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Research Article

Protective Effect of Hydroalcoholic Extract of Dried Fruits of *Helicteres isora* in Dextran Sulfate Sodium Induced Ulcerative Colitis in Experimental Wistar Rats

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ABSTRACT

Ulcerative colitis (UC) is the most common form of inflammatory bowel disease (IBD), which mainly affects colon. The treatment of UC depends upon severity of the diseases. The aim of the present study was to determine the effect of hydroalcoholic extract of dried fruits of *Helicteres isora* (HI) in dextran sulfate sodium (DSS) induced UC in experimental Wistar rats. In this study, Wistar rats of either sex were divided into five experimental groups, where control group received only distilled water. Group 2 was negative control group, which received 4% DSS from drinking water between 15th and 21st days. Group 3 received low dose of hydroalcoholic extract of *H. isora* at a dose 100 mg/kg orally, along with 4% DSS from drinking water between 15th and 21st days. Group 4 received high dose of hydroalcoholic extract of *H. isora* at a dose 200 mg/kg orally along with 4% DSS from drinking water between 15th and 21st days. In group 5, sulfasalazine was used as a standard drug at a dose of 100 mg/kg orally along with 4% DSS from drinking water between 15th and 21st days. Twenty four hours after treatment, animals were sacrificed, and further macroscopical, biochemical, and histopathological evaluation was done, and all the results were compared with control at $p < 0.05$ significant value.

INTRODUCTION

The UC is an inflammatory bowel disease, which is an idiopathic, chronic inflammatory disorder of the colonic mucosa. UC starts initially in the rectum and then extends proximally in a continuous manner into the entire colon UC shows symptoms, like abdominal pain, diarrhea, and hematochezia.^[1,2] UC can be acute or severe, which needs timely recognition, evaluation, and intervention for its management.^[3] There are various which lead to the pathogenesis of UC, such as, genetic factors, intestinal microbiota, host immune system disorders, and various other environmental factors.^[4]

In current study, UC in experimental rats was induced by DSS. The induction of UC by using DSS is one of the most widely used experimental model which causes epithelial

damage. Administration of DSS to experimental rats in a drinking water causes human UC-like pathologies in which it causes toxicity to the epithelial cells of the colon which further cause disturbances in the mucosal barrier function.^[5] There are several types of medications which are used for the treatment of colitis by controlling inflammation or reduce symptoms of colitis. Herbal medicine or traditional medicines includes an extensive range of practices, as well as, therapies. Herbal drugs are safer than synthetic drug, and therefore, preferred widely to treat various ailments. Some potential benefits of herbal drugs that their acceptance rate by the patient is high, they have good efficacy, relatively safe, and have low cost.^[6]

In present study hydroalcoholic extract of *H. isora* dried fruit was used. *H. isora* L. (Malvaceae), is one of

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the medicinally important tree species, which is used in *Ayurveda*. It is commonly known as *Marodphali* due to screw like appearance of its fruit. Fruits of *H. isora* generally mature during late January to early April. *Avartani* was used in ancient days to treat snake bite, diarrhea, and to treat constipation of new born baby. According to previous research, *H. isora* shows its effect as antioxidant, hypolipidaemic, antibacterial, and antiplasmodial activities. There are many researches on its activity on cardiac antioxidant, antiperoxidative potency, brain-antioxidation potency. *H. isora* also shows its effect in anticancer activity, antinociceptive activity, hepatoprotective activity, anti-diarrheal activity, and wormicidal activity.^[7,8] Therefore, based on previous researches *H. isora* was used to find out its effect on UC.

The present study was carried out to find out the protective effect of *H. isora* extract in DSS induced UC in experimental Wistar rats.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Dried fruits of *H. isora* were collected from the local herbal medicine store of Pune and authenticated by M/s Shamantak Enterprises, Dr. Gautam Botanist, Pune.

