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Pharmacological evaluation of Phyllanthus maderaspatensis on acetic acid induced ulcerative colitis in rats

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ABSTRACT

Objective: The present work is aimed to evaluate the hydroalcoholic extract of *Phyllanthus maderaspatensis* (HEPM) against acetic acid induced Ulcerative Colitis (UC) in rats.

Methods: Male wistar rats (150–200 g) were divided into five groups: normal control group animals received normal saline for 8 days, disease control group animals received normal saline for 8 days and acetic acid (1ml of 4% v/v) on day 4 via rectally, standard group animals received sulfasalazine 500 mg/kg orally and acetic acid (1ml of 4% v/v) on day 4 via rectally, test group I and test group II animals received the HAEPM at doses of 200 mg/kg and 400 mg/kg bw respectively orally for 8 days and acetic acid (1ml of 4% v/v) on day 4 via rectally. At the end of study, Disease severity was assessed using the Disease Activity Index (DAI), macroscopic scoring, estimation of biochemical parameters (SOD, GSH, MDA, MPO), and histopathology of colon tissue were performed

Results: Disease control group rats showed the significant weight loss, diarrhea, rectal bleeding, and significant increase in oxidative stress markers (MDA, MPO) while significant reduction in antioxidant levels (SOD, GSH). HAEPM treated rats, particularly at 400 mg/kg, significantly mitigated these effects through improvement in DAI scores, Reduced weight loss, stool consistency abnormalities, and rectal bleeding, macroscopic scoring and normalization of anti – oxidant parameters. Histopathological examination revealed that HAEPM preserved mucosal integrity, reduced edema, comparable to disease control animals. The findings suggest that HAEPM exerts protective effects against Ulcerative colitis through its antioxidant mechanisms, likely due to abundant presence of polyphenolic compounds

Conclusion: Hydroalcoholic extract of *Phyllanthus maderaspatens* is demonstrated significant efficacy in alleviating acetic acid-induced colitis in rats, supporting its potential as a natural therapeutic agent in the treatment of ulcerative colitis. Further studies are needed to validate its post-treatment benefits and explore its efficacy in other experimental colitis models mimicking human IBD.

Keywords: *Ulcerative Colitis, Phyllanthus maderaspatensis, Anti –oxidants..*

1. INTRODUCTION

One of the two main types of inflammatory bowel disease (IBD), ulcerative colitis (UC) is a persistent, recurrent, and remitting inflammatory disorder that affects the gastrointestinal tract [1]. It is generally accepted that a combination of environmental stimuli and genetic susceptibility results in an overactivation of the mucosal immune system, which leads to persistent intestinal inflammation [2] even if the precise molecular basis and pathophysiology of ulcerative colitis remain unknown. As there is currently no specific treatment for IBD, controlling the oxidant/antioxidant balance is the most successful course of treatment.

The plant based treatment for UC has showed potential effects. *Phyllanthus maderaspatensis* (or) Madras leaf flower have number of therapeutic benefits, including as antibacterial, anti- microbial, anti-Cataleptic, other medicinal potentials such as deobstuent stomachic, astringent, febrifugal, diuretic & Antiseptic. In India the whole plant is used against kidney Urinary tract infections and digestive disorders. Some of the potential activities of the plant are antihistaminic, anti-inflammatory, antimicrobial, immunomodulatory activity, cytotoxic activity etc. alkaloids, glycosides, flavonoids & phenols, among the plant's Strong bioactive Components [6].

2. MATERIALS AND METHODS

2.1 Collection of plant

Phyllanthus maderaspatensis L was collected in the premises of Raghavendra Institute of Pharmaceutical Education and Research (RIPER) and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, SV University Tirupathi, Andhra Pradesh. The aerial parts are dried under shady conditions. The dried plant leaves are powdered

2.2 Extraction procedure

The plant material was shade-dried and then crushed into a powder using a mechanical grinder. The powdered material was stored in a air tight container .The powdered plant material of *Phyllanthus maderaspatensis* can be extracted by using soxhlet apparatus .

