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EXPERIMENTAL EVALUATION OF TENDER LEAVES OF BHUMIJAMBU – SYZYGIUM CARYO-PHYLLATUM (L.) ALSTON IN ULCERATIVE COLITIS

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ABSTRACT

Introduction: Traditional knowledge is a part of Ayurvedic science which is gaining importance among many known and unknown subjects of the ancient era. Ayurveda is known to be the treasure of medicine and it always Enlighted a path for acceptance of new drugs and their actions from all possible sources. *Bhumijambu-Syzygium caryophyllatum* (L.) Alston is one such plant (especially the tender leaves) which is extensively used by the folk-lore practitioners of Udupi district Karnataka for watery blood mixed stools. On this account, the present research work has been carried out to evaluate the anti-ulcerative colitis effect of a tender leaf of *Bhumijambu-Syzygium caryophyllatum* (L.) Alston. **Methodology:** Tender leaves of *Bhumijambu-Syzygium caryophyllatum* (L.) Alston was collected from in and around Udupi before the flowering season, morphological features compared with that of local flora. The effect on the disease ulcerative colitis was assessed in the acetic acid-induced ulcerative colitis animal model (Swiss Albino Mice). **Result and Discussion:** The experimental study has shown that it has moderate efficacy in the disease ulcerative colitis. **Conclusion:** The experiment has shown a tender leaf of *Bhumijambu-Syzygium caryophyllatum* (L.) Alston is having a moderate effect on the disease ulcerative colitis. The efficacy can be improved with further refining of the formulations.

Keywords: Acetic acid-induced model, Traditional healers, *Bhumijambu*, Ulcerative colitis

INTRODUCTION

The evidence for experimental practice in animals can be seen in history and they had played an important role in medical research since centuries. Ulcerative colitis is an inflammatory disease of the colon with an unknown etiology that clinically manifests with rectal bleeding, diarrhoea, abdominal pain, and weight loss. Recent reports have shown that there is a steady increase in the global incidence of ulcerative colitis. The therapeutic effects of herbal or plant extracts have been well known for centuries and identifying and characterizing these components can provide alternative treatment options for ulcerative colitis patients. Given some hint of efficacy, it is important to understand the underlying mechanisms behind the prospective therapeutic effects of these herbs and plant extracts by using ulcerative colitis models [1]. Hence an attempt is made to know the efficacy of *Bhumijambu – Syzygium caryophyllatum* (L) Alston in acetic acid-induced ulcerative colitis in Swiss Albino Mice.

Materials and Methods:

Procedure: 30 Swiss Albino mice weighing 30-40g were selected and grouped into 5 different categories. Ulcerative colitis was induced except for the control group by acetic acid administration in mice as described by Martin Gollet et al (1997) with some modification. The initial bodyweight of the selected animals was taken, and the drug (test and standard) was administered for 7 consecutive days. Thereafter on the 4th day after drug administration, Swiss Albino mice were isolated and kept in metabolic cages under fasting and only drinking water was provided. On the 5th day after 1 hour of the drug, administration mice were anesthetized with Ketamine Xyaline and a polypropylene tube with a 2mm diameter was inserted 4cm into the colon. A solution of 0.1mL of acetic acid (3%) in 0.9% saline was instilled into the lumen of the colon. Again, the mice were isolated in metabolic cages for 48 hours. On the 6th day, mice were dosed as usual. On the 7th day, 1 hour after drug administration mice will be sacrificed under deep ether anesthesia followed by excision of the colon, ulcers were scored using a magnifying lens. At last, a part of the colon was transferred to 10% formalin for histopathologic examination. Section of 5µm thickness of tissue was prepared using a microtome and stained with hematoxylin and eosin for microscopic observations. All slides were then evaluated under a light microscope (ZEISS Axio lab A1 India) [2].

1. The standard drug- Sulfasalazine DR:
2. The test drug -*Bhumijambu* (Tender leaves of *Syzygium caryophyllatum)*

Animal grouping- As per Table 1

Table 1: Animal grouping

Group	Intervention	Drug
1	Control	Nil
2	Positive control	Nil (Ulcer induced)
3	Standard group	Sulfasalazine DR (Ulcer induced)
4	Test I	1/10 of LD50 (Ulcer induced)
5	Test II	1/5 of LD50 (Ulcer induced)

Test Drug Preparation: For the extraction of *Bhumijambu* the process followed was cold maceration.

