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EFFECT OF *FICUS HISPIDA* IN INFLAMMATORY BOWEL DISEASE IN EXPERIMENTAL ANIMALS

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ABSTRACT: Background: Inflammatory bowel disease (IBD) is an immune-mediated chronic intestinal condition. Conventional treatment regimens utilizing corticosteroids, anti-inflammatory and other immunosuppressive therapies have major limitations owing to their associated side effects and compliance issues. **Objectives:** To evaluate the effect of ethanolic extract of *Ficus hispida* Linn. in inflammatory bowel disease (IBD) in suitable experimental animal models (albino rats) in comparison with standard drug. **Methods:** Healthy albino rats were divided into 6 groups (control, standard & Test I, II & III) of 6 animals each. Colitis was induced by intra-colonic administration of acetic acid under anaesthesia. Then the animals were fed with gum acacia (control), standard drug (sulfasalazine) and test drug (FH) according to their groups. At the end of 14 days, blood sample was collected for serum LDH estimation. The animals were then sacrificed, colon excised and assessed for disease activity index (DAI), colon mucosal damage index (CMDI). Plant extract was also subjected to NO scavenging activity analysis. **Results:** The results were analysed by one way ANOVA followed by Dunnett's t test. The ethanolic extract of FH produced a significant improvement in both morphological and histopathological score compared to standard drug sulfasalazine. There was also a dose dependent reduction in serum LDH level in the test group. FH also showed NO scavenging activity. **Conclusion:** FH is effective in the treatment of IBD. It is due to its anti-inflammatory and free radical scavenging activity.

INTRODUCTION: Inflammatory bowel disease (IBD) encompasses many chronic, relapsing inflammatory disorders involving the gastrointestinal tract.[1] In IBD, the intestine (bowel) becomes inflamed, often causing recurring abdominal cramps and diarrhoea. Among the pathological findings associated with IBD are increases in certain inflammatory mediators, signs of oxidative stress, a deranged colonic milieu, increased intestinal permeability.[2]

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The available treatment choices have major limits owing to the associated adverse effects and compliance issues.[1] As a result, there is high prevalence of complementary and alternative medicines for treating the mentioned disease.

Ficus hispida (FH) Linn. belongs to the family Moraceae is a shrub, grows up to 5 meters in height.[3] Traditionally, different parts of the plant have been used in the treatment of ulcers, psoriasis, anemia, piles, jaundice, vitiligo, haemorrhage, diabetes, convulsion, hepatitis, dysentery, biliousness and as lactagogue and purgative.[4] Since ages, extracts of leaves, bark and root have been used by the tribals of Assam and Manipur in the treatment of jaundice [5] and diabetes.[6] Mandal SC et al suggested that the plant has anti-diarrhoeal

activity and validates its use in folk medicine for gastrointestinal diseases. [7]

MATERIALS AND METHODS:

The study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences (RIMS) Hospital, Imphal for a period of 2 years (October 2011-August 2013). Fresh aerial parts of the plant *Ficus hispida* Linn. were collected from Lamphel area in the month of october 2011. The plant was identified and authenticated by Prof. P. Kumar Singh, Dept. of Life Sciences, Manipur University. A sample of the plant was deposited at the department herbarium and allocated MUH number 003616. The extraction was done according to the method described by Sivaraman et al using Soxehelet apparatus.[8]

Acute Oral Toxicity Study:

Acute oral toxicity was studied with oral administration of extract, using the Organisation for economic co-operation and development (OECD) 2006 guidelines.[9] As per the limit test, female Wistar albino rats were fasted overnight and given 2000 mg/kg of *Ficus hispida* extract orally, the next day. Animals were observed for 48 hours, with special attention during the first 4 hours, and daily thereafter for a period of 14 days, for any signs of toxicity or mortality. No toxicity was observed at a dose of 2000 mg/kg. The study was approved by the Institutional Animal Ethics Committee (IAEC) 1596/GO/a/12/CPCSEA according to the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Throughout the experiments, animals were handled according to the suggested ethical guideline for the care of laboratory animals.

