OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2016.170.181

Research Article

Ameliorative Effect of Alkaloidal Fraction of Leaves of *Alstonia scholaris* Against Acetic Acid Induced Colitis via Modulation of Oxido-nitrosative and Pro-inflammatory Cytokines

Amit D. Kandhare, Mithun V.K. Patil and Subhash L. Bodhankar

Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, 411038, India

Abstract

Background: Inflammatory Bowel Disease (IBD) is a chronic inflammatory, an idiopathic disorder of intestine with unknown etiology. *Alstonia scholaris* Linn., R.Br. (family Apocynaceae) shown to possesses potent antioxidant and anti-inflammatory activity. **Objective:** To evaluate the effect of an alkaloidal fraction of leaves of *Alstonia scholaris* (AFEAS) against acetic acid induced IBD in laboratory rats. **Materials and Methods:** Colitis was induced in male Wistar rats (180-220 g) by intrarectal administration of acetic acid (2 mL, 4% (v/v)). Rats were either treated orally with AFEAS (20, 40 and 80 mg kg^{-1}) or distilled water (10 mg kg^{-1}) or prednisolone (2 mg kg^{-1}). Various biochemical, molecular and histological parameter were assessed. **Results:** Intrarectal administration of 4% acetic acid resulted in significant modulation ($p<0.05$) of serum alkaline phosphatase, lactate dehydrogenase, SOD, GSH, MDA and MPO content along with colonic NO, XO level and protein carbonyl content in the colonic tissue as well as in blood. The AFEAS (40 and 80 mg kg^{-1}) administration significantly ($p<0.05$) ameliorated these acetic acid induced alteration in serum and colonic biochemical parameters. The decreased level of leptin and increased the level of pro-inflammatory cytokines (TNF-α and IL-1β) after acetic acid administration were significantly inhibited by AFEAS (40 and 80 mg kg^{-1}) treatment. The AFEAS treatment reduced histological insult induced in the colon after intrarectal instillation of acetic acid. **Conclusion:** Treatment of AFEAS ameliorates acetic acid induced colitis by virtue of its anti-inflammatory and anti-oxidant potential via inhibition of production oxido-inflammatory mediator and pro-inflammatory cytokines.

Key words: Acetic acid, *Alstonia scholaris*, inflammatory bowel disease, myeloperoxidase, oxidative stress, protein carbonyl content, vascular permeability, xanthine oxidase, pro-inflammatory cytokines

Received: February 28, 2016 Accepted: March 15, 2016 Published: April 15, 2016

Citation: Amit D. Kandhare, Mithun V.K. Patil and Subhash L. Bodhankar, 2016. Ameliorative effect of alkaloidal fraction of leaves of *Alstonia scholaris* against acetic acid induced colitis via modulation of oxido-nitrosative and pro-inflammatory cytokines. Pharmacologia, 7: 170-181.

Corresponding Author: Subhash L. Bodhankar, Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune-411038 Maharashtra, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inflammatory Bowel Disease (IBD) is comprehended Crohn's disease and ulcerative colitis[1]. It's a large group of circumstances, which mainly involves chronic inflammation of mucosal and sub-mucosal layers of the large intestine and rectum. Hepatic, ocular, articular and cutaneous, which are the extra-intestinal manifestations of ulcerative colitis are frequent and appears in almost 45% patient[2]. The culminating factor of IBD includes bated cellular antioxidant capacity, ensuring inconspicuous colonic inflammation due to the activated oxygen species and concomitant lipid peroxidation[3-5]. The incommensurable inflammation plays an imperative role along with the oxidative stress in the pathogenesis of ulcerative colitis disease. Generation of free radical and remittent antioxidant capability are the key marker of inflammatory bowel disease though the cause of ulcerative colitis is not revealed completely[6]. Excessive ROS production results in oxidative stress in the cell from IBD patient thus the lack of balance among the innate and exogenous antioxidant and ROS may result in DNA damage[7,8]. It has been well reported that release of pro-inflammatory cytokines such as IL-6 and IL-1β, which have the potency to triggers the pathological responses in IBD[9].

The present treatment regimen to treat a patient suffering from IBD includes drugs like antibiotics, steroids and immunosuppressant. However, these treatments are associated with side effects like nausea, anorexia, cytopenia myalgia and malfunction of the kidney, liver and lungs are remains a major problem. Acetic acid induced IBD is widely used, reproducible and well-established animal model for screening of various therapeutic moieties against ulcerative colitis[10,11]. It also mimics most of the clinicopathological feature that is present in human.

Alstonia scholaris Linn., R.Br. (family Apocynaceae) the plant grows throughout India. It has an array of pharmacological activity including wound healing, anti-arthritic[12], anti-cancer, hepatoprotective[13], anti-hyperglycaemic[14], neuroprotective[15], anti-malaria, antiulcer, anti-asthmatic[16] and anti-hypertensive[17], etc. It was also shown to possesses potent anti-oxidant activity[13]. However, its potential against inflammatory bowel disease has not been yet evaluated. Thus, the objective of the study was to determine the potential of an alkaloidal fraction of leaves of *Alstonia scholaris* (AFEAS) in acetic acid induced IBD model in laboratory rats.

MATERIALS AND METHODS

Animals: Male Swiss albino mice (20-30 g) and male Wistar rats (180-220 g) were obtained from the National Institute of Biological Sciences, Pune (India). The animals were housed in groups of 6 in solid bottom polypropylene cages. They were maintained at 24±1°C with a relative humidity of 45-55% and 12:12 h dark/light cycle. The animals were acclimatized for 2 weeks and were kept under pathogen-free conditions. The animals had free access to standard pellet chow (Pranav Agro-industries Ltd., Sangli, India) throughout the experimental protocol, except overnight fasting before induction of the ulcer. The experimental protocol (CPCSEA/62/2014) was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA).

Chemicals: Acetic acid, anesthetic ether, ethanol, formalin, hexadecyltrimethylammonium bromide, O-dianisidine dihydrochloride, hydrogen peroxide, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, carbon tetrachloride, chloroform, ether, hydrochloric acid and concentrated sulphuric acid was purchased from S.D. Fine Chemicals, Mumbai, India. The TNF-α, IL-1β and leptin enzyme-linked immunosorbent assay (ELISA) kit were obtained from Ray Biotech Inc., USA.

Preparation of alkaloidal fraction of *Alstonia scholaris* from ethanolic extract of leaves (AFEAS): One kilogram of dried, coarsely powdered leaves of *Alstonia scholaris* was macerated in 75% ethanol and 25% water for one week with occasional shaking/stirring. The macerate was filtered and the marc was further macerated with 75% ethanol and 25% water for 1 week. This was repeated twice. The obtained extract was pooled and concentrated under reduce pressure to give a hydroalcoholic extract (80 g). Forty grams of hydroalcoholic extract was dissolved in 200 mL of 1% hydrochloric acid. This solution was dissolved in equal volume of 200 mL ammonia water (1:1) maintaining its pH (9-10). The basic solution was partitioned with 400 mL ethyl acetate in separating funnel and collected ethyl acetate fraction which was concentrated under reduced pressure to afford alkaloidal fraction (11 g).

Preliminary phytochemical investigation of AFEAS: The alkaloidal fraction was subjected to qualitative chemical investigation to test for the presence of various

phytochemicals in the extract[18,19]. A test solution of AFEAS was prepared in ethanol with the concentration of 25 mg mL^{-1}.

Acute toxicity testing: Acute Oral Toxicity (AOT) in Swiss albino mice was performed according to OECD guidelines using AOT 425 software[20]. Graded doses of the alkoloidal fraction of *Alstonia scholaris* were dissolved in distilled water were administered orally and the animals were observed for 2 weeks following administration. Body weight, food consumption, fluid intake and psycho-motor activities were recorded daily.