Preparation of Herbal Extract

Dried fruits of *H. isora* (HI) were collected, authenticated, and powdered by using grinding apparatus. Powdered *H. isora* was then packed into Soxhlet apparatus column by using Soxhlet apparatus filter paper and extracted by using ethanol (90%) and distilled water. After extraction process is over, the extract obtained was concentrated with the help of rotary flash evaporator under reduced pressure. Six cycles of extraction process was carried out to obtain the sufficient practical yield of hydroalcoholic extract of *H. isora* dried fruits. The concentrated extract was then stored in airtight container below 100°C.

Experimental Animals

In current study, experimental Wistar rats of either sex weighing 200 to 250 grams were used. The temperature condition was maintained at temperature 22 to 24°C, following 12 hours light/dark cycle. The humidity was maintained at 50 ± 5%. The rats were housed in a sanitized cages and sterile paddy husk individually after randomly divided into experimental and control groups. The animals had free access to food and water *ad libitum* throughout study period. All experimental procedures were in compliance with the Institutional Animal Ethical Committee (IAEC) approved protocol (CPCSEA/IAEC/PT-08/01-2K19).

Experimental Design

In the present study, experimental Wistar rats weighing 180 to 250 grams were used. The study period was 21 days. The Wistar rats of either sex were randomly divided

into five experimental groups, and each group contains six rats. The rats in the control group received distilled water. In the second group, i.e., negative control group rats received 4% DSS in drinking water for seven days from day 15th to 21st days of the study. Third group, i.e., low dose group receive hydroalcoholic extract of dried fruits of *H. isora* (HAEHI) in a dose 100 mg/kg by oral route once daily for 21 days. Fourth group, i.e., low dose group receive hydroalcoholic extract of dried fruits of *H. isora* (HAEHI) in a dose 200 mg/kg by oral route once daily for 21 days. In the fifth group, sulfasalazine was administered to the rats at a dose of 100 mg/kg by gavage once daily for 21 days. For inducing UC to the experimental groups, DSS was added to the drinking water of the rats at a concentration of 4% from 15th day to 21st day. When treatment of 21 days was over after twenty-four hours, the rats were sacrificed under xylazine (10 mg/kg)-ketamine (60 mg/kg) anesthesia. After dissection, 10 cm tissue was taken from the distal colon by washing with physiological saline solution.

Assessment of Disease Activity Index

The assessment of disease activity index was done after the 21 days' treatment was over and animal weight loss, stool consistency, and blood feces were observed, and disease activity was assessed (Table 1).^[9] Results are summarized in Figs. 1 and 2.

Preparation of Colon Tissue Homogenate

After completion of a treatment period rats were euthanized and colonic tissue about 100 mg was dissect out for homogenization. The tissue was then homogenized by using phosphate buffer solution (PBS) pH 7 and centrifuged at 5,000 rpm for 15 minutes. After centrifugation, supernatant was collected and used for determination of lipid peroxidation also referred as malondialdehyde assay (MDA) or TBARS levels, catalase (CAT), and superoxide dismutase (SOD). The



Fig. 1: Macroscopic appearance of rat colon

Table 1: Criteria for scoring UC

Score	Weight loss (%)	Stool Consistency	Blood Feces
0	Negative	Normal	Negative
1	1–9	Soft	Red
2	10–19	Very soft	Dark red
3	< 20%	Diarrhea	Black



concentration of MDA, CAT, and SOD was determined by using spectrophotometric method.

Determination of Lipid Peroxidation or MDA Levels

To determine the concentration of malondialdehyde, 100 microliters of colon homogenate was mixed with 2.5 mL reaction buffer containin 0.37% thiobarbituric acid (TBA), 0.25 M HCl and 15% trichloroacetic acid was taken in a ratio of 1:1:1. This mixture is then heated at 95°C for about 60 minutes, then cooled downed, and finally centrifuged at 5,000 rpm for 20 minutes. The supernatant obtained from above procedure was taken, and the absorbance of supernatant was measured at 540 nm spectrophotometrically. Data were expressed as nM of MDA/mg protein.^[10]

