Effect of Ethanolic Extract of Seeds of *Solanum torvum* in Acetic Acid induced Ulcerative Colitis in Male Wistar Rats

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Abstract: *Objective*: To elucidate the protective effect of ethanolic extract of dried seeds of *Solanum torvum (S. torvum)* in acetic acid-induced ulcerative colitis in male Wistar rats. Methods: The animals were divided into various treatment groups (n=5). Rats were administered with 2ml of acetic acid (4%) via intrarectal route. Prednisolone was used as a standard drug and *S. torvum* was administered at a dose of 100 and 300mg/kg, p.o. Macroscopic score, colon weight to length ratio, colonic superoxide dismutase (SOD), reduced glutathione (GSH), myeloperoxidase (MPO), catalase (CAT), and lipid peroxidation (TBARS) levels and histopathological changes were recorded after the treatment regimen of 11 days.

Results: Intrarectal instillation of acetic acid caused significant ($P<0.05$) increase in colon weight to length ratio, TBARS, and MPO levels. It caused significant ($P<0.05$) decrease in the levels of SOD, CAT and GSH levels. Pretreatment with *S. torvum* (100&300mg/kg, p.o.) exhibited significant ($p<0.05$) increase in the levels of SOD, CAT and reduced GSH and significant decrease in TBARS and MPO levels. Histopathological changes showed that acetic acid treatment caused significant structural damage to colon like inflammation, ulceration with a loss of 40% mucosa which was reversed by *S. torvum* (100&300mg/kg) treatment.

Conclusion: The present investigation demonstrates the potent therapeutic value of *S.torvum* (100, 300 mg/kg, p.o.) in the amelioration of experimental ulcerative colitis in rats.

Keywords: Acetic acid, *Solanum torvum*, Ulcerative colitis, Antioxidants, Histopath.

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease, a condition affecting the gastrointestinal tract. It is an inflammatory disorder of the colonic mucosa characterized by relapses and remission [1]. The exact etiology of the disease is not known but may involve genetic and immunological influence on the gastrointestinal tract and its ability to distinguish foreign from self antigens. Several studies have reported that there is generation of free radicals which aggravate and precipitate pathological changes in colonic mucosa. Localized inflammation and generation of free radicals along with the release of inflammatory mediators have been elucidated to the damage of colonic mucosa [2]. Similar conditions are provided by rectal administration of acetic acid in laboratory animals which resembles human condition. Acetic acid induced ulcerative colitis is a reproducible animal model for the screening of drugs [3]. Other agents which induce ulcerative colitis in laboratory animals includeDextran sodium sulfate (DSS) [4]; 2,4,6-Trinitro benzenesulfonic acid (TNBS) [5]; 2,4-dinitrobenzene sulfonic acid (DNBS) [6]. Despite of a very massive research work, no specific therapy has been developed for the disease [7,8]. The present treatment regimen used to treat ulcerative colitis include aminosalicylates, corticosteroids and immunomodulators which posses a wide range of side effects [9]. Treatment withherbal medicines minimize these side effects. Polyphenols and flavonoids from medicinal plants have been shown to alleviate chronic inflammation in experimental model of ulcerative colitis [10]. *Solanum torvum* (sundakai) is a small shrub of the family Solanaceae which is commonly known as turkey berry. It is widely distributed in India, China, Phillipines and Tropical America. The fruits and leaves have a great medicinal importance. A decoction of fruits is used in case of liver and spleen enlargement [11]. The plant is sedative and diuretic and leaves are used as a haemostatic. The ripened fruits are used in the preparation of tonic and haemopoietic agents and also for the treatment of pain [12]. *Solanum torvum* contains a number of potential pharmacologically active chemicals like isoflavonoid sulfate and steroidal glycosides [13], chlorogenone and neochlorogenone [14], tri-acontane derivatives [15,16], 22-beta –O-spirostanololigoglycosides [17], 26-beta-O-glucosidase [18]. *S. torvum* also possesses antimicrobial [19,20], antiviral [21], immuno-secretory [22], antioxidant [23], analgesic and anti-inflammatory

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ISSN: 1814-8085 / E-ISSN: 1927-5129/19

[24], antiulcerogenic activities [25], cardiovascular [26], nephroprotective [27], antidiabetic [28], angiotensin and serotonin receptor blocking activities [29]. Many medicinal plants like *Bauhinia tomentosa* [30], *Cucumis sativus* [31], *Hibicusrosasinensis* [32], *Lanata camera* [33], have been reported to be effective in the treatment of Ulcerative colitis. The antioxidant property of flavonoids, their ability to inhibit inflammatory mediators and their ability to down regulate a number of cells of immune system could be effective in reducing ulcerative colitis [34]. Since *S. torvum* is known to contain a high percentage of flavonoid content of about 85.26±0.02 µg rutin equivalent/ mg of extract [35], the objective of the present investigation was to unravel the possible protective effect of *Solanum torvum* against acetic acid induced ulcerative colitis in male Wistar rats.

MATERIALS AND METHODS

Collection of Seeds

Dried seeds of *Solanum torvum* were purchased locally from the markets of Chennai, Tamil Nadu and authenticated by Dr. S.C. Pal, NDMVP Samaj's College of Pharmacy, Nashik, India. The voucher specimen (1731) has been deposited at Agharkar Research Institute, Pune, India.

