WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 5, 1323-1334. Research Article ISSN 2277– 7105

DESIGN, FORMULATION AND EVALUATION OF HERBAL RECTAL SUPPOSITORIES FOR THE TREATMENT OF COLITIS

Vaishali Potnis*, Komal Navale, Pooja Ghule and Prachi Takbhate

Jayawantrao Sawant College of Pharmacy and Research, Pune (India).

Article Received on
25 Feb. 2022,

Revised on 15 March 2022,
Accepted on 05 April 2022

DOI: 10.20959/wjpr20225-23764

***Corresponding Author**
Vaishali Potnis
Jayawantrao Sawant College of Pharmacy and Research, Pune (India).

ABSTRACT

Rectal suppositories of ethanolic extract of unripe fruits of *Aegle marmelos* L. Corr. family Rutaceae were prepared for the treatment of colitis. The medicated rectal suppositories formulations composed of *Aegle marmelos* Extract (AME): PEG 6000: Softisan 378 and *Aegle marmelos* Extract: Witepsol W-45. Both placebo and medicated rectal suppositories were characterized for Physical, physicochemical and Organoleptic, dimensions, surface pH, hardness, melting range, liquefaction temperature, liquefaction time, content of drug, dissolution profile *(in- vitro)*. Moreover, the medicated rectal suppositories were characterized for anti-inflammatory efficacy (in-vivo) by using acetic acid induced colitis model. Medicated rectal suppositories possessed good hardness (3 and 4.5 kg/cm2), disintegration time (10-12 and 9-10 min), liquefaction time (10-12 min) and temperature (40-42 and 37-41 0C). Both these formulations also retained >95% AME and demonstrated complete release of contents of AME over a period of 1 h. and remained stable with no gross change in any of the physico-chemical characteristics.

KEYWORDS: Colitis, Anti-inflammatory effect, Medicated Herbal Rectal suppositories.

INTRODUCTION

Colitis is an inflammation of the inner lining of colon and can be associated with diarrhea, abdominal pain and blood in stool. This inflammation response may be due to variety of reasons including infection, loss of blood supply to colon, IBD and allergic reaction. Inflammatory bowel disease (IBDs) is characterized by chronic inflammation of the digestive or gastrointestinal (GI) tract. It is of 2 types, ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis primarily affects colonic mucosa characterized by persistent inflammation, open sores, or ulcers. Crohn's disease (CD) is more commonly found at the

end of the small intestine (the ileum) where it joins the beginning of the large intestine (or colon). UC is usually characterized by severe diarrhea, pain, fatigue and weight loss and could be debilitating. It may lead to life-threatening complications if not controlled. Both the IBDs result in huge loss of over a million workdays per year and impose huge direct medical costs annually, comprising of hospitalcosts and drug costs.

Herbal remedies are increasingly being used for the treatment of IBD. *Aegle marmelos* (Bael), one of the oldest and most popular species in the world belonging to the family Rutaceae, is found abundantly throughout India. Its fruits are useful in gastrointestinal (GI) disorders like diarrhea, dysentery, and constipation. It is also used as a medicine for its anti-inflammatory, immunomodulatory, antibacterial, and antioxidant properties.

Hence, the present study was undertaken to evaluate the effect of AME on IBD in Sprague Dawley rats by formulating rectal suppositories.

MATERIALS AND METHODS

Materials

The drug *Aegle marmelos* unripe fruit powder (Bael fruits powder) was Purchased from Local vendor of Ayurvedic Pharmacy. Polyethylene glycol (PEG) grades were purchased from Research-Lab Fine Chem, Industry, Mumbai while softisan were procured from Cipla Ltd. Pune. Witepsol was a generous gift from IOI Oleo GmbH, Germany. All other reagents and solvents were of analytical grade.

