



**PROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF LEAVES OF
MURRAYA KOENIGII ON TNBS INDUCED ULCERATIVE COLITIS IN
RATS**

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ABSTRACT

Ulcerative colitis is a global disease having increasing incidence and worldwide prevalence. *Murraya koenigii* has been used in various conditions like asthma, diabetes, inflammation etc. & the present study was aimed to study the effect of ethanolic extract of leaves of *Murraya koenigii* (EEMK) in TNBS induced ulcerative colitis in Sprague dawley rats. 2, 4, 6 – trinitro benzene sulphonic acid (TNBS) (20 mg) was introduced intra-rectally in all groups on 1st day except normal control group. Standard control group received sulphasalazine (120 mg/kg) p.o & treatment group received different dose of EEMK (125, 250 & 500 mg/kg) for consecutive seven days. Data findings of present study revealed that EEMK showed significant improved (P<0.05) disease parameters like body weight, stool consistency & rectal bleeding, colon length as compared to disease control group. Treatment with EEMK (250 & 500 mg/kg) shown significantly improved (P<0.05) DAI & CMDI score as compared to DC group. Significantly higher antioxidant parameters like SOD & Catalase and lower NO, MPO & LPO level (P<0.05) were observed in animals treated with EEMK (500 mg/kg) as compared to disease control animals. Present study has revealed that ethanolic extract of leaves of *Murraya koenigii* is having beneficial and curative effect in TNBS induced ulcerative colitis in rats. LCMS data confirmed presence of mahanine, mahanimbine & β -sitosterol in ethanolic extract of leaves of *Murraya koenigii* which might be responsible for its protective effect in ulcerative colitis.

**Keywords: Curry leaves; Ethanolic extract of leaves of *Murraya koenigii*; Sulphasalazine;
TNBS; Ulcerative colitis**

INTRODUCTION

Inflammatory bowel disease comprises of a group of chronic, idiopathic inflammatory condition of the gastrointestinal tract [1].

Two main forms of inflammatory bowel disease are Ulcerative colitis and Crohn's disease [2].

Ulcerative colitis is a chronic disease in which inflammation starts from the distal part of colon which further extend towards proximal part in a continuous manner [3, 4].

Altered luminal bacteria and increased intestinal permeability play an important role in dysfunction of intestinal immune system which ultimately cause gastrointestinal injury [1].

Use of herbal drugs is considered an effective treatment as it is a good source of secondary metabolites. Having traditional importance, *Murraya koenigii* is used as hair tonic, also used as a stimulant, anti-inflammatory and anti-depressant agent [5].

Leaves of *Murraya koenigii* L. are rich source of crystalline glycosides, carbazole alkaloids, triterpenoid alkaloids and sterols [6, 7]. They possesses anti-inflammatory activity which may be because of presence of carbazole alkaloids like mahanine and mahanimbine [7-9]. Anti-ulcerative colitis activity of β -sitosterol has also been reported [10].

Therefore, on the basis of above mentioned evidences, present study was aimed to

evaluate effect of ethanolic extract of *Murraya koenigii* leaves on experimentally induced ulcerative colitis in rats.

MATERIAL AND METHODS

Plant Collection & Authentication

Fresh leaves of *Murraya koenigii* were collected from Manavadar region, District – Junagadh & Authentication of plant was done at Department of BioSciences, Saurashtra University, Rajkot

Preparation of plant extract [8]

Fresh curry leaves were collected and allowed to dry in shade for 3 days & finely powdered. Powder was extracted using ethanol at 60 °C in soxhlet apparatus for 24 hrs, concentrated by evaporation process. Obtained extract was filtered by whatmann no. 1 filter paper into a conical flask, allowed to dry at R.T. at normal atmospheric pressure. Crude extract was stored in closed container below 4 °C.

Phytochemical Screening [11, 12]

Preliminary phytochemical screening was performed using ethanolic extract of *Murraya koenigii* leaves to confirm presence of phyto-constituents.

LC-MS Analysis [13, 14]

Ethanolic solution of ethanolic extract of leaves of *Murraya koenigii* was analysed by using LC-MS to confirm presence of carbazole alkaloids and phytosterol content.