Study Design:

The animals were divided into 6 groups (control, standard & test I, II & III) of 6 animals each and were treated with respective drugs as shown in the table I. Control group included positive control and negative control.

Induction of Colitis:

After overnight fasting and under light ether anesthesia colitis was induced by intra-colonic administration of 1 ml of 4% acetic acid according to the method described by Millar et al to the animals in all the group except negative control. [10] The negative control group was given 0.9% normal saline instead of acetic acid. The animals were treated with respective drugs in each group for 14 days along with normal diet. On the 15th day animals were sacrificed by high ether anaesthesia and dissected open to remove the distal colon and assessed for colon mucosal damage index (CMDI) morphologically and disease activity index (DAI) by histopathology.

Assessment of CMDI & DAI:

Macroscopic scoring was done and evaluated according to the formula of CMDI described by Morris et al ranging from 0-5 (**Table 2**).[11] The histological scoring (**Table 3)** was performed as a combined score of inflammatory cell infiltration (score 0-3) and tissue damage (score0-3). The combined histological score ranges from 0 (no changes) to 6 (extensive cell infiltration and tissue damage).[12]

Biochemical assay:

A. Measurement of Serum LDH

Under light ether anesthesia about 0.5-1 ml of blood was drawn from orbital sinus using capillary tube and centrifuged at speed of 5000 rpm for 10 min and the serum was used for LDH estimation. Serum LDH was assayed according to the method described by King using colorimeter.[13]

B. NO Scavenging Activity:

Nitric oxide scavenging assay was performed using Griess reagent method using colorimeter and absorbance was measured at 546 nm.[14] The % inhibition of OD was calculated by using the formula:

% Increase in absorbance = (Blank OD- Test OD)/ Blank OD multiplied by 100

Disposal of animal carcasses-

The animal carcasses were buried deep in the ground covered with lime and disinfectants after the experiment.[15]

RESULTS:

A. Colon Mucosal Damage Index: Macroscopic scoring was done to the colonic tissue according to

the method described by Morris et al and was given a score from 0-5. There was no macroscopic change (**Fig.1**) in the normal saline group (negative control). Acetic acid group (positive control) showed a score of 4.33±0.51. The standard drug sulfasalazine produced a score of 2.00±0.63. The test drugs FH (800 mg/kg) produced a score of 2.50±0.54 ($P < 0.001$) which is significantly less compared to control (**Table 4**).

TABLE 1: SHOWING THE VARIOUS GROUPS AND THE DRUGS THEY WERE TREATED WITH.

Group I (Positive Control) (Negative Control)	**1ml of 2% gum acacia p.o**
Group III (Standard)	Sulfasalazine 360mg/kg/d p.o
Group IV (Test Drug FH)	i FH 200 mg/kg p.o ii FH 400 mg/kg iii FH 800 mg/kg

TABLE 2: SHOWING CRITERIA FOR SCORING OF GROSS MORPHOLOGIC DAMAGE

Score	Gross Morphology
0	No damage
1	Localized hyperemia, but no ulcers or erosions
2	Ulcers or erosions with no significant inflammation
3	Ulcers or erosions with inflammation at one site
4	Two or more sites of ulceration and/or inflammation
5	Two or more major sites of inflammation and ulceration or one major site of inflammation and ulceration extending >1 cm along the length of the colon

TABLE 3: SHOWING HISTOPATHOLOGIC SCORE WHICH INCLUDES CELL INFILTRATION & TISSUE DAMAGE

INFLAMMATORY CELL INFILTRATION

0	Presence of occasional inflammatory cells in in the lamina propria
1	Increased numbers of inflammatory cells in the lamina propria
2	Confluence of inflammatory cells extending into the submucosa
3	Transmural extension of the infiltrate

TISSUE DAMAGE

0	No mucosal damage
1	Lymphoepithelial lesions
2	Surface mucosal erosion or focal ulceration
3	Extensive mucosal damage and extension into deeper structures of the bowel wall the bowel wall

B. Disease Activity Index:

Histopathological scoring was done on the colonic tissue according to the method described by Siegmund et al and was given a score from 0-6 based on cell infiltration and tissue damage. There was no microscopic change (**Fig.2**) in the normal saline group. Acetic acid group showed a score of 5.50±0.54 (**Table 5**).