Dosages of AFEAS and standard drugs used: Freshly prepared aqueous solution of AFEAS in the three different dosages (20, 40 and 80 mg kg^{-1}) were administered to animals orally for 7 days. On 8th day, the disease was induced by acetic acid. The drug treatment was continued even after administration of acetic acid. Standard drug used for comparison was prednisolone. Prednisolone was not given as pre-treatment. It was given on the day of acetic acid administration at a dose of 2 mg kg^{-1} day^{-1} orally.

Induction of colitis and drug treatment schedule: Colitis was induced according to the previously described procedure[21,22]. Rats were randomly divided into following groups of 6 rats as follows:

- **Group 1: Normal animals (N):** Received 1 mL of distilled water (10 mg kg^{-1}, p.o.) for 12 days
- **Group 2: Acetic acid control animals (AA Control):** Received 2 mL of 4% acetic acid solution (once, intrarectally) and distilled water (10 mg kg^{-1}, p.o.) for 12 days
- **Group 3: Prednisolone (2 mg kg^{-1}) treated animals (Pred 2):** Received prednisolone (2 mg kg^{-1}, p.o., for 5 days) and acetic acid (2 mL of 4% solution, once, intrarectally). Prednisolone and acetic acid treatment were started on the same day
- **Group 4: AFEAS (20 mg kg^{-1}) treated animals (AFEAS 20):** Received 7 days pretreatment with AFEAS (20 mg kg^{-1}, p.o.) and 2 mL of 4% acetic acid solution, intrarectally on the 8th day. Drug treatment was continued until 12th day
- **Group 5: AFEAS (40 mg kg^{-1}) treated animals (AFEAS 40):** Received 7 days pretreatment with AFEAS (40 mg kg^{-1}, p.o.) and 2 mL of 4% acetic acid solution, intrarectally on the 8th day. Drug treatment was continued until 12th day
- **Group 6: AFEAS (80 mg kg^{-1}) treated animals (AFEAS (80):** Received 7 days pretreatment with AFEAS (80 mg kg^{-1}, p.o.) and 2 mL of 4% acetic acid solution, intrarectally on the 8th day. Drug treatment was continued until 12th day

The AFEAS was administered to rats in the three different dosages (20, 40 and 80 mg kg^{-1}) for 12 days. On the 13th day, rats were sequentially anesthetized with anesthetic ether for about 30-40 sec. Blood was withdrawn by retro-orbital puncture. Each blood sample was collected into separate vials for determination of serum parameters. After blood collection, the animals were sacrificed by cervical dislocation, the colon was excised and frozen immediately in liquid nitrogen and stored at -80°C for further biochemical and histopathological examination.

Assessment of colonic damage, ulcer area and ulcer index: The severity of colitis was evaluated by an independent observer who was blinded to the treatment. For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, slightly cleaned in physiological saline to remove fecal residues and weighed. Macroscopic inflammation scores were assigned based on clinical features of the colon. The presence of adhesions (score 0-10) and stool consistency (score 0-4) were evaluated according to the previously reported method[21,22]. The evaluation of ulcer area and ulcer index were performed according to the previously reported method[23,24].

Determination of serum parameters: Serum activities of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were measured using ultraviolet-visible spectrophotometer (JASCO-V-530, JASCO Corp., Tokyo, Japan) and commercially available reagent kits (Accurex Biomedical Pvt. Ltd. and Pathozyme Diagnostics, India).

Biochemical assays: For colon homogenization, tissue segments were mixed with 0.1 M phosphate buffer and homogenized in ice for 60 sec at 10000 rpm in a homogenizer (Remi Equipment Pvt. Ltd., Remi Motors Ltd., Mumbai, India). Supernatant of tissue homogenates were employed to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA content) and nitrite/nitrate (NO) levels as previously described[25-31]. The colonic myeloperoxidase (MPO) assay was determined according to the previously described method[32-37]. The colonic vascular permeability, xanthine oxidase activity and of protein carbonyl contents were determined according to previously reported methods[22].

Determination of serum leptin, colonic TNF-α and IL-1β levels: Serum leptin, colonic TNF-α and IL-1β were quantified by enzyme-linked immunoabsorbent assay according to instructions provided by manufacturer (Ray Biotech Inc., USA).

Histological examination: Freshly excised colon of one animal from each group was washed with saline and preserved in 10% formaldehyde solution for histopathological studies. Paraffin-embedded tissue sections cut at 5 μm thickness were prepared and stained after deparaffination using hematoxylin and eosin stain (H and E). The various changes in histological features were graded as: grade 0 (not present or very slight), grade 1 (mild), grade 2 (moderate) and grade 3 (severe) as described earlier[38]. Photomicrographs were captured at a magnification of 40 and 100x.

Statistical analysis: All the results were expressed as Mean±SEM. Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, CA). Statistical comparisons were made between drug-treated groups and acetic acid control animals. Data analyzed using one-way ANOVA followed by Tukey's multiple range test. Data of macroscopical score and stool consistency was analyzed using nonparametric Kruskal-wallis ANOVA. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Preliminary phytochemical investigation: The alkaloidal fraction obtained after vacuum drying yielded a solid residue which was greenish brown in color. The yield of the AFEAS was 1.1% w/w. Preliminary phytochemical analysis of AFEAS revealed that it contains alkaloids confirmed by Mayer's test, Wagner's test, Hager's test and Dragendroff's test.

Acute toxicity testing: Acute toxicity studies of the AFEAS shows no signs and symptoms such as restlessness, respiratory distress, diarrhea, convulsions and coma and it was found safe up to 2000 mg kg^{-1}.

Acetic acid-induced colitis

Effect of AFEAS on acetic acid-induced alterations in body weight, colon weight, food intake, water intake, ulcer area and ulcer index: The body weight was decreased significantly (197.52±4.88 g, $p<0.05$) and colon weight was increased (3.84±1.01 g, $p<0.05$) significantly in the acetic acid control group as compared to normal group (230.5±4.78 and 1.78±0.55 g). Administration of AFEAS (40 and 80 mg kg^{-1}, p.o.) significantly increase ($p<0.05$) body weight (220.11±3.68 and 228.48±4.78 g) and significantly decreased the colon weight (2.88±0.85 and 2.60±0.48 g) as compared to acetic acid control group. In the present study, acetic acid induced colitis exhibited significantly decreased ($p<0.05$) in food and water intake in acetic acid control rats (64.60±5.69 and 103.00±5.69 mL) as compared with normal (151.4±3.82 and 180.6±3.77 mL). Administration of AFEAS (40 and 80 mg kg^{-1}, p.o.) significantly inhibited ($p<0.05$) acetic acid induced decreased food intake and water intake (99.70±7.22 and 125.8±7.26 g, 146.3±5.64 and 174.3±6.48 mL, respectively) as compared to acetic acid control group (Table 1).

