RESEARCH PAPER

A Comparative Preclinical Efficacy Study of Ayurvedic Ghrita Formulations Prepared by Traditional and Modern Methods for Hemorrhoids (Piles) In Rats

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Abstract:

Background:

Hemorrhoids (piles) are common anorectal disorders characterized by enlargement and inflammation of hemorrhoidal vascular cushions, causing pain, bleeding, irritation, and discomfort. Current treatments include conservative therapy and surgical interventions; however, recurrence and side effects remain concerns. Ayurveda describes hemorrhoids as Arsha and uses Ghrita-based formulations traditionally. Scientific validation of these formulations is limited. Therefore, the present study was designed to prepare, standardize, and evaluate Ayurvedic Ghrita formulations using traditional and modern methods for anti-hemorrhoidal activity.

Aim and Objectives:

The study aimed to prepare and standardize Traditional Ghrita (TG) and Modern Ghrita (MG) containing Ficus racemosa leaf extract and evaluate their anti-inflammatory, antioxidant, safety, and anti-hemorrhoidal activity. The objectives included phytochemical screening, physicochemical evaluation, *in-vitro* antioxidant and anti-inflammatory studies, and *in-vivo* evaluation using a croton oil-induced hemorrhoid model in rats.

Materials and Methods:

Leaves of *Ficus racemosa* were extracted using a hydroalcoholic solvent and subjected to qualitative and quantitative phytochemical analysis. Traditional Ghrita was prepared by classical Ayurvedic method, while Modern Ghrita was prepared using a modified extraction approach to preserve thermolabile compounds. Formulations were evaluated for physicochemical parameters including pH, viscosity, specific gravity, acid value, iodine value, and saponification value. Antioxidant activity was assessed using DPPH free radical scavenging assay and anti-inflammatory activity by protein denaturation method. Acute toxicity study was performed according to OECD guideline 423. Anti-hemorrhoidal activity was evaluated in croton oil-induced hemorrhoid rats through recto-anal coefficient, antioxidant enzyme estimation (SOD and CAT), and histopathological examination.

Results:

Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, glycosides, triterpenoids, carbohydrates, proteins, and amino acids. Total phenolic content and flavonoid content indicated the presence of bioactive compounds responsible for antioxidant and anti-inflammatory effects. Both Ghrita formulations showed acceptable physicochemical properties. It was observed that modern Ghrita formulation showed better quality and efficacy than traditional Ghrita Formulation. pH of Traditional Ghrita and Modern Ghrita were found to be 6.56±0.01 and 6.80±0.03, Specific gravity 0.90±0.005 and 0.92±0.01, Viscosity 6.48±0.02 and 5.28±0.01, Acid Value 6.68±0.02 and 1.11±0.01, Iodine value 3.41±0.01 and 3.17±0.02, Saponification Value 224.4±0.005 and 203.31±0.01respectively which are within pharmacopeial limits for oral administration as well as appropriate for maintaining their uniformity and physiochemical stability.

The formulations exhibited antioxidant and anti-inflammatory activity. Acute toxicity studies showed no mortality or toxic effects up to 2000 mg/kg. In-vivo studies demonstrated reduction in recto-anal coefficient,

improvement in SOD and CAT levels, reduced inflammation, and restoration of anorectal tissue architecture. Modern Ghrita showed comparatively better protective activity than Traditional Ghrita.

Conclusion:

The present study demonstrated that *Ficus racemosa* based Traditional and Modern Ghrita formulations possess significant antioxidant, anti-inflammatory, and anti-hemorrhoidal activities. Modern Ghrita showed improved efficacy by preserving bioactive constituents and providing better histopathological protection. The formulation may serve as a promising herbal therapeutic candidate for hemorrhoidal disease management and requires further clinical evaluation.

Keywords:

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Hemorrhoids; Piles; Ficus racemosa; Ghrita formulation; Anti-inflammatory activity; Antioxidant activity; Anti-hemorrhoidal activity; Ayurvedic formulation.

1. INTRODUCTION:

Hemorrhoids (HEM-o-Royds; Hemo-Blood; Rhodium Flow). Hemorrhoids, referred to as piles, are enlarged and irritated blood vessels located within the anal canal and lower portion of the rectum. This health problem arises as a result of the abnormal enlargement of the normal hemorrhoidal vascular cushion. The main factor that causes this disease is increased pressure in the abdomen, which occurs because of chronic constipation, straining during defecation, sitting for a long period, pregnancy, obesity, aging, and physical inactivity. Prolonged exposure to such risk factors affects the normal circulation, causing harm to the inner wall of blood vessels, releasing of inflammatory chemicals, and a process called venous stasis. Such factors lead to the emergence of hemorrhoids, which can be accompanied by pain, irritation, inflammation, and discomfort during bowel movements. [1]

Haemorrhoids are one of the prevalent anorectal ailments globally, hence haemorrhoidal disease contributes highly towards consultations in gastroenterology and surgical fields. Epidemiologically, up to 50 to 85 percent of the general populace will develop symptoms of haemorrhoid during their lifetime. It is possible that the problem may even be underestimated due to social stigma and self-medication, although the incidence rate has been approximated as high as 1 million patients every year. Prevalence rates in the general populace vary widely, varying between 4.4% to as high as 86% depending on several factors like the demographic profile and methodology adopted.[2] It is estimated that haemorrhoids are 75% prevalent in India, affecting both genders of all ages equally. Prevalence rates vary widely. Whereas generalized estimates indicate that between 75% of people may experience haemorrhoid problems in life, prevalence rates based on community researches stand at around 11% in middle age population groups. Variations in fiber diet intake, sedentary lifestyles, work conditions, defecation habits and healthcare facilities may account for variations in rates [3].

