

MEYNA LAXIFLORA - A TRADITIONAL HERBAL PLANT FOR TREATMENT OF INFLAMMATORY BOWEL DISEASE

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Received on: 31/10/2022

Revised on: 21/11/2022

Accepted on: 11/12/2022

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ABSTRACT

As per WHO Traditional medicine is the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of illness.^[1] World Health Organization define Traditional herbal medicines as naturally occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices.^[2] Meyna is a genus of shrub and the plant is Small or medium size tree, Bark light black, smooth. Leaves opposite or whorled,^[3] distributed in tropical and subtropical region. Earlier *Vangueria spinosa* covers a group of plants from Meyna genus recently the plants has been separated and classified into eleven different species of *Meyna* with the help of molecular phylogenetics.^[4,5] The plant was reported to be used for treatment of diseases of digestive system, anti-inflammatory antioxidant, anti-dysentery and constipation. It also exhibits anti-fungal and anti-bacterial activities which indicate its usefulness as a promising drug in treatment of IBD.

KEYWORDS: anti-inflammatory, dysentery, IBD, meyna laxiflora, constipation.

INTRODUCTION

Inflammatory bowel disease (IBD) is an umbrella term with two most prevalent entities namely Crohn's disease (CD) and Ulcerative colitis (UC), defined as idiopathic chronic, relapsing and remitting inflammatory condition of intestinal tract.^[9] Crohn's disease is exemplified by discontinuous transmural inflammation, involving any portion of the intestinal tract, most commonly colon and ileum; whilst Ulcerative colitis is typified by confluent inflammation of the mucosa, restricted to colon and rectum. Both CD and UC pursue a protracted relapsing and remitting course, usually extending over years. Once it appears IBD continues to remain a lifelong disease. In spite of the vivid difference between UC and CD; typical signs and symptoms include diarrhoea, bleeding ulcers, stomach pain, abdominal pain and cramping, bloody stools, bloating due to bowel obstruction, unintended weight loss and anemia along with extraintestinal manifestations such as arthritis, uveitis, liver disease, pyoderma gangrenosum, primary sclerosing cholangitis.^{11,12} Extra-intestinal manifestations, such as inflammatory reactions in the skin, joints, eyes and hepatobiliary system are sometimes present. More recently described involvements are pulmonary diseases, thromboembolic events, osteopenia and osteoporosis. The incidence of UC is calculated to be 1-25 per 1,00,000 per year and the disease affects people of all ages. Onset of disease in the first decade of life is

unusual but there is a steep increase in incidence during puberty and the following adolescence and young adulthood. The etiology of the disease is yet to be revealed but the pathogenesis is believed to be multifactorial. An observed tendency of UC to cluster to families has implied that genetic components are involved in the development of the disease.^[13]

❖ Plant profile

➤ *Meyna laxiflora*

The Plant *Meyna Laxiflora* was collected from Satpuda hills Akkalkuwa, Dist: Nandurbar, Maharashtra, India. The plant was Authenticated by Dr. Bhanu H. Kakrani, HOD, Department of Botany, Shivam Institute Of Science and Paramedical College, Valasan, Anand (Gujrat). Voucher specimen were deposited with the institute wide reference no. SET/SISPC/2019-112.

Fig. 1: *Meyna laxiflora* a) leaf.Fig. 2: *Meyna laxiflora* b) fruit.

➤ Taxonomical classification

Kingdom:	<i>Plantae</i>
Domain	<i>Eukaryota</i>
Subkingdom:	<i>Viridaeplantae</i>
Phylum:	<i>Tracheophyta</i>
Subphylum:	<i>Euphyllophytina</i>
Infraphylum:	<i>Radiatopses</i>
Class:	<i>Mangoliospida</i>
Subclass:	<i>Asteridae</i>
Superorder:	<i>Gentiananae</i>
Order:	<i>Gentianales</i>
Family:	<i>Rubiaceae</i>
Genus:	<i>Meyna</i>
Specific epithet:	<i>laxiflora</i> – Robyns

➤ Vernacular name

Assamese:	Kutkura, Moin
Bengal:	Mainphal, Muduna, Muyna, Muyuna
English:	May-nuh
Gujrati:	Alu, Atu
Hindi:	Moina, Muduna, Muyuna
Kannad:	Mullakare, gundkare, gobergally
Marathi:	Alu, Huloo, Halawni
Sanskrit:	Pindi, Pinditaka, Pindituka, Pindu, Nagakesarah
Tamil:	Manakkarai

