



## IN-VITRO EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF TRITICUM AESTIVUM (WHEAT GRASS)

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### ABSTRACT

The present investigation was carried out to evaluate the anti-inflammatory potential of solvent extracts of *Triticum aestivum* (wheat grass). The anti-inflammatory activities were assessed through in vitro, the results were found to be very surprising and promising. Ethanol solvent extracts of *Triticum aestivum* (wheat grass) were found to have significant anti-inflammatory activity. The ethanolic fractions of the plant causes significant reduction in inflammation compared to standard anti-inflammatory drug, diclofenac Sodium. Thus results showed that extracts showed significant anti-inflammatory activity in dose-dependent manner. The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane, and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated Neutrophils such as bacterial enzymes and proteases which cause further tissue inflammation and damage. Protein denaturation is also used to assess to anti-inflammatory activity of wheat grass. The results show that decrease in protein denaturation is presented by increase in absorbance. The present investigation indicate that wheat grass possess anti-inflammatory activity.

**KEYWORDS:** *Triticum aestivum* (wheat grass), anti-inflammatory, HRBC, Protein Denaturation.

### INTRODUCTION

Inflammation is a normal protective mechanism to tissue injury and it consist of a complex array of enzyme activation, chemical mediator release, fluid extravasations, cell migration, tissue breakdown and repair.<sup>[1]</sup> In this process, inflammation is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase in denaturation of proteins and membrane modifications.<sup>[2]</sup> In this process, inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes/macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors leads to increased vascular permeability, neutrophils chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Most of these mediators have short - live but cause harmful effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acids (prostaglandins, leukotrienes) and cytokines (tumor necrosis factor and interleukin -1).<sup>[3]</sup> The mechanism of inflammation involves a series of steps such as metabolism of arachidonic acid which leads to formation of inflammatory mediator prostaglandins and thomboxanes in presence of cyclooxygenase and 5-

lipoxygenase respectively. The study of plants that have been used traditionally for curing inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs.<sup>[4]</sup> *Triticum aestivum* Linn belongs to the family Poaceae (Graminae) is used for both human and animal consumption.<sup>[5]</sup> It has potential therapeutic activity indicate the presence of wide variety of chemical constituents such as 70% chlorophyll, vitamins like A, C, E and B complex and The various enzymes responsible for its pharmacological actions are protease, amylase, lipase, cytochrome oxidase, transhydrogenase, super oxide dismutase (SOD).<sup>[6]</sup> Due to its ability to increase in hemoglobin content it known as green blood Thus, wheatgrass, containing about 70% chlorophyll, has been proclaimed to improve blood flow, aid in digestion and in general detoxification of the body.<sup>[7]</sup>

### METARIALS AND METHODS

#### Preparation of extract

The leaves material of *Triticum aestivum*, was collected from surroundings of Aditya college of pharmacy, Kakinada, East Godavari. And the leave material were dried under the shade and ground to fine powder with the help of electrical grinder. The material was stored in airtight container for further studies. Around 100gms of

dried powder of leaves of *Triticum aestivum*<sup>[7]</sup>, is macerated using ethanol for 5 days with intermittent shaking. Then obtained extract was used for present study.

#### Preliminary photochemical studies

- Take few ml of alcoholic extract and add two drops of Dragon Dorff's reagent (Solution of potassium bismuth iodide). It gives reddish brown precipitate.
- Take few ml of alcoholic extract and add two drops of Wagner's reagent (Solution of iodine in potassium iodide) gives reddish brown precipitate.
- Take few ml of alcoholic extract and add two drops of Mayer's reagent (Potassium mercuric iodide) gives cream color precipitate.
- Take alcoholic extract and add two drops of Hager's reagent (Saturated solution of picric acid) gives yellow color precipitate.

#### Saponins

##### • Foam test or frothing test

About 1ml of Alcoholic extract and 20ml distilled water mixed and Shake well in graduated cylinder for 15 minutes. 1cm of persistent foam layer was observed. The frothing is mixed with 3 drops of olive oil and shake it vigorously. Emulsion formation indicates the presence of saponins.

#### Carbohydrates

- To leaf extract add Molisch's reagent ( $\alpha$ -naphthol in Alcohol) and few drops of  $H_2SO_4$  along the sides of test tube. It gives violet color ring.
- To leaf extract add Fehling's solution A and Fehling's solution B (1:1) then heated on water bath. Brick red precipitate of cuprous oxide was observed.
- To leaf extract add 2 ml of Benedict's reagent, mix and then boil for 2 min gives brick red precipitate.

