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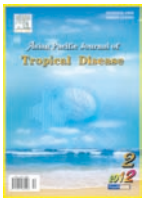

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Effect of *Azadirachta indica* leaves extract on acetic acid-induced colitis in rats:Role of antioxidants, free radicals and myeloperoxidase

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ABSTRACT

Objective: To evaluate the healing effects of extract of dried leaves of *Azadirachta indica* (Neem) on acetic acid-induced colitis in rats. Neem tree is known as 'arishtha' in Sanskrit, meaning 'reliever of sicknesses'. **Methods:** 50% ethanolic extract of *Azadirachta indica* leaves was administered orally, once daily for 14 days in rats after the induction of colitis with acetic acid and 500 mg/kg dose of extract was found to have an optimal effect against acetic acid-induced colonic damage score, weight and adhesions (Macroscopic). Effect of *Azadirachta indica* extract was then further studied on various physical (mucous/blood in stool, food and water intake and body weight changes), colonic mucosal damage and inflammation (microscopic), antibacterial and biochemical parameters viz. i) antioxidants (superoxide dismutase, catalase and reduced glutathione) and ii) free radicals (nitric oxide and lipid peroxidation) and myeloperoxidase (acute inflammatory marker) activities in acetic acid-induced colitis. **Results:** *Azadirachta indica* extract decreased colonic mucosal damage and inflammation (macroscopic and microscopic), mucous/bloody diarrhea, fecal frequency and increased body weight. *Azadirachta indica* extract showed intestinal antibacterial activity and enhanced the antioxidants but decreased free radicals and myeloperoxidase activities. Acute toxicity study indicated no mortality or other ANS or CNS related adverse effects even with 5.0 g/kg dose (10 times of effective dose) indicating its safety. **Conclusions:** *Azadirachta indica* seemed to be safe and effective in colitis by its predominant effect on promoting antioxidant status and decreasing intestinal bacterial load, free radicals and myeloperoxidase responsible for tissue damage and delayed healing.

1. Introduction

Inflammatory bowel disease (IBD) including ulcerative colitis is a chronic incurable disease that damages the gastrointestinal tract of affected individuals, which is a resultant effect of dysregulated innate, adaptive and epithelial immune functions. It is characterized by chronic inflammation in the mucosal membrane of the small and/or large intestine. It is largely a disease of the industrialized world, and is more common in urban areas and northern climates[1]. Ulcerative colitis (UC) is clinically characterized by recurrent inflammatory involvement of intestinal segments with several manifestations often resulting in an unpredictable course. The aetiology remains unknown; however, two primary theories have been proffered focusing on either a specific persistent infectious agent or an abnormal host immune response to ubiquitous antigens in the luminal constituents[2]. Reactive oxygen species (ROS)-mediated injury plays an important role in the pathophysiology of IBD. The increased generation of highly toxic ROS exceeds the limited intestinal antioxidant defense system, thereby contributing to intestinal oxidative injury in IBD/UC patients[3]. Many treatments have been recommended for UC; they are far from treating the cause but are effective only in reducing the inflammation and accompanying symptoms in up to 80% of patients. The current medical management consists of anti-inflammatory and immunosuppressive agents and biologic drugs, as well as surgery. The primary goal of drug therapy is to reduce inflammation in the colon that generally requires frequent intake of high dose of anti-inflammatory drugs [4]. The

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propensity to cause adverse events and lack of effectiveness of standard therapies has diverted towards the use of complementary and alternative medicines, particularly of herbal therapies, for IBD[5].

Azadirachta indica (Malvacea) popularly known as Neem, is an evergreen tree, cultivated in various parts of the Indian subcontinent. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. The Sanskrit name of the Neem tree is 'Arishtha' meaning 'reliever of sicknesses'. Various parts of the Neem tree have been used as traditional Ayurvedic medicine in India from time immemorial. The medicinal utilities have been described, especially for leaf, fruit and bark. Neem has a role in the treatment of disorders like microbial infections, skin diseases, dental disorders, malaria, syphilis, leprosy and has antiseptic property. Anti-inflammatory and immune-stimulant actions have been reported in the extract of *Azadirachta indica*[6].