In vivo animal model for Ulcerative Colitis

Experimental Animals [8]

Male Sprague Dawley rats (300 – 350 gm) were used, maintained at temperature of 22 – 24°C and relative humidity of 45 – 65%. Rats were housed in polypropylene cages with ad libitum access to food and water in a room with a 12:12 light/dark cycle. The present study was approved by IAEC (BKMGP/IAEC/26/RP73/2020)

TNBS Induced Ulcerative Colitis

Study Design [8, 15, 16, 17]

Table 1: Study design for ulcerative colitis [8, 15, 16, 17]

Sr. No.	Group (n=6)	Method of Induction	Treatment
1	Normal Control	-	CMC (p.o)
2	Disease Control	20mg TNBS in 50% ethanol on 1 st day (intra-rectal)	-
3	Standard Control		Sulphasalazine (120 mg/kg p.o) for 7 days
4	125EEMK		125EEMK (125 mg/kg, p.o) in vehicle for 7 days
5	250EEMK		250EEMK (250 mg/kg, p.o) in vehicle for 7 days
6	500EEMK		500EEMK (500 mg/kg, p.o) in vehicle for 7 days

TNBS = 2, 4, 6 – trinitro benzene sulphonic acid, EEMK = Ethanolic Extract of *Murraya koenigii* leaves, p.o = per oral

Evaluation Parameters

Measurement of Body Weight [18]

Body weight, stool consistency & rectal bleeding were monitored daily during 11:00 to 12:00 in the morning. Loss of body weight was calculated as the difference between the initial and final weight. Loss in body weight indicates onset of ulcerative colitis.

Measurement of Colon Length and Weight [19]

Animals were sacrificed on day 8. Each rat was dissected to isolate colon. Colons were separated from the proximal rectum close to its passage under the pelvisternum. Colon was washed immediately with ice-

Animals were divided as six animals in each set. On first day, TNBS was administered intra rectally to all the groups except normal control group for induction of ulcerative colitis. Ethanolic extract of curry leaves was administered through oral route for consecutive seven days. Change in body weight, food and water intake was monitored for 8 days. On day 8, animals were sacrificed and evaluation parameters were performed.

cold phosphate buffer saline (pH 7.4). The length of colon was measured by placing it on a graph paper with standard measured length in centimeters. Weight of colon tissue was measured and compared accordingly to calculate severity.

Disease Activity Index [20]

Weight loss, diarrhoea and rectal bleeding were three major clinical symptoms from which DAI score was derived.

$$\text{DAI} = \text{Weight loss} + \text{rectal bleeding score} + \text{diarrhoea score}$$

Colon Mucosal Damage Index [18]

Distal 10 cm of colon segment was excised, opened by a midline incision and rinsed

with saline. It was observed from luminal side

Evaluation of Antioxidant Parameters

Colon tissue was excised and homogenized in ice-cold tris hydrochloride buffer (10mM, pH 7.4). Formed homogenate was centrifuged at 7000 rpm for 20 min using homogenizer, supernatant was used for estimation of following oxidative stress parameters.

- ✓ **Superoxide Dismutase (SOD) level [21, 22]**
- ✓ **Catalase level [23]**
- ✓ **Nitric Oxide (NO) Activity [19]**
- ✓ **Lipid Peroxidase (LPO) level [23, 24]**
- ✓ **Myeloperoxide (MPO) Activity [17]**

Histopathology [17]

Colon tissue sample was stored in 10% formalin solution, then embedded in paraffin, at 5 μ m thickness stained with haematoxylin and eosin stain. Histopathological evaluation parameters were evaluated using microscope.

Statistical Analysis

All values were expressed as mean \pm SEM. The statistical significance was tested by analysis of variance (ANOVA) followed by Tukey test using Graph Pad Prism 9.0. $P < 0.05$ was considered as significant difference among the two different groups.

RESULTS

% yield of extract

Obtained extract was dark green in color and semisolid in nature. The percentage yield of ethanolic extract of 50 gm of dried powder of leaves of *Murraya koenigii* was found to be 7.76% w/w using soxhlet method of extraction.

Phytochemical Screening

Preliminary phytochemical screening of EEMK confirmed presence of phyto-constituents such as alkaloids, phenols, flavonoids, triterpenoids, anthraquinones, phlobatannins, glycosides, coumarins except saponins.

