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Protective Effects of Ethanolic Extract of *Ageratum conyzoides* on Experimental Induced Inflammatory Bowel Disease

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ABSTRACT

Inflammatory Bowel Disease (IBD) is classified into two subtypes: Ulcerative colitis and Crohn's disease. There is a widespread belief that the herbal products have fewer side effects when compared to allopathic medicine and there are numerous herbal drugs which are contemporarily used in the treatment of IBD. *Ageratum conyzoides* Linn. (Family: Asteraceae) is an annual herb which has been considered in traditional medicine and mainly used in treatment of ulcers, colic, inflammation and analgesia. *Ageratum conyzoides* is also scientifically accepted for its anti-inflammatory, antioxidant, gastro protective activities. Hence, the present study was designed to evaluate the effect of *Ageratum conyzoides* on acetic acid-induced colitis and indomethacin-induced enterocolitis models in rats. The parameters studied includes macroscopic evaluation (scoring of ulcers), microscopic evaluation (histopathology), estimation of biochemical parameters (myeloperoxidase and lipid peroxides) and measurement of physical parameters (change in body weight and colon weight). The pretreatment with ethanolic extract of *Ageratum conyzoides* at a dose of 500 and 750 mg kg^{-1}, p.o. (per oral) and standard group of animals shows significant attenuated results in all above said parameters when compared to disease inducing group. Histopathological study supports the above results by protective and regeneration effects on colonic cells by *Ageratum conyzoides* pretreatment. The finding of the present study provides the evidence that, ethanolic extract of *Ageratum conyzoides* may be beneficial in patients suffering from inflammatory bowel disease.

Key words: *Ageratum conyzoides,* inflammatory bowel disease, acetic acid, indomethacin, histopathology

INTRODUCTION

Inflammatory Bowel Disease (IBD) is a series of chronic idiopathic intestinal and bowel inflammatory conditions. Majority symptoms of IBD include diarrhea, abdominal pain, bleeding stools and weight loss. Crohn's Disease (CD) and Ulcerative Colitis (UC) are the two major types of IBD (Sellin and Pasricha, 2006). CD may either affect a particular part of the intestine or the entire intestine whereas, UC mainly affects the colon and rectum and the inflammation is confined to the intestinal layer (Krzystek-Korpacka *et al*., 2009). Both the disease increases the risk of adrenocarcinoma of the colon in the affected areas (Dudzinski and Serhan, 2008).

The etiology of IBD is unclear but the current literature suggests that multiple immune, genetic and environmental factors influence both the initiation and progression of colitis (Strober *et al.*, 1998). Combination of various factors like oxidative stress, immune deregulation and abnormal gastrointestinal (GI) tract causes IBD. Another major cause for IBD is presence of microorganisms in the GI tract and loop holes in the GI mucosal barrier that allow microorganisms to penetrate into the mucosa (Scaldaferri and Fiocchi, 2007).

IBD was seen at an increasing rate in Europe and North America during late 20th century and is becoming the most common problem in major parts of the world due to the western life style (Sartor, 1997). UC and CD mainly occur in different socioeconomic groups (Loftus and Sandborn, 2002; Andres and Friedman, 1999). As the mechanism, pathophysiology and pathogenesis of the disease occurrence is still not clear, this forms the major barricade in the treatment of IBD. Many drugs which are used in IBD, offer symptomatic or temporary relief (Jagtap *et al.*, 2004). Most of the current therapies for IBD involve treatment with glucocorticosteroids and 5-aminosalicylic acid (Katzung, 2007). Immunosuppressive drugs have also been used to control severe illness, regardless of the more serious complications and toxic side effect associated with them (Shanahan, 2001). Therefore, there is need to develop safe and effective alternative therapeutic agents for the treatment of IBD.

There is a widespread belief that the natural products are less toxic when compared to pure chemicals. Phytobotanical and ethnobotanical research have focused for decades on the search for the single active principle in plants, based on the assumption that a plant has one or few ingredients which determine its therapeutic effects. But the traditional system of medicine like Ayurveda, traditional Chinese medicine or the European pharmacotherapy generally assumes that a synergy of all ingredients of the plants will bring about the maximum therapeutic efficacy (Ulrich-Merzenich *et al.*, 2010). Recent data suggests that 80% drug molecules are Phytochemicals or inspiration from an herbal compound (Bhutani and Gohil, 2010).

Ageratum conyzoides Linn (Family: Asteraceae) is an annual herb distributed throughout India (Kirtikar and Basu, 1993). The ethnopharmacology of *Ageratum conyzoides* listed the uses of the plant in folk remedies which includes treatment of ulcers, febrifuge, colic, anti-inflammatory and anti-diarrheic (Bioka *et al.*, 1993). The *Ageratum conyzoides* has been proven scientifically for its anti-inflammatory, antioxidant, gastroprotective, anti-microbial, herbicidal, hepatoprotective, analgesic, antifungal, hypoglycaemic and antihyperglycaemic activities (Galati *et al.*, 2001; Shirwaikar *et al.*, 2003; Amadi *et al.*, 2007; Akhtar *et al.*, 2001; Ita *et al.*, 2009; Okemy-Andissa *et al.*, 2006; Widodo *et al.*, 2008; Dayie *et al.*, 2008; Nyunaii *et al.*, 2009). To best of knowledge no scientific data regarding the activity of *Ageratum conyzoides* on IBD is available. Hence, this work has undertaken to evaluate the effect of *Ageratum conyzoides* on acetic acid induced colitis and Indomethacin induced enterocolitis in rats.

