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SHORT COMMUNICATION

Wheatgrass (*Triticum aestivum*) Extract Reduces Inflammatory TNF-Alpha Response in Experimentally Induced Gastric Ulceration in Rat Model

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Abstract

Background: The term 'peptic ulcer' refers to the ulcers that occur in either the stomach or the first part of the small intestine that leads out of the stomach, called the duodenum. **Aim:** The present study aims to examine the protective consequence of wheatgrass (*Triticum aestivum*) extract on ethanol-induced gastric ulceration in Albino Wistar rats. **Methods:** The extract of wheatgrass significantly reduced gastric ulceration in rats in comparison to the control ethanol group. **Results:** Consequently, a remarkable decrease in the inflammatory activity of the TNF-α was observed in ethanol-induced ulcerated rats receiving treatment with wheatgrass extract. The gastric volume and ulceration increased with oral administration of ethanol. A drastic decrease in the total acidity and ulcer index was observed in rats treated with wheatgrass extract. In the protective index percentage, a substantial increase was observed with doses of the wheatgrass extract. **Conclusion:** These results suggest the gastro-protective effect of wheatgrass extract.

Keywords: Gastric Ulcer, TNF-α, *Triticum aestivum*, Ulcer-Index, Wheat Grass Extract

1. Introduction

Peptic ulcer is one of the major disorders in the alimentary canal affecting more than 10% of the world's population. The reasons for gastric ulcers have been connected to a variety of causes, including Helicobacter pylori infection, excessive use of Non-Steroidal Anti-Inflammatory Medicines (NSAIDs), smoking, excessive alcohol use and stress[1]. Various anti-ulcer drugs such as proton pump inhibitors[2], H2 receptor blockers, prostaglandin analogues etc, are employed to manage and the treatment of gastric ulcers[3]. However, clinical evaluation of each of these drugs has shown low to severe side effects, prompting a search for a non-toxic, easily accessible and affordable anti-ulcer medication[5]. In recent years medicinal plants have been highly valued and widely used in the traditional system of medicines to cure many diseases including gastric ulcers[6].

Wheatgrass, a young shoot of the common wheat plant (*Triticum aestivum* Linn) is known for its therapeutic and nutritional properties[7,8] as well as for its potential as an anti-inflammatory anti-oxidative and anti-apoptotic agent[9]. Phytochemical screening of wheatgrass has revealed it to be a rich source of flavonoids, polyphenols and major nutrients such as vitamins C and[10-12] its medicinal efficacy in treating gastric ulcers has been studied and documented by few[7,9,13]. Wheat grass's medicinal benefit has been attributed to its rich chlorophyll (70%) and metallo-chlorophyll derivatives reported to possess anti-oxidant and anti-inflammatory properties[14-17].

Among the several pathological factors responsible for gastric ulcer formation, inflammation is an important step, mediated by the signalling and expression of cytokine Tumour Necrosis Factor (TNF, also known as TNF-*α*), identified as a key regulator of

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the inflammatory cell response[18]. Depending on the cellular conditions, TNF-α signalling could mediate cell survival or trigger cell death, thus playing a striking role in maintaining gut homeostasis[9,17]. As wheatgrass has the potential to be anti-inflammatory[9], the present study aims to investigate the medicinal potential of wheatgrass extract in inhibiting the production of TNF-**α** levels in ethanol-induced gastric ulcers in a rat model.

2. Materials and methods

2.1 Preparation of *Triticum aestivum* Linn. Grass Extract (TAE)

Fresh sprouts of wheatgrass (*Triticum aestivum*) plants were obtained from germinating the plant seeds procured from Ozone International, Mumbai. The seeds obtained were first soaked overnight in water and later transferred to the soil for germination under indoor conditions. The soil was sprinkled with water and kept in indirect sunlight for 3-4 hours daily for 7 days. On the seventh day, the wheatgrass grown was harvested and authenticated by Dr. Keshava Chandra, Department of Botany, St. Agnes College, Mangalore, India. The harvested wheatgrass was air-dried and ground to obtain a coarse powder. The powdered wheatgrass was subjected to 70% ethanol extraction using a soxhlet extractor as described previously[19]. The aqueous ethanol extract was filtered and evaporated further with the help of a rotary evaporator at 40°C until the solvent had completely dried. The extract obtained was stored in a refrigerator at 4°C until further use.