Determination of SOD Levels

To determine the concentration of superoxide dismutase, 0.05 mL supernatant was added to 2 mL of carbonate buffer and 0.5 mL of 0.01 Mm ethylenediaminetetraacetic acid (EDTA) solutions. The reaction was started by addition of 0.5 mL of epinephrine and autoxidation of adrenaline to adrenochrome was determined and recorded by using spectrophotometric method at 480 nm. The results are expressed as unit of SOD U/g of wet tissue.^[11]

Determination of CAT Levels

The reaction mixture consisted of 2 mL of phosphate

buffer (pH 7.0), 0.95 mL of hydrogen peroxide (0.019 M) and 0.05 mL of supernatant obtained from the colonic tissue homogenate in a final volume of 3 mL, and absorbance was taken at 240 nm. The results were expressed as units of CAT activity U/g of wet tissue.^[11]

Determination of Serum C-Reactive Protein Levels

C-reactive protein (CRP) is a type of pentameric protein, which is synthesized by the liver. The increase in the level of this protein rise inflammation. There are various causes by which increase in the level of CRP such as various acute, as well as, chronic conditions and these may have infectious or non-infectious etiology. C-reactive protein is also associated with the biomarkers of inflammation, such as, malondialdehyde (MDA).^[12]

Histopathological Analyses

For histopathological analyses, colon tissue of rat of all the experimental groups were processed routinely and fixed in paraffin. The sections of 3 to 5 μ thickness were taken by cutting the colon tissue, and then, the section was stained with hematoxylineosin stain. The section of tissue then placed on the microscopic slides and observed under microscope for assessment of ulceration or any lesions in the colonic tissue of rats.

Statistical Analyses

Statistical analysis was carried out using GraphPad Instat 3. All of the data is expressed as the mean $\pm$ standard error of the mean, i.e., SEM, and all the data were analyzed using one-way analysis of variance (ANOVA) method. Significant differences between the control and experimental groups were determined using Tukey-Kramer test all comparison test and $p < 0.001$ value was considered as significant.

RESULTS

Macroscopic Assessment of Rat Colon

Lipid Peroxidation

The results of lipid peroxidation in homogenate of rat colonic tissue shows significant increase lipid peroxidation level in negative control group significantly, when compared with the standard group and shows oxidant suppressing property, i.e., reduction in lipid peroxidation levels in treatment group such as low dose (HI 100 mg/kg) group, high dose (HI 200 mg/kg) group, and standard group (sulfasalazine 100 mg/kg) (Table 2 and Fig. 3).

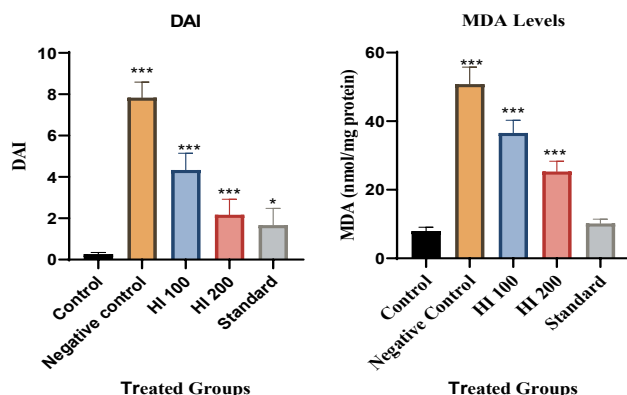


Fig. 2: Effects of *H. isora* extract on DAI in rats; all values were expressed as mean $\pm$ SEM (n = 6); one-way ANOVA (Analysis of Variance) followed by the Tukey Kramer's; (* $p < 0.01$; *** $p < 0.001$ considered significant as compared to control group)

Fig. 3: Effects of *H. isora* extract on MDA level in rats; all values were expressed as mean $\pm$ SEM (n = 6); one-way ANOVA followed by the Tukey Kramer's; (** $p < 0.01$; *** $p < 0.001$ considered significant as compared to control group)

Table 2: Evaluation of lipid peroxidation levels in colon tissue homogenate of rat