The standard drug sulfasalazine produced a score of 1.83±0.75. The test drug FH at dose of 400 and 800 mg/kg produced a score of 3.83±0.40 and 2.50±0.54 which is significantly less compared to control ($P < 0.001$)

C. Serum LDH Estimation:

Serum LDH estimation was done according to the method described by King. The serum LDH level in the normal saline group was 63.33±1.63 IU/ml (Table-6). The level in acetic acid group was 253.67±22.35. The level in standard group was 91.33±8.06. There was a significant reduction in LDH level in FH group with values of 194.33±6.25 ($P<0.01$), 141.33±6.02 and 123.00±6.54 ($P<0.001$) at increasing doses when compared to control.

D. NO Scavenging Activity:

The NO scavenging activity was measured in terms of optical density and the percentage of increase in absorbance was measured among different groups. The standard & FH in all three doses had a significant level of NO scavenging activity. The % inhibition was maximum 72.30% for standard followed by 68.46% for FH at 100 µg/ml (**Table 7**).

TABLE 4: EFFECT OF *FICUS HISPIDA* L. ON MACROSCOPIC SCORE IN COLON IN ACETIC ACID INDUCED COLITIS IN RATS.

Group	Macroscopic Score (Mean±SD)
Negative control	0.00±0
Positive control	4.33±0.51
Sulfasalazine	2.00±0.63**
FH 200 mg/kg	4.00±0.63
FH 400 mg/kg	3.50±0.54
FH 800 mg/kg	2.50±0.54**

One-way ANOVA F= 40.647, df= (10, 55), $p<0.01$

Values are mean±SD, n=6 in each group, *$p<0.01$, **$p<0.001$ when compared to control.

TABLE 5: EFFECT OF *FICUS HISPIDA* L. ON HISTOPATHOLOGICAL SCORE IN COLON IN ACETIC ACID INDUCED COLITIS IN RATS.

Group	Histopathological Score (Mean±SD)
Negative control	0.00±0
Positive control	5.50±0.54
Sulfasalazine	1.83±0.75**
FH 200 mg/kg	4.33±0.52
FH 400 mg/kg	3.83±0.40**
FH 800 mg/kg	2.50±0.54**#

One-way ANOVA F= 51.393, df= (10, 55), p<0.01
Values are mean±SD, n=6 in each group, *p<0.01, **p<0.001 when compared to control. # p<0.01 when compared within the groups.

TABLE 6: EFFECT OF *FICUS HISPIDA* L. ON SERUM LDH IN ACETIC ACID INDUCED COLITIS IN RATS.

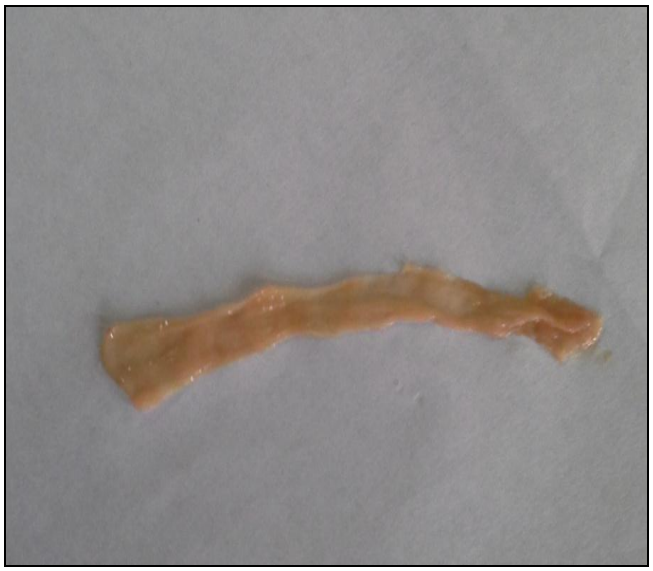
Group	Serum LDH I.U./ml (Mean±SD)
Negative control	63.33±1.63
Positive control	253.67±22.35
Sulfasalazine	91.33±8.06**
FH 200 mg/kg	194.33±6.25*
FH 400 mg/kg	141.33±6.02**#
FH 800 mg/kg	123.00±6.54**#

One-way ANOVA F= 309.127, df= (10, 55), p<0.01
Values are mean±SD, n=6 in each group, *p<0.01, **p<0.001 when compared to control. # p<0.01 when compared within the groups.

