Section A-Research paper
ISSN 2063-5346

PRESENTATION OF THESIS IN SCIENTIFIC FORUM

Anti-haemorrhoidal activity of *Actiniopteris radiata* (Sw.) Link (*Mayurasikha*) paste mixed with milk internally in Wistar Albino Rats.

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DOI: 10.48047/ecb/2023.12.si4.1779

ABSTRACT

Haemorrhoids or piles are the pathological condition, characterized by vasodilation and inflammation in the ano-rectal region, which causes increased permeability of blood vessels and induce oxidative stress in the ano-rectal tissues. Prevalence of Haemorrhoid has been estimated as 50-85% of the total population globally and 75% in India. Although the initial stages of haemorrhoid is manageable with medication i.e., for grades one to three; the disease needs surgical intervention for grade four where in prolapse is irreducible. From time immemorial, through traditional and ethnomedical practice, the disease haemorrhoid has been effectively managed with plant based remedies as well as dietary modifications. *Mayurasikha* is one such drug, having anti-inflammatory and anti-oxidant properties, used by the tribes to treat

haemorrhoids and is botanically identified as *Actiniopteris radiata* (Sw.) Link belongs to the family Pteridaceae. To scientifically evaluate the anti-haemorrhoidal potential of paste of *Actiniopteris radiata* (Sw.) Link (*Mayurasikha*) with milk internally, an in- vivo study has been designed in Wistar Albino Rats.

The study was carried out in six groups namely, Normal control group, Positive control group, Standard drug group, Trial drug paste mixed with un-boiled milk group, Trial drug paste mixed with boiled milk group and Trial drug paste group and containing six rats in each group. For the purpose of inducing haemorrhoids, croton oil preparation was used in all the groups except Normal control group. Standard drug group and trial drug sample groups were given respective treatments for a period of seven days. On the seventh day the animals of each group were sacrificed under deep ether anesthesia and Ano-rectal tissue were collected for histopathological evaluation. The results were statistically analyzed using Wilcoxon signed rank test. Among the groups, only trial drug paste with boiled milk group exhibited statistically significant changes justifying the traditional claim of *Mayurasikha* paste mixed with boiled milk group in the treatment of Haemorrhoids.

Key words: *Actiniopteris radiata* (Sw.) Link, *Mayurasikha*, Anti-haemorrhoidal activity

1. INTRODUCTION

Haemorrhoids or piles are one of the most common pathological conditions characterized by an alteration in the vasculature of anal canal including blood vessels, supporting tissues, muscles and elastic fibers. Its main clinical features include bleeding, prolapse, pain, mucous discharge and anemia[1].

Globally the prevalence of Haemorrhoids ranges from 50-85% and it affects about 75% of the population in India.[2] Based on the degree of prolapse the disease is divided into four. Of which grade one and two are curable by medicinal intervention, grade three, by manual reduction and in grade four by surgical intervention. Pharmacotherapeutics in modern medicine includes the use of drugs having anti-inflammatory and anti-oxidant properties, cortical steroids, surgical intervention, laxatives, dietary recommendations and lifestyle modifications. Haemorrhoids can be correlated to the term *Arshas* mentioned in Ayurveda as "*arivat pranan shrinoti hinasti iti*

arshah". Since the disease demands long-term medication, the *Samhitas* and *Nighantus*, describe many medicinal preparations, *Anushasthra* procedures and pathyahara viharas for its management.

Review on folklore literature also suggests the use of medicinal plants with high therapeutic potential for treating many diseases including *Arshas*. One such drug is *Actiniopteris radiata* (SW.) Link of the family Pteridaceae, which is mentioned in *Ayurveda* classics as *Mayuraśikha*. The present in-vivo study was designed to scientifically evaluate the traditional claim of the efficacy of the whole plant paste of *Actiniopteris radiata* (SW.) with milk in the treatment of experimentally induced Haemorrhoids in Wistar Albino Rats.

2. MATERIALS AND METHODS

2.1. Identification and collection of the trial drug:

The trial drug *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) was identified using taxonomical characters and was confirmed by the Taxonomist before collection of the trial drug. Herbarium specimens of the plants were deposited in the Department of Dravyaguna vijnana (Ayurveda Pharmacolgy), Amrita School of Ayurveda, Amrita Vishwa Vidyapeetham, Amritapuri, India. For phytochemical, pharmacognostical and in vivo studies, pasteurized milk having 3% fat content was procured from the market of Kollam (Kerala) and Udupi.

2.2 Pharmacognostical and phytochemical analysis of trial drugs:

2.2.1. Study Center:

1. ACARA lab, Amrita School of Ayurveda Campus, Vallikavu, Clappana P.O, Kollam-690625, India.
2. CARe KERALAM, Koratty, Thrissur, Kerala.
3. CEPCI LABORATORY & RESEARCH INSTITUTE (The Cashew Export Promotion Council of India) Sponsored by Govt. of India) CASHEW BHAVAN, Mundakkal, Kollam-691 001, Kerala, India.

2.2.2. Methods:

Macroscopic and microscopic evaluation, Physicochemical analysis, Phytochemical analysis, HPTLC and LC-MS QTOF of trial drug samples were conducted.

2.3. In-vivo study:

2.3.1. Study Center:

1. Department of Pharmaceutical Chemistry and Pharmacognosy, SDM Center for Research in Ayurveda and Allied Sciences, Udupi, Karnataka.

2.3.2. Ethical clearance statement:

Animal ethical clearance was obtained with the Approval No: SDMCRA/IAEC/AM-D-02 from SDM Center for Research in Ayurveda and Allied Sciences, Udupi.

2.3.3 Animal selection criteria and maintenance

Healthy male Wistar Albino Rats weighing between 100-300gm were procured and kept under standard conditions of dark and light cycle and temperature. Standard pellet diet and water *ad libitum* were provided throughout the experimental period. Diseased rats and rats under trial of other experiments were excluded.

2.3.4. Study Design

Thirty-six male Wistar Albino Rats were divided into 6 groups, with 6 rats in each group,

Group A- Normal control group,

Group B- Positive control group,

Group C- Standard drug group,

Group D- Trial drug paste mixed with un-boiled milk group,

Group E- Trial drug paste mixed with boiled milk group,

Group F- Trial drug paste group for the experimental study.

The Haemorrhoid was experimentally induced using croton oil preparation in all the groups except Normal control group.

In Normal control group and Positive control group, simple tap water and pellet diet was given throughout the study for a period of seven days.

The dose of the trial drug as well as standard drug was calculated by extrapolating the therapeutic dose to the rat dose on the basis of body surface area ratio by referring to the Paget and Barnes table (1979). (2160mg/individual animal body weight)

In Standard drug group Pilex tablet (200mg/ individual animal body weight) was administered

with distilled water for seven days.

Accordingly, in the test drug groups, freshly prepared paste of the drug *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) mixed with distilled water, boiled milk and unboiled milk was administered to each animal after calculating animal dose.

2.6. Methodology

Rats of all the groups except Normal control group were kept fasting overnight before inducing Haemorrhoid externally with croton oil application. Sterile cotton swabs (about 4mm diameter) soaked in 100ml of croton oil preparation were inserted into the Ano-rectal area.(20mm from anal opening) by using forceps and was kept for 10 seconds and the inflammatory changes were observed.

Then the rats were maintained in individual cages, which were bedded with thick paper and the bedding was changed daily. After five hours, they were provided with water and a pellet diet.