The present study was undertaken to evaluate healing activities of *Azadirachta indica* in experimental colitis with the premise that if it is effective in treating gastric ulcer then it could be effective in treating colitis also. It includes detailed study of the ulcer healing effects of 50% ethanolic extract of dried leaves of *Azadirachta indica* on experimental ulcerative colitis (UC) induced by acetic acid in rats on various physical (mucous/bloody diarrhea, change in body weight, food and water intake), histopathology (macroscopic- colonic mucosal damage score, adhesions and colonic microscopic changes), biochemical (estimation of antioxidants- superoxide dismutase, SOD; catalase, CAT; and reduced glutathione, GSH and free radicals-lipid peroxidation, LPO; and nitric oxide, NO) and Acute inflammatory marker, myeloperoxidase (MPO) in the colonic mucosa of CMC/*Azadirachta indica* extract/SS-treated acetic acid-induced colitis rats.

2. Materials and methods

2.1. Animals

Inbred Charles-Foster albino rats (150-250 g) and mice (25-30 g) of either sex were obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi. They were kept in the departmental animal house at 26±2° C and relative humidity 44-56%, light and dark cycles of 10 and 14 h respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Pashu Acetic acidhar, Ramnagar, Varanasi) and water ad libitum. 'Principles of laboratory animal care' (NIH publication no. 82-23, revised 1985) guidelines were followed. Approval from the Institutional Animal Ethical Committee was taken prior to the experimental work (Notification no.: Dean/2009-10/741 dated 11.12.2009).

2.2. Plant material and preparation of extract

Leaves of *Azadirachta indica* were collected in the months of March to May from Ayurvedic Gardens, Banaras Hindu University. They were dried in shade and powdered. 50% ethanolic extract of *Azadirachta indica* leaves was prepared by adding 200 g of dried fine powder in 1000 ml of ethanol (500 ml) and distilled water (500 ml) mixture. The mixture was shaken at intervals and the extract so obtained was filtered after an interval of two days and the procedure was repeated twice. *Azadirachta indica* extract so obtained each time was mixed and later dried at 40° C in incubator. The yield was about 10.2% (w/w). *Azadirachta indica* extract was stored at -20°C until further use. Enough quantity of the extract was prepared fresh before use.

2.3. Standard drug

Sulfasalazine (SS, 5-Aminosalicylic acid, SAZO, 1000 mg delayed release tablet, WALLACE) was purchased from market and used as standard colitis protective drug in the dose of 100 mg/kg [1].

2.4. Induction of colitis and treatment protocol

Experimental colitis was produced by intracolonic administration of acetic acid (10%, 0.20 ml/rat) given per rectally[7]. The animals were sacrificed 15 days after intra-colonic administration of either normal saline (NS, negative control) or acetic acid and after administration of *Azadirachta indica* extract (test extract) or SS (positive control) in acetic acid-induced colitis groups in rats. *Azadirachta indica* extract /SS suspension was prepared in 0.5% CMC and was administered orally, once daily in the volume of 1 ml/100 g rat for 14 days after the induction of colitis. First dose of *Azadirachta indica* extract /SS was given 4 hours after induction of colitis with acetic acid on day 1 and then administered orally once daily till 14 days for studying various physical parameters while, the rats were sacrificed on 15th day of experiment for estimation of various biochemical parameters and histopathology (macroscopic and microscopic). The results of the treatment were compared with acetic acid-treated group.

2.5. Assessment of frequency of stool and diarrhea

Number of stool and mucous or blood stained diarrhea were observed in NS intra-colonic treated and acetic acid-induced colitis after administration of CMC/*Azadirachta indica* extract/SS. The effects were seen on the 0, 2, 4, 6, 10 and 14th day of the experiment. The result of acetic acid was compared with NS (without colitis) while that of *Azadirachta indica* extract/SS treated were compared with acetic acid -induced group.

