EFFECT OF ETHANOLIC EXTRACT OF FLOWERS OF TECOMA STANS ON ACETIC ACID INDUCED COLITIS IN ALBINO RATS

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ABSTRACT

Aim to evaluate the effect of ethanolic extract of flowers of Tecoma stans on acetic acid induced colitis in albino rats. Ethanolic extract of Tecoma stans (EETS) was prepared by percolation method. Acute toxicity test was done by using Organization for Economic Cooperation and Development guidelines. Albino rats were divided into four groups of five animals each. Groups A and B received 3% gum acacia. Groups C and D received EETS 500 mg/kg body weight (BW) and 5-aminosalisylic acid 100 mg/kg BW respectively. Colitis was induced by transrectal administration of 4% acetic acid on 5th day. All animals were sacrificed after 48 h of colitis induction and distal 10 cm of the colon was dissected. Colon was weighed for disease activity index (DAI) and scored macroscopically and microscopically. Biochemical assessment of tissue myeloperoxidase (MPO), catalase (CAT) and superoxide dismutase (SOD) was done in colonic tissue homogenate and malondialdehyde (MDA) was estimated in serum. T.stans showed significant ($P < 0.05$) reduction in DAI, macroscopic and microscopic lesion score as well as significant ($P < 0.05$) improvement in MPO, MDA, CAT, and SOD level as compared to Group B. The ethanolic extract of flowers of T.stans showed significant amelioration of experimentally induced colitis, which may be attributed to its anti-inflammatory and antioxidant property.

Key words: Antioxidant, Colitis, Tecoma stans.

INTRODUCTION

Inflammatory bowel disease (IBD) including ulcerative colitis is a chronic incurable disease that damages the gastrointestinal tract of affected individuals, which is a resultant effect of dysregulated innate, adaptive and epithelial immune functions. It is characterized by chronic inflammation in the mucosal membrane of the small and/or large intestine. It is largely a disease of the industrialized world, and is more common in urban areas and northern climates [1]. Ulcerative colitis (UC) is clinically characterized by recurrent inflammatory involvement of intestinal segments with several manifestations often resulting in an unpredictable course. The aetiology remains unknown; however, two primary theories have been proffered focusing on either a specific persistent infectious agent or an abnormal host immune response to ubiquitous antigens in the luminal constituents [2]. Reactive oxygen species (ROS)-mediated injury plays an important role in the pathophysiology of IBD. The increased generation of highly toxic ROS exceeds the limited intestinal antioxidant defense system, thereby contributing to intestinal oxidative injury in IBD/UC patients [3]. Many treatments have been recommended for UC; they are far from treating the cause but are effective only in reducing the inflammation and accompanying symptoms in up to 80% of patients. The current medical management consists of anti-inflammatory and immunosuppressive agents and biologic drugs, as well as surgery. The primary goal of drug therapy is to reduce inflammation in the colon that generally requires frequent intake of high dose of anti-inflammatoriy drugs. The propensity to cause adverse events and lack of effectiveness of standard therapies has diverted towards the use of complementary and alternative medicines, particularly of herbal therapies, for IBD [4].

Tecoma stans (common name yellow bell) also known as yellow trumpet bush belongs to the family bignoniaceae. It is an ornamental plant. It is an erect, branched, sparingly hairy or nearly smooth shrub two to four meters in height. The leaves are opposite, odd-pinnate, up to 20 centimeters in length with 5 to 7 leaflets. The leaflets are lanceolate to oblong-lanceolate, 6 to 13 centimeters long, pointed at both ends and toothed on the margins. Trumpet shaped flowers are yellow faintly scented with a melodious fragrance...
and borne in short, dense, terminal clusters. The calyx is green, 5 to 7 millimeters long and 5 toothed. Flowering can begin as early as April and continue in to fall. The flowers are followed by 6 inch long, tan pods that are filled with small, papery winged seeds. [5]

Leaves of *Tecoma stans* contain the alkaloids tecomin and tecostamine are potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the anti diabetic activity. Roots are powerful diuretic and vermifuge [6]. *Tecoma* is not a toxic because this plant is used in latein America as a remedy for diabetes and moreover for feeding cattle and goats in mexico [7]. The preliminary phytochemical screening of methanolic extract of flower extract of *Tecoma stans* showed the presence of flavanioids, phenol, alkaloids, tannins, steroids, triterpenes, anthraquinones and saponins etc. As the flowers of *T.stans* possess anti-inflammatory and antioxidant properties, this study has been undertaken to evaluate the effect of *T.stans* in experimentally induced IBD and to find its probable mechanism of action including its antioxidant potential.