LC-MS Analysis

Ethanolic extract of leaves of *Murraya koenigii* was analysed using LC-MS to confirm presence of β -sitosterol, mahanine and mahanimbine. LC-MS spectra of EEMK shown presence of mahanine, mahanimbine & β -sitosterol at m/z – 348.2, 332.2 & 414, which matches with its molecular weight. This data confirms presence of these constituents in EEMK.

Effect of Ethanolic extract of leaves of *Murraya koenigii* in TNBS induced Ulcerative colitis in rats

1. Change in Body Weight, Food Intake, Water Intake & Colon weight/length ratio:

A significant difference in body weight, food intake, water intake & weight/length ratio of colon was observed as shown in **Figure 1**.

2. Change in Disease Activity Index (DAI) & Colon Mucosal Damage Index (CMDI):

A significant difference in disease activity index and colon mucosal damage index was observed as shown in Figure 2.

Effect of Ethanolic extract of leaves of *Murraya koenigii* on anti-oxidative parameters in TNBS induced Ulcerative colitis in rats

Measurement of Superoxide dismutase (SOD), Catalase, Nitric oxide (NO), Lipoperoxidase (LPO) & Myeloperoxidase (MPO) level

A significant difference in SOD, catalase, NO, LPO & MPO level was observed as shown in Table 2.

Histopathology

Histopathological changes were observed as shown in Figure 3.

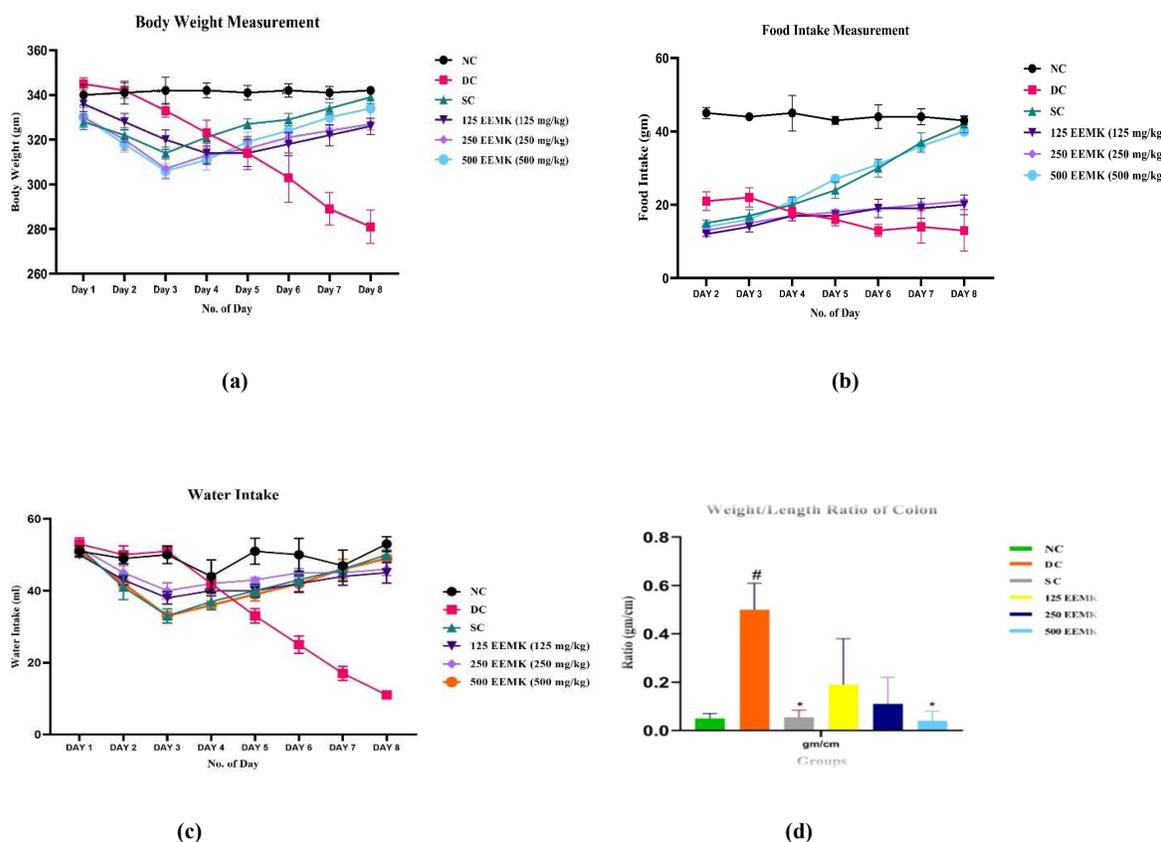


Figure 1: Effect of EEMK on (a) Body weight (b) Food Intake (c) Water Intake and (d) Weight/Length ratio of colon in TNBS induced UC in rats

