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Original Article

Evaluation of Anti-inflammatory Effects of Polysaccharide Derived from *Plantago ovata* Husk on Trinitro Benzenesulfonic Acid-induced Ulcerative Colitis in Mice

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ABSTRACT

Background: Ulcerative colitis, an idiopathic, chronic inflammatory illness of the intestinal mucosa is primarily fuelled by inflammation and oxidative stress. **Aim:** The present study aimed to investigate the anti-inflammatory and antioxidant effects of *Plantago ovata* husk in mice with Trinitro Benzenesulfonic Acid (TNBS)-induced Ulcerative Colitis (UC). **Materials and Methods:** BALB/c mice were exposed to TNBS intrarectally, causing ulcerative colitis. All mice received diet supplemented with *Plantago ovata* husk polysaccharide for 6 days, and their clinical symptoms were identified and evaluated. Following sacrifice, analyses of macroscopic and microscopic damage, intestinal oxidative stress levels, and antioxidant enzyme levels were performed. The colon's histological characteristics were also examined. **Results:** In comparison to the induced group, the highest dose significantly reduced the ulcer index, the histopathologic damage, and levels of NO, LPO, and MPO in tissues. Furthermore, SOD and GSH levels were increased restoring the balance of oxidants in colonic mucosa. The healing of injured tissues was also evident from the histology analysis. **Conclusion:** In higher doses, *P. ovata* husk extract significantly lowers mucosal damage and inflammation in mice with UC. *P. ovata* husk extract showed moderate protective effects and can be beneficial in treating Ulcerative colitis.

Keywords: *Plantago ovata*, Polysaccharide, Ulcerative colitis, TNBS, Inflammation.

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INTRODUCTION

Bowel disease is a recurrent, enduring, and sometimes fatal gastroenteric inflammatory illness. Although, the exact aetiology is undetermined; those who are susceptible have an aberrant mucosal immune response to commensal gut flora, which causes intestinal inflammation.[1,2] Ulcerative Colitis (UC), one of two idiopathic inflammatory bowel diseases, has a persistent, non-specific condition, it occurs only in the large bowel, and the inflammation is confined to the mucosa. Despite the fact that the cause is still unknown,[3] it has been hypothesized that an abnormal mucosal immune response may be responsible for causing inflammation, that draws neutrophils and activates the release of nitrogen species and reactive oxygen along a number of proinflammatory cytokines.[4,5]

Among the drugs that are most commonly used for treating UC, mesalazine is considered a common and safe first-line drug for the treatment of mild to moderate UC.[6,7] For patients who do not react to mesalazine, further treatment choices include corticosteroids and immunosuppressants.[8] Similarly, moderate to severe colitis was treated with additional therapeutic medications such biological and microbiological agents. In spite of the standard treatments for UC, frequent negative side effects and ineffective clinical results have been reported. In order to prevent this, it has been discovered recently that polysaccharides derived from numerous plants, animals, and microbes in nature have benefits such as safety, high therapeutic efficacy, non-toxicity, cheap cost, and good biocompatibility. These substances, which aid in immune system regulation, can be taken orally to reduce discomfort and improve patient compliance.

Researchers have found that polysaccharides including pectin, rhamnogalacturonan, chitosan, fructan, psyllium, glycosaminoglycan, polysaccharides from fungi and bacteria, can safely and successfully treat inflammatory illnesses like UC.[9,10]

Plantago belongs to the family of Plantaginaceae and is generally recognized as isabgol or psyllium. Psyllium is a combination of neutral and acid polysaccharides with galacturonic acid. In the current investigation, we seek to determine if polysaccharides

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isolated from *Plantago ovata* husk can treat mice induced with ulcerative colitis.

MATERIALS AND METHODS

Isolation of polysaccharide

Plantago ovata husk were acquired from marketplace and polysaccharides were isolated based on the procedure by Washi *et al*., and Pawar *et al*.[11,12] In order to completely release the mucilage into the water, the husk of *Plantago ovata* was immersed in distilled water for 48 hr before being boiled for a short period of time. To filter, the substance was forced through cotton cloth. The polysaccharide was then precipitated (by adding an equal volume of acetone to the filtrate), separated, pulverized, sifted, dried, and their physiochemical properties were analysed and furthered for other experiments.

Carbohydrate Estimation

Stock

Sugar concentration of 60-90 µg/mL was obtained from the stock solution (100 µg/mL of glucose in distilled water). To 1 mL of this solution, 1 mL of phenol (5%), and 5 mL of H_2SO_4 was added. The absorbance was measured after 10 min at 490 nm. Distilled water (1 mL) with 1 mL phenol (5%) and 5 mL of H_2SO_4 was considered as blank.

Test

About 10 mg of the obtained polysaccharide powder (*Plantago ovata* husk) was melted in distilled water (100 mL). To estimate the polysaccharide, absorbance of 1 mL phenol, 1 mL *Plantago ovata* husk (test) solution and 5 mL H_2SO_4 was measured after 10 min at 488 nm.

Sulfate Modification and Estimation

To obtain functional polysaccharide from the obtained husk, sulphate is introduced and determined to estimate the change in the physicochemical properties and activities of the obtained polysaccharides.[13] Five milligrams of *Plantago ovata* husk powder was digested in 5 mL of hydrochloric acid (1 mol/L) and incubated for 6 hr at 100°C. A volume of the cooled mixture containing exactly 0.4 mL was taken and transferred into a test tube along with gelatin (0.5% w/v, 5 g/L), TCA (3% w/v, 7.6 mL) and 2.0 mL barium chloride (10 g/L, 1% w/v). Absorbance taken after 15 min was considered as A1 value. A2 values were determined using gelatin (5 g/L, 0.5% w/v, 2.0 mL). A linear regression equation was used to determine the sulphate concentration.

Experimental design

Thirty-six female BALB/c mice, purchased from the NIN, Hyderabad, India, were acclimated, fed with unlimited water, and kept in a Standard Environment in Accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC) of xxxxx. Animals, aged 8–10 weeks and weighing 28–32 g, were arbitrarily allocated to different groups.

Induction of colitis

Mice were separated into 6 groups. The polysaccharide powder (*Plantago ovata* husk) at variable concentration (100, 200, and 300 mg/kg) was mixed with commercial mice feed was provided for mice up to 7 days. In order to cause ulcerative colitis, Trinitrobenzene Sulfonic Acid (TNBS) was utilised as an agent. The mice were given access to water and starved for a day before being inducted. Following anaesthesia with isoflurane, a catheter was used to inject 100 µL of absolute ethanol and 5% TNBS (1:1) intrarectally. For greater TNBS dispersion, the mice were held vertically for 2-3 min after treatment. The mice received the medication for 7 days, after which they were sacrificed with a larger dose of isoflurane. The colon was subjected to histological examination and used in subsequent investigations. The experiment groups were illustrated in Table 1.