Preparation of Extract

Powdered material (500gms) was defatted using petroleum ether (60-80 ˚C) for 18hrs using Soxhlet apparatus. The marc was dried and again extracted using ethanol for 18hrs. The ethanolic extract was collected and allowed to air dry to obtain the product (6% w/w). Appropriate concentrations of the extracts were made using distilled water as a vehicle.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of ethanolic extract was tested forthe presence of flavonoids, alkaloids, tannins, glycosides and saponins.

Animals

Healthy adult male Wistar rats (180-200 g) were obtained from Mahaveer enterprises, Hyderabad, India. They were maintained at 24±1 ˚C, with relative humidity of 45-55% and 12:12 dark/light cycle. The animals were acclimatized for a period of one week in animal house. Commercial pellet diet and water were provided *ad libitum*. The experiments were carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethical Committee.

Drugs and Chemicals

Prednisolone was obtained as a gift sample from Bafna Pharmaceuticals Ltd (Chennai). All the other chemicals were purchased locally.

Dosage of *S. torvum* and Standard Drugs Used

The freshly prepared ethanolic extract of *S.torvum* was administered to animals orally for 7days in two different doses (100 and 300mg/kg). On the 8th day colitis was induced by intra rectal administration of 2ml of 4% acetic acid. The oral administration of extract was continued even after administration of acetic acid. Prednisolone was used as standard drug, but was not given as pre-treatment. Prednisolone was administered at a dose of 2mg/kg orally in vehicle.

Experimental Protocol

Induction of Colitis

Colitis was induced as per Modified Millar Method [36]. 24hrs fasted rats were anaesthetized with diethyl ether.2ml of 4%acetic acid was administered into the rectum of rats using a catheter (3mm) in diameter at a distance of 8cms into colon for 30secs. After 30s acetic acid was withdrawn followed by flushing of colon using 0.9%saline.

Experimental Design

The animals were randomly divided into following experimental groups with 5 animals each.

Group 1 - Vehicle treated: received 1ml of distilled water for 11days.

Group 2 - Acetic acid treated animals: received 2ml of 4%acetic acid solution once intra rectally.

Group 3 - Prednisolone treated animals: received Prednisolone (2mg/kg, p.o., for 3days) and acetic acid (2ml of 4% solution, once, intrarectally). Prednisolone and acetic acid treatment was started on the same day.

Group 4 - Drug treated animals: Pre treated with *S. torvum* (100mg/kg) for 7days and 2ml of 4% acetic acid solution administered intra rectally on 8th day. Drug treatment was continued till 11th day.

Group 5 – Drug treated animals: Pre treated with *S.torvum* (300mg/kg) for 7days and 2ml of 4%acetic acid solution administered intra rectally on 8th day. Drug treatment was continued till 11th day.

On the 11th day animals were sacrificed and colons were collected for macroscopic study and biochemical assays. Portions of colonic specimens were kept in 10% formalin solution for histopathological studies.

Preparation of Tissue Homogenate

The colon tissue was washed with ice-cold 0.9% saline and homogenized with 0.1M tris buffer (pH -7.5) using Remi homogenizer to give 10% homogenate. The homogenate was centrifuged at 10,000rpm for 20min and supernatants were used for estimation of antioxidant enzymes.

Assesment of Colonic Damage

Macroscopic Scoring

The colon was excised and opened longitudinally, rinsed with cold saline and colonic damage was evaluated by independent observer according to scale ranging from 0 to 4 as follows [36]

0 - normal appearance, 1 - mucosal erythema only, 2 - mild oedema, slight bleeding or small erosions, 3 - moderate oedema, bleeding ulcers, 4 - severe ulcerations, erosions, oedema and tissue necrosis.

Colon Weight/Length Ratio (g/cm)

After animals were sacrificed colon was removed, gently flushed with saline placed on ice cold plate cleaned of fat and mesentery and blotted on filter paper to dry. Each colon was weighed and its length was measured [37].

Antioxidant Parameters

Superoxide Dismutase (SOD)

The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome .0.05ml supernatant was added to 2.0ml of carbonate buffer and 0.5ml of 0.01Mm EDTA solution. The reaction was initiated by addition of 0.5ml of epinephrine and autoxidation of adrenaline to adrenochrome was measured at 480nm. The change in absorbance for every minute was measured against blank. The results are expressed as unit of SOD activity (mg/wet tissue) [38].

Catalase (CAT)

The reaction mixture consisted of 2ml of phosphate buffer (pH 7.0),0.95ml of hydrogen peroxide (0.019M) and 0.05ml of supernatant in a final volume of 3ml.Absorbance was recorded at 240nm every 10sec for 1min.One unit of CAT was defined as the amount of enzyme required to decompose 1µmol of peroxide per min at 25˚c .The results were expressed as units of CAT U/g of wet tissue [39].

Reduced Glutathione (GSH)

1ml of homogenate was added to 1ml of 10% TCA and centrifuged.1ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100ml of 1% sodium citrate) and 3ml of phosphate buffer (pH-8). The color developed was measured at 412nm [40].

