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IN VITRO ANTI-INFLAMMATORY ACTIVITY OF PROTEINS ISOLATED FROM PIPPALI (*PIPER LONGUM*)

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ABSTRACT: To investigate the in vitro, Antioxidant and Anti-inflammatory activity of the proteins isolated from Pippali (Piper longum). A protein of the extract was isolated by Ammonium sulphate protein precipitate method. In vitro antioxidant studies were carried out for the Piper longum proteins using superoxide radical scavenging method and anti-inflammatory studies was done using membrane stabilization assay were performed for Piper longum proteins. The superoxide radical scavenging ability of the proteins was compared with standard antioxidant like BHA and Ascorbic acid at a maximum dosage of 8µg, where Piper longum protein showed a maximum inhibition of 48% and BHA and Ascorbic showed 60% respectively. The anti-inflammatory activity of Piper longum proteins were compared with the standard drug Diclofenac sodium, where at a dosage of 1000µg/ml, Piper longum protein showed maximum inhibition whereas the drug Diclofenac Sodium showed at a dosage of 200µg/ml. The results of the present study concluded that the Piper longum proteins possess significant antioxidant and anti-inflammatory activity and it may due to the presence of proteins.

KEYWORDS: *Piper longum*, proteins, Antioxidant, Anti-inflammatory, Drug, Diclofenac sodium

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1.INTRODUCTION

The response of body tissues to harmful environmental stimuli resulting in pain, redness, swelling, loss of function etc is generally known as Inflammation [1][2]. The property of a substance that reduces inflammation or swelling is its anti-inflammant nature. Now a days, non- steroidal anti-inflammatory drugs (NSAID) available in Pharmaceutical market which lighten pain by counteracting the cyclooxygenase (COX) enzyme, where it synthesizes prostaglandins and creating inflammation [3]. The use of NSAID drug commonly have side effects, that, in the long usage of these anti-inflammants, can cause gastric erosions, leads to stomach ulcers and in extreme cases can cause severe haemorrhage, resulting in death[4]. There is a need of searching of nontoxic, inexpensive, easily available herbal sources or medicinal plant sources or dietary sources, which one can have as a part of dietary component as an antioxidant and as well as anti- inflammant. In Ayurveda, Unani, Chinese medicine it is stated that, herbs like Turmeric, Ginger, Pippali and Garlic had antioxidant and antiinflammant properties and these types of preparations are not scientifically evaluated. Pippali reduces excess body weight, it diminishes obesity, clears the skin, smooths wrinkles and slows down aging. It was reported that, Pippali freshens the lungs as it improves the flow of oxygen through enhanced vasodilation. It has been used to fortify the bones and support skeletal integrity. Working from the inside out it heals the bone marrow first then relieves conditions like backpain, arthritis, rheumatism, fractures, sprains and broken or brittle bones. Pippali's warming qualities invigorates the blood and tone the heart muscle. With improved circulation all the vital organs are oxygenated and function more efficiently. It treats heart disease, sciatica, impure blood, numbness and poor circulation. infertility, nausea, parasites These results encouraged us to study the anti-inflammatory activity of Pipalli proteins [5] [6] [7] [8] (Khalaf et al., 2008, Reddy et al., 2014, Karthikeyan and Rani, 2003, Shah et al., 2008).

2. MATERIALS AND METHODS

Extraction of proteins from Piper longum:

10g of cleaned Piper longum collected from authentic source and powdered (British Pharmacopoeia 100 mesh) and stored in glass bottle.The powder mixed with 200 ml of double distilled water and

vortexed for 4 hours at 20^0C using magnetic stirrer. The vortexed mixture is centrifuged at 8000 rpm for 20 minutes, the supernatant was separated. The supernatant was subjected to 55% ammonium sulphate precipitation and vortexed overnight.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 -10^0C for further analysis.

Stability to proteases

The proteins of Piper longum were tested for its ability to withstand with proteases like trypsin, and pepsin. 500µg of Piper longum proteins was incubated at 370C 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 Piper longum 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.[9] with minor modifications. 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 Piper longum 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 Piper longum 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 37^0c for 30 min 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.