2.6. Assessment of changes in body weight, food intake and water intake

The above parameters were measured on the 0, 2, 4, 6, 10 and 14th day of the experiment. Each rat was individually weighed using standard rat weighing machine and their respective weights were noted down. Similarly a measured

weight of food was given to each rat individually in the respective cages at a fixed time of day (10:00 am morning) and next day the amount of food left was weighed again to calculate the amount of food intake by individual rat. To find the water intake, a measured volume of water was given to each rat individually at a fixed time in bottles and next day measured with help of measuring cylinder. The difference in the volume before and after gave the volume of water ingested by individual rat in one day.

2.7. Assessment of colonic damage and inflammation and histopathology

All scorings of damage and excision of tissue samples were performed by an observer unaware of the treatment group. The rats in the various treatment groups were randomized before being sacrificed. The rats were weighed and sacrificed by over dose of ether and proximal 8 cm of colon was removed. The colon was opened by a longitudinal incision, rinsed with tap water and pinned out on a wax block. Macroscopically visible damage was scored on a 0-10 scale using the scoring system as reported by Saleh et al., 2012[8], which takes into consideration the area of involvement and the presence or absence of ulcers, adhesions to surrounding tissue and thickness (signs of inflammation). Subsequently 8 cm of colon were taken for measurement of weight and weight was then expressed as mg/per cm length of individual rat. Histopathology of the colon was done in all the groups on 15th to know the status of healing. A piece of colon was removed and fixed in 10% buffered formalin and paraffin embedded. 4-6 μm thick sections were stained with Hemotoxylin and Eosin stain for histological evaluation and examined under microscope at x100 magnification.

2.8. Estimation of colonic mucosal antioxidants, free radicals and pro-inflammatory marker, myeloperoxidase

Antioxidants, SOD, CAT and GSH, free radicals, LPO and NO, acute inflammatory marker, myeloperoxidase (MPO) and protein were estimated in colonic mucosal homogenates following the standard procedures as reported earlier[9, 10]. SOD, CAT, and MPO were expressed as mU/mg protein while GSH, LPO, NO were expressed as nmol/mg protein.

2.9. Antimicrobial susceptibility and minimum inhibitory concentration (MIC)

Antibacterial susceptibility test of *Azadirachta indica* extract was done against various intestinal pathogens i.e. *Escherichia coli* ATCC 25922, *Shigella boydii*, *Shigella sonnei* and *Shigella flexneri* obtained from the American Type Culture Collection (ATCC) and clinical strain preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India following the disk diffusion method[11] while, MIC was performed by micro dilution method[12,13].

2.10. Acute toxicity study in mice

Swiss strain albino mice of either sex, weighing between 20 to 25g fasted overnight, were used for toxicity study. Suspension of *Azadirachta indica* extract was orally administered at 5 g/kg stat dose (10 times of the optimal effective dose of 500 mg/kg) to mice. Subsequent to *Azadirachta indica* extract administration, animals were observed closely for first three hours, for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 1 week.

2.11. Statistical analysis

Statistical comparison was performed using either unpaired't' test or one way analysis of variance (ANOVA) and for multiple comparisons versus control group was done by Dunnett's test. All statistical analysis was performed using SPSS statistical version 16.0 software package (SPSS® Inc., USA). P value <0.05 were considered statistically significant.

3. Results

3.1. Effects on diarrhea, fecal output and presence of blood or mucous

10% acetic acid when instilled intra-rectally into the colon led to severe diarrhea in all the animals (100%) which was prominent on day 2 and then decreased to 50 % of animals on day 4 and was associated with presence of blood till 4th day and mucous till day 10. They also showed increased stool frequency from day 2 onwards (147.0%). The intensity of stool frequency gradually increased over day 4 onwards (183.9%) and then started declining at day 14 (140.4%) compared with Day 0 (100%) frequency. Administration of *Azadirachta indica* extract (500 mg/kg) once daily for 14 days in acetic acid-treated rats showed decrease in stool frequency from day 4 onwards (Figure 1a).

3.2. Effects on body weight changes and food and water intakes

Acetic acid-induced colitis led to gradual decrease in body weight as observed from day 2 onwards till 14th day of study. Significant decrease on body weight was observed from 6th day onwards as compared with untreated normal animals. Treatment with *Azadirachta indica* extract for 14 days reversed the decrease trend in body weight suggestive of beneficial effects of *Azadirachta indica* extract (Figure 1b). However, little or no change was observed on food and water intake between the acetic acid-treated and *Azadirachta indica* extract and SS treated animals from 0 day to 14th day of study treatments.