The anal cushions' integrity demands an integrated support system consisting of Treitz's muscle, conjoined longitudinal muscle, Park's ligament and connective tissue framework. These cushions that support the cushions against the anal sphincter complex may flatten during defecation and then return to their normal position. Integrity of this support system is necessary for normal anorectal anatomy. These supporting structures progressively weaken and lead to hemorrhoidal disease. Damage to the muscle of Treitz and relaxation of connective tissue, which makes them unable to maintain their anatomical position, results in prolapse and downward displacement of anal cushions. This decline is due to aging, genetics, and repeated straining. The cushions cannot reorganize after defecation, and fibrous tissue grows and loses its elastic rebound. The process of inflammation is another factor that contributes to hemorrhoid pathogenesis. The action of pro-inflammatory cytokines like IL-6 and TNF-α induces damage and localized inflammation in the tissues of the anal cushions. The oxidative stress and inflammatory factors impairing the health of the vascular and connective tissues of the anal cushions also activate certain enzymes such as matrix metalloproteinases. Through damaging the tissue components of the anal cushions, it leads to venous stasis within the hemorrhoidal plexus.[4]

Administration of flavonoids through oral and topical route is considered as the best form of conservative treatment for hemorrhoids and also use of laxatives, topical therapy, sitz bath therapy) can alleviate symptoms, but do not prevent a recurrence. Despite improvements in the outcomes of today's surgical methods (like laser hemorrhoidoplasty, stapled hemorrhoidopexy, rubber band ligation), risks and recurrences remain an issue [5].

Ayurveda, an Indian classical medicine, recognizes hemorrhoids as Arsha, a disease that arises due to abnormality in srotas and Doshas. Kshara Karma and Bheshaja Chikitsa are two forms of treatment of the disease as per Ayurvedic literature. However, medicinal approaches, especially those involving use of Ghrita formulations are less studied scientifically, as compared to chemical cauterization. Scientific research on effectiveness of Ghrita formulation for haemorrhage is limited and

this research aims to provide evidence supporting the integration of Ghrita-based therapies for managing haemorrhagic conditions.Under Ayurvedic principles, blockage of the flow of blood vessels in the anal area such as VATA, PITTA, and KAPH causes the condition known as piles (ARSHA).[6]Among many medicinal plants showing potential in their anti-hemorrhoidal property, the Udmubar (Ficus racemosa) have properties like anti-inflammatory, antioxidant, and wound-healing capabilities of leaves, bark, and fruits help to control bleeding, prevent swelling of vessels and aid in healing of tissues. It has been found from researches that Ghrita increases the tone of the rectal veins and reduces rectal oxidative stress. Compared to traditional treatment, herbs are more efficacious due to minimal side effects and increased efficacy of herbal treatment. The traditional description of ghrita is that of rasayana that helps maintain tissue health, dosha balance, and also improves the bioactivity of herbs. Decoctions made from herbs have been traditionally prepared by boiling them in ghee, but the process might lead to the degradation of heat-sensitive compounds.

This problem is addressed through modern procedures that ensure stability and effectiveness, as a result of preservation of active ingredients in controlled conditions. In this context, the use of ghrita is recommended in the treatment of piles because of its benefits as an effective and traditional therapy tool, as well as due to the need to incorporate such information into the development of economical and efficient alternatives to traditional methods. Through various mechanisms, such as repair of mucosal tissues, improvement of blood vessel tone, reduction of inflammation, and correction of bowel habit. [8] However, medicinal approaches, especially those involving use of Ghrita formulations are less studied scientifically, as compared to chemical cauterization. Scientific research on effectiveness of Ghrita formulation for haemorrhage is limited and this research aims to provide evidence supporting the integration of Ghrita-based therapies for managing haemorrhagic conditions [7].

2. MATERIALS & METHODS

Plant material

Leaves of *Ficus racemosa* were collected and confirmed as one of the Ayurvedic herbs used for making the Ayurvedic Ghrita. Local *Ficus racemosa* leaves were collected. Plant materials collected were first cleaned and then dried in the shade. Plant materials were grounded to coarse powder in the grinder. Identification of source plants was done by the Botanist of Shikshanmantri Dr. Bapuji Salunkhe College, Miraj.

Dried powder of *Ficus racemosa* leaves was defatted using petroleum ether at (40-60 °C). Defatted samples were further Soxhlet extracted with hydro-alcoholic mixture (70:30 ethanol-water ratio). This technique made it possible for simultaneous extraction of both lipid soluble as well as water soluble compounds into concentrated semi-solid extract for further analysis [8] [9].

2.2 Phytochemical Screening:

Qualitative Analysis -

The extract was screened qualitatively for the identification of the different secondary metabolites in the hydro-alcoholic extract. The test of alkaloids, flavonoids, tannins, saponin, steroids, cardiac glycosides, triterpenoids, carbohydrates, phenolic compounds performed. [10] [11].

Quantitative Analysis-

Total phenolics of hydroalcoholic extract was quantified using Folin Ciocalteu reagent, Aluminium chloride method was used for determining the total flavonoid content. [12] [13].

Preparation and Standardization of Ghrita

a) Preparation of Traditional Ghrita (AG)

Freshly collected and dried *Ficus racemosa* leaves and fresh cow ghee were used in preparing Ayurvedic ghrita. Firstly, dried leaves of the plant were collected, dried in shade and powdered. In order to prepare a paste for use in preparation of Kalka, some finely powdered leaves were then triturated with a small quantity of water. Boiling one part of the coarsely powdered leaves with sixteen parts water then concentrating to one fourth of its original quantity resulted in the preparation of kwath.