Test Drug Dose: According to an AOT study based on OECD guidelines 425-Table 2&3 (According to AOT study LD50 is more than 2000 mg/kg)

Table 2: Calculated dose of aqueous extract of *Bhumijambu* of trial 1 group

Mice	**Bodyweight(g)**	**Dose of test 1 as per body weight (ml)**
Head	32	0.32
Neck	39	0.39
Body	30	0.3
Tail	30	0.3
Forelimb	31	0.31
No mark	31	0.31

Table 3: Calculated dose of aqueous extract of *Bhumijambu* of trial 2 group

Mice	**Bodyweight(g)**	**Dose of test 2 as per body weight (ml)**
Head	30	0.3
Neck	30	0.3
Body	35	0.35
Tail	31	0.31
Forelimb	39	0.39
No mark	39	0.39

RESULT:

Ulcer Macroscopic Evaluation:

P value is 0.8912 Nonsignificant

Control v/s Test 2 ns P>0.05

Control v/s Test 1 ns P>0.05

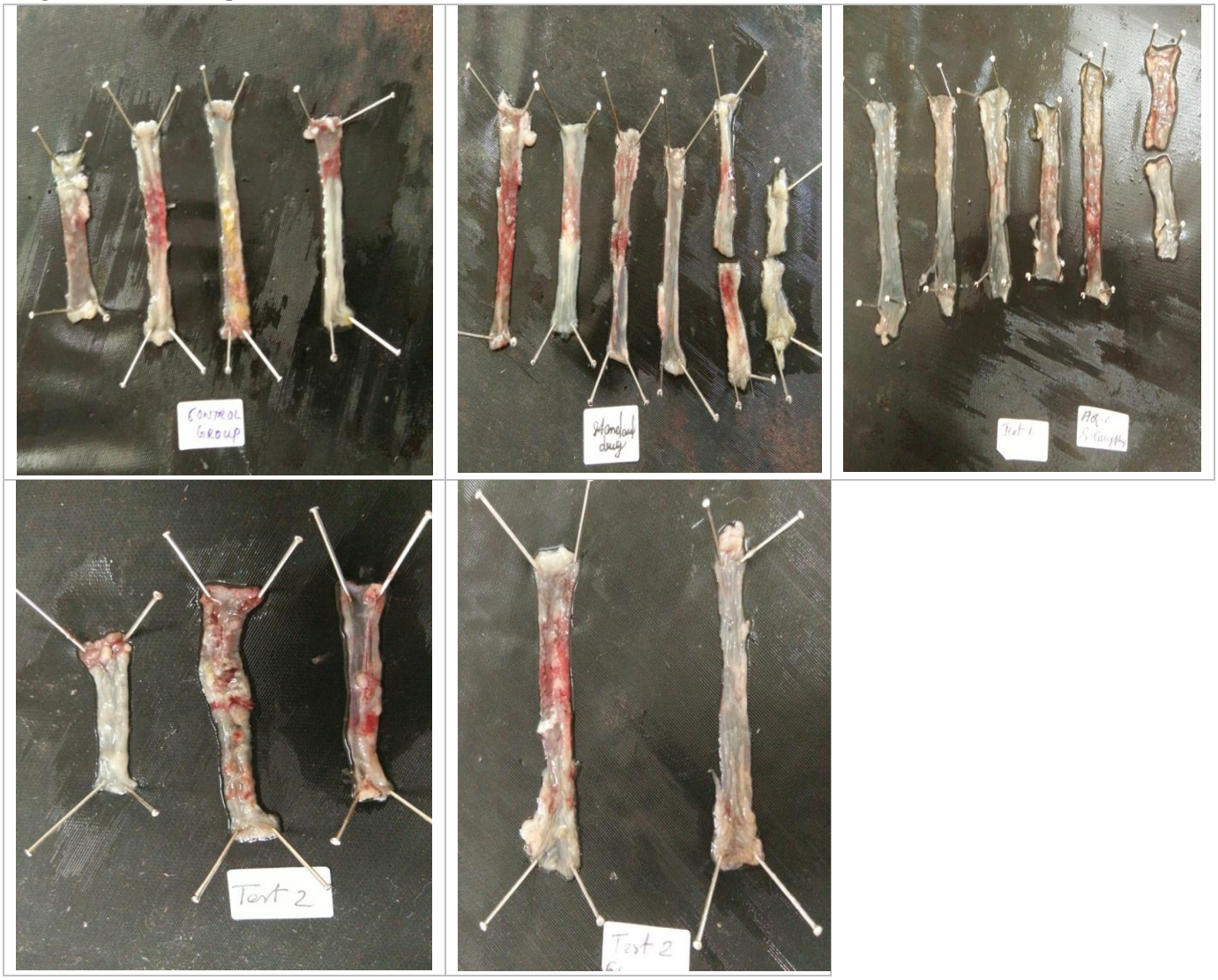
Control v/s Standard ns P>0.05

Table 4: Showing result of the macroscopic evaluation of ulceration (Ulcer score)

Name	±SEM
Control	42±9.30
Standard	28.33±12.15
Test 1	35.62±10.79
Test 2	36±14.33

Note: SEM- Standard Error Mean

Figure 1: Macroscopic view of ulcerative colitis



Histopathology:

Figure. 2: Histopathology result of Group 1- Normal control

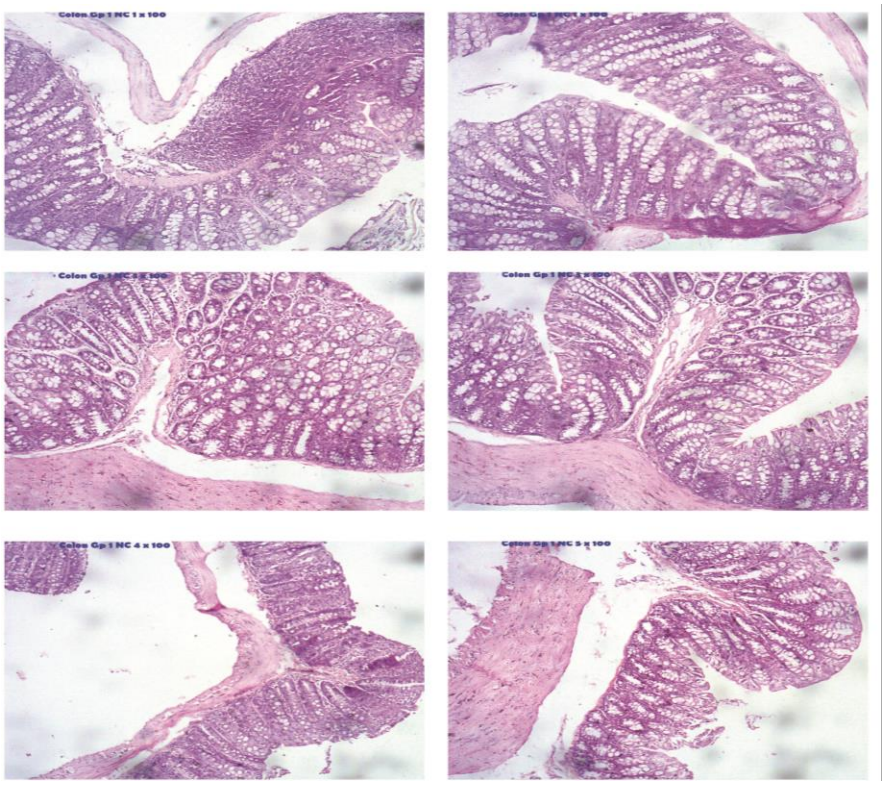


Figure 3: Histopathology result of Group 2- Positive control