Methods

Preparation of EtOH AME

The EtOH extract (AME) of fruit powder of *A. marmelos* L. Correa was prepared by soxhlet extraction method. The major steps involved are enlisted as follows;

1. Weighing of 125 g AM powder.
2. Packing of weighed quantity of powder in coarse filter paper cylinder and transferring into the thimble of Soxhlet assembly.
3. Addition of 1/4thabout (62 ml) of EtOH in small proportions, over the packed AM powder allowing displacement of entrapped air within powder bed and adding the remaining 3/4th volume to leave a layer of liquid the moistened bed of AM powder overnight (10-12h).
4. Addition of volume of EtOH in excess of volume of AM powder.
5. Heating of percolated liquid contents in RBF over a period of 8-9 h. over a

thermostatistically controlled heating mantle (70-75^{0}c).

6. Cooling the contents of extract in RBF for 2-3 h. (20-25^{0}c) using cool water bath.
7. Evaporation of combined liquids obtained by filtering the marc and pressing the marc liquid extract at room temperature (20-25^{0}c) followed by drying to obtain dry extract.
8. Weighing of extract to the nearest constant weight.
9. Calculation of the % yield. (Eq.No.1)
10. Storage of dried extract wrapped in self-sealed plastic bag at 25-30^{0}c till further use

Calculation of % yield of EtOH AME:

Weight of empty porcelain dish=A Weight of porcelain dish with extract= BWeight of extract= C=(B-A)

Eq. No. 1: % yield= C*100/ Volume of liquid extract

Preformulation studies

A stock solution (1000µg/ml) of AME was prepared by dissolving accurately weighed 10mg quantity using EtOH. The λ max and linearity range values of appropriately diluted solutions were noted (Shimadzu-1800).

Compatibility studies

The compatibility of mixtures of AME with suppository bases was ascertained by exposing them to the ambient environmental conditions over 15 days. The detection of changes in any of the physical or physicochemical characteristics of blends was carried out by visual inspection and FT-IR spectrophotometry (Shimadzu-8400).

Evaluation of rectal suppositories

The placebo and herbal rectal suppositories were assessed for Physical, organoleptic characteristics, hardness, liquefaction time, liquefaction temperature, surface pH. Addition to this, these herbal rectal suppositories were also evaluated for contents of AME and anti-inflammatory efficacy (in vivo).

1. **Physical and Organoleptic characteristics:-** For this, each of the suppository units per mould was observed visually with unaided eyes as well as with magnifying glass for detection of structural defects. Moreover, odour was perceived subjectively while Texture of the surface was perceived by touch as well as by observation under magnifying glass.

2. **Hardness:-** For this, 1 suppository unit was introduced in the test tube and carefully aligned vertically with its tapering end touching the bottom of test tube and glass rod length (11cm weight 75g) was placed vertically. Crushing strength/ hardness of each of the suppository unit under the pressure of incremental addition of weights. was calculated in terms of total crushing observed within specific unit time (sec) after addition of last weight.
3. **Liquefaction time:-** For this, slightly modified disintegration test was carried out, wherein the time taken by the individual suppository unit to melt completely when immersed in water bath maintained at constant temperature of 37±30C was noted. For this, an individual suppository unit was placed in upright position (tapering end at bottom) within a small circular cooper wire loop (Photograph No.7.3.). This loop was placed carefully at the centre of bumper tube containing PB (pH 7.4). The bumper tube with suppository unit was submerged into a beaker containing immersion fluid and a thermometer. Subsequently, the contents in the glass beaker were heated using thermostatistically controlled heating mantle and the melting of suppository unit was observed till it melted completely leaving no palpably hard residue inside the bumper tube.
4. **Liquefaction temperature:-** For this, the test procedure described previously for assessment of simulated melting (In-vitro) of suppository was repeated to note down the temperature at which the suppository unit liquefies completely when in contact with the PB pH 7.4.
5. **Surface pH:-** For this, accurately weighed 1 g quantity of the rectal suppository was dispersed into 100 ml of purified water. The dispersion was allowed to stand still for about 30 min, followed by stirring for about 5 min. The pH of supernatant liquid was noted at three different sampling points and average of the same has been noted using previously calibrated (calibration at 9.0, 4.00 and 7.00 pH) digital pH meter.