Preparation of ghrita was done using a ratio of 1:4:16 of Kalka, cow ghee and kwath, respectively. The filtrate obtained after preparation was collected as ayurvedic ghrita. Mixture of the above-mentioned ingredients was then heated until all the aqueous content evaporates to form ayurvedic ghrita. Filtered heated mixture using double layered muslin cloth and collected the clear liquid as ayurvedic ghrita, which was later poured in a clean dry glass jar [14].

b) Preparation of Modern Ghrita (MG)

Ficus racemosa extract of the plant and cow ghee were used to prepare modern ghrita. Since thermosensitive bioactive compounds had to be protected, the dried and ground plant matter was extracted using the Soxhlet technique under low temperatures in a hydroalcoholic solvent. The extracted solution obtained through the Soxhlet technique was then mixed slowly into heated cow ghee that was stirred continuously until all the solvent was evaporated.

Ficus racemosa extract of the plant and cow ghee were used to prepare modern ghrita. Since thermosensitive bioactive compounds had to be protected, the dried and ground plant matter was extracted using the Soxhlet technique under low temperatures in a hydroalcoholic solvent. The extracted solution obtained through the Soxhlet technique 4 part was mixed slowly into 1 part of heated cow ghee that was stirred continuously until all the solvent was evaporated. The final ghrita was then filtered to remove all traces of plant matter before obtaining clear ghrita.

Fig 1. Formulation of Traditional and Modern Ghrita

Physicochemical evaluation of Ghrita

As per established methods described in ayurvedic pharmacopoeia and textbooks, the Ayurvedic samples of Ghrita were analyzed on several organoleptic (color, aroma, taste, texture, feel) and physico-chemical properties (acid number, iodine number, saponification number, peroxide value, rancidity test, refractive index, viscosity, pH value, and specific gravity).

1. pH

A digital pH meter that was already calibrated was used to determine the pH of the sample. The electrode was then cleaned and rinsed with distilled water after which the calibration was carried out using the standard buffers that had pH values includes 4.0, 7.0, and 10.0. After calibrating the instrument, the electrode was immersed in the solution; gently swirled, and when stable reading was achieved, the reading was taken.

2. Specific Gravity

A specific gravity bottle that had been dried and cleaned was used to determine the specific gravity of the sample. First, the empty bottle was weighed carefully. Secondly, the bottle was filled with exactly 10 ml of distilled water at right temperature then weighed. Likewise, 10ml of the sample under test was transferred into another clean dry vial and weighed. The specific gravity of the sample was therefore calculated using the formula below [14]

$$\text{specific gravity} = \frac{Liquid\ mass\ of\ Sample(gm)}{mass\ of\ Water\ in(gm)}$$

3. Viscosity

Viscosity Viscometers or Ostwald viscometers can be used to measure the viscosity of liquids. Glass U-Tube viscometer is the name of the apparatus used. When the liquid sample is inserted into the tube, the time taken to move from point A to point B is then measured.

$$\text{Viscosity} = \eta 1 = \eta 2.\ \rho 1\ t1 / \rho 2\ t2$$

4. Iodine value

Iodine value determination was performed on the fat sample in order to establish the level of unsaturation. Five grams of fat sample was weighed accurately and dissolved in chloroform in the iodination flask marked "Test". After addition of 10 milliliters of iodine monochloride reagent, the mixture was vigorously shaken. The iodination flask was kept in the dark for half an hour so as to allow the reaction to take place. Preparation of blank entailed adding 5ml of chloroform and 10 milliliters of iodine monochloride reagent in another flask which was incubated in the dark for 30 minutes just like test sample. After incubation, potassium iodide solution (5 ml) was added into test sample, distilled water (25 ml) used to wash down the flask walls. Starch indicator (0.5 ml) was added when titration was done using standardized sodium thiosulphate solution until pale straw colored was achieved. Titration process was continued till the blue color disappeared; the point at which blue color disappeared was the end point of titration [15][16].

$$\text{Iodine value} = \frac{(B-S)*N*12.69}{W}$$

5. Acid Value

Acid value is defined as the quantity of potassium hydroxide needed (in mg) to neutralize the acids present in 1 g of a particular material. In the current experiment, 5 g (or 0.5 – 2.5 g for resins) of the material was accurately measured and placed in a 250 ml conical flask. The flask was further filled with 25 ml of alcohol-ether neutral solution (1:1), and 0.5 ml of phenolphthalein indicator solution was added.

The solution was then heated gradually using a water bath until the material melts or dissolves. The solution was titrated with 0.1 N potassium hydroxide until a pink color persists for 15 seconds after constant stirring. The volume of potassium hydroxide required for neutralization was determined. The acid value was obtained from the following equation:

$$\text{Acid value} = \frac{V*N*56.1}{W}$$

6.Saponification Value

The no. of milligrams of potassium hydroxide necessary for the neutralization of the fatty acids produced by the full hydrolysis of one gram of oil or fat is called as saponification value.

A conical flask of 250 mL having been tared was filled with 2 g of the substance under test. To it, a solution of alcoholic potassium hydroxide (25 mL) was added. For its full saponification, a reflux condenser was fixed on the flask, then placed in water bath to heat it for an hour, occasionally

swirling it. After heating, it was cooled down to room temperature. To it, 1 mL of solution of phenolphthalein indicator was added, then it titrated against 0.5 N HCl till end point was achieved; the volume of acid taken for it was noted as a.

A blank test was simultaneously conducted using equal amounts of reagents except the substance; the volume of acid was noted as b.