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

Statistical analysis

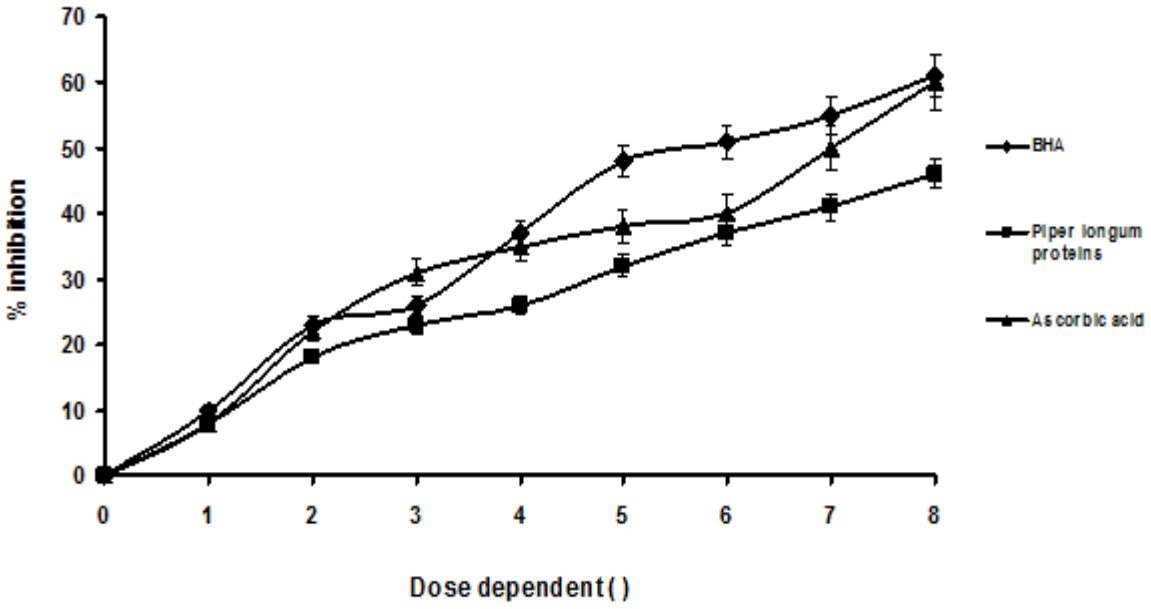
The data were expressed as means ± standard deviations (SD). All the experiments were repeated at least three times and the values are expressed as Mean ± SD. The significance of the experimental observation was checked by student's test and the value of p value.

3. RESULTS AND DISCUSSION

The protein from water extract of Piper longum was done as explained in materials and methods. Further the supernatant was subjected to Ammonium Sulphate precipitation to precipitate proteins, 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., 2015[12]. To confirm the antiprotease activity or protease inhibitory activity of the Piper longum 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 Piper longum proteins or enzyme and the % hydroxyl radical scavenging activity was calculated accordingly (Results not shown). The antioxidant activity of the proteins of Piper longum 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 [13]. Herein, we used the NBT assay system to check Piper longum proteins scavenge superoxide radicals. As shown in Figure-1, a dose dependent study was done towards scavenging superoxide radicals by Piper longum proteins along with other antioxidants like BHA and Ascorbic acid. Here Piper longum protein inhibited NBT

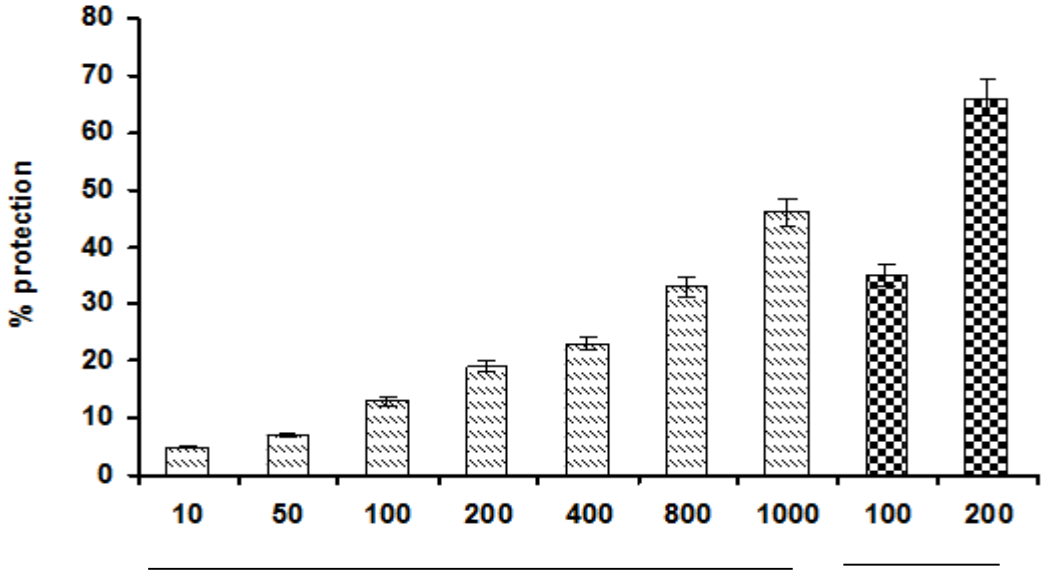
reduction by a maximum dose of 8µg. BHA shows a maximum inhibition at a dosage of 7 µg and Ascorbic shows maximum inhibition of reduction of NBT at a dosage of 8 µg. This observation indicates that Piper longum proteins are superoxide scavengers. Further, the anti-inflammatory activity of the root proteins 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 Piper longum 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 Piper longum proteins 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 Piper longum proteins may stabilize lysosomal membrane. The above results shows that, the proteins of Piper longum proteins 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.