Group C, Group D, Group E and Group F received drug treatment once daily for seven days, twenty-four hours after the induction of haemorrhoids. On the seventh day, about six hours after the medicine administration, the animals of Group C, Group D, Group E and Group F were sacrificed by keeping the animal in deep ether anesthesia. The normal control Group and Positive control Group were also sacrificed in the same way. Immediately after euthanizing the Ano-rectal tissue specimens were collected and kept in a 10% formaldehyde solution for histopathological studies.

2.5. Statistical Analysis:

All the quantitative data obtained from the experimental animal study had been analysed statistically with the help of statistics software SPSS VER 20. The test used for analysis was WILCOXON SIGN RANK TEST.

3. RESULT

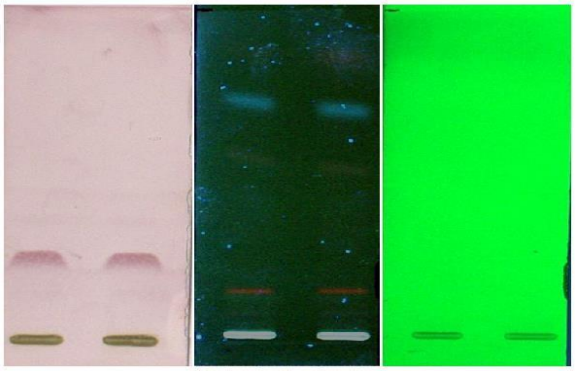
3.1. Result of Phytochemical analysis

TEST	Whole plant paste	Whole plant paste with un-boiled milk	Whole plant paste with boiled milk
Alkaloids	Absent	Absent	Absent

Flavonoids	Absent	Absent	Absent
Glycosides	Absent	Absent	Absent
Saponins	Absent	Present	Present
Carbohydrate	Present	Present	Present
Phenol	Present	Present	Present
Tannin	Present	Present	Absent

3.2. Result of TLC of Trial drug samples.

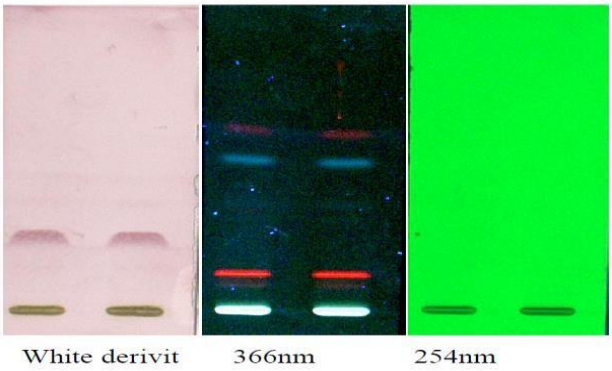
SAMPLE 1: Whole plant paste of *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*)



White derivit 366nm 254nm

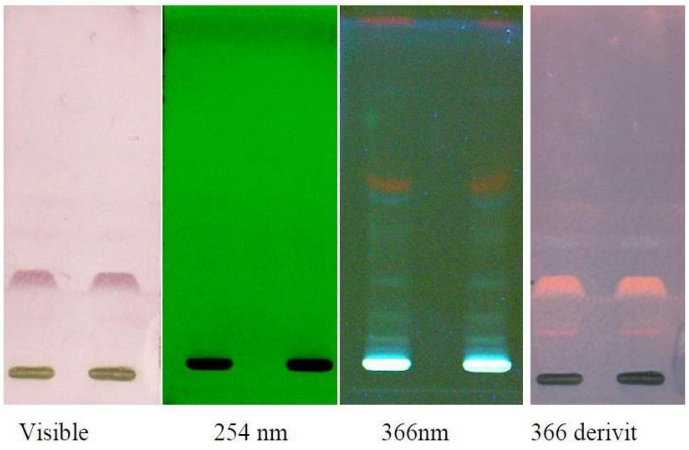
The methanol extract of the drug *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*)with solvent system toluene: chloroform: methanol (8:3:1) on silica 366 showed two major bands at Rf 0.15 & Rf 0.73.

SAMPLE 2: Whole plant paste of *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) with unboiled milk.



The methanol extract of the drug *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*)with solvent system toluene: chloroform: methanol (8:3:1) on silica 366 showed two major bands at Rf 0.15 & Rf 0.73.

SAMPLE 3: Whole plant paste of *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) with boiled milk.

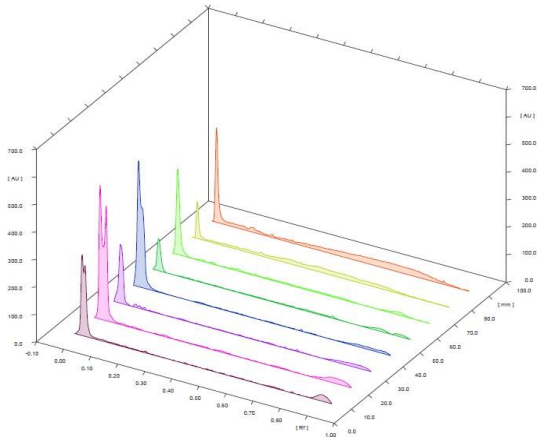


The methanol extract of the drug *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) with solvent system toluene: chloroform: methanol (8:3:1) on silica 366 showed two major bands at Rf 0.15 & Rf 0.73.

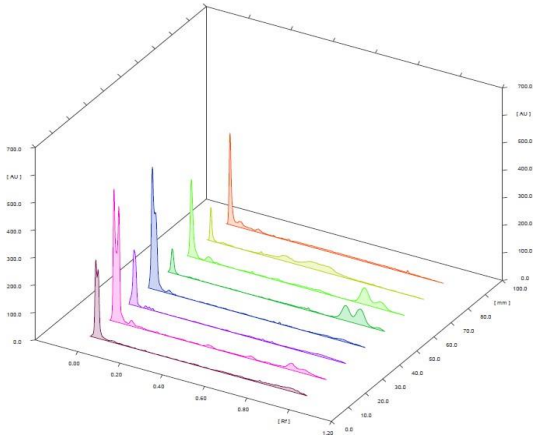
3.3. Result of HPTLC of Trial drug samples.

SAMPLE 1: Whole plant paste of *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*).

SAMPLE 2: Whole plant paste of *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) with unboiled milk.



