IJPSR (2021), Volume 12, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 January 2020; received in revised form, 09 March 2020; accepted, 11 March 2020; published 01 January 2021

EFFECT OF HYDROALCOHOLIC EXTRACT OF *SIDA SPINOSA* L. ON 2,4,6-TRINITRO-BENZENESULFONIC ACID INDUCED ULCERATIVE COLITIS IN RATS

A. V. Kulkarni [*1] and N. S. Vyawahare [2]

Department of Pharmacology [1], Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, Savitribai Phule Pune University, Pune - 411044, Maharashtra, India.
Dr. D. Y. Patil College of Pharmacy [2], Akurdi, Pune, Savitribai Phule Pune University, Pune - 411044, Maharashtra, India.

Keywords:

Sida spinosa L., 2,4,6-trinitrobenzenesulfonic acid (TNBS), ulcerative colitis, Inflammatory bowel disease

Correspondence to Author:
A. V. Kulkarni

Assistant Professor,
Department of Pharmacology,
Dr. D. Y. Patil College of Pharmacy,
Savitribai Phule Pune University,
Pune - 411044, Maharashtra, India.

E-mail: ashishvk1@gmail.com

ABSTRACT: The present study aimed to evaluate the effect of hydroalcoholic extract of *Sida spinosa* L. (HYSS) in colitis induced in rats by intrarectal administration of TNBS by clinical, morphological and biochemical alterations. The HYSS administered at three different concentrations 100, 200 and 400 mg/kg, p.o. and sulfasalazine (50 mg/kg, p.o) as reference standard for 10 days in colitis induced rats. TNBS administration caused induction of colitis resulting in significant reduction in percentage body weight, increased stool consistency score, macroscopic score, colon weight, weight to length ratio, ulcer area, ulcer index *etc*. It also caused elevation of oxidative stress *i.e.* increased MDA, MPO level and depleted GSH level. It also resulted in histological changes in colon architecture suggestive of extensive mucosal damage associated with intermittent inflammatory changes and infiltration of inflammatory cells in mucosa and submucosa. HYSS at 200 & 400 mg/kg significantly restores loss of percent body weight, reduced stool consistency score, ameliorate macroscopic changes, histological changes, colon weight to length ratio, ulcer index, reduced MPO, MDA level and restores GSH level when compared to TNBS induction control group. Results of present study indicates the anti-inflammatory and immunomodulatory potential of HYSS to heal TNBS induced colitis in rats.

INTRODUCTION: Inflammatory bowel disease (IBD) is a chronic immune-inflammatory disorder of the gastrointestinal tract. It consists of Crohn's disease (CD) involving inflammation of small and large bowel and ulcerative colitis (UC) characterize by severe inflammation of the large bowel. The common symptoms are recurrent diarrhea, abdominal pain and some may experience complications like deep ulcerations, bowel obstruction, infections, anemia, weight loss, malnutrition, colon cancer, *etc*. [1]

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(1).450-58

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).450-58

Although the etiology is unknown, evidence from reported literature suggested important features of IBD inflammation are mediated by cells of the acquired immune system like overly aggressive T-cell responses against environmental factors, altered proinflammatory cytokines such as TNF-α, interleukins (IL), anti-inflammatory cytokines (IL-4 and IL-10), glycosaminoglycan content in gastric mucosa, increased oxidative stress, intestinal permeability, *etc*. [2] The incidences and prevalence of IBD have increased worldwide, affecting approximately 0.5% of the general population within the age range of 10 and 30 years [3]. Apart from regular clinical manifestations, loss of education, difficulty in gaining employment, associated psychological alterations are other major issues that suggest immediate address for this complaint.

The conventional treatment of IBD includes the use of anti-inflammatory drugs (*e.g.* 5-aminosalicylic acid), glucocorticoids, immunosuppressants (*e.g.* azathioprine, mercaptopurines, cyclosporine *etc*.), anti-TNF-α agents *etc*. [4] Treatment of IBD is long term treatment [5]; the selection of specific or combination therapy for IBD depends upon disease severity, and the nature and extent of inflammation [6]. Hence, even though many options are available for management of IBD, inconvenient dosing regimen, associated severe side effects like nephrotoxicity, hepatotoxicity, bone marrow depression, hypertension, myalgia, *etc*. as well as the cost of IBD therapy are excessively high, particularly treatment with biologic agents [7]. Therefore, there is a wide scope to discover drugs having efficacy and lower side effects from an alternative system of medicine for the treatment of IBD [8]. Indian traditional system of medicine, *i.e.*, Ayurveda, identified several herbs and plant medicines to treat GI tract disorders. In support of the traditional claims as gastroprotective for these herbs and their active constituents, several preclinical and clinical studies have provided the scientific basis for their effectiveness in treating GI tract disorders [9].

Sida spinosa Linn. (Malvaceae), traditionally claimed as Rasayana plant [10] has been scientifically validated for various activities like antibacterial [11], antioxidant [12], hypoglycemic [13], anti-hyperglycemic and anti-hyperlipidemic [14], wound healing [15], antiulcer [16] suggesting authenticity of it's traditional claims. However, its therapeutic usefulness against IBD has not been experimentally evaluated. Therefore, the present study was carried out to evaluate the potential of hydroalcoholic extract of *Sida spinosa* L. against TNBS-induced experimental colitis in rats.