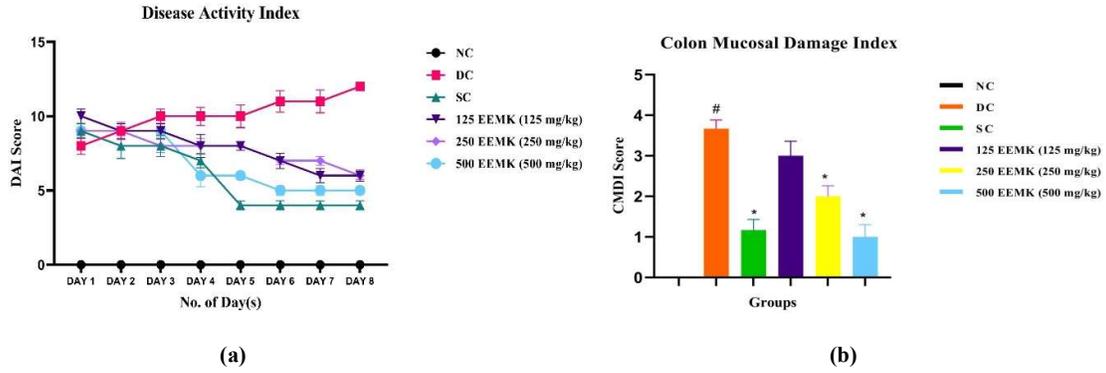
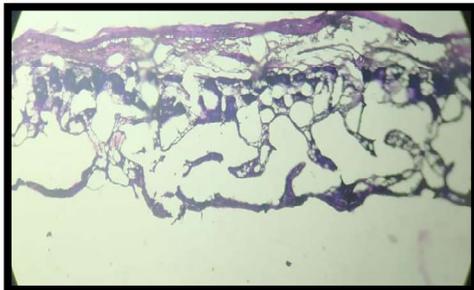


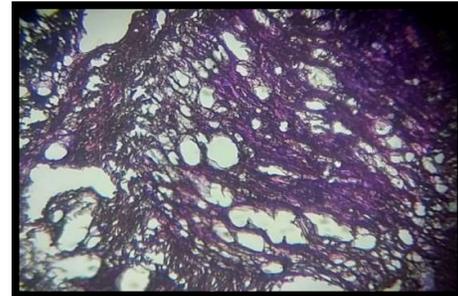
Figure 2: Effect of EEMK on (a) Disease activity index (b) Colon mucosal damage index in TNBS induced UC in rats

Table 2: Effect of EEMK on SOD, Catalase, NO, LPO & MPO level in TNBS induced UC in rats

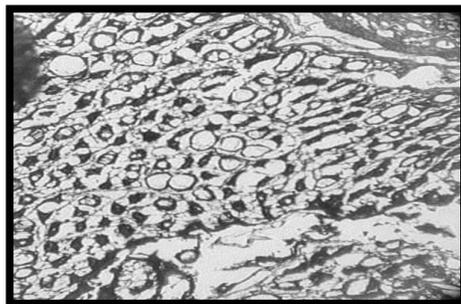
Groups	SOD	Catalase	NO	LPO	MPO
Normal Control	44.24±5.22	2.35±0.17	5.00±0.07	9.16±0.14	1.08±0.13
Disease Control	22.93±3.42 [#]	0.52±0.24 [#]	8.06±0.12 [#]	15.3±1.05 [#]	2.10±0.16 [#]
Standard Control	42.76±3.43 [*]	2.04±0.26 [*]	4.9±0.11 [*]	11.02±0.73 [*]	0.90±0.08 [*]
125 EEMK	29.29±8.89	0.73±0.09	7.91±0.13	14.24±0.44	2.00±0.11
250 EEMK	31.91±6.20	0.93±0.10	7.03±0.17 [*]	13.42±0.93	1.91±0.03
500 EEMK	40.44±3.36 [*]	1.77±0.23 [*]	4.7±0.2 [*]	10.05±0.62 [*]	0.80±0.10 [*]