Rectal administration of 4% acetic acid produced ulcers of the colon of acetic acid control rats. The mean ulcer area of the acetic acid control group was (41.22±2.48 mm^2) showed

Table 1: Effect of AFEAS on acetic acid-induced alterations in body weight, colon weight, food intake, water intake, ulcer area, ulcer index, macroscopic score and stool consistency score of rats

Parameters	Normal	Acetic acid control	Prednisolone (2)	AFEAS (20 mg kg^{-1})	AFEAS (40 mg kg^{-1})	AFEAS (80 mg kg^{-1})
Body weight (g)	230.50±4.78	197.52±4.88#	227.44±3.78*,$	204.63±5.55	220.11±3.68*,$	228.48±4.78*,$
Colon weight (g)	1.78±0.55	3.84±1.01#	2.22±0.48*,$	3.45±0.24	2.88±0.85*,$	2.60±0.48*,$
Food intake (g)	151.40±3.82	64.60±5.69#	131.00±6.21*,$	81.04±9.12	99.70±7.22*	125.80±7.26*,$
Water intake (mL)	180.60±3.77	103.00±5.69#	167.20±5.11*,$	11.20±6.99	146.30±5.64*	174.30±6.48*,$
Ulcer area (mm^2)	0.00±0.00	41.22±2.48#	7.45±0.43*,$	35.11±2.89	24.62±2.78*	10.78±2.55*,$
Ulcer index	0.00±0.00	70.11±1.48#	10.55±1.78*,$	40.12±3.02	28.96±1.11*	15.42±3.02*,$
Inhibition (%)	-	-	88.42±0.00	14.63±0.00	38.96±0.00	80.45±0.00
Macroscopic score	0.00±0.00	7.20±0.11#	1.80±0.37*,$	6.60±0.50	4.20±0.20*	2.80±0.37*,$
Stool consistency score	0.40±0.10	3.60±0.37#	1.00±0.10*,$	3.20±0.10	2.40±0.40*	1.40±0.37*,$

Data are expressed as Mean±SEM (n = 5) and analyzed by one-way ANOVA followed by Tukey's multiple range test, data of macroscopical score and stool consistency was analyzed using nonparametric Kruskal-Wallis ANOVA followed by Tukey's multiple range test, *$p<0.05$ as compared to acetic acid control group, #$p<0.05$ as compared to the normal group as well as sham control group and $$p<0.05$ as compared to one another, AFEAS: Alkaloidal fraction of *Alstonia scholaris* from the ethanolic extract of leaves

significantly high ($p<0.05$) ulcerogenic effect of acetic acid. Administration of AFEAS (40 and 80 mg kg^{-1}, p.o.) significantly decreased the ulcer area (24.62±2.78 and 10.78±2.55 mm^2, $p<0.05$) as compared to acetic acid control group. The mean ulcer index of the acetic acid control group (70.11±1.48) was significantly higher than normal rats. Administration with AFEAS (40 and 80 mg kg^{-1}, p.o.) significantly decreased the ulcer index (28.96±1.11 and 15.42±3.02, $p<0.05$) as compared to acetic acid control group (Table 1).

Effect of AFEAS on acetic acid-induced alterations in macroscopic scores and stool consistency: The colons of the rats were examined macroscopically for signs of hemorrhage and ulceration by an independent blinded observer, unaware of treatment schedule using a previously established scoring system. Colons from acetic acid administered rats showed significantly ($p<0.05$) higher macroscopic score (7.20±0.11) and stool consistency (3.60±0.37) as compared to normal rats. Rats treated with the AFEAS (40 and 80 mg kg^{-1}, p.o.) showed significantly improved macroscopic scores (4.20±0.20 and 2.80±0.37, $p<0.05$) and stool consistency (2.40±0.40 and 1.40±0.37, $p<0.05$) compared with acetic acid control rats. Prednisolone-treated rats (2 mg kg^{-1} day^{-1}) also showed significantly decrease macroscopic score (1.80±0.37, $p<0.05$) and stool consistency (1.00±0.10, $p<0.05$) as compared to acetic acid control rats (Table 1).

Effect of AFEAS on acetic acid-induced alterations in serum LDH, colonic nitrite level, colonic xanthine oxidase and colonic protein carbonyl content: Table 2 summarizes the effect of AFEAS on serum LDH, colonic nitrite level, colonic xanthine oxidase and colonic protein carbonyl content of acetic acid treated animals. The acetic acid control rats showed a significantly increased ($p<0.05$) serum LDH (187.4±12.96 U L^{-1}), colonic nitrite level (67.96±2.62 µg mg^{-1} of wet tissue), colonic xanthine oxidase (5.81±0.35 U mg^{-1} of protein) and colonic protein carbonyl content (1.77±0.09 nmol mg^{-1} of protein) as compared to normal rats. These increased in serum LDH (124.04±7.73 and 84.45±8.65 U L^{-1}), colonic nitrite level (44.40±3.88 and 32.44±2.53 µg mg^{-1} of wet tissue), colonic xanthine oxidase (3.46±0.35 and 2.21±0.22 U mg^{-1} of protein) and colonic protein carbonyl content (1.29±0.10 and 1.11±0.06 nmol mg^{-1} of protein) were significantly attenuated ($p<0.05$) in the AFEAS treated rats (40 and 80 mg kg^{-1}, p.o., $p<0.05$). Prednisolone (2 mg kg^{-1} day^{-1}) treated rats also showed significantly decrease ($p<0.05$) serum LDH (73.52±7.59 U L^{-1}), colonic nitrite level (30.56±2.00 µg mg^{-1} of wet tissue), colonic xanthine oxidase (1.99±0.18 U mg^{-1} of protein) and colonic protein carbonyl content (1.11±0.11 nmol mg^{-1} of protein) as compared to acetic acid control rats (Table 2).

Effect of AFEAS on acetic acid-induced alterations in colonic SOD and GSH concentrations: Induction of colitis produced a significant decrease ($p<0.05$) in colonic SOD and GSH content as compared with the normal group. The AFEAS (40 and 80 mg kg^{-1}, p.o.) treatment significantly increased ($p<0.05$) SOD and GSH content as compared with acetic acid control group. Prednisolone (2 mg kg^{-1} day^{-1}) treatment also significantly inhibited ($p<0.05$) decreased SOD and GSH induced by acetic acid ($p<0.05$) as compared to acetic acid control rats (Fig. 1a, b).

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test. The *$p<0.05$ as compared to TNBS control group, $^{\#}p<0.05$ as compared to normal group and $^{\$}p<0.05$ as compared to one another. Alkaloidal fraction of *Alstonia scholaris* (AFEAS) from the ethanolic extract of leaves.

Effect of AFEAS on acetic acid-induced alterations in colonic malondialdehyde and MPO concentration: Colonic malondialdehyde concentration and MPO level in the acetic acid control group increased ($p<0.05$) as compared to the normal group. When compared with acetic acid control rats, treatment of rats with AFEAS (40 and 80 mg kg^{-1}, p.o.) produced a significant decrease ($p<0.05$) in lipid peroxides concentration and MPO level ($p<0.05$). Prednisolone

Table 2: Effect of AFEAS on acetic acid-induced alterations in serum LDH, colonic nitrite level, colonic xanthine oxidase and colonic protein carbonyl content of rats

Treatments	Serum LDH levels (U L^{-1})	Colonic nitrite level (Wet tissue µg mg^{-1})	Xanthine oxidase (Protein U mg^{-1})	Protein carbonyl content (Protein nmol mg^{-1})
Normal	31.30±5.54	24.78±0.96	1.04±0.25	0.86±0.06
Acetic acid control	187.40±12.96$^{\#}$	67.96±2.62$^{\#}$	5.81±0.35$^{\#}$	1.77±0.09$^{\#}$
Prednisolone (2)	73.52±7.59*,$^{\$}$	30.56±2.00*,$^{\$}$	1.99±0.18*,$^{\$}$	1.11±0.11*,$^{\$}$
AFEAS (20 mg kg^{-1})	149.46±8.54	60.66±3.64	5.14±0.42	1.72±0.07
AFEAS (40 mg kg^{-1})	124.04±7.73*	44.40±3.88*	3.46±0.35*	1.29±0.10*
AFEAS (80 mg kg^{-1})	84.45±8.65*,$^{\$}$	32.44±2.53*,$^{\$}$	2.21±0.22*,$^{\$}$	1.11±0.06*,$^{\$}$