Preparation of rectal suppositories

The rectal suppositories of AME: PEG 6000: Softisan 378 (90:10) was prepared by using pour method. Required quantities of suppository bases were melted by using thermostatically controlled heating mantle then blended at temperature slightly above the corresponding melting ranges. After that molten mass of base composition was poured into individual die of mould. After congealing the suppository unit were removed from mould. “Photograph No.01”

Photograph no. 01: Rectal suppositories of AME with PEG 6000 and Its combination with Softisan 378.

Evaluation of herbal rectal suppositories

6. **Content of AME:-** For this, 3 randomly selected suppositories were taken in 1000 ml volumetric flask containing 100 ml of EtOH. The contents in flask were shaken for sufficient period of time to allow dissolution of AME from suppositories. Absorbance of the resulting solution was noted after appropriate dilutions (if required) at against the dissolution fluid containing placebo suppositories.
7. **Anti-inflammatory efficacy (in vivo):-** For this, the procedure described by Jayanti et al. for testing effect of aqueous extract of unripe fruits of A. marmelos L. Correa on IBD model, was followed (with slight modifications as needed) using healthy, adult, mature female rats. The animals were procured from approved breeding centre and were housed in the facility of Prado Preclinical Laboratory, Pune. The protocol and design of study were approved by IAEC of the Centre (Protocol No-IAEC-1801). The housekeeping conditions for animals were as follows;

Room conditions: Temperature 20- 25 0C and humidity RH 30-70%.

Light conditions: The artificial lighting; the sequence was 12 hours illumination, followed by 12 hours darkness.

Bedding: Rice husk.

Feed: Standard pelleted rat diet.

Water: Purified water from municipal corporation supply (ad libitum in plastic bottle with aluminium nozzles.),

Cages/coding: 3 animals per cage will be housed together in the Polycarbonate cages.

Randomization: routine observations of animal during quarantine, during experiment and after treatment.

Animal details:

Age- 3-4 weeks

Strain – Sprague Dawley **Sex –** Female (adult healthy)**Weight –** 120-140 g

No of animals/ group – 4

No of groups- 06

Table No.7.25: Details of animal groups for testing anti-inflammatory/ wound healing activity ofAME rectal suppository.

Sr. No.	Group	Dose (mg/kg)	Type of treatment	No. of animals/group
1	I	0	Normal control (No disease/ no treatment)	1-4
2	II	0.2ml	Vehicle control (PB, pH 7.4)	5-8
3	III	150	Rectal suppository containing AME (low dose 0.5% w/v)	9-12
4	IV	300	Rectal suppository containing AME (high dose 1% w/v)	13-16
5	V	300	Marketed rectal suppository containing Mesalamine (1% w/w)	17-20
6	VI	0.2ml	Disease control (acetic acid induced UC)	21-24

RESULS AND DISCUSSION

The organoleptic, physicochemical and solubility characteristics of AME supported its identity and purity. Moreover, the spectral characteristics viz. wavelength maxima (292 nm) and linearity of concentration and absorbance range (10-60 µg/ml) further supported the quality and purity of AME “Fig 1”.