In this method 1:5 ratio powder and hydroalcohol (ethanol + water 125ml each) was taken for soxhlet process.50gm of *Phyllanthus maderaspatensis* was taken and kept in thimble and added 250ml of hydroalcohol into a round bottom flask and continued for extraction.Collected the extract and evaporated the solvent by using rotary evaporator at 65 degree centigrade [7]

2. 3. Preliminary phytochemical analysis of *Phyllanthus maderaspatensis extract*

Hydroalcoholic extract of *P. maderaspatensis* was subjected to chemical test for identification of alkaloids, steroids, tannins, phenols, proteins, glycosides, saponins, carbohydrates, flavonoids, amino acids [8]

2. 4. Animlas: Male wistar rats were obtained from Vyas labs, Hyderabad and maintained as of CCSEA guidelines and protocol was approved by institutional animal ethics committee of Raghavendra Institute of Pharmaceutical Education and Research (RIPER) with approval no. IAEC/VII/08/RIPER/2025.

2. 5. Experimental Design

All the animals were divided into 5 groups with six animals in each group and received the below mentioned treatment.

Group I: animals received normal saline orally for 8 days

Group II: animals received normal saline (0.9% w/v) p. o for 8 days and 1 ml of 4.0% v /v acetic acid intrarectally on day 4.

Group III: animals received Sulphasalazine 500 mg/kg p. o. for 8 days and 1 ml of 4.0% v /v acetic acid intrarectally on day 4.

Group IV: animals received the HEPM 200mg/kg, orally for 8 days and 1 ml of 4.0% v /v acetic acid intrarectally on Day 4.

Group V: animals received the HEPM 400mg/kg, orally for 8 days and 1 ml of 4.0% v /v acetic acid intrarectally on Day 4.

The ulcerative colitis activity was evaluated after completion of the treatment period. The animals were sacrificed and 9 cm of distal colon was excised. It was flushed with saline to remove faecal matter. The ulcerative colitis was diagnosed by recording of clinical score, morphological changes in colon.

2. 6. Determination of disease activity index:

The disease activity index (DAI), which uses a scoring system to assess weight loss, stool consistency, and rectal bleeding, was used to quantify disease activity. Every day, the following metrics were noted: Diarrhea (0: normal, 1: soft stool but still formed, 2: very soft stool, 3: mild diarrhea and 4: severe diarrhea), body weight loss (0: none, 1: 1–5%, 2: 6–10%, 3:11–20%, and 4: >20%), and rectal bleeding (0: normal, 1: positive hemoccult, 2: blood traces in stool visible, 3: mild bleeding, 4: severe bleeding). DAI values were computed using the mean values of those parameters [10]

2.7. Determination of macroscopic activity score:

For macroscopic evaluation, the excised colons were placed on white sheet and photographed with a camera. Image J software was used to measure the lesions in each colon as a percentage of total surface area. Each colon was also weighed and measured to determine the weight- to length rations (mg/kg), and Wallace score were used to assess macroscopic damage.

The Wallace scoring system is a semiquantitative scoring system that takes into account the area of inflammation and the presence or absence of ulcers as follows: 0, no ulcer or inflammation; 1, no ulcer with local hyperemia; 2, ulceration without hyperemia; 3, ulceration and inflammation at one site only; 4, two or more sites of ulceration and inflammation; 5, ulceration extending more than 2cm.[11]

2. 8. Estimation of oxidative stress parameters in the isolated colon tissue:

A cold 0.9% w/v saline solution was used to clean the colon of each rat after it had been dissected. After that, the colon was weighed and homogenized in cold phosphate buffer (0.1M, pH 7.4) using a Remi homogenizer. To achieve a 10% w/v concentration, the homogenization process was finished quickly in cold circumstances. After centrifuging the resulting homogenate for ten minutes at 1000 rpm, the supernatant was carefully gathered. superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and myeloperoxidase (MPO) were measured in the supernatant [12].