MATERIALS AND METHODS

Chemicals: Prednisolone and Indomethacin were procured from Microlabs, Bangalore, India. Hexadecyl Trimethyl Ammonium Bromides (HTAB), O-Dianisidine di hydrochloride, Trichloro-barbituric acid and acetic acid were procured by High media Bombay, India. Other chemicals used were of analytical grade.

Plant material: The whole plant, *Ageratum conyzoides* Linn (Family: Asteraceae) were collected in the month of July and August from Khanapur forests of Dharwad district, Karnataka, India.

Then it is identified by Dr. G. R. Hegde, Head of Department of Botany, Karnataka University Dharwad (KUD), Dharwad, India. A voucher specimen (SETCPD/Ph.cog/herb/11/2010) is retained in department for further reference. The collected material was washed with running water. The plant were chopped in to small pieces and dried under shade. Dried parts of plant were coarsely powdered and used for extraction.

Preparation of extracts: The ethanolic extract of *Ageratum conyzoides* (AC) was prepared by taking the dried coarsely powdered plant material (1 kg) and exhaustively extracted with 95% ethanol using Soxhalet apparatus. It was then concentrated in vacuo to syrupy consistency. The suspensions of *Ageratum conyzoides* were prepared by using tragacanth (2%) in distilled water and used for the experiment.

Animals: All the experiments were carried out with Male Wistar rats of 200-250 g after approval from the Institutional Animal Ethical Committee (Approval Number: SETCP/IAEC/2009-2010/396 and Date-09-12-2009). Animals were kept in the animal house of S.E.T's College of Pharmacy, Dharwad, India. Under controlled conditions of temperature (23±2°C), humidity (50±5%) and 12 h light-dark cycle. Animals were fed with rat diet pellet (obtained from venkateshwara Enterprises, Bangalore) and water ad libitum. All the animals were acclimatized for seven days before the study.

Acute toxicity study: Healthy wistar rats of either sex, starved overnight were divided into six groups (n = 6), they were orally fed with the ethanol extract of *Ageratum conyzoides* in increasing dose levels of 100, 500, 1000, 3000, 6000 and 10,000 mg kg^{-1} body weight, results consistent with (Igboasoiyi *et al*., 2007). The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after a period of 24 and 72 h for any lethality or death (Ghosh, 1984; Turner, 2009).

Drug treatment: The ethanolic extract of *Ageratum conyzoides* was administered to animals per orally for 7 days. On the 8th day, the disease was induced by irritants indomethacin/acetic acid. The drug treatment was continued even after administration of irritants.

Preparation of doses: *Ageratum conzyzoides* 500 mg kg^{-1} and 750 mg kg^{-1} is given as suspension made by 2% tragacanth in distilled water. Standard drug used was prednisolone (2 mg kg^{-1} per orally). Prednisolone was not given as pretreatment. It was given on the day of irritant administration. Prednisolone was given in a dose of 2 mg kg^{-1} per day, orally in rats as suspension containing 0.5% of sodium CMC.

Indomethacin-induced enterocolitis in rats: The study comprises five different groups of six wistar rats (200-250 g) each as follows:

- Normal or untreated animals
- Disease Control animals receive only Indomethacin (7.5 mg kg^{-1}, s.c.)
- AC treated animals, receive *Ageratum conyzoides* (500 mg kg^{-1} p.o.) and Indomethacin on 8th and 9th day. AC treatment continued till 11th day

- AC treated animals, receive *Ageratum conyzoides* extract (750 mg kg^{-1} p.o.) and Indomethacin on 8th and 9th day. AC treatment continued till 11th day
- Prednisolone treated group which will receive prednisolone (2 mg kg^{-1} p.o.) on 8th day onwards to 11th day and indomethacin was administered on 8th and 9th day

Animals are pretreated with *Ageratum conyzoides* extract for 7 days followed by administration of Indomethacin (7.5 mg kg^{-1}, s.c.) on 8th and 9th day. Extract was continued up to 11th day. On 11th day after treatment, the animals will be scarified by cervical dislocation and dissected. Ileum and colon were taken out to assess inflammation, based on physical parameters, macroscopy (scoring of ulcer) and microscopic features (histopathology). Quantification of inflammation was done using biochemical assay like myeloperoxidase and lipid peroxides (MPO and LPO) (Jagtap *et al*., 2004; Hager *et al*., 2007).