Tracks at 254nm



Tracks at 366nm

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	1	-0.05 Rf	0.2 AU	0.01 Rf	99.2 AU	92.83 %	0.05 Rf	9.3 AU	707.4 AU	85.87 %
1	2	0.84 Rf	0.8 AU	0.89 Rf	23.1 AU	7.17 %	0.93 Rf	1.4 AU	774.4 AU	14.13 %
2	1	-0.03 Rf	0.6 AU	-0.00 Rf	88.0 AU	51.58 %	0.01 Rf	7.2 AU	928.4 AU	48.43 %
2	2	0.01 Rf	68.8 AU	0.02 Rf	19.9 AU	44.38 %	0.07 Rf	5.3 AU	124.7 AU	40.54 %
2	3	0.81 Rf	6.2 AU	0.86 Rf	19.4 AU	2.05 %	0.87 Rf	3.2 AU	621.1 AU	6.10 %
2	4	0.87 Rf	18.2 AU	0.88 Rf	18.8 AU	1.98 %	0.92 Rf	3.1 AU	501.3 AU	4.93 %
3	1	-0.03 Rf	0.8 AU	0.00 Rf	21.2 AU	00.00 %	0.04 Rf	5.3 AU	296.5 AU	00.00 %
4	1	-0.03 Rf	1.7 AU	-0.00 Rf	65.5 AU	00.00 %	0.07 Rf	7.5 AU	191.0 AU	00.00 %
5	1	-0.04 Rf	2.3 AU	-0.00 Rf	25.3 AU	92.23 %	0.03 Rf	5.2 AU	764.9 AU	82.36 %
5	2	0.77 Rf	2.7 AU	0.81 Rf	10.6 AU	7.77 %	0.86 Rf	0.8 AU	378.0 AU	17.64 %
6	1	-0.03 Rf	4.2 AU	-0.00 Rf	21.2 AU	96.73 %	0.04 Rf	0.0 AU	946.7 AU	90.46 %
6	2	0.76 Rf	2.9 AU	0.81 Rf	10.9 AU	3.27 %	0.86 Rf	1.0 AU	416.3 AU	9.54 %
7	1	-0.05 Rf	4.7 AU	-0.00 Rf	50.9 AU	90.95 %	0.03 Rf	0.9 AU	871.5 AU	82.22 %
7	2	0.67 Rf	14.3 AU	0.68 Rf	15.0 AU	9.05 %	0.72 Rf	3.7 AU	404.7 AU	17.78 %
8	1	-0.05 Rf	5.6 AU	-0.01 Rf	55.3 AU	93.11 %	0.03 Rf	2.2 AU	732.8 AU	75.78 %
8	2	0.71 Rf	26.2 AU	0.71 Rf	26.3 AU	6.89 %	0.78 Rf	7.0 AU	193.1 AU	24.22 %

Wavelength m254nm

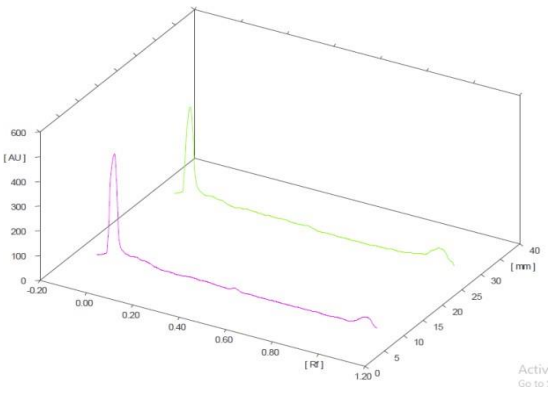
Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	1	-0.02 Rf	0.4 AU	0.01 Rf	82.9 AU	00.00 %	0.06 Rf	3.9 AU	217.2 AU	00.00 %
2	1	-0.02 Rf	0.4 AU	-0.00 Rf	81.7 AU	49.93 %	0.01 Rf	0.5 AU	670.1 AU	44.92 %
2	2	0.01 Rf	51.9 AU	0.02 Rf	22.7 AU	43.81 %	0.05 Rf	9.2 AU	046.9 AU	38.92 %
2	3	0.07 Rf	6.2 AU	0.08 Rf	21.0 AU	2.18 %	0.10 Rf	5.6 AU	349.7 AU	3.36 %
2	4	0.81 Rf	6.1 AU	0.84 Rf	24.9 AU	2.59 %	0.87 Rf	1.1 AU	769.6 AU	7.40 %
2	5	0.87 Rf	11.2 AU	0.90 Rf	14.4 AU	1.50 %	0.94 Rf	4.8 AU	560.4 AU	5.39 %
3	1	-0.02 Rf	3.6 AU	0.00 Rf	01.4 AU	95.07 %	0.04 Rf	0.4 AU	796.3 AU	96.75 %
3	2	0.05 Rf	0.2 AU	0.06 Rf	10.4 AU	4.93 %	0.07 Rf	3.9 AU	94.1 AU	3.25 %
4	1	-0.02 Rf	4.1 AU	-0.00 Rf	43.3 AU	97.23 %	0.06 Rf	3.8 AU	439.6 AU	97.14 %
4	2	0.06 Rf	3.6 AU	0.07 Rf	12.6 AU	2.77 %	0.11 Rf	1.2 AU	218.8 AU	2.86 %
5	1	-0.02 Rf	2.9 AU	-0.00 Rf	88.6 AU	44.06 %	0.03 Rf	0.1 AU	019.8 AU	21.12 %
5	2	0.76 Rf	2.7 AU	0.82 Rf	55.3 AU	27.48 %	0.85 Rf	1.8 AU	859.5 AU	38.51 %
5	3	0.85 Rf	22.3 AU	0.89 Rf	57.2 AU	28.46 %	0.94 Rf	4.6 AU	949.8 AU	40.38 %
6	1	-0.02 Rf	1.7 AU	-0.00 Rf	81.2 AU	73.06 %	0.04 Rf	1.7 AU	236.4 AU	48.70 %
6	2	0.05 Rf	1.2 AU	0.08 Rf	17.8 AU	4.63 %	0.10 Rf	2.8 AU	377.1 AU	5.67 %
6	3	0.76 Rf	2.2 AU	0.82 Rf	60.5 AU	15.71 %	0.86 Rf	5.0 AU	139.1 AU	32.19 %
6	4	0.86 Rf	15.3 AU	0.89 Rf	25.4 AU	6.60 %	0.94 Rf	1.2 AU	892.5 AU	13.43 %
7	1	-0.02 Rf	0.2 AU	-0.00 Rf	21.3 AU	63.53 %	0.02 Rf	2.7 AU	961.3 AU	26.48 %
7	2	0.29 Rf	7.3 AU	0.35 Rf	20.6 AU	10.80 %	0.39 Rf	9.8 AU	991.8 AU	27.32 %
7	3	0.43 Rf	13.1 AU	0.47 Rf	25.1 AU	13.15 %	0.48 Rf	3.9 AU	813.0 AU	22.39 %
7	4	0.54 Rf	23.4 AU	0.55 Rf	23.9 AU	12.51 %	0.60 Rf	7.9 AU	864.6 AU	23.81 %
8	1	-0.02 Rf	0.5 AU	-0.01 Rf	33.0 AU	90.57 %	0.03 Rf	4.5 AU	036.7 AU	80.90 %
8	2	0.03 Rf	14.6 AU	0.04 Rf	21.9 AU	5.95 %	0.07 Rf	9.0 AU	471.2 AU	12.55 %
8	3	0.11 Rf	5.4 AU	0.13 Rf	12.8 AU	3.48 %	0.16 Rf	2.0 AU	245.9 AU	6.55 %

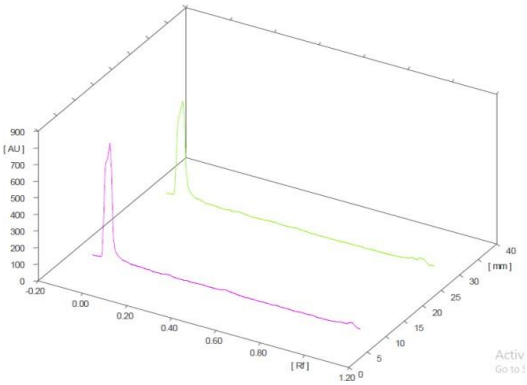
Wavelength 366nm

SAMPLE 3: Whole plant paste of *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) with boiled milk.