➤ Traditional Uses

1. The tribe such as Pawara, Bhil, Tadavi and Vanjara of satpuda uses foilage as food.^[14,16]
2. The tribal community in Western Ghat region of Maharashtra uses young fruits as food and dried fruits as Narcotic and anti-dysentery.^[15]
3. The Chothe Tribe Bishnupur and Chandel districts of Manipur uses fresh leaves as chutney time to time to enhance blood purification and skin texture and fruits for constipation.^[16,17]
4. The Meitei community of the Imphal valley uses leaves as ingredient for preparation of Chinghi an herbal shampoo.^[18] young leaf and fruits uses in treatment of helmenthiasis and hoarseness (abnormal change in voice due to throat infection).^[19]

MATERIALS AND METHODS

➤ Animals

Albino rats (Sprague-Dawley) and aged between 8 - 10 weeks were chosen for the experiments. These mice were acclimatized for 14 days in the laboratory before the commencement of the experiment. The animals were maintained at a relative humidity of 50-55% and temperature 22-24°C with a 12 hr light/ dark cycle. Normal drinking water and the commercially available pellet were given as a diet. Control group animals received same experimental handling as those of test groups except that drug treatment was replaced by administration of appropriate volumes of dosing vehicle. Animal handling and study procedures were followed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on

Animals (CPCSEA), Government of India. (CPCSEA/IAEC/DIPS/06/22/35)

➤ **TNBS-induced Colitis**

Crude extracts of *M. laxiflora* were tested against TNBS induced colitis in rats.

Groups and Drug Treatments

Animals were divided in five groups of six animals each and treated as follows

Group 1: Control: Uninduced untreated

Group 2: Model Control: Induced Untreated

Group 3: ML 10 mg/kg/day –TNBS treated. **ML** was administered in dose 10, mg/kg/day p.o. for 7 days prior to induction of colitis and continued for next 4 days after TNBS treatment.

Group 4: ML 50 mg/kg/day –TNBS treated. **ML** was administered in dose 50 mg/kg/day p.o. for 7 days prior to induction of colitis and continued for next 4 days after TNBS treatment.

Group 5: ML 100 mg/kg/day –TNBS treated. **ML** was administered in dose 100, mg/kg/day p.o. for 7 days prior to induction of colitis and continued for next 4 days after TNBS treatment.

➤ **Induction of colitis**

Trinitrobenzenesulfonic Acid (TNBS) was used as an agent to induce ulcerative colitis. Prior to induction, the mice were starved for a day with access to drinking water. A mixture of 100 μ L of 5% TNBS and absolute ethanol (1:1 ratio) was intrarectally administered using a catheter. After administration, the mice were held vertically for 2-3 min for better distribution of TNBS. Animals were administered test samples at a dose of 100mg/kg for 7 days. At the end of the treatment period the animals were sacrificed by cervical dislocation and the colons were dissected out and cleaned with cold saline.

➤ **Image capturing for morphologic visualization**

Opened colonic samples were flattened and carefully sandwiched between two layers of a transparent plastic folder of A4 size. Specimens within plastic folder were scanned using a scanner and captured image was saved (TIFF format) in computer hard drive.

➤ **Biochemical estimation**

Myeloperoxidase (MPO) assay

The colonic tissues were weighed and homogenized in an ice-cold 50 mM Potassium.

Phosphate buffer (pH 6.0) containing 0.5% Cetyltrimethyl ammonium bromide (CTAB). The tissue homogenate is then mixed with O-dianisidine, 50 mM Potassium Phosphate buffer, and diluted H₂O₂. A yellow compound is formed and it is spectrophotometrically measured at 450 nm.

Lipid Peroxidase assay (MDA)

Add 600 μ L of TBA reagent into each well containing

200 μ L sample. Incubate at 95°C for 60 minutes. Cool to room temperature in an ice bath for 10 minutes Take 200 μ L of the reaction mix (containing MDA-TBA adduct) and add into a 96-well microplate for analysis. Measure absorbance immediately on a microplate reader at OD 532 nm for colorimetric assay.

