



Research Article

IN VITRO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF ROOT AND FRUIT OF GOKSHURA (*TRIBULUS TERRESTRIS* LINN.)

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ABSTRACT

Gokshura (*Tribulus terrestris* Linn, Zygophyllaceae), a medicinal plant mentioned in ayurvedic classics is mainly known for its effect on filtration defects of renal system and urinary tract infections. The present study was performed as an attempt to prove the anti-inflammatory and anti arthritic activity of kashaya (decoction/ aqueous extract) of the two useful part, root and fruit of Gokshura by invitro Protein denaturation and Proteinase enzyme inhibition test. Both root and fruit of Gokshura (*Tribulus terrestris* Linn) were found to be effective in inhibiting protein denaturation. Maximum inhibition of 82.89%% was observed for root kashaya and 74.81% was observed for fruit kashaya at 500 µg/ml concentration. A significant inhibition of 79.94% proteinase enzyme activity was exhibited by fruit of Gokshura and root kashaya showed 45.29% at the concentration of 500 µg/ml. Thus the study revealed the presence of anti-inflammatory and anti arthritic action in both root and fruit of Gokshura. This is a preliminary study and further investigations are required to find active component of the extract and to confirm the mechanism of action.

Keywords: Anti-inflammatory, Anti-arthritic, Gokshura, *Tribulus terrestris* Linn., Protein denaturation, Proteinase inhibition

INTRODUCTION

Gokshura is a medicinal plant mentioned in ayurvedic classics and the source plant is *Tribulus terrestris* Linn of Zygophyllaceae Family. It is a procumbent herb found in the waste lands and dry habitats throughout the warmer regions of India. It is a major ingredient of Ayurvedic formulations like Dasamoola katutrayam kashaya, Dhanwantharam kashaya, Maharasnadi kashaya, Dasamoolarishtam, Yogaraja guggulu and Amritharishtam etc which are very much effective in inflammatory and arthritic conditions. This herb is mainly known for its effect on filtration defects of renal system and urinary tract infections in Indian medicine. The therapeutic indication of Gokshura almost includes the symptoms of vatavyadhi (arthritic conditions) like ruja (pain), sophā (edema), dourbalya (loss of strength) etc.

Inflammation is the body's attempt at self-protection that aims to remove harmful stimuli, including damaged cells, irritants, or pathogens - and to begin the healing process. It is a body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues. Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as, the increase of vascular permeability, increase of protein denaturation and membrane alteration¹. Inflammatory diseases including different types of rheumatoid disease are a major cause of morbidity of the working force throughout the world. Arthritis is acute or chronic inflammation of a joint, often accompanied by pain and structural changes². India has a high prevalence of Arthritis with about 15% people i.e. over 180 million people affected by it³. Arthritis results in inflammation of the joints and, in particular, the synovial membrane that

covers them (synovitis). The inflammation in the joints causes damage to the cartilage and sometimes to the bone ends. If this process is not halted, the cartilage damage can result in severe pain, swelling, stiffness and finally will leads to the deformities or destruction of the joint. The current treatment of arthritis includes minimization of this associated pain and inflammation using non-steroidal anti-inflammatory drugs (NSAIDs) as well as deceleration of disease progression using anti-rheumatic drugs. But beside the target action, NSAIDs are well known for its adverse reactions and arthritic patients usually search for any alternative treatments including traditional methods. Protein denaturation and Proteinase enzyme inhibition test provides basic data on the anti-inflammatory and anti arthritic activity of a drug.

Hence the present study has been performed as an attempt to prove the anti-inflammatory and anti arthritic activity of the two useful part, root and fruit of Gokshura (*Tribulus terrestris* Linn).

MATERIALS AND METHODS

Plant material

The genuine root and fruit of Gokshura (*Tribulus terrestris* Linn) was collected from its natural habitat, cleaned by thorough washing and dried in shade. The root and fruit were coarsely powdered separately and were kept in air tight containers.

Preparation of Kashaya (decoction/ aqueous extract)

48 g of coarsely powdered root was boiled with 768ml water and reduced to 96 ml according to the ratio of kashaya preparation mentioned in Sarngadhara samhitha⁴, a classical

ayurvedic text book. In the same way the kashaya of fruit of Gokshura was also prepared.

Procedure

Inhibition of protein denaturation method⁵

The Test solution consists of 0.45 ml of bovine serum albumin and 0.05 ml of Test solution (decoction of both root and fruit of Gokshura (*Tribulus terrestris* Linn)). The Test control consists of bovine serum albumin and 0.05 ml of distilled water. Product control consists of 0.45 ml of distilled water and 0.05 ml of Test solution. Standard solution consists of 0.45 ml of Bovine serum albumin and 0.05 ml of Diclofenac sodium. All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 minutes and the temperature was increased to 57°C for 3 minutes. After cooling, added 2.5ml of phosphate buffer to the solutions. The absorbance was measured using UV-visible spectrophotometer at 416nm. (Note: Stock concentration 10mg/ml)

Calculation

The percentage inhibition of protein denaturation can be calculated as

$$\% \text{ inhibition} = 100 - \left[\frac{(\text{optical density of test solution} - \text{optical density of product control})}{\text{optical density of test control}} \right] \times 100$$