Wavelength 254nm

Wavelength 366nm





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.12	0.0	-0.05	426.4	85.62	0.02	28.8	9720.0	82.48
2	0.08	23.7	0.09	24.8	4.98	0.13	12.1	578.7	4.91
3	0.44	2.5	0.47	12.0	2.41	0.51	0.2	245.9	2.09
4	0.98	3.3	1.04	34.8	6.98	1.08	2.6	1240.2	10.52

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.09	1.3	-0.04	700.8	94.74	0.06	6.3	15544.1	94.50
2	0.95	2.7	1.00	12.1	1.64	1.01	10.9	312.6	1.90
3	1.02	11.0	1.04	26.8	3.63	1.08	1.3	592.2	3.60

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.11	3.3	-0.05	366.7	78.76	0.01	25.9	8650.4	67.10
2	0.07	22.9	0.08	23.0	4.95	0.11	11.4	464.7	3.60
3	0.42	8.7	0.45	13.1	2.82	0.50	0.2	392.7	3.05
4	0.92	14.4	1.02	62.7	13.47	1.07	20.4	3384.9	26.25

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.09	3.2	-0.05	581.8	90.52	0.01	16.7	13651.3	89.60
2	0.92	7.1	0.99	24.8	3.85	1.00	19.0	703.8	4.62
3	1.01	19.1	1.02	36.2	5.63	1.07	6.0	881.4	5.78

3.4. Result of LC-MS QTOF

1. Whole plant paste of trial drug	**2. Whole plant paste of the trial drug mixed with unboiled milk**	**3. Whole plant paste of the trial drug mixed with boiled milk**
Cinnamic acid	Cinnamic acid	Cinnamic acid
2 Methyl-3-hydroxybutyric acid	2-Methyl-3-hydroxybutyric acid	2-Methyl-3-hydroxybutyric acid
Liquiritigenin	p-Cymene	p-Cymene
Linalool	Linalool	Linalool
Maslinic acid	Mecillinam	Serine
Kanamycin	Tretinoin	Mecillinam
Quercetin	alpha-Pinene	alpha-Pinene
alpha-Pinene	Carvone	Carvone

Carvone	6-Methyl-5-hepten-2-one	6-Methyl-5-hepten-2-one
Afzelin	Caffeic acid	Isoliquiritin
N-Methylcorydaldine	N-Methylcorydaldine	beta-Sitosterol
Tryptophan	Ellagic acid	Tryptophan
beta-Sitosterol	beta-Sitosterol	Sinapic acid
Tricin		Caffeic acid
Genistein		D-alanine
		Luteolin

3.5. Results of the in-vivo study

Histopathological observations are made into table, for Inflammation severity, inflammation extent, and formation of granulation tissues, oedema, congested and thickened blood vessels and also for regenerative changes. Assessment was done by grading the observations, where in grade 0 indicate no change, grade 1 indicate mild, grade 2 indicate moderate and grade 3 indicate severity.

1. Inflammation severity

Table 3.5.1.1: Inflammation severity

Rat	Normal control	Positive control	Standard drug	Trial drug paste with unboiled milk	Trial drug paste with boiled milk	Trial drug paste
1	0	3	2	3	2	3
2	0	3	2	3	2	3
3	0	3	3	3	2	3
4	0	3	3	3	2	3

Table 3.6.1.2 : Inflammation severity- Wilcoxon Sign Rank Test

Ranks					Test Statistics	
		N	Mean Rank	Sum of Ranks	Z	Asymp. Sig. (2-tailed)
Inflammation severity Standard	Negative Ranks	3	2.00	6.00	-1.633	.102

drug - Inflammation severity Positive control	Positive Ranks	0	0.00	0.00		
	Ties	1				
	Total	4				
Ranks					Test Statistics	
Inflammation severity Trial drug paste with unboiled milk - Inflammation severity Positive control	Negative Ranks	0	0.00	0.00	.000	1.000
	Positive Ranks	0	0.00	0.00		
	Ties	4				
	Total	4				
Ranks					Test Statistics	
Inflammation severity Trial drug paste with boiled milk - Inflammation severity Positive control	Negative Ranks	4	2.50	10.00	-2.000	.046
	Positive Ranks	0	0.00	0.00		
	Ties	0				
	Total	4				
Ranks					Test Statistics	
Inflammation severity Trial drug paste - Inflammation severity Positive control	Negative Ranks	0	0.00	0.00	.000	1.000
	Positive Ranks	0	0.00	0.00		
	Ties	4				
	Total	4				

Wilcoxon signed rank test showed that trial drug paste with boiled milk showed statistically significant change with Positive control with Z=-2.000, P value-0.046< 0.05

2. Inflammation extent

Table 3.5.2.1: Inflammation extent

Rat	Normal control	Positive control	Standard drug	Trial drug paste with unboiled milk	Trial drug paste with boiled milk	Trial drug paste
1	0	3	2	2	2	2
2	0	3	3	3	2	2
3	0	3	2	2	3	3
4	0	3	2	3	2	3

Table 3.5.2.2: Inflammation extent - Wilcoxon Sign Rank Test

Ranks					Test Statistics	
Inflammation extent Standard drug - Inflammation extent Positive control	Negative Ranks	0	0.00	0.00	.000	1.000
	Positive Ranks	0	0.00	0.00		
	Ties	4				
	Total	4				
Ranks					Test Statistics	
Inflammation extentTrial drug paste with unboiled milk - Inflammation extent Positive control	Negative Ranks	3	2.00	6.00	-1.732	.083
	Positive Ranks	0	0.00	0.00		
	Ties	1				
	Total	4				
Ranks					Test Statistics	
Inflammation extent Trial drug paste with boiled milk - Inflammation extent Positive control	Negative Ranks	3	2.00	6.00	-1.732	.083
	Positive Ranks	0	0.00	0.00		
	Ties	1				
	Total	4				
Ranks					Test Statistics	
Inflammation extent Trial drug paste - Inflammation extent Positive control	Negative Ranks	2	1.50	3.00	-1.414	.157
	Positive Ranks	0	0.00	0.00		
	Ties	2				
	Total	4				

Wilcoxon signed rank test showed, P value greater than 0.05, the result is not statistically significant.

3. Granulation tissue

Table 3.5.3.1: Granulation tissue

Rat	Normal control	Positive control	Standard drug	Trial drug paste with unboiled milk	Trial drug paste with boiled milk	Trial drug paste
1	0	2	2	2	1	2
2	0	1	2	2	2	2
3	0	1	2	2	3	2
4	0	2	2	3	2	2

Table 3.6.3.2: Granulation tissue - Wilcoxon Sign Rank Test

Ranks					Test Statistics	
Granulation tissue Standard drug - Granulation tissue Positive control	Negative Ranks	0	0.00	0.00	-1.414	.157
	Positive Ranks	2	1.50	3.00		
	Ties	2				
	Total	4				
Ranks					Test Statistics	
Granulation tissue Trial drug paste with unboiled milk - Granulation tissue Positive control	Negative Ranks	0	0.00	0.00	-1.732	.083
	Positive Ranks	3	2.00	6.00		
	Ties	1				
	Total	4				
Ranks					Test Statistics	
Granulation tissue Trial drug paste with boiled milk - Granulation tissue Positive control	Negative Ranks	1	1.50	1.50	-.816	.414
	Positive Ranks	2	2.25	4.50		
	Ties	1				
	Total	4				
Ranks					Test Statistics	
Granulation tissue Trial drug	Negative Ranks	0	0.00	0.00		

paste - Granulation tissue Positive control	Positive Ranks	2	1.50	3.00	-1.414	.157
	Ties	2				
	Total	4				

Wilcoxon signed rank test showed no statistically significant difference in any of the stages since the P value is greater than 0.05.