Lipid Peroxidation (TBARS)

0.1ml of homogenate (Tris Hcl buffer, pH 7.5) is treated with 2ml of (1:1:1) TBA-TCA-HCL reagent and placed in water bath for 15min,cooled and centrifuged at room temperature for 10min at 1000rpm. The absorbance of supernatant was measured against reference blank at 535nm [41].

Myeloperoxidase (MPO)

0.1ml of homogenate (Tris Hcl buffer, pH 7.5) was treated with equal volume of potassium phosphate buffer (pH 7.5) and was centrifuged at room temperature for 10 min at 10000 rpm. The supernatant was treated with 0.5% tetramethylbenzidine. This mixture was oxidized by MPO in presence of hydrogen peroxide and absorbance was measured at 655 nm [42].

Histopathological Examination

The colonic tissues were fixed in 10% formalin. The specimens were then processed for standard procedure and were embedded in paraffin wax in swiss roll model such that the entire area of colon is exposed without missing any lesional and normal areas of colon. The blocks were then sectioned according to hematoxylin and eosin method. Four-micrometer thick histological sections were obtained from the paraffin blocks. The sections were examined under the light microscope and photographs were taken under 40X.

Statistical Analysis

All data were expressed as the mean± SEM. For statistical analysis of the data group means were

compared by one-way analysis of variance (ANOVA) followed by Dunnett's test, P<0.05 was considered significant.

RESULTS

Preliminary Phytochemical Screening

The ethanolic extract of *S. torvum* was found to contain flavonoids, alkaloids, glycosides, tannins and saponins.

Effect of *S. torvum* on Macroscopic Scores

The colons of the rats were examined macroscopically for signs of hemorrhage and ulceration. Acetic acid treated rats showed a significant (P<0.05) increase in mean macroscopic score of (3.9±0.06) as compared to vehicle treated group. Rats pretreated with *S. torvum* (100&300mg/kg, p.o.) had significantly (P<0.05) decreased macroscopic scores (2.3±0.17 and 1.5±0.09 respectively) compared to acetic acid treated group, as did prednisolone (2mg/kg/day, p.o.) pretreated rats (2.74±0.04) (P<0.05) (Table **1**).

Effect of *S. torvum* on Colon Weight to Length Ratio

The ratio of colon weight/length was found to be increased significantly (P<0.05) in acetic acid treated group (0.165±0.01) as compared to vehicle treated group (0.078±0.01). Pretreatment with *S.torvum* (100&300mg/kg, p.o.), significantly (P<0.05) decreased the colon weight to length ratio (0.134±0.014, 0.074±0.005 respectively) as compared to acetic acid treated group in a dose dependent manner (Table **1**).

Effect of *S. torvum* on Colonic SOD

A significant decrease (P<0.05) in colonic SOD concentration was observed in acetic acid treated group [(2.30±0.23) U/mg of wet tissue] as compared with the vehicle treated group [(12.3±0.23) U/mg of wet tissue]. Pretreatment with *S. torvum* (100 and 300mg/kg, p.o) significantly (P<0.05) increased SOD content [(5.41±0.32) and (11.4±0.54) U/mg of wet tissue] as compared to acetic acid treated group in a dose dependent manner. Prednisolone also protected

Table 1: Effect of *S. torvum* (100 and 300mg/kg) on Macroscopic Scoring and Colon Weight to Length Ratio (g/cm) of Rat in Acetic Acid-Induced Ulcerative Colitis

Treatment groups	Macroscopic scoring	Colon weight to length ratio (g/cm)
Vehicle (Distilled water)	0.0±0.00	0.078±0.02
Acetic acid control (4%)	3.9±0.06*	0.165±0.06*
Prednisolone (2mg/kg)	2.7±0.04$^{\#}$	0.100±0.01$^{\#}$
S. torvum (100mg/kg)	2.3±0.17$^{\#}$	0.134±0.01$^{\#}$
S. torvum (300mg/kg)	1.5±0.05*	0.074±0.05$^{\#}$

n=5, All data analyzed by one way ANOVA followed by Dunnet's test. **P*<0.05 as compared to vehicle treated group, $^{\#}$*P*<0.05 as compared to acetic acid treated group.
(0-normal appearance; 1- mucosal erythema only; 2- mild oedema, slight bleeding or small erosions; 3-moderate oedema, bleeding, ulcers; 4-severe ulcerations, erosions, oedema and tissue necrosis).

Table 2: Effect of *S. torvum* (100 and 300mg/kg) on Various Antioxidant Parameters of rat Colon in Acetic Acid Induced Ulcerative Colitis

Treatment groups	SOD (U/mg of wet tissue)	CAT (U/mg of wet tissue)	Reduced GSH (µg GSH/mg of wet tissue)	TBARS (n moles/ mg of wet tissue)	MPO (U/mg of wet tissue)
Vehicle	12.3±0.52	0.53±0.042	22.8±2.899	24.2±0.30	5.19±0.25
Acetic acid (2ml, 4%v/v)	2.30±0.23*	0.28±0.09*	10.8±0.36*	68.2±1.31*	20.3±0.43*
Prednisolone (2mg/kg)	9.31±0.27$^{\#}$	0.41±0.05	17.6±2.11$^{\#}$	31.2±0.79$^{\#}$	7.60±0.29$^{\#}$
S. torvum (100mg/kg)	5.41±0.32$^{*\#}$	0.38±0.01	13.9±0.27*	54.2±1.02$^{*\#}$	15.7±0.37$^{\#}$
S. torvum (300mg/kg)	11.4±0.54$^{\#}$	0.64±0.01$^{\#}$	21.4±0.40$^{\#}$	42.9±1.82$^{*\#}$	10.7±0.39$^{*\#}$

n=5, all data analyzed by one way ANOVA followed by Dunnet's test. **P*<0.05 as compared to vehicle treated group, $^{\#}$*P*<0.05 as compared to acetic acid group.