3. HISTOPATHOLOGY:

Five micrometer-thick slices of the colon were cut after it had been preserved in 10% formalin and covered with paraffin wax. These slices were stained with hematoxylin and eosin dye to aid in histological analysis [13].

4.1 Statistical analysis:

For every six-animal group, the findings were shown as Mean ± SD. For statistical analysis, Graph Pad Prism V 10.2.1 was utilized. Dunnett's multiple comparison test was applied following the completion of a one-way analysis of variance (ANOVA). A P-value of less than 0.05 was deemed statistically significant.

4. RESULTS AND DISCUSSION

Phytochemical analysis

Hydroalcoholic extract of *Phyllanthus maderaspatensis* showed presence of alkaloids, flavonoids and glycosides.

Effect of HEPM against disease activity index

Body weight, stool consistency, and rectal bleeding in the colon is significantly altered by acetic acid in comparison to normal animals ($p<0.0001$, $p<0.0001$, $p<0.0001$). HEPM treated animals at the dose of 200mg/kg, 400mg/kg body weight treated animals showed the significant improvement body weight ($p<0.0001$), significant decrease in stool consistency and rectal bleeding ($p<0.0001$) in dose dependent manner in comparison to acetic acid treated animals (Figure 1)

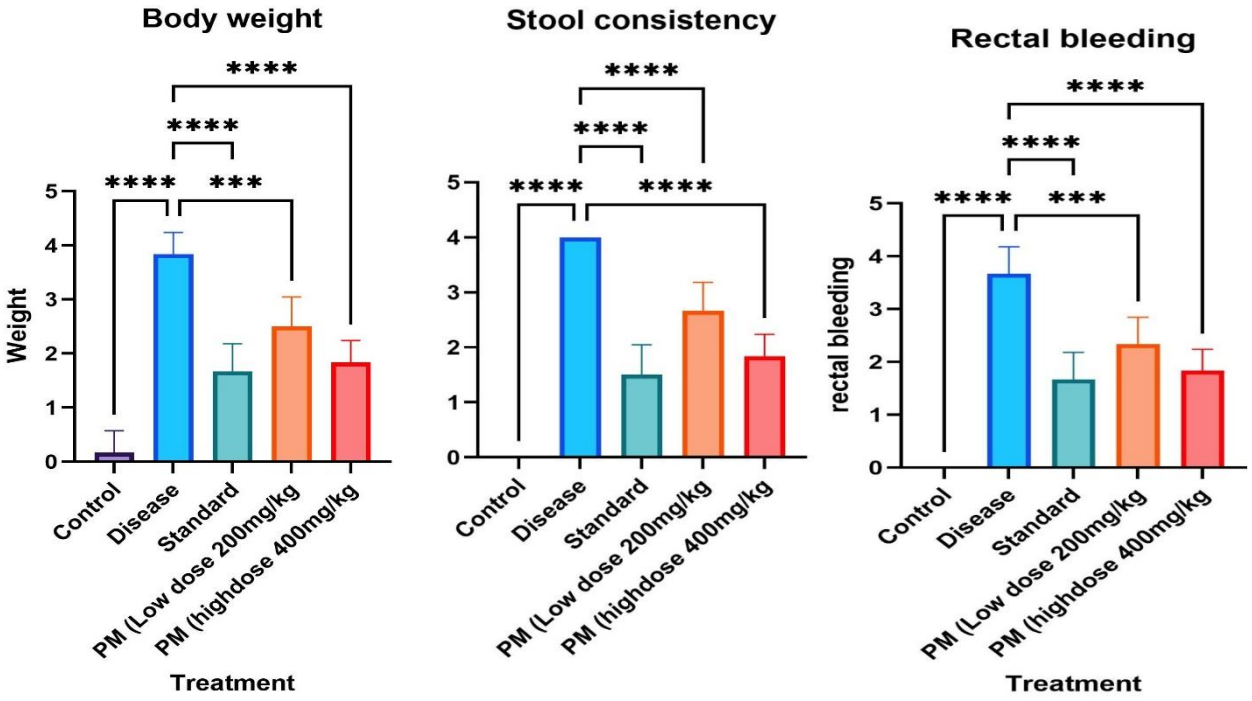


Figure 1: Effect of Hydroalcoholic extract of *Phyllanthus maderaspatensis* against disease activity index.