Acetic acid-induced colitis in rats: The study comprises five different groups of six wistar rats (200-250 g) each as follows:

- Normal or untreated animals
- Disease control animals receive only acetic acid (2 mL of 4% v/v i.p.)
- AC treated animals, receive *Ageratum conyzoides* (500 mg kg^{-1} p.o.) and acetic acid on 8th day. AC treatment was continued till 10th day
- AC treated animals, receive *Ageratum conyzoides* extract (750 mg kg^{-1} p.o.) and acetic acid on 8th day. AC treatment was continued till 10th day
- Prednisolone treated groups receive prednisolone (2 mg kg^{-1} p.o.) for three days (from 8th day to 10th day) and acetic acid on 8th day. Prednisolone treatment was started on the day of acetic acid treatment

Animals will be treated with *Ageratum conyzoides* extract for 7 days and fasted over night. On 8th day, animals were anaesthetized using pentobarbitone sodium and 2 mL of 4% v/v acetic acid solution was instilled into rectum. After 48h animals were sacrificed by cervical dislocation and dissected to isolate colon. Contents of colon were removed with saline gently. Inflammation was assessed based on physical parameters like macroscopic (by scoring of ulcer) and microscopic features (by histopathology). Quantification of inflammation was done using biochemical assay (MPO and LPO) (Jagtap *et al*., 2004; Hager *et al*., 2007).

Evaluation of the disease: The disease induced in experimental animals was evaluated based on its physical parameters, macroscopic and microscopic characteristics. Quantification of inflammation was done using biochemical assay (MPO and LPO).

Evaluation based on physical parameters: The physical parameters like body weight, colon weight, colon length and colon weight/length ratio were used to evaluate the disease status in animals. As the weight loss is one of the clinical symptoms of IBD, so rats from respective groups were weighed each day and the percentage of original weight was evaluated. After sacrifice the colon weight, length and weight/length ratio obtained and utilized for the evaluation of the disease (Jiang *et al*., 2006; Paiva *et al*., 2004). Evaluation based on macroscopic characters (Jagtap *et al*., 2004).

Table 1: The scoring table for macroscopic changes

Score	Macroscopic changes
0	No visible change
1	Hyperemia at sites
2	Lesions having diameter 1 mm or less
3	Lesions having diameter 2 mm or less (number of lesion<5)
4	Lesions having diameter 2 mm or less (number of lesion 5-10)
5	Lesions having diameter 2 mm or less (number of lesion>10)
6	Lesions having diameter more than 2 mm (number of lesion<5)
7	Lesions having diameter more than 2 mm (number of lesion 5-10)
8	Lesions having diameter more than 2 mm (number>10)

Table depicting various macroscopic changes and scores assigned depending on the severity of lesions

Table 2: The scoring table for percentage of area affected

Score	Percentage of area affected
0	00-00
1	01-05
2	05-10
3	10-25
4	25-50
5	50-75
6	75-100

Table depicting the percentage of area affected and scores assigned depending on the increasing percentage. Score for an individual rat was calculated from below table

Ulcers score for ileum and colon: For each animal, the distal 10 cm portion of the colon and ileum was removed and cut longitudinally and slightly cleaned in physiological saline to remove faecal residues. Pieces of rat ileum and colon (10 cm long each) were scored for macroscopic features using following scoring pattern, mentioned in Table 1.

Percentage area affected of rat caecum: Rat caecum was scored for macroscopic features using following scoring pattern mentioned in Table 2.

Evaluation based on microscopic (histological) characters: The colon (in acetic acid-induced colitis in rats) and caecum (in Indomethacin-induced enterocolitis in rats) from each animal was removed after sacrificing the animal and preserved in 10% formalin saline solution (Deshmukh *et al*., 2010). The samples were submitted to Jeevan Regional Diagnostic Health Care and Research Centre Pvt Ltd. (Belgaum, India) for histological examination.

Evaluation based on biochemical parameters

Myeloperoxidase (MPO) assay: Myeloperoxidase assay for quantification of inflammation is carried out by the method of Jagtap *et al*. (2004). One unit of MPO activity is defined as the change in absorbance per minute by 1.0 at room temperature, in the final reaction.

Calculation of MPO activity:

$$\text{MPO activity (U g}^{-1}) = \frac{X}{\text{Weight of the piece of tissue taken}}$$

Where,

$$X = \frac{10 \text{ X change in absorbance per min ute}}{\text{Volume of sup erna tan t taken in the final reaction}}$$

Measurement of colonic lipid peroxides (LPO) concentration: Lipid peroxidation, an indicator of mucosal injury induced by reactive oxygen species was measured as thiobarbituric acid reactive substance by the method of Hager *et al.* (2007) and Mackay *et al.* (2004).

Statistical analysis: All data were expressed as Mean±Standard error of the mean (SEM) of 6 rats per experimental group. Parametric one way analysis of variance (ANOVA) followed by Tukey's post test. Statistical analyses were performed using software Graph pad prism version 5.0. The minimal level of significance was identified at $p<0.05$.

RESULTS

Acute toxicity study: Acute toxicity studies of AC extract was found to be safe up to a dose level of 10,000 mg kg^{-1}. p.o. No signs and symptoms of toxicity were observed up to this dose level, if extract shows/has any kind of toxicity or LD_{50} it might be above this dose level. Hence, we have selected the two safe dose levels of AC 500 mg kg^{-1} and 750 mg kg^{-1} for the experimental study.

