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"Evaluation of *Ulmus Fulva* Extract In Experimentally Induced Inflammatory Bowel Disease In Rats."

Neelam Bangodi*[1], Manu J[1], Mukund Handral[1], Ismail Pasha[2], Shama G Ternikar[3]

1 Department of Pharmacology and Toxicology, PES College of Pharmacy, Bengaluru, Karnataka, India
2 Department of Pharmacology, Orotta College of Medicine and Health Sciences, Asmara University, Eritrea
3 Department of Pharmaceutical Chemistry, Sant Gajanan Maharaj College of Pharmacy, Mahagoan, Kolhapur, Maharashtra, India

Abstract
The intent of the present study was to investigate the effect of Ulmus fulva extract in inflammatory bowel diseased rats. About 30 wistar rats were divided into 5 groups (n=6). After 48hr overnight fasting, under light anaesthesia (Phenobarbitone), colitis was induced by intera-colonic administration 1ml of 4% acetic acid to all group except the normal group. All the animals were treated as per treatment protocol. Animals treated with Ulmus fulva extract produced a significant (P<0.05) improvement in both morphological and histophological score as compared to nearer to control group and standard group (sulphasalazine). There was also a dose dependent reduction in serum lactate dehydrogenase (LDH) level (P<0.001) in the test group as compared to diseased induced group. Ulmus fulva is effective in the treatment of Inflammatory Bowel Disease may be due to the development of a defensive mucosal layer.

Key words: *Inflammatory bowel disease, Ulmus fulva, LDH level, Phenobarbitone and Sulphasalazine.*

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I. Introduction

Inflammatory Bowel Disease is a disorder that involves two conditions Crohn's disease and ulcerative coloitis. The cause of the disease is still unknown. [(1)] Ulcerative colitis only affects the colon (large intestine) and rectum, while Crohn's disease can affect the entire digestive system; from the mouth to the anus. [(2)] Most of the current therapies for inflammatory bowel diseases involve treatment with corticosteroids (Prednisone, Budesonide), 5-aminosalicyclic acid, immunosuppressive drug (Azathioprine, 6-mercaptopurine), antibacterials (Metronidazole, Ornidazole), biologics (Infliximab, Adalimumab etc.) and probiotics (*Saccharomyces boulardii, Lactobacillus sp,*). More frequently prescribed antibiotics (Metronidazole and Ciprofloxacin) have also been used to control severe illness.[(3)] The mechanism action of drugs is likely to have multiple anti-inflammatory effects by inhibition of cyclooxygenase, lipoxygenase, β-cells and several key inflammatory cytokines etc.[(4)] But several antibiotics and vaccines cause damage to intestinal mucosa of the stomach, small bowel and colon.[(5)] Even non-steroidal anti-inflammatory drugs (NSAIDs), immunomodulatory drugs, monoclonal antibody drugs cause severe adverse effects like gastro intestinal injury, hypersensitivity reactions including hepatitis, pneumonitis, arthritis and risk of lymphoma among patients with IBD. [(6)] Thus ulcerative colitis and crohn's disease are due to inflammation and oxidation caused by the synthetic drugs, therefore the herbal plants, plant extracts and their phyto-constituents plays an important role in exhibiting potential anti-inflammatory and anti-oxidant property and moreover is now considered as safe against IBD. [(7)]

Ulmus fulva (slippery elm) is among the oldest cultivated plants, and it used for medicinal application. It is a member of *Ulmaceace* family, which consist of 18 genera and 1753 species.[(8)] It contains a complex assortment of chemical and nutritional compounds including mucilage (hexoses, pentose, methyl pentose, glucose, polyuronides, tannins, starch, fat, phytosterol and various nutrients (calcium, iron, zinc, magnesium and potassium).[(9)] The bark powder are topically applied to treat wound and skin irritation and internally for sore throat, coughs and gastrointestinal conditions.[(10)] This study assumes significance in the context that prolonged use of synthetic drugs in treatment of IBD leads to adverse effects. Hence, to search for new agents that retain therapeutic efficacy and devoid the adverse drug reaction therapies from natural sources to replace currently used drugs which are doubtful in efficacy and safety. Herbs, medicinal plants, vegetables and crude drugs substances are considered to be a potential activity to control the diseases. [(11)]

II. Methodology

Plant Collection

Completely dried bark powder of *Ulmus fulva* was procured from the authenticated supplier, with authentication letter from VHCA Ayurveda LLP (VHCA Herbals), G.T Road, Gharaunda-132114, Karnal (Haryana), India in the month of October, 2017.