For calculating the saponification value, the following equation was used

$$\text{saponification value} = \frac{B - S * N * 56.1}{W}$$

***IN-VITRO* STUDIES:**

Anti-Inflammatory Activity

Activity Against Inflammation The anti-inflammatory activity was assessed by egg albumin denaturation. Eggs from a freshly laid hen were used in the preparation of 1% egg albumin in distilled water. The solution was stirred until it became uniformly dispersed. Since egg albumin will denature on heating, cold distilled water is used to avoid the coagulation of the protein. Suitable vehicle was used in the preparation of the test sample of hydroalcoholic extract and Ghrita for making stock solution of concentration 1 mg/ml. Working solutions were made at concentrations of 200 µg/ml, 400 µg/ml, 600 µg/ml, and 800 µg/ml through dilution of stock solution. Reaction mixture consisted of 1 ml egg albumin, 2.8 ml phosphate buffered saline (PH 6.4), and 1 ml sample of each concentration in test tube.

This was then replaced with distilled water to form the control sample. The normal drug that was used was the Diclofenac sodium drug, and it was dosed in the same manner. Following the process of 15 - 30 minutes of incubation at the temperature of 37 ± 2 °C, all the reaction mixtures were then incubated at 70 °C for five minutes. The UV-visible spectrophotometer was used to determine the absorbance at wavelength 660 nm upon cooling to room temperature [17].

$$\% \text{ Inhibition} = \left[\frac{(A_s - A_b)}{A_c}\right] \times 100$$

DPPH free radical scavenging assay:

The antioxidant activity was determined by measuring the scavenging action of free radicals by 2,2-diphenyl-1-picrylhydrazyl. The antioxidant activity was determined based on the scavenging action of the DPPH free radicals. DPPH solution was prepared by dissolving 3.9 mg of DPPH in 100 ml methanol and stirring overnight at refrigeration conditions (4 °C). This led to the preparation of a DPPH free radical solution of 0.1 mM concentration. The violet-colored solution of DPPH free radicals was kept in the frozen state (-20°C) for further use. Solutions of five different concentrations 100, 200, 300, 400 and 500 µg/ml of methanolic extracts were prepared by diluting the stock solutions. In all cases, 2.5 ml of methanolic DPPH solution was diluted with 0.5 ml of each solution. In case of control experiments, 2.5 ml of methanolic 0.1 mM DPPH solution was mixed with 0.5 ml distilled water. All solutions were incubated for half an hour in the absence of light. Measurements were taken at a wavelength of 517 nm using a blank solution of 0.5 ml distilled water + 2.5 ml of MeOH.

where AS represents Absorbance of sample solution, Ab represents absorbance of blank and AC represents absorbance of control.

$$\% \text{ Scavenging} = \left[\frac{(A_s - A_b)}{A_c}\right] \times 100$$

The concentration of samples needed to scavenge 50% of DPPH radicals is referred to as IC50 values, which were obtained from the graph of the radical scavenging ability in relation to extract concentration.

***IN-VIVO* STUDIES:**

Experimental Animals:

This study was carried out in line with the practice and principles of the institution on the use of experimental clearance and approval by the IAEC set up according to CCSEA, India. Thirty-six female Wistar Albino rats 8–10 weeks old and weighing between 180g and 230 g obtained from the animal house were used for this study. The rats were housed in wire meshed (6 per cage) cages and maintained at 24±2°C temperature and a cycle of 12:12 hours light/dark with free access to food and water at all time. After adaptation into the laboratory conditions for one week, the rats were randomly divided into 7 groups of 6 rats.

Acute oral toxicity study

The OECD (Organization for Economic Cooperation and Development) guideline 423 was referred for determination of acute oral toxicity study of Traditional and Modern ghrita. Overnight fasted Wistar female rats were orally administered with Traditional and Modern ghrita. following the guidelines and were observed individually for about 24 h for any behavioral or neurological abnormalities viz. diarrhoea, feeding behavior, convulsions, tremors, lacrimation, sleep, and salivation as a marker for toxicity. For confirming the mortality rate if any, the rats were kept under observation for two weeks.

Induction of Hemorrhoids (Piles) Disease:

The Wistar rats will be induced with the formation of hemorrhoids through the use of the modified croton oil method. The modified croton oil solution will be prepared through the mixing of deionized water, pyridine, diethyl ether, and 6% croton oil in diethyl ether in the ratio 1:4:5:10. Before the experiment, the rats were starved overnight without restriction in the intake of water. For the induction of hemorrhoids, 6% croton oil will be introduced intra-anally using the sterile cotton which will be inserted into the rectum of the rats to a distance of 1.5 cm for about 30 seconds. The treatments shall commence from the 4th day until the 8th day.

Physical parameters of the animals shall be assessed from day one up to day nine [18]

Group I -Normal control group with only normal saline

Group II-Disease control with application of 100 µL Croton Oil Preparation (Anorectal portion) for 3 days.

Group III (STD) with 200 mg/kg Pilex granules by oral route and

Group IV & V with low dose (200 mg/kg) and high dose (400 mg/kg) of Traditional Ghrita respectively.

Group VI &VII with low (200 mg/kg) and high dose (400 mg/kg) of Modern Ghrita respectively.

Both the test drug and a standard drug would be administered to 5 groups of animals for 5 days. The drugs shall be administered from 4th day to 8th day. Physical characteristics of the animals would be observed from 1st day up to 9th day. Animals would be sacrificed and ano-rectal tissues collected for biochemical assays.