Indomethacin-induced enterocolitis in rats

Effect of *Ageratum conyzoides* (AC) on physical parameter: As the weight loss is one of the clinical symptoms, all the rats from respective groups are weighed each day and the percentage of original weight is used for evaluation. At the end of the experiment, except for Indomethacin induced group, body weight of the other groups shows increase (normal control) as well as retain (drug treated groups and the standard prednisolone groups) in their body weight. Evaluation based on body weight shows that there will be significant decrease ($p<0.001$) in the body weight (which is expressed in percentage of original weight) of the Indomethacin induced group compare with normal group (after induction). The drug treated groups (500 mg kg^{-1} p.o. $p<0.05$ and 750 mg kg^{-1} p.o. $p<0.01$) and the standard prednisolone groups showed significant ($p<0.01$) retain in their total body weight compared with the Indomethacin induced group. AC 500 mg kg^{-1} and 750 mg kg^{-1} shows body weight of 175.0 and 179.16 g, respectively whereas decrease in the body weight of 156.66 g Indomethacin group is observed (Table 3).

Effect of AC on macroscopic (Ulcer) scores: Two days treatment with Indomethacin (7.5 mg kg^{-1}, s.c.) produced severe inflammation in rat intestine. The middle portion of the small

Table 3: Effect of AC on physical parameters (body weight) in Indomethacin-induced enterocolitis

Group	1st day body weight (g)	11th day body weight (g)	Percentage of body weight (%)
Normal	164.16±6.112	189.166±4.729	115
Indomethacin	166.66±3.333	156.66±4.014***	94
AC 500 mg kg^{-1}	164.16±2.007	175.000±2.236#	106.6
AC 750 mg kg^{-1}	167.50±3.096	179.166±3.745##	106.9
Prednisolone	167.50±3.096	180.000±3.651##	108.05

Values are expressed as Mean±SEM of 6 animals;***Represents the statistical significance of $p<0.001$ compared to normal group. ## and # Represents the statistical significance of $p<0.01$ and $p<0.05$, respectively compared to Indomethacin control group was done by One-way ANOVA, followed by Tukey's post test

Table 4: Effect of AC on macroscopic (Ulcer) scores in Indomethacin-induced enterocolitis

Group	Mean of macroscopic scores±SEM
Normal	00.00
Indomethacin	10.17±0.47***
AC 500 mg kg^{-1}	03.83±0.30###
AC 750 mg kg^{-1}	02.50±0.22###
Prednisolone	02.00±0.25###

Values are expressed as Mean±SEM of 6 animals; ***Represents the statistical significance of $p<0.05$, $p<0.01$, $p<0.001$, respectively compared to normal group. ###Represents the statistical significance of $p<0.001$ compared to Indomethacin control group was done by One-way ANOVA, followed by Tukey's post test

Table 5: Effect of AC on MPO and LPO activity in Indomethacin-induced enterocolitis

Group	MPO activity (U g^{-1})±SEM	LPO activity (μmol g^{-1})±SEM
Normal	1.34±0.1	0.264±0.02
Indomethacin	8.44±0.21***	0.680±0.54***
AC 500 mg kg^{-1}	4.41±0.10###	0.508±0.42##
AC 750 mg kg^{-1}	3.09±0.06###	0.469±0.01##
Prednisolone	2.06±0.08###	0.404±0.01###

Values are expressed as Mean±SEM of 6 animals; ***Represents the statistical significance of $p<0.05$, $p<0.01$, $p<0.001$, respectively compared to normal group. ###, ##Represents the statistical significance of $p<0.001$, $p<0.01$, respectively compared to Indomethacin control group was done by One-way ANOVA, followed by Tukey's post test

intestine i.e., jejunum and proximal ileum showed more inflammation compared to proximal portion of the small intestine. Caecum was the most severely affected part, showing hemorrhagic spots. The ileum showed many lesions which were transmural. Evaluation based on macroscopic features showed ulcer score values significantly ($p<0.001$) increased in the Indomethacin induced group as compared with normal control. The standard drug prednisolone (2 mg kg^{-1} p.o.) and AC (500 and 750 mg kg^{-1} p.o.) significantly ($p<0.001$) reduced score values as compared to Indomethacin induced group. Ulcer score values of the AC 500 mg kg^{-1} and 750 mg kg^{-1} group showed 03.83 and 02.50, respectively which were comparable with the ulcer scores obtained in prednisolone treated group of 2.0 (Table 4).

Effect of AC on biochemical parameters

Myeloperoxidase (MPO) activity: The myeloperoxidase assay showed significant increase ($p<0.001$) in MPO activity of Indomethacin induced group compared to normal group. The drug treated AC (500 and 750 mg kg^{-1} p.o.) and Prednisolone treated group showed significant ($p<0.001$) reduction in MPO activity compared to Indomethacin induced group. MPO activity of the drug treated AC 500 mg kg^{-1} and 750 mg kg^{-1} group showed 4.41 U g^{-1} and 3.09 U g^{-1}, respectively which was comparable with the Prednisolone treated group of 2.06 U g^{-1} (Table 5).