MATERIALS AND METHODS:

Drugs and Chemicals: 2,4,6-Trinitrobenzene-sulfonic acid (TNBS) and other chemicals purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Sulfasalazine was provided by Symed Pharmaceuticals Pvt. Ltd, Hyderabad. All other chemicals purchased were of analytical grade.

Plant Material: The aerial part of the *Sida spinosa* Linn. were procured from regions of Tirupati, Andhra Pradesh, and authenticated by botanist, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India (Voucher no. 928).

Preparation of Extracts: The plant material shade dried and coarsely powdered. The powder material (500g) was macerated using a solvent hydroalcoholic mixture (70:30) in 1:8 ratio for 72 h at room temperature. The residue was removed by filtration; the solvent was then evaporated under reduced pressure in a rotary evaporator at 42-45 °C. The concentrated extract was transferred to Petri dishes and dried in a vacuum oven at 40 °C. The solid extract was scraped before complete drying and then dried to a constant weight. The percentage yield was 20%, and the extract was kept in an airtight container until used.

Preliminary Phytochemical Analysis of Extract: HYSS further subjected to preliminary phyto-chemical analysis using standard procedures to record the presence of different classes of phytoconstituents [17].

Animals: Wistar rats weighing 200 to 250 gm of either sex were procured from Lacsmi Biofarm Pvt. Ltd., Pune, and housed in polypropylene cages at 25 ± 2 °C temperature with 60% relative humidity and kept under 12:12 h light-dark cycles. They were fed with standard pellet diet (Nutrivet Life Sciences, Pune) and water *ad libitum*. The rats were allowed to acclimatize to laboratory conditions prior to experimentation. All procedures were carried out in the daylight period. The experiment protocol was approved by the Institutional Animal Ethics Committee (IAEC) (DYPCOP/IAEC/ 2017/01), and care of animals was taken as per guidelines of the Committee for Control and Supervision of Experimentation on Animals (CPCSEA).

Acute Oral Toxicity Study of Extracts: The acute toxicity study for hydroalcoholic extract (HYSS) was performed in female rats using OECD guideline no. 423 [18]. At the limit dose of 2000 mg/kg, no mortality or behavioral changes recorded during the observation period. The dose of 100 mg/kg, 200 mg/kg, and 400 mg/kg dose were selected for further study.

Induction of Colitis and Drug Treatment Schedule: Colitis was induced as per the procedure described by Sadar *et al.*, [19], after some modifications. Before the experiment, 36 rats (who were kept fasting for 24 h with free access to water) were divided into six groups, each consisting of 06 rats. All the rats from group II to VI were anesthetized with ether and subjected to administration of 0.25 ml of TNBS dissolved in ethanol (50%) at 120 mg/ml using 3.5 F polyethylene catheter up to 8 cm proximal to the anus. To distribute TNBS into colon and cecum, rats were held the vertical position for 30 sec after injection. Rats from the group I received 0.25 ml of ethanol (50%) intra-rectally. After TNBS instillation, respective treatment was given for the next 10 day as follows:

Group-I: Vehicle Control: Received 0.25ml ethanol (50%), once intra rectally on day 1, and distilled water 1ml/100g, p.o, from day 1 to day 10.

Group-II: TNBS Induced Control: Received 0.25ml TNBS dissolved in ethanol (50%) at 120mg/ml, once intra rectally on day 1 and distilled water 1ml/100g, p.o, from day 1 to day 10.

Group-III: TNBS induced and Sulfasalazine: Received 0.25ml TNBS dissolved in ethanol (50%) at 120mg/ml, once intra rectally on day 1 and Sulfasalazine (50 mg/kg, p.o) from day 1 to day 10.

Group-IV: TNBS + HYSS-I: Received 0.25ml TNBS dissolved in ethanol (50%) at 120mg/ml, once intra rectally on day 1 and HYSS-100mg/kg, p.o, from day 1 to day 10.

Group-V: TNBS + HYSS-II: Received 0.25ml TNBS dissolved in ethanol (50%) at 120 mg/ml, once intra rectally on day 1 and HYSS 200 mg/kg, p.o, from day 1 to day 10.

Group-VI: TNBS+HYSS-III: Received 0.25ml TNBS dissolved in ethanol (50%) at 120mg/ml, once intra rectally on day 1 and HYSS-400mg/kg, p.o from day 1 to day 10.

Assessment of Colitis:

Body Weight: Bodyweight of each animal from all the groups was recorded daily. Change of body weight and percent weight loss was calculated.

Stool Consistency: Stool consistency of each animal was observed daily and scored as previously reported by Saiyed *et al.*, [20] on scale 0 to 3 as follows; 0:- Normal stool (well-formed pellets), 1: soft stool but still formed, 2: Very soft stool, 3: Diarrhea

On day 11, rats were euthanized under ether anesthesia; the colon was dissected and washed with ice-cold saline, removed fecal matter, and adherent tissue. Colon damage assessed by measuring length, weight, and macroscopic score. Colon edema measured by calculating the weight/length ratio. The tissue immediately stored at -80 °C for further biochemical examination. For histopathological studies, a part of the freshly excised colon of two animals from each group washed with saline and preserved in a 10% formaldehyde solution.