Control	Negative control	Low dose of <i>Helicteres isora</i> extract (100 mg/kg)	High dose of <i>Helicteres isora</i> extract (200 mg/kg)	Standard dose of sulfasalazine (100 mg/kg)
7.96 $\pm$ 0.46	50.81 $\pm$ 2.09***	36.55 $\pm$ 1.51***	23.96 $\pm$ 1.34***	10.18 $\pm$ 0.50

Results are expressed as mean $\pm$ SEM (n = 6); data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Superoxide Dismutase (SOD) Levels

The results of SOD levels in the homogenate of rat colonic tissue show significant decrease in the negative control group. However, after treatment with low dose of extract (HI 100 mg/kg) and high dose of extract (HI 200 mg/kg) as well, standard drug sulfasalazine (100 mg/kg) showed increase in the SOD level (Table 3 and Fig. 4).

Catalase (CAT) Levels

The results of catalase showed significant decrease in negative control group. However, after treatment with low dose of extract (HI 100 mg/kg) and high dose of extract (HI 200 mg/kg) as well, standard drug

sulfasalazine (100 mg/kg) showed increase in the CAT level (Table 4 and Fig. 5).

C-Reactive Protein (CRP) levels

The results of CRP in the blood serum of rat showed significant increase in negative control group, when compared with the control group. However, after treatment with low dose of extract (HI 100 mg/kg) and high dose of extract (HI 200 mg/kg) as well, standard drug sulfasalazine (100 mg/kg) showed increase in blood CRP level (Table 5 and Fig. 6).

Histopathological Analyses

Histopathological analyses of colon of Wistar rats of different experimental group, such as, negative

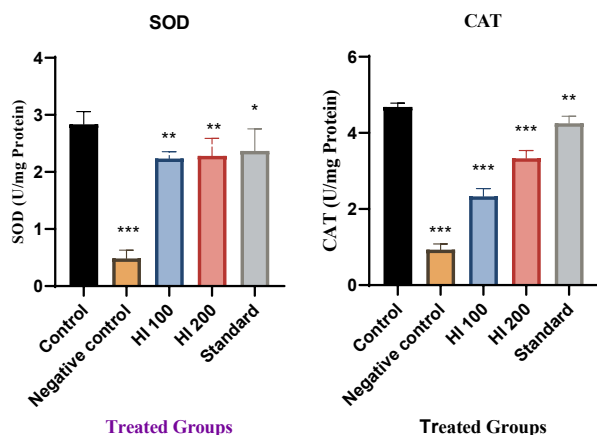


Fig. 4: Effects of *H. isora* extract on SOD level in rats; all values were expressed as mean ± SEM (n = 6); one-way ANOVA followed by the Tukey Kramer's; ** p < 0.01; *** p < 0.001 considered significant as compared to control group

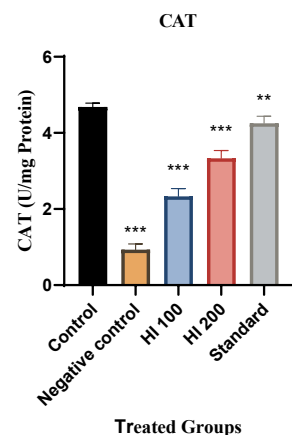


Fig. 5: Effects of *H. isora* extract on CAT level in rats; all values were expressed as mean ± SEM (n = 6); one-way ANOVA followed by the Tukey Kramer's; ** p < 0.01; *** p < 0.001 considered significant as compared to control group

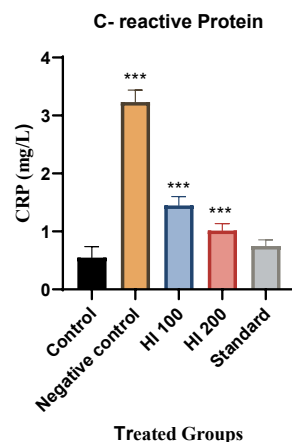


Fig. 6: Effects of *H. isora* extract on blood CRP levels in rats; all values were expressed as mean ± SEM (n = 6); one-way ANOVA followed by the Tukey Kramer's; ** p < 0.01; *** p < 0.001 considered significant as compared to control group