Water and food intake were monitored. Presence of obvious blood in stools, stool consistency and body weight were observed daily for each mouse throughout the trial (Table 2). The Disease Activity Index (DAI) was considered as given below: total score [body weight decrease + stool consistency + rectal bleeding] / 3.

Macroscopic evaluation and scoring UC

After euthanasia, the complete lower gastrointestinal tract was resected. The colon was recognized, dissected, and longitudinally opened. Using normal saline, the luminal content was eliminated. For the purpose of determining the Colonic Macroscopic Damage (CMD), the length and weight of the mice's colons were obtained using the calculation given below.[14]

$$\text{CMD} = \text{Weight of the colon (mg)/Length of the colon (cm)}$$

According to Zeng *et al*.[15] description, visible changes in the distal colon were investigated and graded from 0 to 5 as per the degree of colonic injury (Table 3).

Evaluation of Nitric Oxide (NO)

NO production was evaluated by measuring nitrite (Griess method).[16] Briefly, colon samples were weighed and homogenized with liquid nitrogen, supernatant along with 1% sulphanilamide (Merck) solution and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (Merck) solution was dissolved in 5% phosphoric acid. Nitrite quantity was determined, as µM, after the absorbance was measured at 492 nm.

Evaluation of lipid peroxidation status

Determination of MDA, Malondialdehyde, a by-product, when polyunsaturated fatty acids in cell membranes is broken down is an indicative test of lipid oxidation. The colon sample (0.3 g)

was homogenised in 2 mL of cold KCl buffer (1.15 M, pH 6.0). Lipid peroxidation status was estimated based on the Ohkawa *et al.*, with some modification. To 0.1 mL of homogenate, 1.5 mL of TBA (0.8%), 1.5 mL of acetic acid (20%) and 0.2 mL of SDS (8.1%) were added, incubated for an hour at 95°C and then cooled in ice for 10 min. To this, 2.5 mL of n-butanol pyridine (15:1 v/v) was added, centrifuged (15,000 g, 30 min., 4°C) and the absorbance (532 nm) of the supernatant was recorded.

Evaluation of Myeloperoxidase (MPO) activity

Colonic tissues were weighed and processed in an ice-cold Potassium Phosphate buffer (50 mM, pH 6.0) containing Cetyltrimethylammonium Bromide (0.5%). After that, diluted H_2O_2, O-dianisidine and Potassium Phosphate buffer are added to the tissue homogenate and measured spectrophotometrically at 450 nm at an interval of 30 sec. MPO activity was determined and expressed in U/mg of tissue.[17]

Evaluation of Superoxide Radical (SOD)

The colons were homogenized with PBS and supernatants obtained by centrifugation at 5000 × g for 30 min mixed with PMS (0.06 mM, 0.2 mL), NBT (0.08 mM, 0.2 mL) and NADH (0.25 mM, 0.4 mL)[18] was measured at 560 nm after incubation.

$$\text{Superoxide radical scavenging ability (\%)} = (1 - A1/A0) \times 100$$

A1 - absorbance of reaction solution with the sample and,

A0 - absorbance of untreated group.

Evaluation of Reduced Glutathione (GSH) level

As per the method established by Khan, R *et al.*,[19] 0.5 mL of sulphosalicylic acid (4%) was supplemented to the homogenised sample and incubated at 4°C for an hour. The samples were centrifugated at 3000 rpm for 20 min at 4°C. About 0.4 mL of DTNB (10 mM), 0.4 mL of filtered aliquot, and 0.2 mL of phosphate buffer (pH 7.4, 0.1 M) were added to the supernatant. The mixture was immediately read at 412 nm.

DTNB conjugate formed/gram tissue using molar extinction coefficient of 13.6× 103 M^{-1} cm^{-1}.

Statistical analysis

Mean±Standard Deviation (SD) is used to express the experiment findings. Significance was set, if the *p* value is less than 0.001. One-way ANOVA was used in the statistical analysis (GraphPad Software Inc., San Diego, CA, USA).

Table 1: Experimental design.

	No. of animals/ group	Sex	Concentration	Group Name
Group 1	6	Female	N/A	Control
Group 2	6	Female	50 µL of ethanol	Vehicle Control
Group 3	6	Female	50 µL of 5% TNBS + ethanol	Induced
Group 4	6	Female	TNBS-induced mice with 100 mg/kg of polysaccharide powder (*Plantago ovata* husk).	Treated
Group 5	6	Female	TNBS-induced mice with 200 mg/kg of polysaccharide powder (*Plantago ovata* husk).	Treated
Group 6	6	Female	TNBS-induced mice with 300 mg/kg of polysaccharide powder (*Plantago ovata* husk).	Treated

Table 2: Disease Activity Index (DAI) score used to evaluate the TNBS-induced colitis. DAI index was calculated as total score (body weight decrease + stool consistency + rectal bleeding) divided by 3.

Score	Body weight decrease (%)	Stool consistency	Rectal bleeding
0	None	Normal	No bleeding
1	1-5%	-	
2	5-10%	Loose stool	Slight bleeding
3	10-15%	-	
4	>15%	Watery diarrhoea	Gross bleeding

RESULTS

Carbohydrate and Sulphate estimation of *Plantago ovata* husk powder

Carbohydrate

Based on the absorbance of standard and test, the standard curve was plotted and found to be in lines in the range of 60-90 µg/mL. A correlation coefficient of 0.9980 shows that the concentration and absorbance have a very strong linear connection. Polysaccharide content in husk powder was determined using a regression equation derived from the standard curve. The total polysaccharide content in husk powder was 50.88% w/w (Table 4).

Sulphate

The amount of SO_4^{2-} in *Plantago ovata* husk powder was 47.22%, which demonstrated a positive linear correlation in the range of 12-120 mg.

Clinical observation

Animals were observed for daily clinical signs and symptoms. None of the animals exhibited overt toxic effects or obvious clinical indicators of illness. No animal showed signs of mortality or unexpected illness.

Body weight of experimental animals

Compared with the induced group, treated group showed a significant increase ($p< 0.0001$) in body weights at the end of the experiment (Figure 1). The animals in the induced group exhibited a decreasing trend in body weight and a slight decrease was observed in vehicle group mice reverts to normal at the end of the experiment. In mice treated with polysaccharide, the weight loss reverts back to normal at the end of 7 days. In addition, no difference was found in water and food intake between the group.

Scores (DAI)

The clinical signs of the TNBS mouse model include diarrhoea, bloody stools, and weight loss. All of the aforementioned characteristics are evaluated as part of the DAI, along with the level of inflammation. On days 5 and 6, the score in the induced group reached a maximum of 12 compared to control. The score fell to 1.83±0.68, 1.0±0.9, and 1.0±0.5, respectively, after giving *Plantago ovata* husk powder in doses of 100, 200, and 300 mg/kg, as shown in Figure 2.