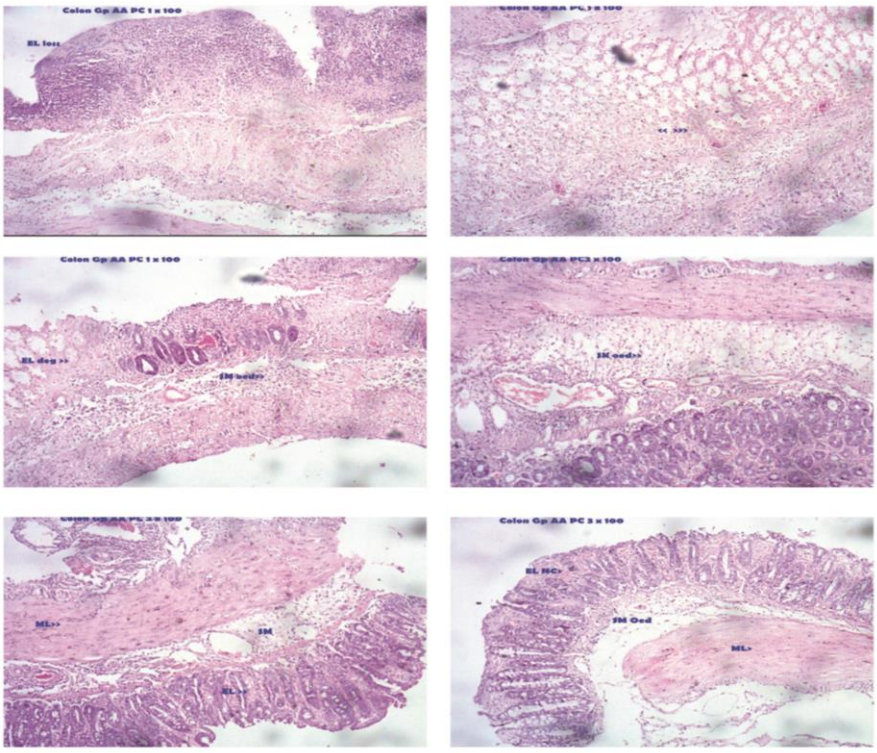


Figure 4: Histopathology result of Group 3- Reference standard

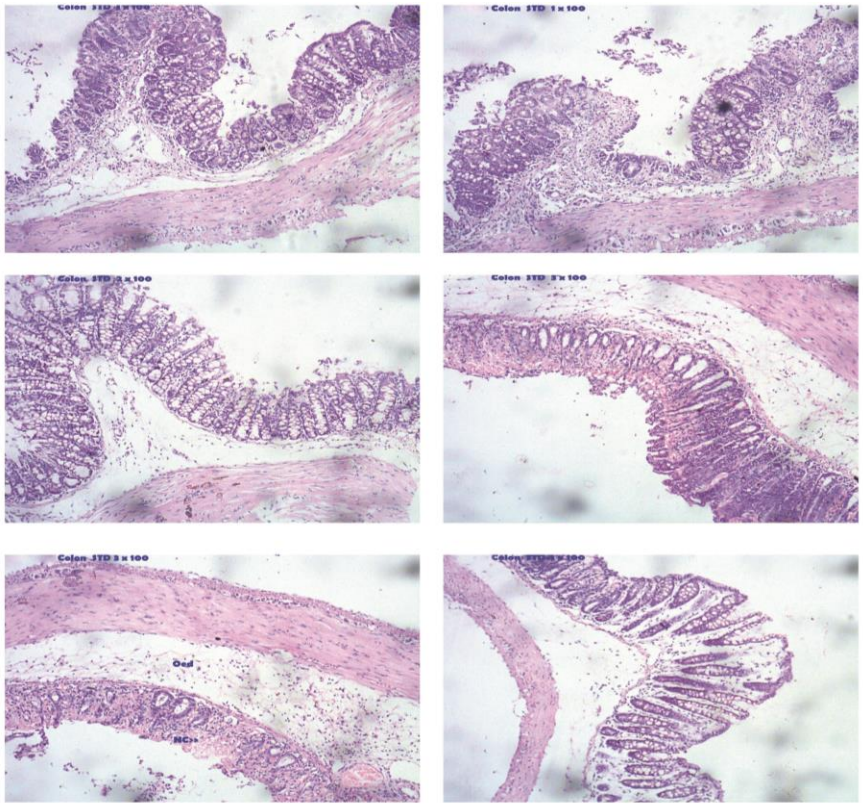


