr>
</tbody>
</table>

Parameters assessment

Evaluation of physical, histological and biochemical parameters

Induction of IBD was confirmed by measuring body weight loss, scoring stool consistency and rectal bleeding\(^{24,25}\). A decrease in body weight was scored as shown in Table I. Stool consistency was given scores as depicted in Table II. Rectal bleeding was scored as described in Table III. The disease activity index is a calculative parameter and was calculated using the following formula:

\[
\text{Disease activity} = \frac{\text{Weight loss score} + \text{Rectal Bleeding Score} + \text{Stool consistency score}}{3}
\]

Assessment of macroscopic damage

Colonic mucosal damage index (CMDI)

Rats were euthanized at the end of 18 days and colonic segments were taken for scoring inflammatory indices. The macroscopic scoring was performed as described in Table IV.

Preparation of tissue homogenates and estimation of biochemical parameters

Colon samples were collected and homogenized and then centrifuged. The supernatant was used for measuring various biochemical parameters like colonic glutathione content (GSH)\(^{27,28}\), lactate dehydrogenase (LDH) assay\(^{26}\), myeloperoxidase (MPO)\(^{29}\) assay and colonic lipid peroxides concentration (LPO)\(^{28}\).

Histopathology\(^{30,31}\)

At the end of the study, a colon sample was collected and stored in a formalin solution and histopathological evaluation was done by preparing hematoxylin and eosin-stained tissue sections. The damage to the colonic mucosa can be seen in histopathology studies.

Statistical analysis

All the results are expressed as mean ± SEM, with P< 0.05 being considered as statistically significant. Statistical analysis was performed using one-way ANOVA, which was followed by Dunn-Bonferroni post-hoc test.

RESULTS

Results of preliminary phytochemical studies

Hydromethanolic extract of A. sativa revealed the presence of carbohydrates, glycosides, alkaloids, flavonoids and tannins.

Ameliorative effect of A. sativa against DNBS-induced IBD in rats

Inflammatory bowel disease was induced after intracolonic administration of DNBS in rats, and it was confirmed with a decrease in body weight (Fig. 2A), stool...
NC: Negative Control, MC: Model control, AS-I (A. sativa 500 mg kg⁻¹ p.o), AS-II (A. sativa 1 g kg⁻¹ p.o), and STD (sulfasalazine 100 mg kg⁻¹, p.o.) Statistical analysis is done by one-way ANOVA followed by the Dunn-Bonferroni post hoc test. Data are presented as mean ± SEM (n = 6). *P < 0.05 as compared to the negative control group. # P < 0.05 as compared to the Model control group.

Fig. 2: Analysis of various parameters (A) Weight loss score (B) Stool consistency score (C) Bleeding score (D) DAI score and (E) CMDI score

consistency score (Fig. 2B), and rectal bleeding score (Fig. 2C). The clinical activity score was significantly increased in MC rats as compared to NC rats. AS-I, AS-II, and sulfasalazine-treated rats showed a significant decrease in clinical activity score as compared to MC rats in all the parameters and the DAI score of the treatment group was significantly improved by the pretreatment with methanolic extract of A. sativa (Fig. 2D). At the end of the study, macroscopic score of the colonic tissue was assessed CMDI score and microscopic evaluation was done by performing a histological study of the colon. There was a significant increase in CMDI score in the MC group
as compared to the NC group, while the CMDI score was significantly decreased in AS-II and sulfasalazine treated rats as compared to the MC group and was seen macroscopically in colonic tissue (Fig. 2E, Fig. 3A). DNBS induced colitis characterized by inflammation of the colon with severe hyperemia, was observed and microscopically stained colonic tissue showed massive necrotic destruction of epithelium (Fig. 3B). Pretreatment with A. sativa showed depletion of the morphological disturbance and reduction of inflammation and mucosal edema associated with DNBS administration (Fig. 3C, D). Standard sulfasalazine-treated rats showed recovered submucosal edema and attenuated the extent and severity of the histological signs of cell damage.

**Effect of A. sativa on biochemical parameters**

MC group showed significant elevation in LDH, MPO, and LPO levels when compared with the NC group. Pre-treatment with AS-I (500 mg kg⁻¹), AS-II (1 g kg⁻¹) and sulfasalazine showed significant depletion of LDH, MPO, and LPO levels (Figs. 4 A, B, D) as compared to the MC group. MC group significantly debased the colonic GSH concentration as compared to the NC group. The reduction in GSH level was significantly prevented by the pre-treatment with AS-I (500 mg kg⁻¹), AS-II (1 g kg⁻¹) and sulfasalazine (Fig. 4C). The results of all biochemical parameters suggested the anti-oxidant effect of methanolic extract of A. sativa.

**DISCUSSION**

Inflammatory bowel disease is a recurrent disorder of the gastrointestinal tract with unclear pathophysiology and etiology. For many decades, chemically induced IBD models have been used for screening newer agents against IBD. The DNBS-induced model produces mild...
Fig. 4: Effect of *A. sativa* on biochemical parameters A) Lactate dehydrogenase (LDH) B) Myeloperoxidase (MPO) C) Glutathione (GSH) D) Lipid peroxidation (LPO)

NC: Negative control, MC: Model control, AS-I (*A. sativa* 500 mg kg⁻¹, p.o.), AS-II (*A. sativa* 1 g kg⁻¹, p.o.), and STD (sulfasalazine 100 mg kg⁻¹, p.o.) Statistical analysis is done by one-way ANOVA followed by the Dunnett post hoc test. Data are presented as mean ± SEM (n = 6). *P < 0.05 as compared to the negative control group. # P < 0.05 as compared to the Model control group.

