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Research Article

PHYTOCHEMICAL ESTIMATION AND ANTI- HEMORRHOIDAL ACTIVITY OF FICUS BENGHALENSIS LINN. PROP ROOT EXTRACT

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ABSTRACT

This present study was conducted to validate the traditional claim of the root extract of *Ficus benghalensis* with black pepper for the treatment of hemorrhoids. The anti-hemorrhoidal property of *Ficus benghalensis* was evaluated by the help of the croton oil induced edema animal experimental model. Preliminary phytochemical screening of ethanolic extract of prop root had shown the presence of alkaloids, glycosides and tannins. Quantitative phytochemical estimation was also performed and total phenolic content was found to be 25.112 mg/g of Gallic acid equivalent (GAE).Hemorrhoids has been induced in the experimental wistar rats by the application of croton oil preparation (COP). The ethanolic prop root extract formulation with black pepper was found to reduce the severity score, the recto anal coefficient, Evans blue dye concentration and levels of TNF-a and IL-6. The extract formulation was found to be effective in the treatment of Hemorrhoids in experimental animals.

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INTRODUCTION

Ficus benghalensis Linn. also known as the Indian Banyan tree is a very sacred plant with good medicinal properties. It is also known as Vata in Ayurveda. A total of 800 species and 2000 species varieties of Ficus are found in the world which are mostly seen in the tropical areas (1). This sacred tree of India has been used to treat a number of pathological conditions namely infections

and inflammations of the Gastrointestinal tract and skin infections. Ficus species are widely distributed all over India and are studied for their medicinal properties (2). Banyan Tree is one of the oldest trees also known for its characteristic hanging prop roots which grow from horizontal branches and touch the ground (3).

The different parts of the plant and their extracts have been known for their analgesic, anti-inflammatory, anthelmentic[4], immune modulatory, and anti-microbial activity (1, 4-5). These plant extracts are also used in different Ayurvedic preparation to treat various disorders (6). The phyto-constituents present bring about these effects.

Hemorrhoids is a pathological condition in which symptomatic enlargement and distal displacement of the normal anal cushions is observed (7). The exact pathophysiology is unknown but sliding anal canal theory best explains this pathological condition. Biochemical changes such as imbalances between endothelium relaxing and constricting factors leading to vascular disordersare also seen (8). A number of marketed formulations are also for the treatment of hemorrhoids such as pilex tablets.

In this present study we are going to investigate the anti-hemorrhoidal property of ethanolic extract of Ficus benghalensis prop root extract. The prop root extracts have been traditionally claimed to be used to treat piles (hemorrhoides) when taken along with *Piper nigrum* dissolved in milk. The plant has been seen to be effective in treating this disorder when taken for seven days. Piperine in *Piper nigrum* acts as a bio enhancer, which may increase the bioavailability of drug in the system thus increasing their efficacy (9, 10).

Methodology

Plant material andauthentication

The prop roots of *Ficus benghalensis* were collected from the tree which is present at the crossing near Rajputana grounds inside the campus of IIT (BHU) Varanasi. The roots were authenticated by Dr. N. K. Dubey, BHU, Varanasi.

Extractionmethod

Microwave assisted soxhlet extraction

Microwave assisted soxhlet extraction of prop roots of *Ficus benghalensis* was done as per standard protocol [11].

Preliminary phytochemical screening of the prop root extracts

Ethanolic extract of *Ficus benghalensis* prop

root extract was taken and subjected to preliminary phytochemical screening for the presence of different phytochemicals using various qualitative reagents (12, 13).

Quantitative Phytochemical estimation

Determination of total phenolic content

For total phenolic content determination, 200 µL of each sample was mixed with 1.4 mL purified water and 100 L of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20 % Na_2CO_3 aqueous solution was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample[15].

