

## Research article

**Influence of laboratory-controlled ethanolic wheatgrass extract on acetic acid-induced changes in biochemical and antioxidant indices in the colitis of Wistar rats**Sainu Susan Oommen<sup>1,3</sup>, Hilda Fernandes<sup>2</sup>, Rajendra Holla<sup>3</sup><sup>1</sup>Department of Anatomy, <sup>2</sup>Department of Pathology, Fr. Muller's Medical College, Mangalore, 575002 Karnataka, India<sup>3</sup>Department of Pharmacology, K.S. Hegde Medical Academy (KSHEMA), Nitte Deemed to be University Mangalore, 575 018, Karnataka, India

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Corresponding author: **Rajendra Holla**. Email: rajendraholla@nitte.edu.in**ABSTRACT**

**Introduction and Aim:** *Triticum aestivum* (wheatgrass) is a good source of mineral nutrients and antioxidant enzymes. It also acts as a detoxifying agent and helps to revive healthy cells. The present study aimed to assess the effects of acetic acid on biochemical and antioxidant indices in the colitis of Wistar rats using an ethanolic extract derived from the dried shoots of wheatgrass maintained under specified growth conditions.

**Materials and Methods:** Aqueous and ethanolic extract of wheatgrass was subjected to preliminary phytochemical screening and in-vitro antioxidant activity. Effects of the ethanolic wheatgrass extract were investigated in the acetic acid-induced rat colitis model. Sulphasalazine was used as the standard anti-inflammatory drug. Malondialdehyde (MDA), myeloperoxidase (MPO), total antioxidant capacity (TAC), catalase (CAT), and reduced glutathione (GSH) levels were estimated in the rat blood. Colitis was quantified with a clinical score and colon length/weight index was measured. Histopathological analyses were also performed on the colon tissue of rats.

**Results:** The presence of phytochemical elements such as saponins, tannins, alkaloids, and terpenes in aqueous and ethanolic wheatgrass extract was discovered. These compounds have the potential to boost antioxidant and anti-inflammatory effects. The ethanolic extract significantly reduced MDA, MPO, and antioxidant levels (TAC, CAT, and GSH) in colon tissue and blood. Biochemical measurements corroborated the conclusions of histopathological investigation.

**Conclusion:** According to macroscopic, microscopic, histological, and other biochemical studies, ethanolic wheatgrass extract significantly inhibits experimental colitis in rats.

**Keywords:** Wheatgrass; acetic acid induced colitis; antioxidant activity; Wistar albino rats; biochemical parameters.

**INTRODUCTION**

Living things constantly produce reactive oxygen species (ROS) as a result of numerous metabolic processes and exposure to various physicochemical factors. They are necessary for cellular processes when present in normal physiological quantities. However, at greater levels, they may be hazardous and cause oxidative stress. They are known to contribute to the initiation and development of many illnesses, including ulcerative colitis, cancer, infectious diseases, atherosclerosis, and aging. They are capable of causing significant cellular component damage (1). For the treatment of inflammatory bowel illnesses, several herbal preparations containing polyphenolics and derivatives of flavonoids have promising results (2). Numerous investigations have demonstrated that ulcerative colitis causes an excessive amount of ROS generation and inflammation, which causes oxidative damage to tissues. As a result, antioxidant therapy is required to decrease the damage caused (3). Treatment for ulcerative colitis might include the use of aloe vera, wheat grass, licorice, slippery elm, and curcumin (4).

The Gramineae family member *Triticum aestivum* Linn., commonly known as wheatgrass, is widely farmed practically everywhere in the globe (5). The term "wheatgrasses" is typically used to describe wheat that germinated during a period of 6 to 10 days. The seeds undergo significant modifications during germination or sprouting. The production of beneficial substances like vitamins and phenolics takes place at this stage. Due to the large percentage of chlorophyll in its chemical makeup (70%), it is often referred to as "green blood" (6). Furthermore, because of its structural closeness to haemoglobin, it results in a high oxygen supply to all tissues (7). Plant extracts with high concentrations of carotenoids and chlorophylls are extremely important because they can exert a variety of protective effects through a variety of methods (5).

Although prior work on wheatgrass has been described (8), no thorough analysis of the serum levels of catalase (CAT), reduced glutathione (GSH), and total antioxidant capacity (TAC) in Wistar rat colitis has been done yet. Therefore, the current study's objective was to assess the effects of acetic

acid on biochemical and antioxidant indices in the colitis of Wistar rats by utilising an ethanolic extract made from dried wheatgrass shoots that had been grown under certain circumstances.