Table 3: Evaluation of SOD levels in colon tissue homogenate of rat

Control	Negative control	Low dose of <i>Helicteres isora</i> extract (100 mg/kg)	High dose of <i>Helicteres isora</i> extract (200 mg/kg)	Standard dose of sulfasalazine (100 mg/kg)
2.8 ± 0.09	0.48 ± 0.060***	2.23 ± 0.04**	2.28 ± 0.12**	2.36 ± 0.15*

Results are expressed as mean ± SEM (n = 6); data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test; * p < 0.05; ** p < 0.01; *** p < 0.001

Table 4: Evaluation of the levels of CAT in the colon tissue homogenate of rat

Control	Negative control	Low dose of <i>Helicteres isora</i> extract (100 mg/kg)	High dose of <i>Helicteres isora</i> extract (200 mg/kg)	Standard dose of sulfasalazine (100 mg/kg)
4.68 ± 0.04	0.93 ± 0.06***	2.33 ± 0.08***	3.33 ± 0.08***	4.25 ± 0.07**

Results are expressed as mean ± SEM (n = 6); data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test; * p < 0.05; ** p < 0.01; *** p < 0.001

Table 5: Evaluation of the blood serum CRP levels of rat

Control	Negative control	Low dose of <i>Helicteres isora</i> extract (100 mg/kg)	High dose of <i>Helicteres isora</i> extract (200 mg/kg)	Standard dose of sulfasalazine (100 mg/kg)
0.55 ± 0.07	3.23 ± 0.08***	1.45 ± 0.06***	1.01 ± 0.04***	0.75 ± 0.04

Results are expressed as mean ± SEM (n = 6); data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test; * p < 0.05; ** p < 0.01; *** p < 0.001



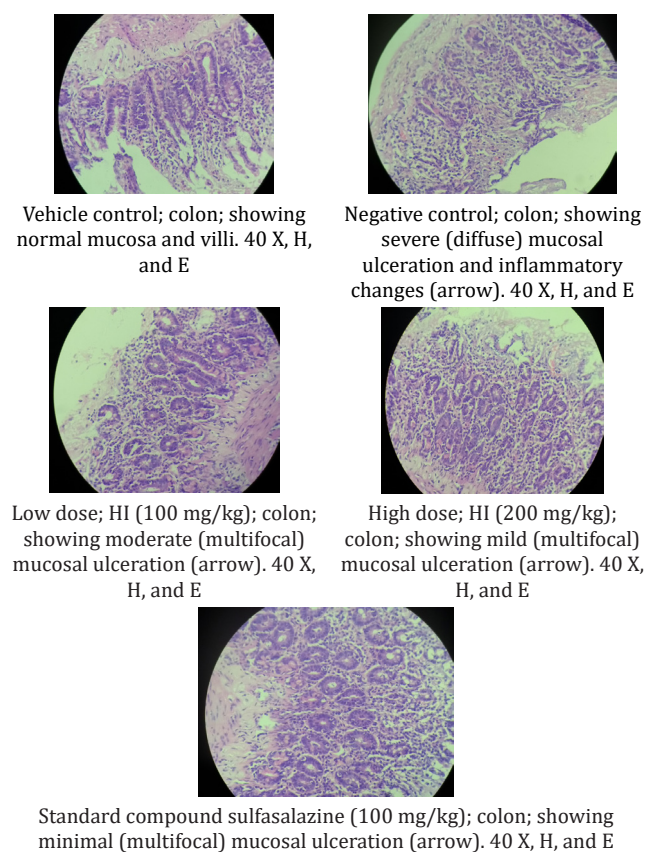


Fig. 7: Histopathological analysis of rat colon

control, low dose, high dose, and standard compound showed mucosal ulceration and inflammatory changes when compared with control group. Negative control group showed severe ulceration, while low dose and high dose groups showed moderate and mild inflammation or ulceration, respectively. However, standard group showed minimal ulceration when compared to the control group. The summarized results are given in Fig. 7