Sacrifice of rats and sample collection

At the end of the study on 9 th day the experimental animals were fasted for 16 hours then taken into a separate room where they were weighed and sacrifice under CO2 Chamber. Then a portion of Ano-rectal tissue was excised, and 10% w/v homogenate was prepared in ice-cold Tris-HCl 50mM solution, respectively, and centrifuged at 3000 rpm for 25min at 4°C. The supernatant obtained was used for the estimation of antioxidant activity (catalase and superoxide dismutase).

Estimation of recto-anal coefficient

For calculating rectoanal coefficient (RAC), the previously weighed recto anal tissues were compared with the body weight of the individual rats and the obtained value represented the RAC, which helps in judgement of severity of inflammation using

the formulae:

Rectoanal coefficient=weight of rectoanal tissue (mg)/Body weight (g)

Antioxidant Analysis:

Antioxidant analysis was performed on the homogenate of rectoanal tissue. The supernatant was used for the estimation of superoxide-dismutase (SOD) activity and catalase activity (CAT) [19].

Histopathology: A 10% formalin solution was used to repair the little section of the anorectal region that had been dissected. After processing it using standard techniques, slices that were 5 micrometer thick were cut out and stained with hematoxylin and eosin. Under a microscope, slides were made and checked for pathological alterations [8]

Statistical Analysis:

The presented value are denoted as Mean ± SEM. The statistical assessment involved employing a one-way ANOVA by Dunnett's test (Graph Pad Prism 11). This study chose level of statistical analysis as $P < 0.05$.

RESULTS

Phytochemical screening of Hydro-alcoholic extract:

The results of the phytochemical screening of the hydro-alcoholic extract are summarized in Table 2. A plethora of secondary metabolites were identified, including alkaloids, flavonoids, tannins, saponin, cardiac glycosides, steroids, triterpenoids, carbohydrates, phenolic compounds, proteins and amino acids is performed.

Table 1 : Phytochemical screening of Hydro-alcoholic extract:

Phytochemicals	Test	Observations Presence (+) /Absence (-)
Alkaloids	Mayer's test	+
	Wagner's test	+
	Dragendroff's test	+
	Hager's test	-
Flavonoids	Ferric chloride test	+
	Lead acetate test	+
	Alkaline test	+
	Sulphuric acid test	-
Tannin	Gelatin test	+
	10% NaOH test	-
	Braymer's test	-
Phenolic compounds	Iodine test	+
	Lead acetate test	+
	Ellagic acid test	+
	Ferric chloride test	+
Phytosterols	Hesse's response	+
	Sulphur test	+
Triterpenoids	Salkowski's test	+
Proteins and amino acid	Biuret test	+
	Xanthoproteic test	-
	Million's test	-
	Benedicts test	+

Carbohydrates	Fehling's test	+
	Legal 's test	+
Glycosides	Cardenolide's test	+
	Killer- killani test	-

In quantitative analysis, Total phenolic content and total flavonoid content were determined and it was found that, total phenolic content was in the range of 100-500 µg/mL, which corresponds to 138 mg GAE/gram to 473 mg GAE/gram. Total Flavonoid content was found to be in the range of 128.95 mg QE/gram to 423.55 of extract. These results suggest that formulation is rich in compounds essential for reducing oxidative stress, inflammation and venous pathology, capillary fragility and hemorrhoids.

Physiochemical Parameters:

Table No. 2: Physiochemical parameters of Ghrita

Parameter	Traditional Ghrita (Mean ±SD)	Modern Ghrita (Mean ±SD)
pH	6.56±0.01	6.80±0.03
Specific Gravity	0.90±0.005	0.92±0.01
Viscosity	6.48±0.02	5.28±0.01
Acid Value	6.68±0.02	1.11±0.01
Iodine Value	3.41±0.01	3.17±0.02
Saponification Value	224.4±0.005	203.31±0.01

Fig.2: Specific Gravity **Fig. 3: Acid value**

Fig. 4: Viscosity

Fig. 5: Iodine value

Fig. 6: Saponification value

***In-Vitro* Study Results:**

Anti-inflammatory activity

The Anti-inflammatory activity observed that where the **Standard (Diclofenac Sodium)** shows anti-inflammatory activity with IC50 value **241 µL/mL** and **Hydroalcoholic extract** shows moderate anti-inflammatory activity with IC50 value **589 µL/mL** The **Traditional ghrita** shows anti-inflammatory activity with IC50 value **247 µL/mL** and **Modern ghrita** more significant inflammatory activity with IC50 value **615 µL/mL**

DPPH Assay

It is observed that the hydroalcoholic extract of *Ficus Racemosa* possesses concentration-dependent antioxidant activity where the % inhibition increases from **48.21 % at 100 µg/mL** to **88.21 % at 500 µg/mL.** With an increase in concentration, its activity becomes closer to the standard antioxidant, ascorbic acid

***In- Vivo* Study Results:**

Acute oral toxicity study

From the results of the acute oral toxicity trial, Traditional and modern Ghrita was found to be safe up to 2000 mg/kg with no evidence of behavioural or neurological toxicity and mortality.

Recto-anal Coefficient (RAC):

The RAC were significantly lower in normal control group compared to the disease control group. In both T-1 and T-2 groups of TG and T-1 and T-2 groups of MG also standard group, RAC were slightly decrease relative to the disease control group.

As the disease progressed, the Recto-anal Coefficient (RAC) of the Disease control group significantly increased and it showed 4.41±0.083, indicating severe hemorrhoidal inflammation and tissue thickening due to hemorrohoid induction. The Normal control group showed 2.81±0.07.