#### PROTEINS

##### Biuret test

Mix 2 ml of extract solution with 4% w/v NaOH and add 1% w/v  $CuSO_4$  drop by drop. Purplish violet or pinkish violet color appears.

##### Xantho protein test

Mix 3 ml of extract with 1 ml of Conc.  $H_2SO_4$ . White precipitate is formed. Boil the contents. Precipitate turns to yellow. Add NaOH till It becomes alkaline, precipitate turns to orange indicates the presence of aromatic amino acids.

##### Millon's test

To 2 ml of extract add equal volume of 10% mercuric sulphate in 10%  $H_2SO_4$  and boil the contents in boiling water bath cool it and the neutralize with 1% sodium carbonate solution and finally add few drops of 1% w/v  $NaNO_2$  and warm it again. Deep red color solution formed indicates the presence of Tyrosine.

#### Tannins

- Drug extract plus dil. ferric chloride gives a blue color. This can be changed to olive green as more ferric acid added. absence of blue color indicates absence of tannins

#### Glycosides tests

- Baljet test; To plant extract and Sodium picrate alkaline gives no orange indicates absence of glycosides.
- Legal test ; To plant extract add Sod. Nitroprusside and pyridine+ alkaline gives no red color indicates the absence of glycosides.

#### Flavanoids

To ethanolic extract (5ml) was added to a conc. sulphuric acid (1ml) and 0.5gm of Mg. There was no pink or red coloration that not disappeared on standing (3 min) indicates the absence of flavanoids<sup>[8]</sup>.

#### Evaluation of in vitro Anti Inflammatory Activity Inhibition of Protein Denaturation method.<sup>[9]</sup>

Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml plant extract (separately) in suitable solvent of different concentrations (100 $\mu$ g/ml, 200 $\mu$ g/ml, and 400 $\mu$ g/ml). Test control solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control solution (0.5 ml) consists of 0.45ml of distilled water and 0.05ml of plant extract in suitable solvent of different concentrations (100 $\mu$ g/ml, 200 $\mu$ g/ml, and 400 $\mu$ g/ml). Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of different concentrations (100 $\mu$ g/ml, 200 $\mu$ g/ml, and 400 $\mu$ g/ml) of diclofenac sodium. All the above solutions were adjusted to  $P^H$  6.3 using 1N hydrochloric acid. The samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, 2.5ml of phosphate buffer was added to the above solutions<sup>[10]</sup>. The absorbance was measured using UV Visible Spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated as,

$$\% \text{ Inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test}) / \text{Absorbance of control}] \times 100}{}$$

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium.

#### HRBC Membrane Stabilization Method.<sup>[9]</sup>

The principle concerned in this method is stabilization of human red blood cell membrane by hypotonicity induced membrane lyses. Blood was collected (2ml) from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl in distilled water) and centrifuged at 3000rpm. The packed cells were washed with isosaline solution and a 10% v/v suspension

was prepared with normal saline. Different concentrations of plant extract (100µg/ml, 200µg/ml, 400µg/ml) diclofenac sodium (100µg/ml, 200µg/ml, 400µg/ml) as standard and control (distilled water instead of hyposaline to produce 100% haemolysis) were separately mixed with 1ml of phosphate buffer, 2ml hyposaline solution and 0.5ml of 10% HRBC suspension was added to prepared reaction mixture. All the assay mixtures were incubated at 37°C for 30min and

centrifuged at 3000rpm for 20 min and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. The percentage of HRBC membrane stabilization or protection was calculated by using the formula.

$$\% \text{ Protection} = \{(\text{Absorbance of control} - \text{Absorbance of test}) / \text{Absorbance of control}\} \times 100$$