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test, *$p<0.05$ as compared to TNBS control group, $^{\#}p<0.05$ as compared to normal group and $^{\$}p<0.05$ as compared to one another, AFEAS: Alkaloidal fraction of *Alstonia scholaris* from the ethanolic extract of leaves

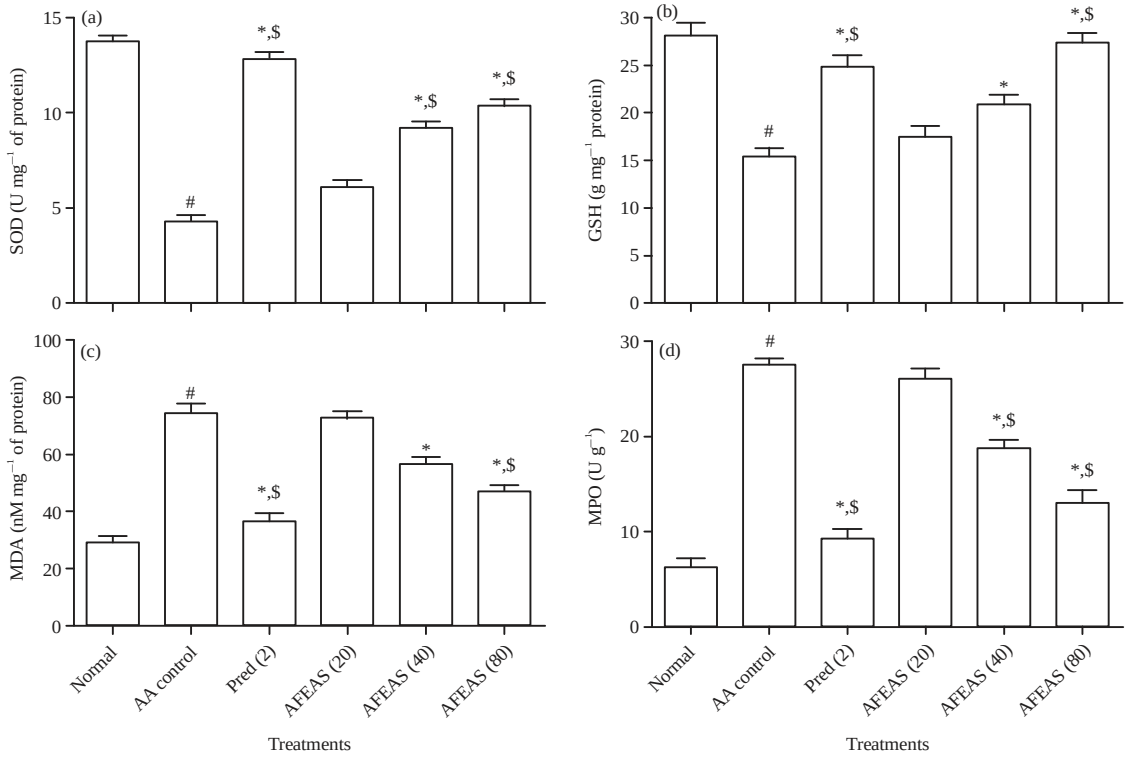

Fig. 1(a-d): Effect of AFEAS on acetic acid-induced alterations in colonic (a) SOD, (b) GSH, (c) MAD and (d) MPO levels of rats

(2 mg kg^{-1} day^{-1}) treatment also showed significant decreased ($p<0.05$) in MDA and MPO levels as compared to acetic acid control rats (Fig. 1c, d).

Effect of AFEAS on acetic acid-induced alterations in colonic vascular permeability: In acetic acid control rats, the level of evans blue was significantly elevated ($p<0.05$) as compared to normal rats. The AFEAS (40 and 80 mg kg^{-1}, p.o.) treated rats showed significant inhibition ($p<0.05$) of this elevated concentration of Evans blue in the colon as compared to acetic acid control rats. Treatment with prednisolone (2 mg kg^{-1} day^{-1}) also significantly attenuated ($p<0.05$) this elevated levels of evans blue as compared to acetic acid control rats (Fig. 2a).

Effect of AFEAS on acetic acid-induced alterations in serum leptin concentrations: The acetic acid colitis was associated with significantly decreased ($p<0.05$) level of serum leptin as compared to normal rats. In the groups treated with AFEAS (80 mg kg^{-1}, p.o.), the serum leptin level was significantly increased ($p<0.05$) as compared with acetic acid control group. Treatment with prednisolone (2 mg kg^{-1} day^{-1}) also significantly increased ($p<0.05$) this serum leptin level as compared to acetic acid control rats (Fig. 2b).

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test. The *$p<0.05$ as compared to TNBS control group, $^{\#}p<0.05$ as compared to normal group and $^{\$}p<0.05$ as compared to one another. Alkaloidal fraction of *Alstonia scholaris* (AFEAS) from the ethanolic extract of leaves.

Effect of AFEAS on acetic acid-induced alterations in colonic TNF-α and IL-1β concentration: Colonic TNF-α and IL-1β levels in the acetic acid control group were significantly increased ($p<0.05$) as compared to the normal group. This elevated levels of TNF-α and IL-1β were significantly attenuated ($p<0.05$) by treatment with AFEAS (40 and 80 mg kg^{-1}, p.o.) as compared to acetic acid-induced colitis group. Prednisolone (2 mg kg^{-1} day^{-1}) treatment also showed significant decreased ($p<0.05$) in colonic TNF-α and IL-1β levels as compared to acetic acid control rats (Fig. 2c, d).

Effect of AFEAS on acetic acid-induced alterations in colon histopathology: Colon tissue from normal rats depicted no inflammatory cells in the lamina propria and the epithelium remained intact. However, it showed presence of mild inflammatory infiltration (Grade 1) and edema (Grade 1) in submucosa (Fig. 3a). The colon tissue from acetic acid control