3.3. Effects on colonic damage, inflammation and adhesions

Untreated rats, receiving 0.5% CMC orally daily, were given normal saline (NS) instead of acetic acid in the colon intrarectally (negative control group) did not show

Table 1.
Effects of graded doses of 50% ethanolic extract of dried leaves of *A. indica* on acetic acid-induced rat colonic mucosal damage score, weight and adhesions

Oral treatment (mg/kg, od x 14 days)		Damage score (0-10)	Colonic weight (mg/cm)	Adhesions (%)
Normal Saline (1% CMC)		0 (0.00±0.0)	171.0±4.80 (65.4±1.8)	0 (0.00)
Acetic acid (1% CMC)		5.78±0.22* (100.0±3.8)	261.2±6.79* (100.0±2.6)	66.7 (100.0)
Acetic acid + *A. indica* extract	250	4.33 ±0.42[b] (74.9±7.3)	207.1±13.1[b] (79.3±5.0)	33.3 (50.0)
Acetic acid+ *A. indica* extract	500	1.67 ±0.17[c] (28.9±2.9)	157.9±7.71[c] (60.5±2.9)	16.7 (25.0)
Acetic acid+ *A. indica* extract	1000	1.50 ±0.22[c] (25.9±3.9)	150.6±5.69[c] (57.7±2.2)	16.7 (25.0)
Acetic acid+ Sulphasalazine	100	1.33 ±0.11[c] (23.0±1.9)	159.1±5.17[c] (60.9±2.0)	16.7 (25.0)

Results are mean±SEM (n=6). Values in parenthesis indicate percent of respective AA value.
*P<0.001 compared to NS group (Unpaired't' test) and [b] P<0.01, [c] P<0.001 compared to respective AA group (one way analysis of variance followed by Dunnett's test).

Table 2.
Effect of *A. indica* extract and sulphasalazine on AA-induced changes in antioxidants (superoxide dismutase, SOD; catalase, CAT and glutathione, GSH) in rat colonic mucosa

Oral treatment (mg/kg, od x 14 days)		Anti-oxidants		
		SOD (mU/mg protein)	CAT (mU/mg protein)	GSH (nmol/mg protein)
Normal Saline (1% CMC)		161.8±23.3	2.49±0.25	12.7±0.66
Acetic acid (1% CMC)		24.8±3.82*	0.96±0.15*	7.18±0.63*
Acetic acid+ *A. indica* extract	500	183.1±13.7[c]	2.49±0.26[c]	10.6±0.37[c]
Acetic acid+ Sulphasalazine	100	294.3±37.9[c]	2.36±0.24[c]	11.8±1.13[b]

Results are mean±SEM (n=6). *P<0.001 compared to respective NS group (Unpaired 't' test) and [b] P<0.01, [c] P<0.001 compared to respective AA group (one way ANOVA followed by Dunnett's test).

Table 3.
Effect of *Azadirachta indica* extract and sulphasalazine on acetic acid-induced changes in free radicals (lipid peroxidation, LPO and nitric oxide, NO) and pro-inflammatory marker, myeloperoxidase (MPO) in rat colonic mucosa

Oral treatment(mg/kg, od x 14 days)		Free Radicals		Myeloperoxidase MPO(mU/mg protein)
		LPO(nmol/mg protein)	NO(nmol/mg protein)	
Normal Saline(1% CMC)		6.26±0.31	4.91±0.61	6.23±1.18
Acetic acid (1% CMC)		10.98±0.77*	9.95±0.60*	74.0±3.55*
Acetic acid+ *A. indica* extract	500	6.17±0.42[c]	7.57±0.63[c]	23.7±2.90[c]
Acetic acid+ Sulphasalazine	100	5.11±0.56[c]	3.54±0.30[c]	8.04±1.63[b]

Results are mean±SEM (n=6). *P<0.001 compared to respective NS group (Unpaired 't' test) and [b] P<0.01, [c] P<0.001 compared to respective AA group (one way ANOVA followed by Dunnett's test).