Determination of total flavanols and flavanoids

It was done by using rutin as the standard reference compound. This method involved the formation of a flavonoid / flavonol-aluminum complex having the absorbitivity maximum at 415 nm and 440 nm for flavonoid and flavonol respectively. The amount of flavonoids and flavonol in plant extracts in Rutin equivalents (RE) was calculated by using the formula:

$X = (A \times mo) / (Aox\ m)$

Where, X is the flavonoid or flavonol content, mg/g plant extract in RE, A is the absorbance of plant extract solution, Ao is the absorption of standard Rutin solution, m is the weight of plant extract in mg and mo is the weight of Rutin in the solution in mg(15).

In vivo studies

Animals

Healthy adult male Wistar rats (8-10 weeks age and 220-250 g weight) were used for the study. The animals were maintained at central animal facility of the institute under standard experimental conditions of temperature (25 ± 1 °C), relative humidity (50 ± 5%) and 12 h light: dark cycle. They were housed in polypropylene cages in a group of 4 animals/cage. They were fed standard rodent chow and water ad libitum. Experiments were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forest, New Delhi after seeking approval by the Institutional

Animals Ethical Committee (IAEC).

Acute toxicity studies

Acute toxicity studies for this plant extract were conducted by the following OECD 423 guidelines. Animals were fasted before dosing and then weighed. The extract was given orally. After the administration, the food was further withheld for further 3-4 hours. Different doses of fraction were administered (100,200, and 400 mg/kg of body weight orally) and was observed after dosing at least once in the first 60 min and then periodically during 24 h for a total of 14 days (16).

Grouping and Treatments

Group 1: Normal control (NC) group without croton oil

Group 2: Hemorrhoid vehicle control (HVC) group received vehicle (1% Tween 80), 5 ml/kg, orally

Group 3: FBEE (150 mg/kg, orally) treated group

Group 4: FBEE (300 mg/kg, orally) treated group

Group 5: FBEE (450 mg/kg, orally) treated group

Group 6: Standard group received Pilex tablets powder (200 mg/kg, orally)

Two experimental group studies are carried out simultaneously. Hemorrhoids were induced in all the groups, except normal control by applying croton oil preparation (COP).All the groups received drug treatments once daily for 7 days, 24 hours after the induction of hemorrhoids. The doses of the extract was based on the toxicity study and approximate LD50 determination carried out in our previous study.[17] The dose of pilex was chosen as reported previously (17).

Induction of hemorrhoid

Hemorrhoids were induced in rats as described previously.[17] COP was prepared consisting of deionized water, pyridine, diethyl ether and 6% croton oil in diethyl ether in the ratio of 1: 4: 5: 10. Rats were fasted over night before application of COP. Sterile cotton swabs (4 mm diameter) were soaked in 100 µl of COP and inserted into the anorectal portion, 20 mm inside from the anal opening, of all the animals of the respective groups and held in that position for a period 10 seconds. A linear development of edema was observed up to 7 to 8 hours after the COP application. For assessment of hemorrhoids, two separate experiments were conducted, employing separate group of animals. In first set, plasma exudation of Evans blue was studied while in next set of experiment biochemical,

hemorrhoidal and histological parameters were assessed.

Assessment of Evans blue exudation

Plasma exudation of Evans blue induced by COP in anorectal tissue was evaluated as described previously (17). Briefly, 30 min before the COP administration, Evans Blue dye (30 mg/kg) was injected intravenously through tail veins of the animals. Twenty-four hours after the induction, all the animals receiving treatment were treated for 7 days. On day 8, animals were sacrificed by deep ether anesthesia and the anorectal tissue specimens of 20 mm in length were dissected, weighed and Evans blue dye in tissue was extracted using formamide. The absorbance of the sample was recorded on UV spectrophotometer (UV 1800, Shimadzu) at 620 nm and quantified using standard curve of Evans blue dye and expressed as micrograms of Evans blue per milligram of ano-rectal tissue.