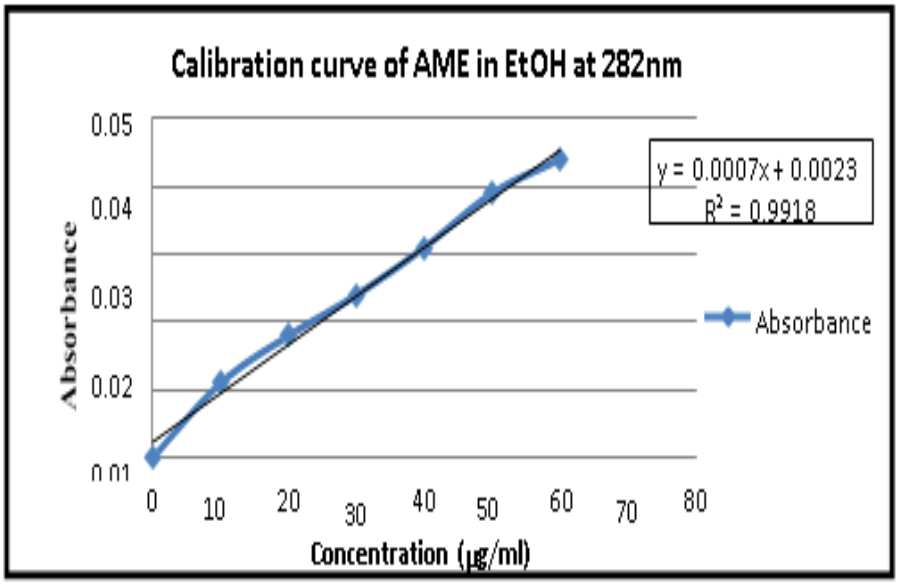


Fig. 1: Calibration curve of AME in EtOH.

Compatibility of AME and formulation excipients

The IR spectra of physical mixture of AME and formulations ingredients did not indicate any gross change in structure of any of them suggesting no effect on their mutual compatibility "Fig. 2" and "Fig. 3".

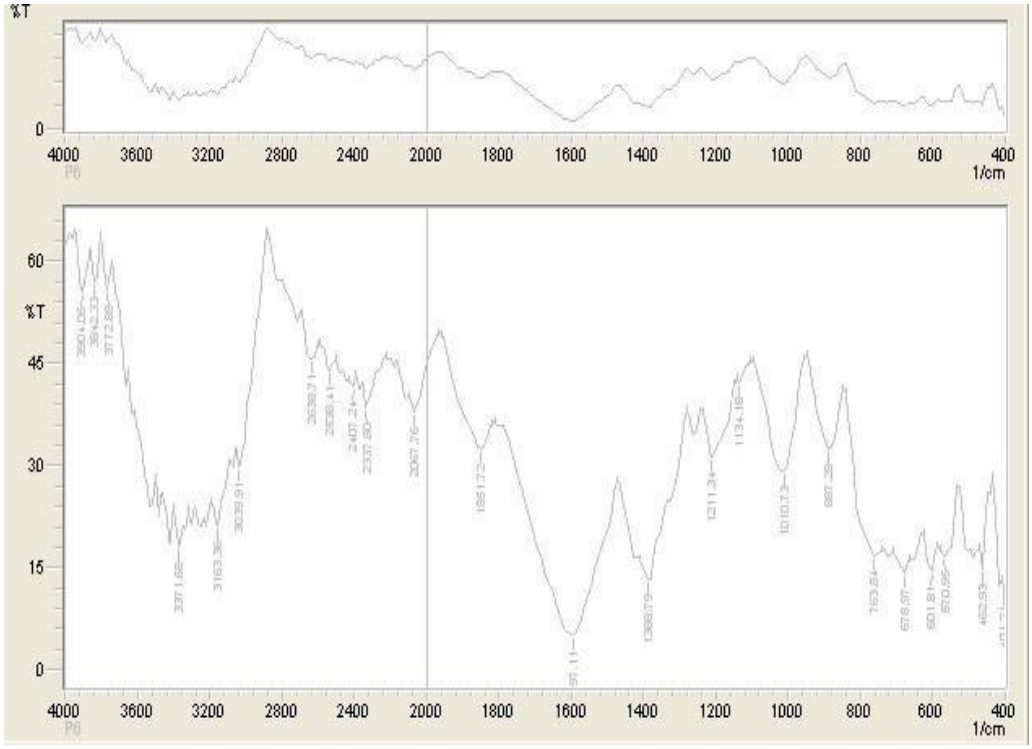


Fig. 2: IR spectrum of AME: PEG 6000 (compatibility sample).