Macroscopic damage and scores of UC

After TNBS induction, the colon's weight increased and its length decreased in contrast to the control groups (0.071±0.004). When compared to the other two dosages, CMD scores were considerably lower (0.110±0.003) at the maximum dose of *Plantago ovata* husk extracts (300 mg/kg) Figure 3 (a).

According to the level of damage, ulcer scores were calculated, as shown in Table 2. There were no macroscopic ulcerations in control or vehicle groups. However, the score significantly increased in UC induced animals via TNBS, reaching 4.06±0.89. When *Plantago ovata* husk extracts were given, this score decreased to 3.6±1.03, 2.86±0.75, and 1.83±0.75, respectively, as shown in Figure 3(b).

Effect of *Plantago ovata* husk extract on Oxidative stress and inflammatory markers

Figures 4, 5, and 6 respectively, show TNBS treatment and induction affects the levels of the inflammatory and oxidative stress markers (NO, LPO and MPO). Nitric oxide levels significantly decreased in the treated group which was given the extract of *Plantago ovata* husks compared to the induced group. Nitric oxide levels were 1.4±0.14 in TNBS-induced animals whereas they were reduced to 1.1±0.001, 0.8±0.02, and 0.6±0.02 µM/mL in a dose-dependent manner (100, 200, and 300 mg/kg).

MDA levels were increased by TNBS. *P. ovata* extracts demonstrated a dose-dependent suppression. While 200 and 300 mg/kg showed a considerable decrease in MDA levels (0.079±0.001 and 0.055±0.011 µM/mL), 100 mg/kg showed a mild decrease (0.1±0.005 µM/mL).

MPO levels in induced groups were substantially greater (1.37±0.06) than in the control group (0.199). The elevation of MPO levels was significantly reduced in the induced group after treatment with the *Plantago ovata* husk extract, with mean values

Table 3: Scoring Ulcer.

Score	Observation
0	No damage.
1	Localized hyperemia with no ulcers.
2	Linear ulcer with no significant inflammation.
3	Linear ulcer with inflammation at one site.
4	Linear ulcer with inflammation, the size of ulcer.
5	Multiple inflammations and ulcers, the size of ulcer >1 cm.

Table 4: Carbohydrate and sulphate estimation.

Estimation	Replicate 1	Replicate 2	Mean±SD
Carbohydrate	49.41	52.35	50.88±2.07
Sulphate	50	44.43	±3.91

of 1.06±0.25, 0.77±0.15 U/mg of tissue, and 0.48±0.062 in a dosage dependent manner for doses of 100, 200, and 300 mg/kg.

Superoxide Radical (SOD) Scavenging Ability

As shown in Figure 7, the SOD activity increased in the *Plantago ovata* husk treated groups in a dose-dependent manner, with mean values of 23.28, 32.52, and 46.47% for doses 100, 200, and 300 mg/kg, respectively.

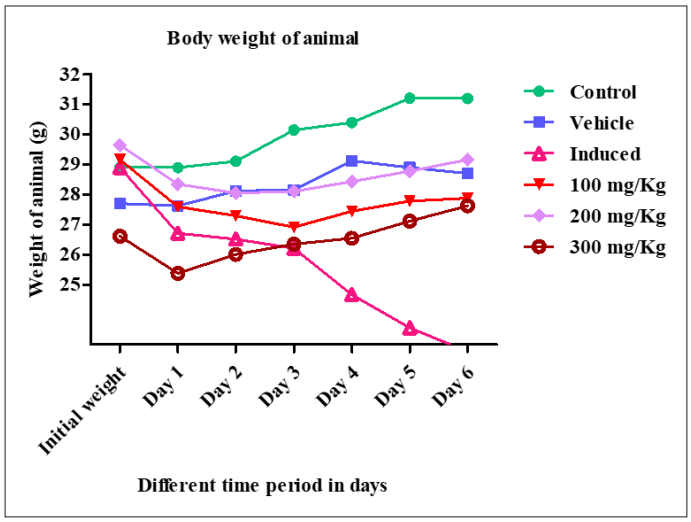

Figure 1: Body weight of animal - *Plantago ovata* husk powder reduced the loss of weight whereas the TNBS induced groups showed significant weight loss.

Effect of Reduced Glutathione (GSH) level

In comparison to the control group (0.76±0.003 mM), the level of GSH in induced group was considerably lower (0.48±0.016 mM). However, as shown in Figure 8, providing *Plantago ovata* husk at doses of 100, 200, and 300 mg/kg elevated GSH levels to 0.50±0.01, 0.53±0.002, and 0.55±0.010 mM, respectively.

Histopathological evaluation

Colon tissue in the control group exhibited normal histological structures, including the typical arrangement of the epithelium, submucosa, and lamina propria, without any immune cell

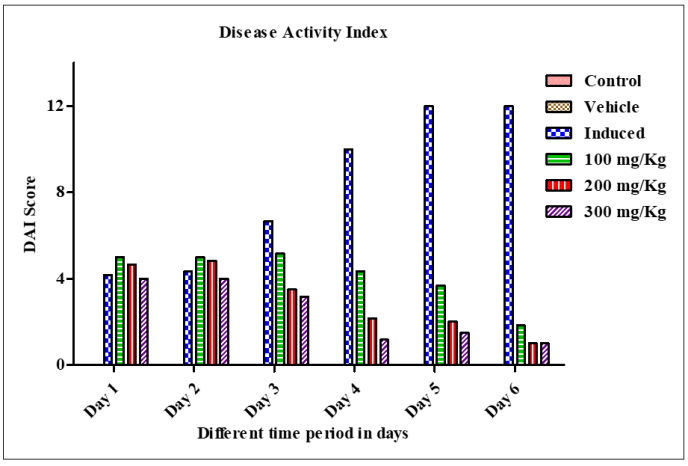

Figure 2: The effects of *Plantago ovata* husk extract on clinical parameters of UC. The DAI index significantly reduced with increase in the dosage. Results are expressed as mean±SD. Treated groups showed high significance compared to induced group (p<0.0001).