2.2 Animals

Albino rats weighing around 200-250g were obtained from the institutional animal facility centre NUCARE, a facility approved by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Mangalore. The rats were maintained in cages at ambient room temperature in a 12-hour light/dark cycle. The rats were fed with pelleted feed and water throughout the experiment. Prior approval was taken from the Institutional Animal Ethics Committee (Reg.115/1999/CPCSEA) for all the experiments.

2.3 Experimentally Induced Gastric Ulcers

The experimental rats were divided into six groups (G1-G6) with six animals in each group. G1-control group received distilled (0.5ml /100g body weight), G2-+ve ulcerogenic control group received 70% aqueous solution (v/v) of ethanol orally (0.5ml/100g body weight), G3 and G5–groups animals were pre-treated with TAE (200mg/kg body weight and 400mg/kg body weight respectively) for eight days with subsequent fasting for 24-hours followed by ethanol induction and sacrifice of animals after 1h. G4 and G6 – animals were subjected to 24-hour fasting, followed by oral administration of ethanol (0.5ml/100g body weight) and subsequent treatment with TAE (200mg/kg body weight and 400mg/kg body weight respectively) for the next 8 days. Post-treatment the animals were sacrificed for further studies.

2.4 Evaluation of Gastric Indices

The sacrificed animals were cut open along the greater curvature. The stomach was opened and gastric content was separated. The stomach was rinsed thoroughly with normal saline and examined macroscopically for ulcerative lesions and their sizes were determined as described previously[20]. The lesion sizes determined were used in calculating the Ulcer Index (UI) and the percentage preventive index using the following equations:

$$\text{Ulcer Index (UI)} = \frac{\text{Total Ulcer Score}}{\text{Number of animals ulcerated}}$$

$$\text{Preventive Index \%} = \frac{UI_{(control)} - UI_{(treated)}}{UI_{(control)}} \times 100$$

Parallely, the gastric content collected was subjected to centrifugation at 2000xg for 5 minutes. The supernatant consisting of the gastric juice was checked for its gastric volume, pH and total acidity. To determine the total acidity 1ml of the gastric juice was first diluted tenfold, followed by the addition of phenolphthalein and subsequent titration with 0.01M sodium hydroxide solution until the colour turned light pink. The total acidity (T_{ac})/litre was calculated from the equation, T_{ac} = n x 0.01x 40 x 1000, where n is the titrated NaOH volume, 0.01 and 40 implying the normality and molecular weight of NaOH[21].

2.5 TNF-α Assay

TNF-α was assayed using Rat TNF-**α** ELISA kit PicoKine™ (Boster Biological Technology, Pleasanton

CA, USA, Catalog # EK0526). Briefly, tubes containing pre-weighed samples suspended in 10mmol/L sodium phosphate buffer (pH 7.4, 1:5 w/v) were first incubated in a shaking water bath at 37°C for 20 minutes, followed by centrifugation at 9000g for 30s at 40°C. The supernatant obtained was quantified for TNF-α based on the manufacturer's instructions. The results are expressed as pictograms/mg of wet tissue.