Table 4.
Antibacterial activity and minimum inhibitory concentration (MIC) of *A. indica* extract

Name of organism	*Azadirachta indica* extract Antibacterial activity (Zone of inhibition in mm)				MIC(mg/ml)
	(50 mg/ml)	(100 mg/ml)	(150 mg/ml)	(200 mg/ml)	
E. coli ATCC 25922	8±0.94	9±0.47	10±0.47	11±0.47	12.5
Shigella sonnie	7±0.47	9±0.81	10±0.94	10±1.24	25
S. boydii	8±0.47	8±0.47	9±0.47	10±0.81	12.5
S. flexneri	9±0.81	9±1.41	10±1.63	11±0.81	25

Values are mean±SEM (n=3).

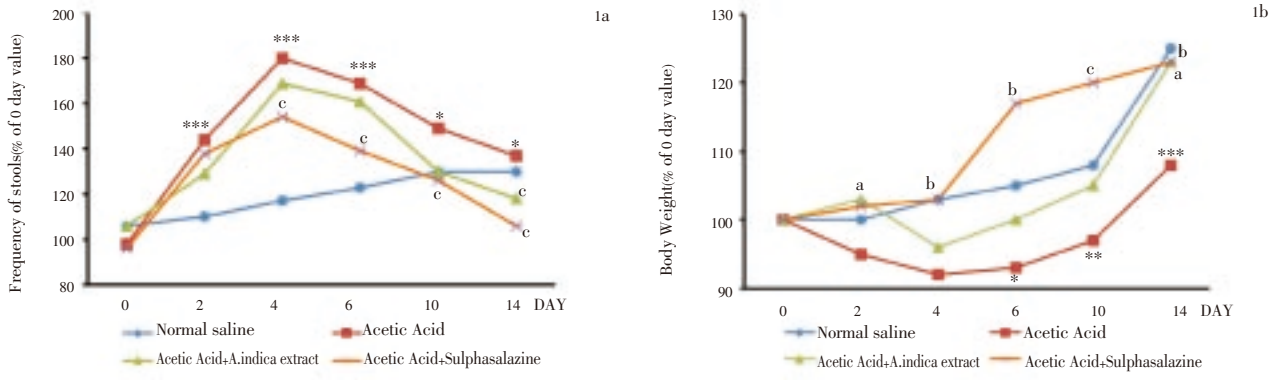
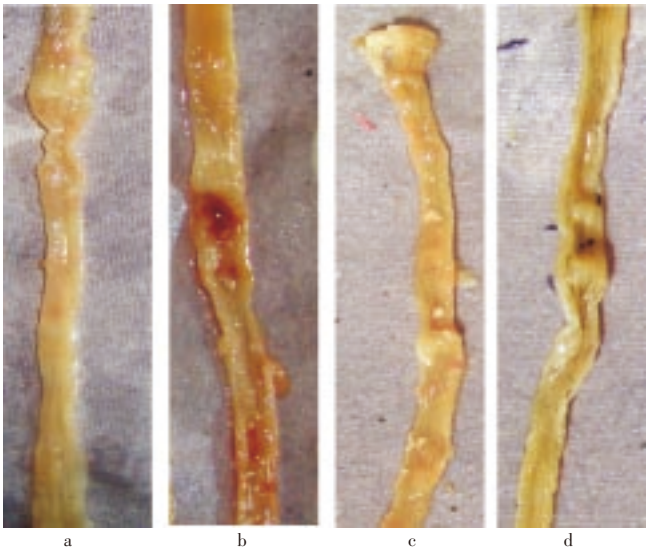


Figure 1. 1a and 1b show changes in the frequency of stool and body weight in *Azadirachta indica* extract- and sulphasalazine-treated acetic acid-induced colitis rats. *P<0.05, **P<0.01, ***P<0.001 compared to NS group and [a] P<0.05, [b] P<0.01, [c] P<0.001 compared to AA group.