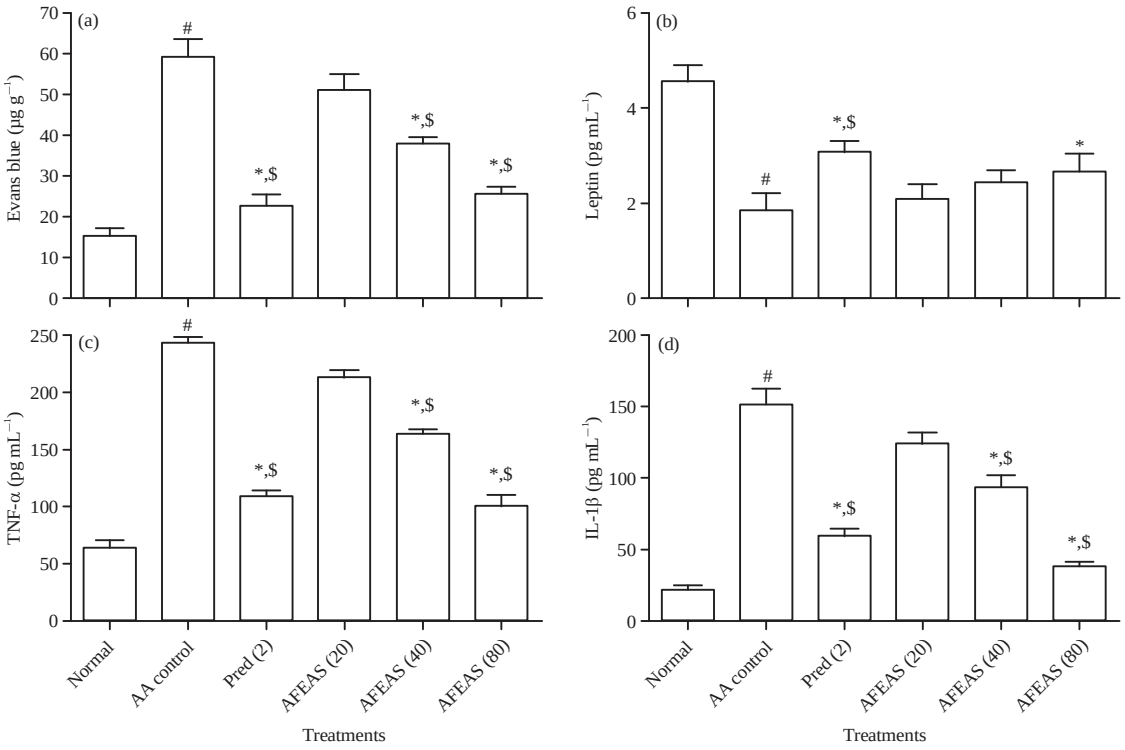

Fig. 2(a-d): Effect of AFEAS on acetic acid-induced alterations in (a) Colonic vascular permeability, (b) Serum leptin, (c) Colonic TNF-α and (d) Colonic IL-1β concentration of rats

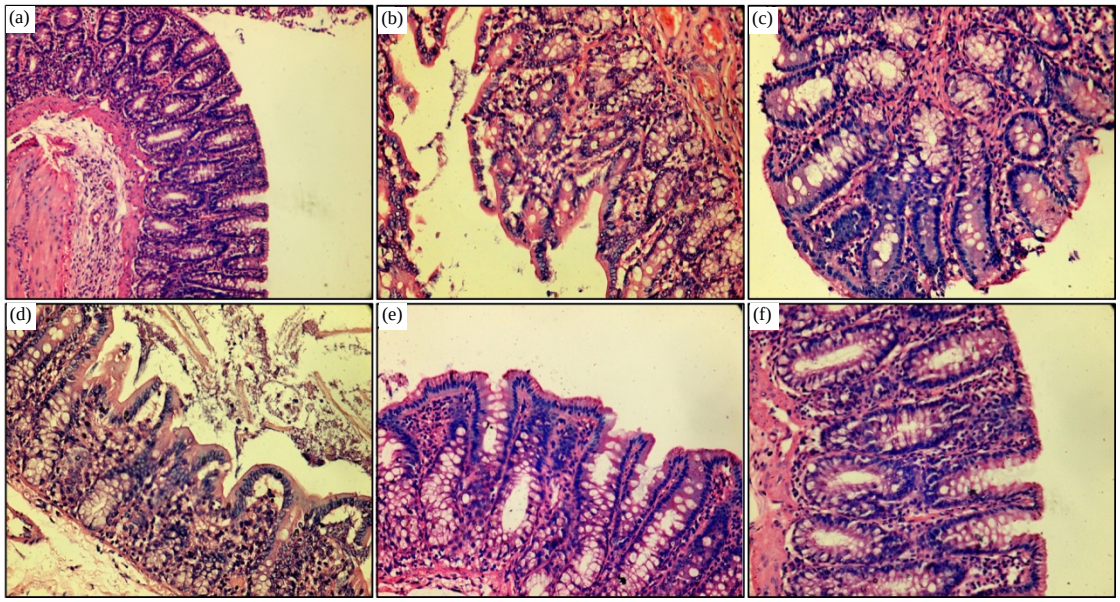

Fig. 3(a-f): Effect of AFEAS on acetic acid-induced alterations in on acetic acid-induced alterations in colon histopathology, photomicrographs of colon sections image of (a) Normal rat, (b) Acetic acid control rat, (c) Prednisolone (2 mg kg^{-1}) treated rat, (d) AFEAS (20 mg kg^{-1}) treated rat, (e) AFEAS (40 mg kg^{-1}) treated rat and (f) AFEAS (80 mg kg^{-1}) treated rat, H and E stain images (at 100x magnifications) are typical representative of each study group, AFEAS: Alkaloidal fraction of *Alstonia scholaris* from the ethanolic extract of leaves

Table 3: Effect of AFEAS on acetic acid-induced alterations in pathological changes of rat colon

	Mucosal epithelium	Crypts	Lamina propria		Submucosa		
Treatments	Ulceration	Mucus depletion	Mononuclear infiltrate	Granulocyte infiltrate	Mononuclear infiltrate	Granulocyte infiltrate	Edema
Normal	0	0	0	0	+	+	+
Acetic acid control	+++	+++	+++	+++	+++	+++	+++
Prednisolone (2)	+	0	+	+	+	+	0
AFEAS (20 mg kg^{-1})	+++	+++	++	++	+++	+++	+++
AFEAS (40 mg kg^{-1})	+++	++	++	++	++	++	++
AFEAS (80 mg kg^{-1})	+	+	0	+	+	++	+

0: None, +: Mild, ++: Moderate, +++: Severe and AFEAS: Alkaloidal fraction of *Alstonia scholaris* from the ethanolic extract of leaves

rats showed histopathological features including edema in the submucosa, cellular infiltration, hemorrhages, necrosis, congested capillaries in lamina propria, dense inflammation of lymphocytes and complete lost goblet cells (Grade 3) (Fig. 3b). When compared with acetic acid control group, prednisolone (2 mg kg^{-1} day^{-1}) treatment also showed a decrease in ulceration, hyperemia, necrosis, edema, cellular infiltration, hemorrhages and goblet cell hyperplasia (Grade 1) (Fig. 3c). However, administration of AFEAS (20 mg kg^{-1}, p.o.) did not show any protection against acetic acid induced colonic damage (Fig. 3d). Colon tissue from AFEAS (40 and 80 mg kg^{-1}, p.o.) treated rats showed attenuated extent and severity of the histological signs of cell damage (Grade 1) induced by acetic acid. However, still it showed moderate inflammatory infiltration (Grade 2) in the submucosa (Fig. 3e, f and Table 3).

DISCUSSION

Although, the pathogenesis of IBD is yet not clear, however, literature is punctuated with evidence that uncontrolled immunological processes and Reactive Oxygen Species (ROS) are responsible to disturbed the normal structure of the tissue and cause the tissue injury in IBD[21]. The failure of the defense mechanism of the antioxidant is likely to be responsible as a causative factor in IBD. The tissue injury is also correlated with the rate of production of the toxic oxidant and the capability of the antioxidant defense mechanism. Acetic acid induced IBD model is well-established and reproducible animal model of ulcerative colitis as it mimics most of the clinicopathological characters of human IBD[10]. Moreover, it also resembles the histopathological appearance that exhibited in the clinical pathophysiology of the ulcerative colitis.

In the present investigation, intrarectal administration acetic acid caused epithelial necrosis, edema which that extended into lamina propria, submucosa and external muscle layer. It has been reported that inflammation occurred mucosa and submucosal layer associated with the arachidonic acid pathway[1]. The significant increase in the colonic mucosal damage assessed by high macroscopical score indicated induction of necrosis and ulceration by intrarectal instillation of acetic acid. Moreover, the elevated wet weight of colon also indicated the edema and inflammation in the colon. However, administration of AFEAS significantly decreased colon weight, ulcer area and macroscopic scores, which may be due to its anti-inflammatory potential. The fecal matter of the rat treated with acetic acid contained mucous and blood in the early phase and the later phase the frequency of the fecal is increased with the blood and mucous. Whereas, administration of AFEAS significantly decreased stool consistency score reflected by decreased in the mucous and bloody stool.