## MATERIALS AND METHODS

### Plant material

Wheatgrass (*Triticum aestivum*) seeds were procured from Ozone International in Mumbai and soaked overnight for cultivation. They were then sprinkled with a little amount of water and indirectly exposed to sunshine for a few hours. The laboratory-controlled wheatgrass grass was collected and authenticated on the seventh day by Dr. Keshava Chandra K. of the Department of Botany at St. Agnes College in Mangalore, Karnataka, India.

### Preparation of wheatgrass extract

The freshly harvested young wheatgrass was washed with tap water followed by distilled water, dried in mid sunlight, and at room temperature of 37 °C. In order to prepare the powder for extraction, the dried leaves were first crushed into a fine powder in a clean electric blender. Then, for 24 hours at 50 °C, a weighed quantity of wheatgrass powder was extracted via hot extraction technique, using a Soxhlet extractor (9). Concentrating the acquired solvent extracts in a rotating vacuum evaporator at 40-60 °C until all solvent was removed, yielding an extract sample. The extracted material was dissolved in gum acacia at a 2% concentration for the animal studies.

### Preliminary Phytochemical analysis

According to established protocols, the initial phytochemical evaluation of aqueous and ethanolic wheatgrass extract was performed (10).

### In-vitro antioxidant activity of Wheatgrass extract

#### Ferric ion reducing antioxidant power (FRAP) assay

The FRAP test was performed to determine the extracts' total antioxidant capability. For aqueous and ethanolic wheatgrass extracts, concentrations of 50, 100, 150, 200, and 250 µg/ml were investigated. The complete method was carried out in accordance with Lokapur et al (11). All samples were examined in duplicate.

#### Phosphomolybdenum (PM) assay

Using Lokapur *et al.*, (2020) with minor adjustments, the total antioxidant activity was calculated using the phosphomolybdenum (PM) test (12). Each test tube included 3 ml of double-distilled water and 1 ml of a solution containing the molybdate reagent, as well as varied quantities of aqueous and ethanolic wheatgrass extract, ranging from 50, 100, 150, 200, and 250

µg/ml. These tubes were incubated for 90 min at 95 °C followed by another 20–30 min at room temperature. The absorbance was measured at a wavelength of 695 nm. Sulphazine was used as the reference standard. All samples were tested in triplicates.

#### 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging ability (DPPH) assay

Using the approach previously described by Jose et al., the free radical scavenging capacity of an aqueous plant extract was assessed (13). The data gleaned throughout the study was provided as IC50 - µg/ml. Triplicates of each sample were examined.

### Study animals

Male Albino rats of Wistar strain weighing 200-300g were obtained from the institutional animal facility centre NUCARE, Mangalore. Six rats per cage were housed together in the autoclaved polypropylene cages. Cage changing was done once weekly. They were maintained at 23 ± 2 °C, with a relative humidity of 45 to 65% and 12:12 hours dark/light cycle. The rats were allowed to acclimatize for 7 days before dosing. During this period, animals were observed daily for clinical signs. The animals were fed with a standard rodent diet and filtered water ad libitum throughout the experimental protocol, except for overnight fasting before induction of experimental colitis. During this period, the animals had access to filtered water only. Approval (Reg.115/1999/CPCSEA) of the institutional Animal Ethics committee (IAEC) of K.S Hegde Medical College, Mangalore, was taken for experimenting.

#### Dosages of wheatgrass extract and standard drugs used

Animals were given two separate doses of the freshly made test solution of ethanol and aqueous wheatgrass extracts (200 mg/kg and 400 mg/kg) orally. Rats were starved for 24 hours on the fourth day, and on the fifth day, rats under anaesthesia were given 2 ml (4% v/v) of acetic acid in normal saline by rectal injection. Drug treatment was continued till the 8th day. Sulphasalazine is used as a standard drug. Sulphasalazine and acetic acid treatment was started on the same day.

#### Induction of acetic acid

Rats were given a 24-hour fast and unrestricted access to water before being given ether anaesthesia. A paediatric catheter was inserted intrarectally for 8 cm, and 2 ml of acetic acid in normal saline was progressively injected into the colon. After that, 2 ml of air was expelled to complete spreading the acetic acid throughout the colon. To stop acetic acid leaks from the anus, rats must be in a head-down position for 30 seconds. Using the same techniques, normal saline was administered to control rats (14).

## Experimental design

A total of 30 rats were divided into five groups (six rats in each group).

**Group 1:** Control group received 0.9% saline orally one time daily for 7 days.

**Group 2:** Colitis was induced in rats by rectal administration of 2 ml (4% v/v) acetic acid.

**Group 3:** Colitis was induced in rats by rectal administration of 2 ml (4% v/v) acetic acid and treated with sulphasalazine (500mg/kg).