Extraction of Plant Material

The freshly prepared *Ulmus fulva* bark powder was placed in a thimble made from the whatman filter paper and placed in a soxhlet apparatus. Ethanol was used as a solvent and heated in the round bottom flask, which vaporizes into the thimble containing sample. Whenever the condensed liquid content reaches the siphon tube the liquid content are emptied to the round bottom flask through siphon tube. This process was continued until the liquid content gets colourless in the soxhlet apparatus. [(12)]

Pharmacological Evaluation

Dose selection

From the literature survey, it is found that ethanolic extract of *Ulmus fulva* is safe at dose level up to 500mg/kg. In the study 250mg/kg and 500mg/kg as low dose and high dose is selected respectively [(13)] for the treatment protocol of IBD induced in rats.

Dose Preparation

Five grams (5gm) of *Umlus fulva* extract was dissolved in 10ml of water gives 500mg in 1ml 500mg in 5ml of water makes 100mg/ml.

Experimental Design

The study was carried by using 30 wistar rats and divided into 5 groups (n=6).

Induction of colitis by acetic acid 1ml (4%):

Prior to induction of colitis, rats were fasted for 24hrs (able to drink *ad libitum*) then anesthetized lightly using phenobarbitone using polyethylene catheter (Instech) colitis was induced by intera-rectal administration of 1ml (4%) acetic acid into the lumen of the colon, in order to minimize leakage of acetic acid animal is kept in an upside-down vertical position for 30-60 seconds, before returning to the cages. The rats were kept under observation for 14 days and were routinely evaluated for the parameters. After the completion of treatment regimen rats were sacrificed by an overdose of phenobarbitone followed by measurement of colon mucosal damage index (CMDI) morphologically and disease activity index (DAI) histo-pathologically. [(14-15)]

Estimation of Lactate dehydrogenase:

Lactate dehydrogenase was estimated by mixing 800µl of reagent 1 with 20µl of the sample in a test tube. For the standard, 800µl of reagent 1 was taken along with 20µl of the standard and for the blank 800µl of reagent 1 and 20µl of distilled water was taken and mixed well, incubated for 1 min at 37˚ C. After incubation, 200µl of reagent 2 was added in each of the test tubes and again mixed and incubated at 37˚ C for 1 min then the initial absorbance was measured for the sample, standard and blank. Then the absorbance change was observed exactly after taking the readings after 1, 2 and 3 min.

III. Results

1. Preliminary Phytochemical Investigation

The preliminary phytochemical investigation was done to confirm chemical constituents present in the *ulmus fulva* powder using standard procedure was positive for Carbohydrates, Alkaloids, Starch, Glycosides, Saponins, Proteins, Mucilage, Flavonoids, Tannins & Phenolic compounds, Steroids & Triterpenoids, Fats & fixed oils.

S. No.	Phytochemical constituents	Inference
1.	Carbohydrates	Positive
2.	Alkaloids	Positive
3.	Starch	Positive
4.	Glycosides	Positive
5.	Saponins	Positive
6.	Proteins	Positive
7.	Mucilage	Positive

8.	**Flavonoids**	**Positive**
9.	**Tannins & Phenolic compounds**	**Positive**
10.	**Steroids & Triterpenoids**	**Positive**
11.	**Fats & fixed oils**	**Positive**

2. Disease Activity Index (DAI)

Evaluation is carried out, based on body weight in acetic acid induced rat.