Lactate dehydrogenase is an enzyme which plays a vital role in energy metabolism and it present in the cell cytoplasm[22,39]. Activation of this enzyme leads to cellular damage (schreiber s). Acetic acid treatment induces epithelial damage which was confirmed by increased in the LDH level[22]. This overproduction of LDH enzyme is counteracted by treatment with AFEAS.

Oxidative stress played a key role in the pathogenesis of ulcerative colitis[40]. The SOD plays a crucial role in systemic protection against inflammation[41-44]. By terminating free radicals, it suppresses the lipid peroxidation in colon via conversion of superoxide into peroxide (H_2O_2)[45-47]. Intrarectal administration of acetic acid resulted in mucosal injury in colon tissue which is the result of the inability of oxidative radical scavenging and detractive SOD activity[22]. The result of present investigation is in accordance with findings of previous investigator where detractive SOD activity in the acetic acid control group showing uncontrolled oxidative stress[22]. However, administration of AFEAS increased the SOD activity by virtue of its free radical scavenging potential.

Glutathione is one of the major constituents of intracellular protective mechanisms, which provide protection against various noxious stimuli, including oxidative stress[48-51]. It is the main component of the endogenous protein and it is capable of repairing membrane lipid peroxides by interacting with free radicals to yield more stable elements[52,53]. Reduced GSH is known to be a major low molecular weight scavenger

of free radicals in the cytoplasm, limiting the propagation of free-radical reactions. Glutathione has the ability to repair membrane by interacting with free radicals and capitulating more stable element[54-56]. In the present investigation, acetic acid induced elevated free radical production, where administration of AFEAS decreased this free radical production via increasing activity of GSH.

Malonaldehyde serves as a marker of lipid peroxidation and it interacts with DNA and protein which leads damage of lipid membrane[57-59]. Furthermore, MPO plays a vital role in the formation of reactive oxygen species and oxidation of biological material together with membranous nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[60-62]. The MPO serves as a hallmark of neutrophil infiltration. The result of present investigation showed that treatment with AFEAS attenuates the elevated MDA and MPO level.

The NO is free radical and plays important role as a universal messenger. It is produced by iNOS in the pathological condition[63,64]. NO produces peroxynitrile radical by interacting with superoxide[65,66]. Overproduction of NO leads to direct cytotoxicity, vasodilation and activation of neutrophils this exacerbate the clinicopathological feature of ulcerative colitis. Various authors demonstrate this elevated NO production after intrarectal acetic acid instillation. In present study also, colonic instillation of acetic acid is associated with elevated NO levels. However, AFEAS treatment reduced thus overproduced NO.

The extent of the mucosal damage is dependent upon the vascular permeability of the colonic mucosa[22]. Increased vascular permeability is a rate limiting step in the mucosal damage produced by acetic acid[67,68]. Hence decreased vascular permeability may play a vital role the mechanism of mucosal protection. Decreased spread of evans blue by treatment of AFEAS exploration it's ulcer protective potential.

Xanthine Oxidase (XO) is the primary biological source of SOD and is considered an important mediator in supplying the active oxygen radical in free radical induced lesions[22]. The level of XO elevated in stressful conditions. The elevated level of protein carbonyl content is a hallmark of oxidation-induced damage. Under oxidative conditions Reactive Oxygen Species (ROS) mediated protein oxidation leads to high levels of Protein Carbonyl Content (PCC) in the tissues[22]. Acetic acid instillation is associated with elevated XO and protein carbonyl content via production of oxygen free radicals. In present study elevated XO and protein carbonyl content were reduced by AFEAS, which may be due to its antioxidant and anti-inflammatory potential.

Leptin is a fat tissue-specific protein and leptin receptor are part of the class 1 cytokine receptor family which reveals the biological action of leptin and this family also includes gp-120 common signal transduction receptor for the IL-6 related family of cytokines[69,70]. The distorted mucin structure and leptin binding or deficit mucin formation is associated with a disturbance in normal intestinal function. Thus, leptin is considered as not only regulatory in food function but also its vital role in other different biological process. In present study leptin level was decreased in serum reflected in decreased food intake whereas, administration of AFEAS increased serum leptin level which in turn increase food intake.

Overproduction of inflammatory mediators (TNF-α and IL-1β) in IBD lead to a positive inflammatory feedback loop which can produce toxic peroxide anions, proteases and oxygen/nitrogen radicals and cause indiscriminate tissue damage[49]. Administration of alkaloidal fraction of *Alstonia scholaris* countered the overexpression of the inflammatory mediators such as TNF-α and IL-1β.

Prednisolone as an anti-inflammatory agent decreases the recruitment of macrophages in the affected area and suppresses the synthesis of many inflammatory mediators, e.g., production of IL-1 from monocytes, production of IL-2 and tumor necrosis factor from lymphocytes[21]. In present study administration of prednisolone suppressed synthesis of inflammatory mediators and also decreased conscription of macrophages in the affected area which may be due to it anti-inflammatory activity.

CONCLUSION

In conclusion, treatment of alkaloidal fraction of *Alstonia scholaris* ameliorates acetic acid induced colitis by virtue of its anti-inflammatory and anti-oxidant potential via inhibition of production oxido-inflammatory mediator and pro-inflammatory cytokines.

ACKNOWLEDGMENTS

The authors acknowledge Dr. S.S. Kadam, Vice-chancellor, Bharati Vidyapeeth University and Dr. K. R. Mahadik, Principal, Poona College of Pharmacy for the keen interest and providing the necessary facilities to carry out the study. The authors also acknowledge Invaluesys Research Group to carry out statistical analysis of the study.

REFERENCES

1. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Anti-inflammatory effect of *Daucus carota* root on experimental colitis in rats. Int. J. Pharm. Pharmaceut. Sci., 4: 337-343.