Nitric oxide scavenging assay

Nitric oxide scavenging activity can be estimated by the use of Griess Ilosvoy reaction. The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with extract of each plant were dissolved in methanol and incubated at 300C for 2 hours. The same reaction mixture without the extract but the equivalent amount of ethanol served as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H₃P₀₄ and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was immediately read at 550nm.

GSH Assay

Glutathione is a key antioxidant that mostly exists in reduced form GSH. Reduced glutathione was determined Colon tissues were homogenized in a potassium phosphate buffer (10 mM). 1-chloro-2, 4-dinitrobenzene (CDNB) was added to the tissue homogenate. CDNB is a substrate that conjugates with GSH present in the sample to form a conjugate that has an absorption maxima at 340 nm. GST is measured in terms of free sulfhydryls (mM).

Measurement of GSH level in the colonic tissue

Colonic tissues were homogenized in ice-cold 125 mmol/L sodium phosphate buffer with 6.3 mmol/L EDTA (pH 7.5, 3 [xL/mg tissue) for 30 s. The crude homogenate was centrifuged at 30 000 g at 4°C for 30 min. Then, 200 iL of 40 g/L sulfosalicylic acid was added to 100 iL of supernatant and allowed to stand on ice for 5 min to precipitate protein. The mixture was centrifuged again at 5000 r/min at 4°C for 10 min. Subsequently, 100 fiL of the de-proteinized supernatant was mixed well with 300 {xL of 125 mmol/L sodiimi phosphate buffer (pH 8) and 2 iL of 10 mmol/L 5,5'-dithiobis-(2-nitrobenzoic acid). The solution was allowed to stand at room temperature for 15 min to develop yellow color. The absorbance was read against the reagent blank at 412 nm in a spectrophotometer. A standard curve of reduced GSH was used for the calculation of the concentration of GSH in the colonic tissues. The final values were expressed as nanomole per milligram protein.

Anti-diarrhoeal activity

Diarrhoea is one of major symptoms of IBD. Crude extract of ML was evaluated for its anti-diarrhoeal potential which will provide symptomatic relief against IBD. ML had anti-diarrhoeal potential and reported in previous literature.

Gastrointestinal Transit

Swiss albino mice of either sex were divided in five groups of six animals each.

Normal defecation

Swiss albino mice of either sex were divided in five groups.

Gastric emptying

Wistar albino rat of either sex were divided in three groups of six animals each, fasted for 24 hrs and treated.

Intestinal fluid accumulation

Wistar albino rat of either sex were divided in three groups of six animals each and treated.

RESULT AND DISCUSSION

Inflammatory bowel disease (IBD) results in a substantial burden to individuals and society, not only because of direct and indirect medical costs, but also by causing disability. The reduction in working capacity, especially in a young and active segment of the population, is the major economic and social burden of disease.^[21]

In current study, aqueous extract of leaves *M. laxiflora* were evaluated for their toxicity and found to be safe in acute and chronic toxicity protocols. This study provides scientific evidence for safety of these plant extracts and showed no mortality or morbidity in rats. These extracts were found to be devoid of any adverse effects when evaluated by morphological haematological and biochemical parameters.^[22]

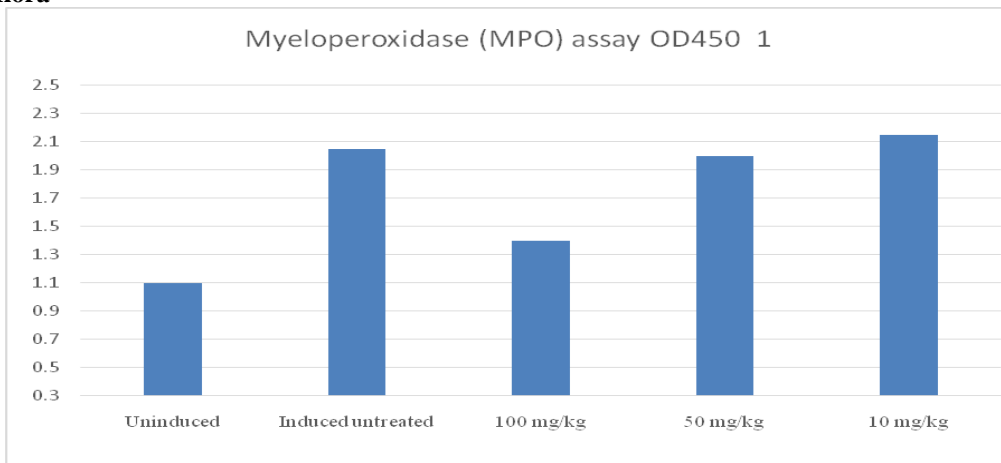
In present study attempt has been made to cope up with the need of alternative herbal medicine for treatment of IBD, which is devoid of side effects, do not show resistance or intolerance and cheaper as compared to current medication available. *Meyna laxiflora* is a traditional herbal medicine in India reported in Indian ancient text "Ayurveda" to possess anti-diarrhoeal and antibacterial activity.^[20] It has been used as an analgesic and anti-inflammatory agent. by traditional medical practitioner This forms a good basis for its use as a curative drug against IBD. TNBS treated rats showed decreased body weight and diarrhoea with pasty stools and presence of blood in stools. Treatment with ML showed reduction in diarrhoea and improved body weight. Anorexia and malnutrition are the symptoms of chronic IBD due to nausea, vomiting, diarrhoea and abdominal pain. It is previously been reported that tissue injury and infection trigger the release of proinflammatory cytokines that alter normal energy