Group (n=6)	Treatment	Initial wt.	5th day	10th day	15th day
1.	**Normal group**	**213.33 ± 8.21**	**198.1 ± 13.3**	**208.8 ± 11.4**	**209.8 ± 10.1**
2.	**Disease group**	**198.6 ± 6.1**	**189 ± 6.7**	**169.3 ± 6.2****	**160 ± 6.8*****
3.	**Test drug (250gm)**	**173.33 ± 11.3****	**147.8 ± 3.5**	**155.3 ± 3.5**	**163 ± 8.8**
4.	**Test drug (500gm)**	**198. 6± 6.0**	**178.3 ± 4.7*****	**184 ± 6.1***	**192.8 ± 14.2****
5.	**Standard drug**	**200.6 ± 6.7**	**193.5 ± 6.5****	**195.3 ± 6.4*****	**198 ± 8.0****

3. Effect of *Ulmus fulva* extract on LDH level

Statistical Comparison: Each group (n=6), each value represents Mean ±SEM. One way Anova followed by Dunnett's test was performed. A P-value ($p<0.001$) denotes comparison of inflammatory bowel disease with normal control and NS - Non significant value of $*P<0.05$, $**P<0.01$, and $***P<0.001$ denotes comparison of all groups with Inflammatory Bowel disease control.

Group name	Serum LDH (U/I)
Normal group	**837.06 ± 14.69**
Disease group	**2223.25 ± 34.19***
Test drug (250gm)	**1652.03 ± 36.50**
Test drug (500gm)	**1430.7 ± 33.87****
Standard drug	**1189.83 ± 21.22*****

The colitis caused by acetic acid was associated with an increase in LDH level. The LDH assay showed significant ($P < 0.001$) increase in LDH level of disease control group compared to normal control group. The animals treated with *Ulmus fulva* extract, and standard group showed significant ($P < 0.001$) decrease in LDH level compared to the disease control group.

4. Colon Mucosal Damage Index (CMDI) Morphologically

The macroscopic rating showed that Group (a) which normal colonic tissue has no sign of ulceration or any colonic damage where as in Group (b) which is acetic acid induced colonic tissue shows signs of hyperaemia, swelling, oedema and ulceration. In contrast the Test groups and standard Groups (c), (d) and (e) showed lesions, where are greatly relieved. In comparison to normal group and disease group showed significant increase ($P<0.05$) in Colonic Mucosal Damage Index (CMDI). However, CMDI in treated Groups (c), (d) and (e) showed significantly decrease ($P<0.05$) as compared to untreated Group (b). The scoring given to treated Groups (c), (d) and (e) respectively and normal Group (a) as mentioned in below table.

Group Names	Weight of Colon (g)	CMDI score
Normal group (a)	**1.2 ± 0.02**	**0**
Induced group (b)	**2.8 ± 0.07**	**6**
Test Drug (250gm) (c)	**2.14 ± 0.05**	**4**
Test drug (500gm) (d)	**1.75 ± 0.20**	**3**
Standard drug (e)	**1.5 ± 0.02**	**2**

5. Disease Activity Index (DAI) Histo-pathologically

Histopathological examination of colon (colonic mucosal sections at 10X and 40X): (A) Normal rat showing normal mucosa with intact epithelial surface, (B) Acetic acid- induced colitis showing massive necrotic destruction of epithelium, (C- D) Treatment with extract of *Ulmus fulva* 250 mg/kg and 500mg/kg showing decreased epithelial damage, regeneration and suppressed inflammatory reaction, (E) Standard group show suppressed inflammatory reaction.