Macroscopic Score: Isolated colons were examined for signs of inflammation by an independent observer. The severity of inflammation observed macroscopically and scored according to a scale ranging from 0 to 4: Score -0:- No macroscopic changes (Normal Mucosa), Score-1:- Mild hyperemia (Mucosal erythema only), Score-2:- Moderate Hyperemia (Mild mucosal edema with slight bleeding or small erosions), Score -3:- Severe Hyperemia (Moderate edema with bleeding ulcer or erosions < 40%), Score-4:- Severe hyperemia, necrosis, and an ulcer (Severe ulceration, erosion edema extending > 40%) [21].

Ulcer Index: [22] The ulcer index was calculated by using the following formula:

Ulcer Index (UI) = Total area of ulcer (mm^2) × 100 / Total area of colon (mm^2)

And the percentage (%) inhibition was calculated using the formula-

Percentage (%) inhibition = ^{UI}(TNBS induced Control) - ^{UI}(Treated) × 100 / ^{UI}(TNBS induced Control)

Biochemical Examination:

Determination of Colonic Myeloperoxidase (MPO) Content: The colonic MPO assay method described by Krawisz *et al.*, [23] carried out as a marker of neutrophil infiltration. Colon tissue removed from -80 °C and placed on ice. 0.5 cm of the colonic segment was homogenized in MPO homogenization buffer (0.5% hexadecyltrimethyl-ammonium bromide in 50 mM potassium phosphate buffer (pH 6.0) to give a 50 mg colon segment/ml of homogenization buffer suspension.

Homogenized solution aliquoted into 1ml portions and centrifuge at 4 °C for 2 min at 10,000 × g. In a cuvette, mixed 0.1 ml aliquot and 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/ml of o-dianisidinedihydrochloride and 0.0005% hydrogen peroxide. Absorbance was measured using a spectrophotometer at 460 nm. MPO activity is defined as the quantity of enzyme degrading 1µmol/min of H_2O_2 to H_2O at room temperature and is expressed in units per mg of tissue.

Determination of Colonic GSH Contents: The colonic GSH assay was performed as per the method previously described by Moron *et al.* [24] Briefly, tissue samples 100 mg homogenized in ice-cold trichloroacetic acid (10%) in a tissue homogenizer. The precipitate was centrifuged at 3000 rpm for 10 min. After centrifugation, 0.5 ml supernatant mixed with 0.2 ml of 0.4 M disodium hydrogen phosphate solution and 2 ml of dithiobisnitrobenzoate (DTNB) (0.4 mg/ml in 1% sodium citrate). The final volume was made upto 3 ml with phosphate buffer. The absorbance was measured using a double beam spectrophotometer at 412 nm immediately after mixing. The amount of reduced GSH was expressed as µg of GSH/mg protein.

Determination of Colonic MDA Contents: MDA levels in the colon tissue were determined by the method of Oshakawa *et al*. [25] Briefly, tissue homogenate was prepared in 5% ice-cold trichloroacetic acid (TCA). To 1 ml of homogenate 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added, and this was incubated at 95 °C for 30 min. The mixture is immediately cooled on ice and centrifuged at 4000 rpm for 10 min. and absorbance of the supernatant was read at 532 nm. The values were expressed as nmol of MDA/mg protein.

Histology: Colon samples were fixed in 10% buffered formalin, paraffin-embedded, sectioned (4µm thick), and stained with hematoxylin and eosin. All tissue sections were examined under a light microscope (an optical microscope with Nikon E200 camera) to obtain a general impression of the histopathology features of a specimen such as thickening of the mucosa, destruction of mucosal epithelium, inflammatory cell infiltration, sub-mucosal edema, necrosis and ulceration [26].

Statistical Analysis: All experimental results mentioned as mean ± SEM. The data analysis carried out using GraphPadInStat software. The statistical significance of the data was evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. $p<0.05$ was considered as statistically significant.

RESULTS:

Preliminary Phytochemical Analysis of Extract: HYSS showed the prominent presence of steroids, glycosides, flavonoids, alkaloids, tannins, and phenolic compounds.

Effect of HYSS on TNBS Induced Alterations in Body Weight in Rats: As shown in **Fig. 1**, the bodyweight of rats of the vehicle control group showed a gradual increase during the entire treatment period, while rats of TNBS treated group resulted into significant ($p<0.001$) reduction in the body weight from day 2 of treatment when compared to the vehicle control group. This decreased in body weight was significantly restored in a dose-dependent manner with the administration of HYSS 200 mg/kg & HYSS 400 mg/kg.

The HYSS 100 mg/kg was not effective. At 200 mg/kg, the significant ($p<0.01$) restoration was continued from 7th day onwards, whereas at 400 mg/kg, it showed statistically significant ($p<0.05$, $p<0.001$) restoration from 4th day onwards with respect to the day of treatment. The reference standard sulfasalazine significantly ($p<0.05$, $p<0.001$) restored the body weight from 2nd day onwards with respect to the day of treatment.

Effect of HYSS on TNBS Induced Alterations in Stool Consistency Score in Rats: TNBS treatment in induction control group resulted into significant ($p<0.001$) increased in stool consistency score when compared to the vehicle control group.

This increased stool consistency score was significantly ($p<0.05$) decreased with the HYSS 400 mg/kg treatment from the 6th day onwards. HYSS 200 mg/kg and 100 mg/kg also reduced the score, but the difference was not found to be statistically significant. The reference standard sulfasalazine significantly decreased the stool consistency score ($p<0.05$) from 4th day, and the effect was more significant ($p<0.01$) from the 6th day onwards **Fig. 2**.