regulatory mechanisms resulting in anorexia, tissue catabolism, increased metabolic rate, and loss of body weight. Similar results were obtained in TNBS induced tissue injury and increased proinflammatory cytokines in rats.^[23] In experimental colitis induced by TNBS, anorexia was reported in model control rats, while there was no significant alteration in food intake in ML treated rats. This effect may be ascribed to protective and anti-inflammatory effects of these extracts. Animals treated with TNBS alone exhibited significant increase in colonic lesion area, compared with control. ML treatment decreased colonic damage induced by TNBS. Histological sections from TNBS treated rats colon showed trans-mural necrosis, along with extensive morphological disorientation, oedema and diffuse leukocyte cellular infiltrate as well as lymphocyte in submucosa. While treatment with ML reduced the disorientation of mucosa, prevented infiltration and oedema in higher dose levels (200 and 400 mg/kg doses). Weight of colon in TNBS treated rats was found to be increased, which is an indicator of oedema and increased fluid deposition in colonic tissue layers. Pretreatment with ML significantly decreased colon weight, compared with model control.

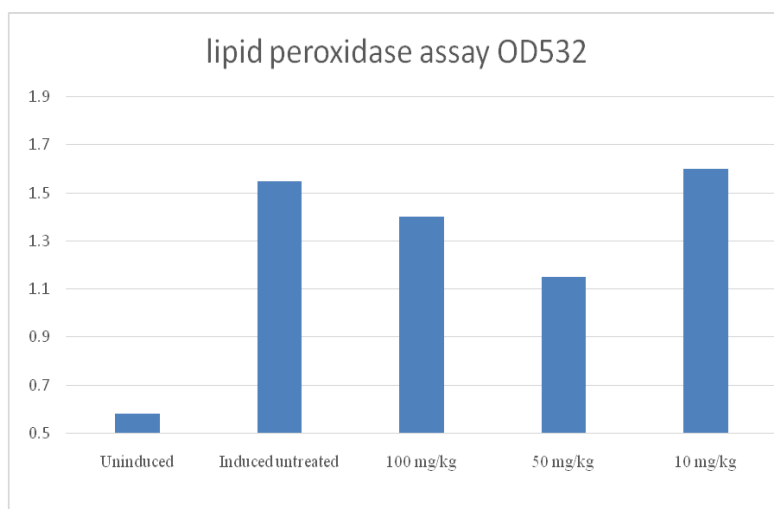
XO (xanthine oxidase) pathway seems to be discrete for UC. Reynolds and co-workers in one of clinical study illustrated that colonic XO activity was not elevated in UC patients.^[24] It was suggested that XO may not be a major source of superoxide and ROS production in UC in present study MPO levels correlated well with severity of symptoms of TNBS induced colitis in rats.^[25] Model control animals showed elevated activity of MPO in colon while pretreatment with ML showed significantly reduction. Previous studies showed that decrease in colonic GSH concentration following colitis induction is due to over-production of ROS that deplete GSH by inhibiting synthetic enzymes for GSH production in colonic tissue. Similar results were reported in current study showing significant decrease of GSH levels in TNBS treated rats. Levels of GSH were improved significantly with treatment of ML treatment was unable to show significant effect.