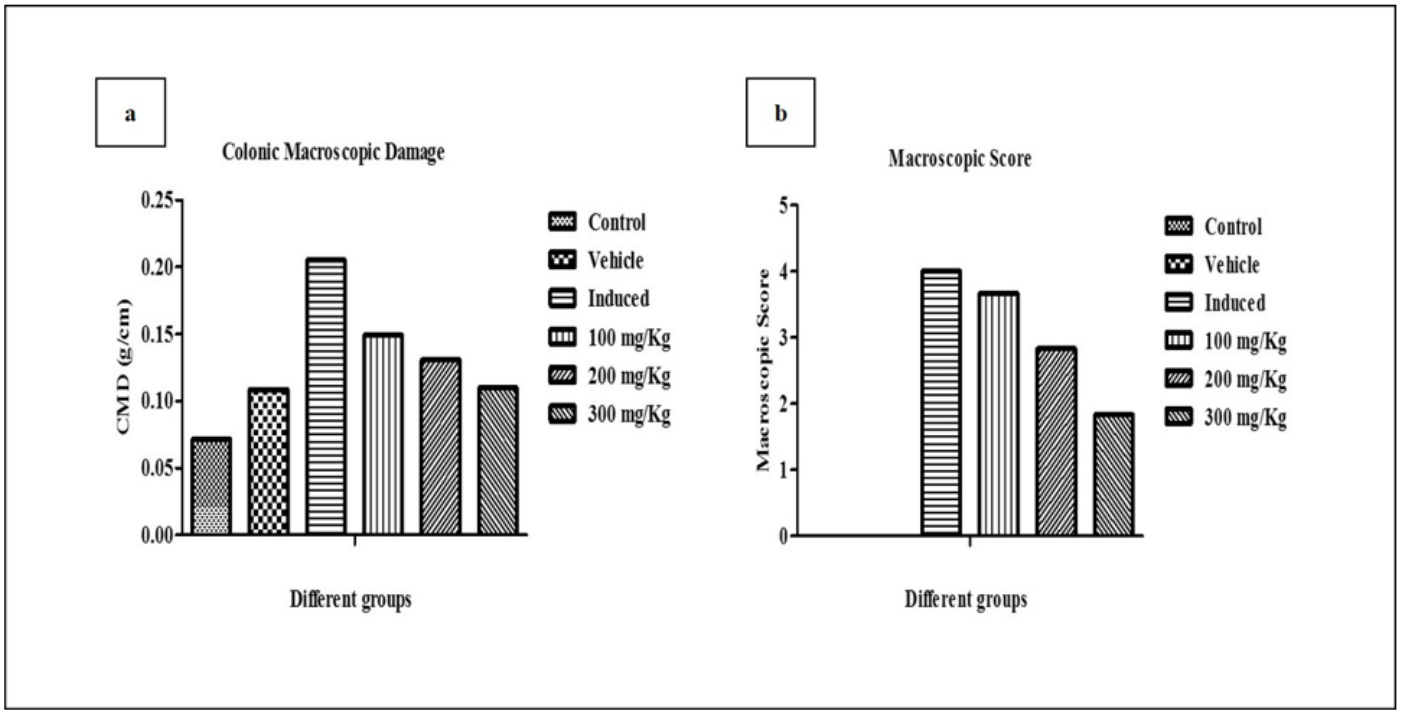

Figure 3: (a). Extracts from the husk of *Plantago ovata* have an impact on colon size and length. When compared to the induced group, the CMD of the treated groups demonstrated great significance (p< 0.0001). The results are given as mean±SD.

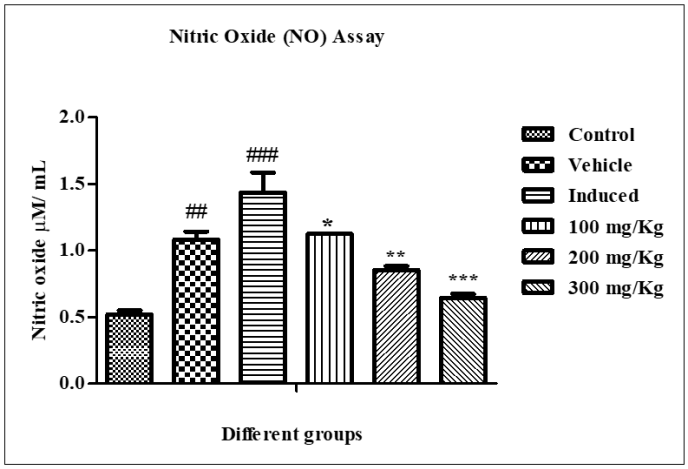

Figure 4: Effect of *Plantago ovata* husk extracts on nitric oxide levels of treated groups at different doses. NO level of treated groups was significantly decreased when compared to induced group and also showed high significance compared to control ($p< 0.0001$). # - Indicates the comparison between control and induced, * - Indicates the comparison between induced and treated groups.

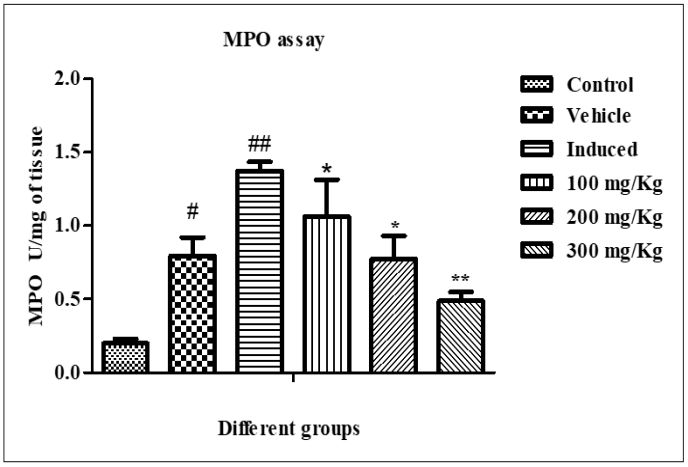

Figure 6: Myeloperoxidase estimation between control, vehicle, induced and *Plantago ovata* husk extracts treated groups were studied. MPO level of treated groups was significantly decreased when compared to induced group. Treated groups showed high significance compared to control ($p< 0.0001$). # - Indicates the comparison between control and induced, * - Indicates the comparison between induced and treated groups.

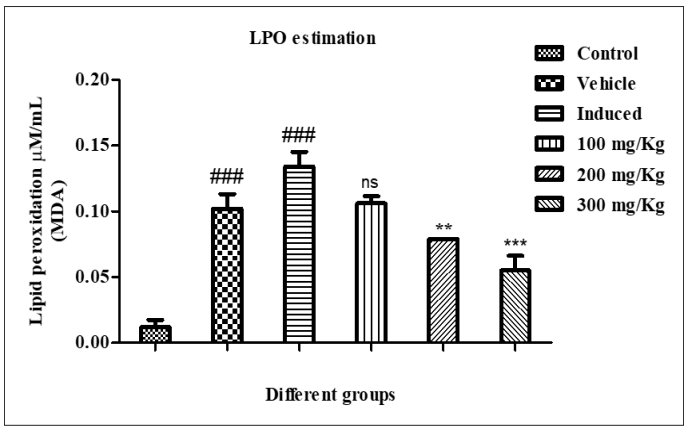

Figure 5: Lipid peroxidation estimation between control, vehicle, induced and *Plantago ovata* husk extracts treated groups. LPO level of treated groups was significantly decreased when compared to induced group and showed high significance compared to control ($p< 0.0001$). # - Indicates the comparison between control and induced, * - Indicates the comparison between induced and treated groups.