The Standard-treated group showing 2.74±0.078 it indicated protection against hemorroidal Similarly, the TG Test-1 group showed (RAC) only a slight increase (RAC) 3.60±0.052. TG Test-2 group showed (RAC) 3.24±0.086 as well as MG Test-1 group showed (RAC) 3.60±0.052 it indicating moderate improvement. MG Test-2 group also maintained near-normal (RAC), 3.05±0.066 increasing (RAC) suggesting better prevention of disease-induced (RAC)

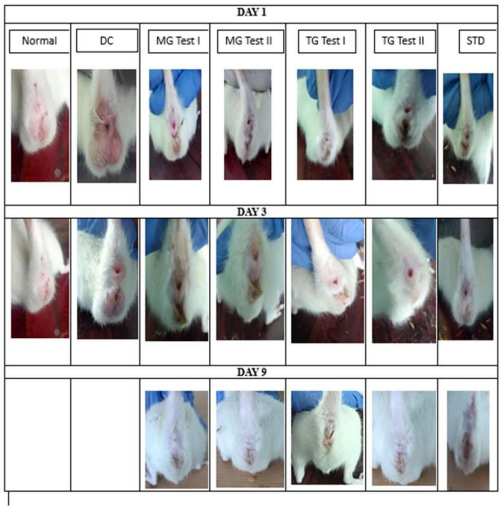
Table 3: RECTO-ANAL COEFFCIENT (RAC)

GROUP	RECTO-ANAL COEFFCIENT (RAC)
NC	2.81±0.077
DC	4.41±0.083
STANDARD	2.74±0.078
TEST 1 (TG)	3.60±0.052
TEST 2 (TG)	3.24±0.086
TEST 1 (MG)	3.60±0.052
TEST 2 (MG)	3.05±0.066

Fig. 7: Recto-anal Coefficient

Values are presented as mean ± SEM, with N = 6 animals per group. Statistical analysis was performed using two-way ANOVA, followed by post hoc Dunnett's multiple comparisons test, with DC group as the reference control for all comparisons. The statistical significance of differences in body weight between treatment

groups and the DC group is indicated by asterisks: p <0.05 (*), p < 0.01 (**), p < 0.0001 (****) "ns" denotes non-significant differences (p > 0.05).



Estimation of Antioxidant Parameters-

The antioxidant enzyme SOD, CAT levels were significantly reduced in the campared to the Disease control group, indicating increased oxidative stress caused by hemorrhoid induction. The Normal control group showed SOD and CAT level of 36.92±0.15 and 0.054±0.0007 U/mg protein. In Disease Control (DC) group significantly decreased SOD, CAT level of 10.78±0.32 U/mg protein and 0.012±0.0003 U/mg protein.

Standard treated group (STD) and MG Test-2 group showed increased anti-oxidant stetus. The Standard treated group showed SOD, CAT level 35.12±0.05 U/mg protein and 0.034±0.0008 U/mg protein. Similarly, the MG Test-1 group shows SOD, CAT level 29.42±0.27 U/mg protein and 0.022±0.0009 U/mg protein. TG Test 1 group shows SOD, CAT level 16.16±0.12 U/mg protein and 0.016±0.0006 U/mg protein. TG Test 2 group shows SOD, CAT level 16.16±0.12 U/mg protein and 0.016±0.0006 U/mg protein. The MG Test-2 group showed SOD, CAT level 34.20±0.09 U/mg protein and 0.028±0.012 U/mg protein, suggesting better protection against oxidative stress induced by hemorrohoids [19].

Table 4: Antioxidant Parameters of Anorectal Tissue Homogenate

GROUP	SOD	CAT
NC	36.92± 0.15	0.054±0. 0007
DC	10.78± 0.32	0.012±0. 0003
STANDARD	35.12± 0.05	0.034±0. 0008
TEST 1 (TG)	16.16± 0.12	0.016±0. 0006
TEST 2 (TG)	29.08±0 .07	0.022±0. 0008
TEST 1 (MG)	29.42±0 .27	0.022±0. 0009
TEST 2 (MG)	34.20±0 .09	0.028±0. 012

Fig. 8: SOD

Fig. 9: CAT

The antioxidant enzyme SOD, CAT levels were significantly reduced in the campared to the Disease control group, indicating increased oxidative stress caused by Hemorrhoid induction. The Normal control group showed SOD and CAT level of 36.92±0.15 and 0.054±0.0007 U/mg protein. In Disease Control (DC) group significantly decreased SOD, CAT level of 10.78±0.32 U/mg protein and 0.012±0.0003 U/mg protein.

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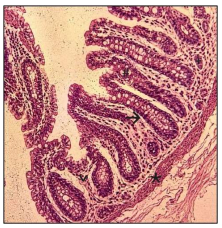
Data represented as mean ± SEM (n=6). ####p< 0.001 when compared with Disease control group and p <0.05 (*), p < 0.01 (**), p < 0.0001 (****), is considered as criteria for significance when compared with disease control using one way ANOVA coupled with Dunnet's comparison test.