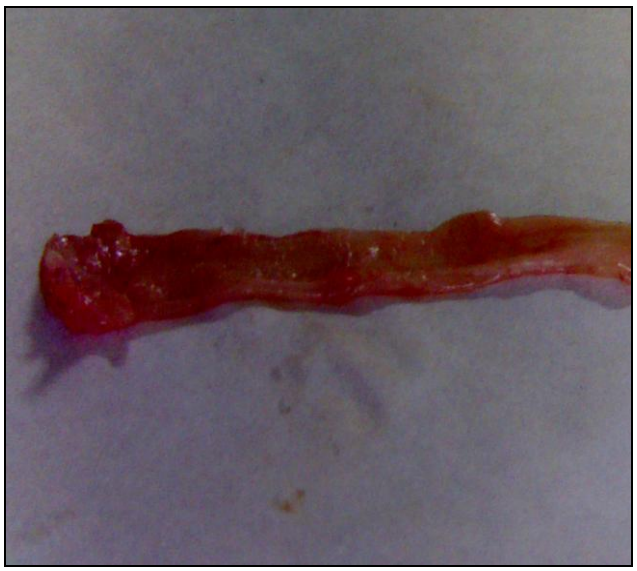
TABLE 7: EFFECT OF *FICUS HISPIDA* L. ON NO SCAVENGING ACTIVITY.

Group	Absorbance (Mean±SD)	% Inhibition
Control	1.30±0.10	------
Standard 50 µg/ml	0.36±0.02*	72.30
FH 25 µg/ml	0.83±0.02*	36.15
FH 50 µg/ml	0.60±0.05*#	53.84
FH 100 µg/ml	0.41±0.02*#	68.46

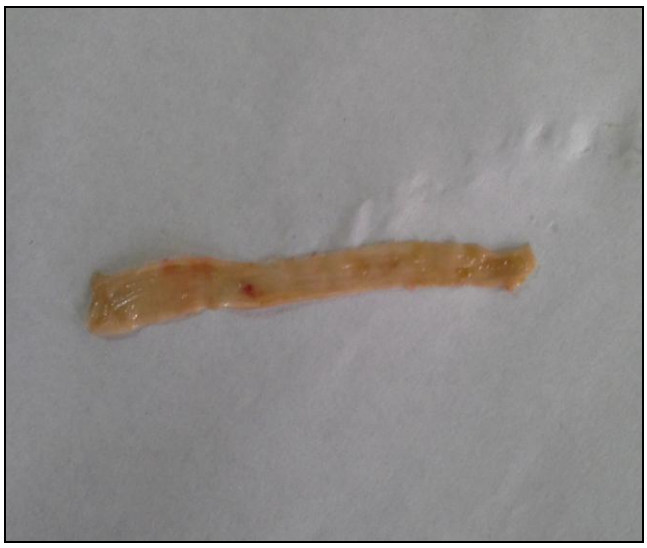
Values expressed as absorbance are the mean ± SEM, n= 3, Significance
* p<0.001 compared to control, # p<0.01 compared within the groups.
Std: Sodium metabisulphate