**MATERIALS AND METHODS**

**Drugs and Chemicals**

5-aminosalislyic acid (5-ASA) and acetic acid were obtained from Merck Limited, Shiv Sagar Estate ‘A’, Dr. Annie Besant Road, Worli, Mumbai - 400 018. The reagents for estimation of myeloperoxidase (MPO) and superoxide dismutase (SOD) were obtained from Sigma Pvt. Limited, Bangalore, India. Thiobarbituric acid was obtained from HiMedia Laboratories Pvt. Limited, Mumbai, India. Malondialdehyde (MAD) bis was obtained from Merck Schuchardt OHG, Hohenbrunn, Germany.

**Preparation of the Extract**

The flowers of *Tecoma stans* were collected in the month of May 2011 from Rasipuram (Namakkal District) Tamil Nadu. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The plant was identified by Dr.G.V.S.Murthy, Joint Director of the Botanical Survey of India, Southern circle, TNAU Campus, Coimbatore, who authenticated the plant from information available in the literature. The flower petals were dried in the shade and then powdered and 100 g of the dried powder was extracted with ethanol using a soxhlet apparatus. The solvent was removed under reduced pressure and controlled temperature using a rotary flash evaporator. Suspension EETS in 2% (v/v) tween-80 was prepared for oral administration by gastric intubation method. The different identification tests were performed to detect the presence of phytoconstituent [8]. The screening revealed the presence of oils and fats, org. acids, flavonoids, triterpenes, steroids, sterols and proteins.

**Animals**

Albino rats of the species *Rattus norvegicus* of either sex, weighing 150-200 g were used. The animals were acclimatized for 1 week under laboratory conditions. They were fed with standard diet, and water was provided *ad libitum*.

**Acute Toxicity Studies**

Acute oral toxicity test for the EETS was carried out as per Organization for Economic Cooperation and Development Guidelines 425 [9]. One arbitrary dose of 500 mg/kg was selected for the study, as the extract was found safe even at doses more than 2000 mg/kg without any sign of toxicity or mortality.

**Experimental Design**

Twenty healthy albino rats were divided into four groups of five animals in each group (n = 5) as follows: Group A (normal control): Received 3% gum acacia 5 ml/kg/day perorally. Group B (experimental control): Received 3% gum acacia 5 ml/kg/day p.o. Group C (test): Received ethanolic extract of *Tecoma stans* (EETS) 500 mg/kg/day p.o. Group D (standard): Received 5-ASA 100 mg/kg/day p.o.

**Induction of Colitis**

The experiment was performed using acetic acid for inducing colitis. [10]. All the animals were pre-treated with the respective drugs (volume of drugs was kept constant at 5 ml/kg) for 5 days, along with the normal diet. On the 5th day, animals were fasted for 12 h (overnight) and IBD was induced the next morning in Groups B, C and D by administration of 1 ml of 4% acetic acid solution transrectally (TR), while Group A (normal control) received 0.9% normal saline TR.

IBD induction was done using an 8 mm soft pediatric catheter which was advanced 6 cm from the anus under low-dose ether anesthesia. Rats were in trendelenburg position during this process and 1 ml of 4% acetic acid or 0.9% normal saline solution was slowly administered TR. The rats were maintained in head-down position for 30 s to prevent leakage. After this process, 2 ml of phosphate buffer solution of pH 7 was administered TR.