These natural antioxidants reduced colonic GSH level is crucial in inducing inflammatory changes.²⁶ Previous studies showed that decrease in colonic GSH concentration following colitis induction is due to over-production of ROS that deplete GSH by inhibiting synthetic enzymes for GSH production in colonic tissue. Similar results were reported in current study showing significant decrease of GSH levels in DNBS treated rats. Levels of GSH were improved significantly with treatment of ML treatment was unable to show significant effect.

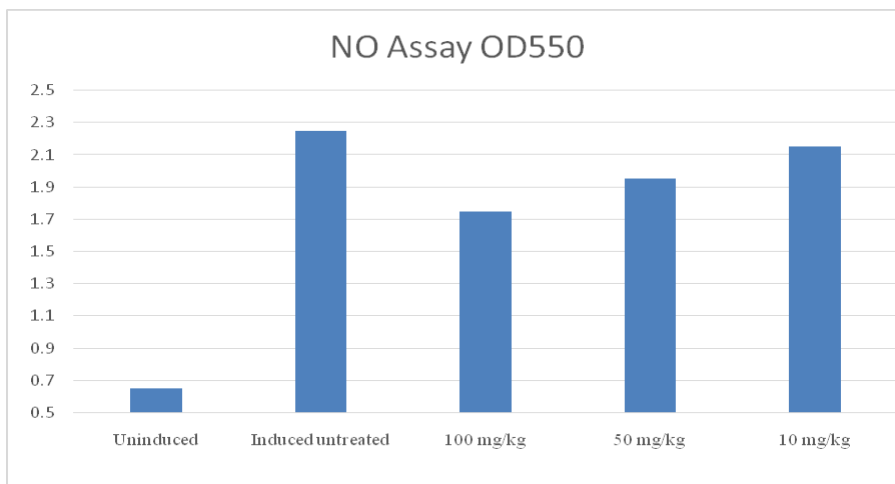
Meyna laxiflora



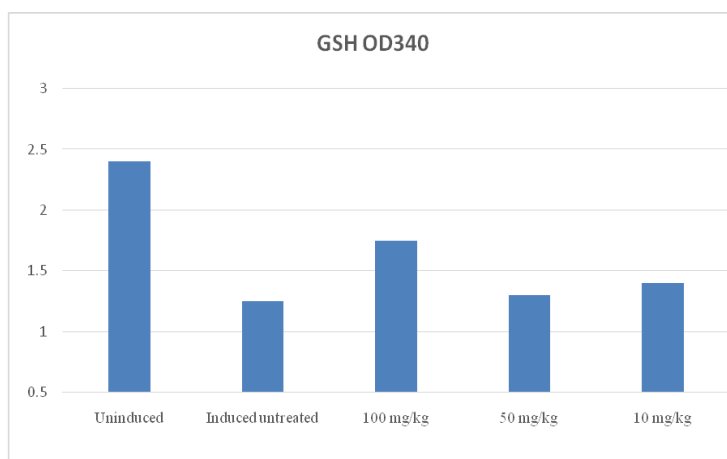
Myeloperoxidase (MPO) assay OD450 1									
Treatment	Animal						Average	STDEV	
	1	2	3	4	5	6			
Uninduced	0.97	1.21	1.14	1.08	0.89	1.04	1.06	0.12	
induced untreated	2.14	2.16	2.24	2.09	2.28	2.31	2.20	0.09	
Meynalaxiflora	100mg/kg	1.62	1.65	1.58	1.12	1.14	1.53	1.44	0.24
	50 mg/kg	1.24	1.85	1.96	2.15	2.08	2.11	1.90	0.34
	10 mg/kg	1.82	2.06	2.24	2.16	2.14	2.35	2.13	2.13



lipid peroxidase assay OD532									
Treatment	Animal						Average	STDEV	
	1	2	3	4	5	6			
Uninduced	0.58	0.48	0.62	0.59	0.52	0.66	0.58	0.07	
induced untreated	1.65	1.47	1.58	1.63	1.42	1.56	1.55	0.09	
Meynalaxiflora	100 mg/kg	1.26	1.18	1.22	1.17	1.13	1.21	1.20	0.05
	50 mg/kg	1.42	1.38	1.36	1.44	1.46	1.39	1.41	0.04
	10 mg/kg	1.56	1.64	1.58	1.62	1.66	1.57	1.61	0.04



