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Antioxidant and Anti-Inflammatory Activity of Proteins Isolated From *Zingiber Officinale* Root

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Abstract: To investigate the *in vitro*, antioxidant and anti-inflammatory activity of the proteins isolated from *Zingiber officinale***,** proteins isolated by Ammonium sulphate precipitation method. *In vitro* antioxidant studies were carried out for the proteins using superoxide radical scavenging method and anti-inflammatory studies was done using membrane stabilization assay. The superoxide radical scavenging ability of the proteins was compared with standard antioxidant like Alpha tocopherol and Ascorbic acid at a maximum dosage of 8µg, where *Zingiber officinale* root protein showed a maximum inhibition of 55% and Alpha tocopherol and Ascorbic showed 55 and 51% respectively. The anti-inflammatory activity of *Zingiber officinale* root proteins were compared with the standard drug Diclofenac sodium, where at a dosage of 1000µg/ml, plant root protein showed maximum inhibition where as the drug Diclofenac Sodium showed at a dosage of 200µg/ml. The results of the present study concluded that the *Zingiber officinale* root proteins possess significant antioxidant and anti-inflammatory activity and it may due to the presence of proteins.

Keywords: *Zingiber officinale*, Ginger, proteins Antioxidant, Antiinflammant.

INTRODUCTION

When body tissues are exposed to harmful environmental stimuli, such as pathogens, damaged cells, or irritants, resulting in pain, redness, swelling, loss of function called as inflammation [1-2]. Anti-inflammatory drugs (NSAID) available in Pharmaceutical market reduce pain by counteracting the cyclooxygenase (COX) enzyme [3].

Clinical surveys reported, in long run, the use of NSAID drug medication, can cause gastric erosions, which can become stomach ulcers and in extreme cases can cause severe haemorrhage, resulting in death [4]. Hence, there is a need of searching of nontoxic, inexpensive, easily available dietary/herb/spices sources which can be used as antioxidant and as well as anti-inflammant [5]. In Ayurveda, Chinese practices, it is referred as Turmeric, Ginger, and Garlic had antioxidant and antiinflammant properties, and a need of scientific evaluation [6]. It is reported that, *Zingiber officinale* (Ginger) has been used for stomach upset, motion sickness, nausea, and vomiting. Some herbal/diet supplement products have been found to contain possibly harmful impurities/additives [7]. These results encouraged us to study the anti-inflammatory activity of the Ginger root.

MATERIALS AND METHODS

Extraction of proteins from *Zingiber officinale* root

10g of cleaned *Zingiber officinale* roots collected from authentic source, cleaned with 0.1% $KMnO_4$ solution, followed with double distilled water, crushed, grinded and mixed with 200 ml of boiling double distilled water and vortexed for 4 hours at 20°C using magnetic stirrer. The vortexed mixture is centrifuged at 10000 rpm for 20 minutes, the supernatant was separated. The supernatant was subjected to 65% ammonium sulphate precipitation and vortexed over night. The mixture was centrifuged at 10000 rpm. The precipitated protein was collected and subjected to dialysis using 2.5kDa molecular cutoff biomembrane against water for 76 hours with an interval of 6 hours. The dialyzed precipitated was separated and stored at -10°C for further analysis.

Stability to proteases

The *Zingiber officinale* proteins were tested for its ability to withstand with proteases like trypsin, and pepsin [8]. 500µg of *Zingiber officinale* root proteins was incubated at 37^0C for 1 hour with 20µg of trypsin in 20mM phosphate buffer pH 8.0 or 20µg of pepsin in 20mM sodium acetate buffer, pH 2.0. The reaction was arrested by keeping the tubes in ice. The incubation mixture contained *Zingiber officinale* root proteins in the presence or absence of proteolytic enzymes in a ratio of 25:1 w/w was used. Aliquots of samples were then subjected to check their antioxidant capacity

Antioxidant activity

Superoxide scavenging activity

The Superoxide radical (O2•-) scavenging activity of was measured according to the method of Lee *et al.* 2002 [9]. The reaction mixture containing 100µl of 30mM EDTA (pH 7.4), 10µl of 30mM hypoxanthine in 50mM NaOH, and 200µl of 1.42mM nitro blue tetrazolium with or without Zingiber officinale root proteins and SOD serving as positive control at various concentrations ranging from 50-300µg. After the solution was pre-incubated at ambient temperature for 3min, 100µl of xanthine oxidase solution (0.5U/ml) was added to the mixture and incubated for one hour at 37°C, and the volume was made up to 3ml with 20mM phosphate buffer (pH 7.4). The solution was incubated at room temperature for 20 min, absorbance was measured at 560 nm. Appropriate controls were included to rule out the artifacts induced reaction. The control was without any inhibitor. Inhibitory effect of *Zingiber officinale* root proteins on superoxide radicals was calculated as-

$$\% \text{ Superoxide radical scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

In vitro anti-inflammatory activity

Membrane stabilization assay

The Human Red Blood Cells (HRBC) membrane stabilization method has been used to study the anti-inflammatory activity [10-11]. Blood was collected from the healthy volunteers and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) and a suspension was made with isosaline (10%v/v). The assay mixture contained 1 ml of Phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%), 0.5ml of HRBC suspension and 1 ml of various concentration of the extract. Diclofenac sodium was used as standard drug. In the control solution, instead of hyposaline, 2ml of distilled water was added. The mixtures were incubated at 37□C for 30 min and centrifuged. The absorbance of the supernatant solution was read at 560nm spectrophotometrically. The % haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization was calculated using the formula.

$$\% \text{ membrane stabilization} = \frac{100 - \text{O.D. of drug treated sample}}{\text{OD of Control}} \times 100$$

STATISTICAL ANALYSIS

The data were expressed as means ± standard deviations (SD). All the experiments were repeated at least three times and the values are expressed as Mean ± SD. The significance of the experimental observation was checked by student's test and the value of p value.