SOD depletion induced by acetic acid [(9.31±0.27) U/mg of wet tissue] (Table **2**).

Effect of *S. torvum* on Colonic CAT

A significant decrease (P<0.05) in colonic CAT concentration was observed in acetic acid control group [(0.28±0.09) U/mg of wet tissue] as compared with the vehicle treated group [(0.53±0.42) U/mg of wet tissue]. Pretreatment with *S.torvum* (100 and 300mg/kg, p.o) significantly (P<0.05) increased CAT content [(0.38±0.01) and (21.4±0.40) U/mg of wet tissue] as compared with acetic acid treated group in a dose dependent manner. Prednisolone protected against CAT depletion induced by acetic acid [(0.41±0.05) U/mg of wet tissue] (Table **2**).

Effect of *S. torvum* on Colonic Reduced GSH

A significant decrease (P<0.05) in colonic reduced GSH concentration was observed in acetic acid treated group [(10.8±0.36) µg/mg of wet tissue] as compared with the vehicle treated group [(22.8±2.86) µ g/mg of wet tissue]. Pretreatment with *S. torvum* (100 and 300mg/kg, p.o) significantly (P<0.05) increased GSH levels [(13.9±0.27) and (21.4±0.40) µg/mg of wet tissue] as compared to acetic acid treated group in a dose dependent manner. Prednisolone also protected GSH depletion induced by acetic acid [(17.6±2.11) µg/mg of wet tissue] (Table **2**).

Effect of *S. torvum* on Colonic TBARS

A significant increase (P<0.05) in colonic TBARS concentration was observed in acetic acid treated group [(68.2±1.31) n moles/mg of wet tissue] as compared with vehicle treated group. Pretreatment with *S. torvum* (100 and 300mg/kg, p.o) significantly (P<0.05) decreased TBARS levels [(54.2±1.02) and (42.9±1.82) n moles/mg of wet tissue] as compared to acetic acid treated group in a dose dependent manner. Prednisolone protection against the elevated TBARS levels induced by acetic acid treatment [(31.2±0.79) n moles/mg of wet tissue] (Table **2**).

Effect of *S. torvum* on Colonic MPO

A significant increase (P<0.05) in colonic MPO concentration was observed in acetic acid treated group [(20.3±0.43) U/mg of wet tissue] as compared with vehicle treated group. Pretreatment with *S. torvum* (100 and 300mg/kg, p.o) significantly (P<0.05) decreased MPO levels [(15.7±0.37) and (10.7±0.27) U/mg of wet tissue] as compared to acetic acid treated group in a dose dependent manner. Prednisolone provided protection against the elevated MPO levels induced by acetic acid treatment [(7.60±0.29) U/mg of wet tissue] (Table **2**).

Histopathological Results

Histopathological examination (40X) of colon tissue of rats treated with acetic acid (4%) showed significant cell inflammation with 40% loss of mucosa whereas *S. torvum* (100&300 mg/kg) treated rats showed prominent round cell collection in mucosa and sub mucosa all through and lymphoid hyperplasia and showed near normal architecture (Figure **1**).

DISCUSSION

Ulcerative colitis is a disorder of GIT with unknown etiology. Although the etiology is unknown the various factors that may lead to UC include multiple immune, genetic and environmental factors which are responsible for initiation and progression of the disease [43]. There is evidence for intense local immune response followed by release of reactive oxygen species (ROS) and other inflammatory mediators resulting in tissue damage [44].

In the present study, ethanolic extract of *S. torvum* (100 & 300mg/kg) has significantly (P<0.05) decreased colon length to weight ratio, TBARS, and MPO levels. It has also significantly (P<0.05) increased the levels of SOD, CAT and GSH levels and reversed the histopathological changes induced by acetic acid treated group. Thus ethanolic extract of *S.torvum* has ameliorated the effects of acetic acid induced Ulcerative colitis in male Wistar rats as proven by its morphological, biochemical and histopathological data. The wet weight of colon is regarded as a reproducible parameter indicating degree of inflammation in colon. The elevation in colon weight to length ratio was inhibited by *S. torvum* depicting its healing property. The gross morphological lesions characterized by ulcer and necrotic area of various sizes were healed indicating protection of microflora from corrosive effects of acetic acid by *S. torvum*.