any colonic mucosal damage or adhesions at 15th day of experiment while, the colonic weight (8 cm of proximal

colon) expressed as mg/cm of colon was found to be 171.0 ±4.8 mg/cm. The acetic acid group rats received 0.5% CMC orally daily as above but were treated with acetic acid intra-rectally led to significant increase in colonic mucosal damage score (5.78±0.22, $P<0.001$) and adhesions (67.7%) and increase in colonic weight to 52.7% (261.2±6.79 mg/cm) compared with NS value (171.0±4.8 mg/cm) indicating an extensive colonic tissue damage, adhesions, inflammation together with oedema. *Azadirachta indica* extract when given in graded doses of 250, 500 and 1000 mg/kg for 14 days, once daily, orally as suspension in 0.5% CMC. *Azadirachta indica* extract showed dose-dependent decrease in damage score from 25.1% to 74.1% ($P<0.01$ to $P<0.001$), colonic weight from 20.7% to 42.3% ($P<0.01$ to $P<0.001$) and tissue adhesions from 50.0% to 75.0% (Table 1). 500 mg/kg of *Azadirachta indica* extract showed optimal protective effects against acetic acid-induced changes on the colonic mucosal weight, damage score and adhesions and its effects were compared with Sulfasalazine (SS, 100 mg/kg), a known drug for treatment of ulcerative colitis (positive control) (Table 1).



a b c d

Figure 2. Rat colonic mucosa (Macroscopic): a: Normal saline enema-treated colon with oral CMC treatment showing normal morphology. b-d: Colon of acetic acid-induced colitis b: rat treated with oral CMC showing necrosis, erosion, hydropsia and ulceration and c and d: rats treated with *Azadirachta indica* extract and sulphasalazine respectively showing reduction in ulceration, hydropsia, erosion and necrosis.

3.4. Histology study

3.4.1. Macroscopic study

The picture in Figure 2a shows the features of colon of orally treated CMC rats after NS enema. The picture in Figure 2b: colon of intra-colonic acetic acid-treated rats on oral CMC treatment showing significant hydropsia, necrosis, erosion and ulceration. The pictures in Figures 2c and d show the colons of acetic acid-induced colitis treated orally with *Azadirachta indica* extract and SS respectively. The severity of hydropsia, necrosis and ulceration were significantly reduced by them. The results of *Azadirachta indica* extract-treated rats were comparable with that of SS-treated rats.

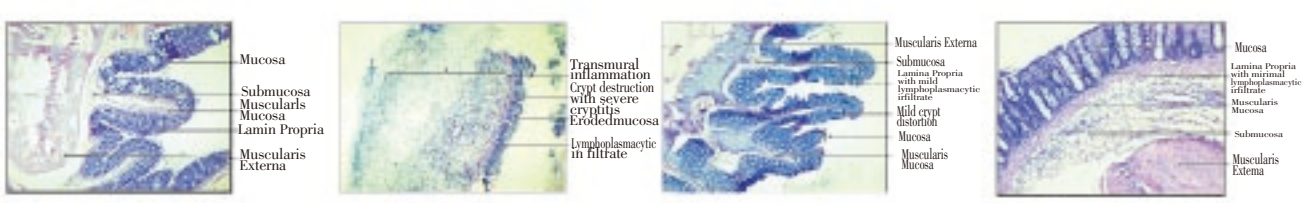
3.4.2. Microscopic study

The photomicrographs of colon shown in Figures 3a-d provided convincing evidence for the protective effects of *Azadirachta indica* extract and SS on colitis induced by acetic acid in the rats. The pictures in Figure 3a showed the morphology of colon of NS enema treated colon of rats treated orally with 0.5% CMC. The structure was relatively normal and clear with intact epithelia and sub mucosa. Figure 3b showed the photomicrographs of the acetic acid-treated colon rat treated orally with 0.5% CMC. There were crypt destruction with severe cryptitis, complete mucosal erosion and transmural inflammation (predominantly-lymphocytes and plasma cells). Figure 3c showed the regenerative mucosa with mild crypt distortion and mild lympho-plasmacytic infiltrate in the lamina propria and 3d: showed intact mucosa with minimal lympho-plasmacytic infiltrate in the lamina propria.