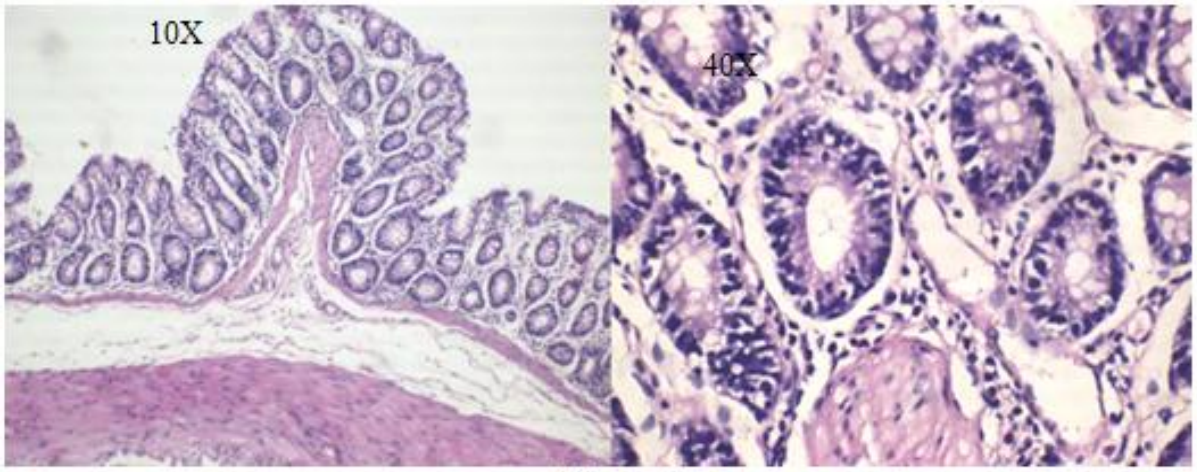

Figure A

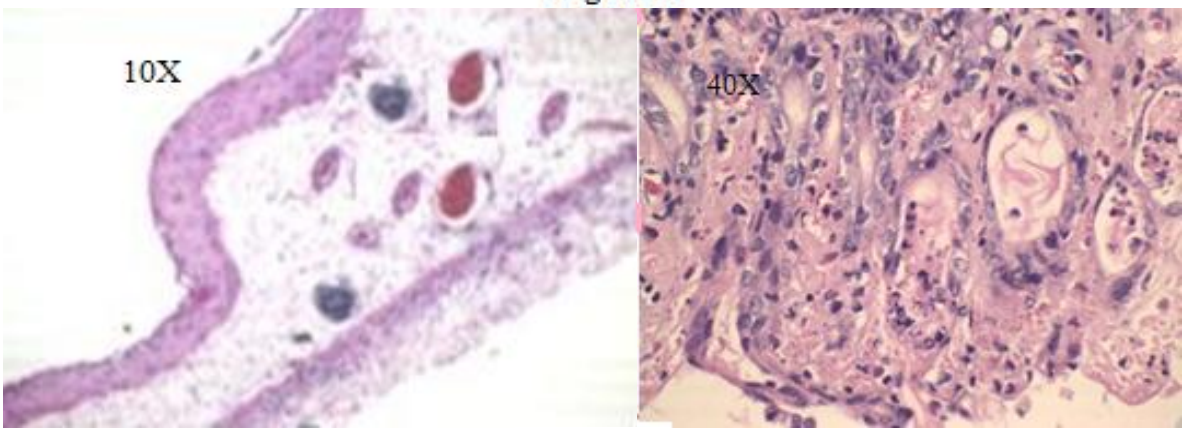

Figure B

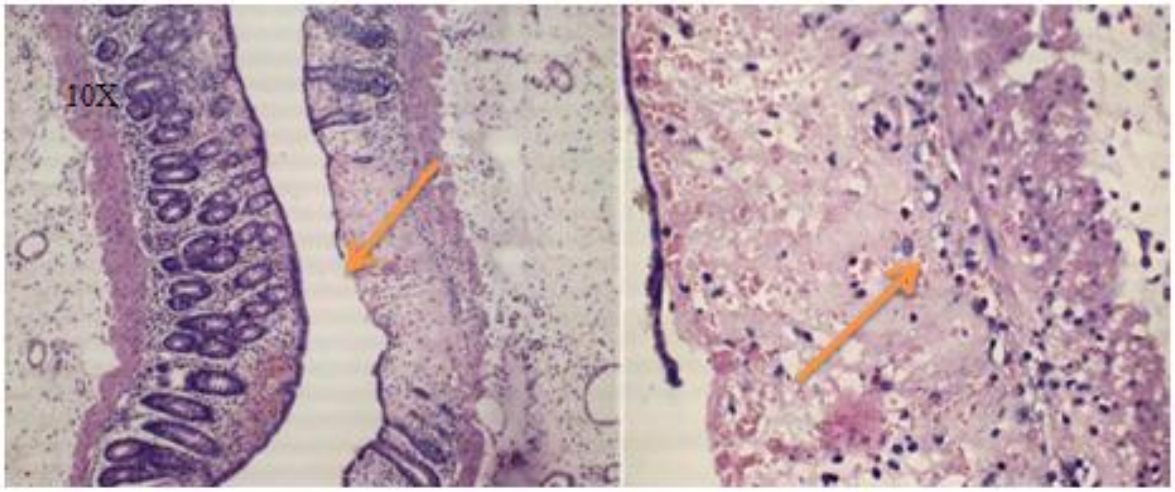

Figure C

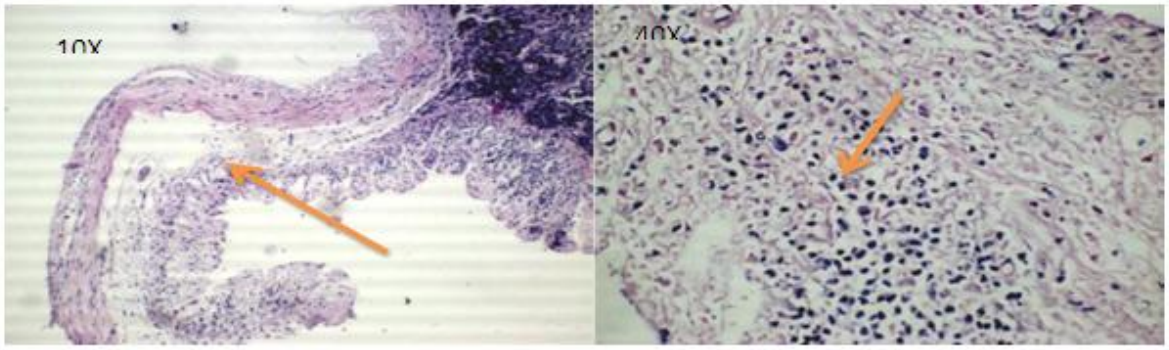

Figure D

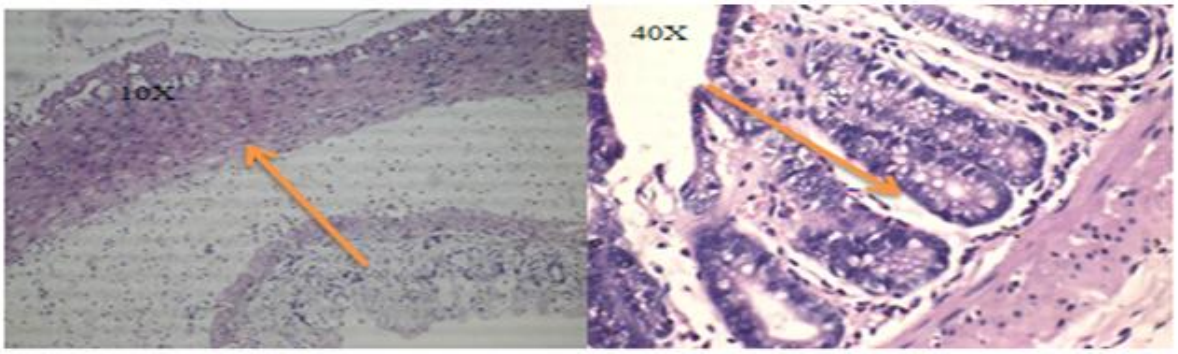

Figure E

IV. Discussion

The present study was aimed to assess the effect of *Ulmus fulva* extract against acetic acid induced inflammatory bowel disease rats. [16] By Intra-rectal administration of 4% (2ml) solution of acetic acid (AA), damages to distal colon portion, the mechanisms by which acetic acid produces inflammation appear to involve the entry of the protonated form of the acid into epithelium, where it dissociates to liberate protons within intracellular acidification that are likely accounts for the epithelial injury.[17] It has been described that acetic acid model of experimental colitis is beneficial for the screening of drugs with anti-colitic activity and has several similarities to pathological and clinical features of the human ulcerative colitis.[18]