4. Congested and thickened blood vessels

Table 3.5.4.1: Congested and thickened blood vessels

Rat	Normal control	Positive control	Standard dr
1	0	3	2

Table 3.5.4.2: Congested and thickened blood vessels - Wilcoxon Sign Rank Test

Ranks					Test Statistics	
Congested and thickened blood vessels Standard drug - Congested and thickened blood vessels Positive control	Negative Ranks	3	2.00	6.00	-1.732	.083
	Positive Ranks	0	0.00	0.00		
	Ties	1				
	Total	4				
Ranks					Test Statistics	
Congested and thickened blood vessels Trial drug paste with unboiled milk - Congested and thickened blood vessels Positive control	Negative Ranks	4	2.50	10.00	-1.890	.059
	Positive Ranks	0	0.00	0.00		
	Ties	0				
	Total	4				
Ranks					Test Statistics	
Congested and thickened blood vessels Trial drug	Negative Ranks	4	2.50	10.00		
	Positive	0	0.00	0.00		

paste with boiled milk - Congested and thickened blood vessels Positive control	Ranks				-1.857	.063
	Ties	0				
	Total	4				
Ranks					Test Statistics	
Congested and thickened blood vessels Trial drug paste - Congested and thickened blood vessels Positive control	Negative Ranks	2	1.50	3.00	-1.414	.157
	Positive Ranks	0	0.00	0.00		
	Ties	2				
	Total	4				
Ranks					Test Statistics	
Edema Standard drug - Edema Positive control	Negative Ranks	4	2.50	10.00	-1.890	.059
	Positive Ranks	0	0.00	0.00		
	Ties	0				
	Total	4				

The results obtained were not statistically significant.

5. Edema

Table 3.5.5.1: Edema

Rat	Normal control	Positive control	Standard drug	Trial drug paste with unboiled milk	Trial drug paste with boiled milk	Trial drug paste
1	0	2	0	2	0	1
2	0	2	1	2	2	1
3	0	2	0	2	2	2
4	0	2	0	0	0	2

Table 3.5.5.2: Edema - Wilcoxon Sign Rank Test

Ranks					Test Statistics	
Edema Trial drug paste with unboiled milk - Edema Positive	Negative Ranks	1	1.00	1.00	-1.000	.317
	Positive Ranks	0	0.00	0.00		

control						
	Ties	3				
	Total	4				
Ranks					Test Statistics	
Edema Trial drug paste with boiled milk - Edema Positive control	Negative Ranks	2	1.50	3.00	-1.414	.157
	Positive Ranks	0	0.00	0.00		
	Ties	2				
	Total	4				
Ranks					Test Statistics	
Edema Trial drug paste - Edema Positive control	Negative Ranks	2	1.50	3.00	-1.414	.157
	Positive Ranks	0	0.00	0.00		
	Ties	2				
	Total	4				

Changes observed were not statistically significant.

6. Mucosal formation (regeneration)

Table 3.5.6.1. Mucosal formation (regeneration)

Rat	Normal control	Positive control	Standard drug	Trial drug paste with unboiled milk	Trial drug paste with boiled milk	Trial drug paste
1	0	0	0	0	1	0
2	0	0	0	0	0	0
3	0	0	0	0	1	0
4	0	0	0	0	1	0

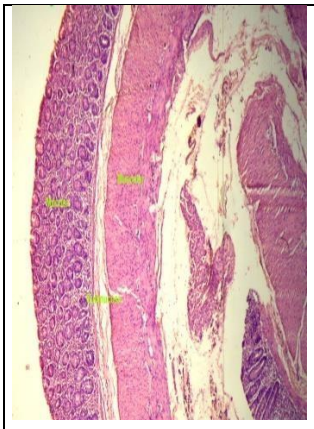
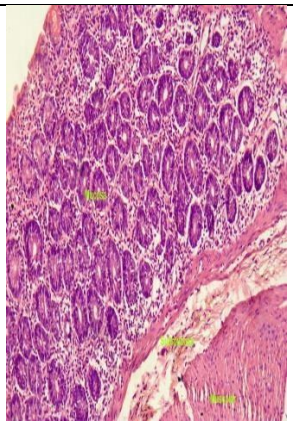
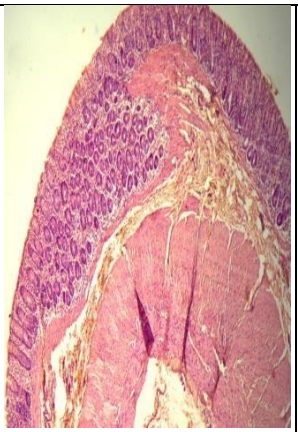
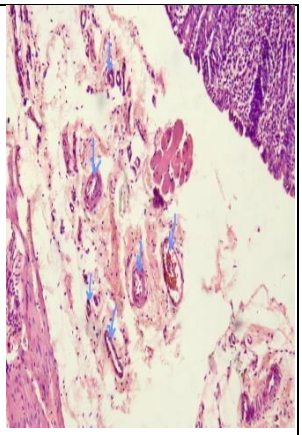
Table 3.5.6.1. Mucosal formation (regeneration) - Wilcoxon Sign Rank Test

Ranks					Test Statistics	
Mucosal formation (regeneration) Standard drug - Mucosal formation (regeneration) Positive	Negative Ranks	0	0.00	0.00	.000	1.000
	Positive Ranks	0	0.00	0.00		

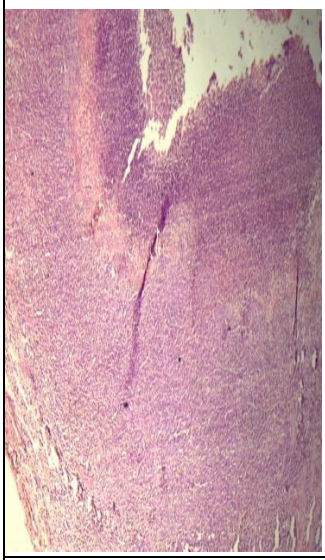
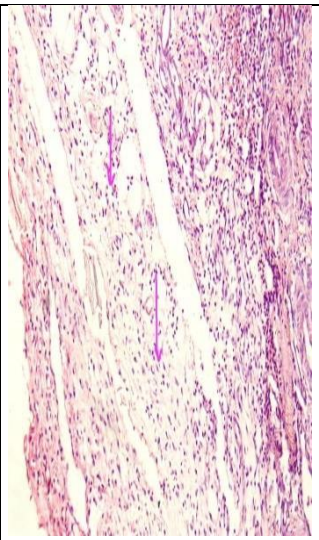
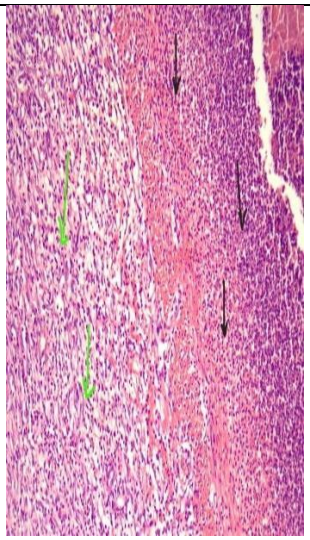
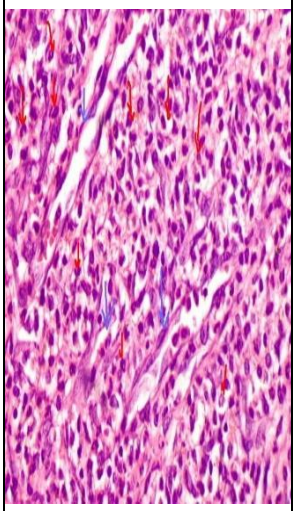
control	Ties	4				
	Total	4				
Ranks					Test Statistics	
Mucosal formation (regeneration) Trial drug paste with unboiled milk - Mucosal formation (regeneration) Positive control	Negative Ranks	0	0.00	0.00	.000	1.000
	Positive Ranks	0	0.00	0.00		
	Ties	4				
	Total	4				
Ranks					Test Statistics	
Mucosal formation (regeneration) Trial drug paste with boiled milk - Mucosal formation (regeneration) Positive control	Negative Ranks	0	0.00	0.00	-1.732	.083
	Positive Ranks	3	2.00	6.00		
	Ties	1				
	Total	4				
Ranks					Test Statistics	
Mucosal formation (regeneration) Trial drug paste - Mucosal formation (regeneration) Positive control	Negative Ranks	0	0.00	0.00	.000	1.000
	Positive Ranks	0	0.00	0.00		
	Ties	4				
	Total	4				