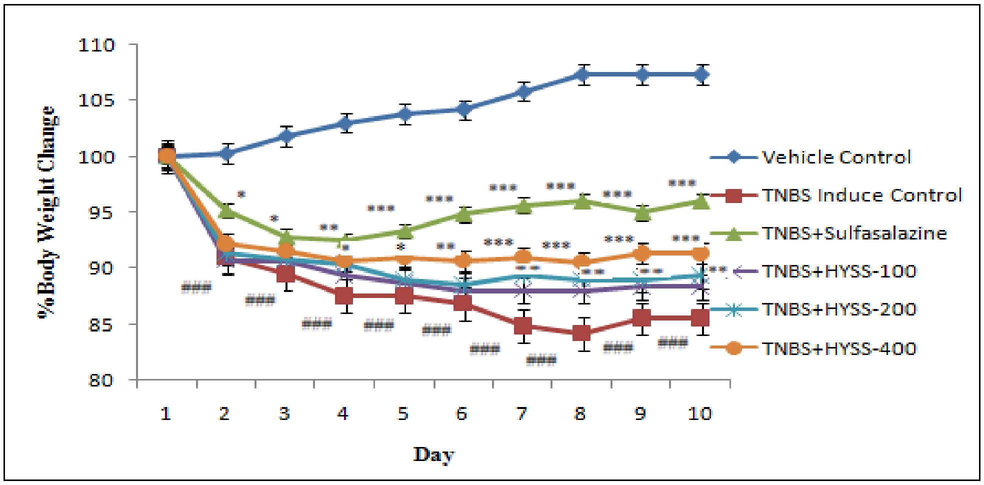

FIG. 1: EFFECT OF HYSS ON TNBS INDUCED ALTERATIONS IN BODY WEIGHT IN RATS. Values are expressed as (Mean ± SEM), n=6, and ### = p<0.001 compared to vehicle control group, *= p<0.05, **=p<0.01 and ***= p<0.001 compared to TNBS induced control group, statistically analyzed by one way ANOVA followed by Bonferroni multiple comparison test

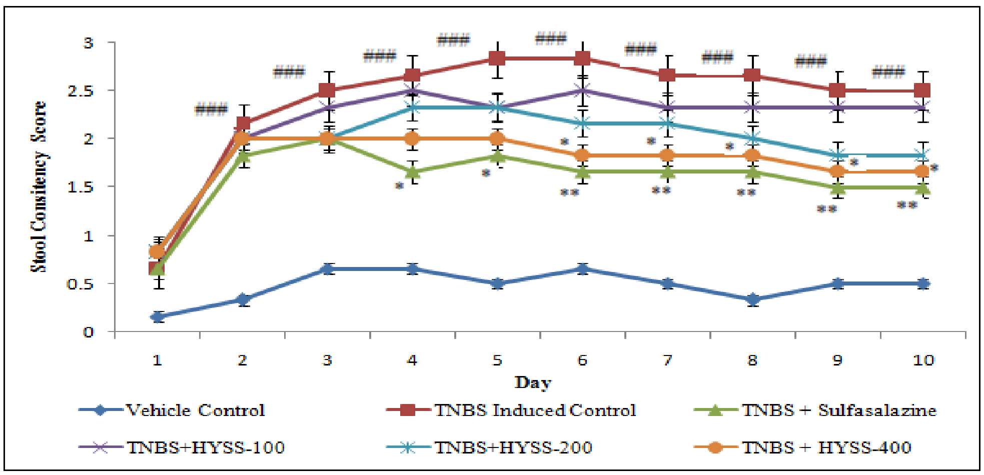

FIG. 2: EFFECT OF HYSS ON TNBS INDUCED ALTERATIONS IN STOOL CONSISTENCY SCORE IN RATS. Values are expressed as (Mean ± SEM), n=6, ### = p<0.001 compared to vehicle control group, *= p<0.05, **=p<0.01 and ***= p<0.001 compared to TNBS induced control group, statistically analyzed by one way ANOVA followed by Bonferroni multiple comparison test

Effect of HYSS on TNBS Induced Alterations in Colon Length, Weight, Weight to Length ratio, Macroscopic Score, Ulcer Area, Ulcer Index in Rats: In this model, TNBS treatment caused significant (p<0.001) decreased in mean colon length while colon weight and weight to length ratio increased significantly (p<0.001) when compared to vehicle control rats. This reduction in colon length was significantly restored in a dose-dependent manner (p<0.01) at HYSS 200 mg/kg and (p<0.001) at HYSS 400 mg/kg. The extract significantly inhibited increased colon weight dose-dependently at HYSS 200 mg/kg (p<0.05) and HYSS 400 mg/kg (p<0.01) whereas extract showed equipotent (p<0.01) reduction in colon weight to length ratio at 200 mg/kg & 400 mg/kg when compared to induction control group. The reference standard sulfasalazine was found most effective and equipotent (p<0.001) in all cases.

The macroscopic score of the colon in the TNBS induction control group significantly (p<0.001) increased when compared to the vehicle control group, whereas equipotent (p<0.01) reduction in macroscopic score was recorded with the treatment of sulfasalazine and HYSS at 400 mg/kg.