3.5. Effects on colonic mucosal antioxidant, free radicals and proinflammatory, myeloperoxidase status

3.5.1 Effect on antioxidants

Acetic acid-treated animals showed significant decrease in SOD, CAT and GSH levels in the colonic mucosal incubates when expressed either as mU (SOD & CAT) or nmol (GSH) per mg protein compared to NS-treated rats. Both *Azadirachta indica* extract and SS when given for 14 days after acetic acid-induction of colitis reversed the above changes in SOD, CAT and GSH levels near to NS group (Table 2).



3a - NS + CMC 3b - Acetic acid+CMC 3c - Acetic acid+ *A. indica* extract 3d - Acetic acid+Sulphasalazine

Figure 3. Photomicrographs of the rat colon stained with H&E stain (x 100). 3a: shows the photomicrographs of saline enema (NS) treated colon of rats with oral CMC. The structure is relatively normal and clear with intact mucosa and sub mucosa. 3b-d: photomicrographs of colon of acetic acid-induced colitis 3b: rat treated with oral CMC shows crypt destruction with severe cryptitis, complete mucosal erosion and transmural inflammation (predominantly-lymphocytes and plasma cells). 3c: rat treated with *Azadirachta indica* extract orally shows regenerative mucosa with mild crypt distortion and mild lympho-plasmacytic infiltrate in the lamina propria and 3d: sulphasalazine oral treated rat shows intact mucosa with minimal lympho-plasmacytic infiltrate in the lamina propria.

3.5.2. Effects on free radicals

Acetic acid enhanced both LPO and NO expressed as nmol/ mg protein compared to NS rats. *Azadirachta indica* extract and SS showed reversal of levels of both LPO and NO near to NS rats. The effect on free radicals by *Azadirachta indica* extract was comparable with SS (Table 3).

3.5.3. Effect on Inflammatory marker, MPO

Acetic acid-treated animals showed significant increase in MPO level in the colonic mucosal incubates when expressed as mU/ mg protein compared to NS rats. *Azadirachta indica* extract and SS when given for 14 days after acetic acid-induction of colitis reversed the above changes in MPO level near to NS group (Table 3).

3.6. Antimicrobial susceptibility and MIC

Azadirachta indica extract showed positive susceptibility test against *Escherichia coli* ATCC 25922, *Shigella boydii*, *Shigella sonnei* and *Shigella flexneri* at 200 mg/ml against all the above gram negative intestinal bacteria showing zone of inhibition $\geqslant$10 mm. MIC value against the above intestinal microorganism ranged from 12.5- 25.0 mg/ml, where the MIC value against E. coli and S. boydii was 12.5 mg/ml and 25.0 mg/ml against S. sonnie and S. flexneri (Table 4).

3.7. Acute toxicity study

Azadirachta indica extract even at 5 g/kg oral dose did not show any acute toxicity manifestation like increased motor activity, salivation, colonic convulsion, coma and death, observed up to a period of 1 week.

4. Discussion

A. indica has been mentioned in traditional system of medicine to be effective in colitis[6] though detailed pharmacological actions related to use in colitis has not been mentioned earlier. The present study was particularly focused on studying the effects of *Azadirachta indica* extract on acetic acid-induced experimental colitis, which is considered a validated model to and drugs potentially active in this disease. Acetic acid-induced colitis is an easily inducible model of IBD and the inflammatory phase bears some resemblance to acute human intestinal inflammation[9]. Our results with intra-colonic administration of acetic acid indicated significant increase in colonic mucosal damage and adhesions (indicative of necrosis and ulcerations) and an increase in colonic weight (indicative of inflammation and hydropsia). The colons of rats treated with *Azadirachta indica* extract showed decrease in the severity of hydropsia in terms of decrease in colonic weight, necrosis and ulceration (decrease in damage score and adhesions) enhanced in acetic acid-induced colitis. Rats showed increase in diarrhea with mucous and blood present in the early phase and increased fecal frequency in the later phase after induction of colitis with acetic acid. This could be due to direct damaging effects of acetic acid as well as alterations in epithelial function produced, either directly or indirectly by products released from activated mast cells[14]. We also found loss of body weight without any significant change in the food and water intake which could be due to alterations in the GIT absorptive functions. Both *Azadirachta indica* extract and SS decreased mucous and bloody diarrhea, and stool frequency but enhanced body weight due to restoration of epithelial functions. Microscopic study of the acetic acid-treated colon indicated extensive epithelial necrosis and edema with crypt destruction, severe cryptitis, complete mucosal erosion and transmural inflammation (predominantly-lymphocytes and plasma cells) while, rats treated with intracolonic normal saline (NS) enema showed intact mucosa and sub mucosa. Treatment with *Azadirachta indica* extract showed regenerative mucosa with mild crypt distortion and mild lympho-plasmacytic infiltrate in the lamina propria and its effect was comparable with SS-treated group. These above effects may be attributed to the anti-inflammatory, immunomodulatory and antiulcer properties of *Azadirachta indica* [6].