Lipid peroxides (LPO) activity: LPO assay showed significant ($p<0.001$) increase in LPO activity of Indomethacin induced group compared to normal group. The drug treated AC (500 and 750 mg kg^{-1} p.o. $p<0.01$) and Prednisolone treated group showed significant ($p<0.001$) reduction in LPO activity compared to the Indomethacin induced group. LPO activity of the drug treated AC 500 and 750 mg kg^{-1} group showed 0.508 μmol g^{-1} and 0.469 μmol g^{-1}, respectively which was comparable with the Prednisolone treated group of 0.404 μmol g^{-1} (Table 5).

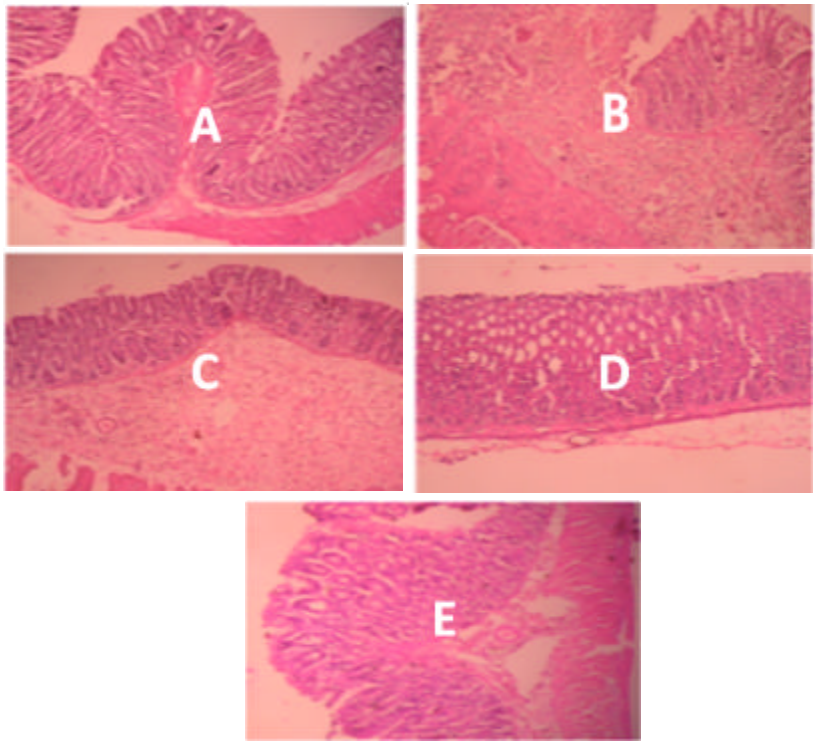

Fig. 1: Histological examination of caecum in Indomethacin-induced enterocolitis Lengends: Histological sections of normal caecum of rat (A), Indomethacin-induced colitis (B) inflamed caecum of rat showing submucosa with severe infiltration. Pre-treatment with AC 500 and 750 mg kg^{-1} (C and D, respectively) caecum of drug treated rat showing decreased submucosal edema and few inflammatory cells and Prednisolone treated rat (E)

Effect of AC on Histological examination: Histological examination of caecum in Indomethacin induced group (B) showed desquamous, inflammatory cellular infiltration, crypt damage, granulation tissue in submucosa and focal ulceration compared to the normal group (A) where, there is no such deformities in the caecum. The drug treated AC group 500 mg kg^{-1} (C) and AC group 750 mg kg^{-1} (D) of an extract showed reduced desquamous, inflammatory cellular infiltration, crypt damage, granulation tissue in submucosa and focal ulceration. The study also supports the protective and regeneration effects. Prednisolone treatment (E) showed suppressed inflammatory reaction (Fig. 1).

Acetic acid-induced colitis in rats

Effect of AC on Physical (body weight) parameter: There will be significant decrease ($p<0.001$) in the body weight (which is expressed in percentage of original weight) of the acetic acid induced group compare with normal group (after induction). The drug treated groups AC (500 mg kg^{-1} p.o. $p<0.05$ and 750 mg kg^{-1} p.o. $p<0.01$) and the standard prednisolone group ($p<0.001$) showed retain in the total body weight compare with acetic acid induced group. AC 500 mg kg^{-1} and 750 mg kg^{-1} shows body weight of 173.33 g and 175.83 g, respectively whereas decrease in the body weight of 180.0 g Indomethacin group is observed (Table 6).