One of the important intestinal tissue damage mechanisms is oxidative stress through an excessive production of reactive oxygen species that include superoxide anion, hydrogen peroxide, hypochlorous acid and hydroxyl radical [45]. The antioxidant enzymes are endogenous systems responsible for detoxification and neutralization of reactive species [46]. SOD is a key enzyme which inactivates

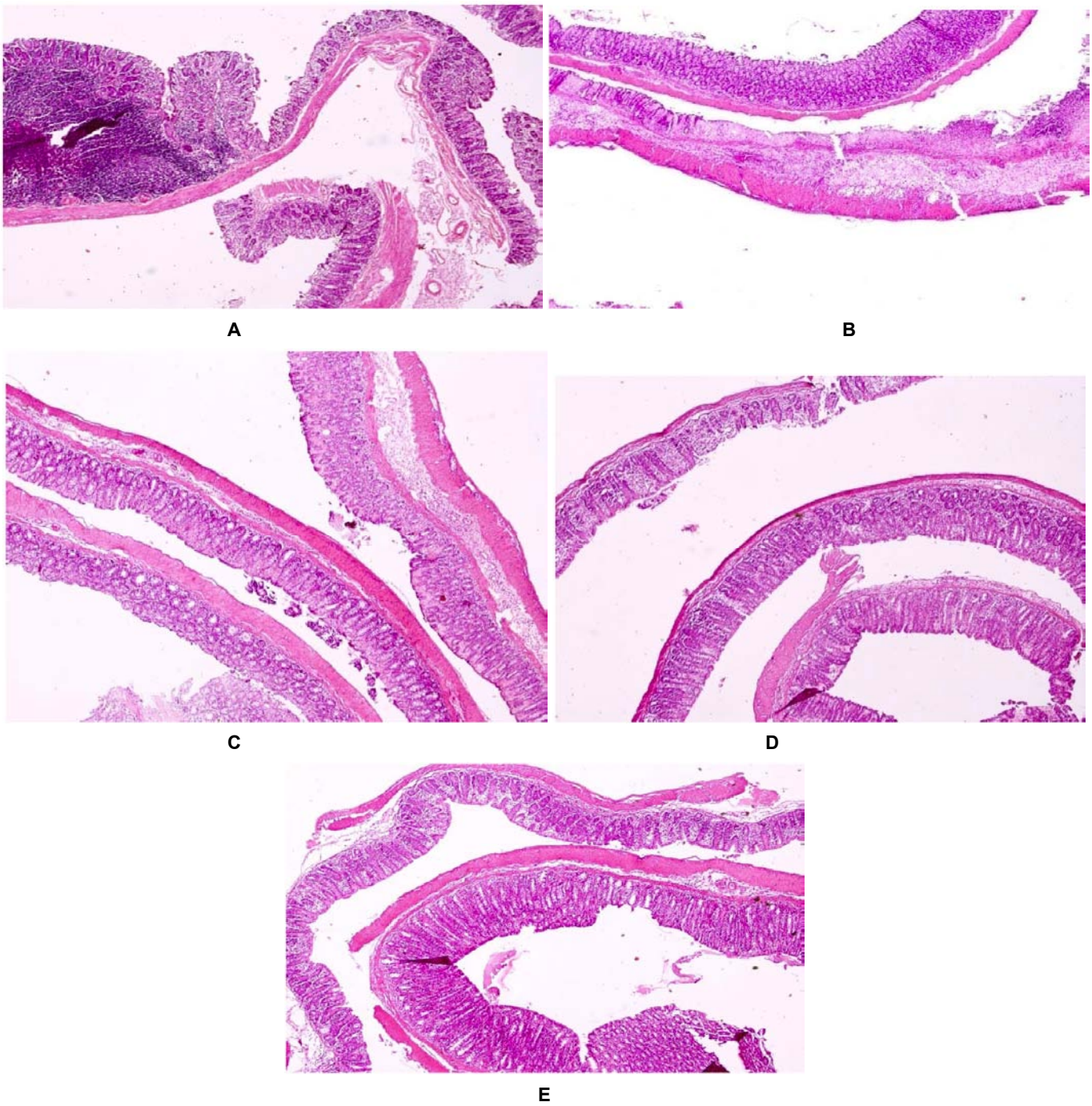

Figure 1: Photomicrographs of histopathological examination (40X) of colon tissue. Section **A**) Control group treated with vehicle shows prominent round cell collection in mucosa and submucosa all through and lymphoid hyperplasia focally, section **B**) Group treated with acetic acid (4% v/v) shows significant cell inflammation with 40% loss of mucosa, **C**) Group treated with prednisolone (2mg/kg) shows scattered round cell collection in mucosa and submucosa, Section **D**) Group treated with extract (100mg/kg) shows prominent round cell collection in mucosa and submucosa all through and lymphoid hyperplasia focally, Section **E**) Group treated with extract (300mg/kg) shows prominent round cell collection in mucosa and submucosa all through and lymphoid hyperplasia focally.

superoxide ion by transforming it into more stable metabolite H2O2- which is converted to water [30]. The SOD level was found to be significantly ($P<0.05$) decreased in Ulcerative colitis. Treatment with *S. torvum* (100&300mg/kg) increased the colonic SOD in a dose dependent manner.