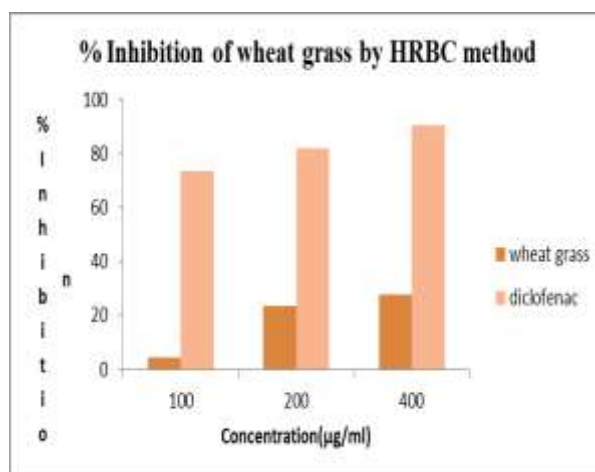
## RESULTS

The Phytochemical examination of the extract was performed by the standard methods and shows the presence of various phytochemical constituents.

| S.NO | Phytochemical constituents | Observation |
|------|----------------------------|-------------|
| 1    | Alkaloids                  | POSITIVE    |
| 2    | Glycosides                 | NEGATIVE    |
| 3    | Saponins                   | POSITIVE    |
| 4    | Flavanoids                 | NAGETIVE    |
| 5    | Carbohydrates              | POSITIVE    |
| 6    | Proteins                   | POSITIVE    |
| 7    | Tannins                    | NEGATIVE    |

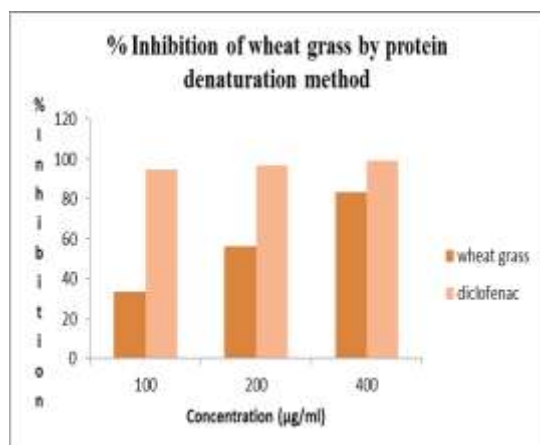
### In Vitro Anti-inflammatory Activity of wheat grass by HRBC Method

| Treatment       | Concentration(µg/ml) | % of membrane stabilization |
|-----------------|----------------------|-----------------------------|
| Wheatgrass      | 100                  | 4.26                        |
|                 | 200                  | 23.41                       |
|                 | 400                  | 27.66                       |
| Diclofenac sod. | 100                  | 73.43                       |
|                 | 200                  | 82.14                       |
|                 | 400                  | 90.40                       |



### In vitro anti-inflammatory activity of wheat grass by protein Denaturation method

| Treatment       | Concentration (µg/ml) | % of protein Denaturation |
|-----------------|-----------------------|---------------------------|
| Wheatgrass      | 100                   | 33.3                      |
|                 | 200                   | 56.3                      |
|                 | 400                   | 83.3                      |
| Diclofenac sod. | 100                   | 94.62                     |
|                 | 200                   | 96.97                     |
|                 | 400                   | 99.03                     |



## DISCUSSION

*Triticum aestivum* L. belonging to family Poaceae is a green commonly found herb in India, although its nativity has been lost today. This plant is believed to be having manifold pharmacological diversities in addition to its nutritional value which are yet to be explored. I decided to work on this plant to find out their usefulness to human being. The present works include screening of anti-inflammatory activity along with its preliminary phytochemical evaluation. There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in vitro assessment of anti-inflammatory property of ethanolic extract of wheatgrass. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

The increments in absorbance's of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by ethanol extract of wheatgrass and reference drug diclofenac sodium. Ethanol extract of wheatgrass. From percentage inhibition in both protein denaturation method and HRBC, it was more active than diclofenac sodium, being effective in lower concentrations.

In HRBC membrane stabilization method the basic principle is that the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane. Therefore as the membrane stabilizers, it interferes with the release and/or action of mediators like histamine, serotonin, prostaglandins, and leukotrienes which are responsible for inflammation.

## CONCLUSION

Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. Further investigations are required in order to isolate more new compounds from the plant extracts and to test their bioactivities with the aim of increasing the drug arsenal currently used in the treatment and prophylaxis of human and animal diseases. It is very necessary to introduce new and biologically safe and active drugs eco-friendly in nature and effective as antibacterial agents.

In this research study, the ethanolic extract of wheatgrass had reported for presence of different phytochemicals. On observation of results for anti inflammatory activity, it was concluded that *Triticum aestivum* has anti inflammatory activity.

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