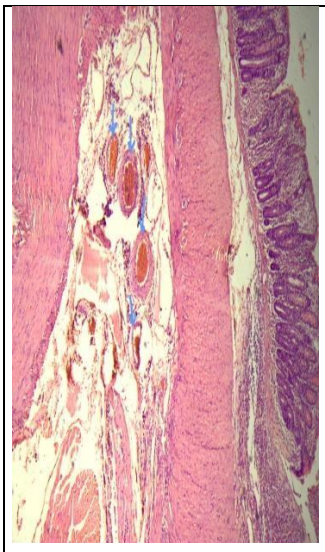
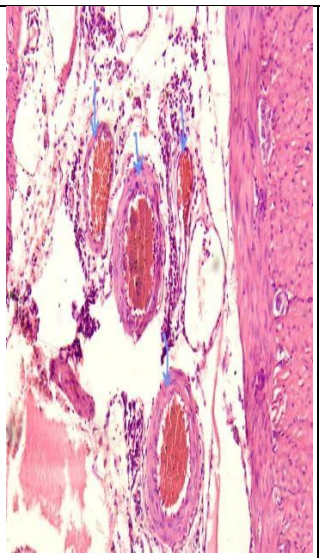
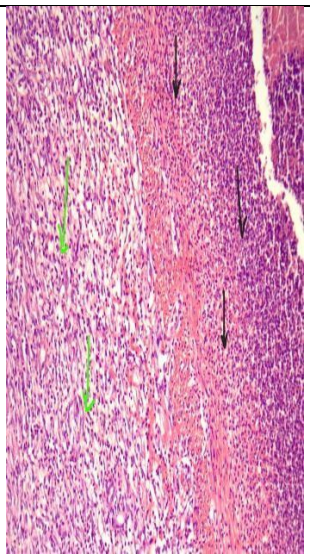
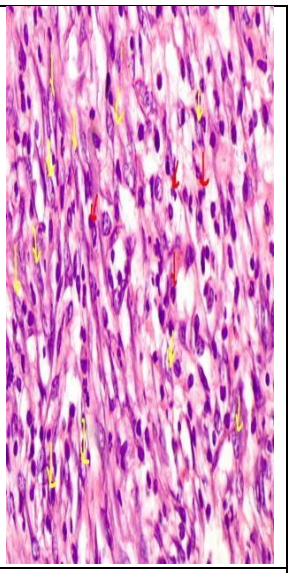
In trial drug paste mixed with boiled milk group, regeneration of mucosal cells were observed, though statistically insignificant.

FIRURE: NORMAL CONTROL GROUP

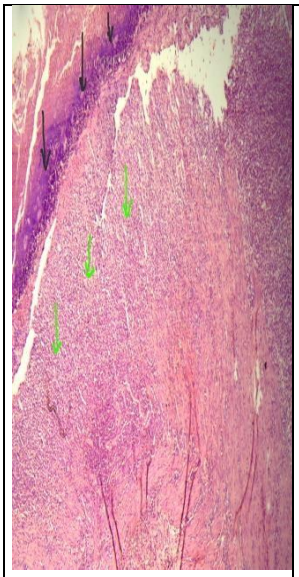
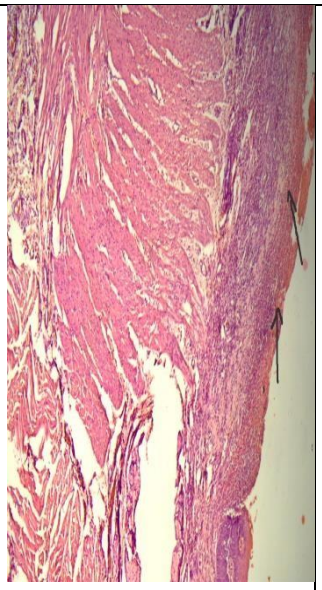
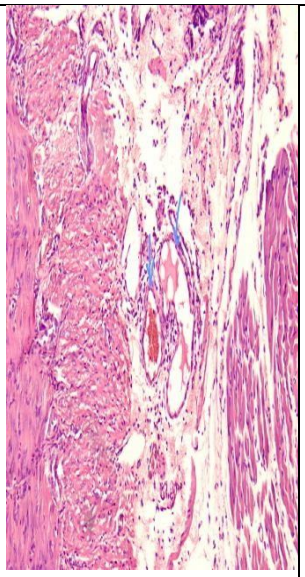
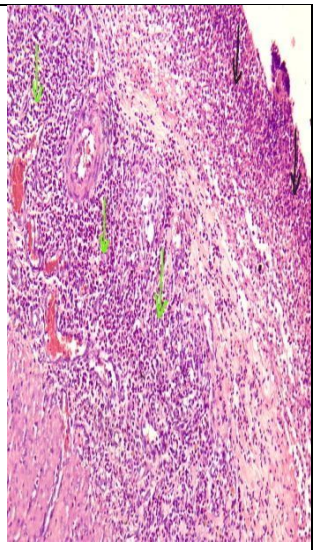
			
LAYERS OF ANO-RECTAL PART			THIN-WALLED BLOOD VESSELS

POSITIVE CONTROL GROUP

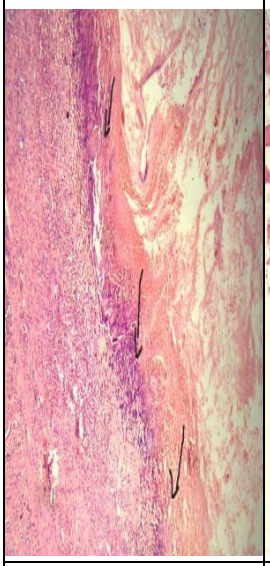
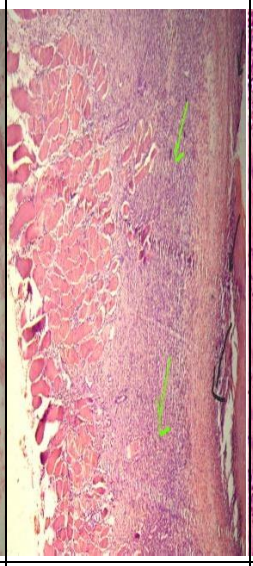
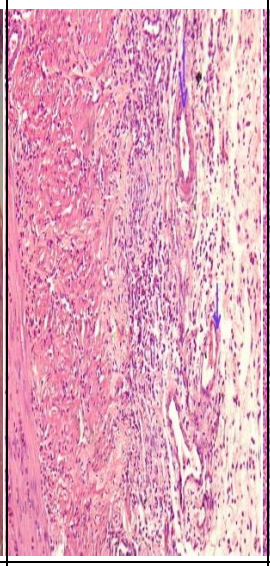
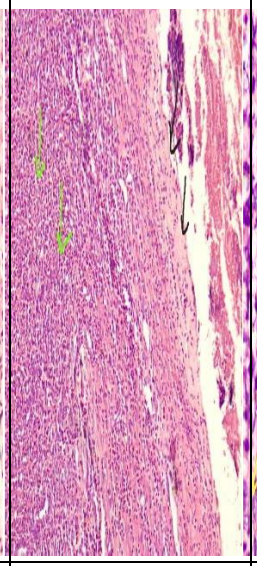
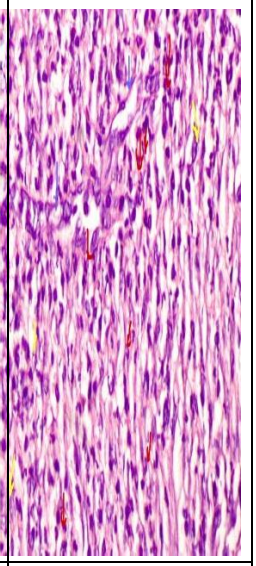
			