Table 1: Effect of Hydroalcoholic extract of *Phyllanthus maderaspatensis* against macroscopic inflammation

Groups	Macroscopic score inflammation
Normal	0.00±0.00
Disease	7.00±0.3651****
Sulfasalazine	1.500±0.223
PM low dose (200mg/kg)	3.500±0.223**
PM high dose (400mg/kg)	1.833±0.307***

All values are expressed as mean±SEM and P<0.0001 vs normal; p<0.01 vs disease; p<0.001 vs disease respectively.

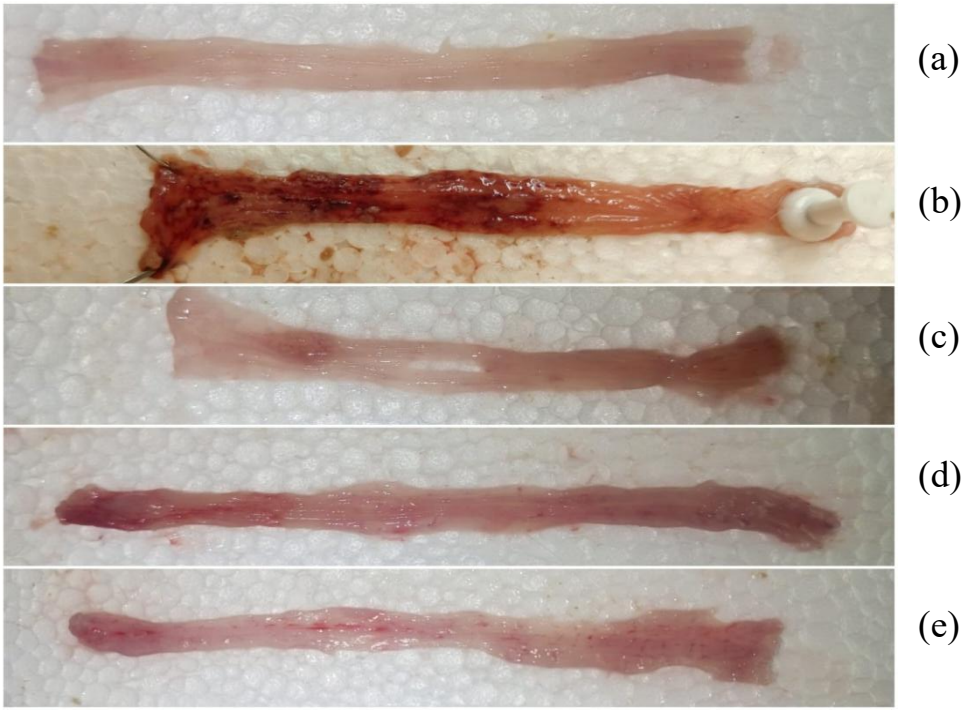


Figure 2: Effect of Hydroalcoholic extract of Phyllanthus maderaspatensis against macroscopic inflammation a: Control group; b: UC group; c: Sulfasalazine; d: HEPM (200 mg/kg) e: HEPM (400 mg/kg)

Effect of HEPM against macroscopic activity score

Significant increase in macroscopic inflammation in the colon was observed in the acetic acid treated animals in comparison to normal control animals (p<0.0001). Rats received HEPM at the dose of 200mg/kg, and 400mg/kg body weight showed the significant decrease in macroscopic inflammation in comparison to acetic acid treated animals (P<0.01, p<0.001 respectively) in comparison to acetic acid treated animals.

Effect of HEPM against myeloperoxidase (MPO) activity

Significant increase in MPO levels were observed in acetic acid treated animals in comparison to normal animals (p<0.0001). HEPM received animals at the dose of 200, 400mg/kg body weight showed the significant decrease in MPO levels (p<0.001, p<0.0001 respectively) in comparison to acetic acid received animals.