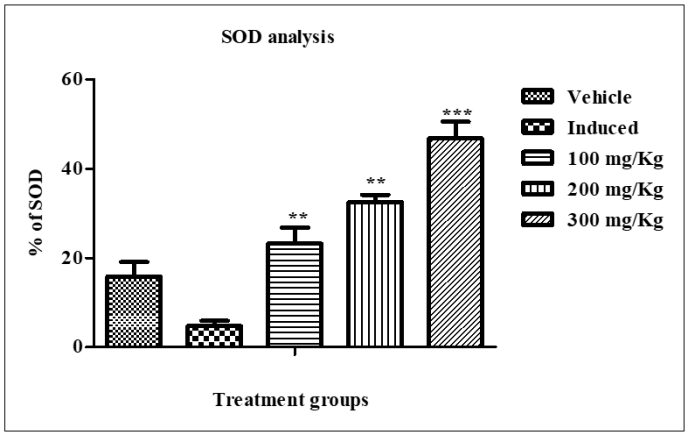

Figure 7: Effect of *Plantago ovata* husk extracts on SOD level of control, induced and treated. Results are expressed as mean±SD. Treated groups showed high significance compared to control ($p< 0.0001$). # - Indicates the comparison between control and induced, * - Indicates the comparison between induced and treated groups.

infiltration (Figure 9A). When 50% ethanol (vehicle) was administered intrarectally, the colonic mucous layer was damaged. No other discernible tissue injury apart from the mucosal layer was observed (Figure 9B). As seen in Figure 9C, tissues of mice which received TNBS, experienced a marked damage in tissue structure, erosion of the epithelium, and an increase in immune cell infiltration. With an increase dosage of *Plantago ovata* husk extract, treated groups displayed a gradual healing of the damaged tissue architecture. On treatment with 200 mg/kg, it was seen that the number of infiltrating immune cells decreased and the mucosal injury was repaired (Figure 9E). The highest dose (300 mg/kg) resulted in reduced tissue damage (Figure 9F).

DISCUSSION

UC is a recurrent, chronic condition that harms the intestinal mucosa and corticosteroids are the chosen possibility for patients with severe UC. Since it is related with irrevocable adverse effects, new therapeutic strategies are being developed with a suitable treatment approach with minimal/no adverse effects.[20]

Plants have the ability to support the mending process naturally and with fewer adverse effects.[21] In the current study, we found that, as compared to untreated control mice, the administration of *P. ovata* husk extract had an effect on the colon's microscopic and macroscopic appearance, tissue oxidative stress indicators, and inflammatory parameters. *P. ovata* husk extracts contain a lot of phytochemicals with potent antioxidant activity. These

phytochemicals work by either scavenging reactive molecules or by enhancing antioxidant molecules and enzymes.

Weight loss, diarrhoea, and bloody stools—symptoms of colitis—were noted and scored after intrarectal TNBS induction. Body weight, food consumption, and water intake all fell noticeably after intra-rectal TNBS delivery. These results agree with the findings of this study[22] that the use of *P. ovata* extracts greatly lessened the colitis-induced loss in body weight. These findings may be explained by the restoration of cellular production and metabolism in animals treated with *P. ovata* extracts.

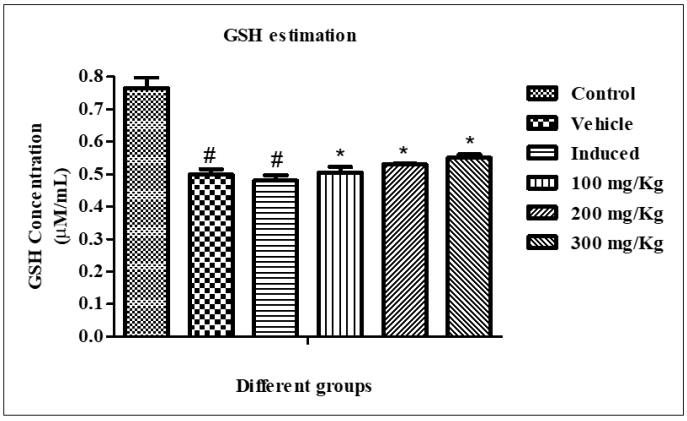

Figure 8: Estimation of glutathione in the control, induced, and groups treated with *Plantago ovata* husk extracts. When compared to the induced group, the GSH level of the treated groups was considerably higher. Compared to the control, the treated groups demonstrated strong significance ($p<0.0001$). # - Indicates the comparison between control and induced, * - Indicates the comparison between induced and treated groups.

These results, in addition to the elevated scores for ulcer and colonic macroscopic damage, strongly suggest an inflammatory environment and significant tissue injury. When *P. ovata* extracts were administered, the diarrhoea was more effectively managed while tissue damage, inflammation, and excessive nutrient loss were all reversed. It is clear from all of these results that Plantago has potent anti-inflammatory qualities because they all decreased the scores.[23] Our findings that *P. ovata* extracts have anti-inflammatory properties are consistent, but with another species of *Plantago* with a different type of cell and different anti-inflammatory response.[24]

The most effective protection against mucosal damage and the suppression of inflammatory and oxidative stress was achieved with treatment using the *P. ovata* husk extract at 300 mg/kg. In physiologically normal circumstances, the tiny molecule NO has anti-inflammatory actions; nevertheless, in abnormal circumstances, it is recognised to be a pro-inflammatory mediator and causes inflammation.[25] By generating highly reactive chemicals like dinitrogen trioxide and peroxynitrite, which damage the colonic mucosa and lead to the development of ulcers, Nitric oxide plays a crucial part in the pathophysiology of UC.[26,27] The results of our investigation revealed that *P. ovata* extracts in doses of 100, 200, and 300 mg/kg can decrease NO production