Figure 5: Histopathology result of Group 4- Test 1

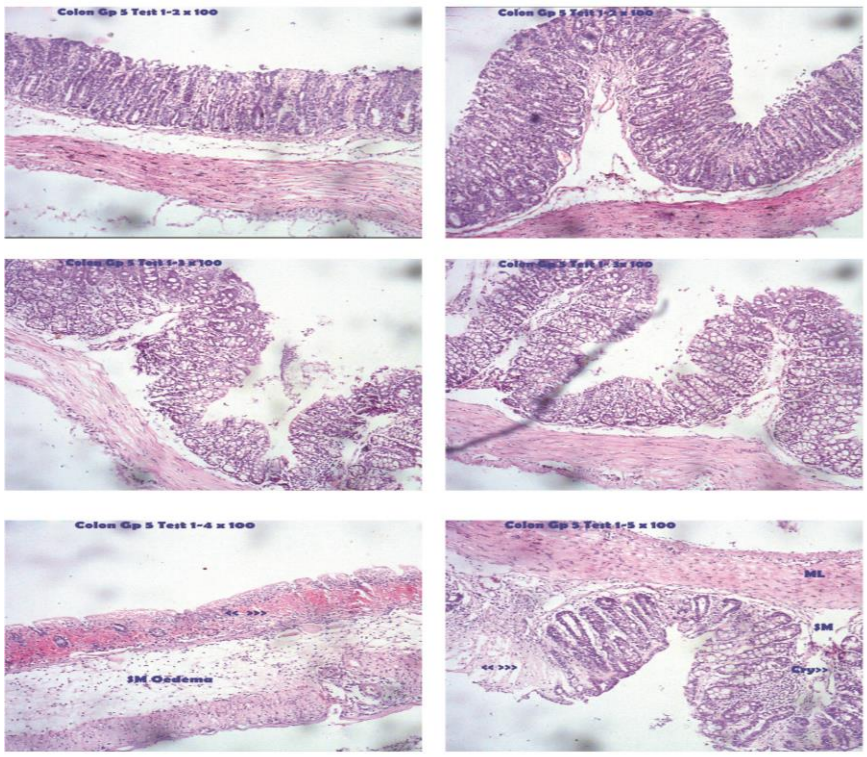
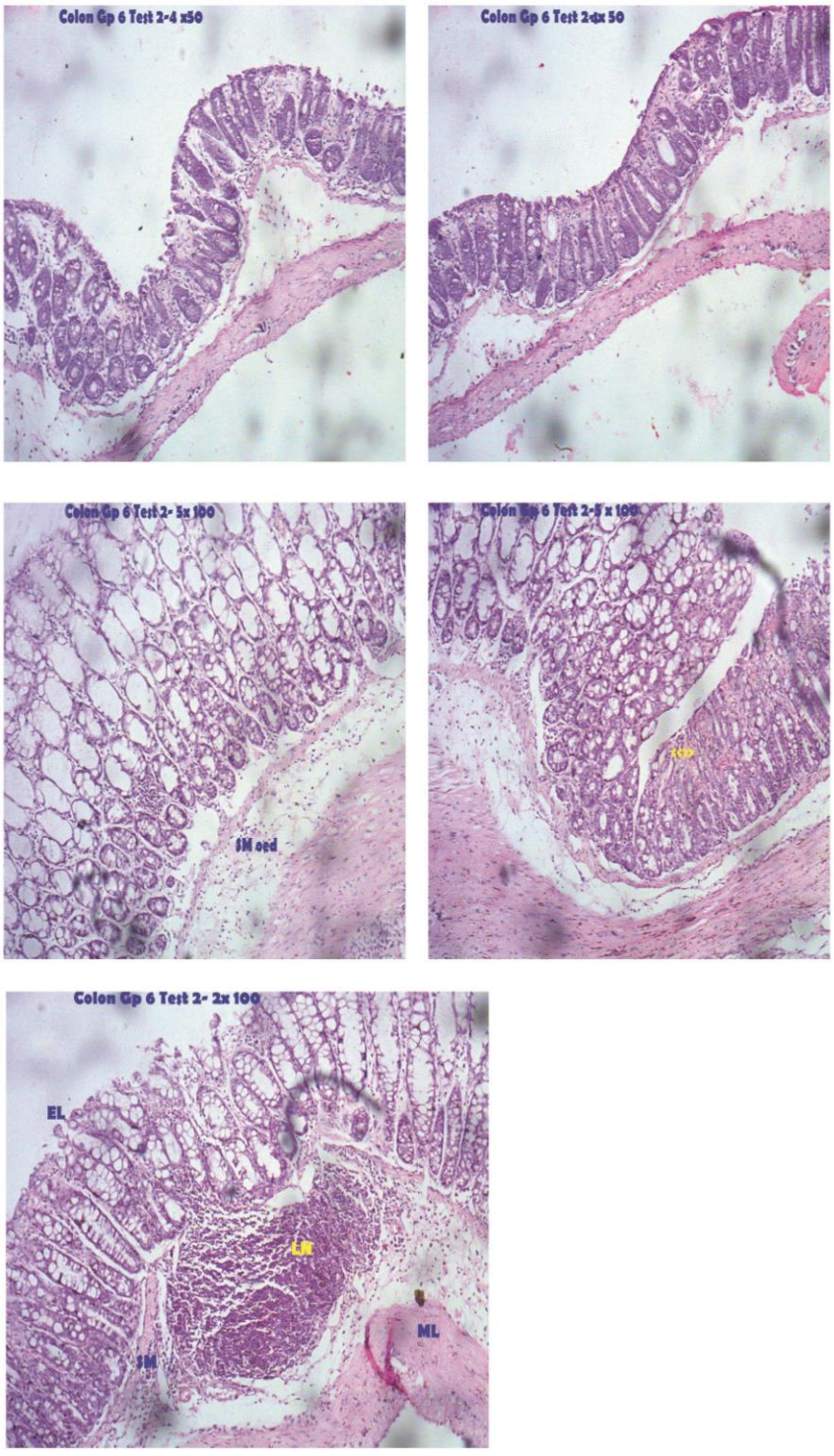