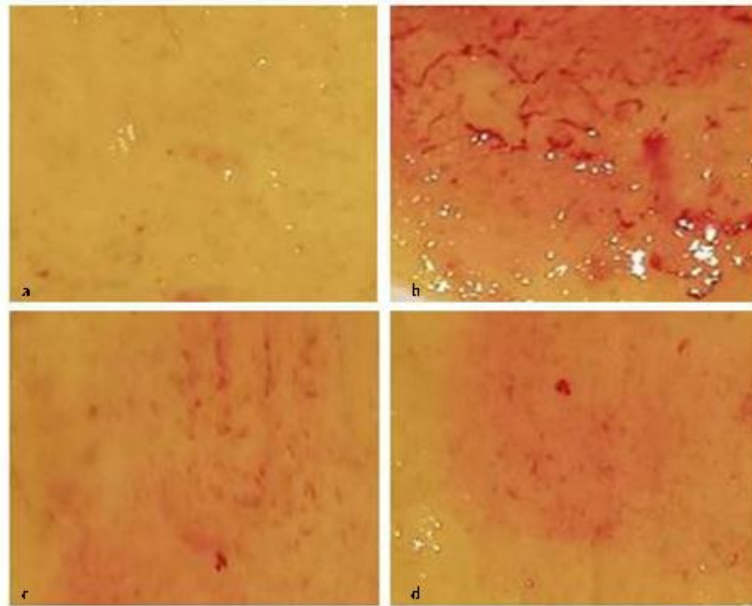
NO Assay OD550									
Treatment	Animal						Average	STDEV	
	1	2	3	4	5	6			
Uninduced	0.75	0.64	0.71	0.58	0.55	0.76	0.67	0.09	
induced untreated	2.14	2.32	2.16	2.24	2.29	2.32	2.27	0.07	
Meynalaxiflora	100 mg/kg	1.85	1.74	1.66	1.68	1.69	1.73	1.73	0.07
	50 mg/kg	2.12	2.18	1.95	1.62	1.66	2.31	1.97	0.28
	10 mg/kg	2.11	2.28	2.31	2.19	2.27	2.17	2.22	0.08



GSH OD340									
Treatment	Animal						Average	STDEV	
	1	2	3	4	5	6			
Uninduced	2.34	2.56	2.31	2.48	2.36	2.41	2.41	0.09	
induced untreated	1.35	1.28	1.17	1.32	1.28	1.21	1.27	0.07	
Meyna laxiflora	100 mg/kg	1.84	1.81	1.76	1.78	1.64	1.69	1.75	0.08
	50 mg/kg	1.32	1.36	1.38	1.42	1.11	1.13	1.29	0.13
	10 mg/kg	1.42	1.5	1.22	1.31	1.36	1.38	1.34	0.07

Gastric Symptom (Diarrhoea, GI transit, defecation, gastric emptying, intestinal fluid accumulation)

Treatment	Animal					
	1	2	3	4	5	6
Uninduced	*	*	*	*	*	*
Induced untreated	***	***	**	***	***	***
Meyna laxiflora	100 mg/kg	**	**	*	**	**
	50 mg/kg	**	***	**	**	***
	10 mg/kg	***	**	***	***	***

Histopathology**Intestinal lumen**

- a) Uninduced untreated;
- b) Induced Untreated;
- c) *Meyna laxiflora* 10mg/kg
- d) *Meyna laxiflora* 50mg/kg

CONCLUSION

1. Aqueous extract of ML was found to be protective against TNBS induced colitis by inhibiting macroscopic and microscopic damage to colon, weight loss, diarrhoea, MPO, MDA and NO levels with increase in natural antioxidant GSH levels in rats. The activity can be ascribed to combined effect of gallic acid found in PML1.
2. ML was found to be safe in acute toxicity study and in limit test performed at dose 1000 mg/kg/day in repeated dose toxicity in rats.
3. ML showed inhibition of macroscopic and microscopic damage to colon, weight loss, diarrhoea, MPO, MDA and NO levels with increase in natural antioxidant GSH levels in rats.
4. Potentially active fraction obtained from ML in dose 100 mg/kg p.o. improved symptoms of Body weight, stool consistency and colonic lesion area was found to be improved significantly. But NF-KB and IL-6 levels in homogenised rat colon were altered slightly.

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