Catalase is an important peroxisomal antioxidant enzyme that metabolizes H2O2 into oxygen and water. It prevents the harmful effects of oxidative radicals and initiation process of lipid peroxidation [47]. The activity of CAT was significantly lower in experimental colitis group as compared to vehicle treated group.

Decreased CAT activity may relate to enzyme consumption due to neutralization of excessive H2O2 in colonic tissue. The tissue CAT levels were significantly (P<0.05) increased in *S. torvum* (100&300mg/kg) treated group compared to acetic acid induced colitis group in a dose dependent manner.

Glutathione (GSH) is a tripeptide and a powerful antioxidant present within the cytosol of cells. It is important in maintaining SH groups in other molecules including proteins and detoxifying foreign compounds and free radicals [48,49]. Treatment with *S.torvum* (100&300mg/kg) significantly (P<0.05) increased colonic GSH levels in a dose dependent manner when compared to levels in acetic acid induced colitis rats.

Colitis induced by acetic acid is known to produce excess ROS [36]. These generated free radicals are involved in lipid peroxidation which leads to oxidative degradation of lipids and cell damage. Enhanced lipid peroxidation is seen in acetic acid treated group which was significantly (P<0.05) reversed by *S. torvum* (100&300mg/kg) in a dose dependent manner.

Myeloperoxidase (MPO) is a peroxide enzyme found in azurophilic granules of neutrophils. Upon activation and degranulation, MPO is released which results in formation of potent oxidants. It enhances the cytotoxic activity of H2O2, catalyzes the reaction of chloride with H2O2 to produce hypochlorous acid which is important for the inflammatory property of neutrophils [50]. This leads to tissue injury and causes some clinical features of UC. MPO levels which were high in acetic acid treated group was significantly (P<0.05) reduced by treatment with *S. torvum* (100&300mg/kg) in a dose dependent manner.

The histopathological changes in colon showed ulceration with 40% loss of mucosa and inflammation in acetic acid treated group. Pretreatment with *S. torvum* (100&300mg/kg) restored histopathological changes in colon.

In general it was reported that the antioxidant property of medicinal plants could be attributed to the presence of flavonoid phytoconstituent [51]. Since oxidative stress is associated with ulcerative colitis flavonoids indeed contribute a majortheurapeutic role in the treatment of ulcerative colitis. The flavonoid concentration was found to be abundant (85.26±0.02 µg rutin equivalent/mg of extract) in seeds of *Solanum torvum*.

In view of the above, the protective effect of ethanolic extract of seeds of *Solanum torvum* against acetic acid induced ulcerative colitis could be attributed to the presence of flavonoids. However further work on isolation and characterization of flavonoid constituent responsible for healing ulcerative colitis is required.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

ACKNOWLEDGEMENT

The authors acknowledge Dr.P.Udaykumar, Scientist E (Deputy Director) & HOD, Pathology Division, NIN (ICMR), Tarnaka, Hyderabad, Andhra Pradesh, India for his guidance to provide histopathology report.

REFERENCES

[1] Langholz E, Munkholm P, Davidsen M, Binder V. Course of Ulcerative Colitis: Analysis of Changes in Disease Activity Over Years. Gastroenterology 1994; 107: 3-11. https://doi.org/10.1016/0016-5085(94)90054-X

[2] Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of Inflammatory bowel disease. Gastroenterology 2011; 140(6): 1756-1767. https://doi.org/10.1053/j.gastro.2011.02.016

[3] Baugmart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. Lancet 2007; 369(9573): 1627-1640. https://doi.org/10.1016/S0140-6736(07)60750-8

[4] Kawada M, Arihiro A, Mizoguchi E. Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. World J Gastroenterol 2007; 13: 5581-5593. https://doi.org/10.3748/wjg.v13.i42.5581

[5] Li XL, Cai YQ, Qin H, Wu YJ. Theurapeutic effect and mechanism of proanthocyanidins from grape seeds in rats with TNBS-induced ulcerative colitis. Can J Physiol Pharmacol 2008; 86: 841-849. https://doi.org/10.1139/Y08-089

[6] Hawkins JV, Emmel EL, Feuer JJ, Nedelman MA, Harvey CJ, Klein HJ, Rozmiarek H, Kennedy AR, Lichtenstein GR, Billings PC. Protease activity in a hapten- induced model of ulcerative colitis in rats. Dig Dis Sci 1997; 42: 1969-1980. https://doi.org/10.1023/A:1018887832465

[7] Patil MVK, Kandhare AD, Bhise SD, Bhale S. A comparative cross sectional descriptive market survey of various brands of levofloxacin. Asian J Pharm Biol Res 2011; 1(2): 174-181.

[8] Patil MVK, Kandhare AD, Bhise SD. Evaluation of patient information leaflets on basis of consumer Psychology and opinion. Int J Phar Pharm Sci Res 2011; 1(2): 87-92. https://doi.org/10.4103/2229-5186.93026

[9] Joshi R, Kumar S, Unnikrishnan M, Mukherjee T. Free radical scavenging reactions of Sulfasalazine, 5-Amino salicylic acid and Sulfapyridine: mechanistic aspects and antioxidant activity. Free Radic Res 2005; 39: 1163-1172. https://doi.org/10.1080/10715760500177880

[10] Rahimi R, Mozaffari S, Abdollahi M. On the use of herbal medicines in management of inflammatory bowel diseases: a systemic review of animal and human studies. Dig Dis Sci 2009; 54: 471-480. https://doi.org/10.1007/s10620-008-0368-x

[11] Simonsma JS, Piluek K. Plant resources of South-East Asia 8 (PROSEA). Indonesia: Bogor, 1994; pp. 412.