Figure 6: Histopathology result of Group 5- Test 2



DISCUSSION

The objective of the present study was to evaluate the efficacy of tender leaves of *Bhumijambu* in Ulcerative colitis in an acetic acid-induced ulcerative colitis model of Swiss albino mice. The efficacy of the test drug was assessed by the macroscopic evaluation of the ulcer scores and histopathological examination of the affected parts. Analysis of the macroscopic

changes in terms of ulcer score shows an apparent decrease in the severity of ulceration. However, due to within-group differences in the severity of ulceration, the observed effect did not reach a statistically significant level. It is to be noted that within each group including reference standard, few mice showed good protection, some moderate and some only mild protection. Further mortality was noted in the positive control, perhaps due to severity or perforation of the ulcerated area, which was not seen in any animals in either test drug or reference standard administered group. Sections of the colon from normal control exhibited normal profile, epithelium well-formed, cryptal arrangement intact, sub-mucosa, and muscular layers normal. Sections from three mice from the acetic acid (positive control) control group exhibited marked degenerative changes in the form of epithelial denudation, severe necrosis, loss of cryptal arrangement, moderate to marked sub-mucosal oedema and the cell infiltration extending even to the muscular layer in the remaining two mice. In colon sections from CH3COOH (Buffer) control-2 group in one mouse, severe degenerative changes were observed, and moderate to severe degenerative changes were observed in sections from two mice. In reference standard group- the degenerative changes were mild with intact general cytoarchitecture, cryptal arrangement, moderate oedema in sub-mucosa, and normal muscular layer in two mice; sections from three mice exhibited mild to moderate degenerative changes while in one rat moderate to severe degenerative changes were observed- indicating moderate to good protection in the majority of the mice. In sections from Test 1 group-almost normal cytoarchitecture with mild necrosis was observed in sections from three mice, in three mice moderate degenerative changes were observed, and in the section from one rat moderate to severe degenerative changes were observed- the inference is good cytoprotection in this group. In sections from Test 2 group-almost normal cytoarchitecture with mild necrosis was observed in sections from two mice, in four mice moderate degenerative changes were observed - the inference is good cytoprotection in this group. Histological examination showed different degrees of cytoprotection which ranged from mild to moderate in both the test drug and reference standard administered groups. Since histological examination provides good evidence for the efficacy or otherwise of a test product it can be suggested that the test drugs do possess at least moderate cytoprotective activity against acetic acid-induced colitis.

CONCLUSION

Traditionally the drug *Bhumijambu* is considered an effective remedy against Ulcerative colitis. Analysis of the macroscopic changes in terms of ulcer score shows an apparent decrease in the severity of ulceration. Histological examination showed different degrees of cytoprotection which ranged from mild to moderate in both the test drug and reference standard administered groups. The experiment has shown a tender leaf of *Bhumijambu-Syzygium caryophyllatum* (L.) Alston is having a moderate effect on the disease ulcerative colitis. The efficacy can be improved with further refining of the formulations.

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