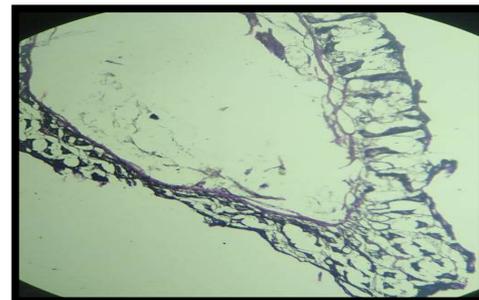
1. NC- Normal histological structure (architecture) of mucosal layer



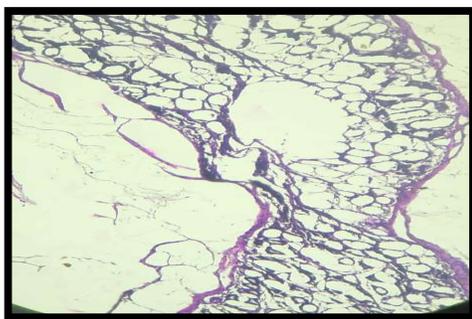
2. DC- Total distortion of mucosal layer with infiltration of neutrophils & ulcers



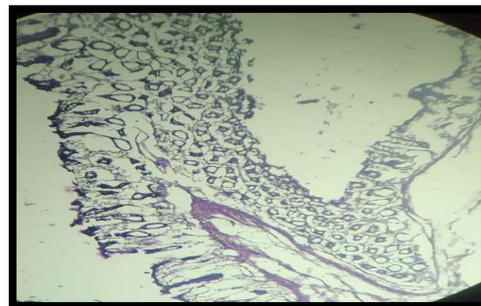
3. SC- Intact mucosal membrane with normal architecture



4. 125 EEMK- Slight dilation of intestinal mucosal glands with mild infiltration of neutrophils



5. 250 EEMK- Decrease in infiltration of inflammatory cells, near normalization of mucosa



6. 500 EEMK- Normal structure of mucosa (architecture) with lesser cell infiltration

Figure 3: Effect of EEMK on histopathology of colon in TNBS induced ulcerative colitis in rats: 1. Normal Control 2. Disease Control 3. Standard Control 4. 125 EEMK (125 mg/kg) 5. 250 EEMK (250 mg/kg) 6. 500 EEMK (500 mg/kg)

DISCUSSION

In the present study, EEMK increase body weight by preventing diarrhoea and improved stool consistency as observed from day 1 to 8. TNBS treated rats having shortened length of colon & higher damage of colon was observed in disease control group. A significant higher ($P < 0.05$) weight/length ratio was observed in disease control group as compared to normal control group. Treatment with EEMK (250 & 500 mg/kg) shown lower weight/length ratio as shown in figure 2. There was significant reduction in DAI score from day 4-8.

In the present study, SOD & Catalase level were found to be higher in TNBS treated rats as compared to NC group this might be due to presence of alkaloids. Treatment with EEMK (250 & 500 mg/kg) resulted in significantly lower nitric oxide (NO), LPO

& MPO as compared to animals of disease control group.

Infiltration of neutrophils, ulcers, distortion of mucosal layer were observed in disease control group.

CONCLUSION

The present study has revealed that ethanolic extract of leaves of *Murraya koenigii* is having beneficial and curative effect in TNBS induced ulcerative colitis in rats. LCMS data confirms presence of mahanine, mahanimbine & β -sitasterol in ethanolic extract of leaves of *M.koenigii* which might be responsible for anti-ulcerative and anti-inflammatory activity. Treatment with EEMK (250 & 500 mg/kg) shown significantly reduced DAI, CMDI & significant higher colon length as compared to disease control group. Significantly higher level of SOD & Catalase while lower level of NO were observed in animals treated with EEMK (500 mg/kg) as

compared to disease control group. Level of LPO & MPO were significantly lower in animals treated EEMK as compared to disease control group. On the basis of above discussion, it can be concluded that ethanolic extract of leaves of *Murraya koenigii* might be effective in the treatment of ulcerative colitis. Further studies are required to confirm active principle responsible for activity & its specific mechanism of action for having protective effect on ulcerative colitis.

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