**Group 4:** Colitis was induced in rats by rectal administration of 2 ml (4% v/v) acetic acid and treated with 200mg/kg BW of wheatgrass extract orally.

**Group 5:** Colitis was induced in rats by rectal administration of 2 ml (4% v/v) acetic acid and treated with 400mg/kg BW of wheatgrass extract orally.

Both wheatgrass and sulphasalazine were administered orally using an oral tube while suspended in 0.9% saline. Rats were starved for 24 hours on the fourth day, and on the fifth day, rats under anaesthesia were given 2 ml (4% v/v) of acetic acid in normal saline by rectal injection. On 8<sup>th</sup> day, blood samples were drawn straight from the heart in order to separate the serum.

## Evaluation of the disease

Rat colons were removed, cleaned with sterile saline, and then utilized to measure biochemical factors, score them macroscopically, and examine their microscopic characteristics (15). Colon length, weight, thickness, and the weight/length index were calculated as previously mentioned (16). The ulcer index was determined by measuring the amount of colonic ulceration in the opened colon.

Ulcer index= Grade of ulcer in positive control- grade of ulcer in test/Grade of ulcer in test – grade of ulcer in normal control\*100

## Biochemical analysis of colon for MPO and MDA level

### Sample preparation

For the biochemical investigation of myeloperoxidase and malondialdehyde level, the proximal 5 cm of the dissected colon sample was employed. The colon sample fragments were cut into small pieces and homogenised using a Polytron homogenizer. centrifuged at 3000 rpm for 20 minutes to extract the supernatant (17).

### Determination of colonic MPO activity

The supernatant sample was mixed uniformly with a citric phosphate buffer solution containing 0.4 mg/mL O-phenylene diamine, 0.015% hydrogen peroxide, and a pH of 5.0. Spectrophotometric analysis was

used to quantify the change in absorbance at 492 nm. Horseradish peroxidase was used to compare the reference dilution's absorbance to the test absorbance. Myeloperoxidase (MPO) was quantified in wet scrapings using (U/gm) units per gram (18).

### Determination of MDA level

With minor adjustments to the methodology described by Ganjare *et al.*, (2011), malondialdehyde (MDA) levels in cell lysate were measured spectrophotometrically to assess lipid peroxidation (19). 0.1 ml of the tissue sample, 0.2 ml of sodium dodecyl sulphate, 8.1%, 1.5 ml of acetic acid, 2%, and 1.5 ml of thiobarbituric acid, 0.8%, were combined in the reaction mixture. A 5 mL mixture of n-butanol and 15% pyridine was then added after the pH of the solution was brought down to 3.5 and the volume eventually increased to 4 ml with distilled water. This mixture underwent an intense shaking. The organic layer's 532 nm absorbance was estimated spectrophotometrically following a 10-minute centrifugation at 4000 rpm.

### Determination of antioxidant parameters in colonic tissue

By performing the conventional phosphomolybdenum (PM) experiment, the total antioxidant capacity (TAC) was calculated (20). Reduced glutathione (GSH), a non-enzymatic antioxidant assay, and the enzyme Catalase (CAT) activity were both assessed using Jose *et al.*, (21).

### Histopathological examination

Tissues from the colon were fixed in 10% formalin buffer. 5 mm thick paraffin tissue blocks were produced for sectioning. For blind microscopic inspection, haematoxylin and eosin (H & E) stain was utilised (22).

### Statistical analysis

For *in vivo* experiments, results were represented as mean±S.E.M for six rats in each group, and statistically significant differences between values were assessed using one-way analysis of variance (ANOVA) followed by Dunnett's test, and for *in vitro* antioxidant activity, two-way ANOVA. GraphPad PRISM 5 software was used to statistically analyse the findings.

## RESULTS

The presence and lack of different key bioactive components in wheatgrass aqueous and ethanol extracts was investigated in this study. The findings revealed the existence of terpenoids, glycosides, alkaloids, saponins, terpenoids, and terpenes (Table 1)