Fig.-1 Superoxide radical scavenging activity of Piper longum proteins: Dose dependent



Dose-dependent Super oxide anions scavenging activity of Piper longum proteins. The control was without protein or BHA or Ascorbic acid. The Super oxide radical scavenging activity was calculated accordingly as described in methods. Results are shown as mean ± SD (n = 5).

Fig. -2: In vitro anti-inflammatory effect of *Piper longum* proteins: Dose dependent



***Piper longum* proteins** **Diclofenac sodium (std drug)**

Dose-dependent anti-inflammatory studies of *Piper longum* proteins (10 to 1000µg/ml) and standard drug Diclofenac sodium (100 and 200µg/ml). Results are shown as mean ± SD (n = 5).

REFERENCES

1. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE, Nielsen, Andersen, Girardin, Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation; Clin. Exp. Immunol; 2007; (2) 147.
2. Khatami M. "Yin and Yang" in inflammation: Duality in innate immune cell function and tumorigenesis.Exp. Opin.Biol. Ther. 2008; 8 :1461–1472.
3. Rock, Kenneth L, Eicke Latz, Fernando Ontiveros, Hajime Kono, The sterile inflammatory response; Annual review of immunology; 2010; 28; 321.
4. Maroon JC, Bost JW, Maroon A. Natural anti-inflammatory agents for pain relief; Surgical Neurology International; 2010;1:80.
5. Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z and Farah H. Antioxidant activity of some common plants. Turkish Journal of Biology. 2008, 19;32(1):51-5.

6. Reddy NJ, Vali DN, Rani M and Rani SS. Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by Piper longum fruit. Materials Science and Engineering: C. 2014, 1;34:115-22.
7. Karthikeyan J and Rani P. Enzymatic and non-enzymatic antioxidants in selected Piper species. Indian journal of experimental biology. 2003, 41(2):135-40.
8. Shah A, Kulkarni M and Juvekar AR. Protective effect of Piper longum L. on oxidative stress induced injury and cellular abnormality in adriamycin induced cardiotoxicity in rats. Indian journal of experimental biology. 2008;46:528-33.
9. Lee JC, Kim HR, Kim J, Jang YS; Antioxidant activity of ethanol extract of the stem of opuntia ficus-indica var. saboten. J. Agri. Food Chem; 2002; 50; 6490-6496.
10. Gandhidasan R, Thamaraichelvan A, Babraj S, Anti-inflammatory action of Lannea coromandelica by HRBC membrane stabilization, Fitoterapia; 1991; 62; 81-83.
11. Latha S, Fatima Grace X, Shanthi S, Chanmundeeswari D, Seethalakshmi S, Uma Maheswara Reddy C, In vitro antioxidant and anti-inflammatory activity of methanol extract of Stereospermum colais (Buch.-Ham. Ex.Dillw), Sri Ramachandra Journal of Medicine; 2011, Vol. 4, Issue 1.
12. Mohamed Azmathulla Khan Y, Dinesha Ramadas, Subhas Chandrappa Mundasada, Santosh Kumar N, Chikkanna D, Antioxidant and free radical scavenging activities of root extracts of Muntingia Calabura, Sch. J. App. Med. Sci; 2015; 3(6B):2309-2312.
13. Liu F, Ng TB, Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Sci. 2000, 66: 725-735.
14. Chou CT, The anti-inflammatory effect of Tripterygium wilforihook on adjuvant induced paw edema in rats and inflammatory mediators release, Phyther Res; 1997; 11; 152-54.