Assessment of hemorrhoidal, biochemical and histological parameters

Twenty-four hours after induction, all the animals were given drug treatments for 7days.On day 8, blood (~1.5 ml) was collected though retro-orbital plexus from overnight fasted rats for estimation of various biochemical parameters. Later on, the animals were sacrificed by deep ether anesthesia and the anorectal tissue specimens of 20 mm in length were dissected and weighed. The tissues were mounted on a plain paper and the inflammation was noted as macroscopic severity score. A small cross section of anorectal tissue was fixed in 10% formaldehyde solution for histology studies and the remaining tissue was stored at -20 ºC till analysis of biochemical parameters. The anorectal coefficient (ARC) was calculated using the formula.

ARC = weight of anorectal tissue/ weight of body

Histology of the anorectal tissue was studied to see the appearance of inflammatory cells and the formation of congestion, hemorrhage, vasodilatation, and medium to high degrees of necrosis (18).

Measurement of macroscopic severity score

The scoring was done as described previously (17). The scoring pattern adopted was from Score 0 to 2.

Estimation of biochemical parameters

Tumor necrosis factor alpha (TNF-α) and interleukin (IL-6) were determined in serum by quantitative enzyme-linked immunosorbent assay kits.

Histomorphological score (17)

A histo morphological scale for evaluating hemorrhoids was validated compared to anorectal tissue of normal animal. Scoring of rat anorectal lesions in various groups was performed in a blinded fashion based on this histo morphologic scale for evaluation of various treatments compared to normal control and reference standard as maximum total score for the best possible outcome (e.g., normal anorectal tissue) was 28.

Results

Phytochemical estimation

The ethanolic extract showed the presence of alkaloids, glycosides and tannins.

Quantitative phytochemical estimation

Estimation of total phenolic content

The results of the estimation are shown below. Standard calibration curve was plotted below by the use of Absorbance vs Concentration (µg/mL).

Table 1: UV spectrometer results

Concentration (µg/ml)	Absorbance (765nm)
5	0.37
10	0.765
15	1.18
20	1.61
25	2.103
30	2.53

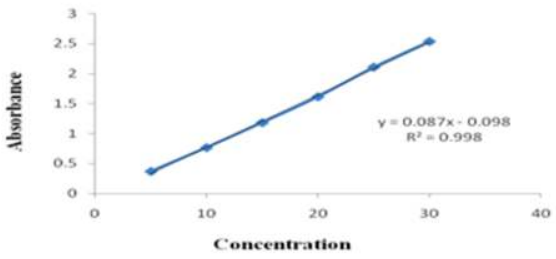

Table 2: Total phenolic content in Gallic acid equivalent (GAE) of plant extract

Ethanolic extract	Absorbance (765 nm)	Total phenolic content in GAE of plant extract
Prop root extract	1.9686±0.04	25.112

Estimation of total flavonoid content

The results for the standard and sample are shown below and the calculation is done using the formulae below.

Table 3: Observation table for standard and sample compounds

Compound	Absorbance (415nm)
Rutin (Standard)	0.364
Ficus benghalensis (Extract)	0.211

Calculations

The amount of flavanoids in plant extracts in Rutin equivalents was calculated by the following formula

$X= (A.m_o)/(A_o.m)$

Where X is the flavonoid content, mg/g plant extract in RE, A is the absorption of plant extract solution, A_o is the absorption of standard Rutin solution, m is the weight of plant extract, mg and m_o is the weight of Rutin in solution mg.

Table 5: Observation table for total flavanoid content

Ficus benghalensis prop root extract	Total flavanoid content in RE mg/g of plant extract
Ethanolic extract	5.44

Here we see the total phenolics and flavanoids content present in the extracts and significant comparison between the two extracts.

Evaluation of biological activity

Ficus benghalensis ethanolic extract (FBEE) of the prop roots was found to be effective in the study done to evaluate the biological activity. Ethanolic extract was used to further carry out the study.

Macroscopic severity score and the recto anal coefficient

COP treatment increased recto anal coefficient ($p<0.05$) as well as the macroscopic severity score in Disease Control as compared to Normal Control Group. Administration of FBEE (150, 300 and 450 mg/kg) cause significant dose dependent reduction in recto anal coefficient and also reduced the recto anal damage. Pilex also showed significant dose dependent reduction compared to the Hemorrhoid Vehicle control.