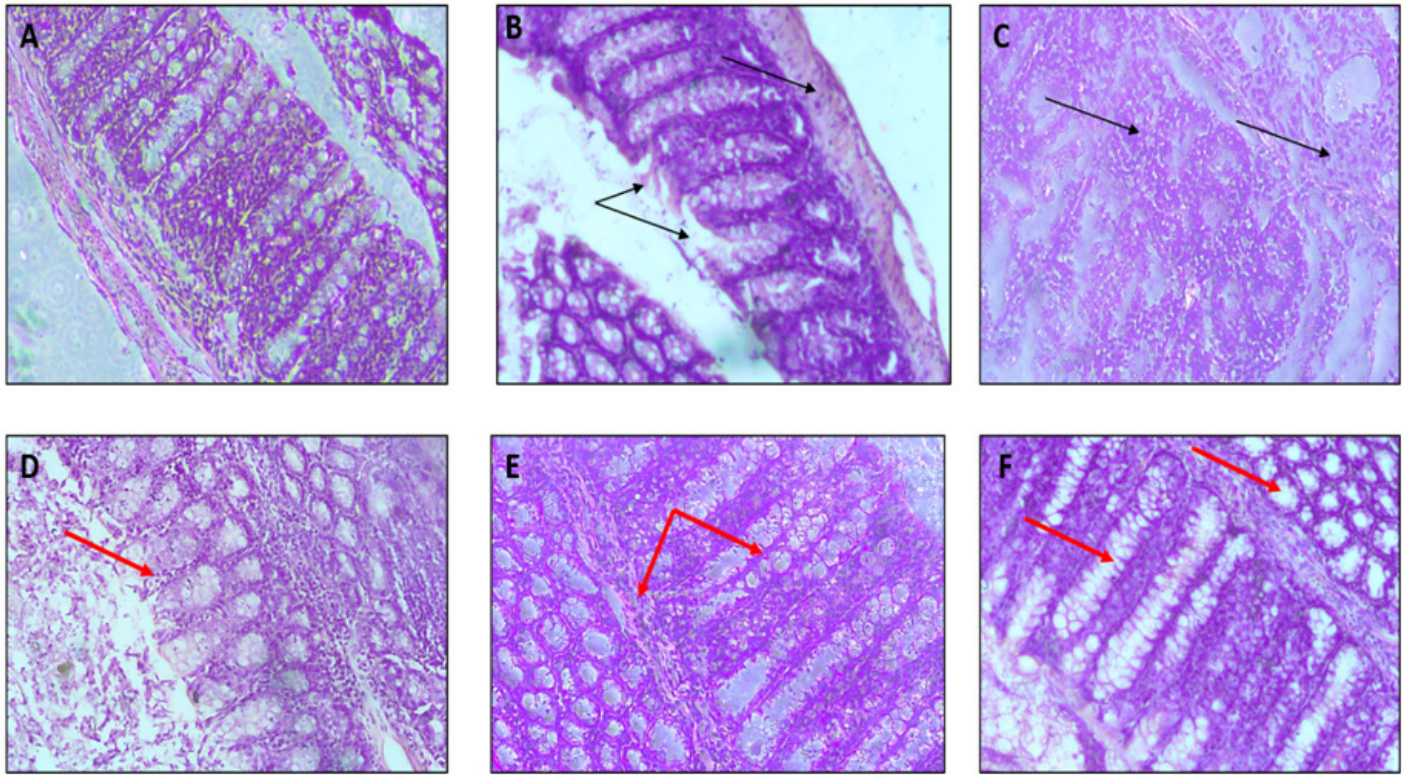

Figure 9: A) Control B) Vehicle C) Induction with TNBS D) 100 mg/Kg E) 200 mg/Kg and F) 300 mg/Kg. The black arrows indicate the mucosal and tissue damage, Neutrophil accumulations in vehicle group and great disintegration of tissues and cells, Neutrophil accumulations in induced group. Red arrow indicates retrieval of tissues in treatment groups as dose dependent manner.

in UC induced mice. There are a few papers demonstrating that several herbs can decrease and enhance NO production[28,29] in certain situations.

Oxidative stress is demonstrated by lipid peroxidation. Free-radicals produce LPO, which degrades the membrane's constituents through oxidation, especially polyunsaturated lipids. Cellular death and DNA damage are outcomes of this process. When lipids are broken down, LPO is generated, and MDA is an indicator of this.[30] In colon tissues, a dose dependant increase in MDA level was observed in treated group. This outcome of LPO might be because of the flavonoids in the plant which are known to prevent the response in LPO.[31]

A tissue level neutrophil infiltration is directed by myeloperoxidase.[32,33] When the body is functioning normally, MPO is released from azurophilic storage granules.[34] Release of this enzyme enhances colonic mucosa scraping MPO, a sign of enhanced infiltration, when an inflammatory shock causes the production of reactive species.[35] *P. ovata* husk extract was found to reduce the level of MPO in a dose dependent manner. MPO activity decreased in response to a dosage increase, which may have reduced inflammation and oxidative stress, thereby proving the ability of the husk extract. The histological analysis of colonic tissue reveals that *P. ovata* husk extract inhibits neutrophil infiltration in colonic tissue.[36]

Superoxide dismutase is an enzyme found in both the mitochondria and the cytoplasm that regulates the tissue's redox balance and prevents vascular endothelial damage.[37,38] An essential physiological element that keeps the mitochondrial energy metabolism functioning is the electron transport chain. The interruption of cellular redox equilibrium was brought on by a raised level of reactive oxygen species.[39] Superoxide dismutase is essential for neutralising harmful free radicals because it converts O_2 to water.[40] In this investigation, it was discovered that SOD levels were lower in the mice in the TNBS induced group, which indicated uncontrolled oxidative stress. Treatment with *P. ovata* husk extract showed a rise in SOD levels, which can help to restore the balance of oxidants in the colonic mucosa.

GSH is a crucial member of the family of antioxidants that scavenges free radicals and changes H_2O_2 into H_2O.[41] GSH protects cells and tissues from the production of free radicals during stressful situations that result from the injection of acetic acid.[42] The transport of amino acids, the detoxification of electrophiles, and DNA synthesis are all significantly influenced by GSH.[43] Therefore, decreased levels of GSH indicate the development of oxidative stress and the loss of the equilibrium between pro- and oxidative enzymes in cells.[44]

Histological changes brought on by TNBS administration, including ulceration, epithelial erosion, and goblet cell depletion in the colonic tissues were restored after administering *P. ovata* husk extract. Oxidative stress induced by TNBS may be the cause of these damages. TNBS also induced infiltration of immune cells causing inflammatory alterations. In the vehicle group, ethanol delivered intrarectally caused damages to the mucosal layer as per our histopathology findings.[45,46] When compared to the induced group, the degree of ulceration is less severe at dose 200 mg/kg, and normal goblet cell histology can be seen at dose 300 mg/kg. The indicators of recovering colon tissue and a decrease in immune cell infiltration in treated groups point to the therapeutic effects of *P. ovata*. Our findings imply that the *P. ovata* husk extract can decrease the development of TNBS-induced colitis in mice by controlling the provocative response and reactivating antioxidant enzymes like SOD and GSH. To fully comprehend the mechanism behind *P. ovata* husk, further investigation is required.

CONCLUSION

The findings from this study show that *Plantago ovata* husk polysaccharide can treat ulcerative colitis induced by TNBS. The DAI scores, colonic macroscopic damage, and ulcer scores were all decreased by this polysaccharide. Moreover, it enhanced antioxidant enzyme activity, decreased immune cell infiltration, attenuated lipid peroxidation, and lowered oxidative stress. These findings significantly support Plantago ovata husk polysaccharide's protective properties and suggest that it may be useful in treating ulcerative colitis.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UC: Ulcerative Colitis; **TNBS:** Trinitrobenzenesulfonic acid; **IBD:** Inflammatory bowel disease; **CD:** Crohn's disease; **DAI:** Disease activity index; **CMD:** Colonic macroscopic damage; **MPO:** Myeloperoxidase; **CTAB:** Cetyltrimethylammonium bromide; **NO:** Nitric oxide; **MDA:** Malondialdehyde; **LP:** lipid peroxidation; **SOD:** Superoxide dismutase; **GSH:** Reduced glutathione; **SD:** Standard deviation; **ANOVA:** Analysis of variance; **CMD:** Colonic microscopic damage; **LPO:** Lipid peroxidation; **ROS:** Reactive Oxygen species; **GI:** Gastro Intestinal.