All the animals were sacrificed after 48 h of IBD induction, by ether overdose. Abdomen was opened and distal 10 cm of colon was excised and opened by a longitudinal incision. After washing the mucosa with saline solution, mucosal injury was assessed macroscopically and borne in short, dense, terminal clusters. The calyx is green, 5 to 7 millimeters long and 5 toothed. Flowering can begin as early as April and continue in to fall. The flowers are followed by 6 inch long, tan pods that are filled with small, papery winged seeds. [5]

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BW) was used to assess the degree of tissue edema and the severity of colonic inflammation, was measured. [12]

A 6-8 mm sample block of the inflamed colonic tissue with full thickness was excised from a region of grossly visible damage for histological analysis. Formalin-fixed tissue samples were embedded in paraffin and stained with hematoxylin and eosin. Colonic tissues were scored for histological damage using the criteria of Wallace and Keenan: 0 = Intact tissue with no apparent damage; 1 = damage limited to surface epithelium; 2 = focal ulceration limited to mucosa; 3 = focal, transmural inflammation and ulceration; 4 = extensive transmural ulceration and inflammation bordered by normal mucosa; 5 = extensive transmural ulceration and inflammation involving entire section. [13]

Biochemical Assessments
Preparation of the sample
The proximal 5 cm of the dissected colon specimen was used for biochemical analysis of MPO, tissue catalase (CAT), SOD. The colonic samples were minced and homogenized using a polytron homogenizer. The supernatant was obtained by centrifuging at 3000 rpm for 20 min.

MPO activity
MPO activity was measured by the method described by Krawisz et al [14].

MDA measurement
MDA was estimated by Satoh method [15].

Assessment of antioxidant status in colonic tissue
CAT was measured by the method of Beers and Sizer [16]. SOD was assayed according to the method of Kakkar et al [17].

Statistical Analysis
For all the above methods, the results were expressed as mean ± SEM. Statistical analysis was done using one-way analysis of variance, followed by Dunnett's multiple comparison test. P < 0.05 was considered significant.

RESULTS
Acute Toxicity Studies
No mortality or sign of toxicity was observed in the animals up to 2000 mg/kg BW. Thus LD50 was calculated more than 2000 mg/kg BW.

Histopathological Findings
Acetic acid administration to the experimental control group caused significant macroscopic ulcerations and inflammation (P < 0.05) along with significant mucosal injury microscopically (P < 0.05) in rat colon, when compared to the normal control group [Table 1], [Figure 1] and [Figure 2]

Biochemical Parameters
A significant derangement of biochemical parameters including tissue levels of MPO, CAT, SOD and serum MDA (P < 0.05), was observed in rats of experimental control group as compared to normal control group [Table 2].

EETS flowers treated rats showed significant improvement in colon architecture both macroscopically as well as microscopically as compared to experimental control rats (P < 0.05). There was significant reduction in ulcer size macroscopically and decreased inflammation microscopically (P < 0.05) [Table 1], [Figure 2] and [Figure 3]. A significant improvement in tissue levels of CAT, SOD (P < 0.05) was also seen [Table 2]. While a significant decrease in the levels of MPO, MDA was observed as (P < 0.05) compared to experimental control [Table 2].

However, rats treated with standard drug 5-ASA, showed significant improvement in all parameters as compared rats receiving T. stans extract (P < 0.05). The 5-ASA showed near normalization of DAI and macroscopic and microscopic scores [Table 1], [Figure 4].