ULCER AREA	EDEMATOUS AREAS	ULCER AREA (BLACK) WITH GRANULATION TISSUE(GREEN)	GRANULATION TISSUE SHOWING ABUNDANCE OF ACUTE INFLAMMATORY CELLS (RED), BLOOD VESSELS(BLUE)

			
DILATED AND CONGESTED BLOOD VESSEL	THICKENED AND CONGESTED BLOOD VESSEL	ULCERATIVE EPITHELIUM (BLACK) WITH GRANULATION TISSUE(GREEN)	GRANULATION TISSUE WITH INFLAMMATORY CELLS (RED), FIBROBLAST (YELLOW)

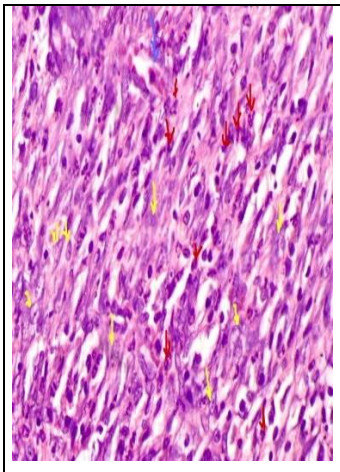
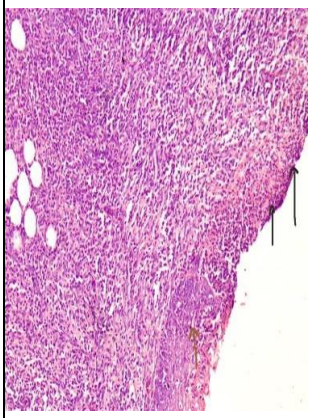
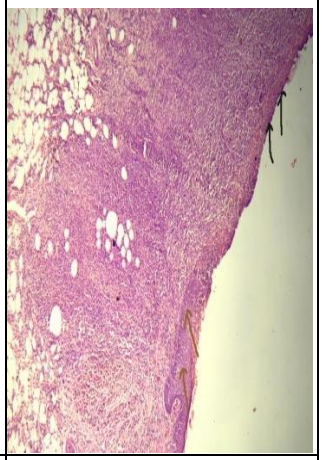
STANDARD GROUP

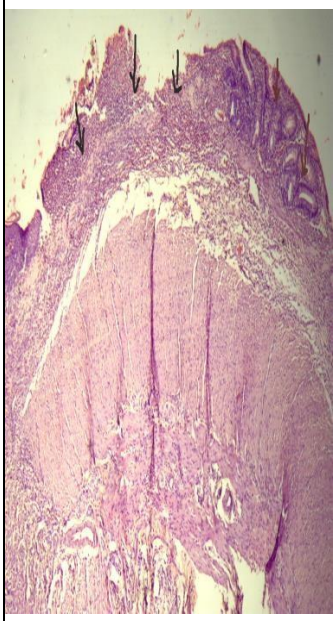
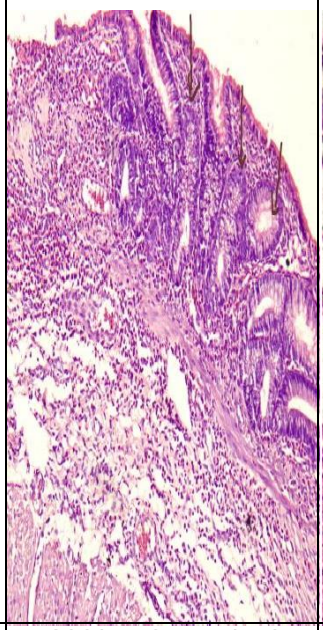
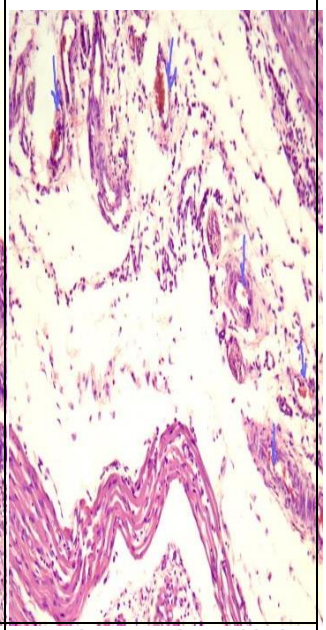
			
ULCER AREA (BLACK) WITH GRANULATION TISSUE (GREEN)	ULCEATIVE EPITHELIUM WITH INTACT MUSCULAR LAYER	REDUCED THICKENING OF BLOOD VESSELS	ULCERATIVE EPITHELIUM (BLACK) WITH GRANULATION TISSUE (GREEN) AND INTACT MUSCLE LAYER

DRUG WITH UNBOILED MILK GROUP

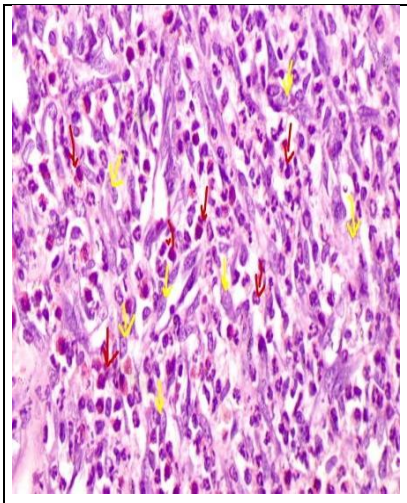
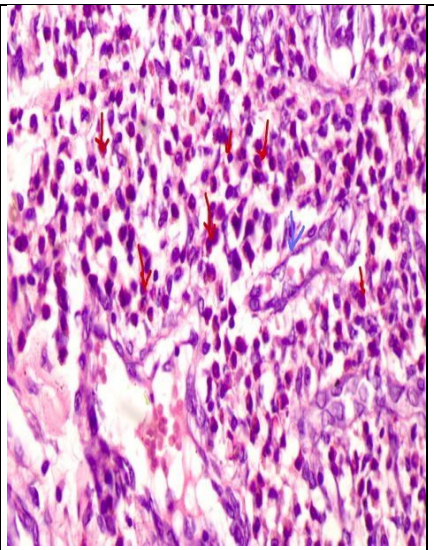
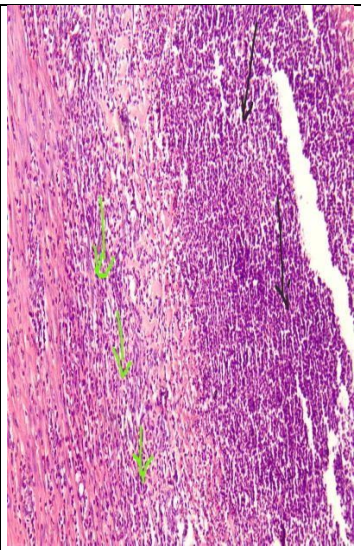
				