Table 6: Effect of AC on physical parameters (body weight) in acetic acid-induced colitis

Group	1st day body weight (g)	10th day body weight (g)	Percentage of body weight (%)
Normal	158.33±4.014	180.83±3.962	113.8
Acetic acid	159.16±1.537	152.50±2.814***	95.8
AC 500 mg kg^{-1}	161.66±3.073	173.33±4.216#	107.2
AC 750 mg kg^{-1}	161.66±2.472	175.83±2.386##	108.4
Prednisolone	162.50±01.708	180.00±6.583###	110.7

Values are expressed as Mean±SEM of 6 animals; ***Represents the statistical significance of p<0.001 compared to normal group. ###,##,#Represents the statistical significance of p<0.001, p<0.01, p<0.05, respectively compared to acetic acid group was done by One-way ANOVA, followed by Tukey's post test

Table 7: Effect of AC on physical parameters in acetic acid-induced colitis

Group	Colon weight (g)	Colon length (cm)	Colon weight/length ratio (mg cm^{-1})
Normal	1.248±0.02	11.82±0.2	105.8±3.13
acetic acid	1.723±0.05***	09.88±0.17***	174.2±4.659***
AC 500 mg kg^{-1}	1.408±0.05##	11.23±0.2##	125.8±5.6###
AC 750 mg kg^{-1}	1.378±0.02##	11.37±0.22##	121.4±3.20###
Prednisolone	1.327±0.09###	11.63±0.39###	114.1±7.539###

Values are expressed as Mean±SEM of 6 animals; ***Represents the statistical significance of p<0.05, p<0.01, p<0.001, respectively compared to normal group. ###, ## Represents the statistical significance of p<0.001, p<0.01, respectively compared to acetic acid control group was done by One-way ANOVA, followed by Tukey's post test

Colon weight measurement (g): In acetic acid group, acetic acid induction increases in the weight of a colon significantly (p<0.001) compare to normal group. Whereas the drug treated AC groups (500 and 750 mg kg^{-1} p.o.) and Prednisolone group decreases the colon weight significantly (p<0.01 and p<0.001) compared with the acetic acid induced group. AC 500 and 750 mg kg^{-1} shows colon weight of 11.23 g and 11.37 g, respectively whereas decrease in the body weight of 11.63 g Indomethacin group is observed (Table 7).

Colon length measurement (cm): The disease induction by instillation of acetic acid intra rectally cause shrinkage in the colon length in the acetic acid induced group significantly (p<0.001) compared with normal control group. The drug treated AC groups (500 and 750 mg kg^{-1} p.o. p<0.01) and Prednisolone group (p<0.001) decreases the shrinkage of colon length significantly when compared to acetic acid induced group, detail data are expressed in Table 7.

Colon weight/length ratio measurement (mg cm^{-1}): The weight/length ratio (mg cm^{-1}) is increased significantly (p<0.001) in acetic acid induced group compared to normal group, whereas drug treated AC groups (500 and 750 mg kg^{-1} p.o.) (p<0.001) and Prednisolone group decreases this ratio significantly (p<0.001) compared to acetic acid group, detail data are expressed in Table 7.

Effect of AC on macroscopic (Ulcer) scores: Intra-rectal instillation of acetic acid caused inflammatory reaction in the colon. The inflammation covered rectum and distal colon portion. The visible changes included severe epithelial necrosis and ulcerated mucosa. Evaluation based on macroscopic features showed ulcer score values significantly (p<0.001) increased in the acetic acid induced group as compared with normal control. Drug treated AC (500 mg kg^{-1} p.o. p<0.01 and 750 mg kg^{-1} p.o. p<0.001) and prednisolone (2 mg kg^{-1} p.o.) treated group showed significant

Table 8: Effect of AC on macroscopic (Ulcer) scores in acetic acid-induced colitis

Group	Mean of macroscopic (Ulcer) scores
Normal	0.00
Acetic acid	4.33±0.50***
AC 500 mg kg^{-1}	2.00±0.44##
AC 750 mg kg^{-1}	1.33±0.30 ###
Prednisolone	0.83±0.49###

Values are expressed as Mean±SEM of 6 animals; *.**.***Represents the statistical significance of p<0.05, p<0.01, p<0.001, respectively compared to normal group. ###, ##Represents the statistical significance of p<0.001, p<0.01, respectively compared to acetic acid control group was done by One-way ANOVA, followed by Tukey's post test

Table 9: Effect of AC on MPO and LPO activity in acetic acid-induced colitis

Group	MPO activity (U g^{-1})	LPO activity (μmol g^{-1})
Normal	0.960±0.09	0.379±0.02
acetic acid	4.180±0.2***	0.770±0.03***
AC 500 mg kg^{-1}	2.320±0.12###	0.555±0.02###
AC 750 mg kg^{-1}	1.570±0.04###	0.510±0.01###
Prednisolone	1.290±0.07###	0.467±0.02###

Values are expressed as Mean±SEM of 6 animals; ***Represents the statistical significance of p<0.05, p<0.01, p<0.001, respectively compared to normal group. ###Represents the statistical significance of p<0.001, compared to acetic acid control group was done by One-way ANOVA, followed by Tukey's post test

(p<0.001) decrease in ulcer score values as compared to acetic acid induced group. The values obtained for the drug treated AC group (especially 750 mg kg^{-1}) is comparable with that of standard prednisolone treated group. Ulcer score values of the AC 500 and 750 mg kg^{-1} group showed 2.0 and 1.33, respectively which were comparable with the ulcer scores obtained in prednisolone treated group of 0.83 (Table 8).