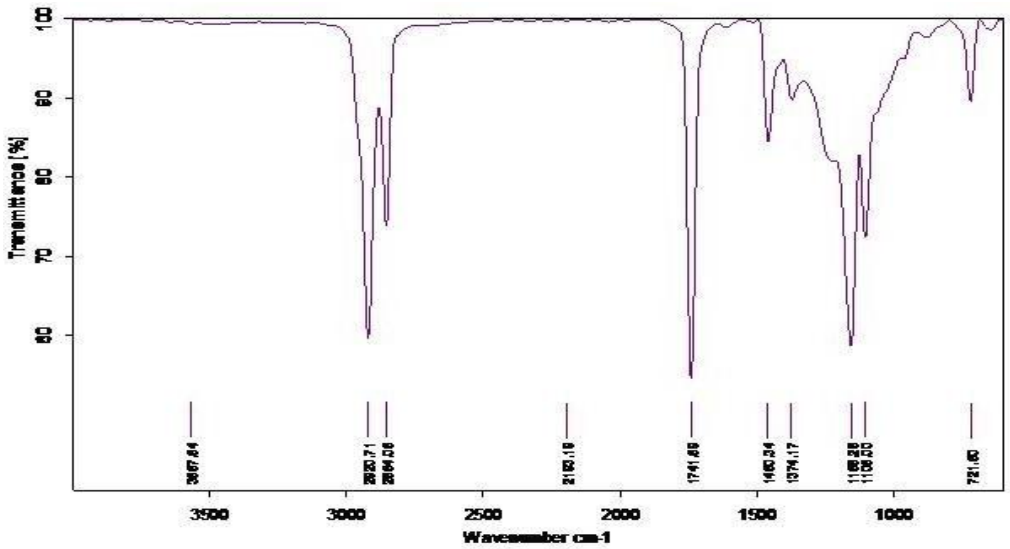


Fig. 3: IR spectrum of AME: Softisan 378 (Compatibility sample).

Evaluation of rectal suppositories of AME: PEG 6000: Softisan 378 (90:10)

The rectal suppositories of AME in PEG 6000 and its combination with hydrophilic and fatty bases revealed consistency in the physical and organoleptic characteristics and dimensions. (Table No. 8.30).

Table No: 8.30: Characteristics of rectal suppositories of AME: PEG6000 and AME: PEG6000: other PEGs andPEG 6000: Softisan 378.

Sr. No.	Characteristics	F1 [PEG 6000]	F2 [PEG 6000: 200 (60:40)]	F3 [PEG 6000: 1500 (60:40)	F4 [PEG6000: Softisan (90:10)]	F5 [PEG6000: Softisan (80:20)]
1. Physical, organoleptic and other characteristics						
1	Appearance	Homogenous				
2	Shape	Pencil shape with blunt tip				
3	Surface texture (magnifyingglass)	Smooth				
4	Colour	Brownish				
5	Odour	Odourless				
2. Dimensions (Average, n=3)						
6	Weight (g)	0.994 ± 0.018	1.024 ± 0.018	1.037 ± 0.018	1.064 ± 0.018	1.032 ± 0.018
7	Length (cm)	1.5± 0.002				
8	Diameter (cm)	0.9± 0.001				
3.Others						
9	Hardness (Kg/cm^2)	**4.0**	3.5	4.5	**3**	3.5
10	Melting range 0 (C)	**45-50**	37-38	37-40	**40-42**	37-39

Anti-inflammatory characteristics of selected experimental rectal suppositories

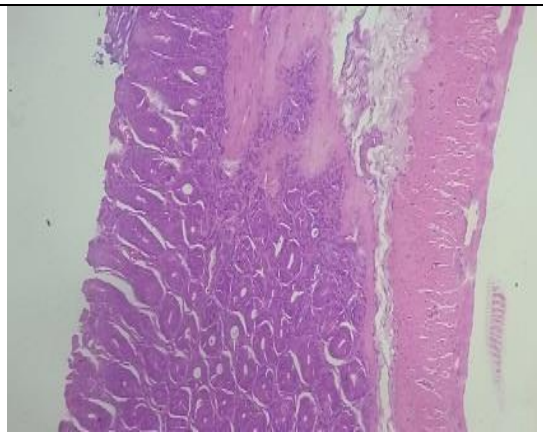
The histological findings of the isolated rectum tissues of the animals belonging to different groups including those treated with experimental rectal suppository of AME: PEG 6000: Softisan 378 (90:10) and that treated with marketed Mesalamine revealed the following distinct features of the target tissue (**Table No. 1** and Photograph No.2,3,4 and 5)