SUMMARY

- This study examined the healing properties of *Plantago ovata* husk polysaccharide in mice with TNBS-mediated ulcerative colitis.

- The polysaccharide prompted curative effects against ulcerative colitis through their ability to reduce the clinical manifestation of the disease, suppress lipid peroxidation, inflammation, and oxidative stress. It also enhances the cytoprotective antioxidant enzymes.
- This study indicates the beneficial effect of the *Plantago ovata* husk polysaccharide in the treatment of TNBS-induced ulcerative colitis.

REFERENCES

1. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(21):2066-78. doi: 10.1056/NEJMra0804647, PMID 19923578.
2. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. Lancet. 2012;380(9853):1606-19. doi: 10.1016/S0140-6736(12)60150-0, PMID 22914296.
3. Zakerska-Banaszak O, Tomczak H, Gabryel M, Baturo A, Wolko L, Michalak M, *et al.* Dysbiosis of gut microbiota in Polish patients with ulcerative colitis: a pilot study. Sci Rep. 2021;11(1):2166. doi: 10.1038/s41598-021-81628-3, PMID 33495479.
4. Ho GT, Porter RJ, Kalla R. Ulcerative colitis: recent advances in the understanding of disease pathogenesis. 2020;9:321:294. doi: 10.12688/f1000research.20805.1, PMID 32399194.
5. Zhang L, Niu Z. Hydroalcoholic *Carthamus tinctorius* L. Extract Attenuates TNBS-induced Ulcerative Colitis in Mice via Downregulation of Inflammation and Oxidative Stress. Indian J Pharm Educ Res. 2022;56(3):780-8. doi: 10.5530/ijper.56.3.128.
6. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. N Engl J Med. 1987;317(26):1625-9. doi: 10.1056/NEJM198712243172603 [internet].
7. Sninsky CA, Cort DH, Shanahan F, Powers BJ, Sessions JT, Pruitt RE, *et al*. Oral mesalamine (Asacol) for mildly to moderately active ulcerative colitis: A multicenter study. Ann Intern Med. 1991;115(5):350-5. doi: 10.7326/0003-4819-115-5-350, PMID 1863024.
8. Buckland A, Bodger K. The cost-utility of high dose oral mesalazine for moderately active ulcerative colitis. Aliment Pharmacol Ther. 2008;28(11-12):1287-96. doi: 10.1111/j.1365-2036.2008.03856.x, PMID 18808444.
9. Wang X, Wang X, Jiang H, Cai C, Li G, Hao J, *et al*. Marine polysaccharides attenuate metabolic syndrome by fermentation products and altering gut microbiota: an overview. Carbohydr Polym. 2018;195:601-12. doi: 10.1016/j.carbpol.2018.05.003, PMID 29805017.
10. Yu Y, Shen M, Song Q, Xie J. Biological activities and pharmaceutical applications of polysaccharide from natural resources: a review. Carbohydr Polym. 2018;183:91-101. doi: 10.1016/j.carbpol.2017.12.009, PMID 29352896.
11. Washi SP, Sharma VD, Jain VK, Sinha P. Plantago ovata: genetic diversity, cultivation,... 1985:3-6. Indian J Nat Prod [cited Jan 27 2023]. Available from: https://scholar.google.com/scholar_lookup?journal=Indian+J+Nat+Prod&title=Plantago+ovata:+genetic+diversity,+cultivation,+utilization+and+chemistry&author=SP+Washi&author=VD+Sharma&author=VK+Jain&author=P+Sinha&volume=1&publication_year=1985&pages=3-6&#d=gs_cit&t=1674819662120&u=%2Fscholar%3Fq%3Dinfo%3AKWjI9o_zg7kJ%3Ascholar.google.com%2F%26output%3Dcite%26scirp%3D0%26hl%3Den. Google Scholar [internet].
12. Pawar H, Varkhade C. Isolation, characterization and investigation of *Plantago ovata* husk polysaccharide as superdisintegrant. Int J Biol Macromol. 2014;69:52-8. doi: 10.1016/j.ijbiomac.2014.05.019, PMID 24854213.
13. Ji CF, Ji YB, Meng DY. Sulfated modification and antitumor activity of laminarin. Exp Ther Med. 2013;6(5):1259-64. doi: 10.3892/etm.2013.1277, PMID 24223655.
14. Olamilosoye KP, Akomolafe RO, Akinsomisoye OS, Adefisayo MA, Alabi QK. The aqueous extract of *Ocimum gratissimum* leaves ameliorates acetic acid-induced colitis via improving antioxidant status and hematological parameters in male Wistar rats. 2019;5(3):220-7 [cited Jan 27 2023]. Available from: https://www.tandfonline.com/doi/abs/10.1016/j.ejbas.2018.05.006. doi: 10.1016/j.ejbas201805006 [internet].
15. Zheng L, Gao ZQ, Wang SX. A chronic ulcerative colitis model in rats. World J Gastroenterol. 2000;6(1):150-2. doi: 10.3748/wjg.v6.i1.150, PMID 11819549.
16. Yaraee R, Ghazanfari T, Eghtedardoost M, Rajabi M, Naseri M. The effect of MS14 on innate and cellular immune responses in BALB/c mice. Immunopharmacol Immunotoxicol. 2011;33(3):509-14. doi: 10.3109/08923973.2010.543687 [internet]. PMID 21284543.
17. Kim JJ, Shajib MS, Manocha MM, Khan WI. Investigating intestinal inflammation in DSS-induced model of IBD. J Vis Exp. 2012;3678(60):1-6. doi: 10.3791/3678, PMID 22331082. Available from: /pmc/articles/PMC3369627.
18. Luo S, Jiang X, Jia L, Tan C, Li M, Yang Q, *et al*. *In vivo* and *in vitro* antioxidant activities of methanol extracts from olive leaves on *Caenorhabditis elegans*. Molecules. 2019;24(4):704-23. doi: 10.3390/molecules24040704, PMID 30781358. Available from: /pmc/articles/PMC6412793.
19. Khan R, Khan AQ, Qamar W, Lateef A, Tahir M, Rehman MU, *et al.* Chrysin protects against cisplatin-induced colon. toxicity via amelioration of oxidative stress and apoptosis: probable role of p38MAPK and p53. Toxicol Appl Pharmacol. 2012;258(3):315-29. doi: 10.1016/j.taap.2011.11.013, PMID 22155348.
20. Suluvoy JK, Sakthivel KM, Guruvayoorappan C, Berlin Grace VM. Protective effect of *Averrhoa bilimbi* L. fruit extract on ulcerative colitis in Wistar rats via regulation of inflammatory mediators and cytokines. Biomed Pharmacother. 2017;91:1113-21. doi: 10.1016/j.biopha.2017.05.057.
21. Shukla G, Rao AS, Sambasiva Rao A. The present status of medicinal plants – aspects and prospects. Res Pharm and v Biomedical Sci. 2011;2(1).
22. Vishwanath M, Patil K, Patil VK, Kandhare AD, Bhise SD. Anti-inflammatory effect of *Daucus carota* root on experimental colitis in rats. Int J Pharm Pharm Sci. 2012;4(1):337-43.
23. Zubair M, Widén C, Renvert S, Rumpunen K. Water and ethanol extracts of *Plantago major* leaves show anti-inflammatory activity on oral epithelial cells. J Tradit Complement Med. 2019;9(3):171. doi: 10.1016/j.jtcme.2017.09.002, PMID 31193927. Available from: /pmc/articles/PMC6544575.
24. Beara IN, Orčić DZ, Lesjak MM, Mimica-Dukić NM, Peković BA, Popović MR. Liquid chromatography/tandem mass spectrometry study of anti-inflammatory activity of Plantain (*Plantago* L.) species. J Pharm Biomed Anal. 2010;52(5):701-6. doi: 10.1016/j.jpba.2010.02.014, PMID 20219312.
25. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharmacology. 2007;15(6):252-9. doi: 10.1007/s10787-007-0013-x, PMID 18236016.
26. Gillberg L, Varsanyi M, Sjöström M, Lördal M, Lindholm J, Hellström PM. Nitric oxide pathway-related gene alterations in inflammatory bowel disease. Scand J Gastroenterol. 2012;47(11):1283-97. doi: 10.3109/00365521.2012.706830, PMID 22900953.
27. Fusco R, Cirmi S, Gugliandolo E, di Paola R, Cuzzocrea S, Navarra M. A flavonoid-rich extract of orange juice reduced oxidative stress in an experimental model of inflammatory bowel disease. J Funct Foods. 2017;30:168-78. doi: 10.1016/j.jff.2016.12.038.
28. Kukongviriyapan U, Luangaram S, Leekhaosoong K, Kukongviriyapan V, Preeprame S. Antioxidant and vascular protective activities of *Cratoxylum formosum, Syzygium gratum* and *Limnophila aromatica*. Biol Pharm Bull. 2007;30(4):661-6. doi: 10.1248/bpb.30.661, PMID 17409498.
29. Wang B, Ma L, Tao X, Lipsky PE. Triptolide, an active component of the Chinese herbal remedy *Tripterygium wilfordii* Hook F, inhibits production of nitric oxide by decreasing inducible nitric oxide synthase gene transcription. Arthritis Rheum. 2004;50(9):2995-303. doi: 10.1002/art.20459, PMID 15457469.
30. Aguirre-Martinez G. Effective biomarkers to assess the toxicity of pharmaceutical residues on marine bivalves. Pharm Mar Coast Environ. 2021;1:419-55.
31. Oladele JO, Anyim JC, Oyeleke OM, Olowookere BD, Bamigboye MO, Oladele OT, *et al. Telfairia occidentalis* mitigates dextran sodium sulfate-induced ulcerative colitis in rats via suppression of oxidative stress, lipid peroxidation, and inflammation. J Food Biochem. 2021;45(9):e13873. doi: 10.1111/jfbc.13873, PMID 34331468.
32. Patil MV, Bhise S, Kandhare A. Pharmacological evaluation of ameliorative effect of aqueous extract of *Cucumis sativus* L. fruit formulation on wound healing in Wistar rats. Chronicles Young Sci. 2011;2(4):207. doi: 10.4103/2229-5186.93026.
33. Patil MVK, Kandhare AD, Bhise SD. Anti-arthritic and anti-inflammatory activity of *Xanthium srtumarium* L. ethanolic extract in Freund's complete adjuvant induced arthritis. Biomed Aging Pathol. 2012;2(1):6-15. doi: 10.1016/j.biomag.2012.01.002.
34. Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity: assessment of inflammation in rat and hamster models. Gastroenterology. 1984;87(6):1344-50. doi: 10.1016/0016-5085(84)90202-6, PMID 6092199.
35. Tahan G, Aytac E, Aytekin H, Gunduz F, Dogusoy G, Aydin S, *et al*. Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid–induced ulcerative colitis in rats. Can J Surg. 2011;54(5):333-8. doi: 10.1503/cjs.013610, PMID 21933527.
36. Kumar VS, Rajmane AR, Adil M, Kandhare AD, Ghosh P, Bodhankar SL. Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. J Biomed Res. 2014;28(2):132-45 [internet]. doi: 10.7555/JBR.27.20120082, PMID 24683411.
37. Abreu MT. The pathogenesis of inflammatory bowel disease: translational implications for clinicians. Curr Gastroenterol Rep. 2002;4(6):481-9. doi: 10.1007/s11894-002-0024-0, PMID 12441038.
38. Bodhankar S, Raygude K, Kandhare A, Ghosh P. The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats. Int J Green Pharm. 2011;5(3):236-43. doi: 10.4103/0973-8258.91233.
39. Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL. Therapeutic role of curcumin in prevention of biochemical and behavioral aberration induced by alcoholic neuropathy in laboratory animals. Neurosci Lett. 2012;511(1):18-22. doi: 10.1016/j.neulet.2012.01.019, PMID 22281447.