ULCER AREA	ULCER AREA (BLACK) WITH GRANULATION TISSUE (GREEN) EXTENDING TO THE MUSCULAR AREA	REDUCED CONGESTION AND THICKENING OF BLOOD VESSELS	ULCER AREA (BLACK) WITH GRANULATION TISSUE (GREEN)	GRANULATION TISSUE SHOWING INFLAMMATORY CELLS (RED), BLOOD VESSEL (BLUE), FIBROBLASTS (YELLOW)

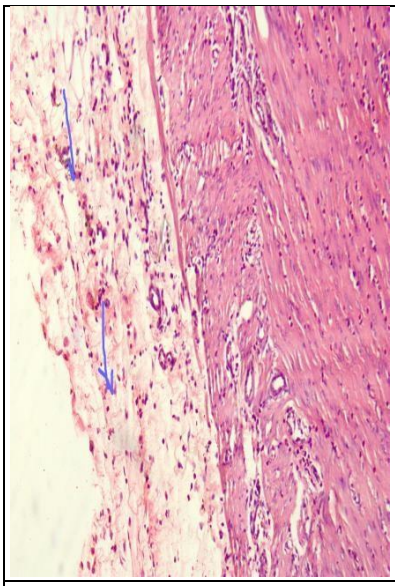
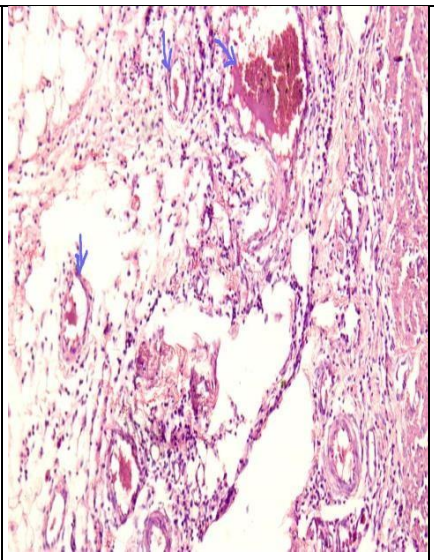
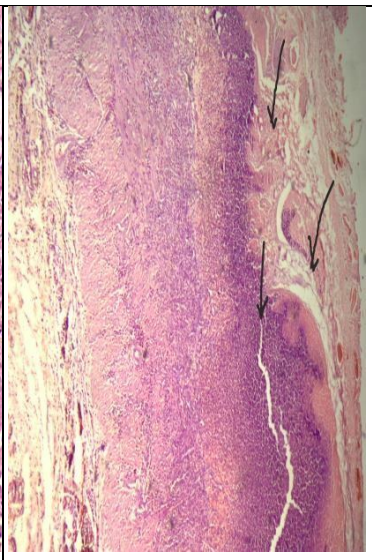
DRUG WITH BOILED MILK GROUP

		
GRANULATION TISSUE WITH INFLAMMATORY CELLS (RED), BLOOD VESSEL (BLUE), FIBROBLAST (YELLOW)	ULCER (BLACK)WITH SMALL AREA OF MUCOSA (BROWN)	ULCER AREA (BLACK)WITH MUCOSA FORMATION (BROWN)

		
ULCER (BLACK)WITH SMALL AREA OF MUCOSAL (BROWN) FORMATION WITH INTACT MUSCULAR LAYER	MUCOSA REGENERATION	REDUCED THICKNESS OF BLOOD VESSELS

DRUG PASTE GROUP

		
GRANULATION TISSUE SHOWING ABUNDANCE OF ACUTE INFLAMMATORY INFILTRATION (RED), FIBROBLAST (YELLOW)	GRANULATION TISSUE CONTAINING INFLAMMATORY CELLS (RED), BLOOD VESSELS (BLUE)	ULCER AREA (BLACK)WITH GRANULATION TISSUE (GREEN)

		
EDEMA	CONGESTED BLOOD VESSEL	ULCER AREA

4. DISCUSSION

The pharmacognostic, physico-chemical and phytochemical evaluation of the drug *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) complied with the data available in databases and confirmed the genuinity and purity of the samples used for the study.

Haemorrhoid induced by croton oil preparation in Wistar Albino Rats were assessed by the histopathological observational parameters like inflammation severity, inflammation extent, edema, congestive and thickened blood vessels, granulation tissue and regeneration of mucosal cell. Compared to the normal control group, the positive control group showed congested blood vessels suggestive of the presence of Haemorrhoids.Standard drug group showed a reduction in size, extension of edema and congestion of blood vessels when compared with the positive control group. Trial drug paste mixed with the unboiled milk group presented a reduction in size of edema, congestion and thickness of blood vessels when compared to the positive control group and Trial drug paste mixed with boiled milk group presented a reduction in size of ulcerative epithelium, acute inflammatory infiltration changes and congested blood vessels. The mucosal regeneration was observed only in the trial drug paste mixed with boiled milk group. The changes in inflammation parameters noted in the trial drug paste group were almost comparable with the positive control group.

Histopathological changes observed in trial drug paste mixed boiled milk showed a reduction in inflammation severity (4 slides) and mucosal regeneration (3slides), when compared to the trial drug paste alone group. The inflammation parameters were graded and the data obtained was analyzed by using statistical tool, Wilcoxon signed rank test. Among the groups, only trial drug paste with boiled milk group exhibited statistically significant changes with Positive control as Z=-2.000, P value-0.046< 0.05. The observed decrease in changes in inflammation severity, inflammation extent, edema and regenerative changes in this group may be attributed to the phytoconstituents like saponins, phenolic compounds, p-Cymene, Mecillinam, 6-Methyl-5-hepten-2-one and Caffeic acid, present in the paste of *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) with boiled milk group.

5. CONCLUSION

The histopathological analysis of the in-vivo study groups suggest that the, whole plant paste of *Actiniopteris radiata* (SW.) Link (*Mayuraśikha*) mixed with boiled milk possess anti-haemorrhoidal activity in Wistar Albino Rats.

Both the boiled and unboiled milk used with trial drug paste showed reduction in histopathology parameters like inflammatory extent, severity, edema, granulation tissue, congestive and thickened blood vessels, suggestive of anti-inflammatory and anti-oxidant properties, when compared to trial drug paste alone group.

Regenerative changes (formation of mucosal cells) were also observed in the trial drug paste mixed with boiled milk group.

6. ACKNOWLEDGMENT

The authors would like to acknowledge Dr. Raiby P Paul, Associate Professor from the Department of Dravyaguna Vigyana, Amrita School of Ayurveda for the constant support, and Mr. Rajagopal, Taxonomist, Amrita School of Ayurveda for botanical identification of the drug. We are grateful to Mr. Sudhakar Bhat, SDM Center for Research in Ayurveda and Allied Sciences, for the assistance and guidance in Animal experimentation and for providing the lab facilities.

7. FUNDING SOURCE

The current study received no specific grants, or funding from any agencies in the public, commercial, or not-for-profit sectors.

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