Effect of AC on biochemical parameters

Myeloperoxidase (MPO) activity (U g^{-1}): The colitis caused by acetic acid was associated with an increase in MPO activity. The MPO assay showed that there is significant increase (p<0.001) in MPO activity of acetic acid induced group compared to normal group. The drug treated AC (500 and 750 mg kg^{-1} p.o.) and prednisolone treated groups showed significant decrease (p<0.001) in MPO activity compared to acetic acid induced group. MPO activity of the drug treated AC 500 mg kg^{-1} and 750 mg kg^{-1} group showed 2.32 U g^{-1} and 1.57 U g^{-1}, respectively which was comparable with the Prednisolone treated group of 1.29 U g^{-1} (Table 9).

Lipid peroxides (LPO) assay (μmol g^{-1}): The Lipid peroxides (LPO) assay showed that there is significant increase (p<0.001) in LPO activity of acetic acid induced group compared to normal group. The drug treated AC (500 and 750 mg kg^{-1} p.o.) and prednisolone treated groups showed significant (p<0.001) decrease in LPO activity compared acetic acid induced group. LPO activity of the drug treated AC 500 and 750 mg kg^{-1} group showed 0.555 and 0.510 μmol g^{-1}, respectively which was comparable with the Prednisolone treated group of 0.467 μmol g^{-1} (Table 9).

Effect of AC on Histological examination: Histological examination of colon in acetic acid induced group (B) showed edema, inflammatory cellular infiltration, crypt damage and ulceration at mucus and submucosal layers compared to the normal group (A) where there is no such

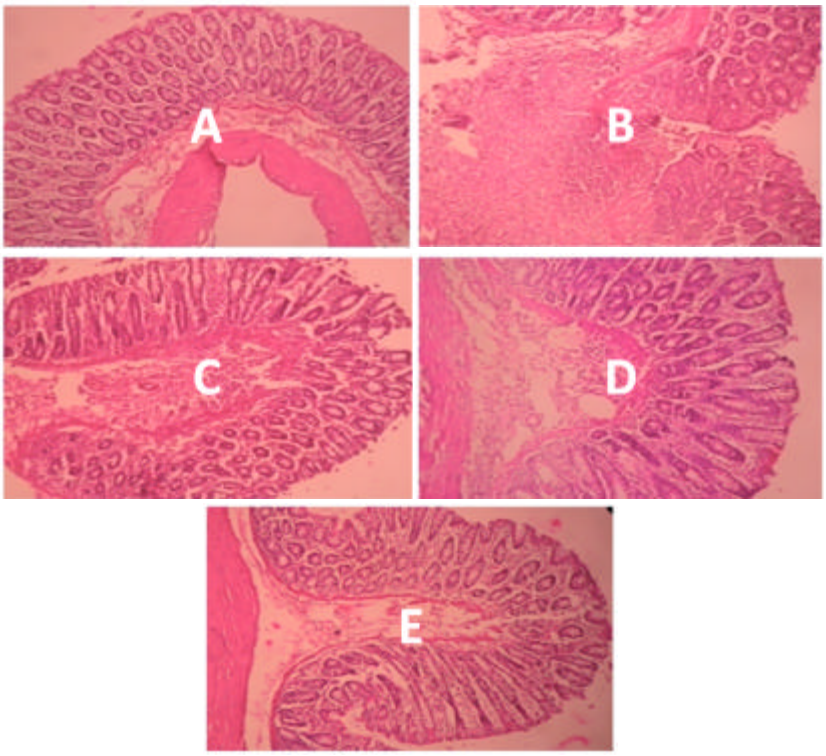

Fig. 2: Histological examination of colon in acetic acid-induced colitis. Legends: Histological colonic mucosal sections of normal rat (A) shows normal mucosa with intact epithelial surface, acetic acid induced colitis (B) showing massive necrotic destruction of epithelium. Pre-treatment with AC (C and D, respectively) shows decreased epithelial damage, regeneration and suppressed inflammatory reaction. prednisolone treated group showed suppressed inflammatory reaction (E)

deformities in the colon. The drug treated AC group (500 mg kg^{-1}) (C) and 750 mg kg^{-1} (D) showed reduced intensity edema, inflammatory cellular infiltration, crypt damage and ulceration at mucus and submucosal layers. The study also supports the protective and regeneration effects on colonic cells. Prednisolone treatment (E) showed suppressed inflammation (Fig. 2).

DISCUSSION

Inflammatory Bowel Disease (IBD) mainly refers to UC and CD, the two relapsing, ulcerative and inflammatory disease of GI tract. The prevalence of IBD is higher in Scandinavian countries, Great Britain, Canada and the United States than in central Europe, Africa and Asia (Abdul-Baki *et al*., 2007). But this gap is becoming narrowing. It is estimated that 1-2 million Americans suffer from IBD with about 30,000 new cases reported each year. Consumption of a Western diet, left-handedness and depression may increase risk for ulcerative colitis (Rigas *et al*., 1993; Kurina *et al*., 2001).