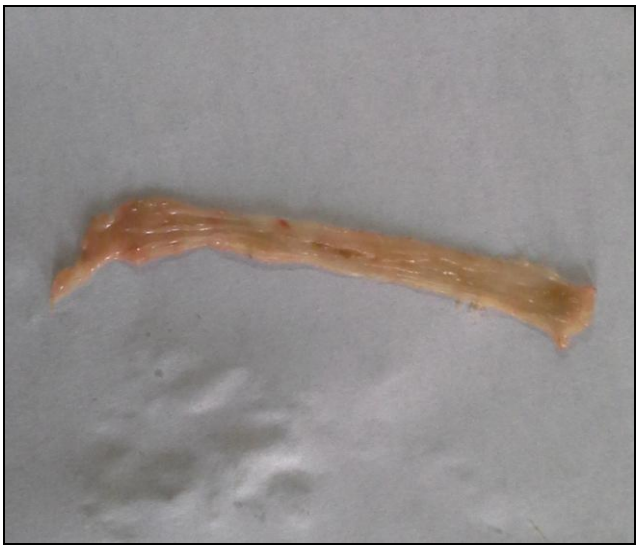
A. NORMAL SALINE GROUP



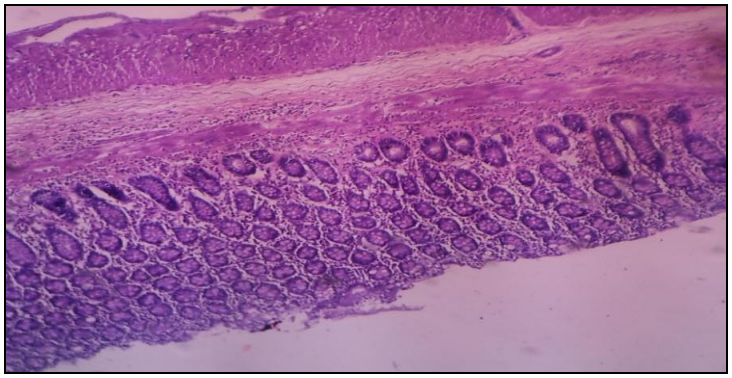
B. ACETIC ACID GROUP LOOKING INFLAMMED & EDEMATOUS



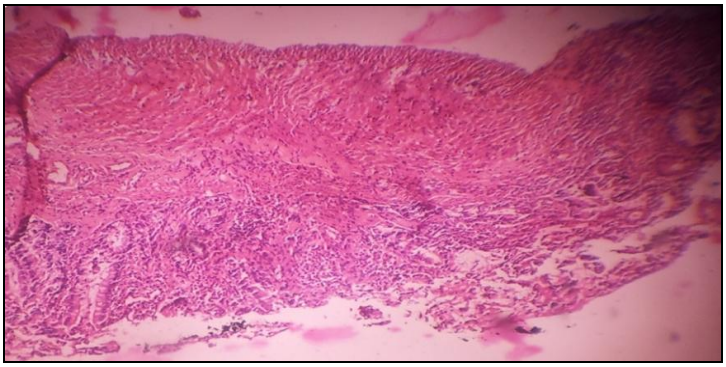
C. SULFASALAZINE TREATED AND



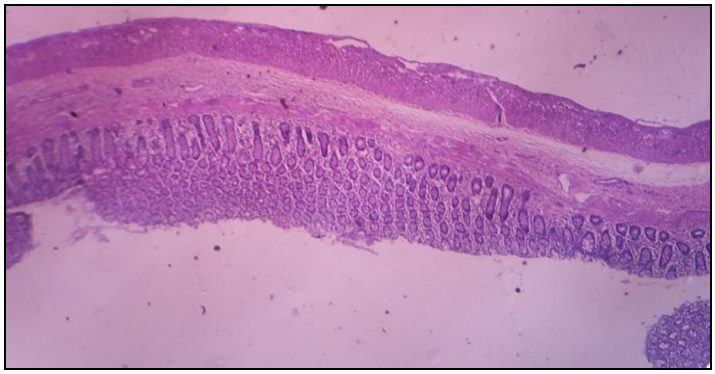
D. FH TREATED (GROSSLY UNREMARKABLE)



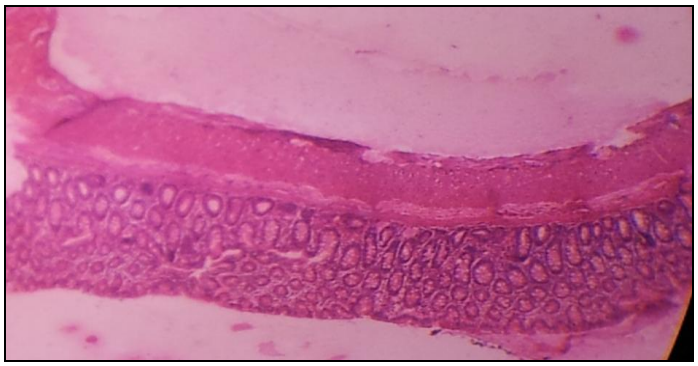
A. H & E STAINED SECTION OF RAT COLONIC TISSUE (NEGATIVE CONTROL) SHOWING NORMAL LARGE BOWEL.