Table no. 1: Histopathological findings of recto-anal tissues of rats used for testing anti-inflammatory (wound healing) efficacy in acetic acid induced colitis model.

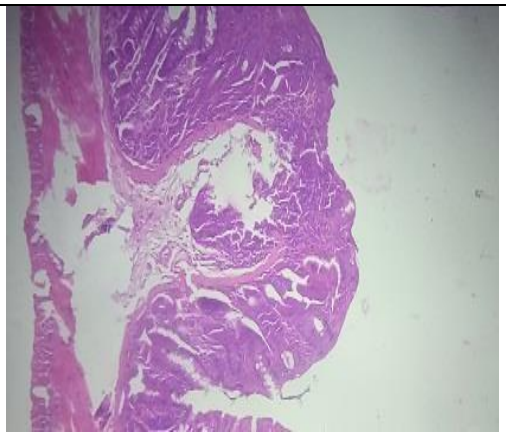
Tissue parameter	Observations	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. IV
Infiltration of cells	**Before**	Normal architecture of colon mucosa, (no infiltration of inflammatory cells)				
	After induction of colitis	No infiltration	**Severe infiltration of inflammatory cells**			
	Post treatment	Normal architecture of colon mucosa	Retained inflamm - atory response in mucosa and sub-mucosa	**Minimum infiltration of inflammatory cells**		
Degeneration of rectal tissue	**Before**	Intact lining of mucosa and sub- mucosal tissue.				
	Post induction of colitis	Retention of intactlining of mucosa	**Severe damage of mucosal and sub-mucosal lining**			
	Post treatment with rectal suppository	No Degeneration of mucosa	Retention of mucosal damage	**Minimal degeneration of mucosa**		
Count of goblet cells	**Before**	Presence of normal count of goblet cells				
	Post induction of colitis	Retention of normal count		Severe depletion		

	Post treatment with rectal suppository	No depletion	Severe depletion	**Increased number of goblet cell count**

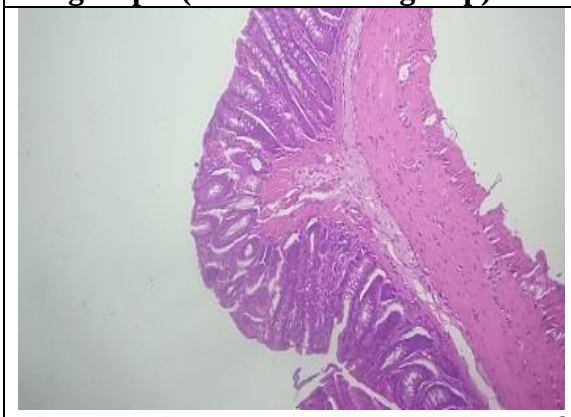
Architecture of crypt of Liburkhun cells.	**Before**		Presence of normal crypts of Liburkhun	
	Post induction of colitis	Normal architectures	Considerable loss of architectural details of crypt of Liberkuhn cells	
	After completion of treatment with experimentalrectal suppository	Normal Crypt of Liberkuhn cells	Retention of loss of architecture of crypt of Liburkhun cells	**Regeneration of normal architecture of crypt of Liburkhun cells**
Inference	Confirmation of non-irritant/non- sensitizing potential of vehicle .		Retention of colitis	**Recovery of colitis wound**