RESULTS AND DISCUSSION

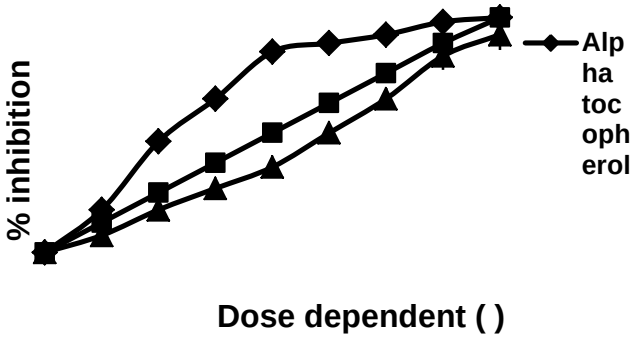

Fig -1: **Superoxide radical scavenging activity of *Zingiber officinale* root proteins: Dose dependent**

Dose-dependent Super oxide anions scavenging activity of *Zingiber officinale* root protein. The control was without protein or Alpha tocopherol or Ascorbic acid. The Super oxide radical scavenging activity was calculated accordingly as described in methods. Results are shown as mean ± SD (n = 3).

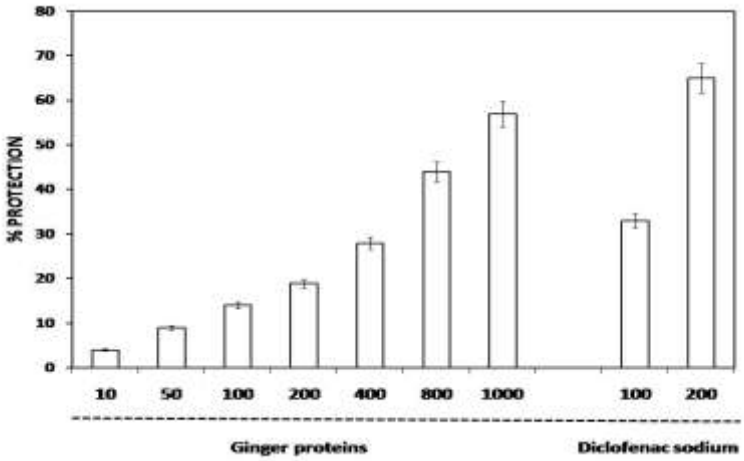

Fig-2: *In vitro* anti-inflammatory effect of *Zingiber officinale* root proteins: Dose dependent

Dose-dependent anti-inflammatory studies of *Zingiber officinale* root protein (10 to 1000µg/ml) and standard drug Diclofenac sodium (100 and 200µg/ml). Results are shown as mean ± SD (n = 3).

The boiling water extraction of *Zingiber officinale* protein was done as explained in materials and methods. Further the supernatant was subjected to Ammonium Sulphate precipitation to precipitate proteins of the root, followed with dialysis against water to removed unwanted salts and confirmed the precipitate rich with proteins by proximate analysis as explained by Mohamed Azmathulla Khan *et al*. [12]. To confirm the antiprotease activity or protease inhibitory activity of the root proteins, the *Zingiber officinale* proteins of 500µg was treated with 20µg of pepsin/trypsin. Hydroxyl radical scavenging activity was done by deoxyribose assay as described in methods. The appropriate controls were included in all the experiments. The control was without any the proteins or enzyme and the % hydroxyl radical scavenging activity was calculated accordingly. The antioxidant activity of the proteins of *Zingiber officinale*, was analyzed by the superoxide radicals scavenging activity studies. Here the superoxide radicals are the free radicals and are generated in a variety of biological systems by auto-oxidation processes or by enzymatic activities [12]. Moreover, superoxide anions produce other kinds of cell damaging free radicals and oxidizing agents [13]. Herein, we used the NBT assay system to check *Zingiber officinale* proteins scavenge superoxide radicals. As shown in Figure-1, dose dependent study was done towards scavenging superoxide radicals by *Zingiber officinale* proteins along with other antioxidants like Alpha tocopherol and Ascorbic acid. Here *Zingiber officinale* proteins inhibited NBT reduction by a maximum dose of 8µg. Alpha tocopherol shows a maximum inhibition at a dosage of 8 µg and Ascorbic shows maximum inhibition of reduction of NBT at a dosage of 8 µg. This observation indicates that *Zingiber officinale* root proteins are superoxide scavengers. It also confirms that, the root proteins are also heat stable.

Further, the anti-inflammatory activity of the *Zingiber officinale* proteins was studied by Membrane stabilization assay. The prevention of hyptonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity as HRBC membrane are similar to lysosomal membrane components [14]. As shown in figure 2, the *Muntingia Calabura* root crude proteins shows maximum anti-inflammatory activity at the concentration of 1000µg/ml which is comparable to that of standard drug Diclofenac sodium (200µg/ml). The anti-inflammatory activity of the crude protein was concentration dependent. The proteins exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is comparable to the lysosomal membrane and hence, its stabilization shows that, the *Zingiber officinale* proteins may stabilize lysosomal membrane. The above results shows that, the proteins of *Zingiber officinale* root proteins extract are good antioxidants when compared to standard antioxidants and also having anti-inflammatory studies. These results need to be confirmed by in vivo anti-inflammatory studies.

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