**Table 1:** Qualitative phytochemical analysis of wheatgrass

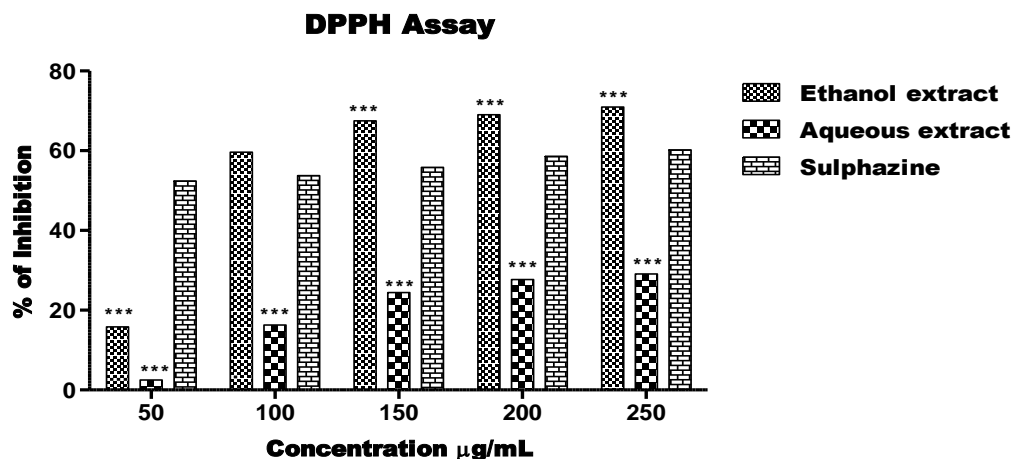
Sl.No.	Chemical tests	Wheatgrass extract	
		Aqueous	Ethanolic
1	<b>Test for triterpenoids and steroids</b> Liebermann Burchard Test	-	-
2	<b>Test for glycosides</b> Keller Killiani's Test	-	+
3	<b>Test for saponins</b> Foam test Froth test	+	+
4	<b>Test for alkaloids</b> Wagner's Test	-	+
5	<b>Test for flavonoids</b> Ferric chloride test Alkaline reagent test Lead acetate solution test	+	+
6	<b>Test for tannins</b> Gelatine test	-	+
7	<b>Test for proteins</b> Biuret test	-	-
8	<b>Test for free amino acids</b> Ninhydrin test	+	-
9	<b>Test for carbohydrates</b> Benedict's test	-	-
10	<b>Test for vitamin C</b> DNPH test	-	+
11	<b>Test for sterols</b> Salkowski test	-	-
12	<b>Test for resins</b>	-	-

+ and - indicates the presence or absence of phytochemicals respectively

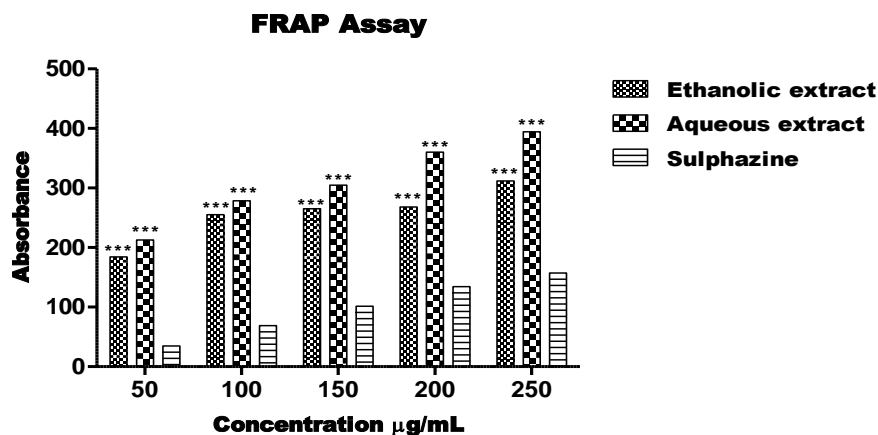
### *In-vitro* antioxidant activity of wheatgrass extracts

In this work, the antioxidant activity of wheatgrass aqueous and ethanolic extracts was examined using the DPPH, FRAP, and PM techniques. Aqueous and ethanolic extracts of wheatgrass showed extremely significant radical scavenging ability as DPPH activity increased with concentration, although standard medication exhibited stronger DPPH scavenging effect at lower concentration (50  $\mu\text{g/mL}$ ). The ethanolic extract had an  $\text{IC}_{50}$  value of 103.9  $\mu\text{g/mL}$  and the aqueous extract had an  $\text{IC}_{50}$  value of