DNBS decreases mucus production, shredding the colonic barrier and hence damaging the mucosa. It leads to neutrophil infiltration and increases the release of several pro-inflammatory mediators, cytokines and chemokines. It is stated that intracolonic administration of DNBS causes acute or subchronic colitis. Leukocyte and neutrophil infiltration leads to tissue necrosis and severe inflammation due to the release of proinflammatory mediators. Hence, DNBS induced model was preferred for the present study.

Administration of DNBS elevated oxidative stress levels which leads to inflammation of mucosa and submucosa leading to its inability to absorb nutrients from food and causing weight loss. A significant decrease in weight loss was noted in MC rats as compared to NC rats. This weight loss was protected by pre-treatment with AS-I, AS-II and sulfasalazine. Similarly, due to damage to the mucosa, stool consistency was reduced and rectal bleeding was increased in rats of the MC group. *A. sativa* helps to improve stool consistency scoring.

The macroscopic assessment proved that DNBS damages the mucosa and causes severe inflammation. *A. sativa* protected colonic damage, ulceration and inflammation. The reduction in mucosal lesion area was significant as compared to MC rats.

Furthermore, colonic MPO is a biomarker depicting the infiltration of neutrophils in the colonic mucosa. DNBS causes mucosal inflammation due to leukocyte trafficking and neutrophil infiltration. Similar results were observed in MC rats. Choi et al. stated that by decreasing the colonic MPO, the mucosal inflammation might be reduced and can be beneficial for controlling inflammation. They also found that seeds of *Raphanus sativus* reduced the colonic...
MPO levels and similarly *A. sativa* at both doses showed depletion in colonic MPO levels.

Yuksel et al stated that oxidative stress worsens the inflammatory condition of ulcerative colitis and Crohn’s disease. Hence, it can be considered as one of the factors responsible for worsening inflammation. Tian et al. stated that reduced GSH is an endogenous antioxidant. DNBS-induced IBD in rats showed a decrease in GSH level as compared to negative control rats. A previous study by Darji et al. shows that hydromethanolic bark extract of *Holarrhena antidysentrica* elevated the GSH level. Similarly, deprivation of GSH was significantly prevented by *A. sativa*. This observation suggests that *A. sativa* could have antioxidant activity.

LPO is a marker of injured mucosa due to excess oxidative stress. DNBS-induced rats showed elevated levels of colonic LPO. Cota et al. stated that the hydroalcoholic extract of *Terminalia arjuna* decreased the MDA level, showing its preventive role in reducing oxidative stress. MDA is a by-product of lipid peroxidation. Similar data are seen in rats treated with *A. sativa*, confirming its antioxidant activity as it reduces LPO levels in colonic tissues. *A. sativa* relaxes the vascular walls and besides its scavenging effect on ROS, it has an anti-inflammatory effect by suppressing ROS generation.

Another biomarker is LDH, which depicts colonic tissue injury. DNBS damages the colonic tissue and LDH level is elevated in rats. In the present investigation, MC rats showed high levels of LDH as compared to NC rats depicting colonic tissue injury due to DNBS. A previous study by Kannan et al. showed that hydromethanolic leaves extract of *Bauhinia tomentosa* prevented colonic damage and this was proved by an increase in LDH level. Similarly, *A. sativa* prevented colon damage, showing its efficacy in treating IBD.

The microscopic findings in IBD depict the severity of inflammation, ulceration, loss of mucosal epithelial layer and presence of crypt abscesses. Histopathology of the colon in DNBS-induced IBD affects the colonic cell architecture. The histopathology shows the damaged goblet cells and mucosal layers. It also shows the infiltration of proinflammatory mediators in MC rats. The damage was protected by sulphasalazine and *A. sativa*.

The study suggests the anticolitic role of *A. sativa* and it may be due to several phytochemicals. Several flavonoids are phytochemicals that possess chemoprotective, immunomodulatory, gastroprotective, neuroprotective and anti-inflammatory activities. Studies have shown that the flavonoid derivatives like dosmalfate is an effective anti-ulcer agent and also show anticolitic activity in acute and chronic experimental colitis. *A. sativa* has anti-oxidant, anti-bacterial, anti-microbial, anti-inflammatory, and anticancer properties due to the presence of flavonoids. Because of these properties, a recent study suggested that *A. sativa* has a beneficial effect on DNBS-induced colitis and can be used as adjuvant therapy in treating inflammatory bowel disease.

**CONCLUSION**

*A. sativa* prevented the weight loss and decrease in colonic GSH, decreased clinical activity score, macroscopic score, LDH level, MPO level, and colonic LPO level. 1 g kg\(^{-1}\) dose of *A. sativa* was found to be more effective as compared to 500 mg kg\(^{-1}\). Histologically, colon damage was restored indicating that *A. sativa* causes mucosal healing and reduces inflammation similar to that of sulfasalazine. Based on the above findings, we can conclude that *A. sativa* may be used in inflammatory bowel disease.

**REFERENCES**