In the TNBS induction control group, ulcer area, and ulcer index was significantly (p<0.001) increased. The equipotent (p<0.001) inhibition of increased level of ulcer area and ulcer index were recorded at higher doses of the extract, *i.e.*, HYSS 200 mg/kg & HYSS 400 mg/kg. The percent inhibition was 34.58% and 54.35% at 200 mg/kg and 400 mg/kg respectively. The reference standard was most effective and significantly (p<0.001) reduced ulcer area and ulcer index. The percentage of inhibition was 85.39%. The lowest dose (HYSS 100mg/kg) was not significant in all cases in **Table 1**.

TABLE 1: EFFECT OF HYSS ON TNBS INDUCED ALTERATIONS IN COLON LENGTH, WEIGHT, WEIGHT TO LENGTH RATIO, MACROSCOPIC SCORE, ULCER AREA, ULCER INDEX IN RATS

Group no.	Nomenclature	Colon Length (cm) Mean ± SEM	Colon Weight (gm) Mean ± SEM	Colon weight to Length ratio (gm / cm) Mean ± SEM	Macroscopic Score Mean ± SEM	Ulcer Area (mm^2) (Mean ± SEM)	Ulcer Index (Mean ± SEM)	% Inhibition
I	Vehicle control	20.01 ±0.44	2.23 ±0.06	0.11 ±0.04	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	--
II	TNBS Induction Control	15.45 ±0.33$^{###}$	3.09 ±0.07$^{###}$	0.20 ±0.04$^{###}$	3.16 ±0.30$^{###}$	32.50 ±2.59$^{###}$	54.16 ±4.32$^{###}$	--
III	TNBS + Sulfasalazine	18.23 ±0.31***	2.56 ±0.06***	0.14 ±0.06***	1.66 ±0.33**	4.75 ±0.70***	7.91 ±1.17***	85.39
IV	TNBS + HYSS-I	15.20 ±0.25^{NS}	3.30 ±0.04 ^{NS}	0.22 ±0.05 ^{NS}	2.66 ±0.33 ^{NS}	29.75 ±1.74	49.57 ±2.90	8.47
V	TNBS + HYSS-II	17.11 ±0.17**	2.82 ±0.07*	0.16 ±0.02**	2.33 ±0.21 ^{NS}	21.33 ±2.12***	35.43 ±3.59***	34.58
VI	TNBS + HYSS-III	17.68 ±0.26***	2.74 ±0.07**	0.15 ±0.08**	1.83 ±0.16**	14.33 ±1.33***	24.72 ±2.76***	54.35

Values are expressed as (Mean± SEM), n=6, HYSS-I, II, III: Hydroalcoholic Extract of *Sida spinosa* Linn. (100, 200, 400 mg/kg respectively). TNBS- 2,4,6-trinitrobenzene sulfonic acid, $^{###}$ = p<0.001 as compared to the vehicle control group, *= p<0.05, **=p<0.01 and ***= p<0.001 compared to TNBS induced control group, Statistically analyzed by one way ANOVA followed by Bonferroni multiple comparison test

Effect of HYSS on TNBS Induced Alterations in Colonic MPO, GSH, and MDA: Myeloperoxidase (MPO) level and MDA level was significantly (p<0.001) increased whereas GSH level was significantly (p<0.001) depleted in TNBS induction control when compared to vehicle control.

HYSS produced dose-dependent reduction in MPO and MDA level at 200 mg/kg (p<0.05) and 400 mg/kg (p<0.01) while it significantly restored GSH level at 200 mg/kg (p<0.01) and 400 mg/kg (p<0.001). Reference standard sulfasalazine produced significant (p<0.001) reduction in MPO and MDA level and significantly (p<0.001) restored GSH level when compared to TNBS control. HYSS 100 mg/kg was found to be non-significant in all parameters in **Table 2**.

TABLE 2: EFFECT OF HYSS ON TNBS INDUCED ALTERATIONS IN COLONIC MPO, GSH, MDA IN RATS

Group no.	Treatment	Colonic MPO (U/mg) Mean ± SEM	Colonic GSH (µg/mg protein) Mean ± SEM	Colonic MDA (nmol/mg of Protein) Mean ± SEM
I	Vehicle control	4.20 ± 0.53	23.23 ± 0.59	1.97 ± 0.10
II	TNBS Induction Control	13.58 ± 1.48$^{###}$	12.63 ± 0.48$^{###}$	3.78 ± 0.15$^{###}$
III	TNBS + Sulfasalazine	6.04 ± 0.57***	20.51 ± 0.54***	2.01 ± 0.10***
IV	TNBS + HYSS-I	11.20 ± 0.37^{NS}	14.54 ± 0.66^{NS}	3.36 ± 0.21 ^{NS}
V	TNBS + HYSS-II	9.63 ± 0.73*	15.90 ± 0.83**	3.13 ± 0.13*
VI	TNBS + HYSS-III	8.66 ± 0.81**	17.49 ± 0.46***	2.98 ± 0.21**

Values are expressed as (Mean ± SEM), n=6, HYSS-I, II, III: Hydroalcoholic extract of *Sida spinosa* Linn (100, 200, 400 mg/kg, respectively). TNBS- 2,4,6-trinitrobenzene sulfonic acid, $^{###}$ = p<0.001 compared to the vehicle control group, *= p<0.05, **=p<0.01 and ***= p<0.001 compared to TNBS Induced Control group, Statistically analyzed by one way ANOVA followed by Bonferroni multiple comparison test

Effect of HYSS on TNBS- Induced Alterations in Colon Histopathology of Rats: In-vehicle control rats **Fig. 3a** showed normal architecture of colon without any abnormal changes while in TNBS installation in induction control **Fig. 3b** and HYSS 100 mg/kg **Fig. 3d** showed significant changes in colon architecture suggestive of extensive mucosal damage associated with intermittent inflammatory changes and infiltration of inflammatory cells in mucosa and submucosa.