40. Kruidenier L, Kuiper I, van Duijn W, Mieremet-Ooms MAC, van Hogezand RA, Lamers CBHW, *et al.* Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. J Pathol. 2003;201(1):17-27. doi: 10.1002/path.1408, PMID 12950013.
41. Sies H. Glutathione and its role in cellular functions. Free Radic Biol Med. 1999;27(9-10):916-21. doi: 10.1016/s0891-5849(99)00177-x, PMID 10569624.
42. Sánchez de Medina F, Gálvez J, Romero JA, Zarzuelo A. Effect of quercitrin on acute and chronic experimental colitis in the rat. J Pharmacol Exp Ther. 1996;278(2):771-9. PMID 8768730.
43. Raygude KS, Kandhare AD, Ghosh P, Ghule AE, Bodhankar SL. Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. Inflammopharmacology. 2012;20(6):331-41. doi: 10.1007/s10787-012-0122-z, PMID 22349996.
44. Hagar HH, El-Medany A, El-Eter E, Arafa M. Ameliorative effect of pyrrolidinedithiocarbamate on acetic acid-induced colitis in rats. Eur J Pharmacol. 2007;554(1):69-77. doi: 10.1016/j.ejphar.2006.09.066, PMID 17112501.
45. Jamwal S, Kumar P. Animal models of inflammatory bowel disease. Animal models for the study of human disease. 2nd ed [internet] [cited Jan 27 2023]. 2017:467-77. Available from: https://www.sciencedirect.com/science/article/pii/B978012809468600019X.
46. Sommansson A, Saudi WSW, Nylander O, Sjöblom M. The ethanol-induced stimulation of rat duodenal mucosal bicarbonate secretion *in vivo* is critically dependent on luminal Cl–. PLOS ONE. 2014;9(7):e1026544–e10265.

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