[12] Kala CP. Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of Indian. J Ethno and Ethnomed 2005; 1: 1-8. https://doi.org/10.1186/1746-4269-1-1

[13] Yahara S, Yamashita T, Nozawa N, Nohara T. Steroidal glycosides from *Solanum torvum*. Phytochemistry 1996; 43: 1069-1074. https://doi.org/10.1016/S0031-9422(96)00396-2

[14] Carabot CA, Blunden G, Patel VA. Chlorogenone and neochlorogenone from the unripe fruits of *Solanum torvum*. Phytochemistry 1991; 30: 1339-1341. https://doi.org/10.1016/S0031-9422(00)95233-6

[15] Mahmood U, Shukla YN, Thakur RS. Non-alkaloidal constituents from *Solanum torvum* leaves. Phytochemistry 1983; 22: 167-170. https://doi.org/10.1016/S0031-9422(00)80080-1

[16] Mahmood U, Agrawal PK, Thakur RS. Torvonin-A, a spirostane saponin from *Solanum torvum* leaves. Phytochemistry 1985; 24: 2456-2457. https://doi.org/10.1016/S0031-9422(00)83069-1

[17] Iida Y, Yanai Y, Ono M, Ikeda T, Nohara T. Three unusual 22β- O-23-hydroxy(5α) –spirostanol glycosides from the fruits of *Solanum torvum*. Chem Pharm Bull 2005; 53: 1122-1125. https://doi.org/10.1248/cpb.53.1122

[18] Arthan D, Svasti J, Kittakoop P, Esen A, Svasti J. Furostanol glycoside 26 –O-β glucosidase from leaves of *Solanum torvum*. Phytochemistry 2006; 67: 27-33. https://doi.org/10.1016/j.phytochem.2005.09.035

[19] Ajaiyeoba EO. Comparative phytochemical and antimicrobial studies of *Solanum macrocarpum* and *Solanum torvum* leaves. Fitoterapia 1999; 70: 184 - 186. https://doi.org/10.1016/S0367-326X(98)00041-0

[20] Chah KF, Muko KN and Oboegbulem SI. Antimicrobial activity of methanolic extract of *Solanum torvum* fruit. Fitoterapia 2000; 71: 187-189. https://doi.org/10.1016/S0367-326X(99)00139-2

[21] Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M and Thebtaranonth Y. Anti-viral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. Phytochemistry 2002; 59: 459-463. https://doi.org/10.1016/S0031-9422(01)00417-4

[22] Israf DA, Lajis NH, Somchit MN and Sulaiman MR. Enhancement of ovalbumin-specific IgA responses via oral boosting with antigen co-administered with an aqueous *Solanum torvum* extract. Life Sci 2004; 75: 397-406. https://doi.org/10.1016/j.lfs.2003.10.038

[23] Siva Priya M, Srinivas L. Isolation and purification of novel antioxidant from the water extract of Sundakai (*Solanum torvum*) seeds. Food Chem 2007; 104: 510-517. https://doi.org/10.1016/j.foodchem.2006.11.060

[24] Ndebia EJ, Kamga R and Nchunga-AnyeNkeh B. Analgesic and anti-inflammatory properties of aqueous extract from leaves of *Solanum torvum* (Solanaceae). AJTCAM 2007; 4: 240-244. https://doi.org/10.4314/ajtcam.v4i2.31214

[25] Telesphore BN, Catherine BF, Gilbert A, Pierre W, Simplice T, Albert DA, Pierre T, Albert K. Anti-ulcerogenic properties of the aqueous and methanolic extracts from the leaves of *Solanum torvum* (Solanaceae) in rats. J Ethnopharmacol 2008; 119: 135-140. https://doi.org/10.1016/j.jep.2008.06.008

[26] Mohan M, Jaiswal BS and Kasture S. Effect of *Solanum torvum* on blood pressure and metabolic alterations in fructose hypertensive rats. J Ethnopharmacol 2009; 126: 86-89. https://doi.org/10.1016/j.jep.2009.08.008

[27] Mohan M,Kamble S, and Kasture S. Protective effect of *Solanum torvumon* doxorubicin-induced nephrotoxicity in rats. Food and Chem Toxico 2010; 48: 436- 440. https://doi.org/10.1016/j.fct.2009.10.042

[28] Gandhi GR, Ignacimuthu S, Paulraj MG and Sasikumar P. Antihyperglycemic activity and antidiabetic effect of methyl caffeate isolated from *Solanum torvum* Swartz. fruit in streptozotocin induced diabetic rats. Eur JPharmacol 2011; 30(23): 623-31. https://doi.org/10.1016/j.ejphar.2011.09.159

[29] Jaiswal BS and Mohan M. Effect of *Solanum torvum* on the contractile response of isolated tissues preparation in fructose fed rat. Int J Pharm Bio Sci 2012; 3(3): 161 - 169.