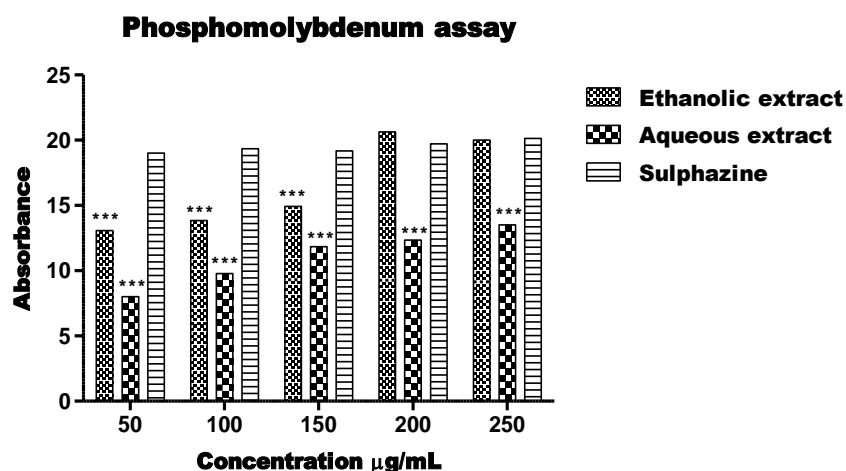
494  $\mu\text{g/mL}$  when the radical scavenging activities of the wheatgrass extracts were compared, whereas the reference medication had an  $\text{IC}_{50}$  value of 37.1  $\mu\text{g/mL}$  (Fig. 1). It was found that both aqueous and ethanolic wheatgrass extract showed stronger FRAP activity compared to standard drug (Fig. 1). Furthermore, phosphomolybdenum activity revealed that ethanolic wheatgrass extract showed highly significant activity compared to aqueous extract (Fig. 3). In addition, Sulphazine was used as standard in this study.



**Fig. 1:** DPPH scavenging activity of aqueous and ethanolic wheatgrass extract. Each value is expressed as means  $\pm$  S.E.M. Concentration ( $\mu\text{g/ml}$ ) taken on the x-axis and percentage inhibition taken on the y-axis. Statistical significance was assessed using two-way ANOVA (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\* $p < 0.001$ ) as compared to the standard group.



**Fig. 2:** FRAP activity of aqueous and ethanolic wheatgrass extract. Data are expressed as mean ± S.E.M (n = 3). Statistical significance was assessed using two-way ANOVA (\* p < 0.05; \*\* p < 0.01; \*\*\*p < 0.001) as compared to the standard group.



**Fig. 3:** PM assay of aqueous and ethanolic wheatgrass extract. Data are expressed as mean ± S.E.M (n = 3). Statistical significance was assessed using two-way ANOVA (\* p < 0.05; \*\* p < 0.01; \*\*\*p < 0.001) as compared to the standard group.

**Acetic acid-induced colitis**

According to the results of this study, compared to the healthy control group, the experimental control group's colon experienced inflammation after receiving 2 ml of 4% acetic acid intrarectally. Animals in the group that were given ethanol extracts had their inflammatory response significantly decreased.

**Effect of ethanolic wheatgrass extract on macroscopic score**

The colons of the rats were macroscopically evaluated for haemorrhage, ulceration, and inflammations by a blinded, independent investigator following intrarectal delivery of 2 ml of 4% acetic acid. When compared to rats in the normal group, the mean macroscopical score in the acetic acid control group was found to be considerably higher (P < 0.01). Wheatgrass extract at 200 and 400 mg/kg showed better benefits in decreasing colon macroscopical lesions when compared to a conventional drug (Table 2).

**Table 2:** Effect of ethanolic wheatgrass extract on colon length, weight, thickness and macroscopic score of rats in acetic acid induced ulcerative colitis

Parameters	Normal	Control (4% Acetic acid induced colitis)	Standard drug-Sulphasalazine pre-treated +AA	Pre-treated with ethanolic extract of 200mg/kg bw of wheatgrass+AA	Pre-treated with ethanolic extract of 400mg/kg bw of wheatgrass +AA
Colon length (cm)	19.66±1.989	15.48 ±1.70 <sup>#</sup>	18.01± 0.877	18.43± 0.69	17.15 ±0.609
Colon weight (g)	1.13±0.114	2.82± 0.39 <sup>#</sup>	1.36± 0.34	1.35± 0.16	2.31 ±0.26 <sup>#</sup>
Colon thickness (cm)	0.44± 0.04	1.123± 0.05 <sup>#</sup>	0.45± 0.06	0.41± 0.058	0.41 ±0.026
Colon weight/length	0.05± 0.005	0.18± 0.02 <sup>#</sup>	0.26 ±0.29	0.07 ±0.010 <sup>#</sup>	0.13± 0.014 <sup>#</sup>
Ulcer index	0	15.78 ± 1.58 <sup>###</sup>	2.6 ± 0.409 <sup>***</sup>	2.65± 0.24 <sup>***</sup>	2.51 ±0.26 <sup>***</sup>
Macroscopic score	0	4.39± 0.59 <sup>###</sup>	0.94± 0.20 <sup>***</sup>	0.95 ±0.175 <sup>**</sup>	1.43± 0.27 <sup>**</sup>

Values expressed as mean ± S.E.M (n=6) and analyse by ANOVA followed by Dunnett's test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 as compared to acetic acid control group; <sup>#</sup>P < 0.05, <sup>##</sup>P < 0.01, <sup>###</sup>p<0.001 when compared to normal group.