Table 6 Effect on macroscopic severity score and recto anal coefficient.

Hemorrhoidal parameters	NC	DC$^{\alpha}$	FBEE$^{\phi}$ 150 mg/kg	FBEE $^{\phi}$300 mg/kg	FBEE $^{\phi}$450 mg/kg	Pilex$^{\phi}$ 200 mg/kg
Macroscopic severity score	0.183± 0.070	1.875± 0.077	1.47±0. 049	1.35±0 .043	1.1±0. 072	0.975±0 .054
Recto anal coefficient	1.183± 0.064	3.61±0 .312	2.32±0. 11	1.61±0 .12	1.19±0 .11	1.19±0. 12

Values are expressed as MEAN±SEM, $^{\alpha}p<0.05$ when compared to NC, $^{\varphi}p<0.05$ when compared to HVC

Assessment of Evans Blue Exudation

COP treatment in DC group causes significant increase in Evans blue concentration in recto anal tissue as compared to Normal control. Treatment with Pilex and FBEE significantly reduced the concentration as compared to the Drug control group.

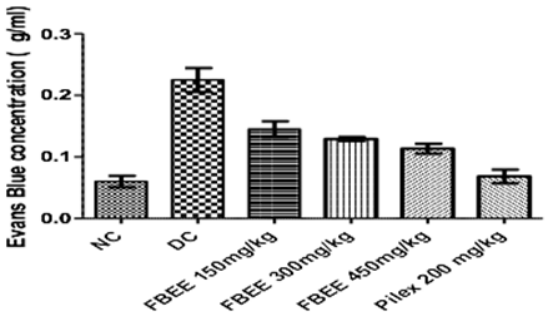

Figure 2: Effect of FBEE on Evans blue concentration

$^{\alpha}p<0.05$ when compared to NC, $^{\phi}p<0.05$ when compared to DC

Effect on Cytokines TNF α and IL- 6

In DC group there was significant increase while the administration of FBEE reduced the levels of both the cytokines as compared to the standard pilex treated group.

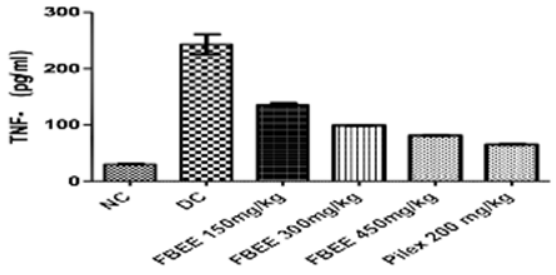

Figure 3: Effect on cytokines. Results are expressed as Mean±SEM.

$^{\alpha}p<0.05$ when compared to NC, $^{\phi}p<0.05$ when compared to DC

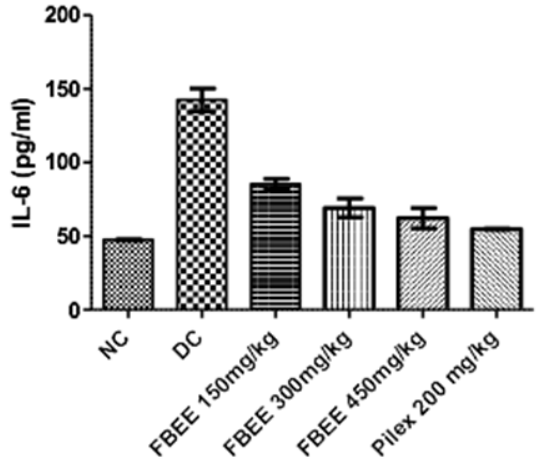

Figure 4: Effect of extract and drug on IL- 6.

Results are expressed as Mean ± SEM

$^{\alpha}p<0.05$ when compared to NC, $^{\phi}p<0.05$ when compared to DC

Effect on Histo- morphological score

COP treated disease control group showed lowest histo-morphological score as compared to the NC group. Treatment with FBEE showed significant reversal of the lesions in the tissue. Lower doses did not show much significant reversal of the lesions as compared to the pilex treated group.