Histopathology:



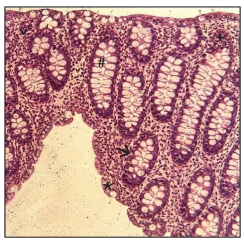


Fig. 6.30: Normal Control Group

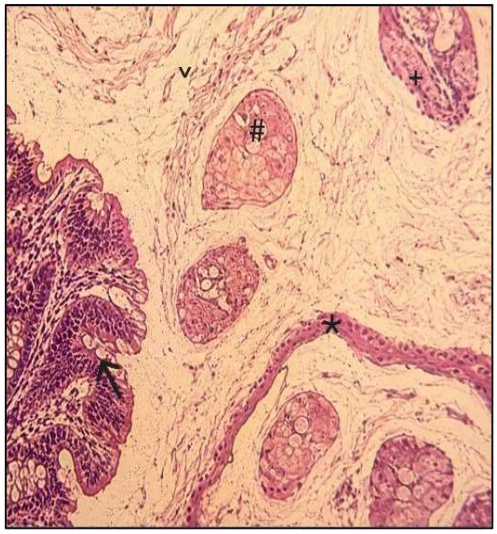


Fig. 6.31: Disease Control Group

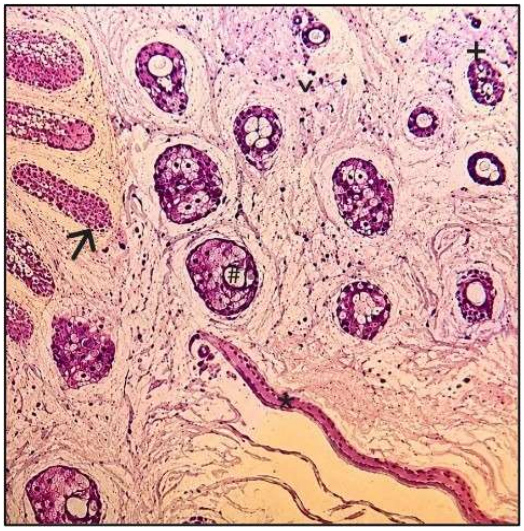


Fig. 6.32: Standard Test-2 Group

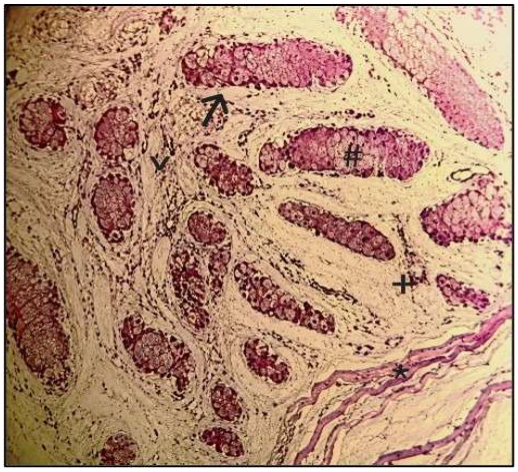


Fig.6.33: TG Test-1 Group

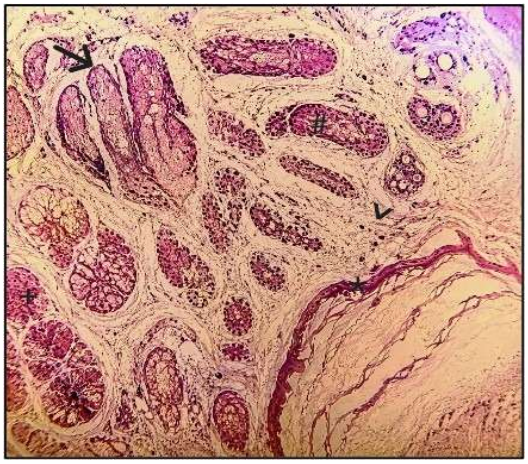


Fig. 6.33: TG

Fig. 6.34: MG Test-1 Group

Fig. 6.35: MG Test-2

Group

The Histopathological observation includes * indicates integrity of epithelium lining and presence or absence of epithelial erosion. # indicates No. of goblet cells and their integrity.

→Aarchitecture of crypt cells. > inflammatory cell infiltration. + indicates vascular congestion. Where, **Disease control Group** showed severe histopathological alterations including epithelial disruption, depletion of goblet cells, distorted crypt architecture, inflammatory cell infiltration, edema, and marked vascular congestion compared with the **Normal control group**, indicating severe piles-induced mucosal injury. Treatment with the **Standard drug** and test formulations significantly improved the tissue architecture. In the case of **TG Test-1** and **TG Test-2**, the moderate restoration of epithelial lining, goblet cells, and crypt formation was observed along with reduced inflammation and congestion. In **MG Test-1**, a relatively better effect in terms of mucosal healing and crypt formation was found, **MG Test-2**, maximum protection was observed by nearly maintaining epithelium, goblet, and crypt cells along with minimal edema and congestion.

DISCUSSION

The present study aimed to prepare and standardize Ghrita formulations by traditional and modern methods and to evaluate their anti-haemorrhoidal (anti-piles) activity of the in rats using the croton oil induced hemorrhoid model. This discussion includes finding across multiple domain including formulations and standardization, physiochemical characteristics, phytochemical profiling. *In-vitro* study includes anti-oxidant activity, anti-inflammatory activity, clotting time determination as well as *in-vivo* study consisting acute toxicity, hemorrohoidal parameters, biochemical analysis and histopathological examination. The Traditional Ghrita was prepared using classical ayurvedic

procedure and Modern Ghrita was prepared by novel approach with slight modification that included lesser heat treatment so as to preserve thermolabile phytoconstituents. It was observed that modern Ghrita formulation showed better quality and efficacy than traditional Ghrita Formulation. pH of Traditional Ghrita and Modern Ghrita were found to be 6.56±0.01 and 6.80±0.03, Specific gravity 0.90±0.005 and 0.92±0.01, Viscosity 6.48±0.02 and 5.28±0.01, Acid Value 6.68±0.02 and 1.11±0.01, Iodine value 3.41±0.01 and 3.17±0.02, Saponification Value 224.4±0.005 and 203.31±0.01respectively which are within pharmacopeial limits for oral administration as well as appropriate for maintaining their uniformity and physiochemical stability.