### Effect of ethanolic wheatgrass extract on colonic MDA and MPO concentrations

A rise in the amounts of MDA and MPO was linked to the colitis brought on by acetic acid. It was shown that wheatgrass ethanol extracts at 200 and 400 mg/kg were the most successful in reducing myeloperoxidase and malondialdehyde activity in tissues stimulated by acetic acid (Table 3).

### Evaluation of serum levels in antioxidant parameters

The mean antioxidant levels in serum of healthy subjects, acetic acid-induced colitis, and rats pre-treated with ethanolic extracts of 200mg/kg BW and 400mg/kg BW of wheatgrass + acetic acid-induced rats revealed that there were differences in the mean levels of total antioxidant capacity (TAC), catalase (CAT), and reduced glutathione (GSH) within the groups, with the healthy group being comparatively higher (Table 4).

**Table 3:** Biochemical parameters of colitis model

Groups	MDA ( $\mu\text{M/L}$ )	MPO (U/ml)
Normal	6.39 $\pm$ 0.86***	15.66 $\pm$ 1.14***###
Control (4% Acetic acid induced colitis)	17.04 $\pm$ 1.94###	69.10 $\pm$ 8.42###
Standard drug- Sulphasalazine pre-treated +AA	6.41 $\pm$ 1.14***	34.56 $\pm$ 5.62***
Pre-treated with ethanolic extract of 200mg/kg bw of wheatgrass +AA	15.83 $\pm$ 2.18###	58.64 $\pm$ 4.76***###
Pre-treated with ethanolic extract of 400mg/kg bw of wheatgrass +AA	11.35 $\pm$ 3.40***###	45.71 $\pm$ 5.91***###

Values expressed as mean  $\pm$  S.E.M (n=6) and analyse by one-way ANOVA followed by Dunnett's test.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  as compared to acetic acid control group; # $P < 0.05$ , ## $P < 0.01$ , ### $p < 0.001$  as compared to normal group.