[30] Narayanan Kannan, Chandrasekharan Guruvayoorappan. Protective effect of Bauhinia tomentosa on acetic acid induced ulcerative colitis by regulating antioxidant and inflammatory mediators. IntImmuno Pharmacol 2013; 16: 57-66. https://doi.org/10.1016/j.intimp.2013.03.008

[31] Mithun Vishwanath K Patil, Amit D Kandhare, Sucheta D Bhise. Effect of aqueous extract of Cucumis sativus Linn. Fruit in ulcerative colitis in laboratory animals. Asian Pac J Trop Biomed 2012; S962-S969. https://doi.org/10.1016/S2221-1691(12)60344-X

[32] Amit D Kandhare, Kiran S Raygude, Pinaki Ghosh, Arvindkumar E Ghule, Tejas P Gosavi, Sachin L Badole, Subhash L Bodhankar. Effect of hydroalcoholic extract of Hibiscus rosasinensisLinn. Leaves in experimental colitis in rats. Asian Pac J Trop Biomed 2012: 337-344. https://doi.org/10.1016/S2221-1691(12)60053-7

[33] Thamotharan G, Sekar G, Ganesh T, Saikat Sen, Raja Chakraborty, Senthil Kumar N. Anti-ulcerogenic effects of Lanta camara Linn. Leaves on *in vitro* models in rats. Asian J Pharm Clin Res 2010; 3: 57-60.

[34] Vaclavikova R, Kondrova E , Ehrilchova DM, Boumendjel A, Kovar J, Stopka P, et al. The effect of flavonoid derivatives on doxorubicin transport and metabolism. Biorg Med Chem 2008; 16: 2034-2042. https://doi.org/10.1016/j.bmc.2007.10.093

[35] Woisky R, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. J Agri Res 1998; 37: 99-105. https://doi.org/10.1080/00218839.1998.11100961

[36] Millar AD, Rampton DS, Chander CL, Claxson AWD, Blades S, Coumbe A, Marris CJ, Blake D. Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using rat model of colitis. Gut 1996; 39: 407-415. https://doi.org/10.1136/gut.39.3.407

[37] Hossam MM, Ramadan A, Hemeida, Ali IM, Farid MA. Prophylactic role of Curcumn in dextran sulphate sodium (DSS) induced ulcerative colitis murine model. Food Chem Toxicol 2009; 47: 1311-1317. https://doi.org/10.1016/j.fct.2009.03.003

[38] Saggu H, Cooksey J, Dexter DA. Selective increase in particular superoxide dismutase activity in parkinsonian subtansianigra. J Neurochem 1989; 53: 692-697. https://doi.org/10.1111/j.1471-4159.1989.tb11759.x

[39] Beers JR, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952; 115: 133-140.

[40] Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959; 82: 70-77. https://doi.org/10.1016/0003-9861(59)90090-6

[41] Niehaus WG, Samuelsson B. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968; 82: 70-77.

[42] Bradley PD, Friebal DA, Christensen RD. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982; 78: 206-209. https://doi.org/10.1111/1523-1747.ep12506462

[43] Strober W. Interactions between epithelial cells and immune cells in the intestine. Ann N Y Acad Sci 1998; 859(17): 37-45. https://doi.org/10.1111/j.1749-6632.1998.tb11109.x

[44] Sunkara S, Swanson G, Forsyth CB, Keshavarzian A. Chronic inflammation and malignancy in ulcerative colitis. Ulcers 2011; 1-8. https://doi.org/10.1155/2011/714046

[45] Kruidenier L, Verspaget HW. Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease–radicals or ridiculous? Aliment Pharmacol Ther 2002; 16: 1997-2015. https://doi.org/10.1046/j.1365-2036.2002.01378.x

[46] Kumar KS, Vaishnaw YN, Weiss JF. Radioprotection by anti-oxidant enzyme activity in aspirin-treated rats. Gen Pharmacol 1988; 26(3): 613-617. https://doi.org/10.1016/0306-3623(94)00234-E

[47] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine 2nd ed., Oxford: Clarendon press 1999.

[48] Mahgoub AA, El-Medany AA, Hager HH, Mustafa AA, El-Sabah DM. Evaluating the prophylactic potential of zafirlukast against the toxic effects of acetic acid on the rat colon. Toxicol Lett 2003; 145: 79-87. https://doi.org/10.1016/S0378-4274(03)00269-8

[49] Demir S, Yilmaz M, Koseoglu M, Aydin A. Role of free radicals in peptic ulcer and gastritis. Turk J Gastroenterol 2003; 14: 39-43.

[50] Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, van derVliet A. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. Nature 1998; 391: 393-397. https://doi.org/10.1038/34923

[51] Coelho RG, Batista LM, Santos LC, Brito ARMS, Vilegas W. Phytochemical study and antiulcerogenic activity of Syngonanthusbisulcatus (Eriocaulaceae) Brazilian. J Pharmaceut Sci 2006; 42(3): 413-417. https://doi.org/10.1590/S1516-93322006000300010

Received on 04-12-2019 Accepted on 21-12-2019 Published on 24-12-2019

https://doi.org/10.29169/1927-5129.2019.15.08