2. Ponder, A. and M.D. Long, 2013. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. Clin. Epidemiol., 5: 237-247.
3. Fabia, R., R. Willen, A. Ar'Rajab, R. Andersson, B. Ahren and S. Bengmark, 1992. Acetic acid-induced colitis in the rat: A reproducible experimental model for acute ulcerative colitis. Eur. Surg. Res., 24: 211-225.
4. Ghosh, P., A.D. Kandhare, D. Gauba, K.S. Raygude and S.L. Bodhankar, 2012. Determination of efficacy, adverse drug reactions and cost effectiveness of three triple drug regimens for the treatment of *Helicobacter pylori* infected acid peptic disease patients. Asian Pac. J. Trop. Dis., 2: S783-S789.
5. Ghosh, P., A.D. Kandhare, K.S. Raygude, D. Gauba, T.P. Gosavi and S.L. Bodhankar, 2012. Cigarette smoking and *H. pylori* infection: A meta-analysis of literature. Der Pharmacia Lettre, 4: 128-134.
6. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Effect of aqueous extract of *Cucumis sativus* Linn. fruit in ulcerative colitis in laboratory animals. Asian Pac. J. Trop. Biomed., 2: S962-S969.
7. Raygude, K.S., A.D. Kandhare, P. Ghosh, T.P. Gosavi and S.L. Bodhankar, 2011. Consumption of alcohol and *H. pylori* infection: A cumulative meta-analysis of literature. Asian J. Biochem. Pharm. Res., 1: 338-345.
8. Dole, S., A.D. Kandhare, P. Ghosh, T.P. Gosavi and S.L. Bodhankar, 2012. Homeopathic analgesic formulations: A critical appraisal of evidence. J. Pharmaceut. Biomed. Sci., 22: 1-6.
9. Sartor, R.B., 1997. Pathogenesis and immune mechanisms of chronic inflammatory bowel diseases. Am. J. Gastroenterol., 92: 5S-11S.
10. Kandhare, A.D., K.S. Raygude, P. Ghosh, T.P. Gosavi and S.L. Bodhankar, 2011. Patentability of animal models: India and the globe. Int. J. Pharm. Biol. Arch., 2: 1024-1032.
11. Yuan, J.H., Y.Q. Li and X.Y. Yang, 2008. Protective effects of epigallocatechin gallate on colon preneoplastic lesions induced by 2-amino-3-methylimidazo[4,5-*f*]quinoline in mice. Mol. Med., 14: 590-598.
12. Yusuf, M., M.A. Wahab, J.U. Chowdhury and J. Begum, 2006. Ethno-medico-botanical knowledge from Kaukhali proper and Betbunia of Rangamati district. Bangladesh J. Plant Taxon., 13: 55-61.
13. Lin, S.C., C.C. Lin, Y.H. Lin, S. Supriyatna and S.L. Pan, 1996. The protective effect of *Alstonia scholaris* R. Br. on hepatotoxin-induced acute liver damage. Am. J. Clin. Med., 24: 153-164.
14. Akhtar, M.S. and H. Bano, 2002. Hypoglycemic effect of powdered *Alstonia scholaris* (Satona). Prof. Med. J., 9: 208-271.
15. Bhattacharya, S.K., R. Bose, S.C. Dutta, A.B. Ray and S.R. Guha, 1979. Neuropharmacological studies on strictamine isolated from *Alstonia scholaris*. Indian J. Exp. Biol., 17: 598-600.
16. Shang, J.H., X.H. Cai, Y.L. Zhao, T. Feng and X.D. Luo, 2010. Pharmacological evaluation of *Alstonia scholaris*: Anti-tussive, anti-asthmatic and expectorant activities. J. Ethnopharmacol., 129: 293-298.
17. Bhogayata, K., P.P. Sharma and B.R. Patel, 2009. A clinical evaluation of Saptaparna (*Alstonia scholaris* L., R. Br.) on essential hypertension. AYU: Int. Quart. J. Res. Ayurveda, 30: 318-322.
18. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2011. Pharmacological evaluation of ameliorative effect of aqueous extract of *Cucumis sativus* L. fruit formulation on wound healing in wistar rats. Chron. Young Sci., 2: 207-213.
19. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Pharmacological evaluation of ethanolic extract of *Daucus carota* Linn root formulated cream on wound healing using excision and incision wound model. Asian Pac. J. Trop. Biomed., 2: S646-S655.
20. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Acute and repeated doses (28 days) oral toxicity study of glycosides based standardized fenugreek seed extract in laboratory mice. Regul. Toxicol. Pharmacol., 72: 323-334.
21. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule, T.P. Gosavi, S.L. Badole and S.L. Bodhankar, 2012. Effect of hydroalcoholic extract of *Hibiscus rosa sinensis* Linn. leaves in experimental colitis in rats. Asian Pac. J. Trop. Biomed., 2: 337-344.
22. Kumar, V.S., A.R. Rajmane, M. Adil, A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2014. Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. J. Biomed. Res., 28: 132-145.
23. Patil, A., A. Guru, A. Mukhrjee, A. Sengupta and S. Sarkar *et al*., 2015. Elucidation of gastro-protective activity of Morin in pylorus ligation induced gastric ulcer via modulation of oxidative stress. Der Pharmacia Lettre, 7: 131-139.
24. Kandhare, A.D., K.S. Raygude, P. Ghosh and S.L. Bodhankar, 2011. The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats. Int. J. Green Pharm., 5: 236-243.
25. Goswami, S., A. Kandhare, A.A. Zanwar, M.V. Hegde and S.L. Bodhankar *et al*., 2016. Oral L-glutamine administration attenuated cutaneous wound healing in Wistar rats. Int. Wound J., 13: 116-124.
26. Kamble, H., A.D. Kandhare, S. Bodhankar, V. Mohan and P. Thakurdesai, 2013. Effect of low molecular weight galactomannans from fenugreek seeds on animal models of diabetes mellitus. Biomed. Aging Pathol., 3: 145-151.
27. Adil, M., A.D. Kandhare, A. Visnagri and S.L. Bodhankar, 2015. Naringin ameliorates sodium arsenite-induced renal and hepatic toxicity in rats: Decisive role of KIM-1, Caspase-3, TGF-β and TNF-α. Renal Failure, 37: 1396-1407.

28. Adil, M., A. Visnagri, V.S. Kumar, A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2014. Protective effect of naringin on sodium arsenite induced testicular toxicity via modulation of biochemical perturbations in experimental rats. Pharmacologia, 5: 222-234.
29. Badole, S.L., S.M. Chaudhari, G.B. Jangam, A.D. Kandhare and S.L. Bodhankar, 2015. Cardioprotective activity of *Pongamia pinnata* in streptozotocin-nicotinamide induced diabetic rats. BioMed Res. Int. 10.1155/2015/403291
30. Raygude, K.S., A.D. Kandhare, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. Inflammopharmacology, 20: 331-341.
31. Ghule, A.E., A.D. Kandhare, S.S. Jadhav, A.A. Zanwar and S.L. Bodhankar, 2015. Omega-3-fatty acid adds to the protective effect of flax lignan concentrate in pressure overload-induced myocardial hypertrophy in rats via modulation of oxidative stress and apoptosis. Int. Immunopharmacol., 28: 751-763.
32. Kandhare, A.D., K.S. Raygude, V.S. Kumar, A.R. Rajmane and A. Visnagri *et al*., 2012. Ameliorative effects quercetin against impaired motor nerve function, inflammatory mediators and apoptosis in neonatal streptozotocin-induced diabetic neuropathy in rats. Biomed. Aging Pathol., 2: 173-186.
33. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Therapeutic role of curcumin in prevention of biochemical and behavioral aberration induced by alcoholic neuropathy in laboratory animals. Neurosci. Lett., 511: 18-22.
34. Aswar, U.M., A.D. Kandhare, V. Mohan and P.A. Thakurdesai, 2015. Anti-allergic effect of intranasal administration of type-a procyanidin polyphenols based standardized extract of cinnamon bark in ovalbumin sensitized BALB/c mice. Phytother. Res., 29: 423-433.
35. Devkar, S., A. Kandhare, A. Zanwar, S. Jagtap, S. Katyare, S. Bodhankar and M. Hegde, 2016. Hepatoprotective effect of withanolide rich fraction in acetaminophen intoxicated rat: Decisive role of TNF-α, IL-1β, COX-II and iNOS. Pharmaceut. Biol., (In Press).
36. Gosavi, T.P., P. Ghosh, A.D. Kandhare, V.S. Kumar, M. Adil, A.R. Rajmane and S.L. Bodhankar, 2012. Therapeutic effect of *H. pylori* nosode, a homeopathic preparation in healing of chronic *H. pylori* infected ulcers in laboratory animals. Asian Pac. J. Trop. Dis., 2: S603-S611.
37. Gosavi, T.P., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2012. Anticonvulsant activity of *Argentum metallicum*, a homeopathic preparation. Der Pharmacia Lettre, 4: 626-637.
38. Kandhare, A.D., P. Ghosh, A.E. Ghule, G.N. Zambare and S.L. Bodhankar, 2013. Protective effect of *Phyllanthus amarus* by modulation of endogenous biomarkers and DNA damage in acetic acid induced ulcerative colitis: Role of phyllanthin and hypophyllanthin. Apollo Med., 10: 87-97.
39. Kandhare, A.D., V.S. Kumar, M. Adil, A.R. Rajmane, P. Ghosh and S.L. Bodhankar, 2012. Investigation of gastro protective activity of *Xanthium strumarium* L. by modulation of cellular and biochemical marker. Orient. Pharmacy Exp. Med., 12: 287-299.
40. Jahanshahi, G., V. Motavasel, A. Rezaie, A.A. Hashtroudi, N.E. Daryani and M. Abdollahi, 2004. Alterations in antioxidant power and levels of epidermal growth factor and nitric oxide in saliva of patients with inflammatory bowel diseases. Dig. Dis. Sci., 49: 1752-1757.
41. Honmore, V., A. Kandhare, A.A. Zanwar, S. Rojatkar, S. Bodhankar and A. Natu, 2015. *Artemisia pallens* alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers. Pharmaceut. Biol., 53: 571-581.
42. Bhilare, N.V., S.S. Dhaneshwar, A.J. Sinha, A.D. Kandhare and S.L. Bodhankar, 2015. Novel thioester prodrug of N-acetylcysteine for odor masking and bio availability enhancement. Curr. Drug Delivery.
43. Devkar, S.T., A.D. Kandhare, B.D. Sloley, S.D. Jagtap and J. Lin *et al*., 2015. Evaluation of the bioavailability of major withanolides of *Withania somnifera* using an *in vitro* absorption model system. J. Adv. Pharmaceut. Technol. Res., 6: 159-164.
44. Dole, S., A.D. Kandhare, P. Ghosh, T.P. Gosavi and S.L. Bodhankar, 2012. The health outcome after homeopathic treatment in cases of BPH and LUTS: A prospective clinical study. J. Pharmaceut. Biomed. Sci., 22: 1-6.
45. Kandhare, A.D., J. Alam, M.V.K. Patil, A. Sinha and S.L. Bodhankar, 2016. Wound healing potential of naringin ointment formulation via regulating the expression of inflammatory, apoptotic and growth mediators in experimental rats. Pharmaceut. Biol., 54: 419-432.
46. Honmore, V.S., A.D. Kandhare, P.P. Kadam, V.M. Khedkar and D. Sarkar *et al*., 2016. Isolates of *Alpinia officinarum* Hance as COX-2 inhibitors: Evidence from anti-inflammatory, antioxidant and molecular docking studies. Int. Immunopharmacol., 33: 8-17.
47. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Prophylactic efficacy and possible mechanisms of oligosaccharides based standardized fenugreek seed extract on high-fat diet-induced insulin resistance in C57BL/6 mice. J. Applied Pharma. Sci., 5: 35-45.
48. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fibrosis in rats: Decisive role of Bax, Nrf2, NF-κB, Muc5ac, TNF-α and IL-1β. Chemico-Biol. Interact., 237: 151-165.
49. Kandhare, A.D., P. Ghosh and S.L. Bodhankar, 2014. Naringin, a flavanone glycoside, promotes angiogenesis and inhibits endothelial apoptosis through modulation of inflammatory and growth factor expression in diabetic foot ulcer in rats. Chemico-Biol. Interact., 219: 101-112.