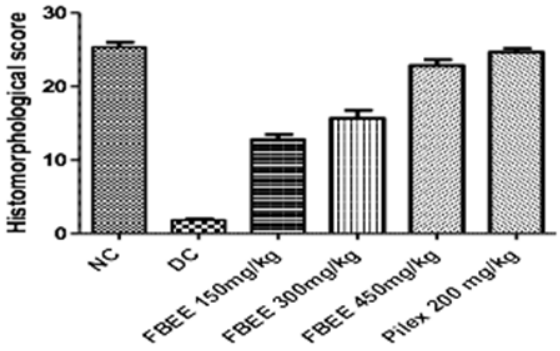

Figure 5: Effect on histomorphological score of recto anal tissue

In the present study, the results revealed that application of croton oil preparation (COP) with exposure for 10 s caused induction of hemorrhoids as indicated by the significant increase in macroscopic severity score and anorectal coefficient as compared to NC animals.The induction of hemorrhoids with COP showed marked increase in the Evans Blue dye concentration, anorectal coefficient and levels of TNF-a and IL-6 as compared to the Normal Control group. The administration of aqueous and ethanolic extracts showed marked reduction in these

parameters as was seen in the standard group treated with Pilex tablets.

DISCUSSION

Hemorrhoids being a very painful pathological condition requires potent medicinal activity which reduces the redness and inflammation which is often seen in the different stages of this disease (19).

The extraction procedures were standardized and microwave assisted extraction has been seen to be very useful. The ethanolic extracts have shown the presence of different phytoconstituents like alkaloids, glycosides and tannins.

TLC profiling of the extracts were done and the solvent system best suited for isolation of phyto constituents was found out Hexane: Ethanol (1:1) which can further be used for isolation of different bioactive components.

Quantitative phytochemical estimation was also performed and total tannin content was found out to be 25.112 mg/g of Gallic acid equivalent (GAE) whereas total flavanoids was about 5.44 mg/g of Rutin equivalent.

In this present in vitro study, the results revealed that application of croton oil preparation (COP) with exposure for 10 seconds causes induction of hemorrhoids as indicated by the significant increase in macroscopic severity score and anorectal coefficient as compared to NC animals.

The induction of hemorrhoids with COP showed marked increase in the Evans Blue dye concentration, anorectal coefficient and levels of TNF-α and IL-6 as compared to the Normal Control group.

The administration of aqueous and ethanolic extracts showed marked reduction in these parameters when seen in the standard group treated with Pilex tablets.

Thus we can see that the extracts have been effective in treating the hemorrhoidal condition in male wistar rats. The standard drug used in this study helps in treating piles by tissue regeneration as it is a polyherbal combination of herbs such as *Terminalia chebula*, *Calendula officianilis*, *Mimosa pudica* etc.[20] Compared with the aqueous and ethanolic fractions they cause a reduction in the biochemical parameters compared to standard pilex tablets (20).

This study helps us to further investigate these pharmacological properties of this plant and prepare formulations along with *Piper nigrum.* Studies on cell lines can also be performed to explore future prospective of this prop root extracts. Also tablet formulations can also be made with *Piper nigrum* powder and prop root extract and

check for further pharmacokinetic properties for use in the treatment of piles. Ficus has shown to significantly reduce inflammation which paves way for its effectiveness in Hemorrhoids. This present study shows that administration of the *Ficus benghalensis* prop root extract has been useful in the treatment of hemorrhoids due to its anti-inflammatory, analgesic, anti- microbial (21, 22) and immunomodulatory effects (21, 23). Traditionally it is useful with when taken with black pepper and a glass of milk due to the presence of the various phytoconstituents like alkaloid flavanoids and glycosides (24). Nanoparticles have also been synthesized using *Ficus benghalensis* extracts (25).

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