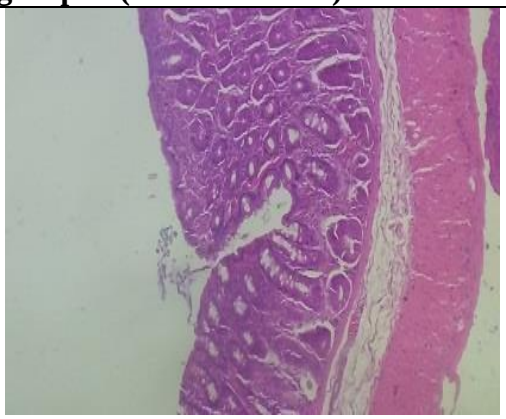
Photograph no. 2: Histopathology of normal recto- anal region of animals in the group-I (vehicle control group)



Photograph no. 3: Histopathology of recto- anal region of animals in group II (disease control)



Photograph no. 4: Histopathology of recto-anal region of animals in group IV (treatment with high dose AME suppositories)



Photograph no. 5: Histopathology of recto-anal region of animals in group V (treatment with marketed Mesalamine suppository)

Hence, it can be concluded that, the experimental rectal suppository composition of base PEG 6000: Softisan 378 (90:10) has successfully delivered the AME locally into the target tissue within ano-rectal site. Both the suppositories containing Mesalamine and AME have demonstrated good anti-inflammatoryand wound healing activity (in vivo).

CONCLUSION

Hence, it can be concluded that, the AME of unripe fruits of Aegle marmelos L. Correa, family Rutaceae has demonstrated good anti-inflammatory efficacy (in-vivo) when formulated as rectal suppository. Moreover, the selected suppository base composition of PEG: Softisan 378 has indicated suitability for rectal delivery of the AMEin the colitis model induced in rats. Hence, this composition F4 may be considered as lead formulation and more extensive studies can be carried out for establishing validity of the claim for its clinical safety and efficacy using different base compositions, different designs of the rectal delivery systems.

ACKNOWLEDGEMENT

The authors are grateful to Sun pharma, Gujrat for the gift sample of Mesalamine, IOI Oleo GmbH, Germany for gift sample of Witepsol and to Prado Preclinical Laboratory, Pune for technical assistance for providing facilities, sophisticated instruments and technical assistance for anti-inflammatory studies.

REFERENCES

1. Akdis M. Healthy immune response to allergens: T regulatory cells and more. Curr Opin Immunol, 2006; 18(6): 738–44.
2. Rubin D, Chronic intestinal inflammation: inflammatory bowel disease and colitis associatedcolon cancer. Front Immunol, 2012; 3: 107-12.
3. Dundas S., Reliability of rectal biopsy in distinguishing between chronic inflammatorybowel disease and acute self-limiting colitis. Histopathology, 1997; 31(1): 60–66.
4. Sanchez M, Epidemiology and burden of chronic constipation. Can J Gastroenterol, 2011; 25.
5. Leone V, Diet, microbes, and host genetics: the perfect storm in inflammatory boweldiseases. Journal ofGastroenterology, 2013; 48(3): 315–21.
6. Mehta RM. Dispensing Pharmacy, Vallabh Prakashan, New Delhi, 2004; 246.
7. El-Majri MA, Sharma RK, Formulation and evaluation of suppositories of piroxicam, Int

J Drug Del, 2010;2: 108-12.

8. Stailling W., The HET-CAM, a useful in vitro assay for assessing the eye irritation properties of cosmetic formulation and ingredients, Toxicology in vitro, Pergamon press/Elsevier, Oxford, 1999; (13): 375-84.
9. Bramhall, Quality of methods reporting in animal model of colitis Inflammatory Bowel Diseases, 2015;21(6): 1248–59.
10. Matthew B, Fraser A, Animal Models of Colitis: Lessons Learned, and Their Relevance to the Clinic, Ulcerative Colitis – Treatments, Special Populations and the Future, 2011: 161.
11. Abraham C., Inflammatory Bowel Disease, New England Journal of Medicine, 2009; 361(21): 2066-78.