50. Ketkar, S., A. Rathore, A. Kandhare, S. Lohidasan, S. Bodhankar, A. Paradkar and K. Mahadik, 2015. Alleviating exercise-induced muscular stress using neat and processed bee pollen: Oxidative markers, mitochondrial enzymes and myostatin expression in rats. Integr. Med. Res., 4: 147-160.
51. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Anti-arthritic and Anti-inflammatory activity of *Xanthium srtumarium* L. ethanolic extract in freund's complete adjuvant induced arthritis. Biomed. Aging Pathol., 2: 6-15.
52. Raygude, K.S., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2012. Anticonvulsant effect of fisetin by modulation of endogenous biomarkers. Biomed. Preventive Nutr., 2: 215-222.
53. Patil, M.V.K., A.D. Kandhare, P. Ghosh and S.D. Bhise, 2012. Determination of role of GABA and nitric oxide in anticonvulsant activity of *Fragaria vesca* L. ethanolic extract in chemically induced epilepsy in laboratory animals. Oriental Pharmacy Exp. Med., 12: 255-264.
54. Kandhare, A.D., S.L. Bodhankar, V. Singh, V. Mohan and P.A. Thakurdesai, 2013. Anti-asthmatic effects of type-A procyanidine polyphenols from cinnamon bark in ovalbumin-induced airway hyperresponsiveness in laboratory animals. Biomed. Aging Pathol., 3: 23-30.
55. Kandhare, A.D., P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2013. Elucidation of molecular mechanism involved in neuroprotective effect of Coenzyme Q10 in alcohol-induced neuropathic pain. Fundam. Clin. Pharmacol., 27: 603-622.
56. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitoterapia, 83: 650-659.
57. Kandhare, A.D., M.V. Patil and S.L. Bodhankar, 2015. L-arginine attenuates the ethylene glycol induced urolithiasis in ininephrectomized hypertensive rats: Role of KIM-1, NGAL and NOs. Renal Fail., 37: 709-721.
58. Kandhare, A.D., V. Shivakumar, A. Rajmane, P. Ghosh and S.L. Bodhankar, 2014. Evaluation of the neuroprotective effect of chrysin via modulation of endogenous biomarkers in a rat model of spinal cord injury. J. Nat. Med., 68: 586-603.
59. Sarkar, S., A. Sengupta, A. Mukhrjee, A. Guru, A. Patil, A.D. Kandhare and S.L. Bodhankar, 2015. Antiulcer potential of morin in acetic acid-induced gastric ulcer via modulation of endogenous biomarkers in laboratory animals. Pharmacologia, 6: 273-281.
60. Arnhold, J., 2004. Properties, functions and secretion of human myeloperoxidase. Biochemistry, 69: 4-9.
61. Visnagri, A., A.D. Kandhare and S.L. Bodhankar, 2015. Renoprotective effect of berberine via intonation on apoptosis and mitochondrial-dependent pathway in renal ischemia reperfusion-induced mutilation. Renal Fail., 37: 482-493.
62. Visnagri, A., A.D. Kandhare, V.S. Kumar, A.R. Rajmane and A. Mohammad *et al*., 2012. Elucidation of ameliorative effect of co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. Biomed. Aging Pathol., 2: 157-172.
63. Kolios, G., V. Valatas and S.G. Ward, 2004. Nitric oxide in inflammatory bowel disease: A universal messenger in an unsolved puzzle. Immunology, 113: 427-437.
64. Visnagri, A., A.D. Kandhare, S. Chakravarty, P. Ghosh and S.L. Bodhankar, 2014. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. Pharmaceut. Biol., 52: 814-828.
65. Cooke, C.L.M. and S.T. Davidge, 2002. Peroxynitrite increases iNOS through NF-κB and decreases prostacyclin synthase in endothelial cells. Am. J. Physiol.-Cell Physiol., 282: C395-C402.
66. Saraswathi, K.Y., A. Muthal, A. Kandhare, S. Rojatkar and S. Bodhankar, 2014. Study of methanolic extract of *Artemisia pallens* wall on endurance of laboratory animals. Pharmacologia, 5: 298-309.
67. Boughton-Smith, N.K., S.M. Evans, B.J.R. Whittle, S. Moncada, C.J. Hawkey, A.T. Cole and M. Balsitis, 1993. Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. Lancet, 342: 338-340.
68. Szabo, S., 1984. Role of sulfhydryls and early vascular lesions in gastric mucosal injury. Acta Physiologica Hungarica, 64: 203-214.
69. Lee, G.H., R. Proenca, J.M. Montez, K.M. Carrol., J.G. Darvishzadeh, J.I. Lee and J.M. Friedman, 1996. Abnormal splicing of the leptin receptor in diabetic mice. Nature, 379: 632-635.
70. Tartaglia, L.A., M. Dembski, X. Weng, N. Deng and J. Culpepper *et al*., 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell, 83: 1263-1271.