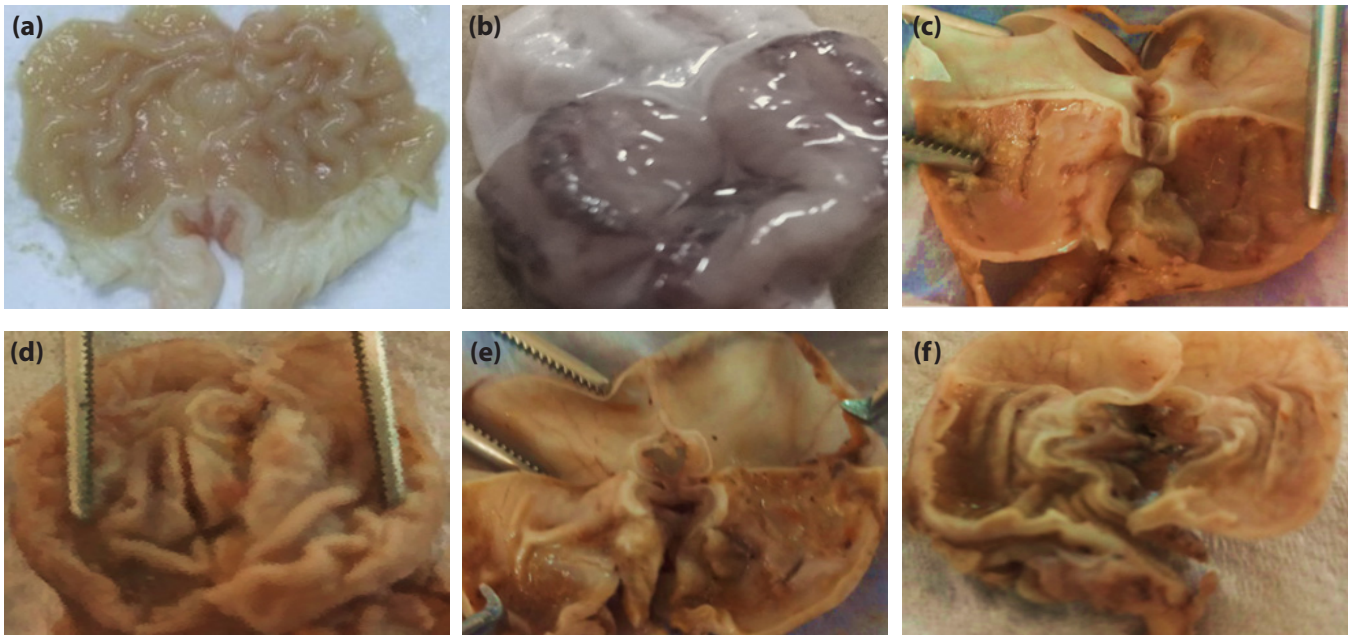
2.6 Statistical Analysis

Data was subjected to a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test/Tukey-Kramer multiple comparison test. The data were analysed using the Statistical Package for the Social Sciences (SPSS, version 18.0). The results are expressed as mean ± standard deviation (SD) and the level of significance was identified at $p > 0.01$.

3. Results

The effect of wheatgrass crude extracts on the gastric indices (pH, total acidity, ulcer index) and Preventive index percentage against ulcers in control and experimental animals are summarised in Table 1. As seen in the table, there was an increase in pH, gastric volume, total acidity and the degree of gastric ulcer index in the G2 group of rats that received oral administration of ethanol in comparison to the G1 (control) group. However, in rats that were inducted with pre and post-ethanol gastric ulcers followed by subsequent treatments with 200 and 400mg of wheatgrass extract (G3-G6 groups), a statistically significant difference was seen in the mean reduction of gastric indices between the positive control group (G2) and the experimentally treated groups ($p<0.01$, Table 1).

Experimentally obtained results were substantiated by macroscopic evaluation, wherein no lesions were observed in rats of the normal control group (Figure 1a), while the rats of the positive control group (G2 group) showed extensive damage to the stomach tissue causing haemorrhage and oedema (Figure 1b). In rats with pre-induced ulcers, followed by treatment with TAE aqueous extracts at 200mg and 400mg/kg respectively, near-normal architecture with no gastric oedema was seen (Figures 1c and 1e). Similar results were also observed in rats pre-treated with TAE followed by ethanol-induced gastric ulceration (Figures 1d and 1f).



(a). Normal control group; **(b).** Ulcer control group; **(c).** Rats with pre-induced ulcers followed by treatment with TAE extract (200mg/kg); **(d).** Rats pre-treated with TAE extract (200mg/kg) followed by post-induced ulcers; **(e).** Rats with pre-induced ulcers followed by treatment with TAE extract (400mg/kg); **(f).** Rats pre-treated with TAE extract (400mg/kg) followed by post-induced ulcers.

Figure 1. The effects of *T. aestivum* extracts on ethanol-induced gastric ulcer.

The extract also showed a gastro-protective effect on both pre and post-ethanol-induced animals with a preventive index of 52 and 55% respectively at a dose of 200mg/kg of wheatgrass extract. An increase in dose to 400mg of TSE provided better protection of 77 and 78% in G5 and G6 group rats respectively. The TNF-**α** levels recorded for control and experimental groups are also presented in Table 1. It was observed that the TNF-**α** levels in gastric-induced (G2 group) rats increased (mean 50.96+3.74) significantly in comparison to rats that received distilled water (G1 group, mean 39.43 ± 7.06). A significant decrease in TNF-**α** values was seen in the G3 and G4 groups treated with 200mg TAE in comparison to the G2 group. However, no difference between pre or post-induction of gastric ulcers and subsequent treatment with 200mg of TAE was seen. A similar reduction in TNF-**α** level was seen in the case of animals in the G5 and G6 groups that were treated with 400mg TAE. Although a marginal increase in levels for TNF-**α** was observed between animals in groups G5 (mean 33.03 ± 5.75) and G6 (mean 30.14 ± 7.54), the overall gastro-protective effect remained almost equally the same i.e 77 and 78% respectively.