Table 1. Effect of Tecoma stans leaves on acetic acid induced colitis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Macroscopic Score</th>
<th>DAI</th>
<th>Microscopic Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (A)</td>
<td>0±0</td>
<td>0.54±0.02</td>
<td>0±0</td>
</tr>
<tr>
<td>Experiment Control (B)</td>
<td>4.68±0.09*</td>
<td>1.74±0.02*</td>
<td>4.44±0.06*</td>
</tr>
<tr>
<td>Test Drug (C)</td>
<td>1.19±0.02**</td>
<td>0.90±0.01**</td>
<td>2.48±0.10**</td>
</tr>
<tr>
<td>Standard (D)</td>
<td>1.41±0.01**</td>
<td>0.68±0.06**</td>
<td>1.42±0.09**</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>F: 988.2</td>
<td>214.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Df: 3.16</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P: &lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values repressed as mean±SEM (n=5), *P < 0.05 when compared to normal control **P<0.05 when to experimental control, ANOVA followed by Dunnet’s test, DAI= Disease activity index, ANOVA=Analysis of variance
Table 2. Effect of Ethanolic extract of *Tecoma stans* on biochemical parameters in acetic acid induced colitis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tissue MPO (U/g)</th>
<th>Serum MDA (nmol/ml of serum)</th>
<th>Tissue CAT (µmol/min/mg of protein)</th>
<th>Tissue SOD (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (A)</td>
<td>0.33±0.02</td>
<td>3.51±0.12</td>
<td>396.18±0.84</td>
<td>7.30±0.05</td>
</tr>
<tr>
<td>Experiment Control (B)</td>
<td>4.12±0.06*</td>
<td>5.49±0.03*</td>
<td>143.6±0.35*</td>
<td>2.64±0.12*</td>
</tr>
<tr>
<td>Test Drug (C)</td>
<td>0.96±0.02**</td>
<td>2.24±0.05**</td>
<td>293.2±1.34**</td>
<td>5.09±0.01**</td>
</tr>
<tr>
<td>Standard (D)</td>
<td>0.90±0.01**</td>
<td>3.01±0.19**</td>
<td>327.6±3.84**</td>
<td>6.18±0.06**</td>
</tr>
</tbody>
</table>

ANOVA

0000F 2020 150 2570 447

Df | 3, 16 | 3, 16 | 3, 16 | 3, 16 |

P  | <0.05 | <0.05 | <0.05 | <0.05 |

Values represent as mean±SEM (n=5). *P <0.05 when compared to normal control **P<0.05 when to experimental control, ANOVA followed by Dunnet’s test, ANOVA=Analysis of variance, SOD=Superoxide dismutase, MPO=Myeloperoxidase, MDA= Malondialdehyde, CAT=Catalase.

DISCUSSION AND CONCLUSION

Acetic acid induced colitis model is similar to human UC in terms of histological features. It affects the distal colon portion and induces non-transmural inflammation, massive necrosis of mucosal and submucosal layers, mucosal edema, neutrophil infiltration of the mucosa and submucosal ulceration. The protonated form of the acid liberates protons within the intracellular space and causes massive intracellular acidification resulting in massive epithelial damage. Inflammation is the pathogenesis of IBD, and several pathways are associated with inflammatory response in IBD due to mucosal intestinal flora. [18].

MPO is an enzyme mainly found in azurophilic granules of neutrophils. It can serve as a good marker of inflammation, tissue injury and neutrophil infiltration in gastrointestinal tissues. Pre-treatment with *T.stans* exhibits...
decrease in polymorphonuclear infiltration demonstrated by significant reduction in MPO activity. Oxidative damage may represent crucial pathogenic factor in IBD because intestinal inflammation is accompanied by increased production of reactive oxygen and nitrogen species.

Oxidative stress is believed to play a key role in the pathogenesis of IBD-related intestinal damage [19]. Intestinal mucosal damage in the IBD is related to both increased free radical production and a low concentration of endogenous antioxidant defense. [20].

MDA is considered as an important indicator of lipid peroxidation, [21] which is found to be increased in rats treated with acetic acid. This might be due to lipid peroxidation. Rat pretreatment with T.stans showed protection against lipid peroxidation characterized by significant decrease in MDA level.

The antioxidant enzymes, mainly SOD and CAT are first line defensive enzymes against free radicals and, ascorbic acid is also known to control oxidative damage [22]. In the present study, it was observed that the T.stans extract significantly increases antioxidant parameters (CAT and SOD) in colitis induced rats. This shows that the T.stans extract can reduce reactive free radicals that might lessen oxidative damage to the tissues.

It is well known that flavonoids are natural antioxidants with significant increase SOD and CAT activities. The elevated levels of SOD and CAT after administration of T.stans extract could be due to the presence of flavonoid. This plant also contains phytochemicals like alkaloids, essential oils, vitamin C, sterol which possesses antioxidant activity. [23] Flavonoids are also reported to possess anti-inflammatory activity [24]. These phytochemicals could be involved in the prevention of the progression of the disease.

Thus it can be concluded that, leaf extract of T.stans possess protective action in experimentally induced IBD that may be due to antioxidant activity.

REFERENCES