Intrarectal instillation of acetic acid in rats affects only the distal colon portion. As this model shares many of the histological features of UC in human beings including mucosal edema, neutrophil infiltration of the mucosa and submucosal ulceration it is one of the frequently using

model for the study of IBD. The mechanism by which acetic acid produces inflammation appears to involve the entry of the protonated form of the acid into epithelium, where it dissociates to liberate protons causing intracellular acidification that most likely accounts for the epithelial injury observed. The inflammatory response initiated by acetic acid includes activation of cyclooxygenase and lipooxygenase pathways and generation of inflammatory mediators like prostaglandins and leukotrienes (Sharon and Stenson, 1985). Excess production of reactive oxygen metabolites e.g., superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid and oxidant derivatives such as N-chloramines are detected in inflamed mucosa and may be pathogenic in IBD. Hence, an anti-inflammatory agent which inhibits prostaglandin synthesis by inhibition of Cyclooxygenase (COX) enzymes mainly COX-2, so gastroprotective is very useful in the therapy of IBD.

Indomethacin, a non-selective COX inhibitor produces enterocolitis in rats on sub cuteneous administration which is characterized by linear ulceration, thickening and transmural inflammation. The mechanisms of indomethacin induced enterocolitis have not been fully illustrated, but previous reports suggest that the inhibition of protective prostaglandins PGE1, PGE2 and prostacyclin (PG12) may be one of the mechanisms by which indomethacin induces injury (Banerjee and Peters, 1990).

In addition, one of the most potential etiological and triggering factors for IBD is Oxidative stress and the role of reactive metabolites of oxygen and nitrogen in the pathophysiology of IBD has been well reported in humans as experimental models of colitis. With all these mechanisms in IBD, the inflammatory process is probably derived from the chronic presence of numerous, activated, myeloperoxidase (MPO)-containing phagocytes in the inflamed intestine. MPO, a biochemical marker of neutrophil infiltration in the damaged tissue which is an abundant heme enzyme released from storage granules following activation of neutrophils by inflammatory stimuli that catalyzes the formation of a number of reactive species. In fact, it has been reported that the production of reactive oxygen species is considerably increased in colonic biopsy specimens from UC and CD patients in comparison with normal control mucosa, in a manner positively correlated with IBD activity. This phenomenon appears to be neutrophil derived. And also there is increased lipid peroxidation that occurs in colonic tissue can initiate a vicious cycle that generate more and more reactive metabolites which exhausts cellular antioxidants and favors the consequent development of further inflammation. Thus, antioxidant therapy can constitute an interesting approach in the down regulation of this inflammatory condition and plant extracts containing antioxidant compounds such as flavonoids and other phenolic compounds may be of prime interest (Cestari *et al*., 2011).

The plant *Ageratum conyzoides* has high variability in the secondary metabolites which includes flavonoids, sterols, alkaloids, cumarins, essential oils and tannins. The ethanolic extract of *Ageratum conyzoides* contains flavonoids like kaempferol and quercetin and in addition to several other flavonoids which has gastroprotective activity (Shirwaikar *et al*., 2003). The sterols like sitosterol, stigmasterol and tannins are known to possess antioxidant activity (Nyunaii *et al*., 2009). The anti-inflammatory activity without gastric lesions led us to believe that the active principle of *Ageratum conyzoides* did not interfere with prostanoid production but they might also act by selective inhibition of COX-2 (Moura *et al*., 2005). These findings uphold the selection of the plant *Ageratum conyzoides*.

So in this study the treatment with ethanolic extract of *Ageratum conyzoides* has shown a decrease in the macroscopic scores for the inflammation. Histopathology examination of drug treated group revealed less damage compared to disease induced groups, results consistent with

Hager *et al.* (2007). Both prednisolone and ethanolic extract of *Ageratum conyzoides* retains total body weight in both the animal models and decreases the colon weight/length ratio, avoids shrinkage of the colon in acetic acid induced colitis model. A significant decrease in MPO and LPO activity was also observed, results consistent with Hager *et al.* (2007) and Jagtap *et al.* (2004), respectively; indicating infiltration of neutrophils and perturbation of the inflammatory system (Krawisz *et al.*, 1984). This fact is documented in both animal models (Akgun *et al.*, 2005) and patients with IBD (Kruidenier *et al.*, 2003). All these observations support the findings that the ethanolic extract of *Ageratum conyzoides* was able to offer significant protection in both the models studied.

The prednisolone which is proved scientifically for anti-inflammatory activity (Essay *et al.*, 2000) has shown significant protection in both the animal models under our study. The ethanolic extract of *Ageratum conyzoides* was found comparable with prednisolone. The ethanolic extract at doses 500 and 750 mg kg^{-1} was as good as prednisolone (at the doses 2 mg kg^{-1}) for rats. It is possible that the *Ageratum conyzoides* acts by the same mechanism as the prednisolone i.e., by decreasing the number of neutrophils and reduction in the synthesis of inflammatory mediators (Jagtap *et al.*, 2004).

CONCLUSIONS

From this study it may be concluded that ethanolic extract of *Ageratum conyzoides* Linn. has shown potent anti-inflammatory activity in both Indomethacin induced enterocolitis and acetic acid induced colitis. Thus this present investigation has opened avenues for the treatment of IBD from the title plant which is beneficial in IBD patients.

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