The HYSS 200 mg/kg **Fig. 3e** and 400 mg/kg **Fig. 3f** reported a dose-dependent recovery in mucosal damage. The 400 mg/kg and reference standard sulfasalazine treatment specimens **Fig. 3c** did not show the presence of the infiltration of inflammatory cells.

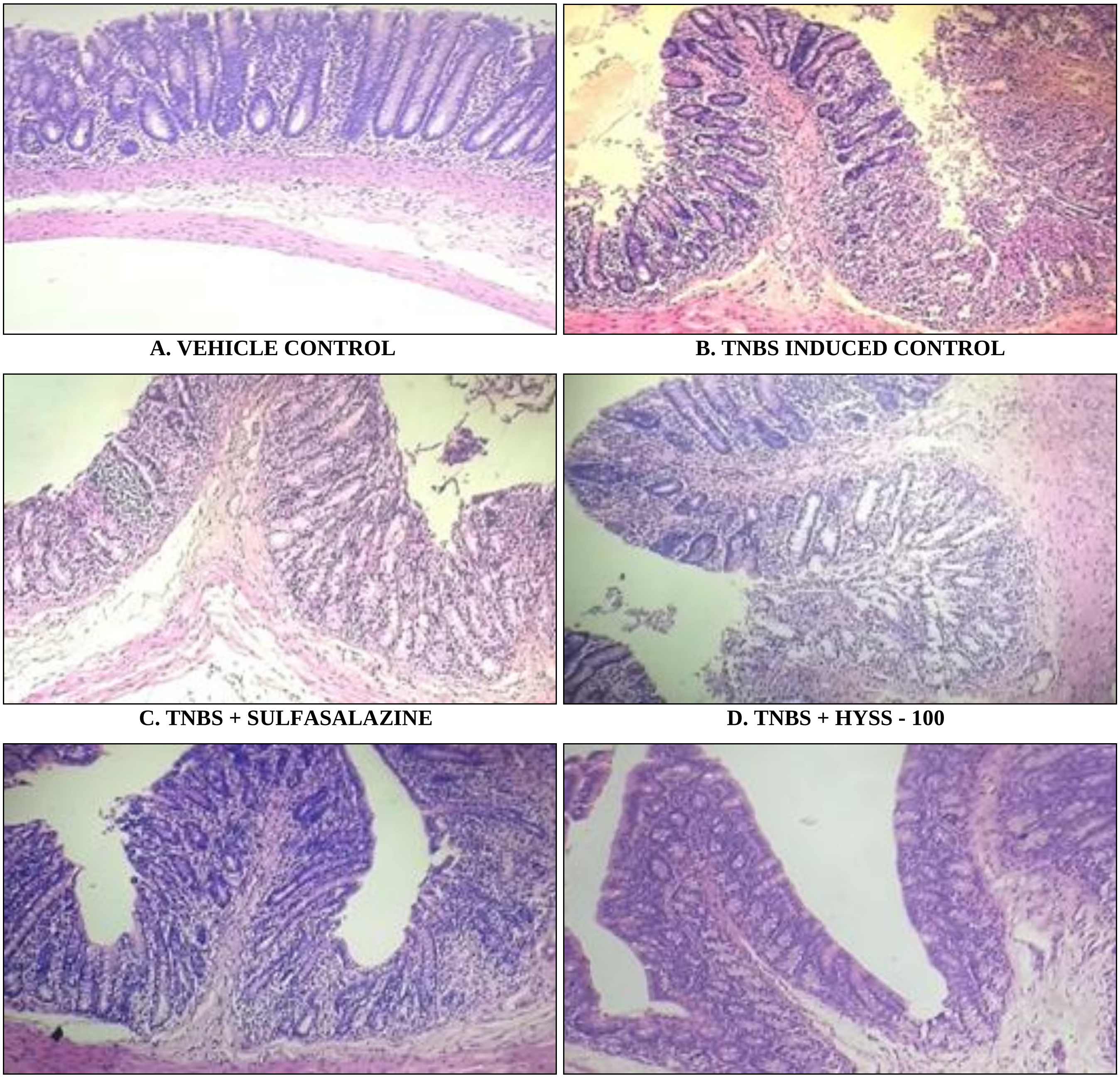

FIG. 3: EFFECT OF HYSS ON TNBS- INDUCED ALTERATIONS IN COLON HISTOPATHOLOGY OF RATS

DISCUSSION: Inflammatory bowel disease (IBD) is characterized by chronic relapsing inflammation in the gastrointestinal (GI) tract, resulting in symptoms of diarrhea, weight loss, abdominal pain, *etc*. The pathogenesis of IBD is multifactorial, involving interactions among the host immune system, genetic susceptibility, and responses to environmental and microbial factors [9]. As per the published literature, it is well established that, the imbalance in the mucosal immune system responsible for the generation of inflammatory mediators such as reactive oxygen species (ROS), cytokines like TNF, IL-1, IL-6, *etc*. [26] which leads to chronic inflammation, ulceration of colonic mucosa and morphologically represented by inflammatory cell infiltration, edema and tissue injury [27]. TNBS induced colitis is simple, reproducible, and widely used preclinical model for screening of drugs efficacy in the treatment of IBD [28]. In the present study, HYSS significantly restored the percent body weight loss in TNBS treated rats in a dose-dependent way. At higher doses, HYSS also showed a significant decreased in stool consistency score, significantly attenuated shortening of the colon, reduced colon weight to length ratio, and macroscopic score suggesting it's anti-inflammatory potential to reduce the clinical symptoms and morphological changes associated with IBD and it's colitis healing property [29]. The significant reduction of ulcer area and ulcer index indicated the antiulcer potential of HYSS and protect the colonic microflora from the corrosive effect of TNBS [22].

Myeloperoxidase (MPO) is an enzyme present in neutrophils. Under stressful conditions, MPO catalyzes the formation of cytotoxic oxidants like hypochlorous acid from H_2O_2 and chloride ions [30]. An Increased level of MPO reflects the neutrophil activation and degree of neutrophil infiltration. Activated neutrophils produce reactive oxygen species in the intestinal mucosa and produce an inflammatory response that causes damage to intestinal epithelial cells [31]. In the present study, results showed that HYSS inhibited the increased MPO activity in a dose-dependent manner but relatively less effective than sulfasalazine.

The reduction of MPO level suggested it's anti-inflammatory potential. This was further supported by histological studies where HYSS at 200 & 400 mg/kg effectively reduces the histological signs of inflammatory cell infiltration, edema, and mucosal damage.

Increased level of Malondialdehyde (MDA) indicated increased lipid peroxidation in inflamed colon tissue [32]. HYSS decreased the MDA content in TNBS treated rats. This may be due to antioxidant property of *Sida spinosa* L., and this was also supported by other studies where the antioxidant potential of *Sida spinosa* L. was documented by Jayasri *et al.,* [12] and Navaneethakrishnan *et al*. [16]