**Table 4:** Serum estimation of antioxidant activity

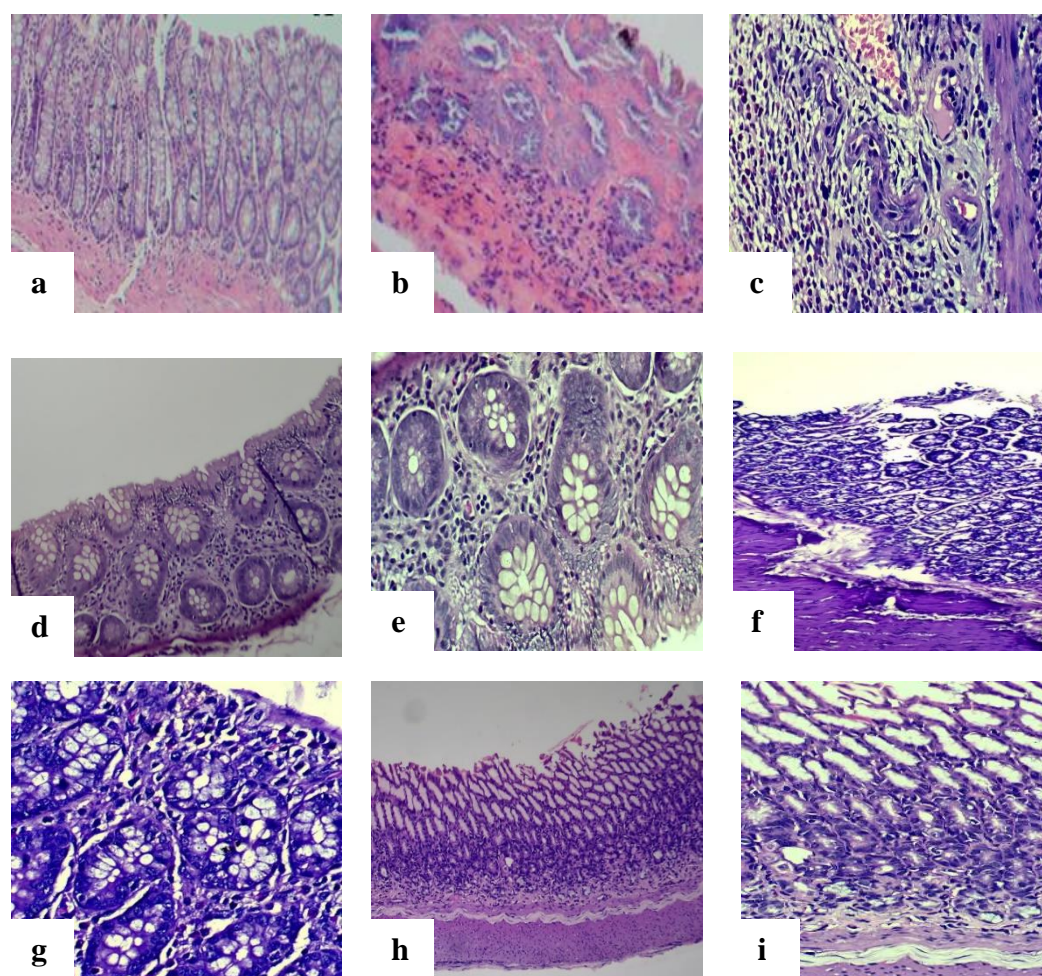
Groups	TAC ( $\mu\text{g/mL}$ )	Catalase (U/ml)	GSH ( $\mu\text{g/min/mg protein}$ )
Normal	0.91 $\pm$ 0.06*	39.76 $\pm$ 5.56***###	2.99 $\pm$ 0.38***###
Control (4% Acetic acid induced colitis)	0.703 $\pm$ 0.084	24.48 $\pm$ 3.87###	0.94 $\pm$ 0.11#
Standard drug- Sulphasalazine pre-treated +AA	0.803 $\pm$ 0.110	42.43 $\pm$ 4.91***	1.94 $\pm$ 0.11*
Pre-treated with ethanolic extract of 200mg/kg bw of wheatgrass +AA	0.367 $\pm$ 0.097***###	30.92 $\pm$ 4.89***###	1.73 $\pm$ 0.29**
Pre-treated with ethanolic extract of 400mg/kg bw of wheatgrass +AA	0.305 $\pm$ 0.06***###	28.70 $\pm$ 3.04###	2.09 $\pm$ 0.52**#

Values expressed as mean  $\pm$  S.E.M (n=6) and analyse by ANOVA followed by Dunnett's test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  as compared to acetic acid control group; # $P < 0.05$ , ## $P < 0.01$ , ### $p < 0.001$  as compared to normal group.

### Histopathological examination of colon

At a microscopic level, the mucosa, submucosa, and mucosa of the colon of a control rat were all present and appeared normal (Fig.4a). In the meantime, colon sections from rats in the model group showed diffused colitis and UC, extensive damage including oedema of the mucosa and submucosa, chronic inflammatory cells infiltrated with widely ulcerating mucosa and haemorrhages, and the presence of a significant neutrophil population (Fig. 4b and c). A few sections from the group that received sulphasalazine showed a little submucosal oedema

(Figure 4(d) and (e)). However, rats' colons pre-treated with 200 mg/kg BW of ethanolic wheatgrass extract and then subjected to acetic acid-induced colitis, stained with H&E to reveal ulceration and crypt loss, showed only mild alterations, including slight submucosal oedema, few inflammatory cells' infiltrations, and congested blood vessels (Figure 4(f) and (g)). Additionally, rats pre-treated with 400 mg/kg BW of ethanolic wheatgrass extract and subsequently given acetic acid to induce colitis did not display any crypt loss in colon sections stained with H&E (Figure 4(h) and (i)).



**Fig. 4:** Photomicrographs of sections of colons from rats. (a) healthy control rat colon section with intact mucosal layer and epithelium in 10X; (b) acetic acid induced colitis model rat in 10X, (c) in 40X; (d) Sulphasalazine (standard drug treatment) treated rat in 10X, (e) in 40X; (f) pre-treated with 200mg/kg Bwt of ethanolic extract of wheatgrass in 10X, (g) in 40X; (h) pre-treated with 400mg/kg Bwt ethanolic extract of wheatgrass in 4X, (i) in 40X.

## DISCUSSION

Although wheatgrass has been traditionally used to treat a number of illnesses, there is currently a dearth of scientific research on the herb. It is obvious that much more scientific research must be done on this plant since it may provide a natural alternative to pharmaceutical medications for a variety of ailments. The growing popularity of phytomedicine is due to its low risk of negative effects (23).