DISCUSSION

UC is an inflammatory bowel disease, which is characterized by relaps and remission. UC causes inflammation in the colorectal mucosa. UC may be seen children, as well as, in adults with different anatomical distribution. The risk factors in UC involved mainly genetic factors, various environmental factors, and also involve autoimmunity, as well as, gut microbiota. For the effective management of UC, proper functional and anatomical status should be tailored, and “disease activity” status should also be considered. In addition, the diagnosis of UC should be accurate depending upon the severity and extent of the inflammation and ulceration for further management of the UC.^[13,14]

There are many pharmacological agents, such as, corticosteroids and aminosalicylates are available for the treatment and maintenance of remission of UC. The proper treatment option should be selected upon disease severity,

efficacy of the drug used, and drugs having minimum adverse effects.^[15]

H. isora L. (Malvaceae) is an ancient *Ayurvedic* medicine which was used traditionally to treat various microbial infections, diabetes, diarrhea, etc.^[8]

In our study, biochemical assays were performed to find out the effect of *H. isora* on oxidative stress. Oxidative stress usually resulted due to an imbalance between the production of reactive oxygen species (ROS) and their elimination by protective mechanisms. This imbalance between production and elimination of ROS results in chronic inflammation. This resulting oxidative stress activates various transcription factors and causes gene expression of various inflammatory pathways, and therefore, causes UC-like inflammatory diseases.^[16]

In this particular study, lipid peroxidation, CAT, and SOD assays were performed. MDA is a marker of inflammation and catalases and SOD are antioxidative enzymes. Increased level of MDA shows colitis formation, and when catalase and SOD level get decrease, it shows inflammation.

Results of our study shows that the hydroalcoholic extract of *H. isora* dried fruit significantly decrease the level of MDA after treatment. It shows that extract of *H. isora* showed anti-inflammatory property when given in low dose (100 mg/kg) and high dose (200 mg/kg). The standard drug sulfasalazine (100 mg/kg) also shows anti-inflammatory properties. However, extract of *H. isora* significantly increases the level of antioxidative enzymes, such as, SOD and CAT after treatment in low (100 mg/kg), as well as, in high dose (200 mg/kg) of extract. The standard drug sulfasalazine (100 mg/kg) also shows anti-inflammatory properties. These results show protective effect of the herbal extract of *H. isora* against DSS induced UC.

The results of CRP increases in negative control group, but after treatment with low dose (100 mg/kg), high dose (200 mg/kg) of *H. isora* extract and standard dose of sulfasalazine (100 mg/kg), it showed significant decrease in the level of serum CRP, thereby, reducing inflammation.

In diagnosis and treatment of UC, histopathological examination plays a most important role.^[17] Results of our histopathological analyses shows normal mucosal layer in the control group animal colon tissue, where mucosal ulceration, as well as, inflammation was seen in the negative control group animal colon tissue. After treatment, low dose group (HI 100 mg/kg) and high dose group (HI 200 mg/kg) shows reduction in the mucosal ulceration and inflammation and provides indication of the hydroalcoholic extract of dried fruits of *H. isora* extract shows protective effect against DSS induced UC in experimental Wistar rats.

CONCLUSION

The Results of our experimental work showed that hydroalcoholic extract of *H. isora* dried fruits have

protective effect in DSS induced UC in experimental Wistar rats.

Dried fruits of *H. isora* have antioxidant properties and therefore, it shows significant results in biochemical analyses, such as, lipid peroxidation levels by decreasing the oxidative stress. *H. isora* extract increases superoxide dismutase and catalase concentration, respectively, and thereby, decreases the oxidative stress and improves the level of antioxidative enzymes. It also reduces the level of CRP and decreases the level of inflammation.

Results of our histopathological analyses shows reduction in the lesion in the mucosal area of colon and shows repairment in the colonic tissue after treatment with the herbal extract of *H. isora* dried fruits.

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