Effect of HEPM against oxidative stress parameters

Significant decrease in anti – oxidants (SOD, GSH) and significant increase in MDA levels were observed in the acetic acid treated animals (p<0.0001, p<0.0001 and p<0.0001) in comparison to normal animals.

HEPM received animals at the dose of 200, 400mg/kg body weight showed the significant increase in SOD (p<0.05, p<0.0001 respectively) GSH (p<0.05, p<0.0001 respectively). HEPM received animals at the dose of 200, 400mg/kg body weight showed the significant decrease in MDA levels (p<0.01, p<0.0001 respectively).

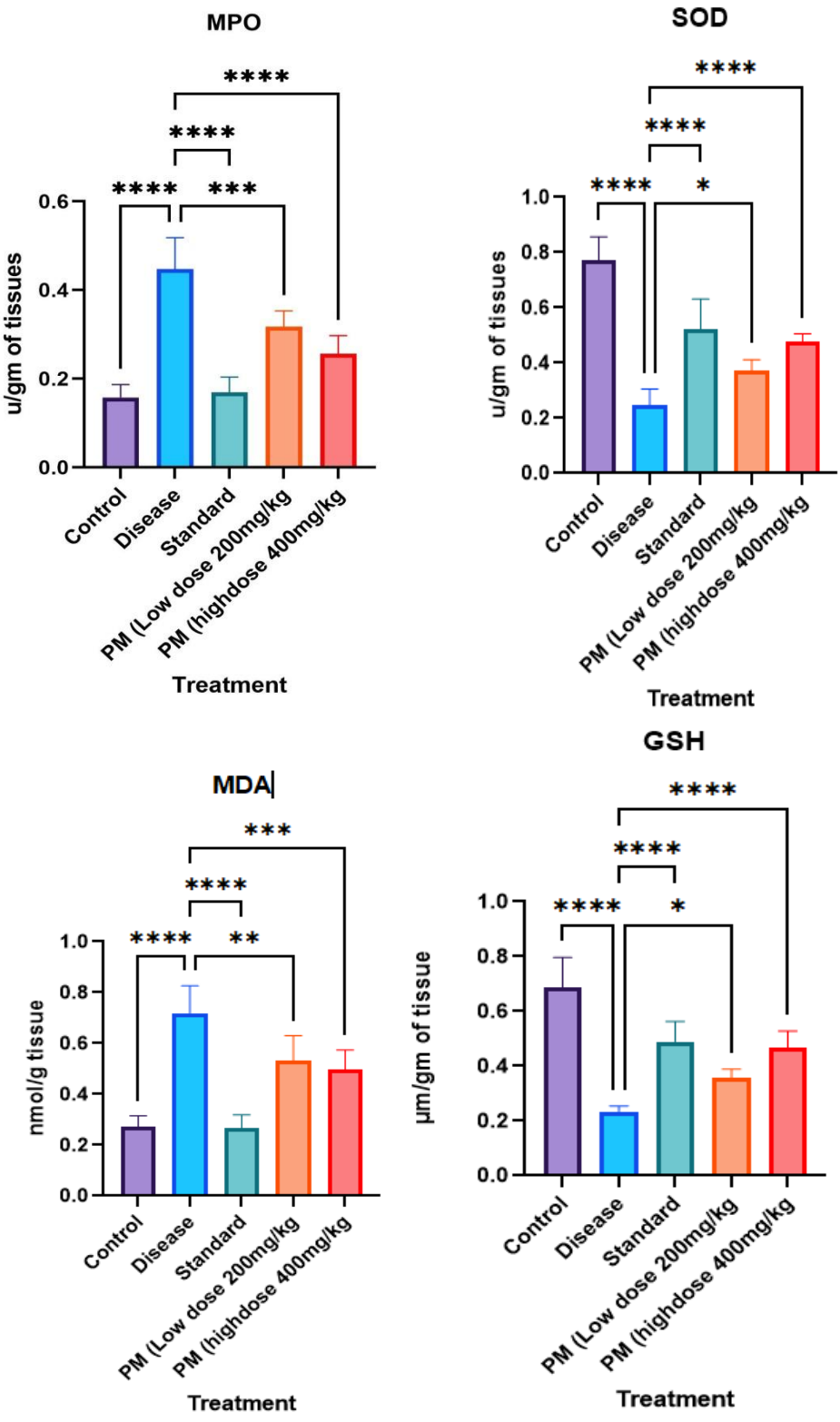


Figure 3: Effect of HEPM against MPO, SOD, GSH and MDA.

Histopathology:

The section of these colon tissue in normal group of rats showing architecture (10X magnification).

Histopathological analysis of all groups in a rat model of ulcerative colitis showed significant differences.

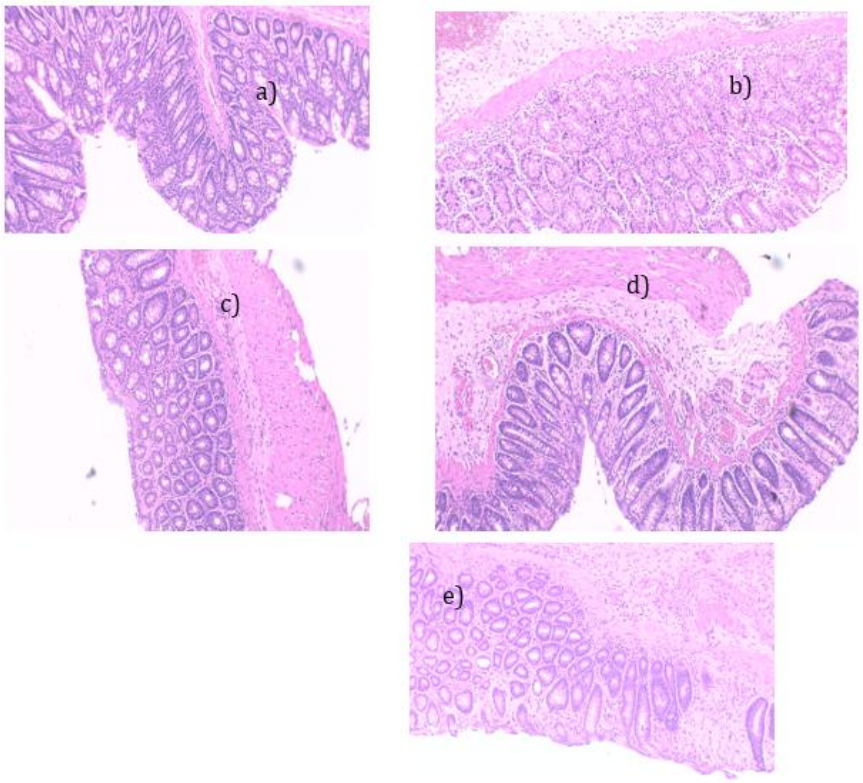
a. Control: Intact epithelium, neatly arranged the crypts, no inflammation.

b. UC Group: Transmural necrosis, submucosal edema, areas of haemorrhages and inflammatory cellular infiltration.

c. Sulfasalazine Group 500mg/kg: There is moderate improvement in mucosal structure with partially restored in the crypts cells.

d. HEPM 200mg/kg: Showing minimal damage of the mucosa with slight submucosal edema, mild inflammatory cell infiltration.

e. HEPM 400mg/kg: Showing normal colonic mucosa.



5. CONCLUSION

The outcomes of the study showed that hydroalcoholic extract of *Phyllanthus maderaspatensis* is capable of protecting male wistar rats from the ulcerative colitis induced by acetic acid in dose dependent manner by inhibiting the disease activity index, macroscopic inflammation, inflammatory marker, myeloperoxidase and by restoring the anti – oxidant parameters. The protective of hydroalcoholic extract of *Phyllanthus maderaspatensis* against ulcerative colitis due to presence of flavonoids and phenolic compounds.

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