Inflamed gut from IBD patients (as well as IBD animal models), are rich in activated macrophages and neutrophils and these inflammatory cells generate excess amounts of ROS with subsequent increases in oxidative stress[15]. These ROS include hydroxyl radicals, superoxide anions, hydrogen peroxide, and nitric oxide. ROS are extremely unstable species due to their high reactivity and may result in excessive generations of free radicals like lipid peroxidation and NO and the oxidation of DNA and proteins[16]. Cellular antioxidants like SOD and NO are protective against the free radicals which are deleterious for the tissue milieu. This antioxidant system was deranged after treatment with acetic acid as found in the colonic mucosal homogenates. Acetic acid-induced colonic injury leads to activation of arachidonic acid pathways leading to production of inflammatory mediators and formation of free radical or ROS. Free radicals and MPO are good markers of the resultant damaging effect and accomplishment of near normal levels of free radicals and reduction in MPO levels is suggestive of significant improvement paramount for attainment of normal homeostasis and tissue healing. Our study demonstrated that *Azadirachta indica* extract treatment ameliorated colonic lesions and histological signs of damage, neutrophils in ltration and decreased myeloperoxidase (acute inflammatory marker), free radicals (LPO and NO) responsible for tissue damage and inflammation and up-regulation of protective antioxidants (CAT, SOD and GSH) as well as improvement in physical parameters when compared with acetic acid group. AI contains important bioactive compounds such as phytosterols (sitosterols, sigmasterol, and campasterol) and flavonoids (Rutin and quercetin)[17]. Flavonoids are most commonly known for their antioxidant activity and are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity.

Some studies have suggested the role of microbial content of the GI tract in the pathogenesis of IBD and it has been shown that the concentration of intestinal bacteria in IBD is higher than normal and increases progressively with disease severity[18]. The complex enteric immune system

plays an important role in interactions between microbial antigens and immune-competent cells. *Azadirachta indica* extract exhibited considerable level of inhibition against the intestinal organisms compared to standard drug. This could be due to the presence of some compounds or groups in the extract with similar mechanism of action to that of standard drug used. The highest activity exhibited by *Azadirachta indica* extract at 200 mg/ml concentration was ⩾10 mm, which have been considered as active dose[12] and this could be due certain phytochemicals including sterols and flavonoids. This could be contributory factors in helping healing of colitis induced by acetic acid in our present study. *Azadirachta indica* extract was also found to have no acute toxicity even with 10 times of the optimal effective dose administered to mice indicating its safety on use.

Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1α (IL-1α), and interleukin-6 (IL-6) signi cantly increased in the colonic mucosa in UC. In particular, TNF-α which is induced, synthesized and secreted from macrophages, lymphocytes, and polymorphonuclear neutrophils, is regarded as the most prominent " first-line" cytokines[19]. Thus the role of AI in experimental colitis vis a vis cytokines will be carried out in future to ascertain the effect of AI if any on cytokines in colitis model of rats. The results of present study with 50% ethanolic extract of AI on various physical and biochemical parameters of colonic damage and inflammation do indicate the effective healing effects in acetic acid-induced colitis.

Conflict of interest statement

We declare that we have no conflict of interest.

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