B. H& E STAINED SECTION OF RAT COLONIC TISSUE SHOWING (ACETIC ACID TREATED) LOSS OF MUCOSAL ARCHITECTURE WITH ULCERATION AND INFLAMMATORY CELL INFILTRATION



C. H & E STAINED SECTION OF RAT COLONIC TISSUE (SULFASALZINE TREATED) SHOWING UNREMARKABLE LARGE BOWEL.



D. H & E STAINED SECTION OF RAT COLONIC TISSUE FH TREATED WITH UNREMARKABLE LINING MUCOSA

DISCUSSION: IBD could be considered as an imbalance between pro-inflammatory and anti-inflammatory mediators. Monocytes/macrophages, polymorphonuclear leucocytes (PMNs) and endothelial cells are mainly involved in inflammatory response and their activation forces them to aggregate and infiltrate the tissue, where they undergo respiratory burst, which increases their oxygen use resulting into oxidative damage to the tissue and triggers the production of pro-inflammatory cytokines, ROS and other mediators of inflammation. Initiation and perpetuation of inflammatory cascade by ROS causes subsequent tissue damage through the activation of nuclear factor kappa B (NF-kB), which is a ubiquitous transcription factor involved in the regulation of several genes in immune and inflammatory responses. Oxidants are potent activators of NF-kB while the groups of structurally diverse anti-oxidants of herbal origin are capable of inhibiting NF-kB activation.[16]

Induction of colitis in rats using acetic acid is a classical method used to produce an experimental model of human IBD. It is both rapid and reproducible and produces diffuse colonic inflammation and definite ulcers resembling many histological characteristics of human UC. In this study colitis was induced by administration of 1 ml of 4 % acetic acid intrarectally. The results confirmed the suitability of the method since an acute and invariably ulcerative colitis was developed in experimental rats.

Several major causative factors in the initiation of human colitis such as enhanced vasopermeability, prolonged neutrophil infiltration and increased production of inflammatory mediators are involved in the induction of this animal model. It affects the distal colon portion and induces non-transmural inflammation, massive necrosis of mucosal and submucosal layers, mucosal edema, neutrophil infiltration of the mucosa and submucosal ulceration.

The protonated form of the acid liberates protons within the intracellular space and causes a massive intracellular acidification resulting in massive epithelial damage. Inflammation is the pathogenesis of IBD and several pathways are associated with inflammatory response in IBD. The inflammatory response initiated by acetic acid includes activation of cyclo-oxygenase and lipo-oxygenase pathways.[17]

The FH treated rats showed a dose dependent decrease in macroscopic and histopathological score by reducing inflammatory cell infiltration and tissue damage. The colonic damage as determined histopathologically paralleled to that of macroscopically visible damage. A significant increase in serum LDH level was observed in control group 253.67±22.35 IU/ml. There was a dose dependent decrease in serum LDH level in FH treated group.

Increased oxidative stress and increased iNOS activity is a notable feature of IBD.[18] Increased iNOS activity yields more oxidative free radicals such as peroxynitrite ($ONOO^-$) to impair the structure and function of the cells. Excess of NO is responsible for the increase in the disease severity by increasing vascular permeability and decrease in the antioxidant defense mechanism by inhibiting the SOD enzyme.[19] In our study FH showed a significant NO free radical scavenging activity with maximum % inhibition at a concentration of 100µg/ml.

CONCLUSION: FH is effective in the treatment of IBD by reducing the DAI, CMDI & serum LDH level. It also has NO scavenging activity. The effect of FH in IBD was due to its anti-inflammatory and free radical scavenging activity. The phyto-chemicals probably responsible for its protective effect are oleanolic acid and β-sitosterol, β-amyrin, hispidin etc.

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