In this study well-established models of induced colitis, the acetic acid model was chosen to detect the morphological and pathological changes associated with colitis. [19] In the present study inflammatory response induced by acetic acid 4% (2ml) includes activation of cyclooxygenase and lipo-oxygenase pathways in rats [20] and also the congestion, haemorrhages, oedema and leukocytic infiltration which result in macroscopic and microscopic pictures and tissue damage after induction of colitis using acetic acid. [21]

Present investigation outlines the anti-ulcerogenic effect of *Ulmus fulva* extract against experimentally induced colitis in rats as a model for IBD. The preventative effect of *Ulmus fulva* extract was confirmed by histological evaluation and also using Sulphasalazine as a standard drug. After fourteen days' treatment with *Ulmus fulva* significantly reduced the AA- induced colonic mucus content and prevented oxidative and inflammatory response in dose dependent manner. [22] In present study, the 4% AA administration resulted a significant increase in colonic weight and induced sever ulceration and tissue necrosis associated with inflammatory infiltrate and goblet cell hyperplasia as indicated in the results of the histopathological estimations. Colonic mucus plays an important protective role against chemically induced ulceration which may also facilitate the repair of the damaged epithelium. [23] Although, there are many numerous pharmacotherapies have been suggested for ulcerative colitis treatment, the side effects or toxicity of these medications are a major clinical problem. [24] That is why naturally occurring products containing such as flavonoids, mucilage is now suggested as an alternative option beside the conventional therapies.

In present study *Ulmus fulva* extract which is used as treatment drug has constituents like of mucilage content, terpenoids and flavonoids that are decreasing the mediators responsible for colonic mucosal damage and provides protective layer to the colonic membrane. [25]

Protection against experimental colitis induced by *Ulmus fulva* was companied by restoration of the increased colon thickening in diseased group, which is an indirect assessment of colon inflammation. By intra-rectal administration of AA (acetic acid) caused an increase in stool consistency; this was more colitis induced

disease group with the scoring no.4 but consistency scores were reduced in treatment group of *Ulmus fulva* extract (250mg/kg), (500mg/kg) and standard group with scoring of 1,0 and 0 respectively.

The *Ulmus fulva* 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 disease control group as compared normal control. There was a dose dependent decrease in serum LDH level in *Ulmus fulva* treated group (250mg/kg and 500mg/kg) and standard drug treated.

Macroscopic scores are useful in assessing gross lesions or alterations during tissue injury, the increase in these scores may be indicative of ulcers and edematous formations; [(26)] and as reported above with such observations which shows, no damage, llocalized hyperemia, but no ulcers or erosions, ulcers or erosions with no significant inflammation, ulcers or erosions with inflammation were scored respectively.

The histopathological sections confirmed the protective action of *Ulmus fulva* extract as it decreased colonic tissue ulceration, necrosis and inflammation in dose dependent manner. The colonic tissue of normal animal showed normal mucosal and sub-mucosal architecture. Acetic acid induced colitis showed submucosal edema, loss of crypt and goblet cells destruction of epithelial architecture infiltration of neutrophils and lymphotes into the mucosa and sub-mucosa. Treatment group *Ulmus fulva* (250mg/kg) and (500mg/kg) and showed moderate improvement in the inflammatory response with slight sub-mucosal edema and infiltration of neutrophils in the mucosa and sulphasalazine standard drug treated group remarkable recovery of colonic mucosa from acetic acid induced colitis damage.

V. Conclusion

The present study, revealed that *Ulmus fulva* extract protects the acetic acid induced ulcerative colitis by inhibiting inflammatory and oxidative bio-markers. Based upon the results outcomes, future clinical usage of *Ulmus fulva* as natural non-toxic effective supplement in IBD can be used. The phyto-chemicals probably responsible for its protective effect are mucilage, flavonoids and tannins etc. which attenuates the colon injury microscopically and macroscopically. Hence these constituents are beneficial for its pharmacological actions. However, further investigations are necessary to evaluate, whether a similar efficacy can be achieved in other experimental models of inflammatory bowel diseases.

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