Qualitative phytochemical evaluation revealed the presence of important secondary metabolites such as alkaloids, flavonoids, phenolics, glycosides, tannins, triterpenoids, saponins and carbohydrates. These constituents are well documented for anti-inflammatory, antioxidant, wound healing and anti-hemorrhoids properties. These properties indicate potential of modern and traditional Ghrita formulations for anti-hemorrhoidal activity. Quantitative analysis confirmed the presence of bioactive compounds. Total phenolic content and total flavonoid content were determined and it was found that the total phenolic content was in the range of 100-500 µg/mL, which corresponds to 138 mg GAE/gram to 473 mg GAE/gram. Total Flavonoid content was found to be in the range of 128.95 mg QE/gram to 423.55 of extract. These results suggest that formulation is rich in compounds essential for reducing oxidative stress, inflammation and venous pathology, capillary fragility and hemorrhoids. Anti-inflammatory activity evaluated by protein denaturation assay showed IC50 value of 615 µL/mL for Modern Ghrita and an IC50 of 589 µL/mL for *Ficus racemosa* extract. The Traditional ghrita shows anti-inflammatory activity with IC50 value 247 µL/mL which is near equivalent to Standard (Diclofenac Sodium). Anti-oxidant activity of the extract and formulations was evaluated using DPPH Scavenging Assay. It showed that IC50 1(17.17 µg/mL) of *Ficus racemosa* extract was lower than Ascorbic Acid (105.17 µg/mL). The clotting time was evaluated by using Lee-White method. The disease control group animals had a higher clotting time as compared to treated animals. Modern and Traditional Ghrita as well as Standard reduced the clotting time but the reduction in clotting time was found to be equivalent to Standard. Acute oral toxicity studies conducted according to OECD 423 guidelines revealed no sign of toxicity and mortality at a dose of 2000 mg/kg, establishing safety of formulation for further pharmacological studies. Anti-hemorrhoidal assessment found that recto-anal coefficient of treated animals (Modern Ghrita, Traditional Ghrita and Standard) was reduced as compared to disease control. Body weight was also increased in treated animals as compared to disease control.

Biochemical estimation further validated the anti-hemorrhoidal activity of formulations. There was a marked improvement in catalase activity at 400 mg/kg of modern Ghrita dose (0.028±0.012 U/mg protein) as well as SOD Activity (34.20±0.09 U/mg protein). This indicated reduced oxidative stress and restoration of antioxidant defence systems in the anorectal region.

Histopathological analysis provided visual confirmation of anti-hemorrhoidal activity. The disease control group showed hemorrhoidal alterations like increased inflammation, vascular congestion, epithelial erosion, edema, dilation of blood vessels, goblet cell depletion, distorted crypt architecture and mucosal damage compared with normal control group. Standard treated group showed improvement in terms of anorectal morphology. Modern Ghrita treated group particularly at 400mg/kg dose, showed maximum protection with intact epithelial architecture, well-preserved goblet cells and crypt cells, minimum edema, congestion and inflammation. Traditional Ghrita also showed improvements in anorectal lesions but not amounting to the efficacy of Modern Ghrita. In conclusion, the Traditional Ghrita and Modern Ghrita formulations demonstrated significant antioxidant, anti-inflammatory and anti-hemorrhoidal activity in both *in-vitro* and *in-vivo* models of hemorrhoidal disease. Their effects were comparable to standard treatment with Pilex Granules, thereby supporting its potential as an alternative or adjunct therapy in the management of hemorrhoidal disease.

CONCLUSION

The current research aimed to prepare and standardize Ghrita formulations by traditional and modern methods and evaluate their anti-hemorrhoidal activity using phytochemical analysis, *In-vitro* antioxidant, anti-inflammatory activity evaluation and *In-vivo* croton oil induced hemorrhoids model approches. The Formulations exhibited acceptable physicochemical characteristics, including appropriate pH, Specific gravity, viscosity, acid value, iodine value and saponification value ensuring their quality, stability and suitability for the oral administration. Phytochemical Analyses revealed the presence of key bioactive compounds such as alkaloids, flavonoids, phenolic acids, glycosides, tannins and carbohydrates which are pharmacologically effective in hemorrhoidal conditions. Quantitative estimation confirmed a high content of total phenolic content and total flavonoid content.Both Traditional and Modern Ghrita showed Anti-inflammatory activity comparable to Standard. DPPH assay showed that *Ficus racemosa* leaf extract had dose dependent free radical scavenging activity.

No toxicity was observed in acute oral toxicity (OECD 423) up to 2000 mg/kg, confirming the formulation safety. Definite and measurable improvements were observed in Clotting Profile of Treated animals as compared to Disease Control Animals embarking upon their efficacy in reducing hemorrhoidal bleeds due to venous congestion and capillary fragility. *In-vivo* croton oil induced hemorrohoids in rats model demonstrated significant improvement in hemorrohoidal condition. The Modern Ghrita treated groups exhibited highest improvement in histopathological lesions as shown by reduced Recto-anal coefficient, oxidative stress, and body weight is increased.

Biochemical parameters further supported the anti-hemorrhoidal activity of the formulation, showing elevated catalase and SOD activity.

In Conclusion the Modern Ghrita formulation demonstrated significant antioxidant, and anti-hemorrhoidal effect comparable to standard treatment with Pilex granules. It would be appropriate to conclude that the Ghrita Formulations containing *Ficus racemosa* leaf extract are a promising candidate for further development as an alternative or complementry treatment for hemorrhoide disease and future clinical validation.

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