GSH is an important antioxidant that protects the cells and tissue from free radicals generated during stressful conditions. GSH also plays a crucial role in electrophile detoxification, transport of amino acid, and synthesis of DNA. Depleted level of GSH reported in chronic and acute inflammation and showed the progression of oxidative stress [33]. Treatment with HYSS significantly restored GSH level and thus preventing the progression of oxidative stress.

Histopathological study showed TNBS installation produces extensive mucosal damage, infiltration of inflammatory cell in mucosa and submucosa. HYSS 200 mg/kg and 400 mg/kg significantly restored the normal architecture and reduced the inflammatory infiltration and mucosal damage.

CONCLUSION: In conclusion, the results obtained in the present study indicated the healing activity of HYSS on the inflamed colon in TNBS induced ulcerative colitis. This could be attributed to the presence of flavonoids, steroids, saponins, phenolic compounds, glycosides, *etc*. acting together on a biological system to inhibit oxidative stress and inflammatory mediators. Therefore extract holds a promise for being used as an immunomodulatory agent as an adjuvant therapy along with conventional therapy in the management of IBD. There is further need to conduct studies on various fractions to determine the most potent fraction and necessary to confirm the mechanism of action of the protective effect *Sida spinosa* L. in IBD like conditions.

ACKNOWLEDGEMENT: The author's thanks to Dr. D. Y. Patil Institute of Pharmaceutical Sciences, Pimpri, and Dr. D. Y. Patil College of Pharmacy, Akurdi, for providing necessary facilities to carry out the study.

CONFLICTS OF INTEREST: The authors declare that they do not have any conflicts of interest

REFERENCES:

1. Rosen M, Dhawan A and Saeed S: Inflammatory bowel disease in children and adolescents. JAMA Pediatrics 2015; 169(11): 1053-60.
2. Lee SH, Kwon JE and Cho ML: Immunological pathogenesis of inflammatory bowel disease. Intest Res 2018; 16(1): 26-42
3. Kaplan GG: The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol 2015; 12: 720-27.
4. Lee HS, Park SK and Park DI: Novel treatments for inflammatory bowel disease. Korean J Intern Med 2018; 33: 20-27.
5. Wilhelm SM and Love BL: Management of patients with inflammatory bowel disease: current and future treatments. Clinical Pharmacist 2017; 9(3.
6. Tripathi K and Feuerstein JD: New developments in ulcerative colitis: latest evidence on management, treatment, and maintenance. Drugs in Context 2019; 8: 1-11.
7. Algieri F, Rodriguez NA and Garrido MN: Intestinal anti-inflammatory activity of the serpylli herbal extract in experimental models of rodent colitis. Journal of Crohn's and Colitis 2014; 8(8): 775-88.
8. Lin SC and Cheifetz AS: The use of complementary and alternative medicine in patients with inflammatory bowel

disease. Gastroenterology & Hepatology 2018; 14(7): 415-25.