A preliminary phytochemical study indicates several naturally occurring antioxidants that are preferred over synthetic antioxidants owing to safety concerns (24). This research is the primary method for detecting secondary metabolites in plant extracts. Aqueous and ethanolic extracts of wheatgrass showed promising results for most of the bioactive compounds that are important to medicine, according to the present study's findings. The issue of oxidative stress has existed for a very long period, and extensive study has been conducted on it. One of the fundamental illnesses that appear to be the mother of other illnesses including diabetes, hypertension, cancer, and ulcers is oxidative stress. To maximize the benefits of

antioxidants from any source, it is essential to employ a range of assays for determining total antioxidant activity. In this study, the antioxidant activity of wheatgrass aqueous and ethanolic extracts was assessed utilizing the FRAP, PM, and DPPH free radical scavenging assays.

It is common practice to evaluate the effectiveness of antioxidants using the stable molecule DPPH, which may be lowered by either removing hydrogen or electrons. Our research indicates that aqueous and ethanolic wheatgrass extracts are free radical scavengers and that variations in the IC<sub>50</sub> value and potential DPPH radical scavenging activity were caused by the presence of secondary metabolites in these extracts. In the FRAP experiment, wheatgrass extracts had a much better capacity to convert Fe<sup>3+</sup> to Fe<sup>2+</sup> than sulphasalazine did at all doses (Fig. 2). To evaluate the overall antioxidant activity of the wheatgrass extracts leaf and sulphasalazine, the phosphomolybdenum technique was also chosen. It is a colorimetric approach that aids in determining the sample's conversion of phosphate-Mo (VI) to phosphate-Mo (V), which leads to the formation of a bluish-green phosphate-Mo (V) complex (25). The

ethanol extract showed the best antioxidant activity in the PM test, and it was shown that these activities were dose-dependent with increasing concentrations (Figure 3). Our findings are consistent with those of the prior paper (26).

One of the most popular experimental models for testing medications to treat ulcerative colitis is the rat model of acetic acid-induced colitis (16). The histological examination in this work demonstrated that elevated levels of both TNF- $\alpha$  and PGE2 resulted in epithelial cell necrosis, oedema, and neutrophil infiltration. According to macroscopic, microscopic, ulcer index, and other biochemical analyses, the ethanolic extract of wheatgrass in the current study had a substantial protective effect against experimental colitis in rats. Ulcer indices were calculated quantitatively. In comparison to normal medication, pre-treatment with wheatgrass ethanol extract exhibited a greater protective effect on ulcer index.

The peroxidase enzyme known as myeloperoxidase (MPO) is often present in neutrophil granulocytes. It is an excellent indicator of tissue inflammation, damage, and neutrophil infiltration. Acetic acid increased colonic Myeloperoxidase levels, indicating neutrophil infiltration; this indicates that neutrophil accumulation causes colitis by inducing oxidative damage. Pre-treatment with wheatgrass ethanol extract reduced neutrophil infiltration as shown by a reduction in colon Myeloperoxidase (MPO) levels and the formation of histological characteristics (27).

Malondialdehyde (MDA), which is observed to be elevated in the colonic tissue of the rats given acetic acid treatment, is a very effective biomarker of lipid peroxidation. A high amount of lipid peroxidation can set off vicious cycles that result in reactive metabolites, decrease cellular antioxidants, and promote the growth of further inflammation. Since wheatgrass ethanol extract was able to considerably lower the malondialdehyde level, it was able to improve colonic oxidative balance in rats with colitis (28).

The serum estimated results (Table 4) showed that the levels of antioxidants (TAC, CAT, and GSH) were relatively greater in the healthy group than in the other groups, indicating that the antioxidants successfully balance the oxidant-related stress in the body. The findings are consistent with those of previous reports (29).

## CONCLUSION

Wheatgrass may be an excellent alternative in the present pandemic crisis when global health awareness appears to be growing and people are looking for quick home treatments and shifting their focus to old Vedic-derived homemade therapeutic medicine. Wheatgrass has the potential to be an effective

immunity booster. The current study's findings revealed that an ethanolic extract of wheatgrass was extremely efficient in preserving mucous membranes from acetic acid-induced damage. The presence of phytochemical components such as saponins, tannins, alkaloids, and flavonoids has been established in ethanolic wheatgrass extract. By inhibiting the production of oxido-inflammatory mediators such as MPO and MDA, these components may enhance antioxidant and anti-inflammatory capabilities against acetic acid-induced experimental colitis. The validity of antioxidant levels as a predictive or diagnostic marker has to be supported by long-term investigations.

## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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