Inhibition of Proteinase enzyme activity⁶

Proteinase inhibitory activity was performed according to the modified method. The reaction mixture (2ml) was containing 0.06mg Trypsin, 1ml 20Mm Tris HCl buffer (pH 7.4) and 1ml Test sample of different concentration 62.5- 500µg/mL from a stock concentration of 10 mg/ml. The mixture was incubated at 37°C for 5 minutes. Then 1ml of 0.8 % (w/v) casein was added. The mixture was incubated for an additional 20 minutes. Then 2ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged at 3000rpm for 10 minutes. The absorbance of the supernatant was read at 200 nm against buffer as blank. The experiment was performed in triplicates. The percentage of proteinase inhibitory activity can be calculated as,

Calculation

$$\text{Percentage inhibition} = \left(\frac{100 - ((A \text{ of test} - A \text{ of product control}) / A \text{ of Control}) \times 100}{A} \right) \times 100$$

A=Absorbance

Table 1. Effect of Root and Fruit of TT on protein denaturation

Concentrations (µg/mL)	Percentage of inhibition		
	Diclofenac sodium Standard	TT Root	TT Fruit
62.5	50.77	61.07	23.46
125	74.27	61.84	40.49
250	81.13	77.52	60.49
500	89.36	82.89	74.81

TT= *Tribulus terrestris* Linn.

Table 2. Effect of Root and Fruit of TT on proteinase inhibitory action

Concentrations (µg/mL)	Percentage of inhibition		
	Diclofenac sodium Standard	TT Root	TT Fruit
62.5	74.67	6.95	14.57
125	81.27	22.31	47.91
250	85.99	42.94	71.00
500	90.66	45.29	79.94

TT= *Tribulus terrestris* Linn.

RESULTS AND DISCUSSION

The root and fruit of Gokshura (*Tribulus terrestris* Linn) were analyzed for invitro anti inflammatory and anti arthritic action by Protein denaturation and Proteinase inhibition test. The high solubility of constituents of both root and fruit in water than that in alcohol supports the selection of dosage form as kashaya.

Inhibition of protein denaturation method

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured¹. Denaturation of tissue proteins may be the cause behind the production of auto-antigens in certain arthritic diseases. So, it may be said that tissue protein denaturation is a marker for inflammatory and arthritic conditions. Thus, protection against protein denaturation, which was the main mechanism of action of NSAIDs postulated by Mizushima (1964) before the discovery of their inhibitory effect on cyclooxygenase (Vane, 1971), may play an important role in the antirheumatic activity of NSAIDs⁷.

From the results of present study, it can be stated that the kashaya of root and fruit of Gokshura (*Tribulus terrestris* Linn) are effective in inhibiting protein denaturation. The present findings exhibited a concentration dependent inhibition of protein denaturation by both the test samples throughout the concentration range of 62.5- 500µg/mL (Table.1). Maximum inhibition of 82.89% was observed for root kashaya and 74.81% was observed for fruit kashaya at 500 µg/ml. Diclofenac sodium, a standard drug showed the maximum inhibition 89.36% at the concentration of 500 µg/ml.

Inhibition of Proteinase enzyme activity

In rheumatoid arthritis and other inflammatory joint diseases, large numbers of leucocytes may enter the synovial fluid⁸. Neutrophils localized at lysosomes are rich source of serine proteinase. During inflammatory reactions leukocytes proteinase play a key role in the development of tissue damage and significant level of protection was provided by proteinase inhibitors⁶. The fruit and root kashaya of Gokshura (*Tribulus terrestris* Linn) exhibited significant antiproteinase activity at different concentrations as shown in Table. 2. Fruit kashaya showed maximum inhibition of 79.94% and root kashaya

showed 45.29% at the concentration of 500 µg/ml. The standard drug Diclofenac sodium showed the maximum inhibition 90.66% at the concentration of 500 µg/ml. Therefore the fruit of Gokshura exhibited higher antiproteinase activity than the root.

So the results of the study reveals that both root and fruit of Gokshura (*Tribulus terrestris* Linn) are capable to inhibit denaturation of protein and are effective as proteinase inhibitor and may be one of the reasons for possessing anti-arthritis activity. Therefore, the in vitro studies on aqueous extract of root and fruit of Gokshura demonstrate the significant anti-inflammatory and anti-arthritis activity. As both of the useful parts are effective use of fruits, which are more available should be preferred, so that destructive harvesting for roots can be avoided.

CONCLUSION

Gokshura (*Tribulus terrestris* Linn) is a common drug in Ayurveda system for inflammatory and arthritic conditions. Here by carrying out Protein denaturation and Proteinase inhibition test, an initial step to validate the pharmacological actions was laid. By Protein denaturation method, maximum inhibition of 82.89% was observed for root kashaya and 74.81% was observed for fruit kashaya at 500 µg/ml concentration. A significant inhibition of 79.94% proteinase enzyme activity was exhibited by fruit of Gokshura and root kashaya showed 45.29% at the concentration of 500 µg/ml. The study revealed the presence of anti-inflammatory and anti arthritic action in both root and fruit of the source plant. This is a preliminary study and further investigations are required to find active component of the extract and to confirm the mechanism of action.

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