4. Discussion

In the present study, oral administration of ethanol was seen to significantly increase the gastric volume as well as ulceration in rats while in wheatgrass extract-treated animals a significant inhibition against ulceration was observed. This could be attributed to the medicinal potential of wheatgrass as being an anti-oxidant and anti-inflammatory agent[10-12].

Ethanol is known to cause severe damage to the gastric mucosa by being cytotoxic and promoting the development of tissue lesions[22]. TNF-**α,** an important pro-inflammatory cytokine, is known to be involved in a wide range of functions and a major factor in the inflammation process[18]. In a normal gut, TNF-**α** plays a fundamental role in maintaining intestinal homeostasis and is not usually detectable in healthy individuals. However, during pathological conditions such as infections, injury or inflammation they are released from activated macrophages as the first line of defence signalling a cascade of events leading to elevated TNF-**α** levels in serum and in intestinal mucosa[23]. In this study, an elevated level of TNF-**α** was observed in ulcerated mice which corroborates with earlier

Table 1. Gastric indices and TNF-α levels in pre and post-induced gastric ulceration in rats and TAE-treated

Treatments	Gastric Indices				Preventive Index %	TNF-α (Picograms/ mg)
	pH	Gastric Volume (ml)	Total Acidity (mEq/l)	Ulcer Index (UI)		
G1 (Normal- Negative Control)	4.55±0.20	2.55±0.31	25.73±0.77	-	-	39.43+7.06
G2 (Gastric Ulcer Induced - Positive Control)	3.48±1.24	5.27±0.21	77.12±1.63	9.16±1.76	-	50.96+3.74
G3 (Pre-induced gastric ulcers followed by 200mg TAE treatment)	5.35±0.54*#	2.27±0.24*#	27.00±0.02*#	3.74±1.34*#	52.0	30.12+5.95*#
G4 (Pre-treated with 200mg TAE followed by post-induced gastric ulcer)	4.82±0.57*#	2.12±0.41*#	28.21±2.64*#	2.19±0.42*#	55.0	30.39+3.82*#
G5 (Pre-induced gastric ulcers followed by 400mg TAE treatment)	4.89±0.35*#	2.45 ±0.27*#	35.39±1.50*#	2.32±0.85*#	77.0	33.03+5.75*#
G6 (Pre-treated with 400mg TAE followed by post-induced gastric ulcer)	4.84±0.23*#	2.56 ±0.45*#	28.12 ±3.60*#	1.54±0.28*#	78.0	30.14+7.54*#

G1-G6 (Groups1-6); TAE= *Triticum aestivum* sprout extract; Values are expressed as mean + standard deviation (n=6 rats). Statistically significant *p<0.01 when compared with normal control; # p<0.01 when compared with positive control.

studies wherein chronic intestinal inflammation was shown to be due to elevated levels of TNF-**α** secreting cells in the intestinal tissue[17,24]. Additionally, several studies involving rat models with induced intestinal inflammation are characterised by an increased TNF-α level in the intestinal mucosa[23,25,26].

Further, treatments with wheatgrass extracts at doses of 200mg and 400mg showed a significant reduction in TNF-**α** levels in pre and post-induced ulceration in rat models (Table 1). This shows that *T.aestivum* grass extract potentially inhibits the production of TNF-**α**, thereby reducing the inflammatory response. The data generated in this study supports earlier studies on the anti-inflammatory potential of wheatgrass[9,16]. Overall, this study supports the anti-inflammatory effects of wheatgrass *in vitro*, implying that its administration could help attenuate inflammatory responses by reducing TNF-**α** expression. Wheatgrass, as a whole, could be regarded as a potential anti-inflammatory therapeutic candidate. This study demonstrated that wheatgrass extract can alleviate the TNF-**α** inflammatory response thereby ameliorating gastric injury in rats.

5. Compliance with Ethical Standards

The experimental protocols were approved by the Institutional Ethical Committee, K. S. Hegde Medical College, Mangalore (Protocol approval No. Reg.115/1999/ CPCSEA). The animals were maintained according to guidelines provided by Control and Supervision of the Experiments on Animals (CPCSEA).

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