9. Lal RU and Singh IP: Review and implications of traditional Indian medicine for inflammatory bowel disease. 2019; 70170: 1-13.
10. Agarwal SS and Singh VK: Immunomodulators: A review of studies on Indian medicinal plants and synthetic peptides. PINSA B65 1999; 3&4: 179-204.
11. Selvadurai SR, Senthamarai T and Vijaya KK: Anitimicrobial activity of ethanolic extract of the whole plant of *Sida spinosa* Linn. (Malvaceae). J Nat Prod Plant Resour 2011; 1(2): 36-40.
12. Jayasri P, Elumalai A, Naik ND and Kalugonda MK: In-vitro antioxidant activity of *Sida spinosa* Linn. J Nat Prod Plant Resour 2011; 1 (4): 35-39.
13. Shaikh I, Kulkarni P, Patel A and Kulkarni V: Hypoglycemic activity of *Sida spinosa* Linn. root extract in normoglycemic rats. International Journal of Phytomedicine 2011; 3: 338-45.
14. Shaikh I, Kulkarni P, Basheerahmed AM and Basel AA: Antihyperglycemic and antihyperlipidemic activity of *Sida spinosa* Linn. root in streptozotocin-induced diabetic rats. British J of Pharm Res 2015; 5(2): 124-36.
15. Naik ND, Suresh B, Priyanka M, Geethanjali B, Yamini K and Malothu R: Formulation and evaluation of herbal ointment consisting Sida spinosa leaves extracts. J Pharm Biol 2012; 2: 40-42.
16. Navaneethakrishnan S, Kumar PS, Shaji G, Satyanarayana T, Vinupama G and Usha V: Preliminary phytochemical screening and anti-ulcer studies of the leaves of *Sida spinosa* Linn. Int J Pharm Pharm Sci 2012; 4: 541-44.
17. Khandelwal KR: Practical Pharmacognocy. Nirali Prakashan, Twentieth Edition 2010: 149-56.
18. OECD iLibrary | Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. https:// www.oecd-ilibrary.org
19. Sadar SS, Vyawahare NS and Bodhankar SL: Ferulic acid ameliorates TNBS induced ulcerative colitis through modulation of cytokines, oxidative stress INOS, COX-2 and apoptosis in laboratory rats. EXCLI Journal 2016; 15: 482-99.
20. Saiyed M, Sachdeva P and Kukkar M: Effectiveness of Ricinus communis root extract against dextran sodium sulfate induced ulcerative colitis in rats. International Journal of Green Pharmacy 2017; 11(2): 84-91.
21. Gravina AG, Prevete N, Tuccillo C, Musis CD, Romano L, Federico A, Paulis A, Argenio GD and Romano M: Peptide Hp(2–20) accelerates healing of TNBS-induced colitis in the rat. United European Gastroenterology Journal 2018; 6(9): 1428-36
22. Kumar VS, Rajmane AR, Adil M, Kandhare AD, Gosh P and Bodhankar SL: Naringin ameliorates acetic acid induce colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. J Biomed Res 2014; 28(2): 132-45.
23. Krawisz JE, Sharon P and Stenson WF: Quantitative assay of acute intestinal inflammation based on myloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology 1984; 87: 1344-50.
24. Moron MS, Depierre JW and Mannervik B: Levels of glutathione, glutathione reductase, and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Acta 1979; 582: 67-68.
25. OshkawaH, Ohishi N and Yagi K: Assay for lipid peroxidase in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-58.
26. Kandhare A, Raygude K, Ghosh P, Ghule A, Gosavi T and Badole S: Effect of hydroalcoholic extract of *Hibiscus rosa sinensis* Linn. leaves in experimental colitis in rats. Asian Pacific Journal of Tropical Biomedicine 2012; 2(5): 337-44.
27. da Silva V, de Araújo A, Araújo D, Lima M, Vasconcelos R and de Araújo Júnior R: Intestinal anti-inflammatory activity of the aqueous extract from *Ipomoea asarifolia* in DNBS-Induced Colitis in Rats. International Journal of Molecular Sciences 2018; 19(12): 1-18.
28. Antoniou E, Margonis G, Angelou A, Pikouli A, Argiri P and Karavokyros I: The TNBS-induced colitis animal model: An overview. Annals of Medicine and Surgery 2016; 11: 9-15.
29. Triantafyllidi A, Xanthos T, Papalois A and Triantafillidis JK: Herbal and plant therapy in patients with inflammatory bowel disease. Annals of Gastroenterology 2015; 28: 210-20.
30. Tian T, Wang Z and Zhang J: Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. Oxidative Medicine and Cellular Longevity 2017; 4535194: 1-18.
31. Honmore V, Kandhare A and Bodhankar S: Artemisia pallens alleviates acetaminophen-induced toxicity via modulation of endogenous biomarker. Pharm Biol 2015; 53: 571-81.
32. Ayala A, Munoz M and Argüelles S: Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. Oxidative Medicine and Cellular Longevity 2014; 1-31.
33. Guan G and Lan S: Implications of antioxidant systems in inflammatory bowel disease. BioMed Research International 2018; 1290179: 1-7.

How to cite this article:

Kulkarni AV and Vyawahare NS: Effect of hydroalcoholic extract of *Sida spinosa* L. on 2,4,6-trinitrobenzenesulfonic acid induced ulcerative colitis in rats. Int J Pharm Sci & Res 2021; 12(1): 450-58. doi: 10.13040/IJPSR.0975-8232.12(1).450-58.